# Studies on *Paris polyphylla* Smith from Sikkim Himalaya: Ecology, Physiology and Regeneration

A Thesis Submitted

To

**Sikkim University**

In Partial Fulfilment of the Requirement for the

**Degree of Doctor of Philosophy**

By

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January, 2021

# DECLARATION

I do hereby declare that the present Ph.D thesis entitled **"Studies on *Paris polyphylla* Smith from Sikkim Himalaya: Ecology, Physiology and Regeneration"** submitted by me for the award of the degree of Doctor of Philosophy (Botany) is a *bona fide* research work carried out be me at the Department of Botany, Sikkim University under the supervision of **Dr. Dhani Raj Chhetri.** The thesis contains no material which has been accepted for a degree or diploma of any other University or Institution, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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## ABBREVIATIONS

| | |
|---|---|
| ABA | Abscisic acid |
| ABTS | 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) |
| BAP | 6-Benzyl amino purine |
| CAT | Catalase |
| DM | Dry Mass |
| DNA | Deoxy ribonucleic acid |
| DW | Dry Weight |
| DPPH | 2, 2-diphenyl-1-picrylhydrazyl |
| EDTA | Ethylene diamine tetraacetate |
| EH | Eastern Himalayas |
| EtOH | Ethanol |
| FW | Fresh Weight |
| $GA_3$ | Gibberellic Acid |
| GAE | Gallic acid equivalents |
| HPLC | High Performance Liquid Chromatography |
| IAA | Indole-3-Acetic Acid |
| IBA | Indole-3-Butyric Acid |
| $IC_{50}$ | Inhibitory concentration (inhibits 50 %) |
| Kn | Kinetin |
| M | Molar |
| NAA | 1-Naphthaleneacetic Acid |
| PCA | Principal Component Analysis |
| PD | Parkinson's disease |
| POX | Peroxidase |
| PVP | Polyvinyl Pyrrolidone |
| QE | Quercetin equivalents. |
| ROS | Reactive oxygen species |
| RT | Retention Time |
| RtE | Rutin equivalents |
| SE | Standard Error |
| SOD | Superoxide Dismutase |
| WHO | World Health Organization |
| WP | Woody Plant |

## 1. INTRODUCTION

The Eastern Himalaya as part of the Indo-Burma Mega biodiversity hotspot is one of the world's 35 critical centers of biological diversity (Sloan et al., 2014). More than 50% of the plant wealth of India has been reported in the northeastern region of Indian sub-continent (Mao, 1993).The Sikkim Himalaya as an integral part of Eastern Himalaya that lies between 27°5'-28°10' N latitudes and 88°4'-88°55' E longitudes covering an area of 7,096 km² and lies sandwiched between the kingdom of Nepal in the West and the kingdom of Bhutan in the East. It is an important component of the biodiversity hot-spot of the Himalayas of India which is characterized by a wide array of climatic zones, a rich floral diversity, many endemic elements and a number of species which have become rare, threatened or endangered (Pandey, 1991). The altitudinal variation of the region ranges from 225m to 8598m (Chib, 1977) and due to its peculiar climatic conditions presents diverse topological conditions, many distinct ecological niches and a plethora of species composition (Dahal et al., 2017). This region is a home to many floral species of high botanical value and rich ethnomedicinal traditions of the locality. Naturally, it is an important place for herbal drug research with the possibilities of future Drug Development.

*Paris polyphylla* (Sm.), belonging to family Melanthiaceae and commonly known as 'Love Apple' or the trade name 'Satuwa' is an economically important medicinal plant. It is a perennial herb growing on moist humus rich soil of the Himalayas. The name '*Paris*' comes from 'Par' meaning equal, and it refers to the symmetry of the flower. While the species '*polyphylla'* means many leaves (Shah et al., 2012). The plant is highly threatened in the Himalayan region, mainly due to anthropogenic activities like habitat destruction, rampant collection prior to seed setting, grazing and also by climate change causing phenomenon like global warming, range-shift

etc. The cumulative destructive effect is mainly bolstered by illegal trade which has threatened this species with extinction if no immediate action is taken. In the state of Sikkim, *P. Polyphylla* is categorized as vulnerable category (Vu-R) under IUCN Status (Ved et al., 2017). However, the situation is much graver and it has since been critically threatened, though there is no such official declaration. *P. polyphylla* had already been categorized as an endangered species in Himachal Pradesh, Jammu and Kashmir and Uttarakhand (Paul et al., 2015).

In the traditional medicine system, the rhizome of *P. polyphylla* is highly valued for its use in the cure of stomach aches, as antispasmodic, digestive, vermifuge, anthelmintic, expectorant and tonic (Baral and Kurmi, 2006). The root paste of the plant is applied as an antidote to insect and snake bite. The root is locally used for curing fever, headache, wounds and burns. It is also chewed to heal wounds inside the throat. In Sikkim Himalaya, the dried rhizome (1.0-1.5 cm) is soaked overnight in a glass of water and the water drank in the following morning to cure bodyache and as a tonic by the Lepcha people. The rhizome pieces are also fed to cattle suffering from diarrhoea and dysentery. Interestingly, the Chinese e-commerce platform *Alibaba.com* lists 97 items for marketing each having *P. polyphylla* as an ingredient (Cunningham et al., 2018).

Steroidal saponins are the major active chemicals found in *P. polyphylla* which are an important class of natural products and composed of a C-27 aglycone moiety and sugar chains. These compounds are classified as spirostanol saponins with sugar chain at C-3 position and furastanol saponins with sugar chains at C-3 and C-26 positions (Sprag et al., 2004). Jun (1989) isolated 12 steroidal saponins from *Paris* spp. and grouped these into three groups viz. diosgenin, pennogenin and 24-hydroxy pennogenin saponins. In general, saponins with diosgenin, pennogenin or

prosapogenin and their congeners as the aglycones constitute the most abundant types of steroidal saponins in *P. polyphylla* rhizome (Man et al., 2013). Other saponins like polyphyllin A-H has been isolated from the rhizome of *Paris polyphylla* of which first six are spirostanol steroidal saponins and remaining two are furastanol steroidal saponins (Singh et al., 1980).

Phytochemical study showed that the main components of *P. polyphylla* steroidal saponins displayed a potential cytotoxicity against various tumour cells, such as CCRF leukaemia cells, ECA109 oesophageal cancer cells etc (Li et al. 2012). *Paris* spp. rhizomes displayed a potent anticancer activity that elicited programmed cell death (PCD) and inhibited metastasis in murine lung adenocarcinoma, due to its diosgenin and pennogenin contents (Man et al. 2011). Pennogenin steroidal saponins not only inhibited cancer cell proliferation and in addition but also induced cancer cell apoptosis. Thus, these saponins may provide clues for designing novel anti-cancer agents (Zhu et al. 2010). The administration of saponins of *P. polyphylla* against lung adenocarcinoma, significantly inhibited the tumour growth and increased apoptosis, the anticancer effect of the compound was dependent on the compounds' structure to a certain extent (Yan et al. 2009). Polyphyllin-D, another major steroidal saponin of *P. polyphylla* acts on breast cancer cell lines, MCF-7(estrogen sensitive) and MDA-MB-231 (estrogen insensitive) and cause their apoptosis, possibly by disturbing the mitochondrial membrane integrity (Lee et al. 2005). Polyphyllin-D also bypasses multidrug resistance (MDR) and induces PCD in multi drug resistant R-HepG2 hepatocarcinoma cells (Lee et al. 2009).

Besides its anticancer effects, the antifungal activity of steroidal saponins from *P. polyphylla* has been seen against *Cladosporium cladosporoides* and *Candida* spp. (Deng et al. 2008). Enterovirus 71 (EV71) and coxsackievirus B3 (CVB3) belong to

the family Picornaviridae and are with single-positive-stranded RNA. EV71 infections are generally mild, usually causing hand-foot-and-mouth disease (HFMD) and acute respiratory disease. In the Asia-Pacific region, EV71 has also resulted in severe central nervous system syndrome and pulmonary edema (Lin et al. 2003; Nolan et al. 2003). CVB3 is an important human pathogen, having a high correlation with serious viral diseases such as myocarditis (Henke et al. 1995). Apoptosis of myocardial tissue in inflamed areas may contribute to CVB3-associated pathogenesis (Henke et al. 2000). The severe pathogenesis of EV71 and CVB3 is mainly caused by the high viral replication rate in the host cells along with the host's immune response cascade (Lin et al. 2003; Wang et al. 2007). *P. polyphylla* exhibited appreciable anti-enterovirus (EV71) and coxsackieviruses (CVB3) activities, and significant enhancement of IL6 levels in both of these types of infected cells (Wang et al. 2011). Total steroidal saponins extracted from the rhizome of *P. polyphylla* possesses direct uterotonic activity and spirostanol saponins, especially penonogenin glycosides are found to be active in stimulating myocardial contractions (Guo et al. 2008). It was also found that diosgenyl saponins showed immune-modulatory activities and the glucoside moieties of diosgenyl saponins are essential for the activation of immunological reactions especially during the period of oxygen consumption such as in the process of inflammation and microbial activity (Zhang et al. 2007).

Carcinogenesis may be characterized as the formation of a cancer, whereby ordinary cells are transformed into cancer cells or the body starts unrestrained development (Tariq et al. 2015). Cancer is a disease complex with more than 100 distinct disorders, involving abnormal cell growth which can invade or spread to other body parts and result in their dysfunction (Sirsat and Kokate, 2019). From an experimental

point of view, a compound is considered carcinogenic when its administration to laboratory animals induces a noteworthy rise in the incidence of one or more histological types of neoplasia, when compared with the animals in the control group which are not exposed to the substance (Gutierrez and Salsamendi, 2001). Among the non-communicable diseases, cancer is the second foremost cause of death, after cardiovascular disease (Desai, 2008). It may be uncontrollable and incurable, and may occur at any time, age and any part of the body (Abdullahi et al. 2018).

According to Tariq et al. (2015), 64 ethno-medicinal plants from the Himalayan region are being used for treating different types of cancer. Bhatia et al. (2011) listed some important Himalayan plants with anti-cancer activity viz., *Podophyllum hexandrum, Rheum emodi, Saussurea costus, Swertia chirayita* and *Taxus baccata* etc. In another survey, a total of 36 species of medicinal plants belonging to 32 genera of 28 families were found in the Indian Himalayan Region (IHR) with anti-cancerous property. These species included *Ageratum conyzoides, Berberis aristata, Dicranopteris linearis, Woodfordia fruticose* and *Streblus asper* (Samarat et al. 2011). The anticancer plants enumerated by Tariq et al. (2015) included plants like; *Arnebia euchroma, Bidens pilosa, Capsella bursapastoris, Potentilla fulgens* and *Verbascum thapsus* etc. Roshan et al. (2017) reported that *Arisaema jacquemontii* Blume which is native to Afghanistan, China, India, Kashmir, Nepal, Pakistan and Sikkim exhibited anticancer activities. The anticancer activity of *Euonymus hamiltonianus,* a medicinal plant collected from Kashmir could be due to the presence of diterpenoid compounds (Mushtaq et al. 2017).

Reactive oxygen species (ROS) are a group of free radicals, and ions that are derived from $O_2$ (Sharma et al. 2012). ROS are generated during mitochondrial oxidative metabolism as well as in cellular response to xenobiotics, cytokines, and bacterial invasion. These reactive species are the by-products of numerous enzymatic reactions in various cell compartments (Forrester et al. 2018) and plays important roles in the modulation of cell survival and other physiological functions (Abdal et al. 2017). Oxidative stress refers to the imbalance due to excess ROS or oxidants over the capability of the cell to mount an effective antioxidant response (Ray et al. 2012). The rapid generation of reactive oxygen species (ROS) is one of the hallmarks of plant responses to various biotic and abiotic stresses (Sewelam et al. 2016). It was concluded that there were approximately 40 antioxidant cum anticancer plants used medicinally in India. Among these around 27 Species from Indian Himalayan Region were exclusively used for their antioxidant properties (Sirsat and Kokate, 2019).

From among the Himalayan plants, the root extracts of *Arnebia benthamii* collected from Kashmir Himalaya, was found to have remarkable antioxidant property (Shameem et al. 2015). *Bergenia ciliata, Riccinus communis* and *Zinziber officinalis* which grows in the Himalayas also possess antioxidant property (Ali et al. 2016). Ayoub, (2018) highlighted a few Himalayan medicinal plants with antioxidant properties viz. *Adiantum capillus veneris, Curcuma longa, Ocimum sanctum, Litsea glutinosa,* and *Arnebia benthamii* etc.

Cancer cells are under continuous oxidative stress (Pervaiz and Clememt, 2004; Kryston et al. 2011) due to the generation of ROS which are in turn involved in the prosses of carcinogenesis. Cancer cells produce ROS at a much higher rate than healthy cells (Lu et al. 2007). Antioxidants play an important role by inhibiting the

initiation step of oxidation of biomolecules as well as scavenging various free radicles (Chejara et al. 2014). Therefore, natural antioxidants are popular for their therapeutic efficacy which ensures prospecting for bioactive plant products an important area of research. Anticancer properties of herbal drugs, in most cases are related to their antioxidant activities (Lepcha et al. 2019). Antioxidants can protect living organisms from DNA/protein damage and lipid peroxidation caused by reactive oxygen species (Li et al. 2007). Antioxidants play a role in ameliorating DNA damage, reducing the rate of abnormal cell division, and decreasing mutagenesis. Therefore, many antioxidant-rich plants possess anticancer activity (Al-Dabbagh et al. 2018).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half a century have been applied towards combating cancer. Some promising anticancer drug obtained from plants are vinblastine, vincristine, campothecin, colchicine, berberine, lycopene, resveratrol, apigenin, curcumin, scutellarin, ajmalicine, podophyllin, podophyllotoxin, 10-deacetyl baccatin and taxol etc. (Iqbal, 2017, Agarwal et al. 2012)

Ecological niche modeling (ENM) uses computer algorithms to generate predictive maps of species distribution in a geographic space. Predicting and mapping the suitable habitat for endangered species is critical for monitoring and restoration of their declining native populations (Kumar and Stohlgren, 2009). In Sikkim Himalaya, *P. polyphylla* is found only in some restricted high-altitude areas and this species was categorized as vulnerable in 2014 (Ved et al. 2017). Moreover, continuous anthropogenic activities within the past few years have drastically decreased the population (Lepcha et al. 2019). In this study, ENM of *P. polyphylla*

has been undertaken to assess the spatial scale of proposed distribution of the species in Sikkim Himalaya to determine the suitable sites for future reintroduction of the species.

Plants produce secondary metabolites as a defense mechanism against attack by pathogens and insects. Plant secondary metabolites are having the great application in human health and are of commercial importance in pharmaceuticals, food additives, flavors, and other industrial materials (Jeong and park, 2005). Abiotic stress, including those imposed various elicitor molecules, often induces the secondary metabolite production in the plants. Elicitors are the chemical compounds from abiotic and biotic sources that can stimulate stress responses in plants, leading to the enhanced synthesis and accumulation of secondary metabolites (Naik and Al–Khayri, 2016). Raomai et al. (2015) observed that the steroidal saponin accumulation of *P. polyphylla* was enhanced by elicitor treatment. The focus of the present study is the elicitor treatment for enhancement of desired secondary product namely diosgenin compound in *P. polyphylla* from two altitudinal regions of Sikkim.

*P. polyphylla* is being used in the Himalaya for various purposes as mentioned earlier (Maity et al. 2004, Jamir et al. 2012, Sharma and Samant, 2014). In addition, it is used in China to treat liver cancer since a long time (Lee et al. 2015). However, there had not been any study on the antioxidant and anticancer properties of the plant from Sikkim Himalaya. Therefore, *P. polyphylla* Smith from Sikkim Himalaya was selected in order to understand its ecological variations, make predictive studies for its reintroduction, devise its conservation and try to modulate the secondary metabolite production by imposing elicitor modulated stress. Study of its antioxidative and anticancer effects where the study is also aimed to understand its therapeutic potential.

**The presented research work on *Paris polyphylla* Smith from Sikkim Himalaya was carried out with the following specific objectives:**

1. To study the different ecotypes and phenology at different stages of *Paris polyphylla* from this region.

2. To map and de-limit the potential distributional areas of *Paris polyphylla* in Sikkim Himalaya.

3. Plant growth regulator induced modulation of biochemical and phytochemical constituents in *Paris polyphylla*."

4. To develop a complete regeneration package for the propagation of the plant.

# 2. LITERATURE REVIEW

## 2.1. Taxonomic description of *Paris polyphylla*

### 2.1.1. Different species belonging to the genus *Paris* L.

The history of the plant, *Paris polyphylla* can be traced to the genus Daiswa proposed by Rafinesque in 1838 to accommodate this single species. However, Takhtajan, (1983) separated *Daiswa* Raf. from *Paris* L. He was of the view that *Paris* is a collective genus consisting of three distinct genera viz., *Paris* (4 species), *Kinugasa* (1 species) and *Daiswa* (15 species). Osaloo and Kawano, (1999) described 20 species of *Paris* distributed globally, though mostly species being restricted to Asia.

Trilliaceae was once a part of the family Liliaceae. However, later on it was concluded that Trilliaceae members are clearly separated from Liliaceae (Sterenson and Loconte, 1995). Consequently, Trilliaceae was placed within a larger clade containing Melanthiaceae. However, based on the difference in its sequences the families were described separately as family Trilliaceae and Melanthiaceae (Baldwin et al. 1995). Naturally, the genus *Paris* L. placed in family Melanthiaceae (Farmer and Schilling, 2002). Li, (2003) described 24 species of *Paris* within family Melanthiaceae of these except for *P. quadrifolia* and *P. incompleta,* all other species are restricted to Asia. As per phylogenetic analysis based on DNA sequence data *Paris* was classified as a single genus rather than 3 genera (*Daiswa, Kinugasa* and *Paris* s. s.) and the genus *Paris* contains two subgenera: subgenus *Paris* and subgenus *Daiswa* (Ji et al. 2006).

In the light of the above discussion, the genus *Paris* is a temperate perennial herb that belongs to Melanthiaceae family as per the latest classification system (Li, 2003) though earlier it was categorized under Liliaceae and Trilliaceae family at different

times. The drug from this plant Paradis Rhizoma was documented in the Anonymous, (1985). The species *P. polyphylla* has been studied since 1960 in the Himalayan region of Eastern Nepal, Darjeeling, Sikkim and Bhutan. The size of the population were small, variations within the populations were minimal but between populations it was wide. Though 20 different species were recognized by Hara, it was opined that the values be treated as infra-specific taxa of one species as the differences among them were not sufficient to demarcate them as individual species (Hara, 1969).

However, subsequently, the genus was proposed to comprise of about 42 taxa containing about 32 species and 10 varieties (Ji et al. 2017; Wang et al. 2017; Yang et al. 2017; Wang et al. 2018) [Table-2.1]. The centre of *Paris* species diversity is located in China (24 species), with 12 endemic species (Cunningham, 2018). With the discovery of a few new species the total number of species reached upto 32 species at present. Some of the new species and varieties reported were; *Paris stigmatosa* (Melanthiaceae) which was described and illustrated from north eastern Yunnan, China. The new species was found growing in wet habitats under bamboo thickets on Yaoshan Mountain of Qiaojia County. Zhang, (2008) and Wen et al. (2012) reported a new variety *P. fargesii* var. *Brevipetala* (PFB). *Paris bashanensis* is widely used in traditional Chinese medicine for the treatment of injuries, fractures and haemorrhage in Hubei and Sichuan Province (Wang et al. 2013). *Paris tengchongensis* can be distinguished from other species in *Paris* sect. *axiparis* by sepals with purple markings, reddish purple petals, an ovoid ovary, and a sub-globose berry (Ji, 2017). The most abundant diversity of *Paris* species has been reported especially from Yunnan Province, China (Wang et al. 2017). The genus *Paris* herb prefebly grows in bamboo forests and temperate forest where *Aresima*

species grows abundantly or rocky slopes along streamside as well as Ravine mossy forests and the altitude ranges from 100–3500m. It ranges from 6 cm (*Paris luquanensis*) to 1.5 m (*Paris delavayi*) with slender or thickened rhizome (1-2.5 cm thick) and the flowers are solitary, bisexual, terminal and pedunculate. Fruit is a berry or a berrylike capsule and is several to many seeded. The rhizome is widely used in traditional Chinese medicine and to some extent in Indian Traditional medicinal practices such as in Ayurveda and Unani for the treatment of injuries, fractures, hemorrhage, antidote during snake bite, food poisoning and more importantly as veterinary purpose. *P. polyphylla* Smith var. *chinensis* and *P. polyphylla* Smith var. *yunnanensis, P. delavayi* are the most widely used species (Liu et al. 2009).

### 2.1.2. Species found in Sikkim

Songyun and Soukup, (2000) reported 7 species or the Taxa of *Paris* from India of which 5 according to them were available in Sikkim Himalaya. Including the species described by Hajra and Verma, (1996); Hara, (1969) and Noltie, (1994), altogether 7 different taxa of *Paris* were reported to be available from Sikkim Himalaya (Table-2.2). Maity et al. (2014) reported one more taxon, *P. polyphylla* Smith var. *nana* H. Li which was originally reported from Sichuan, China. This species was reported from Rishop in Darjelling District and was first report from India. Even within the single species of *P. polyphylla*, several distinct morphological variations have been observed in Sikkim Himalaya [Figure 2.1 (A-O)].

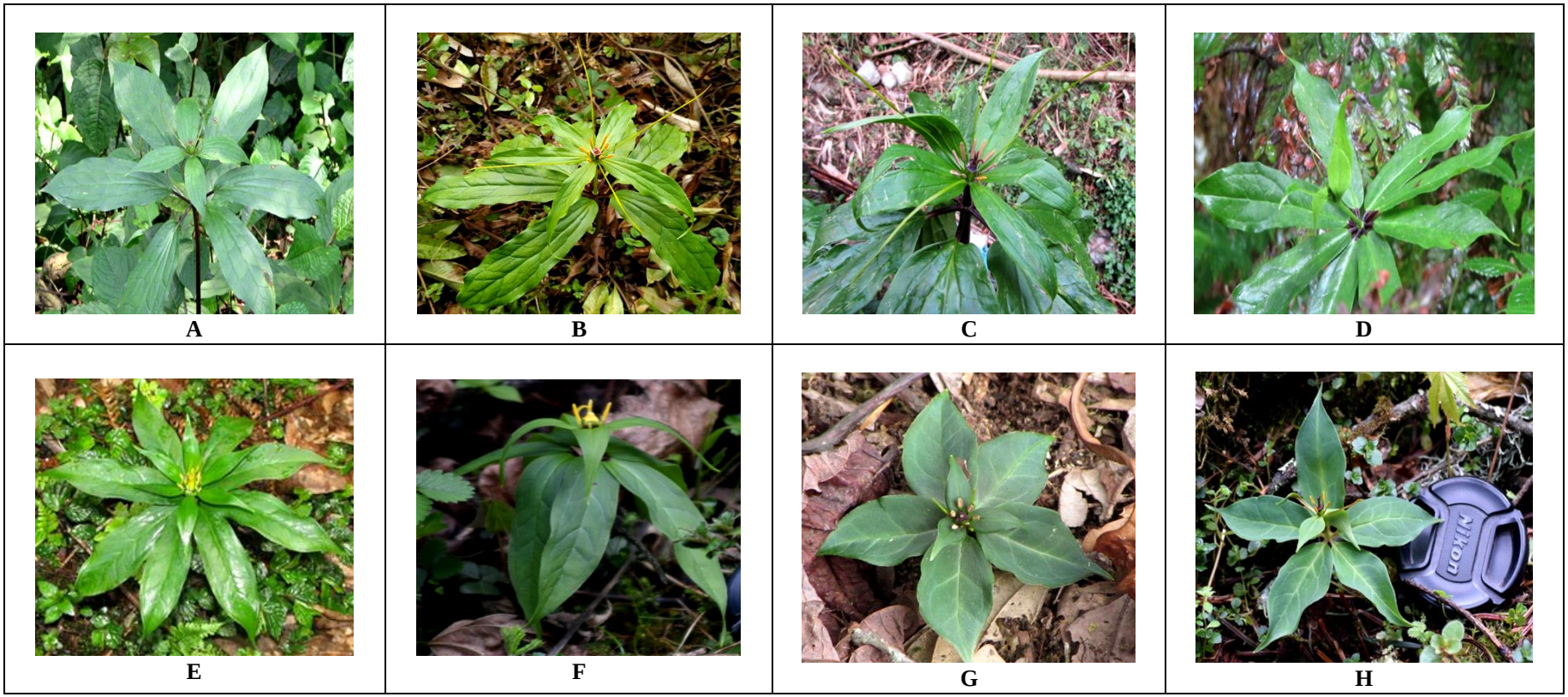


**Figure 2.1** (A–H) Morphological variations within the species of *Paris polyphylla* found in Sikkim Himalaya.

**Contd.**

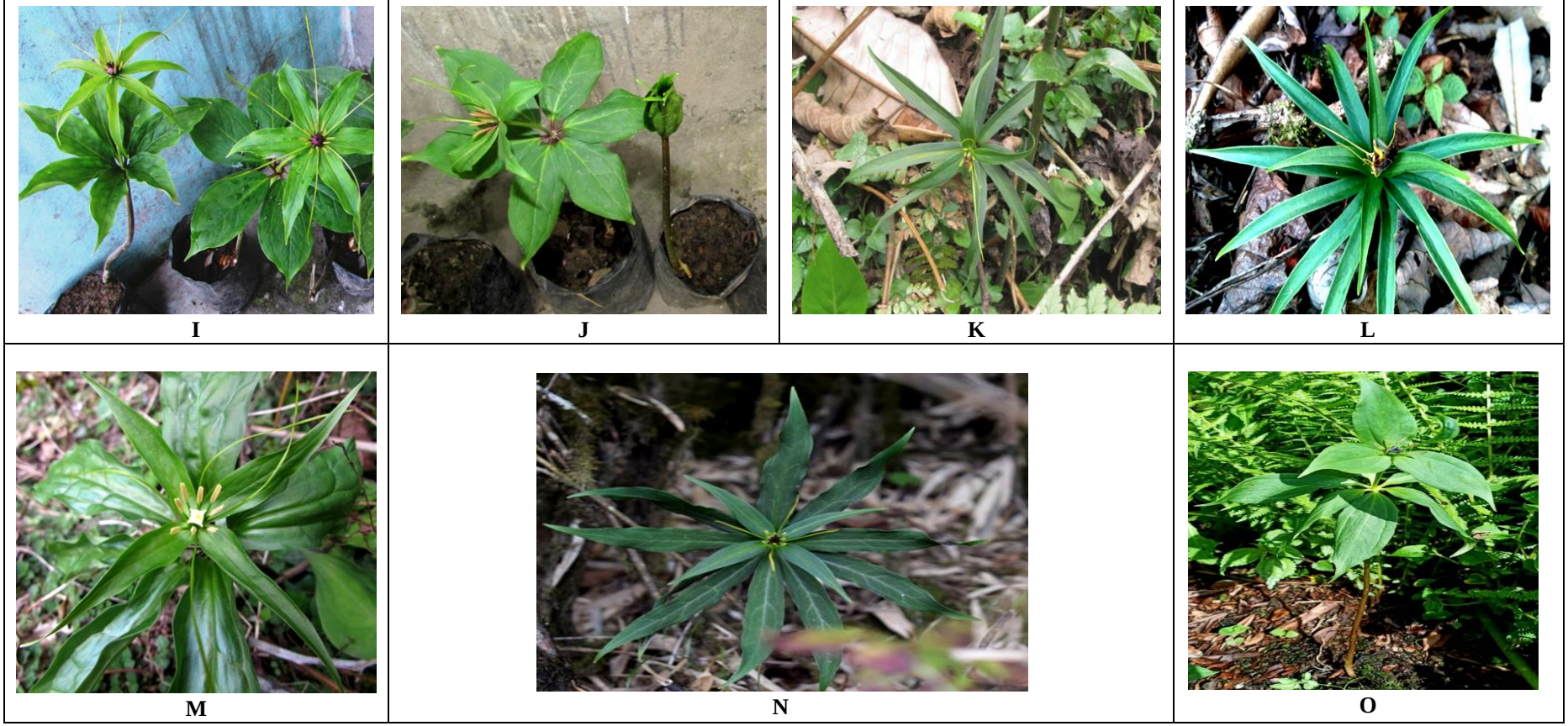


**Figure 2.1** (I–O) Morphological variations within the species of *Paris polyphylla* found in Sikkim Himalaya.

However, according to the herbarium record at Botanical Survey of India (B.S.I), Sikkim Himalayan Regional Centre, Gangtok, different researchers collected and submitted *Paris* samples from different parts of Sikkim at different time e.g. P. K. Hajra (1980), R. C. Srivastava (1991, 1992), A. R. K. Sastry, B. K. Shukla (1999), D. Maity (2000, 2001), A. K. Sahoo (2003) etc. From these collections only two species of genus *Paris* which were identified, *Paris polyphylla* Sm. var. *wallichii* Hara and *Paris polyphylla* Sm. var. *appendiculata* H. Hara. Naturally, these are the species of *Paris* documented and most widely distributed in Sikkim, Eastern Himalaya.

**Table-2.1.** Different species of *Paris* L. described so far along with their synonyms.

| **Sl. No.** | **Species** | **Synonym** | **References** |
|---|---|---|---|
| 1 | *Paris axialis* H. Li. | *Paris axialis* var. *rubra* H. H. Zhou, K. Y. Wu & R. Tao. | Songyun and Soukup, (2000) |
| 2 | *Paris bashanensis* F. T. Wang & Tang. | *Paris quadrifolia* var. *setchuenensis* Fr anch.<br>*Paris setchuenensis* (Franch.) Barkalov<br>*Paris verticillata* var. *setchuenensis* (Franch.) Hand.-Mazz. | Songyun and Soukup, (2000) |
| 3 | *Paris caobangensis* Y.H.Ji, H.Li & Z.K.Zhou | No Synonyms. | Yun-Heng et al. (2006) |
| 4 | *Paris cronquistii* (Takhtajan) H. Li. | *Daiswa cronquistii* Takht. | Songyun and Soukup, (2000) |
| 4.1 | *Paris cronquistii* var. *Cronquistii* | *Daiswa cronquistii* Takht. | Songyun and Soukup, (2000) |
| 4.2 | *Paris cronquistii* var. *xichouensis* H. Li. | *Paris xichouensis* (H. Li) Y. H. Ji, H. Li & Z. K. Zhou. | Songyun and Soukup, (2000), Nga et al.(2016). |
| 5 | *Paris daliensis* H. Li and V. G.Soukup | No Synonyms. | Songyun and Soukup, (2000) |
| 6 | *Paris delavayi* Franchet | *Daiswa delavayi* (Franch.) Takht.<br>*Paris delavayi* var. *delavayi*<br>*Paris henryi* Diels<br>*Paris polyphylla* f. *macrosepala* H. Li | Liang and Soukup, (2000) |

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|---|---|---|---|
| | | *Paris polyphylla* var. *minor* S. F. Wang<br>*Paris polyphylla* var. *pseudothibetica* H. Li. | |
| 7 | *Paris dulongensis* H. Li and Kurita | No Synonyms. | Songyun and Soukup, (2000) |
| 8 | *Paris dunniana* H. Leveille | *Daiswa dunniana* (H. Lev.) Takht.<br>*Daiswa hainanensis* (Merr.) Takht.<br>*Paris hainanensis* Merr. | Songyun and Soukup, (2000) |
| 8.1 | *Paris dunniana* var. *oligophylla* | No Synonyms. | Nohara et al. (1973) |
| 9 | *Paris fargesii* Franchet | *Daiswa fargesii (*Franch.) Takht.<br>*Paris polyphylla var. fargesii* (Franch.) S. Dasgupta<br>*Daiswa fargesii* var. *Brevipetalata* T. C. Huang & K. C. Yang<br>*Paris fargesii* var. *brevipetalata* (T. C. Huang & K. C. Yang) T. C. Huang & K. C. Yang<br>*Paris fargesii* var. *fargesii*<br>*Paris fargesii* var. *latipetala* H. Li & V. G. Soukup<br>*Paris hookeri* H. Lev.<br>*Paris petiolata* var. *membranacea* C. H. Wright<br>*Paris polyphylla* subsp. *fargesii* (Franc h.) H. Hara. | Songyun and Soukup, (2000) |
| 9.1 | *Paris fargesii* var. *fargesii* | *Daiswa fargesii* | Songyun and Soukup, (2000) |
| 9.2 | *Paris fargesii* var. *petiolata* (Baker ex C. H. Wright) F. T. Wang and Tang | *Paris delavayi* var. *ovalifolia* H. Li<br>*Paris delavayi* var. *petiolata* (Baker ex C.H. Wright) H. Li<br>*Paris petiolata* Baker ex C. H. Wright. | Songyun and Soukup, (2000) |
| 10 | *Paris fargesianae* H. Li var. *brevipetalata* | No Synonyms. | Xue et al. 2009 |
| 11 | *Paris forrestii* (Takhtajan) H. Li. | *Daiswa forrestii* Takht.<br>*Paris longistigmata* H. Li. | Songyun and Soukup, (2000) |
| 12 | *Paris guizhouensis* S.Z.He. | No synonyms. | |
| 13 | *Paris incomplete* M. Bieb. | *Demidovia polyphylla* Hoffm*Paris ape tala* Hoffm.<br>*Paris octophylla* Hoffm. | (Osaloo, and Kawano 1999) |
| 14 | *Paris japonica* Franchet | *Kinugasa japonica* (Franch. & Sav.) Tatew. & Suto<br>*Trillidium japonicum* Franch. & Sav.<br>*Trillium japonicum* (Franch. & Sav.) Matsum. | Ji et al. (2006) |
| 15 | *Paris luquanensis* H. Li. | No synonyms. | Songyun and Soukup, (2000) |
| 16 | *Paris mairei* H. Leveille | *Daiswa pubescens* (Hand. - Mazz.) | Songyun and |

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| | | Takht.<br>*Daiswa violacea* (H. Lev.) Takht<br>*Paris polyphylla* var. *pubescens* Hand. - Mazz.<br>*Paris polyphylla* var. *violacea* (H. Lev.) S. Dasgupta<br>*Paris pubescens* (Hand. -Mazz.) F. T. Wang & Tang<br>*Paris violacea* H. Lev. | Soukup, (2000) |
| 17 | *Paris marmorata* Stearn | *Paris polyphylla* subsp. *marmorata* (St earn) H. Hara. | Songyun and Soukup, (2000) |
| 18 | *Paris nitida* G.W. Hu, Z.Wang and Q.F.Wang. | No synonyms. | Wang et al. (2017) |
| 19 | *Paris polyandra* S. F. Wang | No synonyms. | Songyun and Soukup, (2000) |
| 20 | *Paris polyphylla* Smith | *Daiswa polyphylla* (Sm.) Raf.<br>*Paris biondii* Pamp.<br>*Paris debeauxii* H. Lev.<br>*Paris kwantungensis* R. H. Miao<br>*Paris polyphylla* var. *emeiensis* H. X. Yin, Hao Zhang & D. Xue<br>*Paris polyphylla* var. *kwantungensis* ( R. H. Miao) S. C. Chen & S. Yun Liang<br>*Paris polyphylla* var. *polyphylla*<br>*Paris polyphylla* var. *taitungensis* (S. S. Ying) S. S. Ying<br>*Paris polyphylla* var. *wallichii* H. Hara<br>*Paris taitungensis* S. S. Ying. | Songyun and Soukup, (2000), Hong-Xiang et al. (2007) |
| 20. 1 | *Paris polyphylla* var. *alba* H. Li and R. J. Mitchell | *Paris marchandii* H. Lev. | Songyun and Soukup, (2000) |
| 20. 2 | *Paris polyphylla* var. *chinensis* (Franchet) H. Hara. | *Daiswa chinensis* (Franch.) Takht.<br>*Daiswa chinensis* subsp. *brachysepala*<br>*Paris brachysepala* Pamp.<br>*Paris brevipetala* Y. K. Yang<br>*Paris chinensis* Franch.<br>*Paris formosana* Hayata. | Songyun and Soukup, (2000) |
| 20. 3 | *Paris polyphylla* var. *kwantungensis* (R. H. Miao) S.C.Chen and S.Yun Liang | No synonyms. | Songyun and Soukup, (2000) |
| 20. 4 | *Paris polyphylla* var. *latifolia* F. T. Wang and C. | *Paris polyphylla* f. *latifolia* (F. T. Wang & C. Yu Chang) H. Li. | Songyun and Soukup, (2000) |
| 20. 5 | *Paris polyphylla* var. *minor* S. F. Wang. | No synonyms. | Songyun and Soukup, (2000) |
| 20. 6 | *Paris polyphylla* var. *nana* H. Li, Bull. | No synonyms. | Songyun and Soukup, (2000) |

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| 20.7 | *Paris polyphylla* var. *polyphylla* | *Daiswa polyphylla* (Sm.) Raf.<br>*Paris biondii* Pamp.<br>*Paris debeauxii* H. Lev.<br>*Paris taitungensis* S. S. Ying. | Songyun and Soukup, (2000) |
| 20.8 | *Paris polyphylla* var. *pseudothibetica* H. Li, Bull. | No Synonyms. | Songyun and Soukup, (2000) |
| 20.9 | *Paris polyphylla* var. *stenophylla* Franchet | *Paris polyphylla* var. *brachystemon* Franch.<br>*Daiswa bockiana* (Diels) Takht.<br>*Daiswa lancifolia* (Hayata) Takht.<br>*Paris arisanensis* Hayata<br>*Paris bockiana* Diels<br>*Paris hamifer* H. Lev.<br>*Paris lancifolia* Hayata<br>*Paris polyphylla* var. *brachystemon* Franch. | Songyun and Soukup, (2000) |
| 20.10 | *Paris polyphylla* var. *yunnanensis* (Franchet)Handel-Mazzetti | *Daiswa birmanica* Takht.<br>*Daiswa yunnanensis* (Franch.) Takht.<br>*Paris aprica* H. Lev.<br>*Paris atrata* H. Lev.<br>*Paris birmanica* (Takht.) H. Li & Noltie<br>*Paris cavaleriei* H. Lev. & Vaniot<br>*Paris christii* H. Lev.<br>*Paris franchetiana* H. Lev.<br>*Paris gigas* H. Lev. & Vaniot<br>*Paris mercieri* H. Lev.<br>*Paris pinfaensis* H. Lev.<br>*Paris polyphylla* var. *platypetala* Franch.<br>*Paris polyphylla* f. *velutina* H.Li & Noltie<br>*Paris yunnanensis* Franch. | Songyun and Soukup, (2000) |
| 21 | *Paris quadrifolia* Linnaeus | *Paris pentafolia* P. Renault<br>*Paris quadrifolia* var. *angustiovata* D. Z. Ma & H. L. Liu<br>*Paris trifolia* P. Renault. | Songyun and Soukup, (2000) |
| 22 | *Paris qiliangiana* H.Li, J.Yang & Y.H.Wang. | No synonyms. | Yang et al. (2017) |
| 23 | *Paris rugosa* H. Li and Kurita. | No synonyms. | Songyun and Soukup, (2000) |
| 24 | *Paris stigmatosa* | No synonyms. | Zhang et al. (2008) |
| 25 | *Paris tengchongensis* | No synonyms. | Ji et al. (2017) |
| 26 | *Paris tetraphylla* A. Gray. | *Paris tetraphylla* var. *sessiliflora* Makino<br>*Paris tetraphylla* var. *tetraphylla*<br>*Paris tetraphylla* var. *yakusimensis* Masam.<br>*Paris yakusimensis* (Masam.) Masam. | Osaloo and Kawano, (1999), The Plant List, (2013). |
| 26. | *Paris tetraphylla var. pend* | *Paris tetraphylla* f. *penduliflora* (J. | The Plant |

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|---|---|---|---|
| 1 | *uliflora* J.Murata and T.Yamanaka | Murata & T. Yamanaka) H. Hara. | List, (2013). |
| 27 | *Paris thibetica* Franchet | *Paris polyphylla* var. *appendiculata* H. Hara<br>*Daiswa thibetica* (Franch.) Takht.<br>*Paris polyphylla* var. *appendiculata* H. Hara<br>*Paris polyphylla* var. *thibetica* (Franch .) H. Hara<br>*Paris thibetica* var. *thibetica*<br>*Paris wenxianensis* Z. X. Peng & R. N. Zhao. | Songyun and Soukup, (2000) |
| 27.1 | *Paris thibetica* var. *apetala* Hand. -Mazz. | *Paris polyphylla* var. *apetala* (Hand. -Mazz.) F. T. Wang & T. Tang. | The Plant List, (2013). |
| 27.2 | *Paris thibetica* var. *thibetica* | *Daiswa thibetica* (Franch.) Takht. | Songyun and Soukup, (2000) |
| 28 | *Paris undulata* H. Li and V. G. Soukup in H. Li. | No synonyms. | Songyun and Soukup, (2000) |
| 29 | *Paris vaniotii* H. Leveille | No synonyms. | Songyun and Soukup, (2000) |
| 30 | *Paris verticillata* Marschall von Bieberstein | *Paris dahurica* Fisch. ex Turcz.<br>*Paris hexaphylla* Cham.<br>*Paris hexaphylla* var. *manshurica* (Kom.) Vorosch.<br>*Paris hexaphylla* f. *purpurea* Miyabe & Tatew.<br>*Paris manshurica* Kom.<br>*Paris obovata* Ledeb.<br>*Paris quadrifolia* var. *dahurica* (Fisch. ex Turcz.) Franch.<br>*Paris quadrifolia* var. *hexaphylla* (Cham.) B. Fedtsch.<br>*Paris quadrifolia* var. *obovata* (Ledeb. ) Regel & Tiling<br>*Paris verticillata* var. *manshurica* (Kom.) H. Hara<br>*Paris verticillata* subsp. *manshurica* ( Kom.) Kitag.<br>*Paris verticillata* var. *obovata* (Ledeb.) H. Hara<br>*Paris verticillata* f. *purpurea* (Miyabe & Tatew.) Honda. | Songyun and Soukup, (2000) |
| 31 | *Paris vietnamensis* (Takhtajan) H. Li. | *Daiswa hainanensis* (Merr.) Takht. | Songyun and Soukup, (2000) |
| 32 | *Paris wenxianensis* Z. X. Peng and R. N. Zhao. | No synonyms. | Songyun and Soukup, (2000) |

### 2.1.3. The Plant

The plant is bisexual perennial herb with a stout horizontal rhizome and a single shoot which terminates in a single bud that may develops into flower. The flower is solitary, greenish with 4-12 perianth segments outer sepaloid one's green in colour and persistent, inner petaloid segments commonly white, yellowish or purplish thread like segments. Stamen 6-10 or more attached at the base of tepals, anthers basifixed, Ovary superior, ribbed, stigma 4-5 lobed. Fruit globose, bright red coloured berry. Leaves 4-9 in a whorl, elliptic, stalked upto 10 cm in length, with three main leaf veins prominent leaves in whorls of 4-12 elliptic, long to short stalked, sessile in upper parts. Rhizome stout, creeping, bearing annuals scars. Stem erect, plants 30-100 cm in heights (Noltie,1994).

*P. polyphylla* plant starts germinating in April and flowers in April/May. Seeds mature in October. By the beginning of November to mid-November, most of the plant part perishes out and all seeds get dispersed. Before the plant dies and after the seed gets matured, bud sprouts on rhizome which remains underground for the entire winter until the subsequent germination period. Therefore, rhizome is dormant for nearly five months (Madhu et al. 2010). The taxonomic classification of *Paris* has long been in disagreement and appropriate documentation among the species of genus *Paris* poses considerable adversity due to the close similarity in their appearance (Xue et al. 2009).

**Table-2.2.** Different taxa of *Paris* reported from Sikkim Himalaya.

| Sl. No. | Species | Synonym/Variety | Place and Altitude | References |
|---|---|---|---|---|
| 1. | *Paris marmorata* Stearn | *Paris polyphylla* ssp. *marmorata* (Stearn) H.Hara. | Rimbik-Ramam (1900-2400m), Bakhim-Dzongri (2500-3000m) Damthang-Tendong (2200-2600m) | Hajra and Verma, (1996), Songyun and Soukup, (2000), Hara, (1969) |
| 2. | *Paris polyphylla* Smith | *Paris polyphylla* var. *w allichii* H. Hara. | Ramam (2400m), Yoksum (1700m), PenlongLa (2100n), Damthang-Rabongla (2200m), | Noltie, (1994), Songyun and Soukup, (2000), Hajra and Verma, (1996), Hara, (1969) |
| 3. | *Paris polyphylla* var. *appendiculate* Hara | *Paris thibetica* Franchet. | Above Tsoka, Bikbari, Bakhim-Dzongri, Migothang to Nayathang (2500-4000m) | Noltie, (1994), Hajra and Verma, (1996). Hara, (1969) |
| 4. | *Paris polyphylla* var. *polyphylla* | *Daiswa polyphylla* (Sm.) Raf. | Chungthang, Lachen (1300-3960m) Penlongla, Chungthang-Lachen, Fungong, Bakhim-Dzongri, Changu, Sada (1800-3300m) Singhik (1500m) | Noltie, (1994), Songyun and Soukup, (2000) and Hajra and Verma, (1996). Hara, (1969) |
| 5. | *Paris polyphylla* var. *stenophylla* Franchet | *Paris polyphylla* var. *br achystemon* Franch. | Draigchu (*ca* 2500m) | Noltie, (1994), Songyun and Soukup, (2000) and Hajra and Verma, (1996) Hara, (1969) |
| 6 | *Paris thibetica* var. *apetala* Hand. -Mazz. | *Paris polyphylla* var. *ap etala* (Hand. -Mazz.) F. T. Wang & T. Tang. | Chai Bhanjang and Phalut (3100-3500m) | Songyun and Soukup, (2000) and Hajra and Verma, (1996). |

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|---|---|---|---|---|
| 7 | *Paris thibetica* var. *thibetica* | *Daiswa thibetica* (Franchet) Takhtajan; | Data Unavailable | Songyun and Soukup, (2000) |

### 2.1.4. Polymorphism in *Paris polyphylla*

Polymorphism is the existence of number of different forms within a species. It is the occurrence of different forms in a population at the same place and time, the frequency of which cannot be explained on the basis of mutation. Polymorphism in the genus *Paris* is universal (Deb et al. 2015) and an enormous amount of polymorphism in phenology of *P. polyphylla* is evident in the morphological variations observed in some of the *P. polyphylla* in different years even when growing from a single rhizome (Jamir, 2015).

The most striking features of polymorphism in *P. polyphylla* Smith is found in its number of verticillate leaves, length of inner perianth whorls, other floral characters etc. *P. polyphylla* is in a sense extraordinary in the variation of many characters which are often considered stable (Hara, 1969). Not only morphologically, *P polyphylla* is also cytologically heterogenous. Both diploid and tetraploid race have been reported in this genus.

Various enzyme systems have also been applied for polymorphism detection in different organism (Kertadikara et al. 1995) Superoxide dismutase (SOD) are ubiquitous enzyme found in all the aerobes and are involved in neutralizing ROS in cells. Higher plants possess a number of SOD isozymes that can be used for detecting polymorphism (Kertadikara et al. 1995). In *Paris quadrifolia* L. variation in the biophysical characters and phenotypes of SOD bands were detected under

electronic conditions. The polymorphism was based on the population of same species obtained from different locations (Jogaite et al. 2003).

Variation in leaf shape, size and character is one of the important markers of polymorphism in *P. polyphylla*. The leaves may be broad obovate to linear, from cordate to cunnate in the base, and from long petioled to sessile. The inner tepals may be short (1-3 cm) or long (8-10 cm). Some extreme polymorphism in Sikkim Himalayan *P. polyphylla* is found in s. s. *marmorata* (Stearn) in its leaf and stamen characters. Similarly, var. *brachystemon* varies from a typical *P. polyphylla* in having numerous (upto 22), narrow – linear leaves (5 mm) [Hara, 1969].

In *P. polyphylla* found in Northern Yunnan, China var. *stenophylla* was found to be distinct from other varieties in having many (10-15) linear lanceolate leaves. Similarly, var. *pseudothibetica* was unique in its conspicuous free portion of anther connective and in smaller number of apetalous individuals. In addition, the typical specimen of *P. mairei* can be identified by their papillose pubescence. However, specimens from Yaoshan mountain contain little pubescence (Shu-Dong et al. 2008).

Wang et al. (2016) explained that one of the prime reasons for such polymorphism might be because it is an insect pollinated outcrossing species (Li, 1998), which account for its high variations among populations. In addition, the species also exhibit most of the overall genetic variation between, rather than within, its populations. Cheng et al. (2014), conducted an experiment to examine the C-banding pattern of chromosomes of six taxa of *Paris* (*P. polyphylla* var. *chinensis, P. marmorata, P. luquanensis, P. thibetica, P. polyphylla* var. *yunnanensis* and *P.*

*polyphylla* var. *alba*) from Sichuan province, China. They concluded that the observed polymorphism of the bands might be an indicator of their distinctive characters and it was assumed that the C-band might be related with climate and geographical distribution.

### 2.1.5. Chemotaxonomy of *Paris polyphylla*

Chemotaxonomy, also termed as chemosystematics, is an attempt to classify and identify organisms (originally plants) according to verifiable differences and resemblances in their biochemical structures (Hao et al. 2015). In the context of plant science, Chemotaxonomy is basically the classification of plants on the basis of specific class of secondary metabolites and their biosynthetic pathways or the classification on the basis of their chemical constituents. This method of classification is considered to be better in comparison to traditional method due to the ease of working methodology (Singh, 2018). *P. polyphylla* contains a variety of secondary metabolites primarily of saponin group. Saponins are one of the major classes of compounds in natural products chemistry, which have been isolated from plants and marine animals and are biologically active (Negi et al. 2014).

According to the species and distribution, the compositions of steroidal saponins in genus *Paris* vary widely and based on the structure of aglycons they are divided into six main groups: Spirotane, Furostane, Pseudo-spirostanol, Pregnane saponin and other type of Polyhydroxylated saponins (Wang et al. 2017).

Steroidal saponins are the important secondary metabolites isolated from *Paris* sp. The chemotaxonomic significance of these saponins has been worked out (Wang et al. 2013). The compositions of these saponins vary in the *Paris* species based on their distribution. It has been found that Diosgenin, Pennogenin and Furastone type

saponins are abundantly found in *P. polyphylla* var. *chinensis* and *P. polyphylla* var. *yunnanensis*. Polyhydroxylated and other types of saponins are found in *P. polyphylla* var. *chinensis, P. polyphylla* var. *yunnanensis, P. delavayi, P. pseudothibetica* and *P. vietnamensis*. In addition, *P. delavayi* containing 4 diosgenin and 4 pennogenin type saponins is most similar to *P. vietnamensis* in chemical constitution. On the other hand, *P. luquanensis* only contained furostane saponins. It has been proposed that *P. thibetica, P. vietnamensis, P. delavayi* and *P. pseudothibetica* contains more active saponins, as such they could be ideal substitution material for *P. polyphylla* Smith var. *chinensis* and *P. polyphylla* Smith var. *yunnanensis* both of which yields the Rhizome Paradis of commerce (Wang et al. 2013).

The average contents of chemical components like polyphyllin – I, polyphyllin -II, polyphyllin VII and total steroidal saponins may be related to the geographical origin of *Paris.* The samples from Southwestern Yunnan had the highest content of polyphyllin -I and total saponin. While the samples from Guangxi province and Southeast Yunnan had the highest content of Polyphyllin II and polyphyllin -VII respectively. The content of all the metabolites mentioned above were lowest in the samples from Guizhou province. The difference in the chemical compositions may be due to geographical conditions of different areas. Through the combination of NMR analysis, HPLC based determination of metabolites and multivariate analysis accounted for the variation of *Paris* from different geographical locations when 48 populations belonging to 12 species of *Paris* were considered (Zhao et al. 2014).

A slight modification of above method has found chemotaxonomic importance, subsequently, UHPLC chromatography has been used for qualitative and quantitative

estimation of *Paris* metabolites (Kang et al. 2012). UHPLC analysis combined with chemometric analysis may be a useful strategy for the discrimination of different species or species from different geographical regions (Sanchez-Salcedo et al. 2016). Using this method involving UHPLC analysis of polyphyllin -I, and polyphyllin -II and chemometric analysis including principal component Analysis (PCA) and Partial Least Square Discrimination analysis (PLS-DA) have been used to evaluate 38 batches of *Paris* belonging to six species; *P. polyphylla* var. *yunnanensis, P. cronquistii, P. mairei, P. polyphylla* var. *chinensis, P. axialis* and *P. fargesii.* Thus, *P. polyphylla* var. *chinensis* from different geographical regions were found to have different bioactive compounds. The quality of *P. axialis* was similar or better than *P. polyphylla* var. *yunnanensis* in terms of phytoconstituent quantities. Therefore, quantitative analysis combined with chemometric similarity analysis is an efficient method to differentiate different species of *Paris* (Yang et al. 2016).

A metabolomics-based approach involving ultra-high performance liquid chromatography coupled with quadruple time of flight mass spectrometry (HPLC/2-TOF=MS) was optimized for distinguishing the rhizomes of *P. polyphylla* var. *yunnanensis* and *P. vietnamensis.* Of the total of 146 metabolites, 42 new compounds were reported. The data presented 15 new biomarkers to distinguish these two species which were proved to be efficient and accurate (Kang et al. 2017).

In continuation of chromatography-based strategies, a method based on liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with Fourier Transform Infrared spectroscopy (FT-IR) was used to evaluate chemotaxonomic relationship of nine *Paris* species from different geographical regions of China. PCA analysis based or FT-IR data revealed chemical similarities suggesting metabolic constituents influenced by geographical factors and species (Wang et al. 2017). The

different species of *Paris* analyzed were *P. axialis, P. cronquistii, P. mairei, P. polyphylla, P. vietnamensis, P. cronquistii* var. *xichouensis, P. polyphylla* var. *chinensis, P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *stenophylla.* The MS fragmentation pattern of Diosgenin and Pennogenin types saponins were investigated and 22 steroid saponins were described by UHPLC-IT-TOF-MS. Six key metabolites viz., polyphyllin -I, -II, -V, -VI. -VII and gracillin were determined Phyto ecdysones like- β- ecdysones and 5- hydroxy-ecdysones, gracillin as well as open chain steroid saponins may be the key precursor of the biosynthetic pathway. PCA analysis of the FT-IR data demonstrated chemical relationship among the investigated species. Thus, *P. axialis* was separated into a group all alone (Group-1), which was in line with the morphological taxonomy (Li, 1998). *P. cronquistii, P. cronquistii* var *xichouensis* and *P. polyphylla* var. *chinensis* were separated into group – 2. Among these, *P. cronquistii* and *P. cronquistii* var. *xichouensis* have close morphological relationship while *P. cronquistii* var. *xichouensis* and *P. polyphylla* var. *chinensis* were obtained from the same geographical region. This suggests that both geographical difference and species diversity have influence on chemical constituents. The rest of species placed to group- 3 belonged to the same geographical location (Yunnan). Of these *P. cronquistii* was most distant from the other five species which have similar chemical information. The species of *P. polyphylla* var. *yunnanensis*, *P. polyphylla* var. stenophylla and *P. polyphylla* var. *chinensis* are the varieties of *P. polyphylla* in terms of morphology which has both in PCA analysis as well as morphological taxonomy (Wang et al. 2017).

An accurate method of identifying *Paris* species was formulated which was based on analytical techniques (UPLC, FTIR), polyphyllin context and data fusion strategy to established pattern recognition modules including PLS-DA, support vector machine

grid search (SVM) and random forest. These three machine learning algorithms were employed to distinguish different *Paris* species and trace their geographical regions. The mid-level data fusion strategy is PLS-DA model showed the best accuracy to classify *Paris* according to botanical and geographical origin (Wu et al. 2019).

To study the role of geographical origin on the chemical properties of *P. polyphylla* var. *yunnanensis* altogether 183 dried rhizome samples from 16 typical and non-typical natural habitats were analyzed by UV and FTIR methods combined with PLS-DA and hierarchical cluster (HCA) analysis. Such multispectral information fusion strategy could provide correct classification of samples. Consequently, it was found that typical and non-typical natural habitats as well as distantly situated areas within the same habitat could influence the chemical properties of samples (Wang et al. 2019). As per UV and FTIR spectral analysis, the chemical properties of the rhizome varied based on geographical origin. The diversity of steroidal saponins is evident between plants of diverse geographical origin (Yang et al. 2017).

### 2.1.6. Molecular Taxonomy and phylogenetic studies

Most of the molecular study on the plant relates to molecular taxonomy and phylogeny. The classification of *Paris*, long in dispute, is still unresolved despite serious attempts by several taxonomists e.g., Hara, (1969); Li, (1984,1998) and Mitchell, (1987, 1988); Takhtajan, (1983); Dahlgren et al. (1985); Tamura, (1998) and Farmer and Schilling, (2002) etc.

Hara, (1969) divided the 14 species known at the time into three sections: *Paris*, *Kinugasa* and *Euthyra*, based on fruit and seed characters. Thereafter, Li, (1998) recognized subgenus *Paris* (11 species) and subgenus *Paris* was divided into sections *Kinugasa* (one species), *Paris* (five species). Amidst all these confusions,

studies based on DNA sequence data have proved useful for analyzing the phylogeny of *Paris.* Consequently, studies combining sequence data and morphological data supported the division of *Paris* into three genera (*Diaswa*, *Kinugasa* and *Paris* s.s; Farmer and Schilling. 2002). These studies have provided valuable insights for an initial molecular-based evaluation of *Paris* classification.

Even after the shifting of the genus *Paris* to the family Melanthiaceae (Li, 2003) its classification was still cloudy. Various taxonomists have described the genus variously sometimes separately and sometimes bringing together below genera (Hara, 1969; Li, 1984; Takhtajan, 1983; Tamura, 1998). These classifications were mostly based on morphological characters like flower merosity. Needless to say, the confusion only extended. In such situations, phylogenetic studies based on DNA sequence data are careful.

The different molecular phylogenetic studies on undertaken so far are as follows:

#### 2.1.6.1. ITS sequence data and the authentication of *Paris* species

All species of the genus *Paris* were confused with each other and the identification of these species were available only in fragmentary form. The active compounds responsible for the biological activities of *Paris* are a series of steroidal saponins which could either strongly inhibit gastric lesions induced by indomethacin (Madhu et al. 2003) or induced apoptosis in drug resistant HepG2 cells (Cheung et al. 2005).

Among species of *Paris*, biologically active compounds have large variations. Contents of their chemical compounds significant differ from each other (Yin et al. 2007a, b). Therefore, it is necessary to develop an effective and reliable method to authenticate and distinguish *P. polyphylla* from its related species. Liu and Ji, (2012)

describe the analysis of the internal transcribed spacer (ITS) nuclear ribosomal DNA of *P. polyphylla* var. *yunnanensis* and its related species.

In the study, the differential of *P. polyphylla* var. *yunnanensis* and its 11 congeners were investigated through DNA sequence analysis of nuclear internal transcribed spacer (ITS) regions. Based on sequence alignments, it was concluded that the ITS sequence could distinguish *P. polyphylla* var. *yunnanensis* from other *Paris* species. The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) based on ITS sequence can uniquely identify *P. polyphylla* from its adulterants successfully and easily.

DNA sequence data have been used from the ITS region and two regions of the plastid genome (trnL-trnF and psbA-trnH). The study of Ji et al. (2006) supported the classification of *Paris* as a single genus rather than as three genera (*Diaswa, Kinugasa* and *Paris* sensu stricto). The revised infrageneric system of *Paris* includes two subgenus *Paris* and subgenus *Daiswa.* The former comprises sections *Kinugasa* and *Paris,* the latter sections *Axiparis, Thibeticae* and *Euthyra.* All five sections are distinctive on the basis of morphology (Ji et al. 2006).

Thus, DNA studies of ribosomal internal transmitted spacer (ITS) region as well as plastid genes rbcl and mark supported monophylly of *Paris* (Osaloo and Kawanu, 1999; Kazempour et al. 1999). The studies by Farmer and Schilling, (2002) involving such sequence Data provided useful information on molecular phylogeny of *Paris*, But the study was limited to address the issue at generic and infra-generic levels.

To, address the above issue, the classification of *Paris* was tested with DNA sequence data from a larger taxon sample. Such data were used from ITS region as

well as two regions of plastid genome viz. truL-trnF and psbA-trnH. These regions were earlier found suitable for phylogenetic studies at infrageneric level (Soltis and Soltis, 1998). Consequently, *Paris* has been classified as a single genus rather than 3 genera of *Paris* s.s., *Kinugasa* and *Daiswa* as described by Takhtajan, (1903) or the division of subgenus *Paris* into 3 sections- *Kinugasa*, *Paris* and *Axiparis* after the plant was divided into subgenus *Paris* and subgenus *Daiswa* (Li, 1998). The infrageneric classification of *Paris* was revised to divide it into two subgenera; *Paris* (comprising sections *Kinugasa* and *Paris*) and *Daiswa* (comprising sections *Axiparis, Thibiticeae and Euthyra*) [Ji et al. 2006].

**2.1.6.2. Genetic diversity studies through SSR markers**

DNA-based molecular markers have acted as versatile tools in various fields such as taxonomy, physiology, embryology etc. Molecular permit us to make estimates of genetic diversity directly at the DNA level, reducing the interference of environmental factors (Cabral et al. 2011). During recent decades, SSRs (simple sequence repeats), also known as microsatellites, have become the most popular source of genetic markers owing to their high reproducibility, multi-allelic nature, codominant inheritance, high abundance, and wide genome coverage (Sharma et al. 2007; Demir et al. 2010).

Zheng et al. (2012) reported the isolation and characterization of 12 polymorphic microsatellite loci from *P. polyphylla* var. *chinensis* using a biotin-capture method (Bloor et al. 2001), and the analysis of the genetic diversity of *P. polyphylla* var. *chinensis* using SSR molecular markers. Simple sequence repeats (SSRS) using sequence analysis of molecular ITS regions in a phylogenetic study involving *P. polyphylla* var. *yunnanensis* and its 11 (eleven) congeners gave an accurate method

for distinguishing different species of *Paris* and for precise identification of *P. polyphylla* var. *yunnanensis* (Liu and Ji, 2012).

These microsatellites are the most popular source of genetic markers (Sharma et al. 2007). *P. polyphylla var. chinensis* is one of the sources of Rhizoma Paradis, a high value medicine for the analysis of genetic diversity of this plant, 12 microsatellite markers that have been isolated and characterized are important for identification, population genetic studies and conservation of the species (Zheng et al. 2012).

Simple Sequence Repeat (SSR) has been widely used in the study of genetic identification and fingerprint mapping with the characteristics of high polymorphic information content (Abbasi et al. 2014; Zhang et al. 2014; Ghaedrahmati et al. 2014). EST collections can also contribute to the development of molecular markers for further genetic research on *Paris* species. To optimize the conservation and utilization of *P. polyphylla*, the development of Expressed Sequence Tag (EST-SSR) markers is very useful for germplasm identification and research into the genetic diversity of this species. Wang et al. (2016) utilized Illumina HiSeq 2000 second-generation sequencing technology to characterize the root transcriptome of *Paris* and to develop EST-SSR markers. They characterized 8 novel polymorphic EST-SSR markers for this species. These EST-SSR markers provide an important tool for the study of genetic diversity in *P. polyphylla*. Wang et al. (2016) employed the SSR markers to assess genetic diversity. The root transcriptome sequences generated revealed novel gene expression profiles and offer clues for further study of the molecular mechanism of *Paris* root secondary metabolite synthesis. The EST-SSR markers identified may facilitate marker-assisted breeding or genetic engineering schemes involving this species.

### 2.1.6.3. Phylogeny through Chloroplast genome analysis

Chloroplast genome have found extensive applications in plant phylogeny (Dong et al. 2013a; Xue et al. 2012), genome evolution (Dong et al. 2013b), and high-resolution DNA barcode (Dong et al. 2014). The complete chloroplast genome (cpDNA) sequence of the *P. polyphylla* var. *yunnanensis* based on the next-generation sequencing method has been reported. The chloroplast genome was 157.67 kbp in length. It contained 115 genes in total, including 81 protein-coding genes, 30 tRNA genes, and four rRNA genes. Seventy-eight chloroplast genes were used to construct a phylogenetic tree to confirm the location of *P. polyphylla* var. *yunnanensis* by a maximum likelihood analysis with RaxML program (Illuminia Inc., San Diego, CA), and the phylogenetic analysis suggested that genus *Trillium*, *Paris*, *Fritillaria*, and *Lilium* were monophyletic while *P. polyphylla* var. *yunnanensis* was closely related to *Trillium* (Song et al. 2015).

In order to better understand the evolutionary relationship among plant species, the complete chloroplast (cp) genome of 12 *Paris* taxa were sequenced. Comparative analysis of overall genome structure revealed similarly between the species. Phylogenetic analysis based on the cp genome supported the idea of the regeneration of taxa into two genera; *Paris s.s.* and *Daiswa* (Huang et al. 2016). This treatment is also justified by both morphological and geographical evidences.

Gene duplication in cp genomes of higher plants is usually found in tRNA genes (Hipkin et al. 1995), however, such occurrence has not been found in monocots (Do et al. 2014). The cp genome of *P. verticillata* and *P. quadrifolia* is unique in having three copies truL-CAU gene in each, which is not found any other taxa of *Paris*. The

cp genomic phylogeny also established sister relationship between *Paris-Daiswa* and *Trillium* clade.

Generally, the plastid loci rbcL, matK and psbA/trnH are used as universal DNA barcodes are found to have extremely low variability and hence not useful to identify *Paris* s.s. or *Daiswa* species. Consequently, ten plastid DNA regions harboring a high proportion of SNPs which are useful for species identification in *Paris* s.s. and Daiswa have been identified. The study highlighted the potential of cp genome analysis for species identification in phylogenetically difficult plant genera (Huang et al. 2016).

DNA barcoding is a powerful tool for the identification of medicinal plants and their adulterants at species level (Han et al. 2016). However, DNA sequencing of individual species is time consuming and expensive (Osathanunkul et al. 2015). These bottlenecks have been circumvented by high resolution melting (HRM) analysis of ITS2 amplicons. This is a fast, reliable, low-cost PCR closed-tube analysis for the identification and authentication of species (Osathanunkul et al. 2016). Such BAR-HRM analysis has been used to differentiate *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* from their adulterants. The technique could effectively identify the above two authentic *Paris* species and also differentiate their common adulterant species. In view of its fidelity and simplicity, this method has got great future potential (Duan et al. 2018).

### 2.2. Distribution and ecology

#### 2.2.1. Distribution of *Paris polyphylla*

In wild habitat the genus *Paris* was naturally grows in shady, well-moistened places under the dark canopy of Rhododendron forest and sometimes grows under open

canopy of mixed broad-leaved forest. Hara, (1969) reviewed an exceedingly polymorphous species variation in *P. polyphylla* from three different countries viz. Bhutan, India and Nepal where he observed that the size of the population is rather small and uniform in many characters. All the species of genus *Paris* except the European species *P. quadrifolia* L. and the Caucasian species *P. incompleta* M. Bieberstein are endemic to eastern Asia (Osaloo and Kawano, 1999). Liang and Soukup, (2000) reported the distribution of the species in countries like Bhutan, China, India (Sikkim), Japan, Korea, Laos, Mongolia, Myanmar, Nepal, Pakistan, Russia, Thailand, Vietnam and Europe. Yesil and Ozhatay, (2014) described the genus *Paris* L. from Turkey, which was represented by two species viz. *P. incomplete* M. Bieb. and *P. quadrifolia* L. *Paris formosana* Hayata (a Taiwanese native Rhizoma Paradis cultivar) was collected from different areas of Taiwan (Lin et al. 2011).

There are widely known subspecies and varieties of *P. polyphylla* distributed in Bhutan, Laos, Myanmar, Thailand, and Vietnam as well (Mayirnao et al. 2017). According to Kew Royal Botanical Garden's World Checklist China, Tibet, Nepal, Taiwan, Laos, Myanmar, Thailand, Vietnam are the countries where the *P. polyphylla* Smith is found. Cunningham et al. (2018) reported that there was also a single collection from Pakistan particularly from the area of Bagh Tehsil, near Dhuli.

Yonzone et al. (2012) reported that the status of *P. polyphylla* is categorized as sparse in India and listed under endangered category in many Himalayan states like in Himachal Pradesh, Jammu and Kashmir and Uttarakhand (ENVIS, 2010) but the species is well distributed in Uttarakhand region (Tiwari et al. 2010). In IHR, species of genus *Paris* is distributed in Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Mizoram (Lushai Hills), Nagaland, Sikkim, and

Uttarakhand (Paul et al. 2015; Cunningham et al. 2018), Mayirnao et al. 2017). Deb, (2015) conducted detailed study of *P. polyphylla* from one of the Indian North-east states of Nagaland and reported that the plant was available in wild habitats from the areas like Aradura hill, Chida, Longkum and Pangsha area. Jamir et al. (2015) also carried out survey and reported availability of the species from the areas mentioned above. Paul et al. (2015) reported the availability of these species from the areas like Bomdila, Mayudia and Talle Valley of Arunachal Pradesh in one of the Eastern Himalayan Region of India. Similarly, in Manipur, the species was found growing in abundance in the places like Hengbung, Maram, Purul and Makui regions of Senapati district and Puilong in Tamenglong district (Bhat et al. 2017). The species was also distributed in Aka Hills of Assam state (Shah et al. (2012), Sharma et al. 2015; Cunningham et al. 2018).

### 2.2.2. Major threats to the species

*Paris polyphylla* Sm. listed as vulnerable under IUCN threat category in Nepal (Madhu et al. 2010). *P. polyphylla* var. *yunnanensis* was considered under near threatened (NT) in China's Conservation Red List and the data shows that China imported more than 1200 tons dried rhizomes of *Paris* genus in 2015 from Vietnam, Laos, Myanmar, Nepal, India and other few countries (Anonymous, 2016). Thus, illegal import by Chinese companies is one of the main causes of threatening the species all across South-East Asia. Singh et al. (2008) highlighted the issue of exploitation of *Paris* herb in the natural habitat for the need of cash by the villagers as well as local dealers and the herb is threatened with to extinction if the existing trend of unlawful trading is continued without protection and mass production approach. The mass import by China has endangered the wild resources of *Paris* and pushed them to near extinction (Qin et al. 2018). Trampling of *Paris* habitat by

livestock also destroys the plant species as seen in Nepal where the plant is also grazed upon by ungulates. As only a single or few offspring are produced from a single mother plant that too when harvested unscientifically leads to rapid decline in its population (Figure 2.2). Moreover, the local belief that the plant harvested in the first month of local calendar i.e. mid-April is more effective renders the rhizome collected too early i.e. before maturity. Such overharvesting before maturity prevents seed production is consequently a major threat for the regeneration of the species. September-October is the right season for harvesting (Madhu et al. 2010). In addition to overexploitation and increasing demand for medicinal ingredients of *P. polyphylla* rhizomes slow growth of the plant has not been able to match with the market demand particularly the wild stocks of *P. polyphylla* var. *yunnanensis* have been reduced year by year (Meng et al. 2005). The practice of unscientific collection in the wild in where all the underground parts are removed without leaving any fragment might lead the plant to rapid extinction (Madhu et al. 2010). The increasing application has resulted in various problems for the natural resources of *Paris* rhizomes (Huang et al. 2010). The wild *P. polyphylla* populations has been on the edge of extinction during the last few decades because of excessive mining for ethnopharmaceutical usage (Zheng et al. 2012). Unfortunately, slow growth and over-harvesting for the past several years have led to a substantial decline in population and now the species faces the danger of extinction (Zhang et al. 2011). Hence, the species needs to be conserved worldwide in time so as to prevent it from extinction.


**Figure 2.2.** (A-C) Wild animal disturbance and (D-F) Anthropogenic activities especially during unscientific rhizome collection in the wild habitat of *P. polyphylla.*

In the Himalayan region, illegal exploitation of natural resources of this plant is a reality. The plant is being pushed to extinction because of premature collection from its habitat, unsustainable harvesting, illegal border trade, overgrazing, forest fires,

soil erosion etc. The story is similar in Nepal, Manipur, Nagaland, Himachal or Uttarakhand (Chandra et al. 2015). Moreover, the seeds of *P. polyphylla* remains dormant for approximately 18 months in natural habitats and therefore the species requires immediate attention (Zhou et al. 2003).

Bees and flies are the pollinators of this species. Therefore, the loss of these pollinators due to climate change or other anthropogenic activities affects the population of the plant. The plant is very sensitive to environmental vagaries. It is a shade loving plant and if shade is removed due to habitat distribution that affects seed production and also causes wilting of the plant (Deb et al. 2015). Anthropogenic activities like shifting cultivation, road broadening, urbanization, forest resource collection, logging etc. are affecting the wild population of species in Himalayan states like Arunachal. Moreover, rampant collection, grazing and landslides are the hinderances for its regeneration (Paul et al. 2015). Some local tribe in Arunachal also use the shoot of the plant as vegetable (Payum, 2018).

The lack of awareness in usage of wild plants has led to severe dilapidation of the forest bio-diversity and owing to forest degradation, several medicinal plants are threatened and consequently jeopardizing the livelihoods of the local people (Zakir, 2012). A very high industrial and export demand for herbal and cosmetic industry has greatly accelerated herb collection from the wild. Particularly in India, 95% of the demand for medicinal plants of herbal pharmaceutical industries is met from collection from the wild (Tandon, 1997). With the rapid development of the of Chinese Traditional Pharmaceutical industry, the requirement of *P. polyphylla* var. *yunnanensis* produced by the manufacturers is increasing at the rate of 20% per year (Zhang et al. 2004), which leads to the severe decline of the wild resources and

significantly hampers the yields of the pharmaceutical manufacturers and the quality of the products (Li and Yang, 2005).

To maintain the quantity and quality of *P. polyphylla* seed, the collection of seeds may start from the month of September and it is very important to provide shade whenever a cultivation or a domestication attempt is made as it was also observed that the leaf crumble and then die if grown under direct sunlight (Anonymous, 2017).

Spreading awareness about the vulnerability of the plant, its high therapeutic properties, economic importance and getting the participation of the people from various localities of different countries where the plant thrives could increase its population. More importantly, a good conservation strategy could include the species in the priority species list of both the National and State Medicinal Plant Boards for cultivation. This may be helpful for its long-term management and conservation of the species (Puwein and Thomas, 2020).

### 2.2.3. Associated plants

In Nepal Himalaya. the most common associated species of *P. polyphylla* are *Viburnum erubescens, Arisaema* spp. and *Sarcococca coriace.* It was observed and reported that there was a very strong association of *P. polyphylla* with the *Arisaema* species. It was also found that the *P. polyphylla* plant's absence or presence in an area was easily indicated by the presence and absence of *Arisaema* plant in all the natural habitat (Madhu et al. 2010).

The dominant species associated with *P. polyphylla* as reported from Arunachal Pradesh were *Anaphalis busua, Artemisia nilagirica, Arisaema* sp., *Carduus edelbergii, Centella* sp., *Crassocephalum crepidioides, Cyperus cyperoides,*

*Fragaria nubicola, Fragaria vesca, Galearis spathulate, Geranium pretense, Gnaphalium affine,* Grass sp., *Hemiphragma heterophyllum, Hydrocotyle asiatica, Hydrocotyle himalaica, Impatiens* sp., *Podophyllum hexandrum, Polygonatum verticillatum, Polygonum hydropiper, Potentilla plurijuga, Pteris* sp., *Plantago asiatica, Plantago major, Rubia manjith, Rubus calycinus, Rubus nepalensis, Rumex nepalensis, Centella* sp. *Senecio raphanifolius, Selaginella* sp., *Senecio wallichii, Swertia Coptis teeta* and *Viola* spp. (Paul et al. 2015)

In Sikkim Himalaya, *P. polyphylla* is abundantly distributed under the canopy of *Tsuga dumosa*, *Picea smithiana, Schima wallichii* with *Rhododendron arboreum* and under most common shrub and herb species like *Rhododendron niveum, Viburnum erubescens, Piptanthus nepalensis, Ribes* spp., *Arisaema griffithii, Euphorbia sikkimensis, Polygonatum* spp., *Persicaria* spp. and many ferns species (Subba and Lachungpa, 2016). It was also observed that this species thrives well in the presence of some common associated species within 5m perimeter. These common associated species include trees, herbs, and climbers (Puwein and Thomas, 2020). When taken out from its natural environment the herb grows well with associated plants as found in the wild. It was also found that in Sikkim Himalaya 31 different species of plants including *Aconogonum molle, Arisaema griffithii, Arisema ciliatum, Aresima costatum, Artemisia vulgaris, Cantella* sp., *Commelina* sp., *Crassocephalum crepidioides, Cyperus cyperoides, Deparia boryana, Drymeria cordata, Elatostema umbellatum, Fragaria nubicola, Girardinia diversifolia, Impatiens* sp., *Panax bipinnatifidus, Plantago asiatica, Polygonatum varticillutum, Polygonum hydropiper, Pouzolzia hirta, Pteris* sp., *Rubia manjith, Rubus ellipticus, Rumex nepalensis, Sarcococca coriaceae, Selaginella* sp., *Trillium govanianum, Trillium tschonoskii, Urtica dioica, Viburnum erubescens and Viola* sp. etc. of which

*Fragaria nubicola, Sarcococca coriaceae* and *Pteris* sp. are the most dominant species (Lepcha et al. 2019). The associated plants may be used to devise strategies for mass multiplication and conservation (Paul et al. 2016). Similarly, it was found that 12 species of plants were most commonly associated with *P. polyphylla* Smith in different places of Nagaland. These species include *Ageratum conyzoides, Bidens Pilosa, Breynia* sp., *Curculigo capitulate, Dioscorea* sp., *Drymeria cordata, Eupatorium adenophorum, Fagopyrum esculentum, Hottuynia cordata, Lycopodium* sp., *Smilax* sp. and *Spilanthes acmella.* Most of these plants belongs to family Asteraceae among the herbs (Deb et al. 2015), however, the tree species belongs to various species. Interestingly, in Nagaland the species of *Aresima* is not found as an associated species to *P. polyphylla.*

**2.3. Phenology of the plant**

**2.3.1. Seed germination**

*Paris polyphylla* plant starts germinating during the month April and generally flowers in April/May. The germination of seeds is extremely rare in wild and did not even took place in green-house or in laboratory under controlled condition and treating it with different chemicals (Madhu et al. 2010). Joshi, (2012) observed the issue of germination of *P. polyphylla* (Satuwa) and found that the seeds take about 6-7 months to germinate and remains viable for a year. Hence, seeds collected the previous year are sown in the following season of the next year.

After conducting several temperature treatments for germination of *Paris* seeds it was found that Periodic introduction of the seeds to a low-temperature of $4^0$C broke the dormancy in approximately 112 days. The seed coat, predominantly the mesophyll outer coating of the seed skin strongly subdued the germination. After elimination of these coat and exposure of the uncoated seeds to 600 mg/ L $GA_3$ for

48 hrs. before the temperature stratification of 14 days at 4 $^0$C and 14 days at 22$^0$C for 112 days, seed sprouting proportion results as high as 95.3% in about 160 days (Zhou et al. 2003). Altogether six temperatures were considered to stratify the seeds with the goal to discover the effects of the stratifications on the embryo differentiation. The result resolved that the most favorable temperature was between 15ºC and 20ºC where the embryo emergence ratio was around 20.82 ± 5.41 % (Ye et al. 2011).

### 2.3.2. Rhizome growth

Generally, the rhizomes of *P. polyphylla* are horizontal in shape and creeping with transverse bud scale scars (ring) on its rhizome. Every year after the senescence of the above ground part, one ring added to the rhizome but sometimes a plant can also give rise to compound bud, thus predicting the age of the plant by the rings/bud scale scars is irrelevant and not regarded as an accurate method (Deb et al. 2015).

Jamir et al. (2015) found that from a single rhizome can give rise to multiple shoots as the plant gets old and rhizome segments with multiple buds were separated and planted in the field. The *P. polyphylla* plant propagates efficiently with underground rhizomes and this procedure is more prolific than propagation from seeds (Joshi, 2012). The rhizome cuttings and fragmentation (traditional technique without any hormonal usage) showed good plant growth on the prepared soil bed and the plant grows well when more appropriate spacing is given during domestic cultivation. However, the specimen resulted in slow growth rate, presence of high dormancy and the mortality rate of the cut rhizomes was a bit higher (Jamir et al. 2015).

### 2.3.3. Rhizome Sprouting

Before the plant dies and after the seed gets matured, bud sprouts on rhizome which remains underground for the whole winter until the next sprouting season and so, the rhizome is dormant for almost five months (Madhu et al. 2010). *P. polyphylla* usually starts sprouting from February onwards till March and gives rise to new ground above stems, leaves and inflorescence. Throughout the sprouting of the ground above plant body, the leaves and the inflorescence usually develop synchronously and therefore non-reproductive plants are easily identified when the inflorescence lacks in the plant (Deb et al. 2015).

### 2.3.4. Leaf development

*P. polyphylla* is a slow growing perennial herb which prefers to grow under the deep moist temperate canopy forest (Cunningham et al. 2018). Mostly, underground buds from the rhizome start sprouting from February onwards till the month of April and gives rise to new above ground stems and leaves. During the sprouting of the above ground plant body, the leaves and the inflorescence usually develop together (Deb et al. 2015).

After the comprehensive observation in the nursery it was found that the first underground primary root initiations of seeds start around 160-170 days after showing (DAS). Young bud emerges underground from the seed after 310-350 days. But, the first field apical growth starts around 410-430 days (DAS) and eventually turns into one leaf development of about 3-6 primary root initiations at 450-480 (DAS). Finally, after 820-850 (DAS) the plant attains proper shoot with 4-6 leaf and development of about 5-9 roots was observed.

Incase of rhizome cutting propagations the first minute shoot emergence from the main rhizome node starts around 110-150 days after showing (DAS). An active apical growth initiation of distinct whorl leaf (with majority of non-flowering plants) was observed around 150-180 (DAS).

The plant has an aerial herbaceous erect smooth stem with a leaf whorled of 6-12 green leaves which vary in shape from broad obovate to almost linear, from cordate to cuneate (base), and from long petiolate to subsessile parallel reticulate to lanceolate shaped leaves. The peduncle is approximately 5 mm long or up to 60 cm long. The petals (the inner tepals) are filiform and often as long as 8-10 cm, but sometimes much shorter (1-3 cm) than sepals, or totally absent in some populations Hara, (1969); Shah et al. (2012).

### 2.3.5. Flowering and floral characters

The plant flowers generally in March-April and the inflorescence are terminal, solitary. After systematic observation in the nursery it was found that the first flower initiations from seed germinated plants start around 840-870 (DAS). Where young bud formation takes place which later gave solitary-terminal flowers. Subsequently, seed pods formation with few minute red seeds was observed around 970-1010 (DAS).

Incase of rhizome cutting propagations the first flowering bud formation takes place around 560-590 (DAS) which gradually increases the ratio of flowering then non flowering plants.

### 2.3.6. Pollination

Wang et al. (2009) described the process of anther's daily opening and closing in detail, detected the eco-physiological factors that impact on anther opening and closing, and discussed the fitness of this special anther behavior with the fly-pollination mechanism in *P. polyphylla* var. *yunnanensis.* indicated anther opens earlier and closes later in sunny days than cloudy days. Li et al. (2007) reported the same phenomenon in three other species of *Paris*. Faegri and van der Pijl (1979) believed that anthers would not open until the weather was warm and dry enough to facilitate pollination. Wang et al. (2009) further concluded that both opening and closing of the anthers operated through a longitudinal slit on each lateral surface of anther. Though, the two processes underwent with opposite orientations: opening starts from top to bottom and closing starts from bottom to top. Light was detected as the main factor controlling this daily anther opening and closing. Anther opening can be reversed in response to rain and reopens when rain stops minimizes the pollen loss and is highly adaptive. The extended flowering period of the plant may be related to relatively low levels of effective pollinator activity observed in natural populations. Relative humidity (RH) has a slight effect on anther opening and closing.

In *P. polyphylla* var. *yunnanensis* it was found that fly was the main pollinating agent alongside bees which act as an active pollinator during its peak flowering season (Dingkang et al. 2009). Generally, the *P. polyphylla* flowers are self-pollinated. Yet, the probabilities of pollination can occur by small insects such as different types of flies and bees especially during the middle of daytime. Wind may also cause pollination since the pollen grains are very light once the pollination took place, the colour of stigmatic lobes turns from bright to dull purple (Deb et al. 2015).

### 2.3.7. Seed development

After fertilization, the ovary increases forming a round spherical ¾ inch green capsules which split when ripe in late summer to reveal tiny red seeds (Shah et al. 2012; Deb et al. 2015). These tiny red seeds mature in October and subsequently by the beginning of mid-November, most of the aerial plant part dies out and all seeds get dispersed from the plants (Madhu et al. 2010).

Ye et al. (2011) observed that the seeds dispersed from parent plants has only one undifferentiated, round embryo (i.e., proembryo) in a seed. The average embryo length and embryo-emerging ratio of the proembryo were 0.25±0.03 mm and 5.65±0.76% respectively, showing that the embryo was only a clump of undifferentiated cells and the seeds did hold the property of the morphological after ripening of embryos. In *P. polyphylla* seeds are produced during warm weather usually mid-summer but if the plants are exposed to direct sunlight, aborts the seed or seed production is affected significantly (Deb et al. 2015).

## 2.4. Propagation technology of *Paris polyphylla*

### 2.4.1. Land preparation

The plant grows from 2000 to 3000 m in the temperate area (Sharma et al. 2015). A cool, moist, temperate or sub-alpine climate, a temperature ranging from 5-25$^0$C, high humidity are suitable condition for its cultivation. The plant prefers mountain slopes instead of plain land and cannot withstand waterlogging. A gentle slope (North facing) that allows excess water to run off without causing soil erosion with light sandy-loam soil is suitable for its propagation. Moist humus-rich soil slightly acidic to neutral pH 5.6-7.5 under full shade is preferred by the plant (Thomas, 1990; Huxley, 1992). The land should be tilled thoroughly or hoed to obtain a fine tilth. The nursery bed should be about 5-10 cm from the ground, approximately, 5cm of

topsoil should be mixed with sand, forest litter and farmyard manure (1:2:1). The average size of the bed for *P. polyphylla* may be 10m in length and 1m in width in the nursery which may again be divided into 1 x 2m blocks (Joshi, 2012). The plant can be suitably rehabilitated in artificial habitats if similar temperature is maintained at similar altitudes identical to the wild. However, waterlogging was found to be lethal for regions having this *Paris* herb, (Puwein and Thomas, 2020).

**2.4.2. Seed sowing**

It has been found that the *Paris* seeds require at least 2 exposure to low -temperature to break dormancy in a period of 4-6 months (approximately $1/3^{rd}$ of normal dormancy period). Low temperature treatment and treatment with growth regulators significantly enhanced the germination percentage.

Before sowing the seeds are washed to remove their red-sticky pulp and dried under shade. The seeds are then planted approximately 1.25cm deep in the bed with a distance of 5cm between plants and 10cm between rows. Seeds are either planted on September or January-February. Mulching of paddy straw or pine needles is provided which should be 5cm thick above the seed bed and requires watering twice a day (Joshi, 2014). One study suggested that the month of March-April as the best time to sow the seeds which germinates $1^{st}$ at a soil temperature of $23^0$C; The seeds should be soaked in cold water for 1-3 hrs. prior to sowing and the seed bed should have soil and sand composition of 5:2 for better results (Joshi, 2014).

Probert, (2000) described that temperature is one of the most significant environmental factors regulating seed dormancy and germination of most plant species. It was observed that a minimum of two low-temperature incubation periods (which passes through two winters, allowing for germination to occur in spring) was

required to break the seed dormancy of the seeds of *Paris polyphylla* in the natural environment (Li, 1984).

Zhou et al. (2003) established an effective approach for breaking seed dormancy and enhancing seed germination of the medicinal herb *P. polyphylla* var. *yunnanensis,* which is mainly through low-temperature stratification and application of growth regulators (gibberellic acid (GA), kinetin (KT), 6-benzyladenine 3 (6-BA) and ethephon). After various temperature treatments, periodic change of temperature from 14 days at 4 $^0$C. Followed by 14 days at 22 $^0$C was shown to be most effective to break the seed dormancy which leads to 42.8% germination rate after 49 days of experiment. The seed dormancy of *Paris* is due to morphological reasons and the incomplete development of the embryo is the main reason for its longer dormancy. The stratification alternating between 15$^0$C and 20$^0$C promoted the embryo differentiation and development best (Ye et al. 2011). The outer mesophyll layer of the seed coat inhibited germination. Therefore, removal of the seed-coat and exposure of uncoated seeds to 60mg/L $GA_3$ followed by two phase cold-stratification treatment mentioned above gave the best result of 95.3% germination within 160 days (Zhou et al. 2003).

### 2.4.3. Vegetative propagation by rhizome splitting

*P. polyphylla* Smith propagates competently with underground rhizomes and this method is more productive than propagation from seeds. The rhizomes are planted directly on the field maintaining a plant to plant distance of 20cm and row to row distance of 30-60cm. It takes 3-4 months for leaves to come out (Joshi, 2012).

For vegetative propagation (through rhizome splitting) of *P. polyphylla*, the soil texture having soil: loam: sand in 3:2:1 ratio exhibited better sprouting and rooting (76.66% and 73.33% respectively). The percentage of sprouting and rooting work further enhanced when the rhizome cuttings were treated with 100 mg/L each of $GA_3$ and TBA. The rhizome sections of *P. polyphylla* were responsive to large scale vegetative propagation which opens the window of opportunities to meet the demands of the supply raw materials for pharmaceutical industries (Danu et al. 2015). When making rhizome cuttings it is important to note the number of buds and the length of rhizome piece in each cutting. Multiple buds in a cutting is advantageous but if the rhizome length is very short, it will not germinate despite multiple buds (Jamir et al. 2015). Cuttings are planted during October or in March-April with a plant to plant and row to row distance of 45 x 45 cm respectively (Nautiyal and Nautiyal, 2004).

### 2.4.4. Planting

After a year of seed sowing, seedlings are ready for transplanting in the field and the best time for transplanting is May - June. But, before planting Joshi, (2012) recommended for a control burning of the planting site. Ploughing of the nursery field should be done twice before plantations which is followed by the removal of weeds, shrubs, stones from the field. The seedlings are planted 5-10 cm apart on raised soil in the field and the distance between two plant is maintained to approximately about 20-30 cm, while row to row distance is maintained at 60 cm. As *Paris* is a shade-loving plant, shading is required for growth and also to protect the seedbed from heavy rain, direct sunlight, hailstorms etc. So, thatch roof provision is important and alternatively, this may also be made of other materials like bamboo, straw or plastic sheet (Joshi, 2012).

### 2.4.5. Watering and manuring

Watering should be done twice a day for the first-year plant and thrice a day in the hot season in the nursery bed and afterwards occasionally (Joshi, 2012). Regular watering is recommended for the better growth of the seedlings. It is important to maintain well-drained channels in the nursery beds to avoid damping. Intense watering during seedling and early sprouting stages in March-April is needed every 24 hrs. (Nautiyal and Nautiyal, 2004). For manuring mixture of decayed wooden powder, leaves local forest trees and farm manure (cow dung, prefebly a year old) is recommended. Addition of forest litter approx. 30-35 qts/acre above 2500 m and 40-50 qts/acre at 1800-2200 m is beneficial. Mulching annually with decayed manure or forest litter is excellent. Top dressing without mixing the well-decayed manure with soil, during winter or before sprouting is beneficial (Nautiyal and Nautiyal, 2004).

### 2.4.6. Modulation of growth by $GA_3$ treatment

Senescence is subject to regulation by internal factors, such as hormonal status and reproductive development, and by environmental factors, such as extreme temperature, drought, waterlogging, photoperiod, mineral deficiency and pathogenic infection (Munne, 2008). Gibberellin ($GA_3$) treatment is known to delay senescence in numerous plant species (Whyte, (1966); Yu, (2009). In other words, $GA_3$ treatment retarded senescence of the aerial parts and others increased their longevity. Due to the retarded senescence the leaf area is increased and the duration of photosynthesis is prolonged. Therefore, retarded senescence results in increased yield (Masclaux-Daubresse, 2008).

Senescence is an exceedingly regulated and genetically controlled procedure that leads to death of plant parts or whole plants (Nooden et al. 1997). The senescence of leaf discs of *Tropaeolum majus* could be delayed by gibberellic acid treatment

(Beevers, 1966). It has been found that $GA_3$ treatments in case of *P. polyphylla* retards growth, increases rhizome yield and improves rhizome quality in terms of its secondary metabolites content (Yu et al. 2009).

Senescence may be triggered by the oxidation of the plant cells and increased production of reactive oxygen species (ROS). It has been found that ROS may function as signals to activate genetic programming leading to regulated cell suicide (Foyer and Noctor, 2005; Vanacker et al. 2006). During senescence, a decline in endogenous GA and increase in ABA levels takes place (Chin and Beevers, 1970). Application of $GA_3$ delays senescence (Kappers et al. 1998; Rosenvasser et al. 2006) whereas ABA promotes senescence of aerial parts, causes the accumulation of endogenous GA and burns the $H_2O_2$ production while antagonizing ABA. Lowered level of oxidative stress maintenance of antioxidant enzyme activity, decreased lipid peroxidation etc. ultimately leads to retardation of senescence (Yu et al. 2009b).

In *P. polyphylla* exogenous application of $GA_3$ not only resulted in the increase in endogenous GA level and retardation of senescence but also in decreased degradation of chlorophyll. Such treatments inhibited the enzymes involved in chlorophyll breakdown viz. chlorophyllase, Mg-Dechelatase, Peroxidase and Lipoxygenase (Li et al. 2010).

Nitrogen is the most important plant nutrients and is essential for plant growth. Nitrogen 0mobilization from senescing levels and reuse by other plant parts may enhance nitrogen use efficacy (Masclaux-Daubresse et al. 2010). In *P. polyphylla,* treatment with $GA_3$ allowed the senescence retarded the decrease in glutamine synthetase and glutamate dehydrogenase enzymes, and the content of nitrogen. Low nitrogen resorption efficiency during senescence of *P. polyphylla* results from a

sharp decrease in nitrogen remobilization enzyme activity which may be ameliorated by $GA_3$ treatment (Yu et al. 2012).

### 2.4.7. Harvesting

Flowering and seed formation happen after 3-4 yrs. From seed raised plants. Plants raised from vegetative propagation however matures earlier. Seeds ripen in October and harvesting should be done after that (Nautiyal and Nautiyal, 2004). The suitable period for collection of the underground rhizome is after the month of September - October after fruits are fully ripened. The underground parts are dug up carefully by pick-axes or by hand and most importantly, some rhizomes are left in the field itself to facilitate natural regeneration in the next year (Joshi, 2012). Collection, of seeds after its maturity is recommended to ensure good viability (Joshi, 2014).

### 2.4.8. Seed collection

*P. polyphylla* seed collection could be started from September of the year. lf there is any delay from September, the quantity and quality will be compromised as all the good seed will be dispersed first (MAP Programme, Thimphu, 2017). A matured *P. polyphylla* fruit contains about 50-60 seeds (Singh et al. 2008). As per the study conducted at four different localities of Nepal, it was observed that each plant produced an average of 41.77 seeds/plant, with seed production ranging from 28–56.4 seeds per plant and the average seed production was found to be 74.35 seeds per $m^2$ (Madhu et al. 2010). The seeds are dispersed by gravity and dispersal is localized in and around the mother plant (Deb et al. 2015). It has been found that seeds collected during June-July only 20% are viable and those collected during August-September 80% viable (Joshi, 2014).

Joshi, (2012) suggested that for proper collection of the seeds from healthy plants the appropriate timing would be during the month of July to August. After collection the seeds were cleaned by removing the outer coat and dried in sun properly before storing. *P. polyphylla* is a shade loving plant and exposure of the plant to direct sunlight during developmental phase affects seed production. Maintenance of the plants under 50-80% shade gives the best production of seeds (Deb et al. 2015). It appears that *P. polyphylla* growing in Western Himalayan region matures in July - August and the one growing in Eastern Himalaya matures in September – October.

### 2.4.9. *In-vitro* propagation of *Paris polyphylla*

Plant tissue culture was proved to be a suitable tool for the conservation and rapid propagation of pharmaceutically important and rare plants (Baskaran and Jayabalan, 2008) and the only technology for the production of large quantities of elite planting material so as to increase the biomass and productivity (Kehie et al. 2013).

Verma et al. (2012) successfully established in vitro propagations of Twenty-three pharmaceutically important plants belonging from different biodiversity zones of India. Of these, shoot regeneration in *P. polyphylla* was achieved by culturing rhizome explants in MS media supplement with 2.0 mg/L BAP. The shoot culture showed first bud emergence after six months. However, rooting of the plantlets could not be achieved (Verma et al. 2012).

Experimental results have shown that 4°C temperature treatment can effectively reduce the contamination rate of isolated culture of *P. polyphylla* (Yang et al. 2014). However, Teerawatsakul et al. (2004) have found that invitro culture of *P. polyphylla* var. *chinensis* from rhizome shoot tip explants showed best response when cultured between temperature of $14^0$C and $18^0$C which controls the endophytic bacteria and promotes shoot growth.

The thin cell layer technique has been used for mass propagation of some significant medicinal plants species such as *Panax ginseng* (Nhut et al. 2003) and *Spilanthes acmella* (Singh et al. 2009). The technique involves the use of small sized explants excised from different plant organs either longitudinally (lTCL) or transversally (tTCL) (Silva, 2003) and was first described in *Nicotiana tabacum* (Van, 1974). Raomai et al. (2014) reported the micropropagation *of P. polyphylla* through somatic embryogenesis from immature zygotic embryos. Further, they described the effect of cytokinins on mini-rhizome (MR) formation from tTCL followed by analysis of steroidal saponin production in different concentrations of cytokinins.

*P. polyphylla* plant exhibited the highest response (86.6 %) in terms of MR formation was achieved from basal sections cultured on ½ MS medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP). MRs transferred to plant growth regulator free medium gave rise to shoot buds that eventually regenerated into plantlets and were successfully acclimatized with a survival percentage of more than (95 %) under greenhouse conditions (Raomai et al. 2015). Production of MRs via thin layer culture can greatly contribute to year-round production of MRs for large scale shoot regeneration, germplasm storage, international germplasm distribution or exchange and for secondary metabolite production (Raomai, 2015). *P. polyphylla* exhibited recurrent and easy somatic embryogenesis, which could pave the possibility of scaling up the propagation (Choudhary and Tandon, 2014).

### 2.5. Traditional uses of *Paris polyphylla*

#### 2.5.1. Traditional uses in Indian Himalayan Region

The indigenous tribes of Northern part of Sikkim uses the tuber of *P. polyphylla* in the form of paste and poultice, which are externally applied in skin diseases, cuts, wounds and poisonous insect bite. The powder or infusion of the roots is taken orally

for the treatment of diarrhea and dysentery (Maity et al. 2004). Rhizome juice is taken to expel intestinal worms. A piece of rhizome or its powder is taken against food poisoning and fever (Sharma et al. 2015). The rhizome is also used in the treatment of headache, wounds and burns (Pradhan and Bhutia, 2019). In Sikkim Himalaya, the dried rhizome (1.0-1.5cm) is soaked overnight in a glass of water and the water drank in the morning to cure headache and as a tonic by the local Lepcha people (Lepcha et al. 2019). In many states of North-east India, it is commonly used as a stimulant and the rhizome powder is taken as tonic by the local people (Shankar and Rawat, 2013). In Arunachal Himalayas (AH) the local communities use the tuber as antidote for snake and insect poison (Sharma et al. 2015).

In Siang district of Arunachal Pradesh, the rhizome is used against fever by the Adi tribe and the ripened fruits are taken as an edible fruit by some locals. Furthermore, the paste of the rhizome was used against snake bites by the Sherdukpen tribe in West Kameng district of the same province (Paul et al. 2015).

The local inhabitants of Garhwal Himalaya (Uttarakhand, India) use the powdered roots of this plant for the treatment of heart disease, asthma and bronchitis and also used ethno-pediatrics for diarrhoea (Nautiyal and Nautiyal, 2004); Tiwari et al. 2010). Additionally, Pande et al. (2007) reported that the rhizome powder is also used as an ethno-vetinary purpose for treatment of fever, stomach disorders and shoulder wounds of oxen. In Darjeeling Himalaya, the rhizome infusion is used as an antipyretic agent (Chhetri, 2005). In general, in the Indian Himalayan Region (IHR), *P. polyphylla* is considered to be an important folk medicinal plant traditionally used as analgesic, antibacterial, antiphlogistic, antispasmodic, antitussive, hemostatic, depurative, detoxicant, febrifuge and against gastritis, intestinal wounds, rashes, scabies, sleeplessness, snake bite, stomach pain, typhoid, ulcer and wounds

(Farooquee et al. 2004; Maity et al. 2004; Tiwari et al. 2010; Jamir et al. 2012; Lalsangluaii et al. 2013; Pfoze et al. 2013; Mir et al. 2014; Sharma and Samant, 2014 and Paul et al. 2015).

### 2.5.2. Traditional uses in other parts of the world

It is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care requirements (Farnsworth and Soejarto, 1991). The dried rhizome of *P. polyphylla* Smith named 'Chonglou' in Chinese (Rhizoma Paridis), is officially used in traditional Chinese medicine (TCM) as hemostatic, antitumor, antimicrobial and anti-inflammatory agents in China. It is also widely used in Korea and Japan (Wang et al. 2017; Qin et al. 2018).

Tian et al. (1986) first reported the uterine contractile effects of the rhizome of *P. polyphylla* var. *yunnanensis*. Subsequently, a traditional drug was developed for the treatment of Abnormal Uterine Bleeding (AUB) which was frequently available in China (Zhao and Shi, 2005). Bhattarai, (1992) reported the traditional uses of *P. polyphylla* rhizomes by the lay population of Jumla, Mugu and Kalikot districts of Karnali Zone in West Nepal where a decoction of 5 g of the root in 200 ml of water is drunk warm twice a day for 2-3 days as an anthelmintic as well as a febrifuge. The root-paste is also applied to wounds for rapid healing.

In Nepal, the dried rhizome powder of *P. polyphylla* have also been used in insect bites, for narcotic effects, in internal wounds, external wounds, fever, food poisoning, as anti-helminthic and vermifuge in local communities. The root is also fed to cattle to cure diarrhea and dysentery (Dutta, 2007; Baral and Kurmi, 2006; Shah et al. 2012).

In China, *P. polyphylla* var. *chinensis* and *P. polyphylla* var. *yunnanensis* are used traditionally in treating snake bite, traumatic injury, as contraceptive, sedative and for its hemostatic property (Jun, 1989). The paste of Paradis Rhizoma helps to clear heat, resolves toxicity, reduces swellings, and stops pain. It is also used in toxic abscesses and sores, bleeding due to trauma, and pain from stasis and swelling (Bensky et al. 2004). The root of *P. polyphylla* has been used in the treatment of inflammation, infection, jaundice, skin burns and hyperlipidemia in China and Japan (Peng et al. 2013). The boiled liquid preparation of rhizomes of *P. polyphylla* is used by the villagers of Monpa in Medog County (Tibet, China) for treating diabetes. Roots are also used for epilepsy, shock, fever, and vomiting control as antiemetic (Li et al. 2020).

### 2.6. Phytochemistry

#### 2.6.1. Saponins from *P. polyphylla*

Saponins are heterogenous group of glycosides that are widely distributed in plants and composed of glycosides containing one or more sugar chains on a aglycone backbone also called a sapogenin. The aglycone or sapogenin unit consist of either a sterol or the more common triterpene unit. In both the steroid and triterpenoid saponins, the carbohydrate side chain is usually attached to the 3 carbon of the sapogenin.

Saponins in food have traditionally been considered as “antinutritional factors” (Thompson, 1993) and in some cases have limited their use due to their bitter taste (Ridout et al. 1991). However, food and non-food sources of saponins have come to renewed focus in recent years due to increasing evidence of their health benefits such as cholesterol lowering and anticancer properties (Gurfinkel and Rao, 2003).

A single plant species may contain a complex mixture of saponins. The saponin content of plant materials is affected by the plant species, genetic origin, the part of the plant being examined, the environmental and agronomic factors associated with growth of plant, and post-harvest treatments such as storage and processing (Fenwick et al. 1991).

So far scores of steroidal saponins have been isolated and identified from different species of the genus *Paris* (Huang et al. 2009). The structures of the aglycons from the isolated steroidal saponins of these species may have different skeleton types: spirostane, hexacyclic ABCDEF-ring system: furostane, a pentacyclic ABCDE-ring system with sixth open F ring; pregnane, a tetracyclic ABCD-ring system; and pseudo-spirostanol, a hexacyclic ABCDE-ring system with a tetrahydropyrane F ring and polyhydroxylated saponins. Most of the saponin molecules have an oligosaccharidic group may be linked to the aglycon at C-1. The following (Table 2.3) are the main type of saponins found in *P. polyphylla*:

**2.6.1.1. Furostanol saponins**

Furostanol saponins, having a β-glycosyl unit at the C-26 position of the aglycone moiety, are reported to be usually contained in fresh plants as original saponins, which gradually converted into spirostanol saponins during the drying process. Therefore, furostanol glycoside and its spirostanol analogue have been isolated from *Paris*, such as protogracillin and gracillin (Wang et al. 2013). Furostanol saponins generally show weak cytotoxic activity (Man et al. 2010), but a derivative of it have been found to be cytotoxic to HL-60 cells (Mimaki et al. 2001).

Furostanol saponins was reported for the first time from the tubers of *P. polyphylla* by Singh and Thakur, (1982). The two structures of Furostanol saponins (polyphyllins G and H, isolated from the tubers of *P. polyphylla* have been

elucidated as 3-0-{cY-L-rhamnopyranosyl (1+3) [a+arabinofuranosyl (l-+4)]-P-Dglucopyranosyl}-26-O-[/3-D-glucopyranosyl] (25R)-22a-hydroxy-furost-5-en-3P, 26-diol and its 22-methoxyderivative respectively) were reported by Singh et al. (1982). Tribol a novel furostanol saponin was isolated from the aerial parts of *Tribulus terrestris* of Bulgarian origin which were widely use in the traditional medicine of many countries for treatment of cardiac diseases, edema, eye trouble, skin itch and impotence (Conrad et al. 2004).

Braca et al. (2004) demonstrated phytochemical analysis of the polar extracts of the leaves of *Helleborus viridis* (Ranunculaceae) resulted in the isolation of two new furostanol saponins with lasting antialgic, myorelaxant, and blood vessel regulating actions (Kerek, 1981). Furthermore, Dini et al. (2005) isolated eight new furostanol saponins from the seeds of red onion (*Allium caepa* L. var. *tropeana*) of Italy.

From the sister species of *P. polyphylla, P. formosana* Hyata, 10 steriodal saponins have been isolated of which three were furostanol saponins (compound 1-3) (Lin et al. 2011). They used a HPLC method with evaporative light scattering detector (HPLC-ELSD) to simultaneously determine fuiostanol, pennogenyl and diosgenyl glycosides. In the study a maximum of 10.14± 0.67 mg/g dw of furostanol saponins were found in total which varied based on locality.

Furostanol saponins, with a β-glycopyranosyl moiety at C-26 of the aglycone, were isolated from several *Paris* species. 17-Hydroxy and 22-MeO furostanol saponins were found respectively in *P. verticillata* (Huang et al. 2009; Nakano et al. 1981), *P. polyphylla* var. *yunnanensis* (Zhao et al. 2009 and *P. polyphylla* (Miyamura et al. 1982; Singh and Thakur, 1982). The furostanol saponins, with a C-20 = C-22 bond, were isolated by Zhao et al. (2009) from *P. polyphylla* var. *yunnanensis.* Along with

other compounds, these steroidal saponins are the main active compounds in different *Paris* species (Wei et al. 2014).

#### 2.6.1.2. Spirostanol saponins

Spirostane-type saponins contains hexacyclic aglycons namely, diosgenin, pennogenin and pennogenin with additional hydroxylations at positions 23, 24 and 27. In comparing the structures of pennogenin and diosgenin, it is clear that pennogenin has an extra hydroxyl group at CC-17. The aglycone itself is not active in most instances, as demonstrated for diosgenin and their active glycosides (Nakamura et al. 1996). The saponins isolated from the different *Paris* species contain mono-, di-, tri- or tetrasaccharide, which are composed of glucose, rhamnose and arabinose bound through a β- D-glucosyl moiety to the hydroxyl group at C-3 of the aglycone. Physiologically active pennogenin glycosides are abundant in *Paris, Trillium* (Hufford et al. 1988; Hayes et al. 2009) and some *Dioscorea* genera (Mohato et al. 1981; Haraguchi et al. 1994). Pennogenyl saponins with additional hydroxylations at the 23, 24 and 27 positions have been isolated from *P. axialis* and P.P.Y. (Chen and Zhou, 1987; Chen et al. 1995a). Distribution of the same types of saponins amongst the Trilliaceae suggests a close relationship between and *Paris* (Wang et al. 2013).

A total of 11 spirostanol steroidal saponins with diosgenin or pennogenin as the aglycones, were isolated from the rhizomes of *P. polyphylla* var. *chinensis.* Of these one (compound 11) was a new saponin which showed the structure as pennogenin 3-o-{o-a-L-rhamnopyranosyl-(1-~4)-O-a-L-rhamnopyranosyl-(1-r4)-p-D-glucopyranoside}. Acid hydrolysis of compound 11 with 1M HCL in dioxane-$H_2O$ (1:1) liberated D-glucose and L-rhamnose, and several degradation products from the aglycone (Mimaki et al. 2000).

Phytochemical investigation of the steroidal saponins from the rhizome of *P. polyphylla* Smith var. *yunnanensis* led to the isolation of a pair of stereoisomeric spirostanol saponins (1a and 1b). Their structures were elucidated as (25*R*)-spirost-5-en-3b, 7b-diol-3-*O*-a-L- arabinofuranosyl-(1→4)-[a-L-rhamnopyranosyl-(1→2)]-b-D-glucopyranoside (1a), (25*R*)-spirot-5-en-3b, 7a-diol-3-*O*-a-L-arabinofuranosyl-(1→4)-[a-Lrhamnopyranosyl-(1→2)]-b-D-glucopyranoside (1b) respectively (Zhao et al. 2007).

Investigation on the rhizomes of *P. polyphylla* var. *stenophylla* led to the identification of two highly oxygenated spirostanol glycosides named paristenoside A and paristenoside B. The structures were established on the basis of NMR spectroscopy, mass spectrometry, as well as chemical methods. In addition, the cytotoxicity of these two new saponins were tested against two human cancer cell lines HEK293 and HepG2 by MTT method. However, neither of them showed significant anticancer activity (Jin et al. 2016).

Rhizoma paridis saponins (RPS), steroidal saponins, are the main components in *P. polyphylla* (Zhang et al. 2007). It was found that RPS had potential anticancer activities against lung adenocarcinoma *in vivo* (Man et al. 2009b). However, the exact constituent from *P. polyphylla* active against lung cancer was not known. Therefore, it is not only necessary to know the components in RPS, but it is also important to detect which components are absorbed in plasma after oral administration that plays a functional role *in vivo.*

In order to discover the active antitumor components after oral administration of RPS, Man et al. (2010) investigated the metabolism of RPS in rat using ultra-performance liquid chromatography coupled with diode-array detection and

electrospray ionization quadrupole time-of -flight mass spectrometry (UPLC/Q-TOF-MS), which was much more precise and rapid than HPLC-MS. This technique was used for separation, identification and structural analysis of saponins in Rhizoma Paradis Saponins (RPS) and rat plasma after oral administration of RPS. As a result, thirty steroidal saponins from RPS were identified including diosgenyl saponins like gracillin, polyphyllin-C, polyphyllin-E, polyphyllin-F, paris-I, paris II, paris III, paris V etc. and pennogenyl saponins like paris-VI, paris-VII and paris-H etc. This structural data of the chemical constituents of RPS *in vitro* and *in vivo* provided essential data for further pharmacological and clinical studies of *Paris* saponins (Manet al. 2010). Microwave-assisted extraction coupled with countercurrent chromatography is an efficient preparative technique that has been successfully applied for the separation and purification of steroids (Xiao et al. 2014). Using this technique four steroidal saponins, namely polyphyllin VII, gracillin, dioscin, and polyphyllin I, were separated and purified from *P. polyphylla* (Xiao et al. 2014).

Phytochemical investigations on six *Paris* species (*P. polyphylla* var. *yunnanensis, P. verticillata, P. pubescens, P. axialis, P. polyphylla* var. *pseudothibetica,* and *P. fargesii*) also disclosed that steroid saponins are the main constituents of *Paris,* along with many other constituents. In total, 94 steroid saponins were isolated from the genus *Paris.* Among them, the most frequent representatives (more than 50% of saponin) were the Spirostane-type saponins, the diosgenin or pennogenin as aglycones (Wei et al. 2014).

Pharmacological studies showed that the components extracted from *P. polyphylla* var. *yunnanensis* mainly composed of polyphyllin D, paris H, and paris VII, exhibited a powerful antiproliferation effect by inducing apoptosis and inhibiting metastasis (Man et al. 2011).

From one of the *Paris* taxa, *P. vietnamensis,* four new spirostanol saponins, named pavitnosides A-D (1-4), with six known steroidal saponins were isolated from rhizomes. The aglycones of pavitnoside B and pavitnoside C were not reported in previous work. The cytotoxicity of all saponins was evaluated against human glioblastoma U87MG and U251 cell lines. The new spirostanol saponin 1 displayed anti-proliferative activity against U87MG cell line (Liu et al. 2018). Four bioactive spirostanol saponins (paris saponins I, II, VI, and VII) were detected in the total saponins from the rhizomes and above-ground parts of *P. polyphylla* var. *yunnanensis,* which indicated that they may have similar pharmacological properties. The bioactive assays revealed that both the parts of *P. polyphylla* var. *yunnanensis* exhibited the same hemostatic, cytotoxic, and antimicrobial effects (Qin et al. 2018).

*P. polyphylla* afforded 4 saponins from its tubers viz. polyphyllin C ($C_{39}H_{62}O_{12}$), polyphyllin D ($C_{44}H_{70}O_{16}$), polyphyllin E ($C_{51}H_{82}O_{20}$) and polyphyllin F ($C_{51}H_{82}O_{20}$) which are reported to be the spirostanol glycosides of diosgenin (Singh et al. (1980). Zhao et al. (2007) conducted phytochemical investigation on the steroidal saponins from the rhizome of *P. polyphylla* Smith var. *yunnanensis* which led to the isolation of a pair of stereoisomeric spirostanol saponins along with a new cholestane saponin. Liu et al. (2009) isolated a spirostanol steroidal saponin, named maireioside A, together with three known steroidal saponins; hypoglaucin G, paris saponin I and diosgenin-3-O-a-L-rhamnopyranosyl from the rhizomes of *Paris mairei* which were also isolated from *Trillium kamtschaticum* (Ono et al. 2007).

The structure of the 4 other saponins, polyphyllin C, D, E and isolated from tubers of *P. polyphylla* have been elucidated. Acid hydrolysis of each of these compounds yielded glucose and rhamnose in different ratio as common molecules with diosgenin as aglycone (Singh et al. 1982). The total spirostanol saponins extracted

from *P. polyphylla Smith* var. *yunnanensis* displayed stronger activity than some single compounds of spirostanol glycosides in uterine contraction or platelet aggregation, and the synergistic actions observed in the body may have great clinical significance as it can lead to marked potentiation of the uterine contraction and platelet activation in vivo (Yu et al. 2010). Recent phytochemical investigation of the rhizomes of *P. vietnamensis* also afforded four new spirostanol saponins, named pavitnosides A–D with six known steroidal saponins (Liu et al. 2018).

Man et al. (2013) suggested that diosgenyl saponins had the properties to induce mitotic arrest and apoptosis. Diosgenin is the aglycone of diosgenyl saponins and diosgenin has been shown to suppress inflammation, inhibit proliferation and induce apoptosis in a variety of tumor cells (Man et al. 2013). Diosgenin could also suppress proliferation, inhibit invasion, and suppress osteoclastogenesis through inhibiting the expression of NF-κB-regulated gene (Shishodia and Aggarwal, 2006).

**2.6.1.3. Triterpenoid saponins**

Saponins were regarded as the second metabolites which are extensively distributed in the plant kingdom and act as a chemical barrier in the plant defense system to counter pathogens and herbivores (Augustin et al. 2011). Broadly, saponins are divided into two major classes; triterpenoid and steroid glycosides with the variation in their characters are due to the number of sugar units attached at different positions (Hostettmann and Marston, 1995). It has become evident that some of the natural or synthetic triterpenoids are natural proteasome inhibitors that have great potential for use in cancer prevention and treatment (Yan et al. 2013).

Triterpene saponins have been reported to have numerous biological activities including cytotoxic, antibacterial and antiparasitic, anticomplement, antiviral, hypoglycemic and molluscicidal activities (Szakiel et al. 2008; Park et al. 1999; Zhang et al. 2011). Wu et al. (2013) reported the triterpenoid saponins from genus *Paris* (especially from the dried rhizomes of *P. polyphylla* var. *yunnanensis*). These phytochemical investigations of the dried rhizomes, resulted in the isolation of six new oleanane-type triterpenoid saponins, paritrisides, A-F. Triterpenoid saponins were obtained for the first time from genus *Paris* along with nine known triterpenoids saponins. All these triterpenoids saponins were obtained from the genus *Paris.*

**2.6.1.4. Open chain (cholestane) steroidal saponins**

Tian et al. (1986) reported that the total steroidal saponins extracted from the rhizome of *P. polyphylla* Sm. var. *yunnanensis* remarkably reduced the extent of haemorrhage by approximately 95% in case of abnormal uterine bleeding. Several pharmacological investigations have shown that many steroidal saponins have been isolated and identified from Chonglou (the dried rhizomes of *P. polyphylla* Smith var. *yunnanensis* and *P. polyphylla* Smith var. *chinensis* (Li, 1998) and they were considered as the major effective constituents for biological activities, such as the formation of stable foam, hemolysis, neuroprotective, hypocholesterolaemic, antimicrobial, antitumour, antifungal, anticarcinogenic, anti-inflammatory, antioxidant activity, inhibitory activities against platelet aggregation and abnormal uterine bleeding, chronotropic effects on spontaneous beating of myocardial cells and protective effects on ethanol or indomethacin-induced gastric mucosal lesions in rats (Deng et al. 2008; Zhang et al. 2010; Liu et al. 2012).

Open-chain steroidal glycosides are characterized by a C27 cholestrol-drived steroidal skeleton accompanoid by varying numbers of sugar units attached at different positions, and differing from spirostanol and furostanol saponins in lacking the additional heterocyclic ring(s) derived from the C-17 side chain (Challinor and Voss, 2013). Open-chain steroidal saponins have been referred to as "cholestane glycosides", though they encompass far greater structural diversity than this name suggests; additionally, many spirostanol and furostanol saponins can be considered to be cholestane-derived. The presence of open-chain steroidal glycosides is well-documented in a number of marine organisms (Ivanchina et al. 2011).

Over 150 unique open-chain steroidal glycosides have been reported from terrestrial plants to date. All plant open-chain steroidal glycosides possess the C-3 oxygenation that is present in their early biosynthetic precursor cholesterol as a consequence of their biogenesis via the cyclization of 2,3-oxidosqualene, although interestingly glycosides of cholesterol itself have been reported very rarely from plants. To date, plant open-chain steroidal glycosides have been reported with up to five sites of aglycone oxygenation and with up to six accompanying sugar units attached at one (monodesmosidic), two (bisdemosdic) or, rarely, three (trisdesmosidic) separate positions.

Two cholestane-type saponins, parispolyside F and parispseudoside C, were also found in *P. polyphylla* var. *pseudothibetica* (Xiao et al. 2009) and *P. polyphylla* var. *yunnanensis* (Zhao et al. 2007). Three other saponins having homo-aro-cholestane skeletons viz., parispseudoside B, parispolyside E and parispseudoside A were also isolated from *P. pseudothibetica* (Xiao et al. 2009). Parispolyside E was confirmed as a steroidal glycoside, possessing a homo-cholestane skeleton with an aromatized

ring E by Huang et al. (2005) from *P. polyphylla* Smith var. *chinensis* (Franch.) Hara (Zhang et al. 2011).

### 2.6.2. Other glycosides

#### 2.6.2.1. Prototype saponins:

Prototype saponins have one closed loop less than pennogenyl and diosgenyl saponins and if a fragment ion is ascribed to loss of water or methanol from the parent ion, there might be a substituent in prototype saponins (Man et al. (2011). According to Zhang et al. (2011) till now about sixteen prototype saponins were found in genus *Paris* and their basic structural differences between them are due to the substituent groups at C17 and C20. Parisyunnanoside A and Th the two prototype saponin were successfully isolated from *P. polyphylla* var. *yunnanensis* (Zhao, 2007). Some of the other prototype saponins isolated from *P. polyphylla* var. *yunnanensis* are; Protogracillin, dichotomin, parisaponin-I, methylprotosaponin, methylprotogracillin, methyl-dichrotomin etc. (Man et al. 2010).

#### 2.6.2.2. Phenolic and phenylpropanoid glycosides

A new phenylpropanoid glycoside named parispolyside F and a novel derivation of phenolic glycoside named parispolyside G as well as two known flavonoid glycosides were isolated from the rhizome *of Paris polyphylla* var. *yunnanensis* Wang et al. (2007).

#### 2.6.2.3. Saponins with hydroxylated aglycones

Many saponins may have hydroxylated sapogenins of pennogenin, nuatigenin, isonuatigenin, prognienolone etc. (Zhang et al. 2011). This type of saponins may have dihydroxy and sometimes trihydroxy aglycones (Liu et al. 2016). The aglycones, comprising trikamsteroside D are similar to those of T, which could be

valuable for chemotaxonomic markers for the genetic relationship between *Trillium* and *Paris* (Wang et al. 2013). Polyhydroxylated saponins reported from *P. polyphylla* Smith bears a hydroxyl group at C-1 and also glycosylated with the same trisaccharide (Kang et al. 2012). Previously, such hyxylation was found at C-1, C-3, C-21, C-23 and C-24 in trillenogenin from *Trillium kamtschaticum* Pall (Ono et al. 2003). Similarly, 1-dehydro-trillogenin was isolated from *P. quadrifolia* (Nohera et al. 1982).

#### 2.6.2.4. $C_{-22}$ Steroidal lactone glycoside

Qin et al. (2013) demonstrated that the phytochemical investigation on the stems and leaves of *P. polyphylla* var. *yunnanensis* has led to the isolation of three $C_{22}$-steroidal lactone glycosides of which the first two of them are new compounds which have been designated as chonglouoside SL-7 and chonglouoside SL-8 while the third compound was identified as dumoside by the comparison of the observed spectroscopic data (Ahmad et al. 1998). Earlier two C-22 steroidal lactone glycosides, named as dracaenoside A and B, were also isolated from the fresh stem of *Dracaena cochinchinensis* S. C. Chen a traditional medicinal herb from China and which happens to be the first examples that C-22 steroidal lactone glycosides were isolated from plants (Zheng and Yang, 2003).

### 2.6.3. Oligosaccharides from *Paris polyphylla*

Plant polysaccharide from medicinal herbs have attracted considerable interest for their pharmacological effects. Studies have reported that plant polysaccharides possess antioxidant, antihyperlipidemic, anticancer and immune activities (Kan et al. 2015; Ohta et al. 2016).

Oligosaccharides such as pectic oligosaccharides, xyloglucan oligosaccharides, and galactoglucomannan oligosaccharides have been known as an important class of relatively new or 'non-traditional' plant hormones which are capable of modulating plant growth and development and defense responses (Creelman and Mullet, 1997). Oligosaccharide signals play an important role in the regulation of plant development, defenses and other interactions of plants with the environment (Marilynn, 1998). Zhou et al. (2003) isolated two oligosaccharides from the rhizomes of *P. polyphylla* var. *yunnanensis* which stimulated shoot formation *in P. polyphylla* var. *yunnanensis* in culture and saponin production as well as the growth of *P. japonicus* var. *major* hairy roots. Such stimulation of secondary metabolite accumulation in plant tissue and cell cultures by pure or mixture of oligosaccharides is a result of elicitor activity of these compounds. Thus, it was established that natural heptasaccharide and octasaccharide possess growth-regulatory activity in the shoot culture of the same plant species and the hairy root culture of a different species (*P. japonicus* var. *major*) and they also stimulate the growth of *Panax ginseng* hairy roots (Zhou et al. 2007).

A homogenous preparation of polysaccharides has been obtained from *P. polyphylla* leaves (PPLP). The monosaccharide composition of the polysaccharide consisted of L-arabinose and D-galactose with a molar ratio of 4:2:5:8. Structural studies revealed that the backbone of PPLP was comprised of (1→6)-β-D-galactan, and the branched chains mainly consisted of arabinosyl residues which was linked to backbone via (1→3)-linkages. In mouse aging model, the polysaccharide significantly prevented the formation of malondialdehyde (MDA) and significantly improved the levels of antioxidant enzymes and total antioxidant capacity (TAOC) in serum and liver of

experimental animals suggesting the potent antiaging capacity of *P. polyphylla* (Shen et al. 2018).

Liu et al. (2010) reported that four oligosaccharides (penta-, hexa-, hepta and octa-saccharide) derived from *P. polyphylla* var. *yunnanensis* have been synthesized efficiently using a convergent glycosylation strategy. Application of these polysaccharides on cultured tobacco (*Nicotiana tabacum* L.) seedlings showed that they stimulated the growth of the seedlings. Of the four polysaccharides the penta-saccharide had the most significant effect on the growth of tobacco seedling and the effect was concentration dependent between the concentration of 0.1 and 1.0 ppm.

### 2.6.4. Polyphenols and flavonoids from *P. polyphylla*

Polyphenols are being utilized worldwide and considered to be the diverse and unique group of phytochemicals present in various plants, fruits and vegetables and the widespread health benefits made them appropriate for health promotion and prevention of degenerative diseases such as cancer, diabetes, osteoporosis, cardiovascular and neurodegenerative disorders (Fraga et al. 2010; Manach et al. 2004). Polyphenols also possess anti-aging, antitumour, anti-inflammatory and cardiovascular protection properties (Han and Lou, 2007). Therefore, the demand of polyphenols in nutraceutical and pharmaceutical industries is expected to increase by many fold (Lamien-Meda et al. 2008; Anonymous, 2016). Flavonoids are also polyphenolic compounds which possess antihepatotoxic, anti-inflammatory, antitumour, anti-osteoporotic and anti-ulcer activity (Agrawal, 2011).

*P. polyphylla*, widely considered as a source of saponins has not been much studied for polyphenolics and its antioxidant potential. The leaf samples of *P. polyphylla* was analyzed for the first time to look for alternate and renewable source of

polyphenolics compounds where the detection of polyphenolics (namely catechin, caffeic acid, chlorogenic acid, gallic acid and 4-hydroxybenzoic acid) will improve the existing knowledge on metabolic profile and antioxidant activity that can be effectively utilized for the development of functional foods (Mohd et al. 2018).

Six flavonoid glycosides with kaempferol as aglycon, three with isorhamnetin as aglycon, and one with quercetin were isolated from PPY and other species (Zhang et al. 2011). Different authors have reported the presence of 18 different flavonoids from genus *Paris* constituting the second most frequent secondary metabolites from different *Paris* species. Most of these flavonoids are either Kaempferol or isorhamnetin derivatives, but they also included quercetin, rutin, luteoloside, naringenin, myricetin, amentoflavone, kayaflavone etc. (Wang et al. 2007); Nohara et al. 1982; Nakano et al. 1981; Chen et al. 1995). Among them, some were isolated as flavonoid glycosides. Most of the glycoside had the sugar moiety at C (3), and some of the glucose moieties were linked C (7) or another sugar moiety (Wei et al. 2014).

**Table-2.3**. Diversity of saponins reported from different taxa of *Paris* species:

| Sr. No. | Name of the saponin | Molecular Formula | Source plant | References |
|---|---|---|---|---|
| 1. | Polyphyllin G | $C_{51}H_{84}O_{22}$ | *Paris polyphylla* Sm. | Schulten et al. (1984), Singh et al. (1982) |
| 2. | Polyphyllin H | $C_{44}H_{70}O_{17}$ | *Paris polyphylla* Sm. *Paris forrestii* | Singh et al. (1982), Wang et al. (2018) |
| 4. | Trigofoenoside A | $C_{45}H_{74}O_{18}$ | *Paris polyphylla* var. *yunnanensis* | Matsuda et al. (2003) |
| 5. | Paris Saponin II (Formosanin C) | $C_{51}H_{82}O_{20}$ | *Paris polyphylla* var. *yunnanensis* | Yang et al. (2017), Qin et al. (2018) |
| 6. | Paris Saponin V | $C_{39}H_{62}O_{12}$ | *Paris polyphylla* Sm. | Xiao et al. (2009) |
| 7. | Paris Saponin VII | $C_{51}H_{82}O_{21}$ | *Paris polyphylla* Sm. | Xiao et al. (2009), Yang et al. (2017), Qin et al. (2018) |
| 8. | Paris Saponin H | $C_{51}H_{82}O_{21}$ | *Paris polyphylla* Sm. | Xiao et al. (2009) |
| 9. | Diosgenin | $C_{27}H_{42}O_3$ | *Paris polyphylla* var. *yunnanensis,* *Paris polyphylla* var. *chinensis,* *P. polyphylla* var. *pseudothibetica* *Paris mairei* *Paris formosana* *Paris fargesii* var. *brevipetala* *Paris forrestii* | Jun, (1989), Mimaki et al. (2000), Matsuda et al. (2003), Bensky et al. (2004), Devkota et al. (2007), Zhang et al. (2007), Hong-Xiang et al. (2007), Yan et al. (2009), Liu et al. (2009), Lin et al. (2011), Wen et al. (2012), Shah et al. (2012), Wang et al. (2018), Liang et al. (2019) |
| 10. | Pennogenin | $C_{27}H_{42}O_4$ | *Paris polyphylla* var. *yunnanensis,* *Paris polyphylla* var. *chinensis,* *P. polyphylla* var. *pseudothibetica* *Paris formosana* *Paris quadrifolia* *Paris fargesii* var. *brevipetala* *Paris bashanensis* *Paris forrestii* | Jun, (1989), Mimaki et al. (2000), Matsuda et al. (2003), Bensky et al. (2004), Zhang et al. (2007), Guo et al. (2008), Yan et al. (2009), Lin et al. (2011), |

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|---|---|---|---|---|
| | | | | Stefanowicz-Hajduk et al. (2011), Wen et al. (2012), Wang et al. (2013), Wang et al. (2018), Liang et al. (2019) |
| 11. | 24-α- hydroxy pennogenin | $C_{27}H_{42}O_5$ | *Paris polyphylla* var. *yunnanensis*, *Paris polyphylla* var. *chinensis* | Jun, (1989) |
| 12. | Gracillin | $C_{45}H_{72}O_{17}$ | *Paris polyphylla* Sm. | Darbour et al. (2009) |
| 13. | Pseudoproto-Pb | $C_{57}H_{92}O_{25}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007) |
| 14. | Parisyunnanoside B | $C_{50}H_{80}O_{21}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007) Wang et al. (2013) |
| 15. | Parisyunnanoside G | $C_{56}H_{88}O_{29}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007), Kang et al. (2012), Wang et al. (2013) |
| 16. | Parisyunnanosides H | $C_{50}H_{78}O_{24}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007), Kang et al. (2012) Wang et al. (2013) |
| 17. | Parisyunnanosides I | $C_{56}H_{88}O_{29}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007), Kang et al. (2012), Wang et al. (2013) |
| 18. | Parisyunnanosides J | $C_{45}H_{70}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007), Kang et al. (2012), Wang et al. (2013) |
| 18. | Padelaoside B | $C_{50}H_{80}O_{24}$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007), Zhang et al. (2009), Wang et al. (2013) |
| 19. | Trillin | $C_{33}H_{52}O_8$ | *Paris polyphylla* var. *yunnanensis* | Man et al. (2009) |
| 20. | Formosanin C | $C_{51}H_{82}O_{20}$ | *Paris polyphylla* var. *yunnanensis* *Paris formosana* | Man et al. (2009), |
| 21. | Maireioside A | $C_{50}H_{80}O_{24}$ | *Paris mairei* | Liu et al. (2009) |
| 22. | Prosapogenin A | $C_{39}H_{62}O_{12}$ | *Paris polyphylla* var. *yunnanensis* | Yu et al. (2010) |
| 23. | Reclinatoside | $C_{50}H_{80}O_{20}$ | *Paris polyphylla* var. *yunnanensis* | Yu et al. (2010) |
| 24. | Chonglouoside SL-1 | $C_{33}H_{52}O_9$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |
| 25. | Chonglouoside SL-2 | $C_{39}H_{62}O_{14}$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |
| 26. | Chonglouoside SL-3 | $C_{51}H_{82}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |
| 27. | Chonglouoside SL-4 | $C_{51}H_{81}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |
| 28. | Chonglouoside SL-5 | $C_{51}H_{80}O_{21}$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |
| 29. | Chonglouoside SL-6 | $C_{50}H_{80}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Qin et al. (2012) |

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|---|---|---|---|---|
| 30. | Balanitin 7 | $C_{50}H_{80}O_{21}$ | *Paris polyphylla* Sm. | Li et al. (2012) |
| 31. | Paristenosides A | $C_{50}H_{80}O_{25}$ | *Paris polyphylla var. stenophylla* | Jin et al. (2016) |
| 32. | Paristenosides B | $C_{56}H_{88}O_{30}$ | *Paris polyphylla var. stenophylla* | Jin et al. (2016) |
| 33. | Pavitnosides A | $C_{41}H_{64}O_{14}$ | *Paris vietnamensis* | Liu et al. 2018 |
| 34. | Pavitnosides B | $C_{39}H_{62}O_{14}$ | *Paris vietnamensis* | Liu et al. (2018) |
| 35. | Pavitnosides C | $C_{39}H_{60}O_{14}$ | *Paris vietnamensis* | Liu et al. (2018) |
| 36. | Pavitnosides D | $C_{39}H_{62}O_{14}$ | *Paris vietnamensis* | Liu et al. (2018) |
| 37. | Polyphyllin C | $C_{39}H_{62}O_{12}$ | *Paris polyphylla* Sm. | Schulten et al. (1984), Singh et al. (1980) |
| 38. | Polyphyllin D | $C_{44}H_{70}O_{16}$ | *Paris polyphylla* Sm.<br>*Paris forrestii* | Schulten et al. (1984), Ma and Lau, (1985), Lee et al. (2005), Cheung et al. (2005), Wang et al. (2018) |
| 39. | Polyphyllin E | $C_{51}H_{82}O_{20}$ | *Paris polyphylla* Sm. | Schulten et al. (1984), Singh et al. (1980) |
| 40. | Polyphyllin F | $C_{51}H_{82}O_{20}$ | *Paris polyphylla* Sm. | Schulten et al. (1984), Singh et al. (1980) |
| 41. | Hypoglaucin G | $C_{51}H_{82}O_{23}$ | *Paris mairei* | Liu et al. (2009) |
| 42. | Polyphyllin I | $C_{44}H_{70}O_{16}$ | *Paris polyphylla* Sm. | Yang et al. (2016) |
| 43. | Polyphyllin II | $C_{44}H_{70}O_{17}$ | *Paris polyphylla* Sm. | Wang et al. (2016) |
| 44. | Polyphyllin VI | $C_{39}H_{62}O_{13}$ | *Paris polyphylla var. yunnanensis* | Wu et al. (2017) |
| 45. | Polyphyllin VII | $C_{51}H_{84}O_{22}$ | *Paris polyphylla var. yunnanensis* | Wu et al. (2017) |
| 46. | Paristengosides A | $C_{55}H_{86}O_{20}$ | *Paris tengchongensis* | Wang et al. (2020) |
| 47. | Paristengosides B | $C_{44}H_{70}O_{19}$ | *Paris tengchongensis* | Wang et al. (2020) |
| 48. | Paristengosides C | $C_{44}H_{70}O_{18}$ | *Paris tengchongensis* | Wang et al. (2020) |
| 49. | Polyphylloside A | $C_{45}H_{72}O_{17}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 50. | Polyphylloside B | $C_{45}H_{72}O_{17}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 51. | Polyphylloside C | $C_{45}H_{72}O_{17}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 52. | Polyphylloside D | $C_{45}H_{72}O_{17}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 53. | Polyphylloside E | $C_{39}H_{62}O_{15}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 54. | Polyphylloside F | $C_{45}H_{72}O_{19}$ | *Paris polyphylla var. chinensis* | Qin et al. (2020) |
| 55. | Parispseudosides A | $C_{59}H_{92}O_{24}$ | *P. polyphylla* var. *pseudothibetica* | Xiao et al. (2009) |
| 56. | Parispseudosides B | $C_{47}H_{72}O_{16}$ | *P. polyphylla* var. *pseudothibetica* | Xiao et al. (2009) |
| 57. | Parispseudosides C | $C_{57}H_{90}O_{26}$ | *P. polyphylla* var. *pseudothibetica* | Xiao et al. (2009) |
| 58. | Parispolyside F | $C_{51}H_{86}O_{20}$ | *P. polyphylla* var. *pseudothibetica* | Xiao et al. (2009) |
| 59. | Oleanolic acid | $C_{30}H_{48}O_3$ | *Paris polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 60. | Polyphylloside IV | $C_{51}H_{82}O_{23}$ | *Paris polyphylla* Sm. | Wang et al. (2013) |
| 61. | Polyphylloside III | $C_{51}H_{82}O_{22}$ | *Paris polyphylla* Sm. | Wang et al. (2013) |

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|---|---|---|---|---|
| 62. | Trigofoenoside Aprotobioside | $C_{45}H_{74}O_{18}$ | *Paris polyphylla* Sm. | Wang et al. (20130 |
| 63. | Pseudoproto-Pb | $C_{57}H_{92}O_{25}$ | *Paris polyphylla* Sm. | Wang et al. (2013) |
| 64. | Chonglouoside H | $C_{33}H_{52}O_{10}$ | *Paris polyphylla* Sm. | Wang et al. (2013) |
| 65. | Paritrisides A | $C_{41}H_{66}O_{13}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 66. | Paritrisides B | $C_{41}H_{66}O_{13}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 67. | Paritrisides C | $C_{41}H_{64}O_{12}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 68. | Paritrisides D | $C_{41}H_{64}O_{12}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 69. | Paritrisides E | $C_{41}H_{66}O_{12}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 70. | Paritrisides F | $C_{41}H_{66}O_{12}$ | *P. polyphylla* var. *yunnanensis* | Wu et al. (2013) |
| 71. | Parispolyside F | $C_{31}H_{36}O_{16}$ | *Paris polyphylla* var. *yunnanensis* | Wang et al. (2007) |
| 72. | Parispolyside G | $C_{19}H_{24}O_{12}$ | *Paris polyphylla* var. *yunnanensis* | Wang et al. (2007) |
| 73. | Trikamsteroside D | $C_{48}H_{72}O_{24}$ | *Paris polyphylla* Sm. | Wang et al. (2013) |
| 74. | Chonglouoside SL-7 | $C_{40}H_{61}O_{17}$ | | Qin et al. (2013) |
| 75. | Chonglouoside SL-8 | $C_{40}H_{59}O_{16}$ | | Qin et al. (2013) |
| 76. | Parisyunanoside A | $C_{50}H_{82}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Zhao, (2007), Man et al. (2010) |
| 77. | Methylprotodioscin | $C_{52}H_{86}O_{22}$ | *Paris polyphylla* var. *yunnanensis* | Man et al. (2010) |
| 78. | Dichotomin | $C_{57}H_{94}O_{26}$ | *Paris polyphylla* var. *yunnanensis* | Man et al. (2010) Yu et al. (2010) |
| 79. | Nuatigenin | $C_{27}H_{42}O_4$ | *Paris polyphylla* var. *yunnanensis* | Zhang et al. (2011), Liang et al. (2019) |
| 80. | Isonuatigenin | $C_{27}H_{42}O_4$ | *Paris polyphylla* var. *yunnanensis* | Zhang et al. (2011) |
| 81. | Pregnenolone | $C_{21}H_{32}O_2$ | *Paris polyphylla* var. *yunnanensis* | Zhang et al. (2011) |
| 82. | Th (thr-his) | $C_{10}H_{16}N_4O_4$ | *Paris polyphylla* var. *yunnanensis* | Zhao et al. (2007) |
| 83. | Protogracillin | $C_{51}H_{84}O_{23}$ | *Paris polyphylla* Sm. | Matsuda et al. (2003) Man et al. (2010) Wang et al. (2013) |
| 84. | Pari saponin I | $C_{50}H_{82}O_{22}$ | *Paris polyphylla* var. *yunnanensis* *Paris mairei* | Matsuda et al. (2003), Liu et al. (2009), Yang et al. (2017), Qin et al. (2018) |
| 85. | Methylprotogracillin | $C_{52}H_{86}O_{23}$ | *Paris polyphylla* var. *yunnanensis* | Man et al. (2010) |

## 2.7. Biological activities of *Paris polyphylla*

### 2.7.1. Haemostatic activity and prevention of abnormal utrine bleeding

Abnormal uterine bleeding (AUB) is one of the major fields of concern for gynecologists worldwide. Approximately one third of all gynecological consultations are carried out for AUB which rises to 70% in premenopause and postmenopause women (Oehler et al. 2003). The rhizomes of *P. polyphylla* have been widely used in TCM for the treatment of traumatic and abnormal uterine bleeding (Tian et al. 1986). As per Shah et al. (2012) the crude drug of *P. polyphylla* var *yunnanensis* had shorter coagulation time than *P. polyphylla* var *chinensis* and they suggested that saponins of pennogenin type were the main components confirming haemostatic activity to *Paris* species.

A comparative haemostatic, cytotoxic and haemolytic activity of six *Paris* species was reported and concluded that *P. delavayi* var. *delavayi* and *P. bashanensis* could be used as the resources of hemostatic drugs (Liu et al. 2012). *P. bashanensis* belonging to the genus *Paris* was found to contain high levels of pennogenin saponins, compared with the *P. polyphylla* Smith var. *chinensis* and *P. polyphylla* Smith var. *yunnanensis* which may responsible for its higher hemostatic activity and could be used as the best resource of hemostatic drug (Wang et al. 2013). Negi et al. (2014) suggested that pennogenin glycosides with spirostanol structure may be the active ingredients of *P. polyphylla* that promotes hemostasis.

Some spirostanol glycosides representing uterine contractile agonist have been isolated from *P. polyphylla.* The total steroidal saponins from the rhizome extract of *P. polyphylla* var. *yunnanensis* shows uterotonic activity justifying their usage in the therapy of AUB. The spirostanol-type pennogenin glycoside [Pennogenin-3-O-α-L-arabinofuranosyl (1→4) [α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside

(PARG)] from *P. polyphylla* var. *yunnanensis* showed a strong effect on stimulating rat myometrial contractions (Guo et al. 2008) which increases significantly with advancing pregnancy in rat myometrium. Experiment on rat *in-vivo* demonstrated a strong contraction activity of uterine muscle by two saponins isolated from *P. polyphylla* named as saponin 6 and saponin 7. These two saponins have been used successfully to cure genealogical hemorrhage (Jun, 1989). The cytotoxicity and hemolytic activity of *Paris* were investigated and it was found that the two activities are not related to the contents of saponins and concluded that the hemolytic activity of diosgenyl saponins were stronger than that of pennogenyl glycoside (Liu et al. 2014).

### 2.7.2. Anthelmintic activity

With advancing time, an increasing anthelmintic resistance, drug residues in animal products and high cost of conventional anthelmintics has attracted medicine practitioners towards medicinal plants as an alternative source of anthelmintics (Lone et al. 2017).

Wang et al. (2010) first reported the *in vivo* anthelmintic activity of the crude methanolic extracts and pure compounds from the rhizomes of *P. polyphylla*. The methanol extract from rhizomes of *P. polyphylla* showed significant anthelmintic activity against *Dactylogyrus intermedius.* Two saponins, Dioscin and polyphyllin – D having diosgenin as the aglycone have already been found responsible for various biological activities (Hufford et al. 1988; Ikeda et al. 2000; Sautour et al. 2004; Kim et al. 1999). These two compounds from *P. polyphylla* were mainly responsible for the anthelmintic activity against the helminth D. intermedins (Wang et al. 2010). Further, study on anthelmintic efficacy of formosanin - C and polyphyllin VII were examined against the monogenean parasite, *D. intermedius* and it was found that both

the compounds were effective in controlling the parasite. However, formosanin -C was more effective in its anthelmintic action and it probably acted upon the parasite by directly inactivating its mitochondria (Li et al. 2013).

### 2.7.3. Antimicrobial activity

The issue of bacterial resistance to antibiotics has become a serious problem of public health that concerns almost all antibacterial agents and a novel antimicrobial compound against new bacterial targets and drug resistant activity against bacteria is urgently needed (Chan et al. 2012).

Zhao et al. (2009) reported that the volatile oils present in *P. polyphylla* showed strong inhibitory effects on Micrococcus, Xanthomonas, Aerobacter and Brevibacterium. It has been reported that out of 25 compounds present in stem and leaves of *P. polyphylla* 11 compounds exhibited potent antibacterial activity against *Propionibacterium acnes* (Qin et al. 2012). *P. polyphylla* extracts of the aerial parts showed activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Salmonella flexinerai*, while the rhizome extract was active only against *S. aureus* (Chhetri et al. 2012). The stem and leaves of *P. polyphylla* are rich in steroidal saponins which may have exerted the antimicrobial effect (Qin et al. 2012). By using double-layer technique, the antimicrobial activities of fungal isolates from the South China Sea were thoroughly investigated and the result showed that comparatively high quantity (56 %) of fungal isolates exhibited antimicrobial activity against at least one pathogenic bacterium or fungus microbes which might be of potential use to modern medicine, industry and agriculture (Zhang et al. 2013).

The roots of *P. polyphylla* have also shown anti-bacterial action against *Bacillus* spp., *B. typhi, B. paratyphi, E. coli, Staphylacoccus aureas, Haemolytic streptococci, Meningococci* etc. (Sharma et al. 2015). The total saponins from *P. polyphylla* var. *yunnanensis* showed weak antibacterial activities with MIC value of 156 μg/mL whereas the four spirostanol saponins (paris saponin I, paris saponin V, dioscin, and paris saponin II) displayed substantial antifungal activities against *C. albicans* (5314) and *C. albicans* (Y0109) with an MIC value of 1.95 μg/mL (Qin et al. 2018).

Deng et al. (2008) evaluated the anti-fungal activity of *Paris* where the hexane and 80 % ethanol extracts of dried rhizomes of *P. polyphylla* were tested against *Cladosporium cladosporioides* and *Candida albicans.* In the study, the ethanol extract was shown to have antifungal activity. The three pennogenin steroidal saponins isolated from the plant exhibited moderate antifungal activities against *Saccharomyces cerevisiae* and *Candida albicans* (Zhu et al. 2010). A certain compound isolated from *Paris polyphylla* var. *yunnanensis* had antifungal activities against *Saccharomyces cerevisiae* Hansen and *Candida albicans* (Sharma et al. 2015). The phytochemical constituents from the above ground parts of *P. polyphylla* var. *yunnanensis* might be an alternative resource for *P. polyphylla* rhizomes as hemastatic, anticancer and antifungal agents (Qin et al. 2018).

A study on in vitro activity of *P. polyphylla* against Enterovirus 71 (EV71) and Coxsackie virus B3(CVB3) was carried out (Wang et al. 2011). They reported the prevention of viral infection, viral inactivation, and anti-viral replication effects by 95% ethanol extract against both EV71 and CVB3. Of the different anti-viral activities, the anti-viral replication effect was found to be dominant (Wang et al. 2016).

Antiviral activity of *P. polyphylla* saponin I on influenza A virus both *in vitro* and *in vivo* has also been reported (Pu et al. 2015). It was further revealed that *P. polyphylla* saponin I, at a dose of 5 and 10 mg/kg, prolonged the survival rate of mice infected with influenza A virus, from 8 to 13 days (Bhat et al. 2017). The discoveries of new cell receptors of Enterovirus 71 (EV71) have shed light into the pathogenesis of the virus and might be considered a promising, putative drug target against viral infection (Pourianfar and Grollo, 2013).

### 2.7.4. Immunomodulatory properties

*P. polyphylla* rhizome has long been associated with the regulation of body's immune system (Bian et al. 2002). Diosgenyl saponins having the glucoside moieties is needed for the commencement of immunological reactions, particularly during the period of oxygen consumption such as microbicidal activity and inflammation. Although Diosgenin could also stimulate the macrophage phagocytosis including elimination of foreign or denatured matter. The three diosgenyl saponins isolated from *P. polyphylla* stimulates the activities of phagocytosis, respiratory burst, and nitric oxide production. These saponins with sugar moiety possess immunomodulatory activities (Zhang et al. 2007). *P. polyphylla* saponins significantly increased in the serum IFN-r level and reduced the IL-4 level in mice, thereby activating immune cells to generate immune response (Li et al. 2013). It is possible that the tumor inhibition effect of *Paris* saponin is achieved in the body by activation of immune factors (Li, 2008).

### 2.7.5. Antioxidant activity

Reactive oxygen species (ROS) play a chief role in the advancement of oxidative stress which leads to many illnesses including diabetes, cardio vascular diseases, degenerative diseases, inflammation, anemia, cancer and ischemia (Cai et al. 2004). Excessive free radicals can cause many diseases, such as cancer, cardiovascular

disease and atherosclerosis. In cases, where conventional medicines for antioxidant and cancer treatment have limited efficacy and significant toxicity (Li and Zhou, 2007), the plant polysaccharides may have great use because of their antioxidant activities (Xu et al. 2009). Many such plants belonging to Traditional Chinese Medicine along with *P. polyphylla* is reported to possess antioxidant effects which is most possibly imparted by phenolics, flavonins and pure metals like Zn, Mg and Se (Ravipati et al. 2012).

However, when the extraction was carried on for 4.8 hrs. the extracted polysaccharides exhibited strong antioxidant effects on DPPH, Hydroxyl and superoxide radicals. Antioxidant activity of ethyl acetate extracts of *P. polyphylla* was tested in refined peanut oil and the antioxidant capacity was determined by calculating the peroxide value. *P. polyphylla* which exhibited 'very low antioxidant activity compared to other species like *Caesalpinin sappon* and *Lithosparmun erythroxizon* (Yingming et al. 2004). Similarly, the salt extracts of *P. polyphylla* Smith, *Taxus mairei* and *Camptotheca acuminata* had little capacity to remove superoxide anion radical as compared to *Trillium tschonoskii* Maxim (Li and Zang, 2012).

The methanolic rhizome extract of *P. polyphylla* species was examined for its antioxidant activity against the DPPH radicals and it showed a strong antioxidant activity ($IC_{50}$ = 1.09 mg/ml) owing to the presence of fair amount of phenolics and flavonoids in its rhizome (Mayirnao et al. 2017). Indeed, the number of polyphenolic compounds viz. catechin, caffeic acid, chlorogenic acid, gallic acid, benzoic acid etc. have been isolated from the leaves of *P. polyphylla*. These compounds may be harnessed for their antioxidant potential. Thus *P. polyphylla* leaves have great potential as a sustainable source of nutraceutical product formulation (Mohd et al.

2018). Among the reactive oxygen species, hydroxyl radical is considered to be one of the most potent oxidants which can easily cellcross membranes and readily react with most biomacromolecules, such as carbohydrates, proteins, lipids and DNA. The scavenging activity showed higher in case of *P. polyphylla* collected from higher altitude ($IC_{50}$ = 2.38 ± 0.04) of Sikkim Himalaya as compared to the one from lower altitude at ($IC_{50}$ = 1.97 μg/ml) (Lepcha et al. 2019).

### 2.7.6. Anticancer activity

#### 2.7.6.1. Gastric cancer

Gastric cancer is reported to be the most common cause of death from cancer worldwide (Jemal et al. 2011). A novel steroidal saponin along with 12 known steroidal compounds were isolated from the rhizomes of *P. polyphylla* var. *chinensis.* The isolated steroidal compounds were evaluated for their cytotoxic activity on human gastric cancer cell lines HepG2, SGC7901 and BxPC3. Diosgenin-3-O-α-L-rhamnopyranosyl (1--2) [α-L arabinofuranosyl(1-4)] -β-Dglucopyranoside exhibited the most potent cytotoxic activity among the isolated steroids (Lee et al. 2005).

Steroidal saponin from *P. polyphylla* may have gastroprotective effects. Infact, it was found that four spirostanol type steroid saponins isolated from *P. polyphylla* var. *yunnanensis* methanolic rhizome extract strongly inhibited gastric lesions induced by ethanol and indomethacin (Matsuda et al. 2003). One of the steroid saponin of *Paris chinensis*, dioscin had an antiproliferative effect on human gastric cancer SGC-7901 cells is a dose and time dependent manner. After treatment with the dioscin, the SGC-7901 cells showed apoptosis (Gao et al. 2011). Polyphyllin-I, a small steroidal saponin isolated from the rhizomes of *P. polyphylla* has shown anticancer effects (Kong et al. 2010). When used against the fresh gastric tumor tissue, polyphyllin-I exhibited moderate inhibition of growth. However, most importantly, it enhances the

anticancer effects of commonly used chemotherapy agents in gastric cancer therapy, PF (Cisplatin/oxaliplatin) and 5-FU. Gastric cancer patients under this therapy may benefit from polyphyllin-I from *P. polyphylla* (Yue et al. 2013).

#### 2.7.6.2. Breast cancer

Breast cancer is a devastating disease in female around the world causing significant healthcare burden. In many cases, breast cancer has shown resistance to chemotherapy, radiation and hormonal therapy. Therefore, development of new, cost-effective affordable treatment method is the need of the hour (Iqbal et al. 2018). In this regard the use of phytochemicals have been proven as a powerful preventive approach in breast cancer. Numerous plants have played significant role in several types of cancer therapies (Zheng et al. 2016). It has been shown that mushrooms, soy products, cruciferous vegetables and fruits are associated with reduced breast cancer risk (Farvid et al. 2016). Lee et al. (2005) reported that polyphyllin D, the steroidal saponin of *P. polyphylla* is useful for breast cancer treatment. The antitumor activity of polyphyllin D was also investigated by evaluating its cytotoxicity on MCF-7 and MDA-MB-231 human breast cancer cell lines xenografted on BALB/c nude mice. The result showed that polyphyllin D caused apoptosis on both cell lines and of nude mice bearing MCF-7 cells by 50% in terms of size and weight of tumor (Negi et al. 2014; Puwein et al. 2018). It was determined that polyphyllin – D exhibits apoptosis through mitochondrial dysfunction (Lee et al. 2005).

#### 2.7.6.3. Liver cancer

Among different types of cancer, liver cancer is a major cause of mortality in the world. Persistant infection with hepatitis virus and contact with hepatocarcinogen is are the main causes of liver cancer (Henry et al. 2002). Polyphyllin -D, a saponin extracted from *P. polyphylla* has been used in China to treat liver cancer since a long

time. It has been found that polyphyllin-D induces apoptosis in HepG2 cells. In addition, this compound can overcome drug resistance in R-HepG2 cells by inducing programmed cell death via mitrocondrial dyffunction (Cheung et al. 2005).

Rhizoma paridis refers to the rhizome of *P. polyphylla* var. *yunnanensis* (Franch.) Hand. – Mazz. or *P. polyphylla* var. *chinensis* (Franch.) Hara and the total saponin obtained from this shows antitumor effects (Lee et al. 2005). Global analysis of protein alterations after drug treatment of cells may provide valuable insights to understand the drug action mechanisms. Therefore, a proteomic analysis was carried out with Rhizoma paridis total saponin treatment on HepG2 cells. Twelve proteins were significantly altered due to the treatment of which six proteins were down-regulated and six upregulated. These proteins were mainly associated with tumor initiation, promotion and progression. These findings offer insights into the mechanism of anti-tumor effect of total saponins from Rhizoma paridis in HpeG2 cells. Further, studies on these lines may provide new drug targets for cancer chemotherapy (Cheng et al. 2008).

Resistant to drugs is a crucial factor that limits the effectiveness of chemotherapy. In hepatocellular carcinoma, multi drug resistance (MDR) may be induced by the over expression of P-glycoprotein which is the most important drug transporter responsible for the efflux of a variety of drugs from the cells. Polyphyllin D is obtained from *P. polyphylla* has been found to be a potent anti-cancer agent that bypass multi drug resistance and induces PCD in R-HepG2 cells overexpressing P-glycoprotein. Therefore, polyphyllin-D may be suggested for the treatment of liver cancer with MDR (Lee et al. 2009). It has been reported that water extract of *curcuma* significantly increases the anti-tumor effects of Rhizoma paridis saponin. The effect of curcuminoids was similar to that of P-glycoprotein inhibitor, cyclosporin-A. Thus,

these drug combinations have potential application as effective anticancer agents (Man et al. 2013).

The anticancer activity of *P. polyphylla* was also found to produce a promising result in mice that were artificially induced with diethyl nitrosamine (DEN). When mice were injected with DEN (70mg/kg body weight), it induced hepatocarcinogenesis which was similar to those of human hepatocellular carcinoma (HCC). After two months, the injected mice developed liver inflammation and cirrhosis (Qi et al. 2008; Tan et al. 2012).

When Rhizoma paridis saponins (RPS) of *P. polyphylla* var. *yunnanensis* was administered to the DEN-induced mice, showed the levels of protein expression reduced, thereby the liver injury attenuated and the development of liver cancer was inhibited (Liu et al. 2016; Puwein et al. 2018)

#### **2.7.6.4.** O**varian cancer**

Steroidal saponins isolated from Rhizoma paridis including saponin-I (PS-I) and saponin -II (PS-II), also known as polyphyllin -D and formosanin-C respectively, exhibit comparable inhibitory effects against tumor cell growth. While PS-I has been extensively studied for its anticancer effects, the anticancer studies involving PS-II is relatively new (Man et al. 2011). The antitumor efficacy of PS-II in human SKOV3 ovarian cancer xenografts in athymic mice has been studied. It was found that PS-II treatment significantly inhibited the growth of xenograft tumors. It has been found that PS-II targets tumor cells via multiple mechanism including modulation of ERIK ½ activity, induction of cell-cycle arrest and activation of mitochondrial apoptotic pathway (Xiao et al. 2013).

The anti-angiogenesis property of PS-II have been seen in SKOV3 and HOC ovarian carcinoma cell lines. In the xenograft mouse models having the above cell lines, the PS-II treatment reduced tumor weights, tumor sizes and tumor inhibitory effect on neovascularity and dramatically reduced blood vessel diameter and micro vessel density. VEGF is known for its pro-angiogenic effects (Coultas et al. 2005). It was found that PS-II treatment led to the down regulation of VEGF expression in a dose-dependent manner in both the SKOV3 and HOC-7 cells. Thus, it may be concluded that PS-II is an inhibitor of angiogenesis and that it compromises on VEGF- signaling mediated angiogenesis. The metabolite offers a promising therapeutic potential in ovarian cancer treatment (Xiao et al. 2014).

The aqueous extract of *P. polyphylla* (AEPP) acted as a potential suppressor of ovarian carcinoma cell line (OVCAR – 3 cells). The viability of the OVCAR – 3 cells which were treated with AEPP for 24h was found to be <50% as compared to the control and showed that AEPP reduced the viability of OVCAR – 3 cells through the induction of apoptosis. The AEPP effectively inhibits the proliferation of the cancer cell lines through the suppression of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha (Wang et al. 2016; Puwein et al. 2018).

#### **2.7.6.5.** L**ung cancer**

During preliminary screening of anti-proliferation activity of steroidal saponin of *P. polyphylla*, it was found that the compounds inhibited the growth of LA 795-lung adenocarcinoma cell line. Yan et al. (2009) isolated eight steroid saponins from *P. polyphylla* and investigated their anticancer activity on inbred strains of laboratory mice (T739) that carried LA795 metastatic lung cancer. The results showed that the steroid saponins caused remarkable cytotoxicity and induced apoptosis in a dose dependent manner. The oral administration of compound -I (Diosgenin-3-α-L-

arabinofuronosyl (1→4)-(α-L-rhamnopyranosyl (1→2) ]-β-D-glucopyranoside) and its aglycone, diosgenin significantly inhibited tumor growth. The structure of spirostanol 3-0-glycoside and the number of glycosides were the main factor responsible for the anticancer activity against lung adenocarcinoma cell line (Yan et al. 2009).

*P. polyphylla* var. *yunnanensis* saponins (RPS) have been investigated to determine its role in pulmonary metastatics. Effects of RPS was evaluated for its anti-lung-cancer activity in T-739 mice and LA 795 lung cancer cells, RPS treatment inhibited cancer cell proliferation and induced cell death. TUNEL assay showed that RPS caused an intense inhibition of cell growth and apoptosis of cells in vivo. The results demonstrated that RPS has a powerful anti-proliferation effect against lung-adenocarcinoma by inducing apoptosis and inhibiting pulmonary metastasis by reducing the expression of MMP-2 and MMP-9 while upregulating the expression of TIMP-2 (Man et al. 2009). Effects of RPS on Lewis bearing-C57BL/6 mice in terms of immunostimulation and induction of apoptosis in A549 lung cancer cells have been studied. The treatment with RPS significantly inhibited the tumor volume and weight in C57BL/6 mice while the levels of inflammatory cytokines including TNF-α, IL-8 and IL-10 were decreased in serum. RPS induces nuclear changes in A549 cells with DNA condensation and fragmentation as well as induction of apoptosis. It is suggested that the inhibition of tumor growth by RPS ins associated with the amelioration of inflammation responses, induction of apoptosis as well as the decrease in ROS levels (Li et al. 2013).

It has been demonstrated that Paris saponin I (PS-I) inhibited the growth of lung cancer PC-9 cells in a dose and time dependent manner by causing $G_2$/M arrest, inducing apoptosis and regulating B-cell lymphoma 2 (Bcl-2) / $Bcl_2$ associated x protein (Bax) ratio (Jiang et al. 2014). In a further experiment, the role of PS-I in combination of hyperthermia was evaluated on non-small cell lung cancer cell (NSCLC). It was found that PS-I in combination with hyperthymia at $43^0$C caused $G_2$/M phase arrest and significantly induced apoptosis, the expression levels of Bcl-2 decreased while the expression levels of Bax and cerpsage-3 was significantly increased. PS-I in combination with hyperthermia may be an important anti lung tumor therapy since it causes the cell death and tumor inhibition in NSCLC cells (Zhao et al. 2015).

PVI and PVII of *P. polyphylla* showed to be an effective alternative treatment for lung cancer (Jiang et al. 2014). When A549 cells were treated with PVI and PVII for 48h, the proteins expression level of p21 Wafi1/Cip 1 and cyclin B1 were reduced, whereas p53, Fas, DR3, and DR5 expression were upregulated (Lin et al. 2015). The MTT assay and proliferation analysis of LA795 cancer cells lines also produce similar patterned results (Man et al. 2010).

**2.7.6.6. Other biological activities**

Leishmaniasis is a parasitic disease caused by the invasion of intracellular parasite known as Leishmania in the reticulo-endolethial system of the host. These unicellular parasites are transmitted from host to host by the bite of the vector, sand fly (Ram and Nath, 1996).

A WHO technical report estimated that 53 million people, all over the world are at risk of acquiring leishmaniasis, and about 12 million new cases of leishmaniasis occur each year (Anonymous. 1990). Many novel compounds isolated from various

medicinal plants have been screened for their leishmanicidal potential in the past few years and some of them have shown significant antileishmanial activity (Tandon et al. 1991; Moretti et al. 1998). As no safe and effective vaccine is yet accessible for leishmaniasis, chemotherapy is the only means of monitoring the disease. Devkota et al. (2007) conducted a search for bioactive natural products having leishmanicidal potential and screened *P. polyphylla* for their antileishmanial activities in rapid *in vitro* assays. The result showed mild to moderate antileishmanial activities of the known diosgenyl compounds; diosgenin-3-0- [α-L-rhamnopyranosyl] (1-3) β-D-glucopyranoside, diosgenin-3-0- [α-L-rhamnopyranosyl-($1_{Rha}$ -$2_{Glu}$)- α- L-arabinofuranosyl-($1_{Ara}$-$4_{Glu}$)] (3-D-glucopyranoside and diosgenin-3-0- [α-L-rhamnopyranosyl($1_{Rha}$ – $2_{Glu}$) -α-L- rhamnopyranosyl-($1_{Ara}$-$4_{Glu}$)-β-D-glucopyranoside], isolated from the plant.

Diosgenin, activates very critical signaling target for anti-Alzheimer's disease therapy, the 1,25D3-MARRS pathway. Diosgenin is a memory-enhancing drug and its administration increased the object detection memory deficit and reduced several signs of neuronal degeneration including presynaptic disintegration combined with amyloid plaques in the cortex, axonal degeneration associated with amyloid plaques in the cortex and hippocampus. It also reduces the cortex and PHF-tau expression associated with and distal to amyloid plaques in the cortex and hippocampus (Tohda et al. 2012).

Tyrosinase is a multifunctional enzyme containing copper which is widely dispersed in plants and animals. It acts like a catalyst in the oxidation of monophenols, O-di phenols, and O-quinones which is also a vital enzyme in melanin biosynthesis in plants and animals (Sharma et al. 2015). Additionally, tyrosinase is involved in the molting procedure of insects and adhesion of marine organisms (Shino et al. 2001). *P.*

*polyphylla* is used for the treatment of some skin-related disorders associated with melanin hyper pigmentation. It has been reported that activity of enzyme tyrosinase was inhibited by chloroform, ethyl acetate, and butanol extracts of the plant (Devkota et al. 2007).

# 3. MATERIALS AND METHODS

## 3.1. Experimental plant

*Paris polyphylla* Smith; Family: Melanthiaceae; Trade Name: Satuwa (N); Local name: Tuktokbe Sungtee (L) from Sikkim Himalaya is the experimental plant (also called *P. polyphylla* in this thesis).

*P. polyphylla* specimens were collected from two altitudinal zones: the higher sub-alpine zone of 'Tholung' (2700 m asl approx., 27°39′218′′N and 88°27′435′′E) and lower temperate zone of 'Uttaray'(1900 m asl approx., 27°16′115′′N and 88°08′306′′E) respectively (Figure 3.1 and 3.2). The two morphologically distinct samples were collected from its natural habitats in the month of October 2016, India. The samples collected from Uttaray is hereafter called PPU and those from Tholung is hereafter called PPT.

## 3.2. Identification and deposition of the plant specimen

The healthy and mature specimen having flowers and fruit were collected in polythene bags and brought to the laboratory for further processing and identification. Plants were identified by Dr. A. Chettri, Department of Botany, Sikkim University. The voucher specimens of both the samples, PPU and PPT have been deposited at the herbarium in Botanical Survey of India (B.S.I.), Sikkim Himalaya Regional Centre, Gangtok, East Sikkim which were assigned with accession numbers, VS No. 0187 and 0189 respectively.

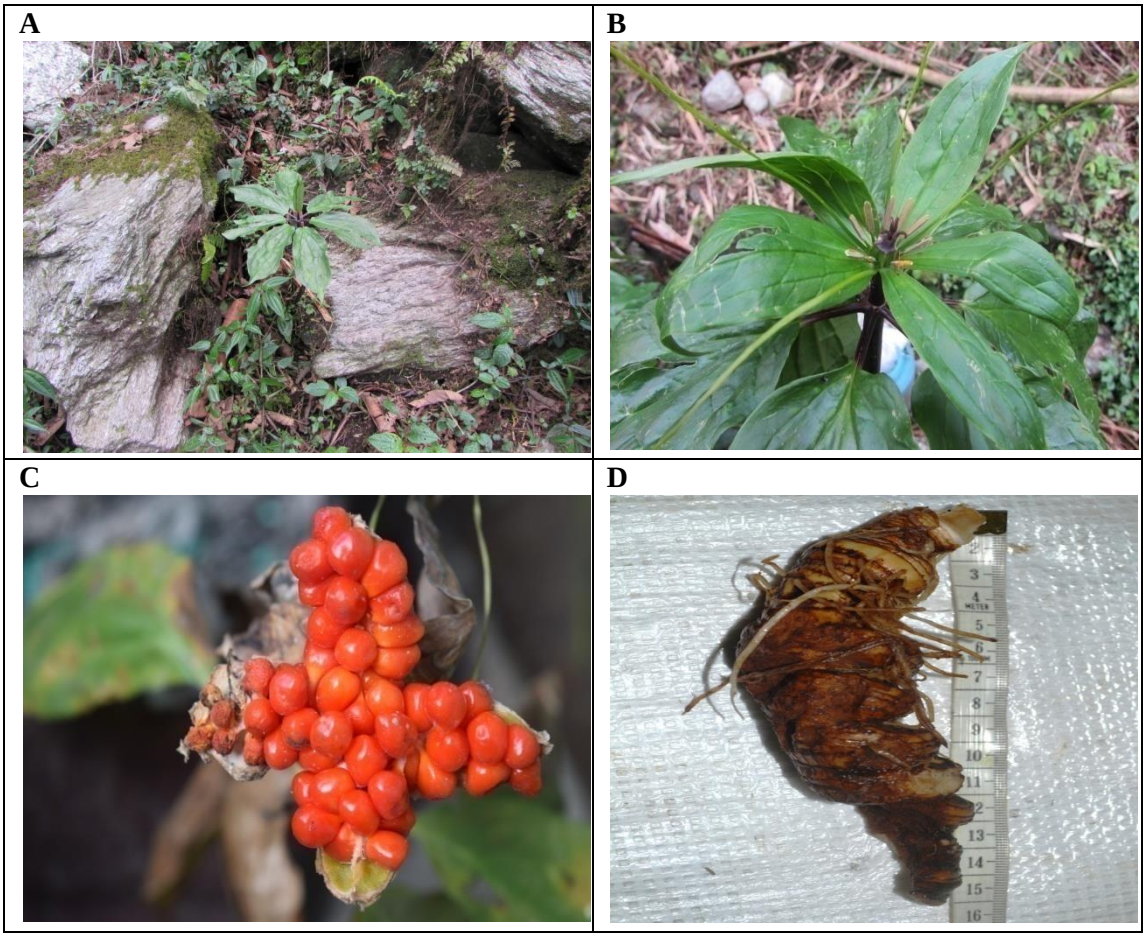



**Figure 3. 1.** *P. polyphylla* from temperate zone of Sikkim Himalaya (PPU): A) Whole plant, B) Plant with flower, C) Mature fruits with exploded pod and D) Sprouting rhizome.

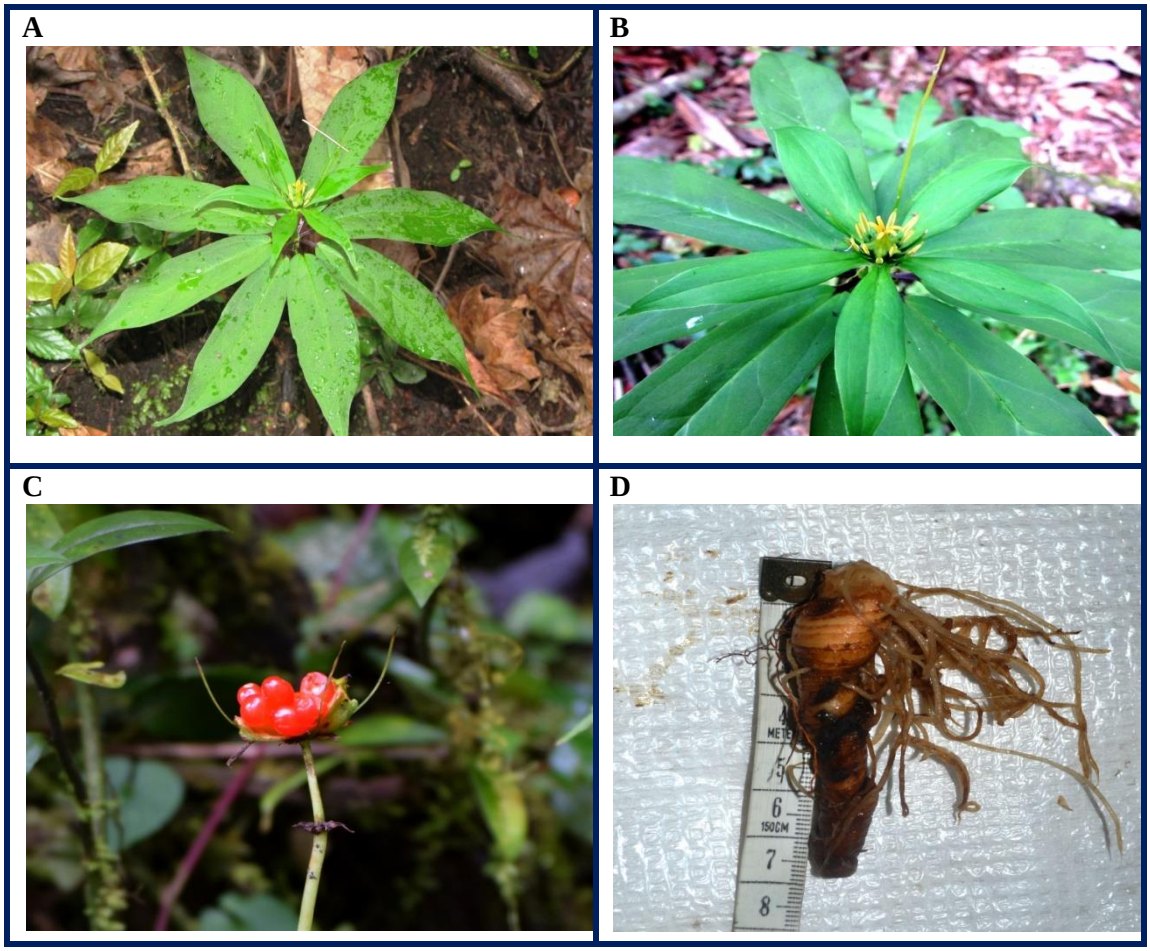



**Figure 3. 2.** *P. polyphylla* from sub-alpine zone of Sikkim Himalaya **(**PPT): A) Whole plant, B) Plant with flower, C) Mature fruits with exploded pod and D) Sprouting rhizome.

### 3.3. Nursery management and plant propagation

For nursery raising of the plant, the methods of Joshi (2012) have been followed with some modification according to the locally available knowledge and resources (Figure 3. 3). The rhizome material having young emerging buds were planted in the pre-prepared nursery bed during November-December. Initially, roots, shrubs and other unwanted weeds from the nursery site were removed followed by a controlled burning in the selected site. The nursery bed was raised to about 10cm from the ground level by mixing forest soil, sand and farmyard manure (1:2:1) unless otherwise. The rhizome segments with the growing buds were sown at a depth of 5cm at a plant to plant and row to row spacing of 30 x 30 cm. Mulching of the bed was done by covering with bamboo and pine leaves. A well-drained channel in the nursery beds was maintained to avoid damping. Regular weeding and irrigation were assured to optimize for the proper development of the seedlings.

For propagation from seeds, fully ripened seeds were collected from healthy plants during August-September. Seeds were sown during January-February. Before sowing the seeds were soaked overnight in cold water. Before sowing in the field the seeds are grown in polybags having forest soil, sand and farmyard manure (1:2:1, unless otherwise mentioned). Decayed wood powder was thinly spread over the planted spolybags. It took 6-7 months for the development of primary roots. After more than a year of growth, the seedlings were transferred to the field in May-June. About 10 cm raised bed was prepared and the seedlings were planted at a distance of 30 x 30 cm between the plants and the rows. Regular watering was done for the better growth of the seedlings. Raised roofing (about 1.25m high) of bamboo sticks and reed and leaf thatch was provided for shading and protection from direct rain (Figure 3.4).

For the treatment with PGRs, the planting material (seeds and rhizome segments) were dipped for 24 hrs in different concentrations of the hormones before planting. Before soaking in the PGRs, the seed material was also soaked overnight in cold water (Danu et al., 2015; Jamir et al., 2015). For all the hormonal concentrations, 12 numbers of cuttings were considered for the experimental set up along with control without hormones.

**A**

**B**

**C**

**D**


**Figure 3. 3.** Experimental cultivation of *P. polyphylla* in Sikkim Himalaya; A-B) Inter- cropping with beans and maize, (C-D) Co-cultivation with Large Cardamom, (E-I) Different stages of cultivation of *P. polyphylla* by the villages in Sikkim Himalaya J) Successful trial of *P. polyphylla* cultivation at a homestead garden.


**Figure 3. 4.** Nursery Development and Propagation: A) Plantation in open terrace field, B) Nursery management with bamboo and thatch roof and (C-D) Mulching with locally available dry leaves.

### 3.4. Mapping and de-limitation of potential distributional areas

Field survey was conducted in all possible sites where *P. polyphylla* grows naturally in the four districts of Sikkim, India. G.P.S. points were collected from the area of its occurrence to generate distribution mapping. Collections of the plant was only made when it was required for the study.

#### 3.4.1. Species occurrence data and environmental variable

The occurrence data for *Paris polyphylla* Smith were collected from the field using Garmin GPS 78s. The environmental variables used in the study were downloaded from the Worldclim database (http://www.worldclim.org) (Hijmans et al. 2005). In order to remove the highly correlated variable (r>0.9) we performed multicollinearity using ENM Tools 1.3 (Warren et al. 2010). Thus, out of 19 bioclimatic variables, six were selected for modeling (Lepcha et al., 2019).

#### 3.4.2. MaxEnt modelling and model validation:

Maxent software (MaxEnt ver. 3.3k;http://www.cs.princeton.edu/~schapire/maxent/) (Philips et al. 2006) were used to model the distribution of *P. polyphylla* in Sikkim. We executed 10 replicated model runs for the species to validate the model robustness with 10 percentile training presence logistic threshold. Other parameters were set default as the program is already calibrated on a wide range of species datasets (Phillips and Dudik, 2008). From the replicated runs, average, maximum, minimum, median and standard deviation were generated. The quality of the model was evaluated based on AUC value, a value below 0.5 was interpreted as a random prediction; 0.5-0.7 indicates poor model performance; 0.7-0.9 indicates moderate performance; and a value above 0.9 is considered to have good discrimination abilities (Peterson et al. 2011). Further, the potential area of distribution was categorized into five classes based on 10 percentile training presence logistic

threshold i.e. very-high (0.717-1), high (0.550-0.717), medium (0.385-0.550), low (0.218-0.385) and very low (0-0.218).

### 3.4.3. Population status and model thresholds:

Thirty random quadrates of 1 m x 1 m were laid in each study sites (viz. Tholung, Lachung, Lingthem, Chungthang (North Sikkim), Barsey, Uttaray (West Sikkim), Pangthang, Zuluk (East Sikkim) and Ravangla (South Sikkim) to study community characteristics using quantitative analytical methods. During survey, much care was taken to reflect different vegetation types, landform, elevation, and other important ecological characteristics so as to provide data of the species found in both the altitudinal range (Bhadra et al. 2016). During the study period, average temperature ranged from 10-22ºC, and annual rainfall was between 2000-2650mm (Meteorological Station Gangtok, Sikkim). The density, frequency and other values of these parameters were calculated and Importance Value Index (IVI) following (Curtis, 1959 and Mishra, 1968). The formula used was;

Relative frequency = (Frequency of a species / Sum of frequency of all species) × 100

Relative density = (Density of a species / Sum of density of all species) × 100

Importance Value Index (IVI) for Herb = Relative Frequency + Relative Density.

The population status of *P. polyphylla* was determined through quadrate studies which were then correlated with model threshold to ascertain whether the region covered in the very high and high suitability thresholds of the model maintain higher populations or not and *vice-versa*.

### 3.4.4. Soil property analyses:

Fresh soil samples from each study site (0-15cm depth) were collected with soil corer (Diameter-5.5cm). Different soil parameters such as soil moisture by gravimetric oven dried method (105$^{o}$C for 24 hours), soil temperature by soil thermometer, soil pH by digital pH meter (Cos-Lab), etc. were analyzed. Further soil samples were air dried, crushed and passed through a sieve (1mm) to separate out coarse material and gravel and live roots. The sieved soil samples were used to determine soil organic carbon (SOC) by colorimetric methods (Anderson and Ingram, 1993). Total nitrogen was determined by using Kjeltec 8500 (FOSS), and available phosphorus was determined by ammonium molybdate-stannous chloride method (Devi and Yadava, 2006).

## 3.5. Pharmacognostic evaluation

Pharmacognostic studies were determined according to the standard methods prescribed in Indian Pharmacopeia (1996) which mainly include the study of morphological characters, microscopical characters and powder microscopy. It also includes physico-chemical constants like ash value; extractive values and loss on drying of the rhizome powder were carried out.

### 3.5.1. Macroscopic characters

The morphology of the plant was studied thoroughly including all its characteristics related to underground rhizome were studied. It also includes the evaluation of drug by colour, odour, taste, size, shape in specific features like touch, texture etc. (Yasodamma et al. 2014).

### 3.5.2. Microscopic Characters

Microscopy include evaluation of crude drugs such as authentication of crude drugs, study of powdered drugs etc. Microscopic features the samples like secondary phloem and secondary xylem were observed. Powder microscopy of rhizome consisted of the evaluation of fibers, vessel elements and xylem parenchyma. Free hand section of both the rhizomes samples of PPU and PPT, *Trillium govanianum* and *Panax bipinifidus* were cut and stained according to the prescribed method (Khandelwal, 2008).

### 3.5.3. Anatomical studies

The fresh rhizomatous specimens were cut into thin transverse section using sharp blade. The sectioned samples were observed in digital microscope attached with computer system (Olympus Mic–D) for the distinguishing characters of the tissue system of rhizome. Photographs were taken which shows the distinguishing characters of the of the rhizome (Figure 3.5 and 3.6) [Reddy at al., 2012].

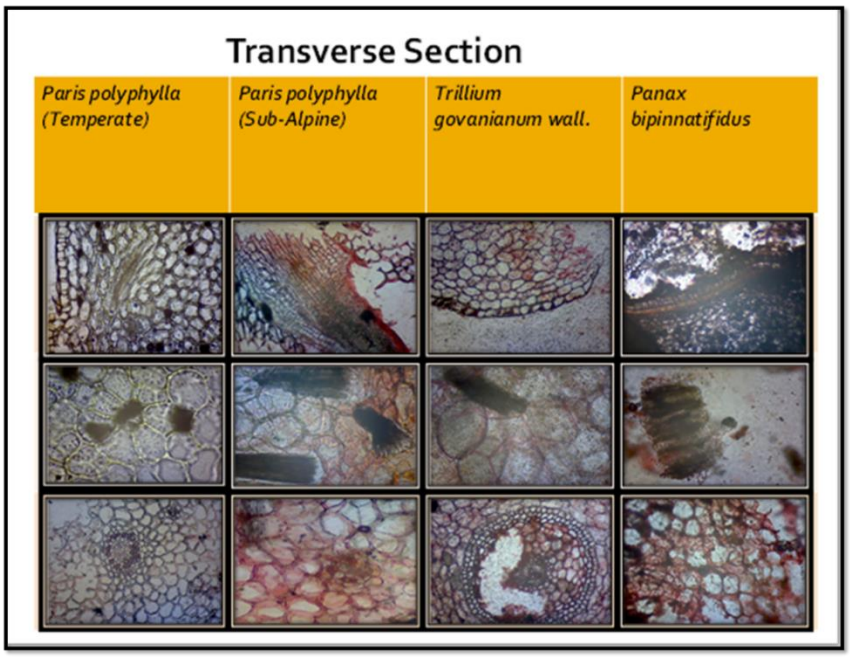



**Figure 3. 5.** Pictorial representation of transverse section of *P. polyphylla*, *Trillium govanianum* and *Panax bipinnatifidus* rhizomes from Sikkim Himalaya

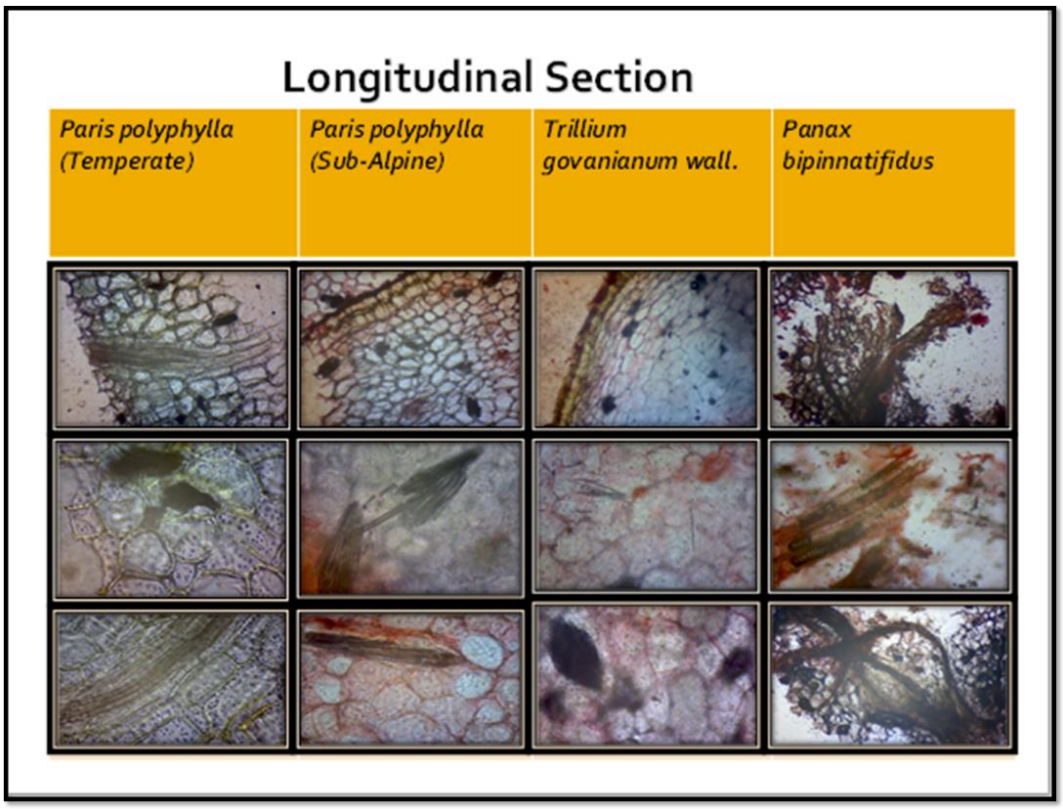



**Figure 3. 6.** Pictorial representation of longitudinal section of *P. polyphylla*, *Trillium govanianum* and *Panax bipinnatifidus* rhizomes from Sikkim Himalaya

### 3.5.4. Powdered drug microscopy

Rhizome of *P. polyphylla*, and two important associate plant which is being frequently used as an adulterant of *Paris* rhizome i.e. *Trillium govananium* and *Panax bipinifidus* were also collected for the pharmacognostic evaluations. All the three collected samples were cleaned well to eliminate dust and adhering materials and then dried under shade for few days and powdered separately for powdered drug detection. All powders were evaluated for microscopic structures, each of them were separately stained with the reagents such as phloroglucinol 1% and conc. HCl (for lignified structures), $H_2SO_4$ (350g/l) (for calcium oxalate crystals), iodine solution (for starch granules), sudan red G (for cuticular cell walls) and sudan red G in acetic acid and

ethanol (for essential oils, resins, fats and fatty oils) were used on bleached powders (Figure 3.7). All samples were observed under microscope (10x followed by 40x) and significant identifying characters were recorded with the help of camera (Tripathi et al. 2014) [Figure 3.7].

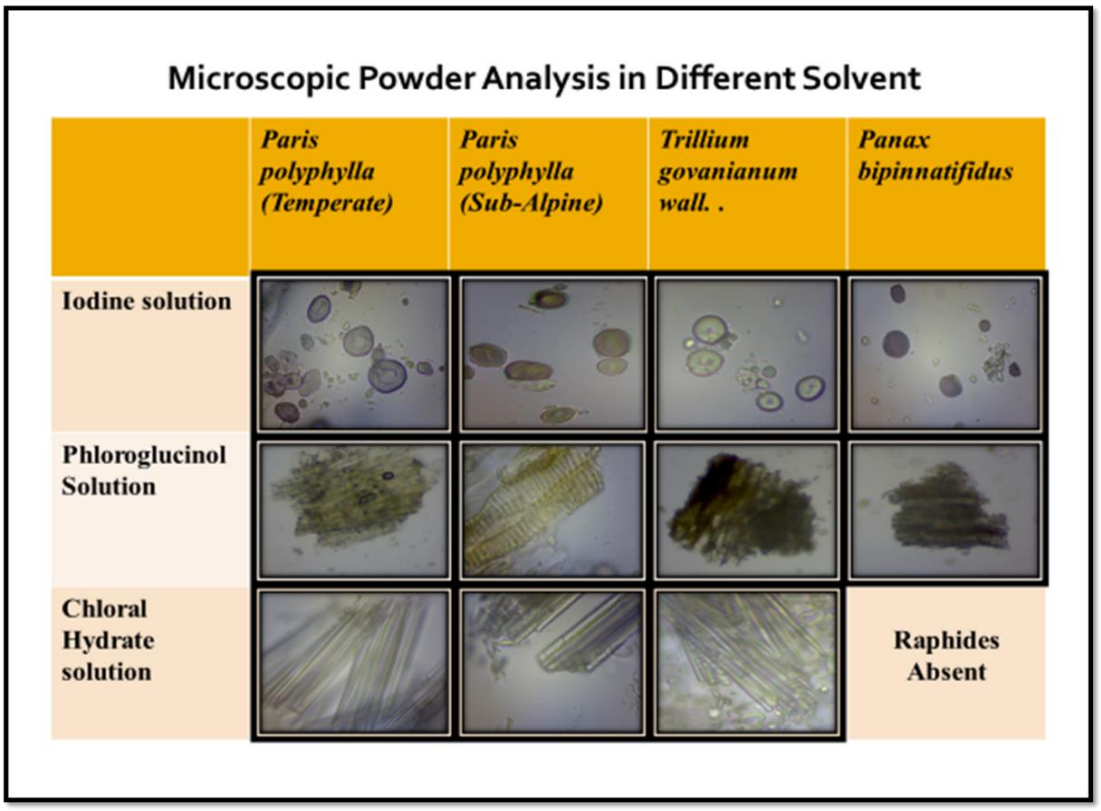



**Figure 3. 7.** Microscopic details of *P. polyphylla*, *Trillium govanianum* and *Panax bipinnatifidus* rhizome powder where Iodine solution shows starch grains, Phloroglucinol Solution shows spiral vessel and Chloral hydrate solution shows raphidian cells.

### 3.5.5. Fluorescence Analysis

Powdered rhizome of the *P. polyphylla* was subjected to various qualitative tests through the determination of fluorescent colour for the identification of phytochemical constituents. The evaluations included tests like: acetic acid test, Barfoed's test, Wagner's test, Mayer's test, Hager's test, iodine test, phloroglucinol test, sodium nitroprusside test, ninhydrin test, methanol test etc. A small quantity of

dried and finely powdered rhizome was placed on a grease free clean microscopic slide and added 1- 2 drops of the freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light of short (254nm) and long (365nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded (George et al. 2014).

The organic constituents absorb light usually over a specific range of wave length and re-emit such radiations. When the re-emission persists until the light source is there, the phenomena is referred to as fluorescence. Fluorescence analysis is a reliable qualitative evaluation method, since certain natural products only fluoresce upon the application of exciting rays, which do not visibly fluoresce in day light. The fluorescence produced at both short and long wave lengths were observed and summarized.

### 3.6. Phytochemical screening of the plants from two altidues

#### 3.6.1. Preparation of crude powder

For making dry powder, the plant material (leaf and rhizome) was reduced into small pieces and oven dried at 60ºC until they are completely dried and get constant weight. The dried samples were ground into a fine powder using a mechanical grinder sieved through No. 20-sieve. The powder samples were placed in cleaned, labelled plastic bottles and stored at room temperature in desiccators until extraction or phytochemical analysis.

#### 3.6.2. Extraction for phytochemical analysis

Method of extraction as per Kahkonen et al. (1999) was followed with slight modifications. 500mg of grounded dry plant material (leaf and rhizome) from each

treatment was taken in a test tube to which 10 ml of 80% aqueous methanol was added and the suspension was gently stirred. The tubes were sonicated for 10 minutes and centrifuged at 1500g for 10 minutes more. The resulting supernatant was collected. Again 10 ml. of 10 ml of 80% aqueous methanol was added to the pellet, stirred, sonicated and centrifuged as before for 2 times more. The supernatant of 3 extractions combined and evaporated in a water bath or rotavac till the volume becomes 1 ml. This concentrated extract was lyophilized and weighed. The extract was resuspended in 10% DMSO (100mg leaf and rhizome extract in 100 ml of 10% DMSO).

### 3.6.3. Qualitative phytochemical analysis

Preliminary chemical tests were carried out for aqueous methanolic extract (80%) of the dried leaf and rhizome powder to identify different phytoconstituents (Harborne, 1973; Parekh and Chanda, 2007). A sign (+) denotes low concentration when a slight opaqueness was produced, (++) sign denotes medium concentration when definite coloration was produced and (+++) sign denotes a high concentration if the addition of reagent produces strong colouration sometimes with flocculation.

#### 3.6.3.1. Determination of alkaloids

The presence of alkaloids was determined by the method of Harborne (1973). 200mg of methanolic extracts of plant material were weighed accurately and dissolved in 10 ml of methanol and then filtered using filter paper. 1ml of the filtrate was then mixed with 6 drops of Wagner's reagent. A creamish, brownish-red or orange precipitate indicated the presence of alkaloids.

### 3.6.3.2. Determination of flavonoids

The crude powder and methanolic extract were treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids (Boham and Kocipai, (1994).

### 3.6.3.3. Determination of tannins

The presence of tannins was determined by ferric chloride test (Segelman et al., 1969). About 0.5 g of the extracts were boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for greenish black coloration and precipitation.

### 3.6.3.4. Determination of phlobatanins

Harborne, (1973) method was followed for the determination of phlobatannins. The crude powder methanolic extract was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanins.

### 3.6.3.5. Determination of triterpenes

Methanolic extract was treated with concentrated sulphuric acid ($H_2SO_4$). Appearance of reddish-brown ring indicated the presence of triterpenes (Harborne, 1973).

### 3.6.3.6. Determination of steroids

Liebermann-Burchard reaction was performed for the presence of steroids. The methanolic extract was treated with the acetic anhydride and few drops of concentrated $H_2SO_4$ were added down the side of test tubes. A blue green ring indicated the presence of steroids (Shetty and Vijayalaxmi, 2012).

### 3.6.3.7. Determination of saponins

The presence of saponins was determined by frothing test. The methanolic extract (1ml) was taken in a test tube and vigorously shaken with distilled water (10ml) and was allowed to stand for 10 minutes and classified for saponin content as follows: no froth indicates absence of saponins, stable froth upto a height of 0.5 cm indicated low saponin (+), upto a height of 1cm indicated moderate (++) and 1.5 cm indicated the presence of high saponin (+++) content (Kapoor et al.,1969).

### 3.6.3.8. Determination of glycosides

To determine glycosides, 0.5 ml methanolic extracts of plant were added with 2ml of 50% hydrochloric acid. The mixtures were hydrolyzed for 2 hrs. on a water bath. After that 1 ml pyridine, few drops of 1% sodium nitroprusside solution, and 5% sodium hydroxide solution were added. Pink to red colour designated the presence of glycosides Method: Kumar et al., 2009).

### 3.6.3.9. Determination of cardiac glycosides

Keller-kiliani test was performed for the presence of cardiac glycosides. The methanolic extract each were treated with 1ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated $H_2SO_4$ were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides (Ajaiyeobu, (2002).

### 3.6.3.10. Determination of anthraquinones

1ml methanolic plant extracts were evaporated and dissolved in 2ml chloroform. 2ml of ammonia was added. Occurrence of red colour suggested the presence of anthraquinones (Kumar et al. (2009).

### 3.6.3.11. Determination of resins

0.5ml of extract was evaporated and dissolved in 2ml of petroleum ether, 2ml of 2% copper acetate solution was then added and the mixture was shaken vigorously and allowed to separate, A green colour indicated the presence of resins (Trease and Evans, 1983).

### 3.6.3.12. Determination of amino acids

The presence of amino acid was determined by Ninhydrin test. To a small quantity of sample extract, two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added. Appearance of purple colour indicated the presence of amino acids (Yasuma and Ichikawa , 1953).

### 3.6.3.13. Determination of proteins

Biuret test was performed to determine the presence of proteins. A small quantity of extract was treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink coloured layer indicated the presence of protein (Gahan, 1984).

### 3.6.3.14. Determination of carbohydrates

The presence of carbohydrates was determined by Molisch test. To a small quantity of extract a few drops of alcoholic alpha-naphthol solution was added. Further, 0.2 ml conc. sulphuric acid was added slowly along the sides of test tube, Appearance of violet coloured ring at junction confirms the presence of carbohydrates. (Joseph et al., 2013)

## 3.7. GC-MS spectral analysis

Gas-Chromatography Mass Spectrometry (GC-MS) of methanolic extract was performed using GC-MS instrument equipped with glass column having capillary

dimension of 30m x 0.25mm x 0.25µ. The operating conditions were: Oven temperature program 80°C to 260°C, inlet and interface temperatures were 250 °C and 200 °C respectively and the final run was kept for 50.07 min. The injector temperature was maintained at 260°C, the volume of injected sample was 0.3µl; pressure 73.3 kPa, total flow 16.3 mL/min, column flow 1.21 mL/min, linear velocity 40.1 cm/sec, purge flow 3.0 mL/min, split ratio: 10.0; ion source temperature 230°C; scan mass range of m/z 40-650 and interface line temperature 270°C. Carrier gas was helium at a flow rate of 1.0 ml/min. The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, US) and WILEY 8.

### 3.8. Determination of anticancer activity

#### 3.8.1. Extraction for anticancer activity assay

The completely shade-dried rhizomes of *P. polyphylla* were pulverized using a waring blender, powdered and sieved (0.1 mm mesh). The powdered plant material (10 g) was soaked in 10 vol. (100 ml) of 70% methanol for 24 h at room temperature with intermittent shaking and the supernatant decanted. The extraction was repeated thrice using fresh solvents and the extracts were pooled together and defatted by partitioning with hexane, the MeOH layer was filtered through a Whatman no. 1 paper, evaporated at low temperature. The residue thus obtained was dissolved in DMSO prior to use.

#### 3.8.2. Cell lines and cultures

The human carcinoma cells, HeLa (cervical cancer), PC3 (prostrate cancer) and HepG2 (hepato carcinoma) were used to investigate the cytotoxic activity evaluation of the methanolic extract of the sample. The cells were cultured to reach confluence in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 unit/ml penicillin

and 100 µg/ ml streptomycin and maintained at 37°C in a humidified atmosphere with 5% $CO_2$ incubator.

### 3.8.3. Cytotoxicity assay by MTT method

Cytotoxic activity was assessed by determining the cell viability under the influence of a methanolic extract by the MTT assay as described by Mosmann (1983). For the experiment, the cells were treated with various concentration of *P. polyphylla* rhizome extract for 72 hr. After the incubation 1:10 volume of MTT solution (5 mg/ml) was added to each well and incubated for 4 hr in dark. Then the medium was carefully removed and the formazan formed in the wells was dissolved for homogenous measurement in 150 µl of dimethyl sulfoxide, the plates were kept for 5 min on a plate shaker. The absorbance was measured at 570 nm using a microplate reader. For the control, SDYB medium (pH 7.4) and Dulbecco's PBS were used in place of the rhizome extract.

Cytotoxicity was calculated as the percent reduction in absorbance relative to the control (DMSO). Half maximal inhibitory concentration (IC50) was determined graphically by plotting percentage of inhibition against the concentration of drug. The percentage inhibition is calculated, from, the data using the formula:

$$\% \text{ Inhibition} = \frac{\text{Mean OD of untreated cells (control)} - \text{Mean OD of treated cells (sample)}}{\text{Mean OD of untreated cells (control) x 100\%.}} \times 100$$

### 3.9. Analysis of physiological and biochemical parameters

#### 3.9.1. Foliar spray of plant growth regulators

For every treatment, 12 numbers of uniformly grown plant seedlings were grown in selected plot and treated with the growth promoters or regulators. For the treatment 3 different growth promoters, *viz*., Gibberellins ($GA_3$), Indole Acetic Acid (IAA) and Kinetin (KIN) and 3 growth retardants, *viz.*, Maleic Hydrazide (MH), Chlorocholine Chloride (CCC) and Abscisic Acid (ABA) were directly sprayed at appropriate stage of the plants (Figure 3.8). The following concentrations of the PGRs were used for the treatments:

i. IAA - 100 and 200 µg/ml (first dissolved in a few drops of 4%NaOH or ethyl alcohol) [$IAA^{100}$ and $IAA^{200}$].
ii. $GA_3$ - 100 and 200 µg/ml (first dissolved in a few drops of ethyl alcohol) [$GA_3^{100}$ and $GA_3^{200}$].
iii. Kinetin – 100 and 200 µg/ml (first dissolved in a few drops of 4%NaOH) [$KIN^{100}$ and $KIN^{200}$]
iv. Maleic hydrazide - 50 and 100 µg/ml (dissolved directly in water) [$MH^{50}$ and $MH^{100}$].
v. Chlorocholine chloride - 50 and 100 µg/ml (dissolved directly in water) [$CCC^{50}$ and $CCC^{100}$]
vi. Abscisic acid – 25 and 50 µg/ml (first dissolved in a few drops of 4%NaOH) [$ABA^{25}$ and $ABA^{50}$].

Foliar applications with the aqueous solutions of the above PGRs were carried out at 60DAE, 90 DAE and 120DAE. The PGRs contained 0.5% Tween-20 as a surfactant. The field was divided into subplots for each variety and each treatment. In each of the subplots 12 plants were transplanted for each treatment. The transplanted plants were irrigated at a regular interval. (Note. *All the analysis involving fresh plant parts were carried out between 4-10 days after foliar spray of PGR. For all analysis involving dried leaves and rhizomes-these parts are harvested within 4-10 days after foliar spray of PGR, dried, ground into fine powder and analysed within 3 months*).


**Figure 3. 8.** A) Preparation of poly bags for phase wise plantation, B) Addition of decayed wood powder after planting, (C-D) Saplings ready for foliar spray and (E-F) Administration of foliar spray with different growth hormones.

### 3.9.2. Growth analyses

For study of growth attributes of the plants, the plants were uprooted at the appropriate time and the physiological parameters like shoot length, root lengthand rhizome diameter were recorded for each plant. Data were recorded from the mean values of five uniformly grown plants for each treatment.

### 3.9.3. Measurement of relative growth index

The relative growth index (RGI) was measured by the method of (Paliouris and Hutchinson, 1991) with slight modifications. 10 seedlings were taken for the estimation. Relative growth index (RGI) was calculated using the formula:

$$RGI = \frac{\text{Average dry matter of seedling in a treatment}}{\text{Average dry matter of seedling in control soln.}} \times 100$$

### 3.9.4. Determination of relative water content

This parameter was analyzed by the method of Smart and Bingham, (1974). For the determination of relative water content (RWC) ten discs from 2$^{nd}$ and 3$^{rd}$ leaves from the top of were collected. After wet mass determination, the leaf discs were floated on distilled water for 24 h at 25°C. The hydrated discs were weighed to determine the turgid mass (TM). The leaf tissues were subsequently dried in an oven at 60 °C for 48 h and weighed to determine the dry masses. RWC was calculated as per the following formula:

$$RWC\ (\%) = \frac{(WM-DM)}{(TM-DM)} \times 100$$

where, WM = wet mass, DM = dry mass and TM = turgid mass

### 3.10. Biochemical analysis

#### 3.10.1. Membrane lipid peroxidation (MLP)

The method of Heath and Packer (1968) was performed with slight modifications in order to determine the membrane lipid peroxidation. 200mg of sample was taken. Homogenised in 5ml of 0.1% TCA (trichloroacetic acid) then centrifuged at 10,000 rpm for 5 minutes and the supernatant taken as sample extract.

The concentration was calculated as per the following formula:

$$\text{Concentration of unknown} = \frac{\text{Absorbance on unknown at 530 nm}}{\text{Diameter of cuvette x 155}} \text{ moles / litre}$$

Finally, the MLP content was expressed in n mole/$g^{-1}$ dry mass of tissue.

#### 3.10.2. Determination of chlorophyll-a, chlorophyll-b and carotenoids:

Photosynthetic pigments were determined as per Lichtenthaler and Welburn (1983) with slight modifications. 50 mg leaf tissue was homogenised with 5 ml 96% ethanol, centrifuged at 5,000 rpm for l0 minutes and the supernatant taken as pigment extract. The absorbance of the pigment extract was measured at 665, 649 and 470 nm. The different pigment contents were calculated using the following formula:

Chlorophyll-a = (13.95 x $A_{665}$ – 6.88 x $A_{649}$) µg/ml

Chlorophyll-b = (24.96 x $A_{649}$ – 7.32 x $A_{665}$) µg/ml

Carotenoids = (1000 x $A_{470}$ – 2.05 x chl.a – 11.48 x chl.b) µg/ml

Chl a, Chl.b and carotenoids were finally expressed in terms of mg/g of tissue.

### 3.10.3. Free amino acid content

The free amino acid content was determined by the method of (Moore and Stein, 1948). 100mg of fresh sample was homogenized with 5ml of distilled water then centrifuged at 10,000rpm for 15 minutes and the supernatant collected, volume made upto 10ml with distilled water, it was served as the crude amino acid extract. 1ml of the crude extract was taken in three replicates, followed by1ml of ninhydrin reagent, incubated at 100 $^0$C for 15 minutes, then 2ml of 90% ethanol was added and incubated at room temperature for 20 minutes. The colour developed was then measured at the 570nm with a Spectrophotometer.

### 3.10.4. Proline content

The sample proline contents were estimated by the method of Bates et al. (1973). 200mg of fresh sample was homogenized in 10 ml of 3% sulfosalicylic acid, centrifuged at 4,000 rpm for 10 minute and the supernatant taken as crude sample extract. Each of the above extract was made into triplicate then 2ml of water was added followed by the addition of 2ml of glacial acetic acid ,then 2ml of acid Ninhydrin reagent was added and incubated in boiling water bath at 100C for 1hour followed by incubation in ice bath for 10 minutes,thereafter stirred for 30 second. The colour developed was measured at 520nm and the proline content was determined from a standard curve of proline (0-100 µg/ml).

### 3.10.5. Soluble protein content

The soluble protein content of the sample was determined by the method of Lowry et al. (1951) with slight modifications. 100 mg of fresh samples were homogenized in 5 ml of distilled water, centrifuged at 10, 000 rpm for 10 minutes and the supernatant collected. The volume made up to 10 ml with distilled water. This was taken as crude protein extract. 1ml of crude sample was taken in triplets and 0.9 ml of protein

reagent-A was added and incubated at room temperature for 15 minutes then 0.1 ml of protein reagent-B was added and incubated at room temperature for 30 minutes after which 2.5ml of water was added and finally the absorbance was measured at 650nm. The content of the soluble protein was estimated from a standard curve prepared from BSA solution (0-100 µg/ml).

### 3.10.6. Determination of soluble and insoluble carbohydrates

Carbohydrate contents of the samples were determined by the method of McCready et al. (1950) with slight modifications. 100mg of fresh sample was homogenized in 5ml of boiling 80% ethanol, centrifuged at 5,000 rpm for 20 minutes and the supernatant taken in a watch glass. Again 5 ml of 80% ethanol was added and supernatant collected x 2. Pooled supernatant was taken in a watch glass and evaporated to dryness; chlorophyll was removed by washing with solvent ether. For soluble carbohydrate estimation, residual material in the watch glass washed 3 times with 80% ethanol and the volume made upto 10 ml with the same. The sample was source of soluble carbohydrate. And for insoluble carbohydrate estimation, 10 ml of 25% $H_2SO_4$ was added to the pellet and stirred with a glass rod. The mixture was heated at 80°C for 30 and cooled to room temp. This served as the sample for insoluble carbohydrate. For both the parameters, 1 ml of crude extract was taken in a triplicate, then 1ml of distilled water was added followed by 3ml of 0.2% Anthrone reagent and incubated for 15 minutes and the absorbance was measured at 610nm. The calculation of carbohydrate content was made from a standard curve prepared from glucose ((0-50 µg/ml).

### 3.10.7. Determination of ascorbic acid

Different sample tissues were re-extracted with meta-phosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1.0 mL) was mixed with 9 ml of 2,6-dichloroindophenol (0.8 g/1000 ml) and the absorbance was measured within 30 minutes at 515nm. Ascorbic acid contents were calculated on the basis of calibration curve of L-ascorbic acid (Klein and Perry, 1982).

### 3.10.8. Estimation of the catalase activity

The catalase activity of the samples were estimated by the method of (Snell and Snell, 1971). For the estimation, 1 g of fresh tissue was homogenized with 5 ml 0.2mM Na-phosphate buffer (pH-6.8) containing 1% PVP, centrifuged at 5,000 rpm for 10 minute and the supernatant taken as crude enzyme extract. The sample extract was placed into two set one control and a reaction sets. In control set 2ml of 1%$TiSO_4$ was added followed by the addition of 2ml of $H_20_2$ and 1ml of enzyme extract. The mixture was incubated for 15 minutes at 37c. In the reaction set, 1% $TiSO_4$ was added after the incubation. The absorbance of the both the sets were measured at 420nm . Finally the enzyme activity was calculated by the formula of Fick & Qualset (1975):

$$\text{Enzyme activity} = \frac{\Delta A \times Tv}{t \times v} \quad \text{Units/min/g FW}$$

Where, $\Delta A$= OD difference of Reaction Set – Control set;

tv = total volume of enzyme extract (5 ml)

t = time of incubation (15 minutes)

v = volume of enzyme extract taken for reaction (1 ml)

### 3.10.9. Estimation of peroxidase activity

To estimate the peroxidase activity, 1 g of fresh tissue was homogenized with 5 ml 300 µm Na-phosphate buffer (pH-6.8), centrifuged at 1,000 rpm for 10 minute and the supernatant taken as crude enzyme extract. This enzyme extract was then taken in triplicate and added with 1ml each of phosphate buffer (pH 6.8), pyrogallol and $H_2O_2$ solution before incubating for 15 minutes at 37ºC. In the control set also, the same operation was undertaken, however, in this case 5% $H_2SO_4$ was added prior to the 15 min incubation period, while in the reaction set it was added only after the incubation. Finally the absorbance was measured at 430nm (Kar and Mishra, 1976) and the enzyme activity was calculated by the formula of Fick and Qualset, (1975) as follows:

$$\text{Enzyme activity} = \frac{\Delta A \times Tv}{t \times v} \quad \text{Units/min/g fresh wt.}$$

Where, ΔA= OD difference of reaction set – control set

tv = total volume of enzyme extract (5 ml)

t = time of incubation (15 minutes)

v = volume of enzyme extract taken for reaction (1 ml).

### 3.10.10. Estimation of superoxide dismutase (SOD) activity

The method of Giannopolitis and Ries (1977) was followed for the estimation of superoxide dismutase (SOD). For the estimation, 500 mg fresh tissue was homogenized with 10 ml 0.1 M Na-phosphate buffer (pH-6.8) containing 1% PVP and centrifuged at 6,000 rpm for 10 minutes. The supernatant was taken as crude enzyme extract. The same sample extract was divided into two sets, control set and the reaction set. In the control set 1ml of water was added followed by the addition of 0.5ml of $Na_2CO_3$ , 0.1 ml EDTA, 0.5ml NBT, 0.5ml Riboflavin and 0.4ml $H_2O$ and placed in a dark room. In reaction set, in place of the first $H_2O$ 1 ml of enzyme extract

was taken followed by the sequential addition of $Na_2CO_3$, EDTA, NBT, Riboflavin and $H_20$ as mentioned above. The entire sets were exposed to fluorescent lamp (40w for 30 minutes) and the absorbance was measured at 560nm. Finally the enzyme activity was calculated by the formula of Fick and Qualset, (1975).

$$\text{Enzyme activity} = \frac{\Delta A \times Tv}{t \times v} \quad \text{Units/min/g fresh wt.}$$

Where, ΔA= OD difference of reaction set – control set

tv = total volume of enzyme extract (5 ml)

t = time of incubation (30 minutes)

v = volume of enzyme extract taken for reaction (1 ml).

### 3.11. Estimation of PGR induced changes in phytoconstituents

#### 3.11.1. Estimation of total phenolic contents

The total phenolic contents were estimated as per Lin et al. (2011). Aliquots of 1.0 mL of extracts prepared as per 3.6.2 were mixed with 5 mL of 10-fold diluted Folin-Ciocalteau reagent and 4 mL of 7.5% $Na_2CO_3$. The mixture was allowed to stand for 90 minutes at room temperature before the absorbance was measured at 760 nm in a UV-vis spectrophotometer. The amount of phenol content was determined using gallic acid as standard and expressed as gallic acid equivalent (mg GAE/g DW) while the calculations were done by using the following formula:

$TPC = C \times V/m$

Where, TPC= total phenol content

C= concentration of Gallic acid (mg/ml)

V= volume of plant extract (ml) and

m= weight of pure plant extract (g)

### 3.11.2. Estimation of total flavonoid contents

About 5 ml of extracts prepared as per 3.6.2 was transferred to test tube, mixed with the 0.3 ml of 5% sodium nitrite and incubated for 5 minutes. Then 0.3 mL of 10% aluminium chloride was added. After 6 min, the reaction was stopped by the addition of 2 ml of 1M NaOH. The mixture was further diluted with distilled water up to 10 ml and the absorbance of the mixture was immediately measured at 510 nm (Lin et al., 2011). Quercetin was used as standard and the flavonoid contents were calculated and expressed as quercetin equivalents (mg QE/g DW).

### 3.11.3. Estimation of total flavanols

About 2.0 ml of sample extract prepared as per 3.6.2 was taken and 2.0 ml of 2% $AlCl_3$ and 3 mL sodium acetate (50 g/l) solutions were added. The absorption was measured at 440 nm after 2.5 h at 20°C. Total flavanol contents were calculated as Rutin equivalents (mg RtE/g DW) which was used as standard (Kumaran and Karunakaran, 2006).

### 3.11.4. Crude alkaloid estimation

2.5 g of the dried sample powder was weighed into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

### 3.11.5. Estimation of crude saponin

10 g of dried sample powder was defatted with petroleum ether for 1 h at 40 ºC in water bath and then extracted with methanol for 1 h with mild heating. It was then filtered and centrifuged at 5000 g for 10 min. In order to get the crude saponin extract, the methanolic extract was dissolved in a mixture of methanol and acetone (1:5 v/v) to precipitate the saponins. It was filtered and dried. Crude saponin was collected and weighed. Crude saponin content is expressed in mg/g of dried sample powder (Yan et al., 1996).

### 3.11.6. Estimation of diosgenin by HPLC

#### 3.11.6.1. Preparation of the sample and chromatographic conditions

The dried plant materials were subjected to hydrolysis in 2N HCL for 4 hrs. The pH of the post-hydrolysis was neutralized to 7.0 with 2N NaOH. The extract was centrifuged and the residue was dried at 55ºC for 36 hours in hot air oven. The dried extracts were subjected to soxhlet extraction with absolute methanol (HPLC grade) at 80 ºC for 4 hours and then re-distilled at 80 ºC. On post- distillation the sample was completely dried by roto-evaporation at 50ºC and cooled. The dried plant extract was dissolved in HPLC grade methanol. The yield of the extract was calculated. The extract was filtered using 0.2µm filters and analysed on HPLC.

Diosgenin fractions were analyzed in an UHPLC (Dionex- UltiMate 3000 UHPLC system (Germany) at a temperature of 30°C was carried out as previously described with some modifications (Li et al. 2012 and Desai et al. 2015). A Thermo Hypersil – C18 column (4.6×250mm, 5µm particle size) was used and the analysis of diosgenin was carried out by isocratic elution with HPLC-grade acetonitrile: water (90:10, v/v) as a mobile phase. The injection volume was 20 µl, a flow rate of mobile phase was maintained at 1ml/min and the elution was monitored at 210nm. Diosgenin had a

retention time of 6.35 ± 0.003 min. All data were analysed using Chromeleon software version 6.80 SR13. Standard of diosgenin was procured from Sigma-Aldrich (USA). The content of diosgenin was expressed as % DW of tissue.

### 3.11.6.2. Standard run and isolation of diosgenin

A standard stock solution of Diosgenin ($mg^{-1}$ $ml^{-1}$) was prepared in HPLC grade methanol, from which working standard solutions range from 1 to 5µg was prepared by serial dilution of the stock solution. Calibration curve was prepared based on peak areas of 5 concentration runs in triplicates. The chromatographic peaks of diosgenin showed maximum absorption at 210nm. The peak was resolved with an isocratic mobile phase of absolute methanol (Figure 3.9). The fractions of diosgenin were collected according to the retention time from calibrated curve with standard (Diosgenin; SIGMA).

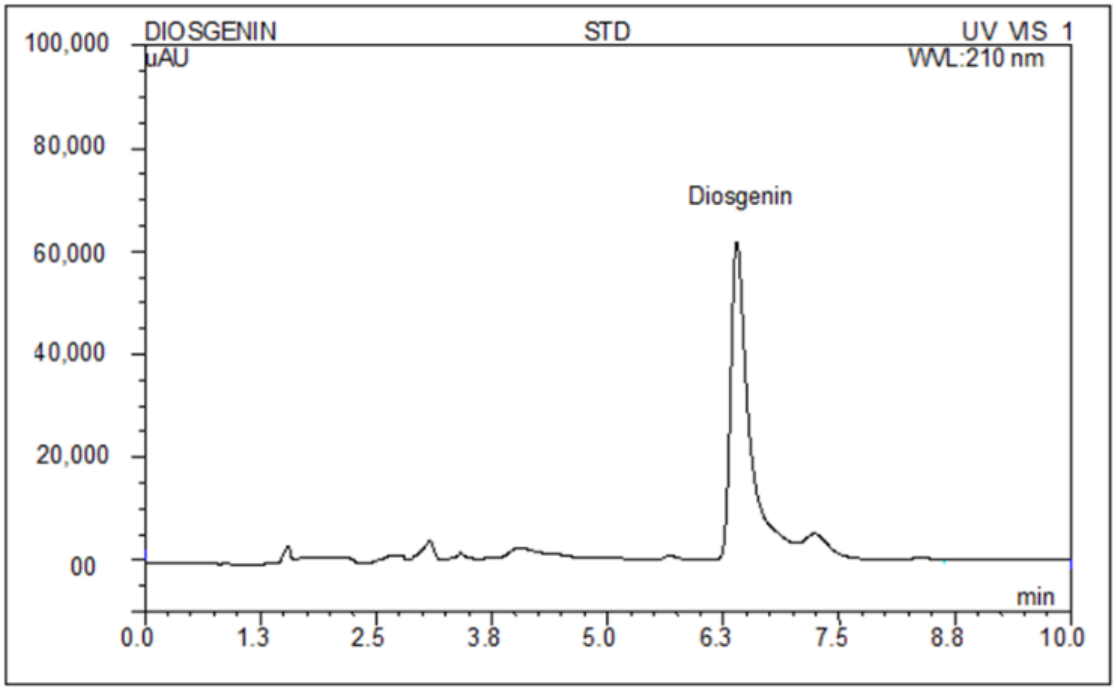



**Figure 3.9.** HPLC chromatogram for standard (Diosgenin: Sigma-Aldrich).

### 3.12. Statistical Analysis:

For ENM studies, in order to remove the highly correlated variable (r>0.9) we performed multicollinearity using ENM Tools 1.3 (Warren et al. 2010). The data obtained from the phytochemical estimation assays were presented as means of triplicate determinations standard error (SE). Cytotoxic activity of the sample extracts was determined from quadruplicate observations and these data were analyzed using Microsoft Excel 2010 (Microsoft Corporation, WA, USA). For HPLC analysis, all data were analyzed using Chromeleon software version 6.80 SR13.

# 4. RESULTS

## 4.1. Meteorological data during the study period (2015-2017)

The climate of the Sikkim Himalaya is monsoonal. State experiences three distinct seasons, i.e. summer (March, April and May), rainy (June, July, August, September, October) and winter (November, December, January and February) season (Meteorological Station Gangtok, Sikkim). Maximum annual rainfall during the study period across all 4 (four) districts was observed in North district (3348.60mm) and minimum rainfall was recorded in the South district (1986.10 mm). Rainy season experiences more than 60% of monthly or seasonal rainfall. Highest average rainfall was recorded during the month of July (519.17 mm) and lowest in the month of December (4.04 mm) during the study period (2015-2017).

Likewise, the average mean temperature is recorded highest in the month of August (21.50°C) and lowest in the month of January (9.98°C) during the study period (Table 3.1.6). Among all the districts during the study period, the mean annual temperature was highest in the West district (21.08°C) and was found lowest in the East district (14.58°C). Climate data of all four district study sites during study period is placed on (Figure 4.1).

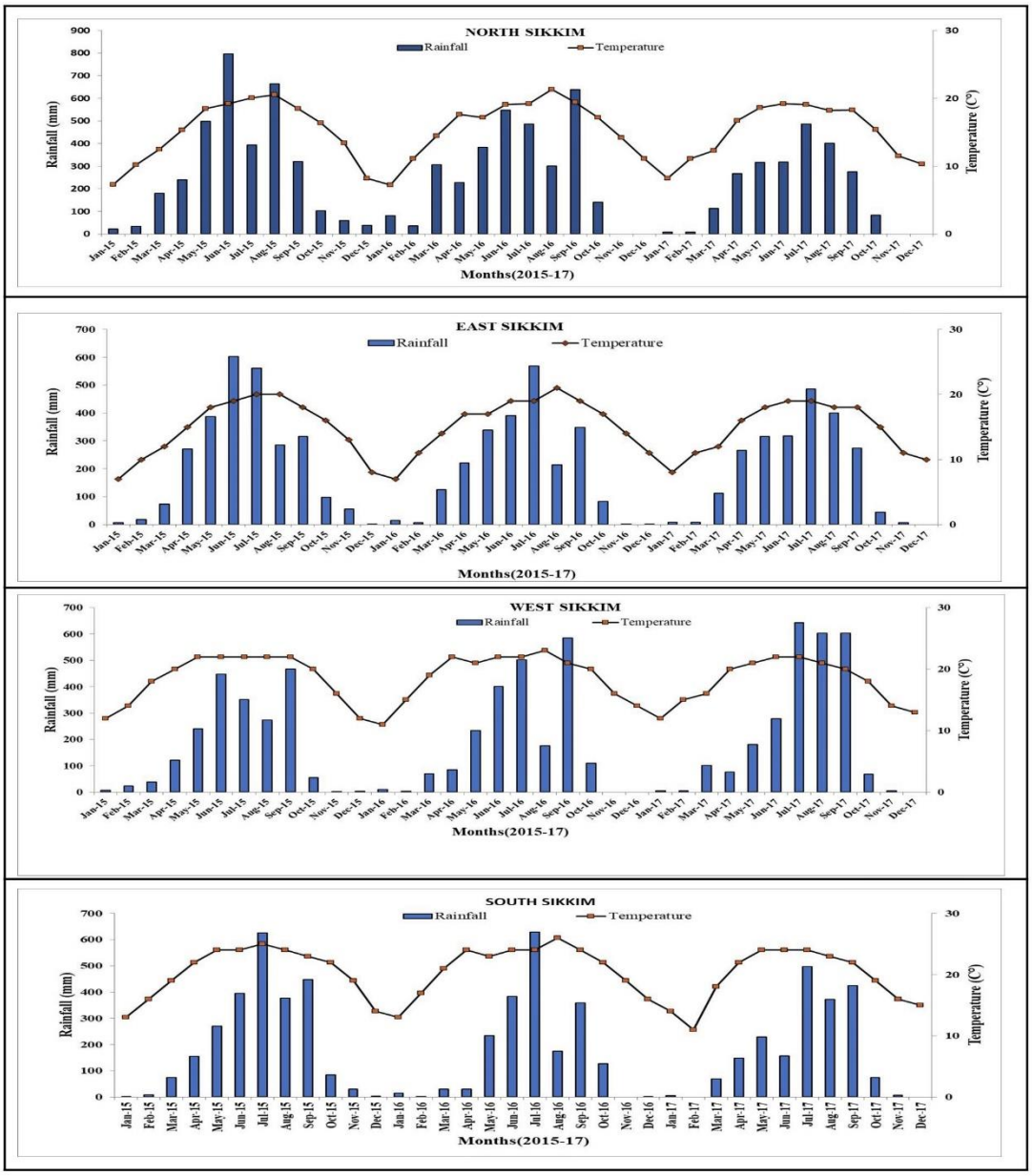



**Figure 4.1.** Climate data of North, East, West and South district of Sikkim during study period (2015-17).

## 4.2 (A). Phenological changes of *P. polyphylla* raised from seeds

Phenology of the plant during different phases of the life of the plants were constantly monitored over a period of three year (this included germination, vegetative phase, flowering stage, seed setting and senescence). The detailed phenological changes in the life cycle of *P. polyphylla* has been studied from 10 uniformly grown plants raised

from seeds at Lingthem, Dzongu, North Sikkim. It was observed that the seedlings collected from both Uttaray and Tholung show active apical growth only during the second year. As young buds and few primary root emerges from the seed but majority of which remains dormant underground in the first year. From third year only a proper well-developed plant can be seen (Table 4.1.1 and 4.1.2). The whole life cycle, runs slow in the plants from Uttaray (PPU) as compare to the Tholung (PPT).

### 4.2 (B). Phenological changes of *Paris polyphylla* raised from rhizome cuttings

A comprehensive phenological change in the life cycle of *P. polyphylla* raised from rhizome collected from Uttaray and Tholung has been presented in Table 4.1.3 and 4.1.4. Unlike seeds germination, here the active apical growth initiates during the first year itself. From second year proper well-developed plant and in some cases with appropriate seed pods formation could be seen in the plants. In case, rhizome the segment raised plants, the entire life cycle is reduced by four months to one year and the biomass of the rhizomes is almost double in the rhizome segments raised plants harvested after the same period of growth.

### 4.3. Predictor environmental variables to the MaxEnt model

Population assessment through field survey followed by ENM revealed a positive correlation between predicted suitable habitats with the actual sites of its occurrence, except in disturbed habitats (Figure 4.2). In order to remove the highly correlated variable ($r>0.9$) we performed multicollinearity using ENM Tools 1.3 (Warren et al. 2010). Thus, out of 19 bioclimatic variables, six were selected for modelling.

**Table-4.1.1** Different phenological changes in the life cycle of *Paris polyphylla* Smith from Uttaray in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from seeds at Lingthem.

| Sl. No. | Phases in the life cycle | Days required after sowing (DAS) | Remarks |
|---|---|---|---|
| 1 | Underground emergence phase | 160-170 | Primary root initiations |
| 2 | Seed germination phase | 310-350 | Young bud emerges from the seed but majority of which remains dormant underground till next germination period |
| 3 | 1st Field emergence phase | 410-430 | Active apical growth starts |
| 4 | Sapling phase | 450-480 | Young shoot with one leaf and development of about 3-6 primary root initiations |
| 5 | 1st Senescence phase | 610-640 | Gradual drying of whole plant |
| 6 | 2nd Field emergence phase | 790-820 | Active apical growth starts |
| 7 | Mature plant phase | 820-850 | Young shoot with 4-6 leaf and development of about 5-9 root initiations |
| 8 | Flower initiation phase | 840-870 | Bud formation takes place which later gave solitary-terminal flowers |
| 9 | Seed formation and maturation phase | 970-1010 | Seed pods formation with few minute seeds |
| 10 | 2nd Senescence phase | 990-1020 | Gradual drying of whole plant |
| 11 | Death phase | 1030-1060 | Above ground plant parts dried and underground rhizome remains dormant throughout the winter. |

**Table-4.1.2** Different phenological changes in the life cycle of *Paris polyphylla* Smith from Tholung in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from seeds at Lingthem.

| Sl. No. | Phases in the life cycle | Days required after sowing (DAS) | Remarks |
|---|---|---|---|
| 1 | Underground emergence phase | 90-120 | Primary root initiations |
| 2 | Seed germination phase | 180-220 | Young bud emerges from the seed but majority of which remains dormant underground till next germination period |
| 3 | 1st Field emergence phase | 270-300 | Active apical growth starts |
| 4 | Sapling phase | 300-360 | Young shoot with one leaf and development of about 3-6 root initiations |
| 5 | 1st Senescence phase | 390-450 | Gradual drying of whole plant |
| 6 | 2nd Field emergence phase | 480-540 | Active apical growth starts |
| 7 | Mature plant phase | 540-600 | Young shoot with 4-6 leaf and development of about 5-9 root initiations |
| 8 | Flower initiation phase | 630-660 | Bud formation takes place which later gave solitary-terminal flowers |
| 9 | Seed formation and maturation phase | 730-770 | Seed pods formation with few minute seeds |
| 10 | 2nd Senescence phase | 770-800 | Gradual drying of whole plant |
| 11 | Death phase | 800-840 | Above ground plant parts dried and underground rhizome remains dormant throughout the winter. |

**Table-4.1.3**. Different phenological changes in the life cycle of *Paris polyphylla* Smith from Uttaray in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from rhizome cuttings at Lingthem.

| Sl. No. | Phases in the life cycle | Days required after planting (DAP) | Remarks |
|---|---|---|---|
| 1 | 1st Field emergence phase | 110-150 | Minute shoot emergence from the main rhizome node |
| 2 | Sapling phase | 150-180 | Active apical growth, initiation of distinct whorl leaf (with majority of non-flowering plants). |
| 3 | 1st Senescence phase | 310-340 | Gradual drying of whole plant body which starts from the leaf |
| 4 | 2nd Field emergence phase | 420-450 | Minute shoot emergence from the main rhizome node |
| 5 | Mature plant phase | 480-560 | Active apical growth, initiation of distinct whorl leaf |
| 6 | Flower initiation phase | 560-590 | Bud formation takes place which gradually increases the ratio of flowering then non flowering plants |
| 7 | Seed formation and maturation phase | 590-630 | Seed pods formation with numerous minute seeds |
| 8 | 2nd Senescence phase | 630-670 | Gradual drying of whole plant body and colour of the main stem became golden brown with blackish dry leaves |
| 9 | Death phase | 670-700 | Above ground plant parts dried and underground rhizome remains dormant throughout the winter. |

**Table-4.1.4.** Different phenological changes in the life cycle of *Paris polyphylla* Smith from Tholung in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from rhizome cuttings at Lingthem.

| Sl. No. | Phases in the life cycle | Days required after planting (DAP) | Remarks |
|---|---|---|---|
| 1 | 1st Field emergence phase | 80-100 | Minute shoot emergence from the main rhizome node |
| 2 | Sapling phase | 130-140 | Active apical growth, initiation of distinct whorl leaf (with majority of non-flowering plants) |
| 3 | 1st Senescence phase | 290-330 | Gradual drying of whole plant body which starts from the leaf |
| 4 | 2nd Field emergence phase | 390-420 | Minute shoot emergence from the main rhizome node |
| 5 | Mature plant phase | 480-510 | Active apical growth, initiation of distinct whorl leaf |
| 6 | Flower initiation phase | 520-550 | Bud formation takes place which gradually increases the ratio of flowering then non flowering plants |
| 7 | Seed formation and maturation phase | 560-590 | Seed pods formation with numerous minute seeds |
| 8 | 2nd Senescence phase | 630-650 | Gradual drying of whole plant body and colour of the main stem became golden brown with blackish dry leaves |
| 9 | Death phase | 670-750 | Above ground plant parts dried and underground rhizome remains dormant throughout the winter. |

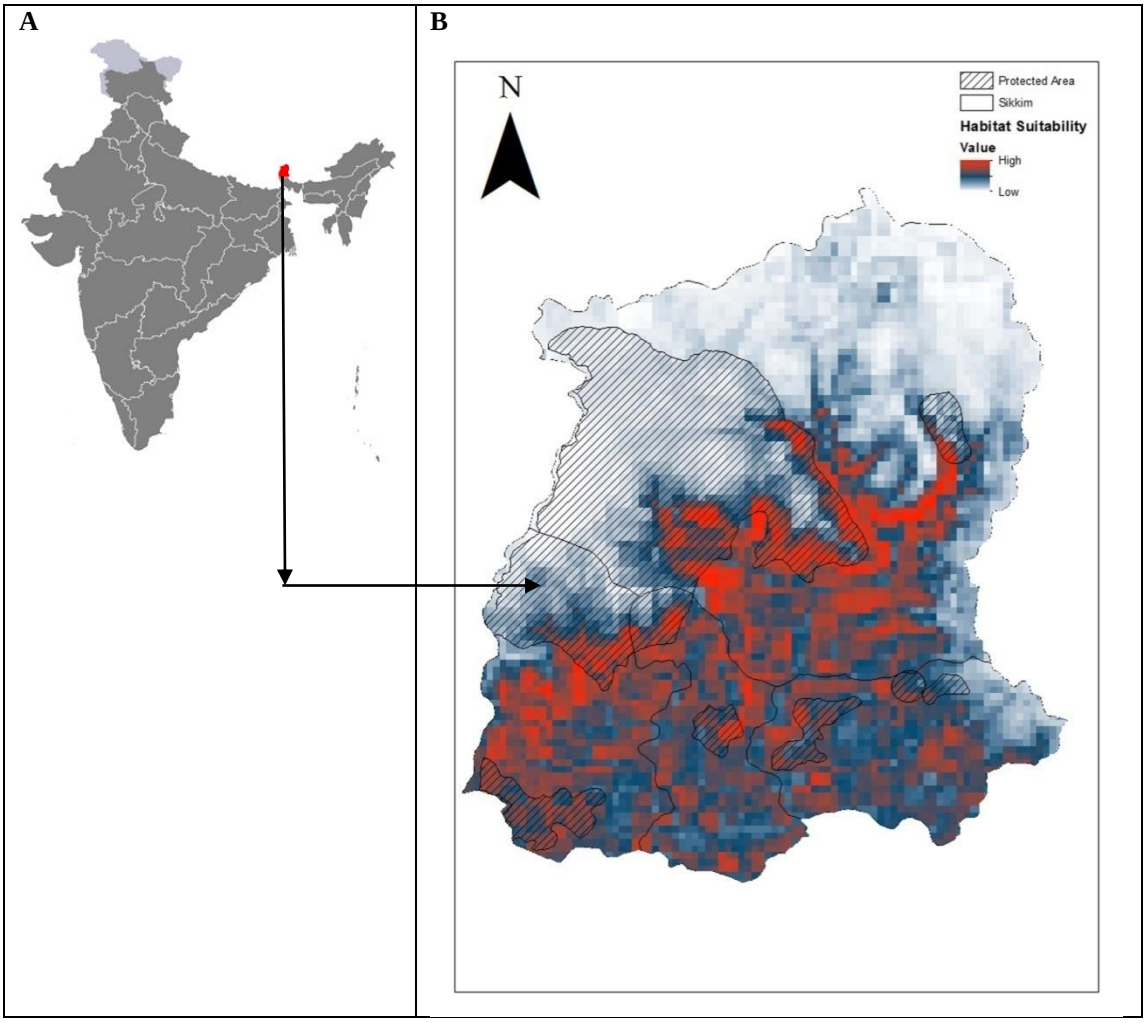
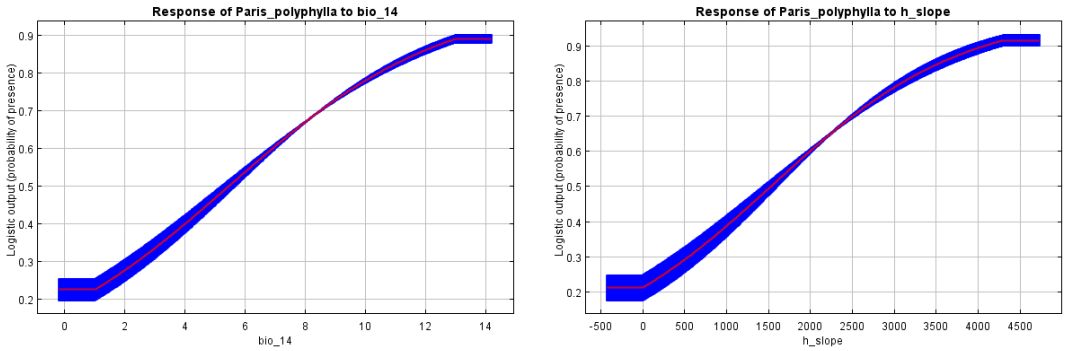



**Figure 4.2.** A) Study site in Sikkim, India (in red) and B) Distribution mapping of *Paris polyphylla* in Sikkim, India.

Amongst all the six variables used precipitation of driest month (Bio 14) [41.9 %] and slope (30 %) had the highest contribution to the MaxEnt model [Figure 4.3 (A) and (B)]. These two variables collectively contributed 71.9 % to the model output (Table 4.1.5). Followed by other variables such as Mean diurnal temperature range (17.1%) and Annual mean temperature (6.6 %) [Table 4.1.5].




A B

**Figure 4.3** (A). Response curves showing the relationships between the probability of presence and Precipitation of driest month (Bio 14) (values shown are average over 10 replicate runs: blue margins show SD calculated over 10 replicate run). (B). Response curves showing the relationships between the probability of presence and Slope (values shown are average over 10 replicate runs: blue margins show SD calculated over 10 replicate run).

### 4.4. Ecological details of *P. polyphylla* habitats in Sikkim Himalaya.

Field survey revealed that the density of the plants varied between 0.45 (pl/m2) and 3.89 (pl/m2) and the frequency varied from 36% to 76%. The IVI for *P. polyphylla* ranged between 2.68 to 8.66 based on locations. On the other hand, the IVI of associated species varied from 3.57 to 18.14 based on species. Density, frequency of occurrence and Importance Value Index (IVI) of the study species is presented in Table 4.1.6. The observation revealed that in Uttaray the mean density of *P. polyphylla* was lowest at 0.45 (pl/m2) with an IVI of 2.68. In contrast, the Tholung forest area recorded the highest density of the plant at 3.81 (pl/m2) and the highest IVI (8.66). This IVI was followed by that in Barsey (6.53) which may be because both of those locations falls under protected areas. Despite showing the Medium and High 'Habitat Suitability' by the MaxEnt model run, the two lowest IVI recorded were by Uttaray (2.68) followed by Pangthang (2.83). Present average population density of *P. polyphylla* was found similar to the reported density (1.78 individual m-2) from Nepal

(Madhu et al. 2010) and higher than the population density reported from Arunachal Pradesh (1.07 individuals m-2) (Paul et al. 2015).

Soil nutrients like organic carbon, total nitrogen and available phosphorous of soil of *P. polyphylla's* habitat were analyzed. In terms of organic carbon, the amount was found slightly higher in Tholung (4.26± 0.29 %) and Lachung (4.08± 0.49 %) forest area as compared to the other sites. In general, in all the *Paris* habitats the soil was characterized by acidic pH (4.38- 5.70 %), high organic carbon (2.86-4.26%) and low soil temperature which ranges from (10.29-15.76$^0$C). Moreover, the available phosphorous was uniformly low in all the sites (Table 4.1.7). Finally, Total nitrogen was recorded highest in Lachung (0.58± 0.13%) whereas it was found lowest at Chungthang forest area (0.31± 0.08 %) (Lepcha et al.2019). Maps showing the distribution of *P. polyphylla* in different areas of Sikkim Himalaya is presented in Figure 4.4 (A-D). Figure 4.5 (A-D) presents the occurrence of *P. polyphylla* with its different indicator species.

**Table 4.1.5.** Percentage contributions of the predictor environmental variables to the MaxEnt model of *Paris polyphylla* Smith habitats in Sikkim Himalaya.

| **Sl. No.** | **Environmental Variables** | **% Contribution** |
|---|---|---|
| 1 | Annual mean temperature (ºC, Bio1) | 6.6 |
| **2** | Mean diurnal temperature range<br>[mean of monthly (max temp – min temp)] (ºC, Bio2) | 17.1 |
| 3 | Precipitation of driest month (mm, Bio14) | 41.9 |
| 4 | Precipitation seasonality (CV) (Bio15) | 4.2 |
| 5 | Slope (%) | 30 |
| 6 | Aspect (º) | 0.2 |

**Table-4.1.6.** Ecological details of different *Paris polyphylla* Smith habitats in Sikkim Himalaya.

**Note:** NS= North Sikkim, ES= East Sikkim, SS= South Sikkim, WS= West Sikkim.

| Site No. | Place of collection/ locality | Geographical coordinates | Probability value (0-1) | Habitat suitability | Quadrates of occurrence | Frequency (%) | Relative frequency | Density (pl/m2) | IVI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Chungthang (NS) | 27°37′131′′N 88°40′017′′E | 0.60 | High | 17 | 56 | 3.44 | 1.76 | 5.21 |
| 2 | Lingthem (NS) | 27°31′515′′N 88°29′542′′E | 0.46 | Medium | 19 | 63 | 3.80 | 2.38 | 6.19 |
| 3 | Tholung forest area (NS) | 27°39′218′′N 88°27′435′′E | 0.62 | High | 23 | 76 | 4.85 | 3.81 | 8.66 |
| 4 | Lachung (NS) | 27°42′450′′N 88°44′573′′E | 0.72 | Very high | 14 | 46 | 3.57 | 1.67 | 5.24 |
| 5 | Pangthang area (ES) | 27°22′222′′N 88°36′201′′E | 0.54 | Medium | 11 | 36 | 2.14 | 0.69 | 2.83 |
| 6 | Zuluk valley (ES) | 27°15′153′′N 88°46′367′′E | 0.47 | Medium | 13 | 43 | 3.18 | 1.51 | 4.69 |
| 7 | Ravangla forest area (SS) | 27°18′410′′N 88°21′508′′E | 0.73 | Very high | 16 | 53 | 3.22 | 1.48 | 4.71 |
| 8 | Uttaray (WS) | 27°16′115′′N 88°08′306′′E | 0.64 | High | 11 | 36 | 2.22 | 0.45 | 2.68 |
| 9 | Barsey *Rhododendron* sanctuary (WS) | 27°12′625′′N 88° 08′470′′E | 0.62 | High | 22 | 73 | 4.36 | 2.16 | 6.53 |

**Table-4.1.7.** Physio-chemical properties of soil collected from different sites of *Paris polyphylla* Smith occurrence in Sikkim Himalaya. (values are mean ± SE, n=3).

| Site No. | Place of collection/ locality | Altitude (m) | Soil pH | Soil moisture (%) | Soil temperature ($^0$C) | Organic carbon (%) | Total nitrogen (%) | Available phosphorous (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Chungthang (NS) | 1800-1900 | 4.83± 0.58 | 30.79± 1.91 | 13.96± 0.54 | 3.30± 0.30 | 0.31± 0.08 | 0.04± 0.01 |
| 2 | Lingthem (NS) | 1500-1700 | 5.00± 0.94 | 30.40± 3.37 | 15.76± 3.06 | 3.55± 0.31 | 0.49± 0.13 | 0.06± 0.04 |
| 3 | Tholung forest area (NS) | 2500-2900 | 4.48± 0.14 | 40.40± 5.42 | 11.77± 1.20 | 4.26± 0.29 | 0.51± 0.23 | 0.05± 0.02 |
| 4 | Lachung (NS) | 2800-3100 | 5.00± 0.16 | 28.68± 5.89 | 10.29± 0.19 | 4.08± 0.49 | 0.58± 0.13 | 0.04± 0.02 |
| 5 | Pangthang area (ES) | 1800-1900 | 5.70± 0.58 | 33.87± 3.25 | 14.21± 0.81 | 3.79± 0.11 | 0.34± 0.12 | 0.04± 0.04 |
| 6 | Zuluk valley (ES) | 2500-2600 | 4.38± 0.50 | 30.91± 2.88 | 10.84± 0.35 | 3.05± 0.34 | 0.51± 0.10 | 0.03± 0.02 |
| 7 | Ravangla forest area (SS) | 1800-1900 | 5.26± 0.24 | 42.35± 1.27 | 13.02± 0.73 | 3.40± 0.60 | 0.31± 0.10 | 0.05± 0.02 |
| 8 | Uttaray (WS) | 1800-2000 | 5.51± 0.55 | 32.81± 4.93 | 14.70± 0.05 | 2.86± 0.31 | 0.37± 0.09 | 0.06± 0.02 |
| 9 | Barsey Rhododendron sanctuary (WS) | 1800-1900 | 4.99± 0.42 | 31.37± 3.17 | 13.04± 0.73 | 3.18± 0.06 | 0.46± 0.13 | 0.05± 0.03 |

**Note:** NS= North Sikkim, ES= East Sikkim, SS= South Sikkim, WS= West Sikkim.

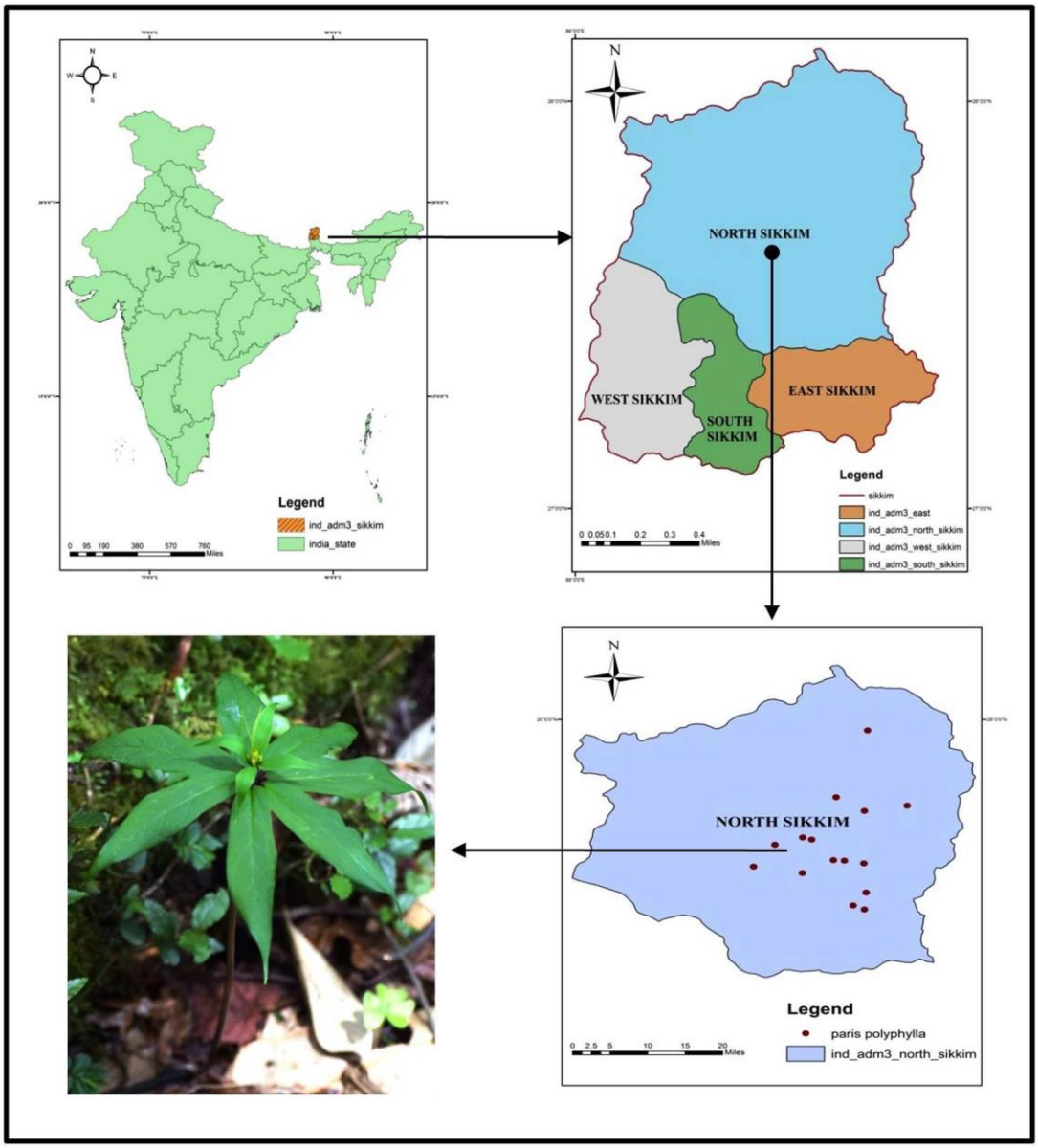



**Figure 4.4 (A).** Distribution of *P. polyphylla* in North Sikkim; Dots represents different areas viz., Kabi, Phensang, Chawang, Namok, Lingthem, Tholung, Suffo, Naga, Chungthang, Lachen and Lachung.

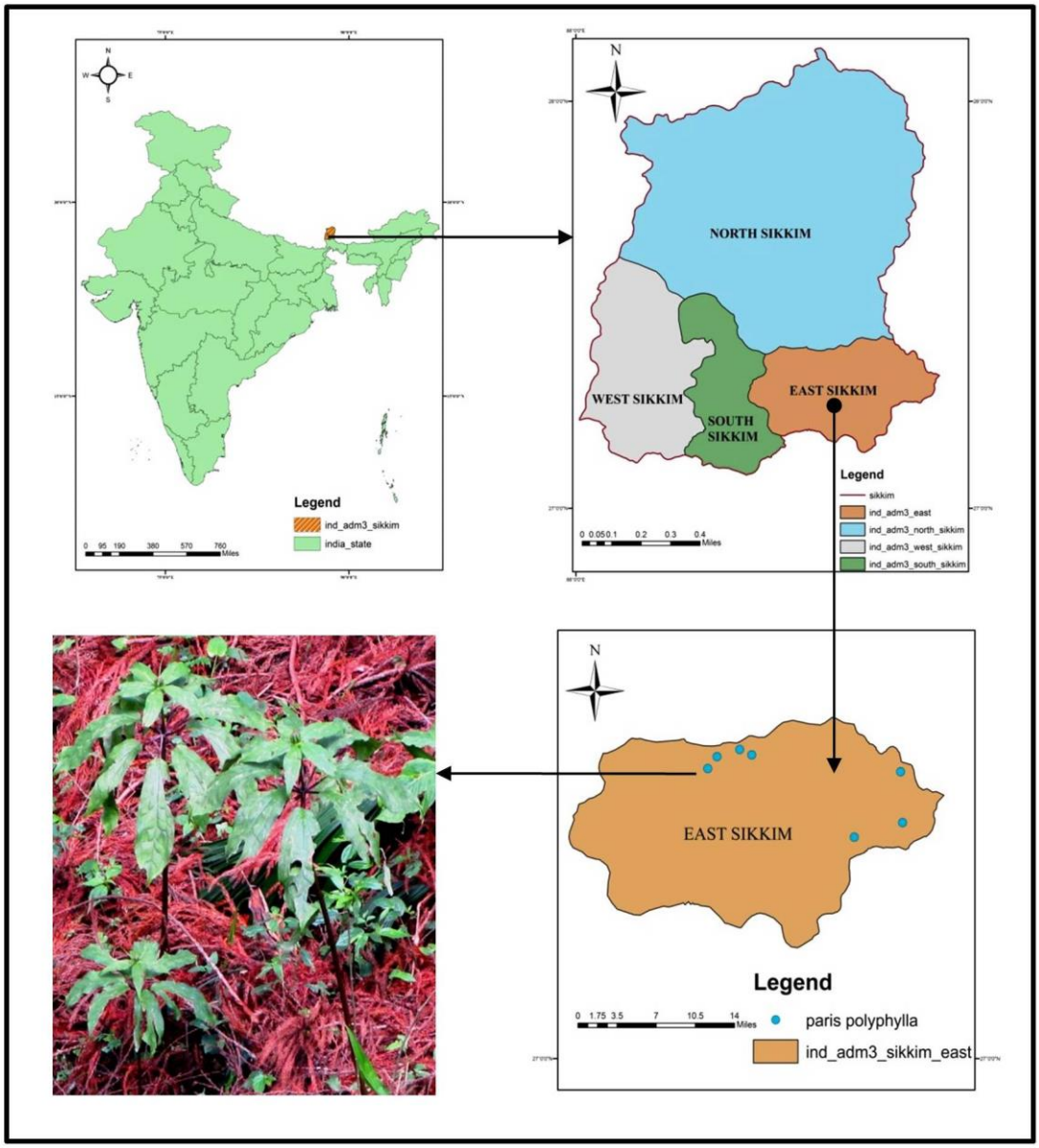



**Figure 4.4 (B).** Distribution of *P. polyphylla* in East Sikkim; Dots represent different areas viz., Pangthang, Fambonglho wildlife sanctuary, on the way to Changu, Pangalokha wildlife sanctuary, and Zuluk area.

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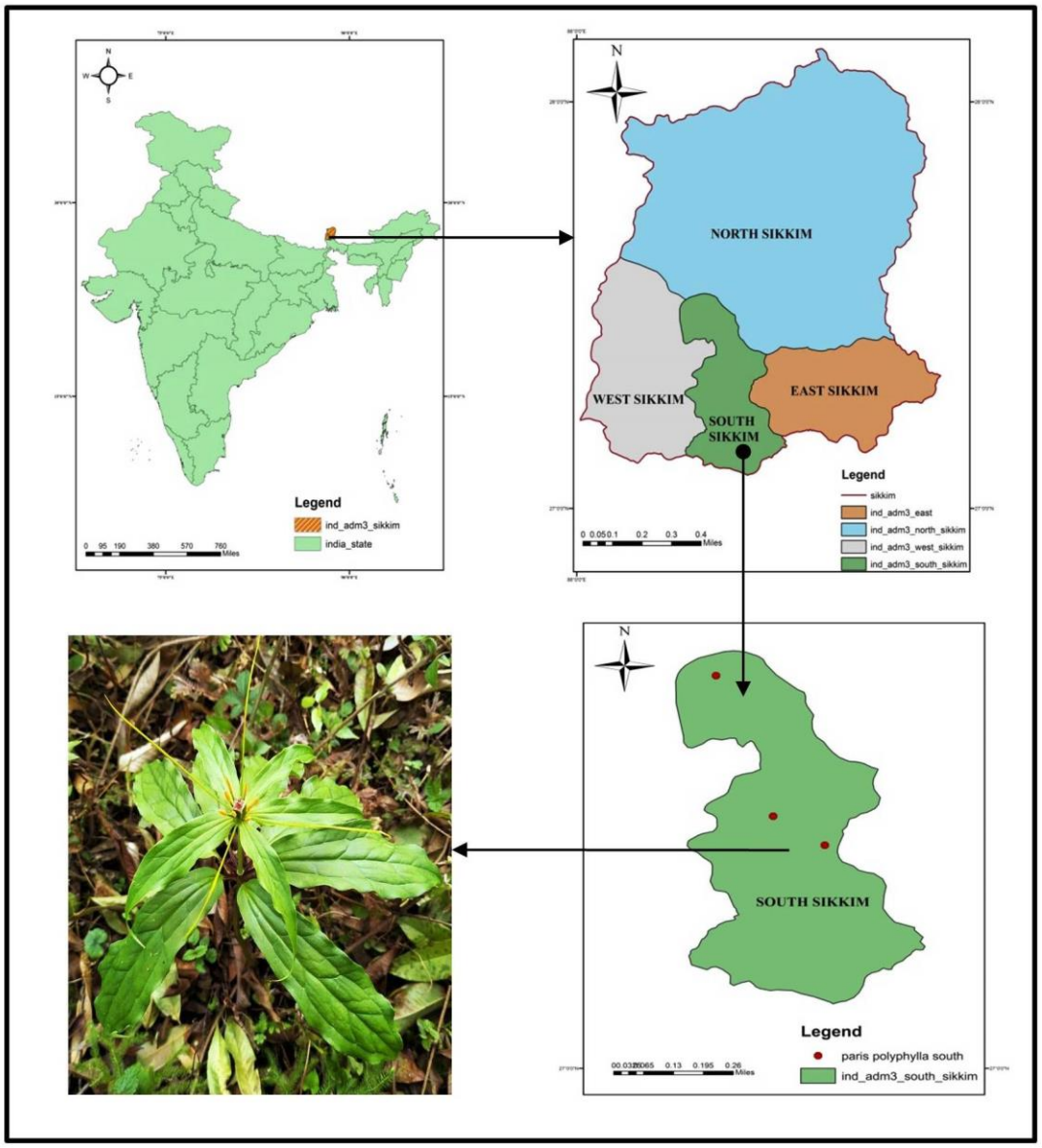



**Figure 4. 4 (C).** Distribution of *P. polyphylla* in South Sikkim; Dots represent different areas viz., Ravangla, Tendong Forest Reserve and Meanam Wildlife Sanctuary.

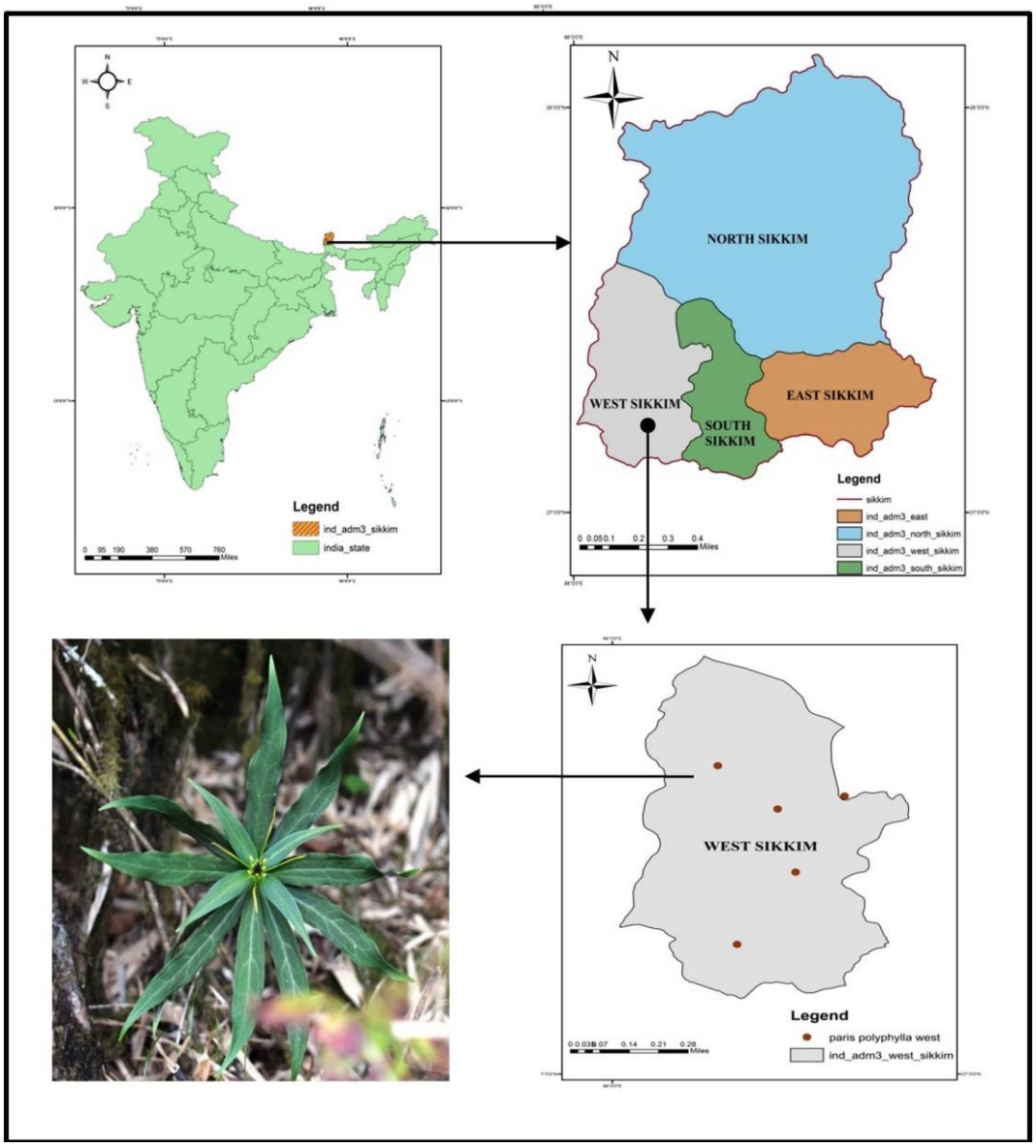



**Figure 4. 4 (D)**. Distribution of *P. polyphylla* in West Sikkim; Dots represent different areas viz., Uttaray, Barsey Rhododendron sanctuary, Dzongri, Bakhim and Yuksom.

The IVI analysis of *P. polyphylla* and 30 other closely associated plants was carried during the survey (Table 4.1.8.) Among all these species the top three dominant associated species with maximum IVI. includes *Fragaria nubicola* (Density;13.54 pl/m$^2$ and IVI:18.14), *Pteris* sp. (Density; 4.44 pl/m$^2$ and I.V.I;8.45) and *Sarcococca coriaceae* (Density; 10.03 pl/m$^2$ and IVI: 14.21) (Figure 4.5 (A-D). And among thirty selected species the least contributor was recorded with *Viburnum erubescens* (Density; 0.73 pl/m$^2$ and I.V.I; 3.90) and *Artemisia vulgaris* (Density; 0.88 pl/m$^2$ and IVI: 3.57).

**Table-4.1.8.** List of associated plant species with *Paris polyphylla* with their ecological parameters.

| Sl. No. | Associate species | Quadrates of occurrence | Frequency (%) | Relative frequency | Density (pl/m2) | IVI |
|---|---|---|---|---|---|---|
| 1 | *Aconogonum molle* | 13 | 45 | 2.89 | 2.47 | 5.37 |
| 2 | *Arisaema griffithii* | 13 | 45 | 2.89 | 2.47 | 5.37 |
| 3 | *Arisema ciliatum* | 17 | 58 | 3.72 | 1.44 | 5.17 |
| 4 | *Aresima costatum* | 24 | 80 | 4.80 | 3.43 | 8.24 |
| 5 | *Artemisia vulgaris* | 13 | 43 | 2.68 | 0.88 | 3.57 |
| 6 | *Cantella* sp. | 19 | 65 | 4.02 | 8.97 | 12.99 |
| 7 | *Commelina* sp. | 19 | 66 | 4.13 | 2.88 | 7.01 |
| 8 | *Crassocephalum crepidioides* | 19 | 65 | 3.90 | 1.70 | 5.60 |
| 9 | *Cyperus cyperoides* | 20 | 68 | 4.13 | 1.86 | 6.02 |
| 10 | *Deparia boryana* | 19 | 65 | 4.02 | 2.63 | 6.66 |
| 11 | *Drymeria cordata* | 19 | 65 | 4.14 | 8.33 | 12.47 |
| 12 | *Elatostema umbellatum* | 19 | 64 | 4.12 | 5.34 | 9.46 |
| 13 | *Fragaria nubicola* | 21 | 72 | 4.59 | 13.54 | 18.14 |
| 14 | *Girardinia diversifolia* | 20 | 67 | 4.19 | 2.18 | 6.38 |
| 15 | *Impatiens* sp. | 22 | 73 | 4.67 | 3.11 | 7.79 |
| 16 | *Panax bipinnatifidus* | 13 | 43 | 3.02 | 1.67 | 4.70 |
| 17 | *Plantago asiatica* | 15 | 51 | 3.63 | 1.88 | 5.52 |
| 18 | *Polygonatum varticillutum* | 14 | 47 | 3.40 | 2.79 | 6.20 |
| 19 | *Polygonum hydropiper* | 19 | 63 | 4.04 | 8.09 | 12.14 |
| 20 | *Pouzolzia hirta* | 20 | 69 | 4.36 | 3.30 | 7.66 |
| 21 | *Pteris* sp. | 19 | 63 | 4.02 | 4.44 | 8.45 |
| 22 | *Rubia manjith* | 19 | 65 | 4.14 | 1.86 | 6.12 |
| 23 | *Rubus ellipticus* | 16 | 56 | 3.62 | 1.19 | 4.82 |
| 24 | *Rumex nepalensis* | 18 | 60 | 4.27 | 3.15 | 7.42 |
| 25 | *Sarcococca coriaceae* | 17 | 58 | 4.17 | 10.03 | 14.21 |
| 26 | *Selaginella* sp. | 21 | 71 | 4.58 | 10.01 | 14.60 |
| 27 | *Trillium govanianum* | 19 | 63 | 4.10 | 1.81 | 5.81 |
| 28 | *Trillium tschonoskiin* | 15 | 51 | 3.68 | 1.88 | 5.57 |
| 29 | *Urtica dioica* | 16 | 53 | 3.64 | 3.11 | 6.75 |
| 30 | *Viburnum erubescens* | 15 | 50 | 3.17 | 0.73 | 3.90 |
| 31 | *Viola* sp. | 15 | 52 | 3.24 | 1.17 | 4.41 |


**Figure 4. 5 (A-D).** Indicator species of *P.polyphylla* in the wild habitat. A) *Aresima cialatum* B) E*latostema lineolatum* C) *Mohonia nepalensis* and D) *Sarcococca coriaceae.*

### 4.5. Fluorescence characteristics of *Paris polyphylla*

The fluorescence characteristics of *P. polyphylla* from Uttaray (PPU) rhizome powder were studied under ultraviolet radiation (254 nm and 365 nm) and visible radiations after treating with different chemical reagents. The powdered drugs showed important characteristic behaviour with different chemical reagents, and the detailed result is presented in the Table 4.1.9. Similarly, the powder prepared from dry rhizomes of the plant from Tholung (PPT) was observed. The powdered drugs showed important but somewhat different characteristic behaviour with respect to treatments with different chemical reagents (Table 4.1.10).

### 4.6. Qualitative phytochemical analysis of *P. polyphylla*

The preliminary phytochemical analysis of methanolic extracts of *P. polyphylla* leaf and rhizome samples collected from Uttaray (PPU) is presented in (Table 4.1.11.). The extracts revealed the presence of various bioactive components like alkaloids, flavonoids, tannins, phenolics, phlobatanins, saponins, resins, amino acids, carbohydrates and proteins. The samples from Tholung (PPT) also revealed identical bioactive components (Table 4.1.12.). All the data recorded from leaf and rhizomes collected from plants after 220 days of field emergence.

### 4.7. Biologically active compounds isolated through GC-MS analysis

The methanolic extract GC-MS chromatogram of *P. polyphylla* from both the altitudinal ranges viz. PPU and PPT displays around 47 peaks indicating the presence of at least forty-seven selected vital phytochemical constituents (Figure 4.6 and 4.7). On comparison of the mass spectra of the constituents with the NIST 11 library and Willey 8 library, these phytocompounds were categorized and acknowledged as given in Table 4.1.13 and 4.1.14 respectively. The photocompositions of the methanolic rhizome powder extract reveals that this plant possesses a numbers of valuable compounds which are useful for the treatment of numerous health complications such as Parkinson's disease, tumour formation, anticancer, antidote, fertility problems, to increase immunity etc. and some of the compounds are also useful for flavouring beverages, personal care goods and household products. The result also shows that it has an insect repellent property and also controls soil-born insects in agriculture. During the study 4 novel compounds were found in PPH while 6 new compounds were reported from PPT. Interestingly, none of the novel compounds were common between the PPU and PPT.

**Table-4.1.9.** Fluorescence nature of *Paris polyphylla* Smith rhizome powder under ultra violet (UV) and visible radiations. Data recorded from the powder prepared from dry rhizomes of the plant from Uttaray in Sikkim Himalaya.

| **Treatments** | **Fluorescence colour** | | |
|---|---|---|---|
| | **Long UV (365 nm)** | **Short UV (254 nm)** | **Visible** |
| **Blank** | Light green | Creamish white | Greenish white |
| **Mayer's** | Violet | Creamish white | Violet |
| **Hager's** | Dark green | Light green | Dull green |
| **Dragendorff** | Black | Dark green | Black |
| **Iodine sol.** | Blackish | Dark olive green | Greyish |
| **1 (N) $HNO_3$** | Dark brown | Light olive green | Light green |
| **50 % $HNO_3$** | Dark brown | Light olive green | Light green |
| **Phloroglucinol** | Greenish + dark | Light olive green | Dark yellowish |
| **Barfoed's** | Black | Dull green | Yellowish |
| **Sodium Nitroprusside** | Blackish | Light olive green | Light yellowish brown |
| **Ninhydrin** | Violet | Light green | White |
| **$FeCl_3$** | Dark Green | Light green | yellowish green |
| **1 (N) NaOH** | Greenish brown | Light green | Light yellow |
| **Acetic Acid** | Light brown | Greenish | Greenish grey |
| **1 (N) HCl** | Blackish | Dull green | Greyish |
| **Methanol** | Violet | Grey | Grey |
| **1 (N) NaOH in Methanol** | Brown | Light dull green | White |

**Table-4.1.10.** Fluorescence nature of *Paris polyphylla* Smith rhizome powder under ultra violet (UV) and visible radiations. Data recorded from the powder prepared from dry rhizomes of the plant from Tholung in Sikkim Himalaya.

| **Treatments** | **Fluorescence colour** | | |
|---|---|---|---|
| | **Long UV (365 nm)** | **Short UV (254 nm)** | **Visible** |
| **Blank** | Brown | Light brown | Creamish |
| **Mayer's** | Violet | Creamish white | Creamish |
| **Hager's** | Dull Brown | Light green | Light brown |
| **Dragendorff** | Black | Black | Brown |
| **Iodine sol.** | Dull brown | Light green | Greyish |
| **1 (N) $HNO_3$** | Dark brown | Light olive green | Dull + yellowish |
| **50 % $HNO_3$** | Light brown | Light green | White |
| **Phloroglucinol** | Brown | Light green | Light yellow |
| **Barfoed's** | Dull green | Dark+ dull green | Dull green |
| **Sodium Nitroprusside** | Dull brown | Dull grey | Cinnamon |
| **Ninhydrin** | Dull brown | Greyish yellow | Greenish |
| **$FeCl_3$** | Dull greenish brown | Yellowish green | Yellowish green |
| **1 (N) NaOH** | Greyish green | Light green | Light brown |
| **Acetic Acid** | Dull grey | Greenish grey | Greenish yellow |
| **1 (N) HCl** | Dull brown | Greenish grey | Light brown |
| **Methanol** | Dull-yellow + Brown | Light yellow | Brown |
| **1 (N) NaOH in Methanol** | Dull grey | Greenish yellow | Light yellow |

**Table-4.1.11.** Preliminary screening analysis of *Paris polyphylla* from Uttaray in Sikkim Himalaya for the qualitative determination of different phytochemical constituents. Data recorded from leaves and rhizomes collected from plants after 220 days of field emergence respectively.

| Phytoconstituents | Type of test performed | Intensity of visual change | |
|---|---|---|---|
| | | Leaf extract | Rhizome extract |
| **Alkaloids** | Wagner's test | + | ++ |
| **Flavonoids** | Alkaline reagent test | + | + |
| **Tannins** | Ferric chloride test | + | + |
| **Phlobatanins** | Hydrochloric acid test | ++ | + |
| **Triterpenes** | Sulphuric acid test | + | - |
| **Steroids** | Liebermann-Buchard test | + | + |
| **Saponins** | Frothing test | + | +++ |
| **Glycosides** | Legal's test | - | + |
| **Cardiac glycosides** | Keller-killiani test | + | + |
| **Anthraquinones** | Ammonia test | ++ | - |
| **Resins** | Copper acetate test | + | + |
| **Amino acids** | Ninhydrin test | ++ | + |
| **Proteins** | Biuret test | ++ | ++ |
| **Carbohydrates** | Molisch's test | + | + |

**Table-4.1.12.** Preliminary screening analysis of *Paris polyphylla* from Tholung in Sikkim Himalaya for the qualitative determination of different phytochemical constituents. Data recorded from leaves and rhizomes collected from plants after 220 days of field emergence respectively.

| Phytoconstituents | Type of test performed | Intensity of visual change | |
|---|---|---|---|
| | | Leaf extract | Rhizome extract |
| **Alkaloids** | Wagner's test | + | ++ |
| **Flavonoids** | Alkaline reagent test | + | + |
| **Tannins** | Ferric chloride test | + | + |
| **Phlobatanins** | Hydrochloric acid test | ++ | + |
| **Triterpenes** | Sulphuric acid test | + | - |
| **Steroids** | Liebermann-Buchard test | + | + |
| **Saponins** | Frothing test | + | +++ |
| **Glycosides** | Legal's test | - | + |
| **Cardiac glycosides** | Keller-killiani test | + | + |
| **Anthraquinones** | Ammonia test | ++ | - |
| **Resins** | Copper acetate test | + | + |
| **Amino acids** | Ninhydrin test | ++ | + |
| **Proteins** | Biuret test | ++ | ++ |
| **Carbohydrates** | Molisch's test | + | + |

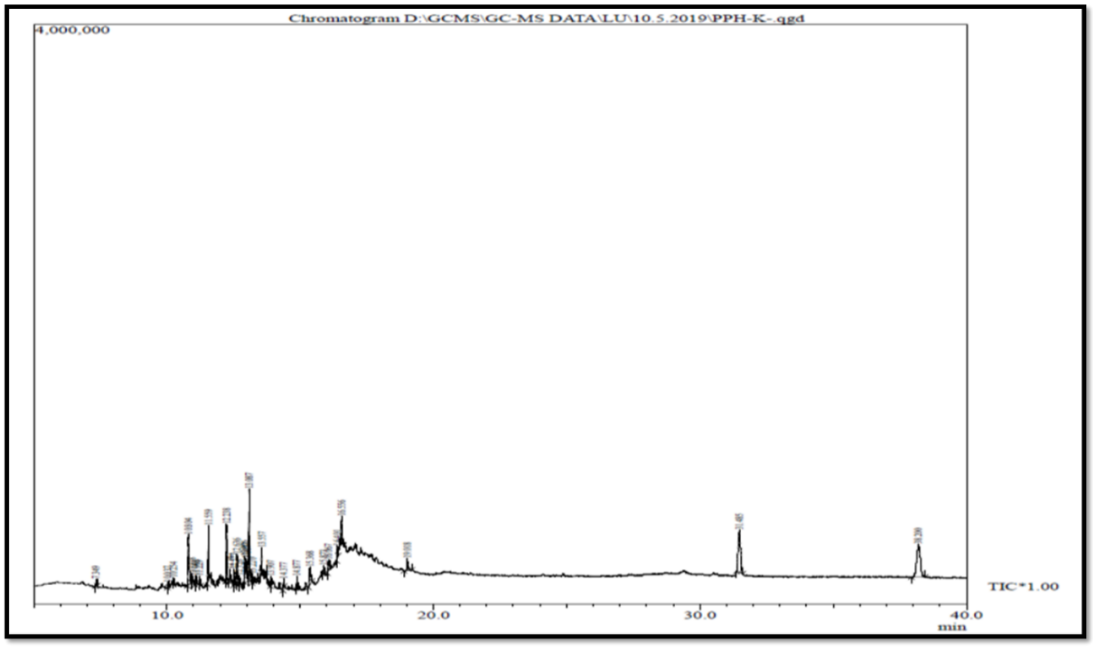



**Figure 4.6.** GC-MS chromatogram of methanolic extract of *P. polyphylla* Tholung (PPT) from Sikkim Himalaya.

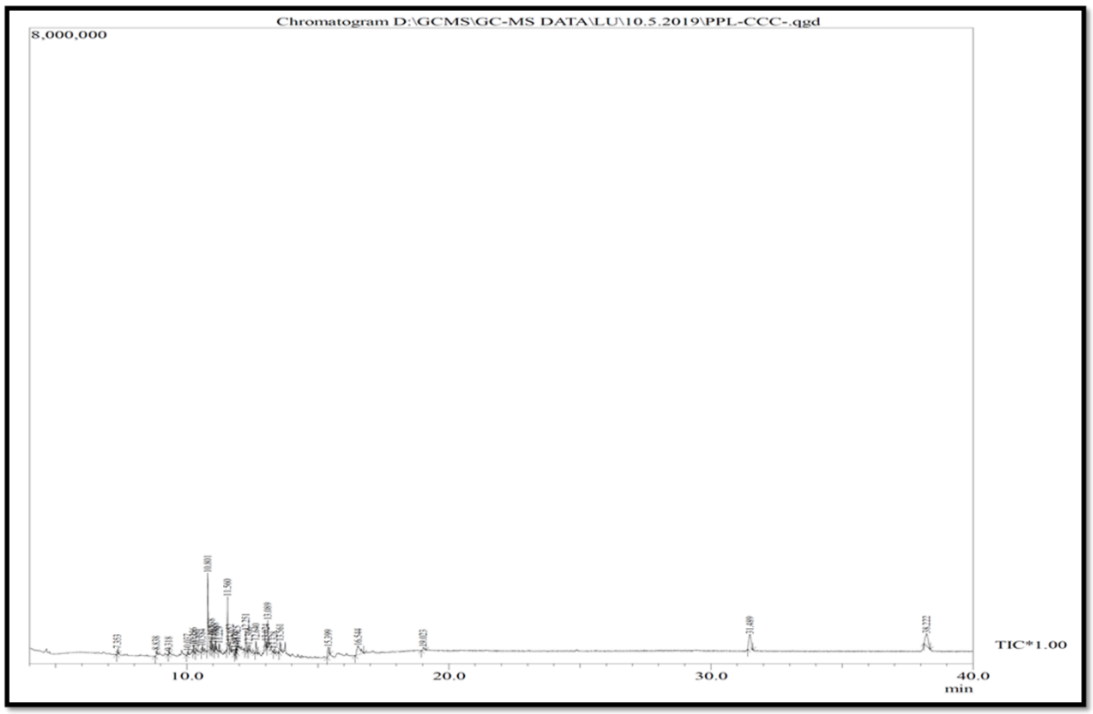



**Figure 4.7.** GC-MS chromatogram of methanolic extract of *P. polyphylla* Uttaray (PPU) from Sikkim Himalaya.

Amongst the whole compounds, Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite was found to cover highest area percentage with (31.49 %), followed by 1,7,7-Trimethyl-bicyclo [2.2.1] heptan-2-ol (17.58 %), Phytol (16.39 %), Chlorpyrifos (15.82 %) and least area percentage withE-11(12Cyclopropyl)do-decen-1-olacetate(0.15%).

**Table-4.1.13.** Major biologically active compounds present in *P. polyphylla* Smith from Uttaray in North Sikkim as per GC-MS analysis.

| **Peak** | **Area (%)** | **Compound** | **Activity** |
|---|---|---|---|
| 1 | 0.59 | Cyclohexene, 1-Methyl-4-(1-Methylethenyl) | Cyclohexane has also been shown to be a beta-oxidant. It has been revealed to have antibiotic effects and also act as a pain reliver. It is commonly used as a dietary supplement, fragrance ingredient for cosmetics products and also used as botanical insecticide and organic herbicide. It is also used in food manufacturing and medicines and other personal care products. |
| 8* | 2.57 | (9Z)-Octadec-9-enoic acid | It is used to induce lung damage in certain types of animals, for the purpose of testing new drugs and other means to treat lung diseases. Also used during the treatment of Parkinson's disease and helps to rise zinc bioavailability (Payum, 2018). |
| 9 | 5.07 | 2(3h)-Furanone, Dihydro-5-Pentyl | Used as an Antidote, Anti-HIV integrase, Haemoglobin inducer, Hepatoprotective and HIV-RT inhibitor (Payum, 2018). |
| 11* | 11.08 | Cyclohexanol | An important feedstock in the polymer industry as a precursor to nylons and minor amounts are used as a solvent. |
| 15 | 8.24 | Methyl palmitate | Used in the preparation of detergents, emulsifiers, resins, lubricants and animal feeds also exhibits an anti-inflammatory and anti-fibrotic agent (Tang et al, 2015) |
| 16* | 0.27 | Hydroxy-2 | Antidote, Hepatonic Hormone balancing and also helps to increase T-helper cells (Payum, 2018). |

| | | | |
|---|---|---|---|
| 18* | 1.58 | Decanoic Acid Ethyl Ester | Impede uric acid production, Arachidonic acid-inhibitor to stop tumour cell (Payum, 2018). |
| 26 | 0.95 | 1,E-6,Z-11-Hexadecatriene | Has anti-cancer and Antidote properties and it also increase zinc bioavailability (Payum, 2018). |
| 27 | 31.49 | Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite | Extensively used stabilizer in polymers where it functions as an antioxidant. The compound is a phosphite ester derived from di-tert-butylphenol. |
| 29 | 4.32 | 17-Hydroxy-4,4-Dimethyl estran-3-One | Helps to prevent breast, ovarian and endometrium cancers and prostate cancer too. |
| 32 | 5.78 | Undecylenic acid | An active constituent in medications for skin infections specifically to relieve itching, burning and irritation related with skin complications. Primarily used for the production of Nylon-11 and in the treatment of fungal infections of the skin but it is also a precursor in the manufacture of many pharmaceuticals, personal hygiene products, cosmetics and perfumes. |
| 40 | 16.39 | Phytol | Mainly used in the fragrance industry (cosmetics shampoos, toilet soaps, household cleaners, and detergents) and also used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K. It has shown antinociceptive, anti inflammatory, antioxidant activities as well as and antiallergic effects (Santos et al, 2013) |
| 41 | 0.15 | E-11(12 Cyclopropyl) do-decen-1-ol acetate | Decrease of C-teleopeptide excretion (to reduce risk of Peget bone disease), fertility enhancing, endocrine protective and Endothelium derived relaxing factor promoter (Payum, 2018). |
| 44 | 17.58 | 1,7,7-Trimethyl-bicyclo [2.2.1] heptan-2-ol | Used in Traditional Chinese Medicine (TCM). It is a component of many essential oils and act as a natural insect repellent. |
| 47 | 13.69 | 5-Hexyldihydro-2(3H)-furanone | Frequently used as a flavouring for beverages, personal care, pharmaceutical and household goods, as well as a food additive. |

* Unique compounds

**Table-4.1.14.** Major biologically active compounds present in *P. polyphylla* Smith from Tholung in North Sikkim as per GC-MS analysis.

| Peak | Area (%) | Compound | Activity |
|---|---|---|---|
| 1 | 0.59 | Cyclohexene, 1-Methyl-4-(1-Methylethenyl) | Cyclohexane has also been shown to be a beta-oxidant. It has been revealed to have antibiotic effects and also act as a pain reliver. It is commonly used as a dietary supplement, fragrance ingredient for cosmetics products and also used as botanical insecticide and organic herbicide. As the main fragrance of citrus peels, it is used in food manufacturing and medicines, such as a flavouring to mask the bitter taste of alkaloids and as a fragrance in perfumery, aftershave lotions, bath products and other personal care products. |
| 3* | 2.16 | Bicyclopropyl | Anti-cancer and Antidote properties, increase zinc bioavailability which is needed for the body's immune system, also helps fertility enhancing and endocrine protective. |
| 7* | 10.03 | Sulfurous acid | Used as reducing agents and as disinfectants. |
| 9 | 5.07 | 2(3h)-Furanone, Dihydro-5-Pentyl | Having an important property such as anti-HIV integrase, Antidote, Haemoglobin inducer, Hepatoprotective and HIV-RT inhibitor (Payum, 2018). |
| 14* | 13.17 | Diethyl phthalate | Used to bind cosmetics and fragrances and act as an insect repellent for mosquitoes and flies. But, it also possess some toxicity which may cause damage to the nervous system as well as to the reproductive organs of both male as well as females. |
| 15 | 8.24 | Methyl palmitate | Used in the preparation of detergents, emulsifiers, resins, lubricants and animal feeds also exhibits an anti-inflammatory and anti-fibrotic agent (Tang et al, 2015) |
| 17* | 2.36 | Butyl hydroxyl anisole | Used in the treatment of Parkinson's diseases (Payum, 2018). |

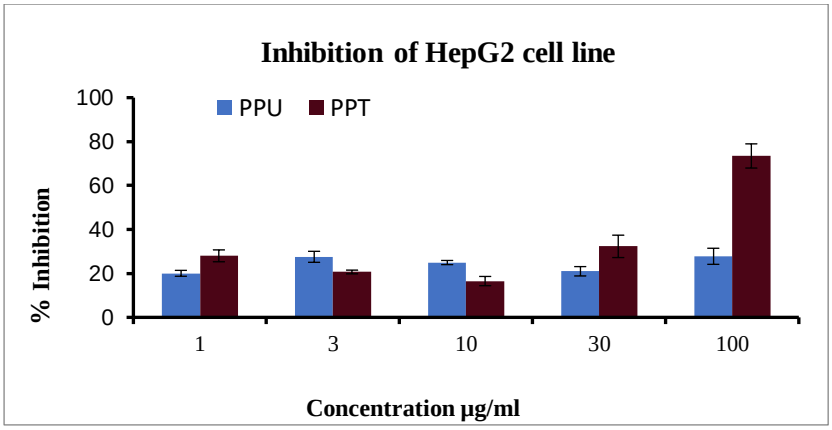
| | | | |
|---|---|---|---|
| 20 | 2.47 | Methyl ester | Used in the treatment of Parkinson's disease and helps to avoid renal failure. |
| 21 | 2.83 | 1,4-Dimethyl-3-(2-Methyl-1-Propenyl)-4-Vinyl | Helps to prevent breast, ovarian and endometrium cancers. |
| 22* | 15.82 | Chlorpyrifos | Used to control insects in agricultural (for control of soil-born insects). |
| 27 | 31.49 | Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite | Commonly used stabilizer in polymers where it functions as an antioxidant. |
| 25* | 16.06 | 1-Dodecanol | Used to make surfactants, lubricating oils, pharmaceuticals, in the formation of monolithic polymers and as a flavour enhancing food additive. In cosmetics, it is used as an emollient. |
| 29 | 4.32 | 17-Hydroxy-4,4-Dimethyl estran-3-One | Helps to prevent breast, ovarian and endometrium cancers and prostate cancer (Payum, 2018). |
| 30 | 8.23 | Palmitin, 2-mono | Extensively used in the treatment of depression and inhibitors of this enzyme may also find application in treatment of hypercholesterolemia (Belter et al. 2011). |
| 32 | 5.78 | Undecylenic acid | An active ingredient in medications for skin infections specially to relieve itching, burning, and irritation associated with skin problems. It is also a precursor in the manufacture of many pharmaceuticals, personal hygiene products, cosmetics and perfumes. |
| 36 | 0.95 | 1,E-6,Z-11-Hexadecatriene | Possess anti-cancer and Antidote properties, correspondingly increase zinc bioavailability (Payum, 2018). |
| 40 | 16.39 | Phytol | Mainly used in the fragrance industry (cosmetics, shampoos, toilet soaps, household cleaners, and detergents) and also used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K. It has shown antinociceptive, anti-inflammatory, antioxidant activities as well as and antiallergic effects (Santos et al, 2013) |
| 41 | 0.15 | E-11(12 Cyclopropyl) do-decen-1-ol acetate | It helps to reduce risk of Peget bone disease, fertility enhancing, endocrine protective and endothelium derived relaxing factor promoter (Payum, 2018). |

| | | | |
|---|---|---|---|
| 43 | 10.24 | Naphthalen-1-ol | Precursor to a variety of insecticides including carbaryl and pharmaceuticals including nadolol as well as for the antidepressant sertraline and the anti-protozoan therapeutic. |
| 43 | 17.58 | 1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-ol | It is a component of many essential oils and act as a natural insect repellent. |
| 47 | 13.69 | 5-Hexyldihydro-2(3H)-furanone | Frequently used as a flavouring for beverages, personal care, pharmaceutical and household goods, as well as a food additive. |

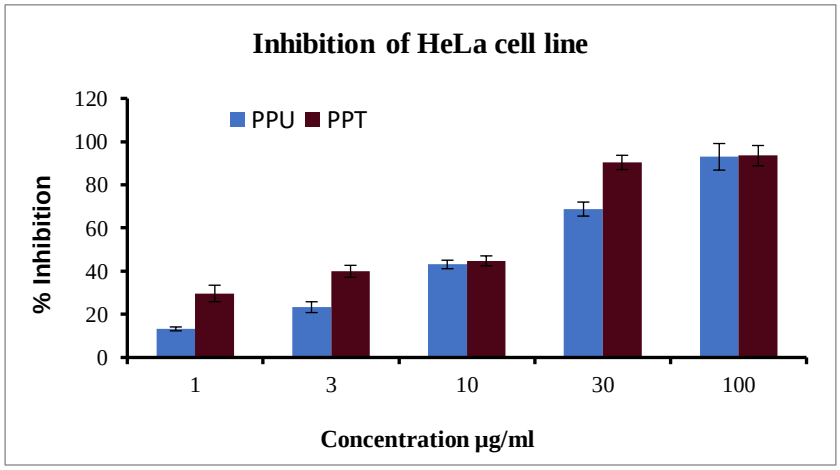
*Unique compounds

### 4.8. Inhibition of cell proliferation

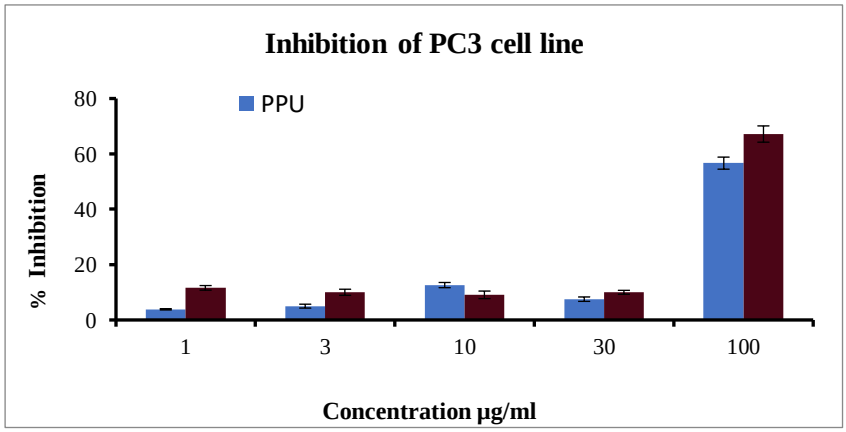
The effect of methanolic rhizome extract on the inhibition of cell growth was investigated by the MTT assay in a dose dependent manner. Among the three cancer cells lines studied, the effect of the methanolic extract had an extremely high dose-dependent inhibition of HeLa cell growth reaching >90% inhibition at 100 µg/ml concentration within 72 hrs of treatment. Both the extracts, PPT and PPU had a relatively moderate effect on HepG2 cells growth upto 30µg/ml concentration, however, PPT showed its significant inhibition (73.47%) at 100 µg/ml concentration. Similarly, PC3 cell line was resistant to both PPU and PPT upto a certain extent, but again both the extracts showed appreciable inhibition (>50%) at a high concentration of 100 µg/ml (Figure 4.7). All the anticancer activities of methanolic extracts were significantly higher than those of control, suggesting the role of extracts in enhancing these attributes (Lepcha et al. 2019).




**A**




**B**




**C**

**Figure 4. 8.** Percentage inhibition in growth of: A) HepG2, B) HeLa and C) PC3 cancer cell lines in presence of different concentration of methanolic rhizome extracts of PPU and PPT as determined by MTT assay. Values were expressed as Mean ± SE of quadruplicate independent observations. The % inhibition was calculated as percent difference between growth in DMSO (control) and growth in presence of extracts at 72 h of incubation.

### 4.9. Analysis of Physiological and Biochemical changes

#### 4.9.1 Effect of PGRs on different growth parameters

The difference in Relative growth index (RGI) of *P. polyphylla* plant samples from Uttaray (PPU) analysed. Between different treatments against control (no spraying) on plants by plant growth regulators and growth retardants after 120 days after emergence (DAE) were observed (Table 4.2.1). The maximum RGI were recorded in treatment $CCC^{100}$ (93.98 %) data followed by $IAA^{100}$ (87.95%) whereas the minimum RGI were recorded in treatment $GA3^{100}$ (48.97%) and $CCC^{50}$ (57.83%) in PPU. The maximum RGI for the samples of Tholung (PPT) were recorded in treatment $MH^{50}$ (86.15%) data followed by $IAA^{100}$ (81.54%) whereas the minimum RGI were recorded in treatment $CCC^{50}$ (52.31%) and $KIN^{100}$ (53.85%) (Table 4.2.2). The data were recorded from 10 uniformly grown plants raised from seeds at Lingthem, Dzongu.

#### 4.9.2. Effect of PGRs on Relative Water Content (%)

The Relative Water Content (RWC) of the leaves *P. polyphylla* Uttaray (PPU) was recorded maximum with 93.65 % against control 91.11 % and minimum of 54.66 % from the sample treated with $ABA^{25}$ (90 DAE) and $MH^{50}$ (60 DAE) respectively. Likewise, the leaf samples from *P. polyphylla* Tholung (PPT) was recorded maximum with 89.11 (%) and minimum of 66.58 (%) from the sample treated with $IAA^{100}$ (90 DAE) and $CCC^{50}$ 120 (DAE) respectively. Overall results are presented in Table 4.2.3.

#### 4.9.3. Effect of PGRs on Membrane Lipid Peroxidation

The Membrane Lipid Peroxidation (MLP) of the leaves *P. polyphylla* Uttaray (PPU) was recorded maximum with 0.063±0.04 n mole/g DM against control 0.042±0.03 n mole/g DM and minimum of 0.012±0.01 n mole/g DM against 0.047±0.02 n mole/g

DM (control) from the sample treated with $MH^{100}$ (120 DAE) and $GA3^{100}$ (90 DAE) respectively. Similarly, the leaf samples from *P. polyphylla* Tholung (PPT) recorded maximum membrane lipid peroxidation with 0.064±0.04 n mole/g DM and minimum of 0.042±0.01 n mole/g DM against 0.051±0.01 n mole/g DM (control) from the sample treatment of $IAA^{100}$ (60 DAE) and $MH^{50}$ 60 (DAE) respectively. Overall results are presented in Table 4.2.4. This suggests a significant variability of Membrane Lipid Peroxidation at 0.05 level of significance between the PPT and PPU after PGR treatment.

**Table-4.2.1** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on different growth parameters of *Paris polyphylla* Smith from Uttaray (PPU) in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from seeds at Lingthem, Dzongu, North Sikkim 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Shoot length (cm) | Stem circumference (cm) | Root length (cm) | Relative growth index (RGI) |
|---|---|---|---|---|
| **Control** | 4.8±0.23 | 0.6±0.08 | 7.5±0.01 | --- |
| **$IAA^{100}$** | 5.2±0.45 | 0.8±0.09 | 6.2±0.06 | 87.95 |
| **$IAA^{200}$** | 6.5±0.21 | 0.9±0.04 | 5.2±0.33 | 67.47 |
| **$GA_3^{100}$** | 5.3±0.13 | 0.8±0.11 | 8.4±0.14 | 48.97 |
| **$GA_3^{200}$** | 7.5±0.03 | 1.1±0.10 | 9.3±0.20 | 78.31 |
| **$KIN^{100}$** | 6.2±0.18 | 0.7±0.13 | 7.1±0.51 | 77.11 |
| **$KIN^{200}$** | 6.6±0.25 | 0.6±0.12 | 8.1±0.22 | 80.72 |
| **$MH^{50}$** | 4.3±0.26 | 0.8±0.02 | 7.5±0.25 | 83.13 |
| **$MH^{100}$** | 4.1±0.01 | 0.7±0.12 | 7.5±0.12 | 69.88 |
| **$CCC^{50}$** | 4.0±0.11 | 0.8±0.13 | 6.2±0.01 | 57.83 |
| **$CCC^{100}$** | 3.9±0.15 | 0.7±0.25 | 5.8±0.28 | 93.98 |
| **$ABA^{25}$** | 4.2±0.18 | 0.6±0.10 | 5.9±0.17 | 79.52 |
| **$ABA^{50}$** | 3.8±0.11 | 0.7±0.24 | 5.5±0.08 | 85.54 |

**Table-4.2.2** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on different growth parameters of *Paris polyphylla* Smith from Tholung (PPT) in Sikkim Himalaya. Data recorded from 10 uniformly grown plants raised from seeds at Lingthem, Dzongu, North Sikkim after 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Shoot length (cm) | Stem circumference (cm) | Root length (cm) | Relative growth index (RGI) |
|---|---|---|---|---|
| **Control** | 4.0±0.02 | 0.7±0.03 | 6.9±0.06 | --- |
| **$IAA^{100}$** | 5.2±0.11 | 0.5±0.01 | 7.2±0.05 | 81.54 |
| **$IAA^{200}$** | 6.5±0.13 | 0.5±0.12 | 6.3±0.07 | 61.54 |
| **$GA_3^{100}$** | 5.3±0.17 | 0.6±0.21 | 6.8±0.01 | 56.92 |
| **$GA_3^{200}$** | 6.5±0.22 | 0.8±0.55 | 7.9±0.06 | 76.92 |
| **$KIN^{100}$** | 5.2±0.15 | 0.7±0.21 | 6.2±0.08 | 53.85 |
| **$KIN^{200}$** | 4.5±0.34 | 0.6±0.01 | 6.0±0.15 | 63.08 |
| **$MH^{50}$** | 4.0±0.03 | 0.8±0.06 | 6.5±0.03 | 86.15 |
| **$MH^{100}$** | 3.7±0.15 | 0.7±0.05 | 5.8±0.16 | 73.85 |
| **$CCC^{50}$** | 3.9±0.26 | 0.5±0.22 | 6.4±0.38 | 52.31 |
| **$CCC^{100}$** | 3.8±0.88 | 0.9±0.07 | 5.4±0.40 | 76.92 |
| **$ABA^{25}$** | 3.6±0.14 | 0.8±0.03 | 5.3±0.18 | 67.69 |
| **$ABA^{50}$** | 3.3±0.15 | 0.6±0.01 | 4.9±0.29 | 70.77 |

### 4.9.4. Effect of PGRs on Chlorophyll-a and Chlorophyll-b Content

In PPU the maximum of chlorophyll-a content from the leaves was recorded at 0.189±0.12 mg/g FW against the control value of 0.149±0.02 and the minimum at 0.046±0.02 mg/g FW against 0.085±0.04 mg/g FW (control) from the sample treated with $Kin^{200}$ (60 DAE) and $CCC^{50}$ (90 DAE) respectively. Likewise, chlorophyll-b content of the leaves was recorded maximum with 0.965±0.35 mg/g FW and minimum of 0.652±0.05 mg/g FW against 0.784±0.14 mg/g FW (control) from the sample treatment of $CCC^{100}$ (120 DAE) and $KIN^{100}$ (120 DAE) respectively (Table

4.2.5). Similarly, the chlorophyll-a content of the leaves from PPT was recorded maximum with 0.521±0.11 mg/g FW and minimum of 0.074±0.05 mg/g FW against 0.112±0.07 mg/g FW (control) from the sample treated with $CCC^{100}$ (120 DAE) and $IAA^{100}$ (120 DAE) respectively. The chlorophyll-b content of the leaves in this case was recorded maximum with 0.981±0.43 mg/g FW and minimum with 0.659±0.22 mg/g FW against 0.914±0.25 mg/g FW (control) from the sample treated with $IAA^{100}$ (60 DAE) and $CCC^{50}$ (60 DAE) respectively (Table 4.2.6). This result suggested the significant variability of chlorophyll-a content between the PPT and PPU after treatment. However, there was no significant variability observed for chlorophyll-b content between the samples from the two places.

**4.9.5. Effect of PGRs on Carotenoids Content**

The carotenoids content of the leaf samples from Uttaray (PPU) was recorded maximum with 189.23±0.28 mg/g FW and minimum of 124.22±1.03 mg/g FW against 139.23±0.23 mg/g FW (control) from the sample treated with $ABA^{25}$ (60 DAE) and $IAA^{200}$ (60 DAE) respectively. In the same way, the Carotenoids content of the leaf samples from Tholung (PPT) was recorded maximum with 168.42±1.42 mg/g FW against control 154.25±1.48 mg/g FW and minimum of 122.15±0.56 mg/g FW against 128.14±0.36 mg/g FW (control) from the sample treatment of $GA_3^{200}$ (90 DAE) and $MH^{100}$ (60 DAE) respectively. The results are presented in Table 4.2.7.

**4.9.6. Effect of PGRs on Free Amino Acid Content**

The Free Amino Acid (FAA) content of the leaves of PPU was recorded maximum with 0.456±0.11 mg/g FW against control 0.148±0.04 mg/g FW and minimum of 0.114±0.01 mg/g FW against 0.123±0.09 mg/g FW (control) from the sample treated with $MH^{50}$ (120 DAE) and 60 (DAE) respectively. Similarly, for rhizome samples the maximum was recorded with 0.157±0.06 against control 0.052±0.03 mg/g FW

**Table-4.2.3** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Relative Water Content (%) of *Paris polyphylla* from Uttaray (PPU) and Tholung (PPT) in Sikkim Himalaya. Data recorded from 60, 90 and 120days old leaves collected from 5 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim.

| Treatments (µg/ml) | Relative Water Content (%) | | | | | |
|---|---|---|---|---|---|---|
| | PPU | | | PPT | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 90.11 | 91.12 | 82.89 | 93.39 | 94.23 | 84.63 |
| **$IAA^{100}$** | 88.51 | 85.63 | 78.32 | 88.32 | 89.11 | 76.33 |
| **$IAA^{200}$** | 83.48 | 84.63 | 82.36 | 84.21 | 76.23 | 68.78 |
| **$GA_3^{100}$** | 87.23 | 86.23 | 84.32 | 86.06 | 88.22 | 75.45 |
| **$GA_3^{200}$** | 82.11 | 83.61 | 78.23 | 78.60 | 81.20 | 74.89 |
| **$KIN^{100}$** | 80.32 | 77.63 | 78.54 | 72.44 | 73.56 | 70.12 |
| **$KIN^{200}$** | 73.46 | 75.63 | 68.78 | 82.67 | 83.54 | 83.57 |
| **$MH^{50}$** | 54.66 | 65.32 | 62.14 | 71.76 | 72.54 | 68.59 |
| **$MH^{100}$** | 82.12 | 83.21 | 75.63 | 87.33 | 88.25 | 77.83 |
| **$CCC^{50}$** | 90.09 | 92.67 | 86.37 | 78.59 | 74.63 | 66.58 |
| **$CCC^{100}$** | 81.20 | 83.23 | 78.02 | 84.68 | 81.25 | 79.14 |
| **$ABA^{25}$** | 92.26 | 93.65 | 87.89 | 80.99 | 79.17 | 76.27 |
| **$ABA^{50}$** | 85.99 | 86.59 | 76.56 | 81.15 | 83.64 | 75.12 |

**Table-4.2.4** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Membrane Lipid Peroxidation (n mole/g DM*) of *Paris polyphylla* from Uttaray (PPU) and Tholung (PPT) in Sikkim Himalaya. Data recorded from 60, 90 and 120days old leaves collected from 5 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Membrane Lipid Peroxidation (n mole/g DM* content) | | | | | |
|---|---|---|---|---|---|---|
| | PPU | | | PPT | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.044±0.01 | 0.047±0.02 | 0.042±0.03 | 0.051±0.01 | 0.063±0.02 | 0.054±0.01 |
| **$IAA^{100}$** | 0.055±0.01 | 0.062±0.05 | 0.052±0.02 | 0.064±0.04 | 0.069±0.05 | 0.063±0.05 |
| **$IAA^{200}$** | 0.048±0.12 | 0.039±0.02 | 0.026±0.01 | 0.057±0.03 | 0.062±0.04 | 0.055±0.01 |
| **$GA_3^{100}$** | 0.047±0.02 | 0.012±0.01 | 0.039±0.01 | 0.052±0.02 | 0.057±0.04 | 0.053±0.04 |
| **$GA_3^{200}$** | 0.044±0.01 | 0.051±0.04 | 0.041±0.02 | 0.055±0.04 | 0.057±0.02 | 0.054±0.02 |
| **$KIN^{100}$** | 0.046±0.02 | 0.048±0.02 | 0.052±0.04 | 0.061±0.04 | 0.055±0.02 | 0.056±0.03 |
| **$KIN^{200}$** | 0.040±0.01 | 0.039±0.02 | 0.045±0.02 | 0.048±0.02 | 0.052±0.02 | 0.047±0.01 |
| **$MH^{50}$** | 0.044±0.02 | 0.049±0.02 | 0.047±0.01 | 0.042±0.01 | 0.049±0.01 | 0.051±0.01 |
| **$MH^{100}$** | 0.049±0.03 | 0.054±0.04 | 0.063±0.04 | 0.057±0.02 | 0.061±0.05 | 0.060±0.04 |
| **$CCC^{50}$** | 0.045±0.01 | 0.049±0.02 | 0.035±0.02 | 0.051±0.04 | 0.056±0.02 | 0.055±0.03 |
| **$CCC^{100}$** | 0.046±0.01 | 0.031±0.01 | 0.042±0.01 | 0.053±0.04 | 0.049±0.01 | 0.048±0.02 |
| **$ABA^{25}$** | 0.044±0.02 | 0.053±0.03 | 0.051±0.04 | 0.053±0.01 | 0.049±0.03 | 0.048±0.02 |
| **$ABA^{50}$** | 0.046±0.03 | 0.039±0.03 | 0.041±0.03 | 0.059±0.03 | 0.056±0.01 | 0.054±0.02 |

*DM=Dry Mass

and a minimum of 0.045±0.03 against 0.049±0.08 mg/g FW (control) from the sample treated with $IAA^{200}$ (90 DAE) and $CCC^{50}$ (60 DAE) respectively. The results are presented in Table 4.2.8.

In PPT, the free amino acid content of the leaves was recorded maximum with 0.274±0.11 mg/g FW and minimum of 0.163±0.02 mg/g FW against 0.172±0.06 mg/g FW (control) from the sample treated with $IAA^{200}$ (60 DAE) and $ABA^{25}$ (60DAE) respectively. Similarly, for rhizome samples the maximum content of free amino acid was recorded with 0.215±0.0705 mg/g FW and minimum of 0.023±0.0805 mg/g FW against 0.069±0.05 mg/g FW (control) from the sample treated with $IAA^{200}$ (60 DAE) and $ABA^{25}$ 60 (DAE) respectively. Results are presented in Table 4.2.9. which suggested a significant variability of free amino acid content in both leaves and rhizomes between the PPT and PPU after PGR treatment.

**4.9.7. Effect of PGRs on Proline Content**

The Proline content was also influenced by PGR treatment. The leaves from PPU recorded maximum proline content of 0.416±0.05 mg/g FW and a minimum of 0.223±0.14 mg/g FW against 0.236±0.06 mg/g FW (control) in the sample treated with $ABA^{25}$ (60 DAE) and $MH^{50}$ (60DAE) respectively. Similarly, for rhizome samples the maximum was recorded with 0.171±0.05 mg/g FW from the sample treated with $KIN^{200}$ (120 DAE) and minimum of 0.115±0.01 mg/g FW ($MH^{50}$ 60 DAE) against control 0.132±0.08 mg/g FW and 0.126±0.05 mg/g FW respectively (Table 4.2.10). In the Tholung samples (PPT), the Proline content (PC) of the leaves was recorded maximum with 0.547±0.22 mg/g FW from the sample treated with $IAA^{200}$ (90 DAE) and minimum of 0.269±0.14 mg/g FW from the sample treated with $IAA^{100}$ (60DAE) against 0.254±0.06 mg/g FW and 0.263±0.12 mg/g FW (control) respectively. Similarly, for rhizome samples the maximum was recorded

with 0.176±0.02 mg/g FW from the sample treated with $GA_3^{100}$ (120 DAE) and the minimum of 0.136±0.06 mg/g FW from the sample treated with $MH^{50}$ 60 (DAE) against control 0.152±0.03 mg/g FW and 0.147±0.08 mg/g FW respectively. The results are presented in Table 4.2.11. that suggested a significant variability in proline content (both leaves and rhizomes) between the PPT and PPU after treatment.

**4.9.8. Effect of PGRs on Soluble Protein Content**

The soluble protein content of the leaves from PPU was recorded maximum with 0.367±0.05 mg/g FW and minimum of 0.174±0.02 mg/g FW against 0.227±0.06 mg/g FW (control) from the sample treated with $ABA^{25}$ (120 DAE) and $IAA^{100}$ (120DAE) respectively. Similarly, for rhizome samples the maximum was recorded with 0.193±0.05 from the sample treatment of $GA_3^{100}$ (120 DAE) and minimum of 0.130±0.08 [$ABA^{50}$ 60 (DAE)] against control 0.146±0.14 mg/g FW (Table 4.2.12). It appears that the leaves contain more soluble proteins than the rhizomes and the growth retardants increase much more protein synthesis as compared to the promoters. The soluble protein content of the leaves from PPT recorded maximum with 0.396±0.06 mg/g FW and minimum of 0.263±0.05 mg/g FW against 0.356±0.10 mg/g FW (control) from the sample treatment of $CCC^{100}$ (90 DAE) and $GA_3^{200}$ (90DAE) respectively. Similarly, for rhizome samples the maximum was recorded with 0.334±0.08 mg/g FW from the sample treatment of $GA_3^{200}$ (90 DAE) and minimum of 0.236±0.08 mg/g FW [$MH^{50}$ 120 (DAE)] against control 0.234±0.08 mg/g FW and 0.214±0.05 mg/g FW respectively (Table 4.2.13). The result suggested the significant variability of soluble protein content (for leaf sample) between the PPT and PPU after treatment such variations was not observed in rhizome sample.

**Table-4.2.5** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Chlorophyll-a and Chlorophyll-b Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from leaves collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Chlorophyll-a (mg/g FW) | | | Chlorophyll-b (mg/g FW) | | |
|---|---|---|---|---|---|---|
| | Days after emergence | | | Days after emergence | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.149±0.02 | 0.085±0.04 | 0.087±0.02 | 0.854±0.10 | 0.894±0.26 | 0.784±0.14 |
| **$IAA^{100}$** | 0.156±0.08 | 0.079±0.05 | 0.063±0.01 | 0.798±0.11 | 0.874±0.24 | 0.859±0.16 |
| **$IAA^{200}$** | 0.163±0.01 | 0.064±0.02 | 0.078±0.02 | 0.789±0.16 | 0.856±0.25 | 0.845±0.16 |
| **$GA_3^{100}$** | 0.126±0.02 | 0.056±0.02 | 0.083±0.01 | 0.845±0.08 | 0.764±0.35 | 0.785±0.11 |
| **$GA_3^{200}$** | 0.133±0.05 | 0.049±0.01 | 0.058±0.03 | 0.811±0.16 | 0.822±0.42 | 0.766±0.12 |
| **$KIN^{100}$** | 0.178±0.11 | 0.063±0.01 | 0.079±0.06 | 0.912±0.17 | 0.769±0.14 | 0.652±0.05 |
| **$KIN^{200}$** | 0.189±0.12 | 0.085±0.06 | 0.126±0.05 | 0.741±0.14 | 0.697±0.18 | 0.684±0.45 |
| **$MH^{50}$** | 0.136±0.06 | 0.058±0.02 | 0.109±0.08 | 0.763±0.23 | 0.654±0.25 | 0.958±0.63 |
| **$MH^{100}$** | 0.139±0.05 | 0.102±0.06 | 0.095±0.01 | 0.659±0.21 | 0.841±0.42 | 0.691±0.41 |
| **$CCC^{50}$** | 0.177±0.03 | 0.046±0.02 | 0.057±0.01 | 0.812±0.15 | 0.729±0.25 | 0.847±0.42 |
| **$CCC^{100}$** | 0.187±0.05 | 0.051±0.01 | 0.105±0.08 | 0.845±0.11 | 0.736±0.16 | 0.965±0.35 |
| **$ABA^{25}$** | 0.154±0.05 | 0.068±0.02 | 0.098±0.06 | 0.689±0.05 | 0.857±0.52 | 0.852±0.22 |
| **$ABA^{50}$** | 0.165±0.12 | 0.065±0.05 | 0.111±0.08 | 0.782±0.04 | 0.820±0.11 | 0.741±0.23 |

*FW=Fresh Weight

**Table-4.2.6** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Chlorophyll-a and Chlorophyll-b (mg/g FW*) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from leaves collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | PPT | | | | | |
|---|---|---|---|---|---|---|
| | Chlorophyll-a (mg/g FW) | | | Chlorophyll-b (mg/g FW) | | |
| | Days after emergence | | | Days after emergence | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.263±0.10 | 0.195±0.09 | 0.112±0.07 | 0.914±0.25 | 0.895±0.24 | 0.856±0.19 |
| **$IAA^{100}$** | 0.189±0.11 | 0.171±0.08 | 0.074±0.05 | 0.981±0.43 | 0.821±0.21 | 0.843±0.18 |
| **$IAA^{200}$** | 0.178±0.15 | 0.166±0.06 | 0.124±0.06 | 0.765±0.34 | 0.756±0.47 | 0.782±0.11 |
| **$GA_3^{100}$** | 0.174±0.18 | 0.152±0.04 | 0.185±0.08 | 0.925±0.52 | 0.844±0.35 | 0.877±0.16 |
| **$GA_3^{200}$** | 0.182±0.09 | 0.141±0.04 | 0.178±0.02 | 0.761±0.14 | 0.811±0.54 | 0.801±0.14 |
| **$KIN^{100}$** | 0.174±0.14 | 0.133±0.05 | 0.148±0.03 | 0.734±0.02 | 0.863±0.25 | 0.854±0.06 |
| **$KIN^{200}$** | 0.221±0.13 | 0.165±0.01 | 0.163±0.04 | 0.856±0.32 | 0.769±0.24 | 0.785±0.01 |
| **$MH^{50}$** | 0.213±0.15 | 0.152±0.05 | 0.145±0.02 | 0.874±0.24 | 0.785±0.17 | 0.896±0.13 |
| **$MH^{100}$** | 0.145±0.12 | 0.142±0.11 | 0.158±0.06 | 0.841±0.23 | 0.963±0.53 | 0.862±0.12 |
| **$CCC^{50}$** | 0.152±0.14 | 0.147±0.14 | 0.452±0.12 | 0.659±0.22 | 0.697±0.12 | 0.756±0.12 |
| **$CCC^{100}$** | 0.176±0.12 | 0.121±0.13 | 0.521±0.11 | 0.785±0.24 | 0.842±0.10 | 0.844±0.46 |
| **$ABA^{25}$** | 0.196±0.10 | 0.125±0.12 | 0.114±0.05 | 0.956±0.15 | 0.873±0.13 | 0.863±0.15 |
| **$ABA^{50}$** | 0.178±0.09 | 0.127±0.14 | 0.129±0.06 | 0.845±0.14 | 0.856±0.18 | 0.825±0.42 |

**Table-4.2.7** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Carotenoids content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) and *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from leaves collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | PPU | | | PPT | | |
|---|---|---|---|---|---|---|
| | Carotenoids (mg/g FW) | | | Carotenoids (mg/g FW) | | |
| | Days after emergence | | | Days after emergence | | |
| | 60 | 60 | 60 | 60 | 90 | 120 |
| **Control** | 139.23±0.23 | 128.14±0.36 | 128.14±0.36 | 128.14±0.36 | 154.25±1.48 | 154.23±1.36 |
| **$IAA^{100}$** | 125.21±1.25 | 127.21±1.25 | 127.21±1.25 | 127.21±1.25 | 153.44±1.54 | 158.63±2.36 |
| **$IAA^{200}$** | 124.22±1.03 | 134.23±1.05 | 134.23±1.05 | 134.23±1.05 | 156.31±2.07 | 155.28±1.41 |
| **$GA_3^{100}$** | 163.12±0.24 | 143.54±1.08 | 143.54±1.08 | 143.54±1.08 | 154.12±1.09 | 152.39±2.08 |
| **$GA_3^{200}$** | 127.36±1.25 | 147.33±2.36 | 147.33±2.36 | 147.33±2.36 | 168.42±1.42 | 153.22±0.54 |
| **$KIN^{100}$** | 136.31±0.25 | 135.37±1.15 | 135.37±1.15 | 135.37±1.15 | 153.41±1.65 | 161.26±1.47 |
| **$KIN^{200}$** | 173.65±1.28 | 133.65±2.28 | 133.65±2.28 | 133.65±2.28 | 158.37±2.12 | 162.59±2.64 |
| **$MH^{50}$** | 124.28±1.28 | 128.28±1.36 | 128.28±1.36 | 128.28±1.36 | 162.18±0.47 | 158.25±1.66 |
| **$MH^{100}$** | 127.98±0.58 | 122.15±0.56 | 122.15±0.56 | 122.15±0.56 | 161.38±2.52 | 156.22±0.39 |
| **$CCC^{50}$** | 174.56±2.28 | 144.51±2.18 | 144.51±2.18 | 144.51±2.18 | 164.15±0.54 | 164.32±1.21 |
| **$CCC^{100}$** | 162.14±0.28 | 142.52±2.63 | 142.52±2.63 | 142.52±2.63 | 159.11±0.69 | 163.22±0.54 |
| **$ABA^{25}$** | 189.23±0.28 | 136.23±1.47 | 136.23±1.47 | 136.23±1.47 | 158.44±2.35 | 150.36±0.64 |
| **$ABA^{50}$** | 156.22±0.28 | 131.78±1.22 | 131.78±1.22 | 131.78±1.22 | 156.26±1.25 | 152.62±1.25 |

### 4.9.9. Effect of PGRs on Soluble Carbohydrate Content

Different PGR treatment influences the content of soluble carbohydrates. The soluble carbohydrate content of the leaves from PPU was found to increase considerably with growth promoter treatment showing maximum with 0.279±0.16 mg/g FW from the sample treated with $IAA^{200}$ (90 DAE). However, the minimum of 0.152±0.18 mg/g FW was shown by the treatment with $ABA^{50}$ (120 DAE) against control 0.110±0.09 mg/g FW and 0.123±0.13 mg/g FW respectively. Contrarily, for rhizome samples the maximum content of soluble carbohydrate was recorded with 0.249±0.06 mg/g FW from the sample treated with $ABA^{25}$ (120 DAE) and minimum of 0.122±0.12 mg/g FW from the sample treated with $MH^{50}$ (120 DAE) against control 0.176±0.03 mg/g FW respectively (Table 4.2.14).

The soluble carbohydrate content of the leaves from PPT was recorded maximum with 0.291±0.15 mg/g FW from the sample treated with $KIN^{100}$ (90 DAE) and minimum of 0.158±0.14 mg/g FW [$IAA^{200}$ 120 (DAE)] against control 0.254±0.06 mg/g FW and 0.263±0.18 mg/g FW respectively. Similarly, for rhizome samples the maximum was recorded with 0.434±0.04 from the sample treatment of $KIN^{200}$ (90 DAE) and minimum of 0.264±0.08 [$IAA^{200}$ 90 (DAE)] against control 0.356±0.01 mg/g FW respectively. Thus, in this case, KIN and IAA behaved in similar manner between the leaves and rhizomes (Table 4.2.15). The results exhibited significant variability of soluble carbohydrate content for the leaf sample between the PPT and PPU.

**Table-4.2.8** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Free Amino Acid Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Free Amino Acid Content (mg/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.123±0.09 | 0.136±0.08 | 0.148±0.04 | 0.049±0.08 | 0.052±0.03 | 0.059±0.22 |
| **$IAA^{100}$** | 0.179±0.04 | 0.184±0.02 | 0.168±0.05 | 0.055±0.05 | 0.058±0.07 | 0.061±0.02 |
| **$IAA^{200}$** | 0.265±0.03 | 0.262±0.02 | 0.279±0.06 | 0.142±0.29 | 0.157±0.06 | 0.149±0.15 |
| **$GA_3^{100}$** | 0.248±0.02 | 0.263±0.06 | 0.312±0.02 | 0.049±0.19 | 0.057±0.07 | 0.062±0.02 |
| **$GA_3^{200}$** | 0.121±0.05 | 0.174±0.09 | 0.189±0.08 | 0.046±0.02 | 0.055±0.06 | 0.057±0.05 |
| **$KIN^{100}$** | 0.137±0.12 | 0.163±0.05 | 0.184±0.02 | 0.049±0.03 | 0.054±0.05 | 0.057±0.03 |
| **$KIN^{200}$** | 0.172±0.03 | 0.184±0.01 | 0.149±0.03 | 0.058±0.24 | 0.063±0.02 | 0.071±0.02 |
| **$MH^{50}$** | 0.114±0.01 | 0.127±0.12 | 0.456±0.11 | 0.060±0.03 | 0.052±0.14 | 0.057±0.01 |
| **$MH^{100}$** | 0.120±0.02 | 0.136±0.03 | 0.157±0.06 | 0.049±0.12 | 0.052±0.02 | 0.057±0.02 |
| **$CCC^{50}$** | 0.187±0.06 | 0.223±0.05 | 0.234±0.03 | 0.045±0.03 | 0.051±0.04 | 0.058±0.06 |
| **$CCC^{100}$** | 0.143±0.05 | 0.154±0.10 | 0.136±0.02 | 0.054±0.02 | 0.061±0.02 | 0.063±0.08 |
| **$ABA^{25}$** | 0.175±0.24 | 0.192±0.15 | 0.205±0.04 | 0.049±0.01 | 0.048±0.05 | 0.055±0.06 |
| **$ABA^{50}$** | 0.167±0.04 | 0.164±0.02 | 0.196±0.06 | 0.047±0.06 | 0.052±0.01 | 0.052±0.06 |

**Table-4.2.9** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Free Amino Acid Content (mg/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Free Amino Acid Content (mg/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.172±0.06 | 0.187±0.06 | 0.181±0.08 | 0.069±0.05 | 0.075±0.06 | 0.062±0.05 |
| **$IAA^{100}$** | 0.262±0.12 | 0.239±0.11 | 0.253±0.09 | 0.072±0.06 | 0.076±0.02 | 0.071±0.01 |
| **$IAA^{200}$** | 0.274±0.11 | 0.261±0.08 | 0.263±0.04 | 0.215±0.07 | 0.211±0.01 | 0.213±0.08 |
| **$GA_3^{100}$** | 0.263±0.02 | 0.226±0.05 | 0.247±0.06 | 0.142±0.04 | 0.138±0.04 | 0.132±0.03 |
| **$GA_3^{200}$** | 0.226±0.06 | 0.231±0.05 | 0.243±0.11 | 0.092±0.06 | 0.098±0.01 | 0.086±0.12 |
| **$KIN^{100}$** | 0.263±0.08 | 0.223±0.12 | 0.265±0.13 | 0.034±0.04 | 0.039±0.01 | 0.042±0.06 |
| **$KIN^{200}$** | 0.273±0.07 | 0.276±0.02 | 0.264±0.02 | 0.047±0.02 | 0.049±0.33 | 0.041±0.04 |
| **$MH^{50}$** | 0.254±0.05 | 0.263±0.07 | 0.267±0.12 | 0.051±0.03 | 0.063±0.01 | 0.065±0.02 |
| **$MH^{100}$** | 0.263±0.05 | 0.265±0.05 | 0.274±0.05 | 0.025±0.04 | 0.061±0.05 | 0.056±0.05 |
| **$CCC^{50}$** | 0.226±0.10 | 0.224±0.01 | 0.235±0.08 | 0.120±0.04 | 0.114±0.02 | 0.098±0.08 |
| **$CCC^{100}$** | 0.232±0.12 | 0.236±0.04 | 0.229±0.06 | 0.121±0.06 | 0.112±0.09 | 0.101±0.08 |
| **$ABA^{25}$** | 0.163±0.02 | 0.169±0.03 | 0.172±0.04 | 0.023±0.08 | 0.085±0.05 | 0.081±0.02 |
| **$ABA^{50}$** | 0.172±0.03 | 0.212±0.05 | 0.254±0.07 | 0.059±0.06 | 0.083±0.04 | 0.076±0.01 |

### 4.9.10. Effect of PGRs on Insoluble Carbohydrate Content

The insoluble carbohydrate content of the leaves from PPU was recorded maximum with 0.278±0.17 mg/g FW from the sample treated with $ABA^{25}$ (90 DAE) and minimum of 0.122±0.12 mg/g FW in the sample treated with $IAA^{100}$ (120 (DAE) against control 0.251±0.04 mg/g FW and 0.263±0.13 mg/g FW respectively. Similarly, for rhizome samples the maximum was recorded with 0.354±0.14 from the sample treatment of $ABA^{25}$ (90 DAE) and minimum of 0.232±0.05 [$GA_3^{100}$ 90 (DAE)] against control 0.302±0.06 mg/g FW and 0.314±0.04 mg/g FW respectively (Table 4.2.16).

The insoluble carbohydrate content of the leaves from PPT was recorded maximum with 0.231±0.02 mg/g FW from the sample treated with $GA_3^{100}$ (90 DAE) and minimum of 0.112±0.03 mg/g FW with $CCC^{50}$ (90 (DAE) against control 0.158±0.03 mg/g FW. Similarly, for rhizome samples the maximum was recorded with 0.185±0.02 from the sample treated with $ABA^{25}$ (90 DAE) and minimum of 0.106±0.06 mg/g FW from the sample treated with $KIN^{100}$ 90 (DAE) against control 0.163±0.08 mg/g FW (Table 4.2.17). The results suggested significant variability of insoluble carbohydrate content (for leaf sample) between the PPT and PPU after treatment with PGRs but showed no significant variability in rhizome samples.

### 4.9.11. Effect of PGRs on Ascorbic Acid Content

The ascorbic acid content in the leaves and rhizomes of PPU was observed to be influenced by PGR treatment. In both the cases the growth retardants caused relatively higher level of ascorbic acid, but the effect tend to be detrimental after 90 days of treatment. The ascorbic acid content of the leaves from PPU was recorded maximum with 0.360±0.09 mg/g FW from the sample treated with $CCC^{100}$ (90 DAE) and minimum of 0.136±0.09 mg/g FW in the sample treated with $MH^{50}$ 960 (DAE)

**Table-4.2.10** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Proline Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Proline Content (mg/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.236±0.06 | 0.242±0.14 | 0.224±0.09 | 0.126±0.05 | 0.134±0.02 | 0.132±0.08 |
| **$IAA^{100}$** | 0.324±0.15 | 0.312±0.06 | 0.319±0.06 | 0.139±0.02 | 0.142±0.03 | 0.143±0.08 |
| **$IAA^{200}$** | 0.239±0.15 | 0.243±0.03 | 0.242±0.04 | 0.142±0.08 | 0.147±0.04 | 0.151±0.04 |
| **$GA_3^{100}$** | 0.363±0.11 | 0.369±0.09 | 0.371±0.02 | 0.136±0.06 | 0.134±0.02 | 0.133±0.05 |
| **$GA_3^{200}$** | 0.414±0.16 | 0.413±0.08 | 0.410±0.25 | 0.147±0.07 | 0.152±0.05 | 0.156±0.05 |
| **$KIN^{100}$** | 0.301±0.08 | 0.315±0.09 | 0.306±0.06 | 0.132±0.02 | 0.137±0.08 | 0.138±0.07 |
| **$KIN^{200}$** | 0.347±0.09 | 0.342±0.05 | 0.339±0.02 | 0.158±0.06 | 0.163±0.07 | 0.171±0.05 |
| **$MH^{50}$** | 0.223±0.14 | 0.234±0.11 | 0.237±0.05 | 0.115±0.01 | 0.119±0.06 | 0.127±0.03 |
| **$MH^{100}$** | 0.256±0.12 | 0.263±0.14 | 0.261±0.03 | 0.128±0.04 | 0.134±0.04 | 0.139±0.08 |
| **$CCC^{50}$** | 0.265±0.06 | 0.267±0.06 | 0.251±0.05 | 0.163±0.06 | 0.167±0.06 | 0.158±0.09 |
| **$CCC^{100}$** | 0.274±0.06 | 0.271±0.02 | 0.276±0.03 | 0.148±0.03 | 0.157±0.02 | 0.141±0.01 |
| **$ABA^{25}$** | 0.416±0.05 | 0.402±0.24 | 0.411±0.09 | 0.157±0.02 | 0.151±0.03 | 0.147±0.05 |
| **$ABA^{50}$** | 0.389±0.11 | 0.385±0.06 | 0.375±0.04 | 0.136±0.07 | 0.141±0.01 | 0.135±0.06 |

**Table-4.2.11** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Proline Content (mg/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Proline Content (mg/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.263±0.12 | 0.254±0.06 | 0.249±0.06 | 0.147±0.08 | 0.154±0.12 | 0.152±0.03 |
| **$IAA^{100}$** | 0.269±0.14 | 0.274±0.15 | 0.271±0.14 | 0.162±0.03 | 0.168±0.13 | 0.169±0.04 |
| **$IAA^{200}$** | 0.551±0.05 | 0.547±0.22 | 0.523±0.25 | 0.156±0.08 | 0.149±0.08 | 0.152±0.06 |
| **$GA_3^{100}$** | 0.421±0.02 | 0.423±0.02 | 0.402±0.06 | 0.173±0.05 | 0.172±0.09 | 0.176±0.02 |
| **$GA_3^{200}$** | 0.369±0.06 | 0.362±0.03 | 0.358±0.07 | 0.146±0.07 | 0.154±0.04 | 0.158±0.07 |
| **$KIN^{100}$** | 0.436±0.07 | 0.428±0.02 | 0.441±0.09 | 0.166±0.09 | 0.164±0.06 | 0.161±0.08 |
| **$KIN^{200}$** | 0.403±0.06 | 0.405±0.09 | 0.412±0.08 | 0.173±0.02 | 0.168±0.07 | 0.164±0.09 |
| **$MH^{50}$** | 0.302±0.05 | 0.311±0.08 | 0.315±0.02 | 0.136±0.06 | 0.142±0.05 | 0.148±0.07 |
| **$MH^{100}$** | 0.321±0.01 | 0.319±0.07 | 0.326±0.03 | 0.148±0.02 | 0.152±0.07 | 0.151±0.02 |
| **$CCC^{50}$** | 0.278±0.06 | 0.274±0.09 | 0.276±0.21 | 0.162±0.05 | 0.138±0.10 | 0.154±0.08 |
| **$CCC^{100}$** | 0.281±0.14 | 0.286±0.05 | 0.284±0.04 | 0.154±0.01 | 0.156±0.13 | 0.161±0.02 |
| **$ABA^{25}$** | 0.276±0.12 | 0.271±0.06 | 0.276±0.09 | 0.151±0.02 | 0.154±0.11 | 0.149±0.06 |
| **$ABA^{50}$** | 0.314±0.23 | 0.317±0.04 | 0.309±0.05 | 0.166±0.02 | 0.162±0.12 | 0.170±0.07 |

against control 0.166±0.06 mg/g FW. Similarly, for rhizome samples the maximum was recorded with 0.935±0.01 mg/g FW from the sample treated with $CCC^{100}$ (90 DAE) and minimum of 0.069±0.06 mg/g FW in the sample treated with $GA_3^{100}$ (120 (DAE) against control 0.096±0.08 and 0.068±0.03 mg/g FW respectively (Table 4.2.18).

The ascorbic acid content of the leaves from PPT was recorded maximum with 0.310±0.06 mg/g FW from the sample treated with $CCC^{100}$ (120 DAE) and minimum of 0.132±0.01 mg/g FW from the treatment with $IAA^{100}$ (120 DAE)] against control 0.152±0.01 mg/g FW and 0.135±0.06 mg/g FW respectively. Similarly, for rhizome samples the maximum was recorded with 0.148±0.06 mg/g FW from the sample treated with $ABA^{25}$ (90 DAE) and minimum of 0.042±0.03 mg/g FW with the sample treated with $IAA^{200}$ (90 DAE)] against control 0.075±0.04 and 0.062±0.02 mg/g FW respectively (Table 4.2.19). The results suggested a significant variability of ascorbic acid content (for leaf and rhizome sample) between the PPT and PPU after treatment. In this case, the content of the metabolite in question showed continued increase even beyond 90 day period.

**4.9.12. Effect of PGRs on Catalase Activity**

The catalase activity of the leaves from PPU was recorded maximum with 0.852±0.05 units/min/g FW from the sample treatment of $GA_3^{200}$ (90 DAE) and minimum of 0.385±0.02 units/min/g FW by $MH^{50}$ treatment (120 DAE) against control 0.539±0.04 units/min/g FW and 0.474±0.11 units/min/g FW respectively. Similarly, for rhizome samples the maximum was recorded with 0.715±0.02 units/min/g FW from the sample treated with $CCC^{100}$ (120 DAE) and minimum of 0.325±0.09 units/min/g FW by the sample treated with $MH^{100}$ (120 DAE) against control 0.426±0.06 units/min/g FW 0.461±0.04 units/min/g FW respectively (Table

4.2.20). In both the leaves and rhizomes MH effected in the decrease of catalase activity.

The catalase activity of the leaves from PPT was recorded maximum with 0.772±0.06 units/min/g FW from the sample treated with $CCC^{100}$ (90 DAE) and minimum of approx. 0.436±0.06 units/min/g FW by the samples treated with $IAA^{100}$ (60 DAE), $KIN^{200}$ (60 DAE) and $IAA^{200}$ (120 DAE) respectively. For rhizome samples from PPT the maximum was recorded with 0.691±0.34 from the sample treated with $MH^{50}$ (90 DAE) and minimum of 0.389±0.01 units/min/g FW by the sample treated with $ABA^{50}$ (120 DAE) (Table 4.2.21). The results suggested significant variability in ccatalase activity between the PPT and PPU especially in the leaf samples.

**4.9.13. Effect of PGRs on Peroxidase Activity**

The peroxidase level of PPU was seen to be positively influenced by KIN, MH and CCC and negatively by ABA. Thus, the peroxidase activity of the leaves from PPU was recorded maximum with 0.438±0.02 units/min/g FW from the sample treated with $MH^{100}$ (90 DAE) and minimum of 0.143±0.02 units/min/g FW by the sample treated with $ABA^{50}$ (120 (DAE) against control 0.223±0.02 units/min/g FW and 0.267±0.11 units/min/g FW respectively. Similarly, for rhizome samples, the maximum was recorded with 0.425±0.05 from the sample treated with $MH^{100}$ (90 DAE) and minimum of 0.156±0.03 units/min/g FW by the sample treated with $ABA^{50}$ (120 DAE) against control 0.355±0.08 units/min/g FW and 0.311±0.17 units/min/g FW respectively (Table 4.2.22). Thus, MH was responsible for maximum positive effect on the activity of peroxidase and ABA minimum negative effect on the same in both leaves and rhizomes of PPU.

**Table-4.2.12** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Soluble Protein Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Soluble Protein Content (mg/g FW) from PPU | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.263±0.08 | 0.227±0.06 | 0.146±0.14 | 0.152±0.14 |
| **$IAA^{100}$** | 0.183±0.02 | 0.174±0.02 | 0.173±0.04 | 0.181±0.15 |
| **$IAA^{200}$** | 0.272±0.03 | 0.276±0.14 | 0.155±0.03 | 0.164±0.11 |
| **$GA_3^{100}$** | 0.285±0.15 | 0.271±0.12 | 0.193±0.05 | 0.185±0.08 |
| **$GA_3^{200}$** | 0.341±0.23 | 0.312±0.14 | 0.167±0.02 | 0.172±0.01 |
| **$KIN^{100}$** | 0.296±0.11 | 0.284±0.15 | 0.163±0.08 | 0.136±0.06 |
| **$KIN^{200}$** | 0.342±0.04 | 0.363±0.26 | 0.173±0.09 | 0.145±0.01 |
| **$MH^{50}$** | 0.354±0.09 | 0.354±0.22 | 0.167±0.07 | 0.172±0.06 |
| **$MH^{100}$** | 0.311±0.03 | 0.305±0.14 | 0.154±0.06 | 0.163±0.02 |
| **$CCC^{50}$** | 0.314±0.08 | 0.296±0.05 | 0.134±0.04 | 0.145±0.06 |
| **$CCC^{100}$** | 0.326±0.06 | 0.317±0.06 | 0.155±0.06 | 0.157±0.14 |
| **$ABA^{25}$** | 0.364±0.07 | 0.367±0.05 | 0.164±0.08 | 0.146±0.11 |
| **$ABA^{50}$** | 0.315±0.04 | 0.321±0.08 | 0.130±0.08 | 0.145±0.03 |

**Table-4.2.13** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Soluble Protein Content (mg/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Soluble Protein Content (mg/g FW) from PPT | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.356±0.10 | 0.364±0.12 | 0.234±0.08 | 0.214±0.05 |
| **$IAA^{100}$** | 0.361±0.23 | 0.372±0.08 | 0.264±0.05 | 0.254±0.05 |
| **$IAA^{200}$** | 0.374±0.02 | 0.357±0.07 | 0.262±0.01 | 0.237±0.06 |
| **$GA_3^{100}$** | 0.287±0.06 | 0.291±0.05 | 0.241±0.02 | 0.252±0.08 |
| **$GA_3^{200}$** | 0.263±0.05 | 0.275±0.05 | 0.334±0.08 | 0.298±0.02 |
| **$KIN^{100}$** | 0.366±0.04 | 0.372±0.06 | 0.254±0.09 | 0.261±0.03 |
| **$KIN^{200}$** | 0.358±0.02 | 0.365±0.04 | 0.267±0.05 | 0.254±0.24 |
| **$MH^{50}$** | 0.377±0.03 | 0.374±0.03 | 0.245±0.02 | 0.236±0.08 |
| **$MH^{100}$** | 0.361±0.04 | 0.363±0.02 | 0.252±0.11 | 0.258±0.08 |
| **$CCC^{50}$** | 0.376±0.05 | 0.371±0.06 | 0.263±0.15 | 0.241±0.02 |
| **$CCC^{100}$** | 0.396±0.06 | 0.381±0.24 | 0.254±0.12 | 0.248±0.02 |
| **$ABA^{25}$** | 0.381±0.07 | 0.376±0.13 | 0.264±0.06 | 0.272±0.08 |
| **$ABA^{50}$** | 0.378±0.09 | 0.368±0.11 | 0.285±0.04 | 0.271±0.04 |

The peroxidase activity of the leaves from PPT was recorded maximum with 0.392±0.02 units/min/g FW from the sample treatment of $CCC^{100}$ (90 DAE) and minimum of 0.161±0.06 units/min/g FW by the treatment with $ABA^{50}$ (120 DAE) against control 0.254±0.06 units/min/g FW and 0.278±0.14 units/min/g FW respectively. Similarly, for rhizome samples the maximum peroxidase activity was recorded with 0.411±0.03 units/min/g FW from the sample treatment with $IAA^{100}$ (60 DAE), $KIN^{100}$ (60 DAE) and $MH^{100}$ (90 DAE) and the minimum of 0.187±0.04 units/min/g FW [$MH^{50}$ 120 (DAE)] against control 0.261±0.04 units/min/g FW respectively (Table 4.2.23). The results suggested minute variability of peroxidase activity (for both leaf and rhizome sample) between the PPT and PPU which may not be considered significant.

**4.9.14. Effect of PGRs on Superoxide Dismutase Activity**

Foliar treatment with PGR also gently influences the superoxide dismutase activity. Among the growth parameters it is positively influenced by $IAA^{200}$, and $GA3^{200}$ and among the growth retardants it is positively influenced by $CCC^{100}$ and $MH^{100}$.The superoxide dismutase activity of the leaves from PPU was recorded maximum with 0.756±0.03 units/min/g FW from the sample treated with $GA_3^{100}$ (120 DAE) and minimum of 0.254±0.01 units/min/g FW by $IAA^{200}$ (60 DAE) against control 0.352±0.06 units/min/g FW and 0.487±0.08 units/min/g FW respectively. Similarly, for rhizome samples the maximum was recorded with 0.565±0.08 units/min/g FW from the sample treatment of $GA_3^{200}$ (120 DAE) and minimum of 0.288±0.02 units/min/g FW with $ABA^{50}$ (120 DAE) against control 0.341±0.04 units/min/g FW (Table 4.2.24).

In the leaves of PPT the PGRs, $IAA^{200}$ and $CCC^{100}$ were almost equally effective as that of $GA_3^{200}$ in raising the activity of superoxide dismutase. Almost equal

detrimental effect has exhibit as that of $ABA^{50}$ was shown by $CCC^{100}$ treatment for the same period. Similarly, superoxide dismutase activity of the leaves from PPT was recorded maximum with 0.428±0.16 units/min/g FW from the sample treated with $GA_3^{200}$ (120 DAE) and minimum of 0.162±0.02 units/min/g FW with $ABA^{50}$ (120 DAE) against control 0.345±0.14 units/min/g FW. Similarly, for rhizome samples the maximum was recorded with 0.414±0.04 units/min/g FW from the sample treated with $GA_3^{200}$ (120 DAE) and minimum of 0.187±0.02 units/min/g FW by the treatment with $ABA^{50}$ (120 DAE) against control 0.263±0.02 units/min/g FW (Table 4.2.25). The results suggested no significant variability of superoxide dismutase activity especially for the leaf sample between the PPT and PPU after treatment with PGRs.

## 4.10. PGR induced changes in phytochemical constituents

### 4.10.1. Effect of PGRs on Total Phenolics Content

The total phenolics content (TPC) for the samples from PPU was determined from the regression equation of the calibration curve obtained from gallic acid equivalent (GAE)/g Dry weight (DW). The TPC was found to be more in the aqueous methanol (90 and 120 DAE) leaves samples with $MH^{50}$ as well as $CCC^{100}$ (90 and 120 DAE treatment giving approx. 23-24 of mg GAE/g DW sample.) In comparison to $ABA^{25}$ (60 DAE) treated sample gives the lowest TPC content of (11.35±0.22 mg GAE/g DW) against control 11.76±0.11 mg GAE/g DW respectively. Likewise, in rhizome samples the TPC was found to be maximum in the (120 DAE) samples treated with $CCC^{100}$ (10.15±0.10 mg GAE/g DW) in comparison to $GA_3^{100}$ of 60 (DAE) sample (04.55±0.56 mg GAE/g DW) against control of 04.47±0.34 mg GAE/g DW (Table 4.3.1).

**Table-4.2.14** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Soluble Carbohydrate Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Soluble Carbohydrate Content (mg/g FW) from PPU | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.110±0.09 | 0.123±0.13 | 0.193±0.04 | 0.176±0.03 |
| **$IAA^{100}$** | 0.260±0.11 | 0.275±0.14 | 0.164±0.05 | 0.154±0.02 |
| **$IAA^{200}$** | 0.279±0.16 | 0.272±0.16 | 0.124±0.14 | 0.136±0.04 |
| **$GA_3^{100}$** | 0.256±0.15 | 0.263±0.15 | 0.159±0.13 | 0.165±0.09 |
| **$GA_3^{200}$** | 0.234±0.02 | 0.227±0.03 | 0.215±0.11 | 0.196±0.06 |
| **$KIN^{100}$** | 0.247±0.09 | 0.241±0.03 | 0.212±0.11 | 0.218±0.11 |
| **$KIN^{200}$** | 0.253±0.06 | 0.261±0.11 | 0.245±0.15 | 0.231±0.15 |
| **$MH^{50}$** | 0.184±0.06 | 0.178±0.12 | 0.126±0.16 | 0.122±0.12 |
| **$MH^{100}$** | 0.164±0.0 | 0.156±0.06 | 0.139±0.11 | 0.167±0.13 |
| **$CCC^{50}$** | 0.178±0.06 | 0.181±0.15 | 0.233±0.02 | 0.224±0.13 |
| **$CCC^{100}$** | 0.169±0.07 | 0.166±0.12 | 0.245±0.08 | 0.236±0.18 |
| **$ABA^{25}$** | 0.176±0.12 | 0.181±0.13 | 0.247±0.06 | 0.249±0.06 |
| **$ABA^{50}$** | 0.158±0.13 | 0.152±0.18 | 0.234±0.08 | 0.208±0.05 |

**Table-4.2.15** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Soluble Carbohydrate Content (mg/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Soluble Carbohydrate Content (mg/g FW) from PPT | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.254±0.06 | 0.263±0.18 | 0.356±0.01 | 0.361±0.22 |
| **$IAA^{100}$** | 0.176±0.04 | 0.158±0.14 | 0.264±0.08 | 0.314±0.29 |
| **$IAA^{200}$** | 0.245±0.03 | 0.265±0.14 | 0.374±0.06 | 0.421±0.24 |
| **$GA_3^{100}$** | 0.274±0.08 | 0.281±0.03 | 0.369±0.05 | 0.394±0.18 |
| **$GA_3^{200}$** | 0.267±0.08 | 0.278±0.06 | 0.364±0.04 | 0.381±0.23 |
| **$KIN^{100}$** | 0.291±0.15 | 0.284±0.09 | 0.402±0.06 | 0.415±0.25 |
| **$KIN^{200}$** | 0.285±0.14 | 0.264±0.08 | 0.434±0.04 | 0.411±0.25 |
| **$MH^{50}$** | 0.236±0.13 | 0.227±0.16 | 0.361±0.12 | 0.354±0.14 |
| **$MH^{100}$** | 0.254±0.06 | 0.256±0.01 | 0.347±0.26 | 0.368±0.08 |
| **$CCC^{50}$** | 0.271±0.07 | 0.273±0.02 | 0.391±0.25 | 0.394±0.06 |
| **$CCC^{100}$** | 0.234±0.04 | 0.285±0.01 | 0.387±0.23 | 0.382±0.02 |
| **$ABA^{25}$** | 0.264±0.06 | 0.247±0.08 | 0.370±0.14 | 0.364±0.15 |
| **$ABA^{50}$** | 0.236±0.04 | 0.244±0.06 | 0.358±0.13 | 0.372±0.03 |

**Table-4.2.16** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Insoluble Carbohydrate Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Insoluble Carbohydrate Content (mg/g FW) from PPU | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.251±0.04 | 0.263±0.13 | 0.314±0.04 | 0.302±0.06 |
| **$IAA^{100}$** | 0.124±0.02 | 0.122±0.12 | 0.233±0.13 | 0.247±0.18 |
| **$IAA^{200}$** | 0.136±0.02 | 0.142±0.03 | 0.251±0.07 | 0.258±0.11 |
| **$GA_3^{100}$** | 0.157±0.06 | 0.162±0.14 | 0.232±0.05 | 0.241±0.03 |
| **$GA_3^{200}$** | 0.163±0.06 | 0.168±0.06 | 0.254±0.02 | 0.256±0.02 |
| **$KIN^{100}$** | 0.213±0.04 | 0.227±0.11 | 0.269±0.05 | 0.278±0.05 |
| **$KIN^{200}$** | 0.236±0.03 | 0.241±0.12 | 0.267±0.05 | 0.289±0.08 |
| **$MH^{50}$** | 0.202±0.03 | 0.210±0.04 | 0.234±0.06 | 0.257±0.08 |
| **$MH^{100}$** | 0.216±0.01 | 0.218±0.08 | 0.315±0.09 | 0.326±0.02 |
| **$CCC^{50}$** | 0.247±0.11 | 0.251±0.21 | 0.322±0.08 | 0.336±0.16 |
| **$CCC^{100}$** | 0.243±0.03 | 0.248±0.06 | 0.324±0.02 | 0.328±0.18 |
| **$ABA^{25}$** | 0.278±0.17 | 0.256±0.07 | 0.318±0.12 | 0.354±0.14 |
| **$ABA^{50}$** | 0.218±0.12 | 0.225±0.04 | 0.305±0.11 | 0.314±0.18 |

**Table-4.2.17** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Insoluble Carbohydrate Content (mg/g FW) of *Paris polyphylla* from and Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Insoluble Carbohydrate Content (mg/g FW) from PPT | | | |
|---|---|---|---|---|
| | Leaves (Days after emergence) | | Rhizomes (Days after emergence) | |
| | 90 | 120 | 90 | 120 |
| **Control** | 0.158±0.03 | 0.174±0.08 | 0.163±0.08 | 0.158±0.01 |
| **$IAA^{100}$** | 0.215±0.04 | 0.214±0.05 | 0.145±0.63 | 0.152±0.06 |
| **$IAA^{200}$** | 0.221±0.05 | 0.224±0.01 | 0.112±0.18 | 0.162±0.07 |
| **$GA_3^{100}$** | 0.231±0.02 | 0.211±0.06 | 0.168±0.14 | 0.157±0.07 |
| **$GA_3^{200}$** | 0.196±0.01 | 0.185±0.09 | 0.159±0.05 | 0.163±0.09 |
| **$KIN^{100}$** | 0.204±0.02 | 0.184±0.04 | 0.106±0.06 | 0.154±0.06 |
| **$KIN^{200}$** | 0.211±0.01 | 0.184±0.06 | 0.136±0.07 | 0.174±0.02 |
| **$MH^{50}$** | 0.151±0.03 | 0.162±0.04 | 0.172±0.03 | 0.184±0.15 |
| **$MH^{100}$** | 0.163±0.01 | 0.174±0.03 | 0.123±0.01 | 0.163±0.11 |
| **$CCC^{50}$** | 0.112±0.03 | 0.136±0.04 | 0.126±0.02 | 0.149±0.08 |
| **$CCC^{100}$** | 0.154±0.04 | 0.182±0.02 | 0.174±0.08 | 0.161±0.06 |
| **$ABA^{25}$** | 0.163±0.04 | 0.142±0.11 | 0.185±0.02 | 0.151±0.04 |
| **$ABA^{50}$** | 0.162±0.04 | 0.158±0.07 | 0.162±0.12 | 0.168±0.05 |

The total phenolics content (TPC) for the samples from PPT was found to be maximum in the methanolic extract of (120DAE) leaf samples treated with $CCC^{100}$ (27.13±0.05 mg GAE/g DW). In addition, other treatments like $KIN^{100}$, $KIN^{200}$, $MH^{100}$, and $CCC^{50}$ also gave almost equal TPC after 120 days. $GA_3^{200}$ of 60 (DAE) sample (16.84±0.18 mg GAE/g DW) gave less TPC than the control 16.27±0.11 mg GAE/g DW. Similarly, in rhizome samples, the TPC was found to be most influenced by the treatment with $CCC^{100}$ (27.22±0.09 mg GAE/g DW), while the treatments like $IAA^{100}$ and $KIN^{200}$ did not cause any changes of value against the control (Table 4.3.2).

### 4.10.2. Effect of PGRs on Total Flavonoid Content

The total flavonoid content (TFC) for the samples from PPU was determined from the regression equation of the calibration curve obtained from quercetin acid (QE)/g Dry weight (DW). Results are presented in Table 4.3.3. The flavonoid levels of powdered leaf samples were recorded highest from the 120 (DAE) treatment samples of $KIN^{200}$ with 31.68±0.21 mg QE/g dry weight in comparison to the lowest from 90 (DAE) sample treatment of $IAA^{100}$ (18.13±0.03) against control 23.57±0.05 mg QE/g dry weight and 18.42±0.04 mg QE/g dry weight respectively. $GA_3^{200}$ treated sample also showed similar flavonoid content as that treated with $KIN^{200}$. Likewise, the flavonoid levels of rhizome samples were recorded the highest content with the treatment of $KIN^{200}$ at 34.52±0.43 mg QE/g dry weight followed by $GA_3^{200}$ treatment (120 DAE). The lowest TFC was found in IAA and ABA treatment with $IAA^{100}$ (21.19±0.09 mg QE/g dry weight) against control 21.16±0.58 mg QE/g dry weight. At the treatment period of 60 DAE the treatment like $IAA^{200}$, $MH^{50}$ and $ABA^{25}$ did not show any effect.

**Table-4.2.18** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Ascorbic Acid Content (mg/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from leaves and rhizomes of 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Ascorbic Acid Content (mg/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.166±0.06 | 0.154±0.07 | 0.142±0.06 | 0.096±0.08 | 0.075±0.04 | 0.068±0.03 |
| **$IAA^{100}$** | 0.175±0.06 | 0.162±0.05 | 0.155±0.02 | 0.102±0.06 | 0.108±0.01 | 0.075±0.04 |
| **$IAA^{200}$** | 0.168±0.15 | 0.141±0.09 | 0.156±0.01 | 0.113±0.04 | 0.116±0.06 | 0.084±0.08 |
| **$GA_3^{100}$** | 0.183±0.25 | 0.171±0.04 | 0.136±0.09 | 0.106±0.01 | 0.123±0.01 | 0.102±0.06 |
| **$GA_3^{200}$** | 0.164±0.06 | 0.156±0.03 | 0.151±0.05 | 0.102±0.04 | 0.106±0.04 | 0.069±0.06 |
| **$KIN^{100}$** | 0.189±0.04 | 0.163±0.08 | 0.154±0.06 | 0.135±0.03 | 0.102±0.06 | 0.094±0.01 |
| **$KIN^{200}$** | 0.176±0.05 | 0.165±0.08 | 0.151±0.01 | 0.122±0.04 | 0.106±0.04 | 0.088±0.01 |
| **$MH^{50}$** | 0.204±0.01 | 0.217±0.03 | 0.190±0.01 | 0.170±0.03 | 0.132±0.02 | 0.130±0.02 |
| **$MH^{100}$** | 0.245±0.03 | 0.229±0.06 | 0.202±0.05 | 0.185±0.02 | 0.168±0.04 | 0.110±0.06 |
| **$CCC^{50}$** | 0.246±0.01 | 0.250±0.02 | 0.218±0.03 | 0.178±0.09 | 0.170±0.07 | 0.095±0.01 |
| **$CCC^{100}$** | 0.330±0.01 | 0.360±0.03 | 0.280±0.03 | 0.225±0.01 | 0.235±0.01 | 0.192±0.02 |
| **$ABA^{25}$** | 0.154±0.04 | 0.148±0.11 | 0.136±0.04 | 0.063±0.02 | 0.099±0.01 | 0.185±0.02 |
| **$ABA^{50}$** | 0.243±0.08 | 0.261±0.08 | 0.275±0.02 | 0.059±0.19 | 0.202±0.02 | 0.262±0.03 |

**Table-4.2.19** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Ascorbic Acid Content (mg/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from leaves and rhizomes of 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Ascorbic Acid Content (mg/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.152±0.01 | 0.147±0.07 | 0.135±0.06 | 0.075±0.04 | 0.062±0.02 | 0.054±0.06 |
| **$IAA^{100}$** | 0.165±0.08 | 0.154±0.01 | 0.142±0.09 | 0.084±0.08 | 0.056±0.06 | 0.063±0.01 |
| **$IAA^{200}$** | 0.173±0.02 | 0.146±0.04 | 0.132±0.01 | 0.088±0.06 | 0.042±0.03 | 0.045±0.07 |
| **$GA_3^{100}$** | 0.162±0.09 | 0.151±0.06 | 0.135±0.05 | 0.079±0.02 | 0.073±0.08 | 0.057±0.06 |
| **$GA_3^{200}$** | 0.124±0.08 | 0.163±0.07 | 0.146±0.02 | 0.092±0.04 | 0.071±0.09 | 0.061±0.05 |
| **$KIN^{100}$** | 0.183±0.03 | 0.158±0.08 | 0.237±0.09 | 0.075±0.04 | 0.086±0.07 | 0.056±0.06 |
| **$KIN^{200}$** | 0.172±0.07 | 0.149±0.05 | 0.253±0.05 | 0.081±0.06 | 0.064±0.06 | 0.064±0.05 |
| **$MH^{50}$** | 0.165±0.05 | 0.195±0.06 | 0.224±0.03 | 0.085±0.08 | 0.118±0.02 | 0.090±0.01 |
| **$MH^{100}$** | 0.215±0.01 | 0.235±0.01 | 0.260±0.04 | 0.090±0.07 | 0.123±0.01 | 0.070±0.03 |
| **$CCC^{50}$** | 0.182±0.05 | 0.188±0.06 | 0.195±0.02 | 0.086±0.06 | 0.077±0.02 | 0.095±0.05 |
| **$CCC^{100}$** | 0.208±0.04 | 0.239±0.04 | 0.310±0.06 | 0.110±0.08 | 0.125±0.02 | 0.145±0.02 |
| **$ABA^{25}$** | 0.176±0.02 | 0.181±0.08 | 0.208±0.07 | 0.130±0.02 | 0.148±0.06 | 0.136±0.01 |
| **$ABA^{50}$** | 0.202±0.02 | 0.213±0.02 | 0.245±0.01 | 0.133±0.01 | 0.130±0.03 | 0.141±0.02 |

The total flavonoid content (TFC) for the samples from PPT is presented in Table 4.3.4. The flavonoid levels of leaf samples were recorded highest from the $CCC^{100}$ (120 DAE) samples with 35.15±0.02 mg QE/g dry weight. $KIN^{200}$ (120 DAE) treated sample also showed equivalent TFC content against the control 25.57±0.05 mg QE/g dry weight. Over a 60 day treatment none of the PGRs showed any effect. However, TFC tends to increase with instrumental treatment time. The flavonoid levels of powdered rhizome samples were recorded highest from the 120 (DAE) treated samples of $GA_3^{200}$ with 38.27±0.01 mg QE/g dry weight against control 24.46±0.08 mg QE/g dry weight. None of the PGRs caused any change in TFC at 60 DAE and the change even after 90 days was negligeable (Table 4.5.4)

**4.10.3. Effect of PGRs on Total Flavonol Content**

The total flavonol content for the samples from PPU was determined from the regression equation of the calibration curve obtained from rutin (mg RtE/g Dry weight). Results are presented in Table 4.3.5. The flavonol levels of the leaf samples were recorded highest from the 120 (DAE) treatment samples of $ABA^{50}$ with 25.52±0.02 mg RtE/g dry weight, $CCC^{100}$ showed equivalent effect in flavonol content after 90 and 120 days of treatment. In case of rhizome samples also $ABA^{50}$ (120 DAE) showed 23.45±0.05 mg RtE/g dry weight against the content in control treatment being 16.74±0.09 mg RtR/g dry weight. Other treatments both in leaves and rhizomes did not cause any difference.

The total flavonol content for the samples from PPT was presented in Table 4.3.6. The flavonol levels of leaf samples were recorded highest from the $KIN^{200}$ (120 DAE) treatment with 29.16±0.06 mg RtE/g Dry weight followed by 120 days treatment with $CCC^{100}$ and $ABA^{50}$. Any of the PGRs could not evoke significant response at a 60 day period of treatment but the least response was observed by

$IAA^{200}$ treatment for 120 days. The flavonol levels of powdered rhizome samples were recorded highest from the 90 (DAE) treatment of samples with $ABA^{50}$ with 27.17±0.12 mg RtE/g dry weight which is followed by 120 (DAE) treatment with $ABA^{50}$ and $KIN^{200}$. The flavanol content could not be influenced by PGR till 60 (DAE) period except by Kinetin, $CC^{100}$ and $ABA^{50}$. However, the lowest effect was caused by $GA_3^{100}$ at 13.38±0.32 mg RtE/g dry weight against the control value of 12.26±0.11 mg RtE/g dry weight. The results suggested significant variability of total flavonol especially in rhizomes between the PPT and PPU after treatment.

**4.10.4. Effect of PGRs on Crude Alkaloid Content of leaves and rhizomes**

The crude alkaloid content of leaves of PPU did not show any trend upto a 90 day period of treatment. However, after 120 DAE the content showed a significant increase with KIN, CCC and MH treatment. The crude alkaloid content of the leaves sample from PPU was recorded maximum with $CCC^{100}$ (120 DAE) treatment showing 22.45±0.06 mg/g FW against control 16.83±0.09 mg/g FW. Similarly, for rhizome samples also showed the same tendency where $MH^{100}$ (120 DAE) gave maximum with 36.60±0.29 mg/g FW against control 25.24±0.08 mg/g FW. In this case of course the effect of $MH^{100}$ was seen to progressively increase with the duration of treatment (Table 4.3.7). The crude alkaloid content of the leaves sample of PPT was recorded maximum at 26.73±0.36 mg/g FW with $MH^{100}$ (120 DAE) treatments against control 21.33±0.49 mg/g FW. $KIN^{200}$ and $CCC^{100}$ (120 DAE) also showed a comparable value of alkaloid content between 24.5 and 26.5 mg/g DW. The results are more visible only after 90 days of treatment. For the rhizome samples the maximum was recorded with 39.40±0.06 against control 22.24±0.05 mg/g in the sample treated with (Table 4.3.8).

**Table-4.2.20** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Catalase Activity (units/min/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Catalase Activity (units/min/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.488±0.16 | 0.539±0.04 | 0.474±0.11 | 0.425±0.06 | 0.533±0.02 | 0.461±0.04 |
| **$IAA^{100}$** | 0.421±0.06 | 0.535±0.11 | 0.602±0.08 | 0.451±0.02 | 0.536±0.03 | 0.561±0.06 |
| **$IAA^{200}$** | 0.452±0.14 | 0.467±0.01 | 0.586±0.17 | 0.501±0.02 | 0.548±0.01 | 0.572±0.02 |
| **$GA_3^{100}$** | 0.412±0.06 | 0.479±0.06 | 0.517±0.23 | 0.496±0.02 | 0.512±0.25 | 0.578±0.14 |
| **$GA_3^{200}$** | 0.750±0.01 | 0.852±0.05 | 0.509±0.11 | 0.635±0.10 | 0.660±0.11 | 0.595±0.01 |
| **$KIN^{100}$** | 0.559±0.01 | 0.664±0.11 | 0.558±0.15 | 0.588±0.14 | 0.546±0.05 | 0.624±0.03 |
| **$KIN^{200}$** | 0.522±0.17 | 0.623±0.21 | 0.532±0.11 | 0.521±0.05 | 0.530±0.29 | 0.652±0.03 |
| **$MH^{50}$** | 0.458±0.12 | 0.440±0.26 | 0.385±0.02 | 0.405±0.06 | 0.388±0.05 | 0.356±0.31 |
| **$MH^{100}$** | 0.530±0.11 | 0.418±0.28 | 0.389±0.06 | 0.454±0.05 | 0.378±0.37 | 0.325±0.29 |
| **$CCC^{50}$** | 0.630±0.06 | 0.691±0.02 | 0.492±0.04 | 0.525±0.02 | 0.556±0.21 | 0.611±0.02 |
| **$CCC^{100}$** | 0.685±0.05 | 0.701±0.01 | 0.538±0.02 | 0.624±0.26 | 0.715±0.02 | 0.594±0.01 |
| **$ABA^{25}$** | 0.558±0.25 | 0.514±0.05 | 0.492±0.02 | 0.453±0.02 | 0.554±0.09 | 0.607±0.02 |
| **$ABA^{50}$** | 0.437±0.02 | 0.472±0.03 | 0.384±0.05 | 0.384±0.05 | 0.358±0.04 | 0.405±0.11 |

**Table-4.2.21** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Catalase Activity (units/min/g FW) of *Paris polyphylla* and Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Catalase Activity (units/min/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.634±0.05 | 0.685±0.14 | 0.654±0.09 | 0.574±0.04 | 0.623±0.17 | 0.615±0.13 |
| **$IAA^{100}$** | 0.436±0.06 | 0.521±0.26 | 0.548±0.08 | 0.521±0.11 | 0.596±0.23 | 0.603±0.24 |
| **$IAA^{200}$** | 0.523±0.14 | 0.557±0.22 | 0.436±0.15 | 0.563±0.09 | 0.541±0.14 | 0.612±0.56 |
| **$GA_3^{100}$** | 0.605±0.23 | 0.589±0.06 | 0.567±0.13 | 0.485±0.02 | 0.512±0.25 | 0.568±0.14 |
| **$GA_3^{200}$** | 0.660±0.12 | 0.708±0.02 | 0.612±0.23 | 0.655±0.13 | 0.668±0.36 | 0.485±0.22 |
| **$KIN^{100}$** | 0.479±0.16 | 0.534±0.18 | 0.568±0.12 | 0.478±0.15 | 0.526±0.24 | 0.574±0.35 |
| **$KIN^{200}$** | 0.436±0.17 | 0.463±0.16 | 0.512±0.14 | 0.517±0.28 | 0.521±0.29 | 0.542±0.45 |
| **$MH^{50}$** | 0.723±0.36 | 0.721±0.15 | 0.678±0.45 | 0.683±0.14 | 0.691±0.34 | 0.610±0.08 |
| **$MH^{100}$** | 0.641±0.13 | 0.754±0.01 | 0.551±0.13 | 0.603±0.05 | 0.651±0.25 | 0.478±0.07 |
| **$CCC^{50}$** | 0.751±0.12 | 0.691±0.02 | 0.662±0.25 | 0.576±0.01 | 0.636±0.21 | 0.621±0.09 |
| **$CCC^{100}$** | 0.631±0.17 | 0.772±0.06 | 0.579±0.02 | 0.588±0.01 | 0.678±0.23 | 0.525±0.06 |
| **$ABA^{25}$** | 0.515±0.02 | 0.481±0.08 | 0.440±0.03 | 0.463±0.02 | 0.433±0.09 | 0.405±0.02 |
| **$ABA^{50}$** | 0.490±0.01 | 0.483±0.07 | 0.380±0.03 | 0.495±0.08 | 0.458±0.21 | 0.389±0.01 |

**Table-4.2.22.** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Peroxidase Activity (units/min/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Peroxidase Activity (units/min/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.284±0.02 | 0.223±0.11 | 0.267±0.12 | 0.251±0.21 | 0.355±0.08 | 0.311±0.17 |
| **$IAA^{100}$** | 0.241±0.02 | 0.266±0.01 | 0.264±0.12 | 0.262±0.02 | 0.314±0.02 | 0.302±0.06 |
| **$IAA^{200}$** | 0.283±0.05 | 0.216±0.02 | 0.286±0.02 | 0.268±0.06 | 0.287±0.11 | 0.264±0.05 |
| **$GA_3^{100}$** | 0.233±0.03 | 0.247±0.09 | 0.268±0.01 | 0.218±0.16 | 0.247±0.02 | 0.245±0.15 |
| **$GA_3^{200}$** | 0.295±0.02 | 0.382±0.01 | 0.375±0.16 | 0.272±0.01 | 0.403±0.05 | 0.441±0.03 |
| **$KIN^{100}$** | 0.224±0.06 | 0.258±0.15 | 0.259±0.18 | 0.312±0.14 | 0.262±0.05 | 0.312±0.01 |
| **$KIN^{200}$** | 0.321±0.03 | 0.302±0.05 | 0.341±0.13 | 0.365±0.11 | 0.384±0.25 | 0.377±0.02 |
| **$MH^{50}$** | 0.285±0.06 | 0.365±0.05 | 0.291±0.26 | 0.280±0.15 | 0.196±0.14 | 0.316±0.04 |
| **$MH^{100}$** | 0.391±0.01 | 0.438±0.02 | 0.314±0.01 | 0.315±0.09 | 0.425±0.05 | 0.249±0.02 |
| **$CCC^{50}$** | 0.258±0.01 | 0.265±0.02 | 0.315±0.08 | 0.324±0.08 | 0.317±0.05 | 0.342±0.04 |
| **$CCC^{100}$** | 0.325±0.19 | 0.381±0.06 | 0.287±0.22 | 0.227±0.08 | 0.271±0.02 | 0.252±0.12 |
| **$ABA^{25}$** | 0.232±0.05 | 0.214±0.05 | 0.204±0.06 | 0.236±0.03 | 0.215±0.09 | 0.176±0.05 |
| **$ABA^{50}$** | 0.209±0.01 | 0.184±0.03 | 0.143±0.06 | 0.215±0.01 | 0.199±0.04 | 0.156±0.01 |

**Table-4.2.23.** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Peroxidase Activity (units/min/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from the leaves and rhizomes collected from 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Peroxidase Activity (units/min/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.264±0.09 | 0.254±0.06 | 0.278±0.14 | 0.361±0.11 | 0.285±0.23 | 0.261±0.04 |
| **$IAA^{100}$** | 0.254±0.06 | 0.266±0.05 | 0.284±0.16 | 0.412±0.02 | 0.325±0.25 | 0.304±0.28 |
| **$IAA^{200}$** | 0.271±0.05 | 0.256±0.07 | 0.265±0.14 | 0.368±0.21 | 0.314±0.14 | 0.274±0.14 |
| **$GA_3^{100}$** | 0.263±0.08 | 0.247±0.09 | 0.291±0.04 | 0.345±0.16 | 0.298±0.13 | 0.285±0.25 |
| **$GA_3^{200}$** | 0.274±0.08 | 0.354±0.08 | 0.358±0.11 | 0.374±0.24 | 0.403±0.24 | 0.441±0.03 |
| **$KIN^{100}$** | 0.271±0.06 | 0.268±0.15 | 0.289±0.18 | 0.412±0.14 | 0.312±0.26 | 0.265±0.01 |
| **$KIN^{200}$** | 0.281±0.03 | 0.251±0.05 | 0.274±0.13 | 0.395±0.16 | 0.284±0.63 | 0.267±0.06 |
| **$MH^{50}$** | 0.285±0.02 | 0.314±0.04 | 0.261±0.17 | 0.286±0.15 | 0.216±0.14 | 0.187±0.04 |
| **$MH^{100}$** | 0.358±0.01 | 0.375±0.07 | 0.284±0.01 | 0.315±0.09 | 0.411±0.16 | 0.285±0.08 |
| **$CCC^{50}$** | 0.258±0.04 | 0.275±0.06 | 0.285±0.08 | 0.314±0.08 | 0.375±0.56 | 0.241±0.14 |
| **$CCC^{100}$** | 0.325±0.13 | 0.392±0.02 | 0.187±0.09 | 0.287±0.08 | 0.348±0.14 | 0.212±0.12 |
| **$ABA^{25}$** | 0.251±0.04 | 0.214±0.05 | 0.189±0.06 | 0.294±0.03 | 0.256±0.09 | 0.235±0.21 |
| **$ABA^{50}$** | 0.223±0.11 | 0.189±0.01 | 0.161.10 | 0.285±0.15 | 0.265±0.02 | 0.247±0.01 |

**Table-4.2.24** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Superoxide Dismutase Activity (units/min/g FW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Data recorded from leaves and rhizomes of 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Superoxide Dismutase Activity (units/min/g FW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.487±0.08 | 0.365±0.03 | 0.352±0.06 | 0.312±0.06 | 0.324±0.14 | 0.341±0.04 |
| **$IAA^{100}$** | 0.367±0.05 | 0.542±0.04 | 0.256±0.15 | 0.345±0.14 | 0.356±0.06 | 0.352±0.03 |
| **$IAA^{200}$** | 0.254±0.01 | 0.345±0.06 | 0.365±0.13 | 0.512±0.07 | 0.369±0.02 | 0.352±0.24 |
| **$GA_3^{100}$** | 0.523±0.23 | 0.614±0.01 | 0.756±0.03 | 0.325±0.02 | 0.362±0.08 | 0.414±0.01 |
| **$GA_3^{200}$** | 0.495±0.25 | 0.668±0.09 | 0.695±0.16 | 0.425±0.08 | 0.502±0.01 | 0.565±0.08 |
| **$KIN^{100}$** | 0.512±0.06 | 0.493±0.18 | 0.386±0.11 | 0.321±0.02 | 0.322±0.06 | 0.361±0.07 |
| **$KIN^{200}$** | 0.491±0.06 | 0.354±0.14 | 0.366±0.16 | 0.363±0.14 | 0.315±0.09 | 0.352±0.06 |
| **$MH^{50}$** | 0.455±0.01 | 0.481±0.23 | 0.502±0.14 | 0.365±0.16 | 0.384±0.05 | 0.432±0.02 |
| **$MH^{100}$** | 0.463±0.03 | 0.589±0.14 | 0.351±0.16 | 0.385±0.14 | 0.461±0.04 | 0.334±0.09 |
| **$CCC^{50}$** | 0.458±0.21 | 0.424±0.16 | 0.464±0.14 | 0.331±0.14 | 0.387±0.03 | 0.403±0.07 |
| **$CCC^{100}$** | 0.465±0.28 | 0.547±0.14 | 0.361±0.08 | 0.481±0.17 | 0.502±0.21 | 0.324±0.05 |
| **$ABA^{25}$** | 0.396±0.01 | 0.338±0.13 | 0.332±0.06 | 0.322±0.14 | 0.312±0.08 | 0.328±0.09 |
| **$ABA^{50}$** | 0.374±0.02 | 0.345±0.08 | 0.310±0.05 | 0.311±0.01 | 0.295±0.01 | 0.288±0.02 |

**Table-4.2.25** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Superoxide Dismutase Activity (units/min/g FW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Data recorded from leaves and rhizomes of 10 uniformly grown plants raised from rhizomes at Lingthem, Dzongu, North Sikkim after 60, 90 and 120 days of emergence. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Superoxide Dismutase Activity (units/min/g FW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 0.265±0.08 | 0.314±0.16 | 0.345±0.14 | 0.231±0.08 | 0.285±0.03 | 0.263±0.02 |
| **$IAA^{100}$** | 0.252±0.06 | 0.287±0.02 | 0.364±0.16 | 0.325±0.16 | 0.235±0.01 | 0.236±0.05 |
| **$IAA^{200}$** | 0.361±0.05 | 0.324±0.14 | 0.356±0.09 | 0.254±0.11 | 0.326±0.06 | 0.354±0.02 |
| **$GA_3^{100}$** | 0.278±0.01 | 0.351±0.16 | 0.352±0.08 | 0.215±0.16 | 0.356±0.03 | 0.215±0.09 |
| **$GA_3^{200}$** | 0.353±0.08 | 0.286±0.15 | 0.428±0.05 | 0.232±0.12 | 0.354±0.14 | 0.414±0.04 |
| **$KIN^{100}$** | 0.301±0.06 | 0.265±0.15 | 0.315±0.24 | 0.202±0.03 | 0.362±0.15 | 0.318±0.14 |
| **$KIN^{200}$** | 0.274±0.16 | 0.312±0.02 | 0.354±0.16 | 0.236±0.17 | 0.254±0.16 | 0.281±0.23 |
| **$MH^{50}$** | 0.256±0.03 | 0.286±0.14 | 0.298±0.14 | 0.284±0.01 | 0.312±0.14 | 0.356±0.14 |
| **$MH^{100}$** | 0.335±0.01 | 0.366±0.09 | 0.324±0.14 | 0.311±0.08 | 0.359±0.18 | 0.218±0.21 |
| **$CCC^{50}$** | 0.288±0.06 | 0.359±0.02 | 0.177±0.03 | 0.256±0.05 | 0.354±0.16 | 0.274±0.13 |
| **$CCC^{100}$** | 0.269±0.05 | 0.365±0.02 | 0.245±0.08 | 0.242±0.09 | 0.352±0.14 | 0.189±0.13 |
| **$ABA^{25}$** | 0.246±0.04 | 0.223±0.03 | 0.245±0.05 | 0.265±0.17 | 0.261±0.02 | 0.235±0.08 |
| **$ABA^{50}$** | 0.256±0.04 | 0.189±0.02 | 0.162±0.02 | 0.212±0.06 | 0.206±0.15 | 0.187±0.02 |

### 4.10.5. Effect of PGRs on Crude Saponin Content

The crude saponin content of the leaves sample from PPU was influenced by some of the PGRs used with maximum at 26.44±0.03 mg/g FW against control 19.25±0.37 mg/g FW shown by treatment with $CCC^{50}$ (120 DAE). The growth promoters $GA_3$ and KIN also showed comparable effects towards increasing the crude alkaloid content. However, they acted only at the higher concentration used for the longest time. For rhizome samples the maximum was recorded with 35.25±0.02 against control 27.51±0.31 mg/g FW the sample treated with $CCC^{50}$ (120 DAE) (Table 4.3.9). Interestingly, in this case IAA, KIN, MH and ABA had similar but negligible effect.

The crude saponin content of the leaf sample from PPT was recorded maximum with 25.14±0.02 mg/g FW against control 16.36±0.07 mg/g FW from the sample treated with $CCC^{50}$ (120 DAE). The effect of $GA_3^{200}$ (120 DAE) treatment was also appreciable. However, IAA, MH and ABA had no effect. Similarly, for rhizome samples the maximum was recorded with 37.87±0.02 mg/g FW from the sample treated with $CCC^{50}$ (120 DAE) In this case, also $GA_3^{200}$ (120 DAE) was the next best treatment for increasing the content while IAA, KIN, MH and ABA had significant effect in changing the crude alkaloid content (Table 4.3.10). Though CCC treatment appeared to be suitable for enhancing the alkaloid content, it appeared that at 100 µg/ml and beyond it become toxic.

### 4.10.6. Effect of PGRs on Diosgenin Content of leaves and rhizomes

Diosgenin is the universal metabolite across all the taxa of *P. polyphylla* that could be used as a marker chemical for the identification and determination of quality of the species in terms of medicinal usage. The diosgenin content of the leaves sample from PPU was recorded maximum with 2.301±0.16 mg/g FW on $GA_3^{200}$ treatment

**Table-4.3.1** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Phenolics Content (mg GAE/g DW*) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Phenolic Content (mg GAE/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 11.76±0.11 | 13.54±0.12 | 13.72±0.08 | 04.47±0.34 | 05.65±0.07 | 06.11±0.44 |
| **$IAA^{100}$** | 12.19±0.12 | 13.83±0.22 | 14.32±0.19 | 05.19±0.14 | 06.13±0.06 | 06.83±0.09 |
| **$IAA^{200}$** | 12.51±0.03 | 13.88±0.15 | 14.29±0.03 | 05.11±0.03 | 05.88±0.11 | 06.29±0.04 |
| **$GA_3^{100}$** | 13.35±0.51 | 13.69±0.21 | 13.84±0.09 | 04.55±0.56 | 06.69±0.22 | 06.64±0.05 |
| **$GA_3^{200}$** | 12.84±0.98 | 13.72±0.27 | 15.04±0.10 | 05.84±0.98 | 06.12±0.24 | 06.74±0.04 |
| **$KIN^{100}$** | 13.11±0.02 | 14.13±0.29 | 13.83±0.14 | 05.21±0.11 | 06.13±0.27 | 07.13±2.22 |
| **$KIN^{200}$** | 19.19±0.11 | 20.13±0.08 | 21.39±0.22 | 05.26±0.05 | 06.33±0.34 | 06.99±0.13 |
| **$MH^{50}$** | 12.85±0.16 | 23.79±0.36 | 24.18±1.05 | 06.05±0.02 | 07.85±0.36 | 10.13±0.14 |
| **$MH^{100}$** | 18.15±0.11 | 22.58±0.41 | 14.14±0.12 | 06.04±0.08 | 09.19±0.12 | 07.14±0.09 |
| **$CCC^{50}$** | 19.19±0.14 | 19.78±0.39 | 21.24±0.14 | 05.39±0.04 | 06.33±0.16 | 07.11±2.03 |
| **$CCC^{100}$** | 22.15±0.33 | 23.59±0.22 | 24.18±0.02 | 07.89±0.52 | 08.58±0.15 | 10.15±0.10 |
| **$ABA^{25}$** | 11.35±0.22 | 14.23±0.11 | 13.10±0.13 | 05.35±0.12 | 06.89±0.36 | 07.10±1.08 |
| **$ABA^{50}$** | 11.94±0.24 | 14.42±0.24 | 13.74±0.19 | 05.24±0.03 | 07.21±0.09 | 06.09±0.06 |

*DW=Dry Weight.

**Table-4.3.2** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Phenolics Content (mg GAE/g DW*) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Phenolic Content (mg GAE/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 16.27±0.11 | 19.35±0.18 | 19.34±0.08 | 9.41±0.05 | 15.20±0.31 | 16.29±0.09 |
| **$IAA^{100}$** | 18.12±0.18 | 19.23±0.06 | 20.83±0.49 | 9.89±0.22 | 16.13±0.09 | 16.93±0.49 |
| **$IAA^{200}$** | 17.11±0.03 | 19.58±0.06 | 20.29±0.03 | 9.91±0.18 | 16.32±0.08 | 17.21±0.33 |
| **$GA_3^{100}$** | 17.35±0.56 | 20.69±0.41 | 21.10±0.79 | 10.35±0.12 | 16.19±0.31 | 17.10±0.15 |
| **$GA_3^{200}$** | 16.84±0.18 | 20.32±0.08 | 22.14±1.54 | 10.84±0.05 | 16.42±0.02 | 17.14±0.56 |
| **$KIN^{100}$** | 18.21±0.11 | 21.83±0.67 | 23.19±0.02 | 10.52±0.37 | 11.36±0.22 | 14.53±0.06 |
| **$KIN^{200}$** | 21.85±0.56 | 22.51±0.35 | 25.20±0.06 | 9.61±0.06 | 16.18±0.03 | 17.89±0.41 |
| **$MH^{50}$** | 17.25±0.16 | 21.09±0.09 | 20.17±0.19 | 10.35±0.08 | 16.64±0.02 | 16.72±0.54 |
| **$MH^{100}$** | 18.14±0.08 | 22.68±0.26 | 23.15±0.08 | 11.04±0.06 | 20.57±0.35 | 21.63±0.45 |
| **$CCC^{50}$** | 19.19±0.24 | 21.10±0.06 | 24.81±0.13 | 10.21±0.18 | 19.41±0.05 | 22.84±0.06 |
| **$CCC^{100}$** | 19.21±0.13 | 24.39±0.15 | 27.13±0.05 | 10.15±0.13 | 16.78±0.11 | 27.22±0.09 |
| **$ABA^{25}$** | 18.15±0.16 | 20.15±0.08 | 22.17±0.29 | 10.35±0.06 | 15.79±0.04 | 17.77±0.09 |
| **$ABA^{50}$** | 16.84±0.08 | 21.72±0.10 | 21.19±0.55 | 10.07±0.08 | 16.28±0.08 | 17.34±0.08 |

*DW=Dry Weight.

against control 1.402±0.01 mg/g FW and all the other treatments remained only marginally effective. However, for the rhizome samples treatments with $GA_3^{200}$, $CCC^{50}$, $ABA^{25}$ and $ABA^{50}$ were all appreciable and equally effective in increasing the diosgenin level raising it to between 3.0 and 3.3 mg/g dry weight (Table 4.3.11). Similarly, the diosgenin content of the leaves sample from PPT was recorded maximum with 2.436±0.04 mg/g dry weight against control 1.427±0.03 mg/g dry weight with $GA_3^{200}$ treatment (120 DAE). Of course, in this case, $CCC^{100}$ and $ABA^{50}$ over a 120 day period also showed appreciable. However, for the rhizome samples, the maximum diosgenin content was recorded with 3.708±0.88 mg/g dry weight with $ABA^{50}$ (120 DAE) against control 2.594±0.12 mg/g dry weight. $GA_3^{200}$, $CCC^{100}$, $CCC^{50}$ and $ABA^{25}$ also gave appreciable effect with 3.0-3.4 mg/g DW of diosgenin content. Though these PGRs are effective in sealing up diosgenin production, there is no significant variability of its content between the PPT and PPU after treatment [(Table 4.3.11 and 4.3.12 and Figure 4.8 (A-D)].

**Table-4.3.3** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Flavonoid Content (mg QE/g DW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Flavonoid Content (mg QE/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 18.21±0.02 | 18.42±0.04 | 23.57±0.05 | 21.16±0.58 | 21.13±0.03 | 22.89±0.01 |
| **$IAA^{100}$** | 19.19±0.14 | 18.13±0.03 | 23.83±0.09 | 21.19±0.09 | 22.17±0.07 | 23.73±0.49 |
| **$IAA^{200}$** | 19.11±0.03 | 19.38±0.03 | 24.29±0.33 | 21.31±0.04 | 22.18±0.12 | 24.19±0.03 |
| **$GA_3^{100}$** | 19.35±0.56 | 19.69±0.21 | 23.10±0.07 | 22.15±0.12 | 22.61±0.36 | 24.10±0.79 |
| **$GA_3^{200}$** | 23.59±0.12 | 27.34±0.02 | 31.45±0.14 | 24.84±0.09 | 30.68±0.02 | 31.55±0.02 |
| **$KIN^{100}$** | 20.14±0.10 | 22.28±0.16 | 24.08±0.49 | 22.19±0.08 | 25.50±0.29 | 27.34±0.01 |
| **$KIN^{200}$** | 23.68±0.09 | 27.97±0.32 | 31.68±0.21 | 24.62±0.33 | 27.78±0.15 | 34.52±0.43 |
| **$MH^{50}$** | 19.89±0.56 | 20.69±0.21 | 24.10±0.79 | 22.21±0.05 | 23.09±0.08 | 24.60±0.43 |
| **$MH^{100}$** | 21.84±0.98 | 22.38±0.05 | 24.14±0.54 | 22.84±0.04 | 24.12±0.02 | 25.20±0.14 |
| **$CCC^{50}$** | 20.11±0.14 | 23.15±0.01 | 26.38±0.06 | 22.47±0.06 | 23.35±0.15 | 23.98±0.02 |
| **$CCC^{100}$** | 20.15±0.03 | 25.65±0.19 | 28.35±0.06 | 22.21±0.08 | 25.60±0.31 | 23.21±0.07 |
| **$ABA^{25}$** | 19.95±0.56 | 20.16±0.21 | 25.16±0.79 | 21.85±0.13 | 22.14±0.23 | 22.90±0.03 |
| **$ABA^{50}$** | 20.14±0.98 | 20.42±0.02 | 24.18±0.54 | 21.74±0.17 | 22.22±0.05 | 23.17±0.56 |

**Table-4.3.4** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Flavonoid Content (mg QE/g DW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Flavonoid Content (mg QE/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 24.22±0.02 | 24.92±0.03 | 25.57±0.05 | 23.16±0.12 | 24.13±0.21 | 24.46±0.08 |
| **$IAA^{100}$** | 24.69±0.14 | 25.13±0.03 | 25.83±0.49 | 23.19±0.16 | 25.10±0.43 | 24.83±0.49 |
| **$IAA^{200}$** | 24.54±0.03 | 25.38±0.03 | 25.69±0.33 | 24.11±0.03 | 25.02±0.63 | 24.69±0.33 |
| **$GA_3^{100}$** | 25.35±0.56 | 25.62±0.21 | 24.72±0.05 | 24.35±0.56 | 25.62±0.05 | 24.70±0.72 |
| **$GA_3^{200}$** | 25.34±0.98 | 29.34±0.02 | 31.65±0.44 | 24.84±0.98 | 27.78±0.11 | 38.27±0.01 |
| **$KIN^{100}$** | 25.19±0.14 | 27.39±0.12 | 29.16±0.05 | 24.14±0.14 | 27.78±0.58 | 31.68±0.22 |
| **$KIN^{200}$** | 26.01±0.03 | 26.68±0.05 | 35.14±0.03 | 26.34±0.03 | 29.15±0.08 | 33.51±0.01 |
| **$MH^{50}$** | 25.39±0.56 | 25.09±0.21 | 25.19±0.02 | 24.11±0.56 | 25.73±0.07 | 25.17±0.06 |
| **$MH^{100}$** | 25.34±0.98 | 25.28±0.02 | 26.04±0.54 | 25.06±0.98 | 25.02±0.09 | 25.44±0.11 |
| **$CCC^{50}$** | 24.89±0.14 | 28.63±0.03 | 31.32±2.01 | 26.09±0.14 | 26.31±0.22 | 26.25±0.02 |
| **$CCC^{100}$** | 25.08±0.03 | 31.38±0.01 | 35.15±0.02 | 28.12±0.01 | 27.34±0.19 | 28.15±0.06 |
| **$ABA^{25}$** | 26.15±0.56 | 25.61±0.21 | 26.10±0.79 | 25.95±0.56 | 24.72±0.20 | 24.83±0.02 |
| **$ABA^{50}$** | 25.24±0.98 | 25.07±0.02 | 25.09±0.54 | 23.81±0.98 | 25.36±0.06 | 25.06±0.06 |

**Table-4.3.5** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Flavonol Content (mg RtE/g DW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Flavonol Content (mg RtE/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 10.11±0.17 | 12.32±0.08 | 13.42±0.04 | 12.26±0.11 | 13.41±0.06 | 16.74±0.09 |
| **$IAA^{100}$** | 11.19±0.14 | 12.13±0.03 | 13.53±0.49 | 13.39±0.14 | 13.12±0.03 | 16.83±0.41 |
| **$IAA^{200}$** | 11.13±0.03 | 13.38±0.03 | 13.29±0.33 | 13.24±0.06 | 13.88±0.05 | 17.21±0.08 |
| **$GA_3^{100}$** | 11.35±0.56 | 14.65±0.08 | 16.98±0.06 | 14.32±0.52 | 19.35±0.11 | 19.08±0.02 |
| **$GA_3^{200}$** | 11.84±0.18 | 15.65±0.45 | 17.89±0.24 | 16.89±0.18 | 18.39±0.08 | 21.64±0.15 |
| **$KIN^{100}$** | 10.29±0.14 | 13.15±0.03 | 13.83±0.49 | 12.69±0.19 | 14.13±0.13 | 17.14±0.19 |
| **$KIN^{200}$** | 11.31±0.03 | 17.35±0.02 | 19.13±0.02 | 13.11±0.08 | 16.54±0.19 | 17.63±0.31 |
| **$MH^{50}$** | 13.35±0.16 | 12.69±0.21 | 12.10±0.79 | 13.47±0.16 | 14.62±0.11 | 17.61±0.79 |
| **$MH^{100}$** | 13.14±0.08 | 12.56±0.02 | 14.14±0.54 | 13.81±0.28 | 14.28±0.08 | 16.84±0.04 |
| **$CCC^{50}$** | 13.12±0.14 | 18.15±0.09 | 19.33±0.06 | 13.64±0.64 | 16.39±0.08 | 17.33±0.21 |
| **$CCC^{100}$** | 12.09±0.03 | 19.44±0.03 | 24.35±0.11 | 14.21±0.03 | 20.16±0.13 | 22.51±0.02 |
| **$ABA^{25}$** | 11.55±0.16 | 13.64±0.21 | 13.14±0.79 | 13.24±0.26 | 14.22±0.07 | 17.28±0.18 |
| **$ABA^{50}$** | 14.32±0.06 | 20.13±0.02 | 25.52±0.02 | 18.97±0.34 | 20.76±0.02 | 23.45±0.05 |

**Table-4.3.6** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Total Flavonoid Content (mg QE/g DW) of *Paris polyphylla* from Tholung (PPT0 in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Total Flavonol Content (mg RtE/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 17.11±0.17 | 19.32±0.06 | 19.42±0.07 | 12.26±0.11 | 14.41±0.04 | 15.24±0.09 |
| **$IAA^{100}$** | 17.52±0.12 | 18.15±0.08 | 18.55±0.05 | 14.22±0.04 | 15.10±0.03 | 15.84±0.46 |
| **$IAA^{200}$** | 18.21±0.03 | 20.88±0.27 | 18.45±0.03 | 13.54±0.06 | 15.27±0.05 | 16.45±0.02 |
| **$GA_3^{100}$** | 18.09±0.06 | 20.64±0.35 | 19.65±0.19 | 13.38±0.32 | 16.32±0.16 | 16.36±0.09 |
| **$GA_3^{200}$** | 18.15±0.11 | 21.63±0.06 | 19.78±0.64 | 13.54±0.18 | 14.83±0.08 | 17.27±0.04 |
| **$KIN^{100}$** | 18.28±0.14 | 23.37±0.04 | 20.34±0.16 | 17.65±0.02 | 19.66±0.38 | 23.17±0.06 |
| **$KIN^{200}$** | 19.61±0.05 | 26.03±0.01 | 29.16±0.06 | 18.87±0.08 | 22.61±0.27 | 24.13±0.01 |
| **$MH^{50}$** | 19.08±0.06 | 18.91±0.02 | 18.42±0.05 | 13.54±0.16 | 15.55±0.55 | 16.54±0.19 |
| **$MH^{100}$** | 20.34±0.08 | 19.54±0.52 | 20.33±0.07 | 14.51±0.02 | 15.89±0.06 | 16.08±0.04 |
| **$CCC^{50}$** | 19.36±0.12 | 19.26±0.33 | 19.62±0.19 | 13.84±0.14 | 14.85±0.02 | 16.39±0.21 |
| **$CCC^{100}$** | 18.12±0.04 | 24.45±0.03 | 27.69±0.25 | 19.35±0.03 | 22.68±0.11 | 23.16±0.13 |
| **$ABA^{25}$** | 18.59±0.08 | 21.56±0.36 | 24.59±0.12 | 13.57±0.06 | 20.68±0.08 | 22.15±0.10 |
| **$ABA^{50}$** | 18.82±0.06 | 22.35±0.14 | 26.23±0.09 | 20.05±0.02 | 27.17±0.12 | 25.65±0.07 |

**Table-4.3.7** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Crude Alkaloid Content (mg/g DW) of leaves and rhizomes of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Crude Alkaloid Content (mg/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 10.19±0.03 | 15.13±0.02 | 16.83±0.09 | 22.38±0.06 | 26.29±0.35 | 25.24±0.08 |
| **$IAA^{100}$** | 11.08±0.18 | 15.52±0.45 | 16.23±0.49 | 22.48±0.14 | 26.34±0.63 | 27.02±0.36 |
| **$IAA^{200}$** | 10.27±0.03 | 15.32±0.08 | 16.89±0.07 | 22.56±0.15 | 26.41±0.03 | 26.63±0.38 |
| **$GA_3^{100}$** | 09.69±0.56 | 15.42±0.21 | 17.09±0.79 | 22.63±0.08 | 26.41±0.21 | 27.10±0.09 |
| **$GA_3^{200}$** | 10.81±0.58 | 16.02±0.02 | 17.32±0.05 | 22.72±0.06 | 27.13±0.02 | 27.32±0.56 |
| **$KIN^{100}$** | 11.36±0.14 | 17.30±0.09 | 21.56±0.21 | 22.51±0.04 | 26.53±0.03 | 26.83±0.04 |
| **$KIN^{200}$** | 13.43±0.02 | 16.68±0.14 | 22.35±0.27 | 23.09±0.04 | 27.03±0.32 | 26.21±0.37 |
| **$MH^{50}$** | 11.62±0.56 | 14.72±0.23 | 19.54±0.06 | 21.51±0.56 | 23.21±0.11 | 30.37±0.10 |
| **$MH^{100}$** | 17.36±0.11 | 18.31±0.02 | 21.75±0.06 | 26.81±0.02 | 31.72±0.09 | 36.60±0.29 |
| **$CCC^{50}$** | 11.06±0.14 | 17.43±0.08 | 20.45±0.49 | 23.08±0.14 | 27.18±0.03 | 28.29±0.13 |
| **$CCC^{100}$** | 18.60±0.28 | 21.64±0.12 | 22.45±0.06 | 22.36±0.03 | 27.42±0.21 | 31.23±0.06 |
| **$ABA^{25}$** | 10.63±0.06 | 15.28±0.22 | 17.05±0.17 | 22.76±0.16 | 27.52±0.21 | 27.13±1.04 |
| **$ABA^{50}$** | 10.21±0.23 | 14.28±0.03 | 17.23±0.54 | 23.06±0.05 | 27.30±0.24 | 26.45±0.54 |

**Table-4.3.8** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Crude Alkaloid Content (mg/g DW) of leaves and rhizomes of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Crude Alkaloid Content (mg/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 12.19±0.11 | 13.23±0.08 | 21.33±0.49 | 18.38±0.12 | 20.19±0.34 | 22.24±0.05 |
| **$IAA^{100}$** | 13.19±0.14 | 13.28±0.03 | 22.08±0.02 | 18.55±0.06 | 22.01±0.21 | 23.81±0.09 |
| **$IAA^{200}$** | 13.21±0.05 | 13.56±0.03 | 21.24±0.45 | 19.05±0.08 | 20.38±0.02 | 23.02±0.05 |
| **$GA_3^{100}$** | 13.08±0.56 | 13.54±0.21 | 22.13±0.02 | 19.21±0.56 | 20.62±0.21 | 24.10±0.72 |
| **$GA_3^{200}$** | 13.14±0.98 | 13.22±0.02 | 23.12±0.54 | 19.64±0.02 | 20.29±0.06 | 23.14±0.47 |
| **$KIN^{100}$** | 14.09±0.14 | 17.23±0.10 | 24.41±0.06 | 19.28±0.14 | 21.18±0.06 | 22.13±0.21 |
| **$KIN^{200}$** | 16.28±0.05 | 19.65±0.41 | 26.53±0.32 | 19.61±0.02 | 21.27±0.08 | 23.21±0.27 |
| **$MH^{50}$** | 13.08±0.56 | 19.54±0.36 | 23.37±0.16 | 18.71±0.06 | 24.56±0.21 | 29.60±0.02 |
| **$MH^{100}$** | 19.64±0.15 | 23.45±0.02 | 26.73±0.36 | 23.61±0.02 | 27.35±0.08 | 39.40±0.06 |
| **$CCC^{50}$** | 13.19±0.14 | 17.31±0.11 | 21.53±2.49 | 19.24±0.14 | 23.74±0.23 | 29.61±0.21 |
| **$CCC^{100}$** | 17.22±0.09 | 21.45±0.07 | 24.50±0.02 | 19.07±0.04 | 27.42±0.08 | 32.35±0.07 |
| **$ABA^{25}$** | 13.32±0.57 | 13.64±0.54 | 21.74±0.38 | 18.56±0.51 | 21.14±0.53 | 23.37±0.79 |
| **$ABA^{50}$** | 13.27±0.98 | 13.26±0.02 | 21.62±0.18 | 19.62±0.08 | 21.24±0.06 | 23.10±0.74 |

**Table-4.3.9** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Crude Saponin Content (mg/g DW) of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Crude Saponin Content (mg/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 14.45±0.11 | 16.35±0.17 | 19.25±0.37 | 24.44±0.12 | 26.76±0.05 | 27.51±0.31 |
| **$IAA^{100}$** | 16.24±0.14 | 16.56±0.03 | 21.23±0.09 | 25.11±0.14 | 27.14±0.03 | 28.32±0.24 |
| **$IAA^{200}$** | 14.71±0.09 | 17.58±0.23 | 20.41±0.33 | 25.31±0.03 | 27.08±0.03 | 28.22±0.13 |
| **$GA_3^{100}$** | 15.41±0.16 | 18.09±0.21 | 22.45±0.08 | 24.65±0.16 | 27.69±0.11 | 30.31±1.06 |
| **$GA_3^{200}$** | 14.72±0.08 | 17.42±0.12 | 24.15±0.14 | 25.24±0.18 | 29.32±0.02 | 32.65±0.14 |
| **$KIN^{100}$** | 15.19±0.19 | 17.13±0.03 | 21.88±0.36 | 25.62±0.14 | 27.13±0.03 | 28.83±0.05 |
| **$KIN^{200}$** | 15.52±0.09 | 19.27±0.12 | 23.05±0.01 | 24.71±0.03 | 27.38±0.11 | 28.59±0.33 |
| **$MH^{50}$** | 16.32±0.56 | 17.62±0.08 | 20.36±0.09 | 25.31±0.06 | 27.19±0.22 | 28.30±0.14 |
| **$MH^{100}$** | 16.44±0.08 | 17.23±0.02 | 21.23±0.04 | 25.62±0.08 | 28.09±0.12 | 28.34±0.04 |
| **$CCC^{50}$** | 18.89±0.05 | 21.35±0.03 | 26.44±0.03 | 24.96±0.14 | 29.61±0.13 | 35.25±0.02 |
| **$CCC^{100}$** | 15.81±0.03 | 17.37±0.23 | 20.24±0.33 | 25.51±0.03 | 27.24±0.23 | 28.56±0.13 |
| **$ABA^{25}$** | 15.32±0.16 | 16.92±0.21 | 20.35±0.14 | 25.55±0.06 | 26.84±0.21 | 28.32±0.09 |
| **$ABA^{50}$** | 15.26±0.08 | 17.24±0.32 | 21.18±0.34 | 25.12±0.08 | 27.62±0.22 | 28.44±0.04 |

**Table-4.3.10** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Crude Saponin Content (mg/g DW) of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. (values are mean ± SE, n=3).

| Treatments (µg/ml) | Crude Saponin Content (mg/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 14.45±0.11 | 16.15±0.05 | 16.36±0.07 | 25.44±0.12 | 27.12±0.09 | 28.51±0.45 |
| **$IAA^{100}$** | 16.34±0.17 | 17.09±0.03 | 16.56±0.09 | 27.04±0.16 | 28.21±0.06 | 28.82±0.24 |
| **$IAA^{200}$** | 15.11±0.09 | 17.24±0.23 | 19.08±0.33 | 25.33±0.03 | 28.27±0.08 | 28.86±0.13 |
| **$GA_3^{100}$** | 15.24±0.06 | 17.45±0.36 | 19.63±0.51 | 26.15±0.16 | 28.31±0.11 | 29.08±0.22 |
| **$GA_3^{200}$** | 16.68±0.02 | 20.14±0.35 | 22.56±0.34 | 27.46±0.21 | 31.34±0.09 | 33.04±0.02 |
| **$KIN^{100}$** | 14.74±0.19 | 16.45±0.45 | 20.77±0.45 | 25.63±0.12 | 28.04±0.03 | 30.02±0.05 |
| **$KIN^{200}$** | 15.78±0.09 | 19.67±0.05 | 21.35±0.06 | 25.78±0.06 | 28.12±0.11 | 28.78±0.78 |
| **$MH^{50}$** | 14.68±0.56 | 17.07±0.08 | 16.76±0.09 | 26.08±0.06 | 27.45±0.03 | 29.21±0.14 |
| **$MH^{100}$** | 15.41±0.08 | 17.63±0.02 | 16.76±0.04 | 26.12±0.02 | 28.31±0.12 | 28.54±0.08 |
| **$CCC^{50}$** | 17.76±0.04 | 21.45±0.33 | 25.14±0.02 | 29.62±0.32 | 32.55±0.25 | 37.87±0.02 |
| **$CCC^{100}$** | 15.25±0.03 | 17.46±0.27 | 20.19±0.11 | 25.75±0.03 | 27.74±0.45 | 29.16±0.65 |
| **$ABA^{25}$** | 15.06±0.16 | 17.08±0.21 | 16.55±0.71 | 26.05±0.21 | 27.54±0.21 | 29.08±0.09 |
| **$ABA^{50}$** | 14.87±0.21 | 16.44±0.02 | 16.71±0.34 | 25.73±0.42 | 27.62±0.22 | 28.73±0.06 |

**Table-4.3.11** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Diosgenin Content (mg/g DW) of leaves and rhizomes of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. The analysis was performed through HPLC system using pure Diosgenin as standard. (Values are mean of three replicates ± SE, Control=distilled water).

| Treatments (µg/ml) | Diosgenin Content (mg/g DW) from PPU | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 1.212±0.01 | 1.354±0.07 | 1.402±0.01 | 2.517±0.04 | 2.508±0.02 | 2.663±0.04 |
| **$IAA^{100}$** | 1.268±0.03 | 1.481±0.09 | 1.563±0.06 | 2.595±0.03 | 2.733±0.05 | 2.776±0.04 |
| **$IAA^{200}$** | 1.521±0.03 | 1.635±0.09 | 1.998±0.36 | 2.852±0.47 | 3.015±0.42 | 2.990±0.26 |
| **$GA_3^{100}$** | 1.487±0.05 | 1.750±0.56 | 1.892±0.35 | 2.593±0.01 | 2.950±0.11 | 3.305±0.03 |
| **$GA_3^{200}$** | 1.534±0.01 | 1.835±0.32 | 2.301±0.16 | 2.985±0.39 | 3.155±0.09 | 3.305±0.84 |
| **$KIN^{100}$** | 1.567±0.02 | 1.534±0.08 | 1.624±0.42 | 2.534±0.06 | 2.647±0.02 | 2.790±0.02 |
| **$KIN^{200}$** | 1.376±0.05 | 1.467±0.04 | 1.628±0.02 | 2.595±0.08 | 2.665±0.03 | 2.743±0.01 |
| **$MH^{50}$** | 1.399±0.11 | 1.553±0.05 | 1.414±0.69 | 2.501±0.07 | 2.532±0.21 | 2.319±0.60 |
| **$MH^{100}$** | 1.327±0.02 | 1.314±0.01 | 1.221±0.34 | 2.259±0.25 | 2.332±0.14 | 2.250±0.34 |
| **$CCC^{50}$** | 1.439±0.13 | 1.690±0.05 | 1.990±0.19 | 2.509±0.05 | 2.722±0.09 | 3.348±0.09 |
| **$CCC^{100}$** | 1.481±0.12 | 1.684±0.04 | 1.950±0.15 | 2.538±0.11 | 2.759±0.02 | 3.139±0.02 |
| **$ABA^{25}$** | 1.472±0.05 | 1.478±0.21 | 1.541±0.03 | 2.524±0.05 | 2.931±0.18 | 3.000±0.01 |
| **$ABA^{50}$** | 1.518±0.01 | 1.713±0.26 | 1.924±0.68 | 2.548±0.05 | 3.115±0.21 | 3.255±0.96 |

**Table-4.3.12** Effect of PGRs, IAA, $GA_3$, KIN (100 and 200 µg/ml), MH, CCC (50 and 100 µg/ml) and ABA (25 and 50µg/ml) on Diosgenin Content (mg/g DW) of leaves and rhizomes of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. The analysis was performed through HPLC system using pure Diosgenin as standard. (Values are mean of three replicates ± SE, Control=distilled water).

| Treatments (µg/ml) | Diosgenin Content (mg/g DW) from PPT | | | | | |
|---|---|---|---|---|---|---|
| | Leaves (Days after emergence) | | | Rhizomes (Days after emergence) | | |
| | 60 | 90 | 120 | 60 | 90 | 120 |
| **Control** | 1.268±0.02 | 1.338±0.02 | 1.427±0.03 | 2.386±0.01 | 2.604±0.03 | 2.594±0.12 |
| **$IAA^{100}$** | 1.332±0.03 | 1.489±0.02 | 1.595±0.05 | 2.483±0.01 | 2.638±0.02 | 2.905±0.25 |
| **$IAA^{200}$** | 1.346±0.03 | 1.497±0.01 | 1.679±0.02 | 2.489±0.02 | 3.154±0.63 | 3.300±0.23 |
| **$GA_3^{100}$** | 1.384±0.02 | 1.635±0.56 | 1.792±0.04 | 2.567±0.02 | 2.840±0.22 | 3.110±0.15 |
| **$GA_3^{200}$** | 1.375±0.01 | 1.822±0.54 | 2.436±0.04 | 2.597±0.04 | 3.250±0.09 | 3.463±0.14 |
| **$KIN^{100}$** | 1.564±0.03 | 1.616±0.07 | 1.734±0.08 | 2.465±0.02 | 2.693±0.01 | 2.732±0.01 |
| **$KIN^{200}$** | 1.587±0.04 | 1.639±0.09 | 1.845±0.02 | 2.461±0.02 | 2.633±0.04 | 2.786±0.01 |
| **$MH^{50}$** | 1.487±0.05 | 1.468±0.02 | 1.384±0.58 | 2.681±0.05 | 2.513±0.03 | 2.751±0.01 |
| **$MH^{100}$** | 1.477±0.02 | 1.032±0.05 | 1.113±0.16 | 2.135±0.58 | 2.000±0.01 | 1.856±0.88 |
| **$CCC^{50}$** | 1.521±0.03 | 1.567±0.10 | 1.905±0.14 | 2.475±0.06 | 2.703±0.04 | 3.000±0.20 |
| **$CCC^{100}$** | 1.564±0.04 | 1.578±0.11 | 2.076±0.08 | 2.493±0.07 | 2.689±0.02 | 3.265±0.09 |
| **$ABA^{25}$** | 1.368±0.02 | 1.547±0.02 | 1.995±0.07 | 2.502±0.08 | 2.913±0.09 | 3.110±0.14 |
| **$ABA^{50}$** | 1.375±0.01 | 2.032±0.06 | 2.115±0.45 | 2.509±0.05 | 2.947±0.58 | 3.708±0.88 |

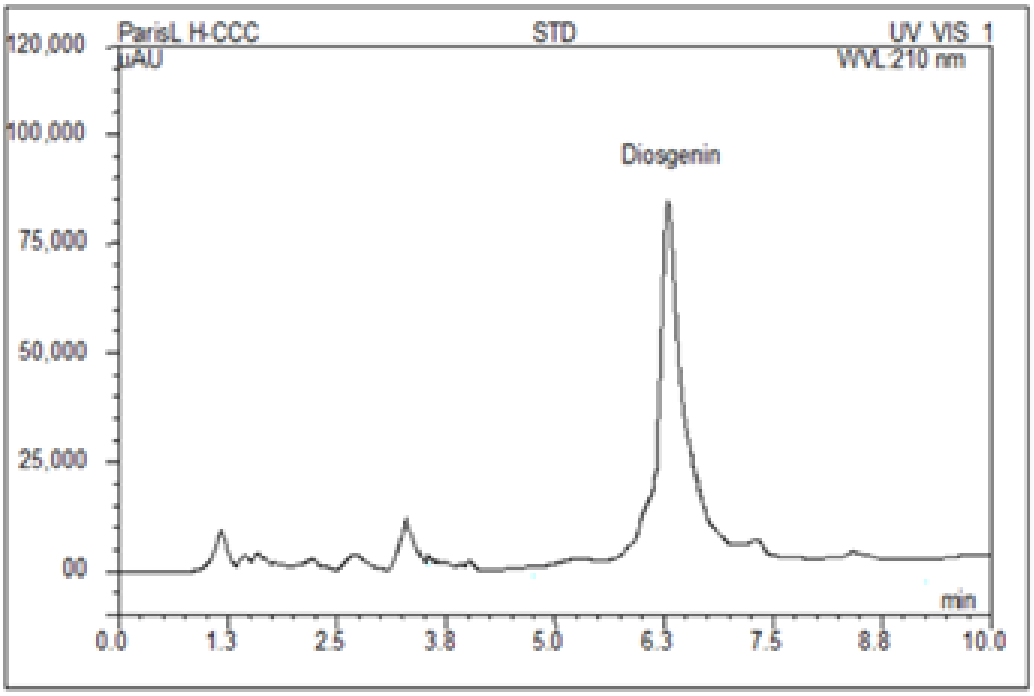



**Figure 4.8 (A).** HPLC chromatogram obtained from the leaf samples of *P. polyphylla* from Tholung (PPT).

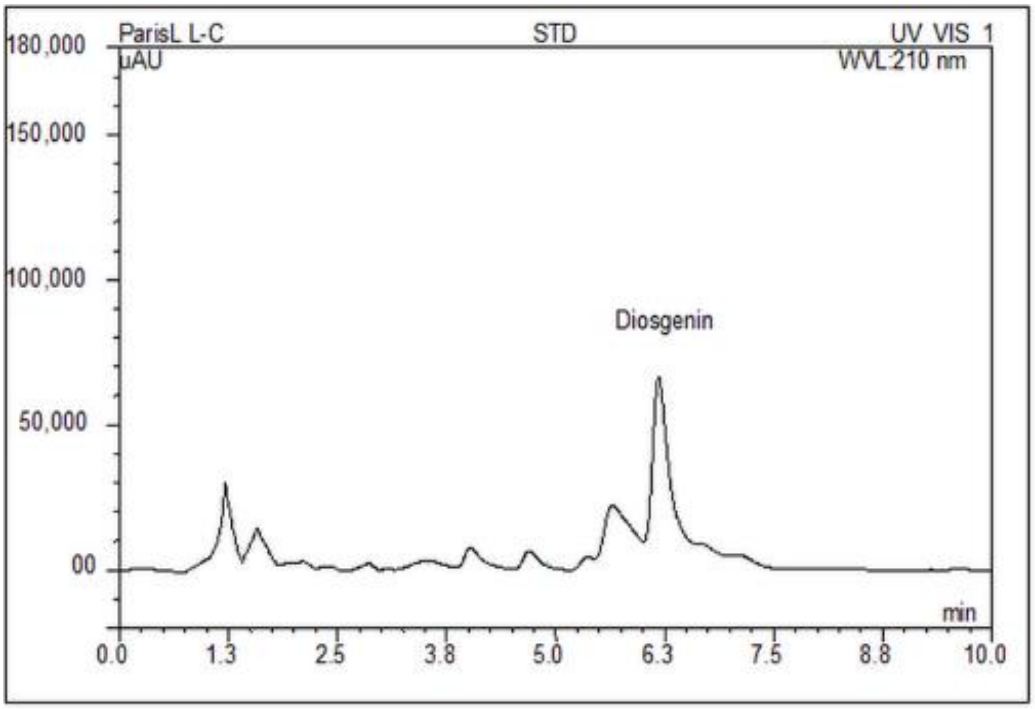



**Figure 4.8 (B).** HPLC chromatogram obtained from the Leaf samples of *P. polyphylla* from Uttaray (PPU).

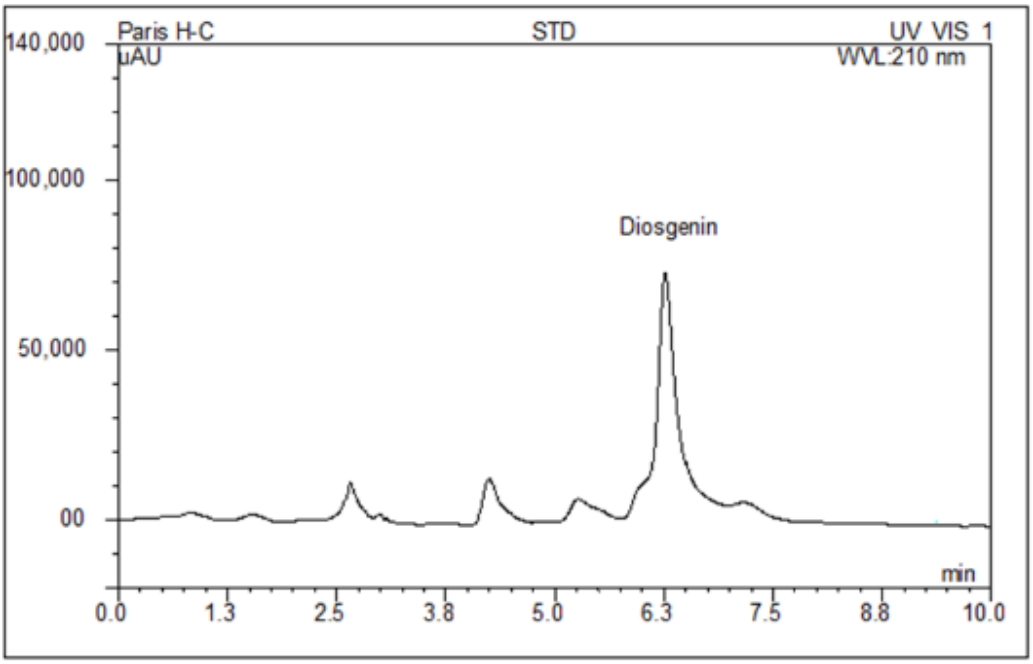



**Figure 4.8 (C).** HPLC chromatogram obtained from the rhizome samples of *P. polyphylla* Tholung (PPT).

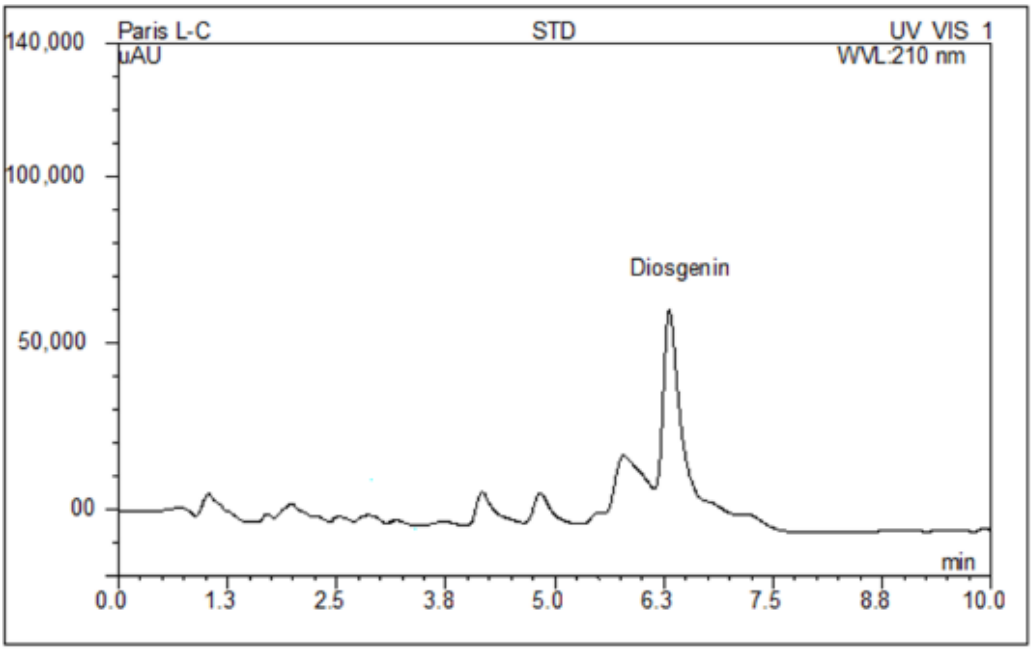



**Figure 4.8 (D).** HPLC chromatogram obtained from the rhizome samples of *P. polyphylla* from Uttaray (PPU)

## 4.12. Propagation of the plant: Regeneration strategy for Sikkim Himalaya

### 4.12.1. Effect of planting medium on germination of seeds and sprouting of rhizomes

Different planting medium were used to regenerate *P. polyphylla* plants from seeds and rhizome cuttings. The locally availablematerial were used during the experiment viz., garden soil, farmyard manure, decayed wood powder and sand. The plants were grown in poly-bags and subsequently transferred to nursery. It was observed that the best planting medium for both seed germination and rhizome sprouting was garden soil: forest litter: sand (1:1:1) resulting in 39 % seed germination and 54% rhizome sprouting in case of PPU. A combination of garden soil, farmyard manure, decayed wood powder and sand also gave good result in case of PPU seed and rhizome cuttings [Figure 4.9 (A)].

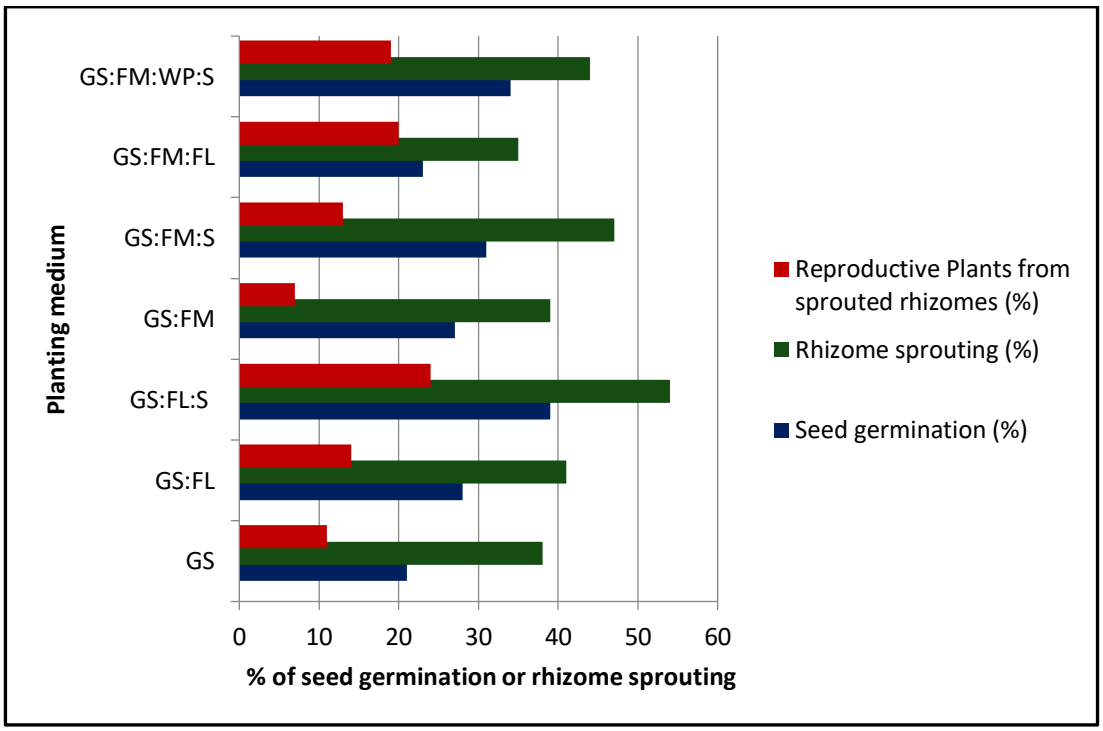



**Figure 4.9 (A).** Effect of planting medium on germination of seeds and sprouting of rhizomes of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya. Seed germination was determined 180 days after sowing and rhizome sprouting was determined 120 days after plantation (GS=garden soil, FL=forest litter, S=sand, FM=farmyard manure, WP=decayed wood powder). All planting medium were used in equal proportion.

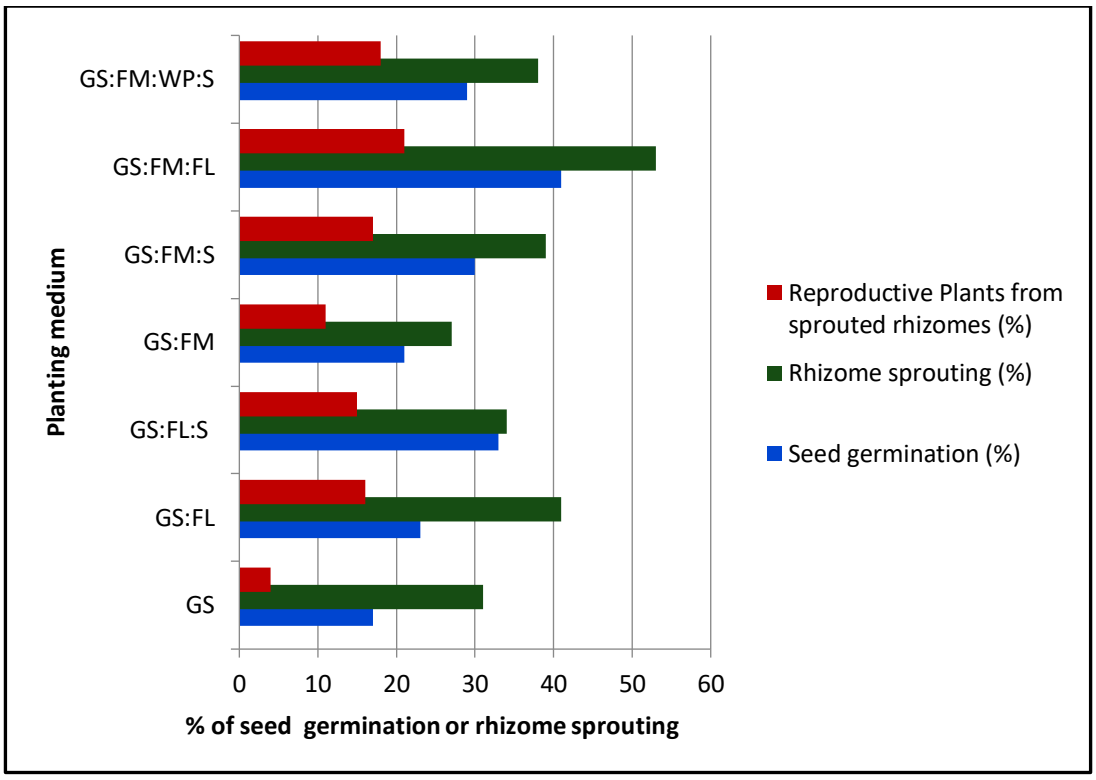



**Figure 4.9 (B).** Effect of planting medium on germination of seeds and sprouting of rhizomes of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya. Seed germination was determined 180 days after sowing and rhizome sprouting was determined 120 days after plantation. (GS=garden soil, FL=forest litter, S=sand, FM=farmyard manure, WP=decayed wood powder). All planting medium were used in equal proportion.

Same planting medium were used to regenerate the samples from Tholung (PPT) from seeds and rhizome cuttings. It was observed that the best planting medium for both seed germination and rhizome sprouting for PPT was garden soil: farmyard manure: forest litter (1:1:1) with 41±0.35 seed germination and 53±0.17% rhizome sprouting. The result is presented in Figure 4.9 (B). Different phases of the growth of plants may be seen in Figure 4.10 and 4.11.

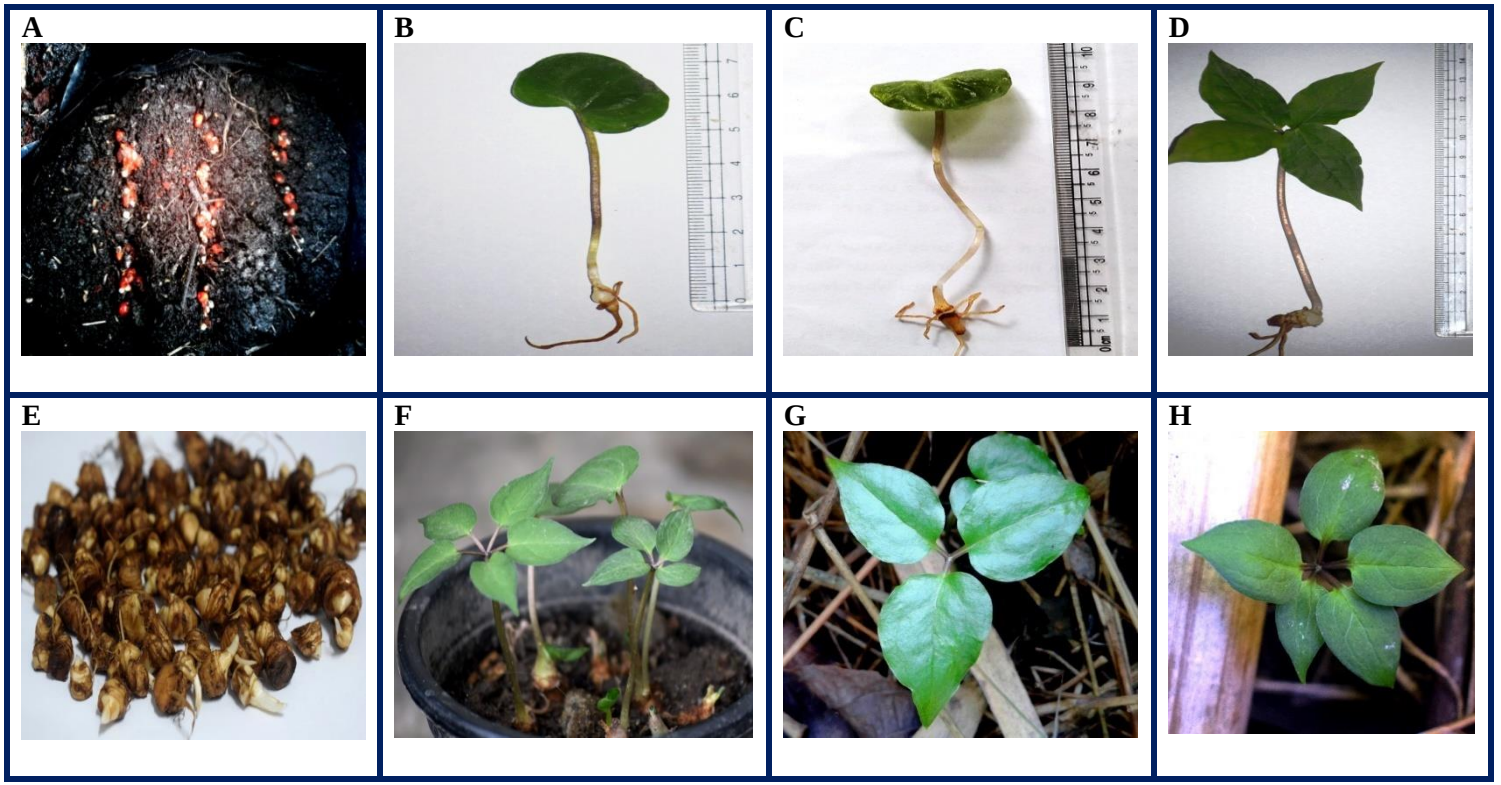



**Figure 4. 10.** Growth phases of *P. polyphylla* (PPT) raised from seeds: A) Seed sowing B-D) different growing phases of seedling raised from seeds, E0. Rhizome cuttings F-H) different growing phases of seedlings raised from rhizome cuttings

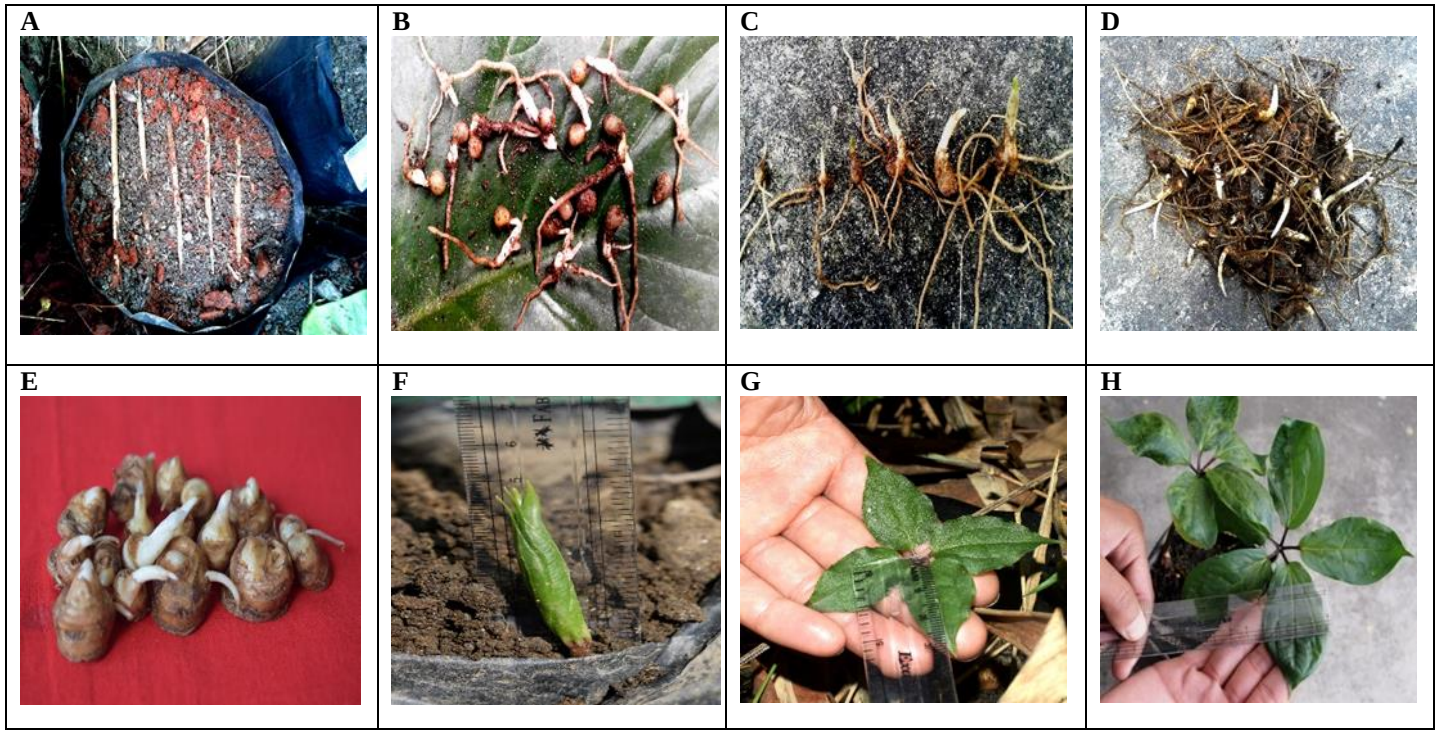



**Figure 4.11.** Growth phases of *P. polyphylla* (PPU) raised from seeds: A) Seed sowing B-D) different growing phases of seedling raised from seeds, E0. Rhizome cuttings F-H) different growing phases of seedlings raised from rhizome cuttings

Along with the regeneration experiment, observation was also made at the same time to look for natural germination in the wild. The percentage germination was very low and in case of natural population of PPT, rodents eat up or take away the seeds after they fall to the ground on maturity [Figure 4.12 (A-D)]. Thus, there was only a minimal chance of natural propagation from the wild seeds. Comparatively, propagation was much better in PPU from the rhizome as well as from seeds. However, in general, *P. polyphylla* faces several challenges for its germination and growth to maturity [Figure 4.13 (A-D)]


**Figure 4.12.** A) Matured *P. polyphylla* plants in the wild and (B-D) seeds are taken away by rodents to their holes.

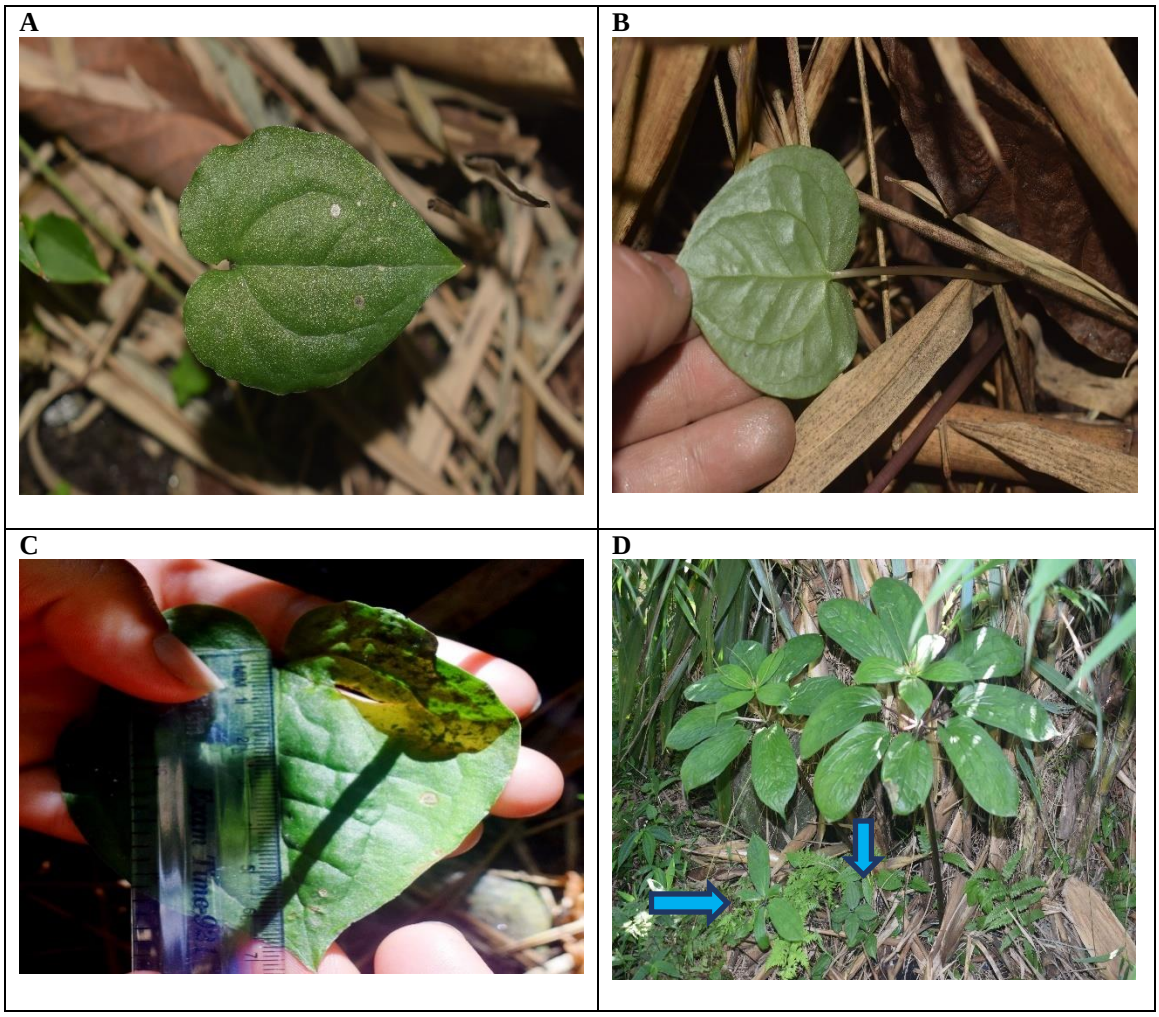



**Figure 4.13 (A-D).** (A&B) Plants germinated from seeds in the wild habitat, C) Fungal infection in the wild saplings and D) A young healthy plant grown from seed.

### 4.12.2. Effects of different PGRs on germination and physical parameters

Experiments on regeneration through seeds using different Plant Growth Regulators (PGRs) were conducted in the nursery and subsequently, the germination rate, shoot length, root length and rhizome diameter was determined. It was observed that the maximum (%) germination of seed was in the sample treatment with $GA_3^{200}$ (74 %) followed by $IBA^{200}$ (62 %). These very chemicals were responsible for maximum shoot

length and root length also. With $GA_3^{200}$ giving a shoot length of (3.30±0.12cm) and maximum root length was recorded also with $GA_3^{200}$ (3.04±0.02cm) treatment. The same trend was observed with rhizome diameter where the maximum diameter was recorded also with $GA_3^{200}$ treatment (Table 4.4.1).

After obtaining all the field data, it was observed that the maximum (%) germination of seeds for the PPT too followed the similar trend as above treatment with $GA_3^{200}$ treatment giving 66 % seed germination. The maximum physical growth in terms of shoot length, root length and rhizome diameter was again due to the seed treatment with 200 µg/ml $GA_3$ followed by IBA of the same concentration (Table 4.4.2).

**4.12.3. Effects of different PGRs on rhizome sprouting and physical parameters**

The regeneration through rhizome cuttings using different plant growth regulators (PGRs) for PPU samples were conducted in the nursery and physical parameters such as sprouting rate, shoot length, root length, rhizome diameter were estimated. It was observed that the sprouting rate (%) of rhizomes was influenced by all the hormones used with the most positive influence being with the treatment with $GA_3^{200}$ (69 %), $GA_3^{100}$ (65%) and $BAP^{200}$ (55%) against the control (31 %). In the same way, these very hormones also influences the shoot length and root length in a positive way with maximum shoot length and root length afforded by $GA_3^{200}$ treatment. The rhizome diameter was also influenced by all the hormones used with $GA_3^{200}$ (2.33±0.02cm) giving maximum diameter of rhizome. The complete screening results for PPU are exhibited in Table 4.4.3.

It was observed that the maximum sprouting rate (%) of rhizomes of PPT was influenced positively by all the PGRs tested with $GA_3^{200}$ (70 %) showing the maximum against the

control (27%). Physical growth parameters like shoot length, root length and rhizome diameter are influenced by all the PGRs but $GA_3$ of both concentrations had the maximum positive effect with $GA_3^{200}$ µg/ml giving a shoot length of 7.83 ±0.26 cm and root length of 4.18 ±0.12 cm against the control values of 4.36 ±0.32 and 1.52 ±0.10 cm respectively. Similarly, the maximum rhizome diameter was also obtained with the treatment of $GA_3^{200}$ml (2.00±0.06cm) and minimum with 1.14±0.14 cm (IBA-100 µg/ml) against control (0.56±0.02 cm). The complete screening results for PPT samples are presented in Table 4.4.4.

**Table-4.4.1.** Germination in *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya through seeds: Effects of IBA, BAP and $GA_3$ (100 and 200 µg/ml) on germination rate, shoot length, root length, rhizome diameter. Germination rate was determined 6 months after sowing and other measurements were calculated 2 months after germination (values are mean ± SE, n=5).

| Treatment (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | Rhizome diameter (cm) |
|---|---|---|---|---|
| **Control** | 39.00 | 1.46±0.08 | 1.73±0.12 | 0.42±0.16 |
| **IBA-100** | 58.00 | 2.61±0.12 | 2.36±0.08 | 0.63±0.09 |
| **IBA-200** | 62.00 | 2.86±0.24 | 2.41±0.07 | 0.73±0.23 |
| **BAP-100** | 45.00 | 1.40±0.21 | 2.24±0.16 | 0.61±0.62 |
| **BAP-200** | 53.00 | 1.35±0.40 | 2.15±0.04 | 0.69±0.45 |
| **$GA_3$-100** | 41.00 | 2.91±0.32 | 2.85±0.16 | 0.87±0.52 |
| **$GA_3$-200** | 74.00 | 3.30±0.12 | 3.04±0.02 | 0.89±0.36 |

**Table-4.4.2.** Germination in *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya through seeds: Effects of IBA, BAP and $GA_3$ (100 and 200 µg/ml) on germination rate, shoot length, root length, rhizome diameter. Germination rate was determined 6 months after sowing and other measurements were calculated 2 months after germination (values are mean ± SE, n=5).

| Treatment (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | Rhizome diameter (cm) |
|---|---|---|---|---|
| **Control** | 28.00 | 1.36±0.08 | 1.32±0.12 | 0.36±0.32 |
| **IBA-100** | 54.00 | 2.03±0.05 | 1.73±0.08 | 0.54±0.15 |
| **IBA-200** | 56.00 | 2.93±0.33 | 2.16±0.07 | 0.70±0.09 |
| **BAP-100** | 41.00 | 1.33±0.29 | 1.38±0.16 | 0.45±0.06 |
| **BAP-200** | 46.00 | 1.28±0.40 | 1.46±0.14 | 0.57±0.13 |
| **$GA_3$-100** | 61.00 | 2.75±0.56 | 2.27±0.21 | 0.78±0.38 |
| **$GA_3$-200** | 66.00 | 2.88±0.27 | 2.54±0.36 | 0.92±0.08 |

**Table-4.4.3.** Regeneration of *Paris polyphylla* from Uttaray (PPU) in Sikkim Himalaya through rhizome cuttings: Effects of different PGRs on sprouting rate, shoot length, root length, rhizome diameter, % of reproductive plants and % of dormant plants. Sprouting rate was determined 120 days after planting: measurement of vegetative parts was done after 60 days after sprouting and percentage of dormant and reproductive plants were calculated 240 days after sprouting. (values are mean ± SE, n=5).

| Treatment (ppm) | Sprouting (%) | Shoot length (cm) | Root length (cm) | Rhizome diameter (cm) |
|---|---|---|---|---|
| **Control** | 31.00 | 5.36±0.09 | 2.52±0.03 | 0.86±0.05 |
| **IBA-100** | 41.0 | 6.22±0.22 | 4.73±0.12 | 1.61±0.03 |
| **IBA-200** | 49.00 | 6.49±0.06 | 5.36±0.08 | 1.84±0.02 |
| **BAP-100** | 42.0 | 5.71±0.47 | 3.92±0.06 | 1.44±0.14 |
| **BAP-200** | 55.00 | 6.05±0.23 | 4.00±0.16 | 1.65±0.06 |
| **$GA_3$-100** | 65.00 | 7.35±0.02 | 5.88±0.08 | 1.95±0.03 |
| **$GA_3$-200** | 69.00 | 7.73±0.39 | 6.35±0.21 | 2.33±0.02 |

**Table-4.4.4.** Regeneration of *Paris polyphylla* from Tholung (PPT) in Sikkim Himalaya through rhizome cuttings: Effects of different PGRs on sprouting rate, shoot length, root length, rhizome diameter, % of reproductive plants and % of dormant plants. Sprouting rate was determined 120 days after planting; measurement of vegetative parts was done after 60 days after sprouting and percentage of dormant and reproductive plants were calculated 240 days after sprouting. (values are mean ± SE, n=5).

| Treatment (ppm) | Sprouting (%) | Shoot length (cm) | Root length (cm) | Rhizome diameter (cm) |
|---|---|---|---|---|
| **Control** | 27.00 | 4.36±0.32 | 1.52±0.10 | 0.56±0.02 |
| **IBA-100** | 43.00 | 5.23±0.09 | 2.69±0.05 | 1.14±0.14 |
| **IBA-200** | 46.00 | 6.79±0.25 | 3.40±0.12 | 1.62±0.09 |
| **BAP- 100** | 42.00 | 5.74±0.41 | 1.79±0.16 | 1.54±0.05 |
| **BAP- 200** | 49.00 | 6.10±0.13 | 2.56±0.10 | 1.49±0.07 |
| **$GA_3$-100** | 56.00 | 7.32±0.01 | 3.24±0.05 | 1.74±0.01 |
| **$GA_3$-200** | 70.00 | 7.83±0.26 | 4.18±0.02 | 2.00±0.06 |

## 5. DISCUSSION

### Phenological and ecological parameters

Himalayan medicinal plants have increasingly been an attraction for the treatment of human maladies. Medicinal plants are available all over, but the occurring is naturally stressed conditions produces more secondary metabolites as compared to the ones growing in the so-called normal environment. The Himalayan ecosystems is having climatically challenging environment faces great stresses which may be critical for producing active ingredients in them, and thereby reducing these plants medicinally more important. Thus, we have more than a dozen plants effective for cancer treatment from Sikkim Himalaya alone. *P. polyphylla* is such a plant although it has not got many other biological activities. *P. polyphylla* and its congeners have been widely researched in other parts of the world. However, there is a dearth of knowledge regarding this plant from this part of the world and whatever little information is available, it mostly deals with eco-taxonomy works on other fields including biochemistry, phytochemistry, physiology etc. are lacking. Therefore, this work sets the tone on *P. polyphylla* research and provides baseline data on the plants for future work. The phenological studies in general and flowing phenology in particular are useful in planning out the conservational strategies as well as formulating measures for cultivation (Barngrdello et al. 2001). The study of temporal behavior of a species is generally termed phenology, and it means the study of seasonal appearance and timing of life cycle events of a plants. Observations on different phenophases of these species in existing natural habitats along an altitudinal gradient are needed to assess the role of environmental factors in *ex-situ* conservation and domestication (Dhyani et al. 2010). Air and soil temperature are known to control

different phenophases (Diekmann, 1996), and they vary with latitude, altitude, type of community, and growth of plant thereby influencing the distribution of species.

In the present investigations phenological changes has been observed in *P. polyphylla* plants in its natural habitats. Two habitats, relatively lower (Lingthem) and relatively higher (Tholung) and plant raised from two different propagules (seeds and rhizomes) have been considered. From the plants growing from seeds in lower altitude showed the completion of life cycle at about 3-years while those growing in the relatively higher altitude of Tholung the life cycle is every out three months earlier. It is because the underground emergence stage requires a longer period in lower altitude. Maybe, the underground conditions have to reach sufficient colder period before emergence to take place. After emergence also every phenological events gets delayed in the saplings growing at Lingthem. However, it was observed that in the plants raised from rhizome cuttings the life cycle was complete almost at the same time in both the locations. Of course, the first field emergence field was delayed at Lingthem (lower altitude) in this case also but the plants caught up rhythm at the flowering stage. The phenology of vegetative phases is important as leaf emergence and its unfolding is intimately related to plants water status (Raich, 1995). The emergence phase in the present context was quite asynchronous and the asynchrony perimeters to later developmental stages two when considered in terms of differences in altitudes and that of propagules. However, in rhizome grown plants from both altitudes there is some semblance of uniformity. The physiological episodes of plants are governed by the eco-edaphic conditions is grows in which in turn dictate the strategies of the plants in general and alpine herbs in particular (Wani et al. 2006). In the Sikkim Himalaya also the differences in edaphic factors in two

distinct altitudinal zones may have been responsible for the asynchronous phenological behavior in *P. polyphylla.* A Himalayan plant, *Lilium polyphyllum* (Liliaceae) requires about 100 days for germination and bulblet production (Dhyani et al. 2010) which is also similar in this case although the species varies widely. In this plant also the growth period lasts from one to several years based on growing conditions (Baranova, 1987). The time of invitation for a particular phase often varied considerably from place to place with temperature being the prime controlling factor. However, different phenophases of this plant may be determined by altitudes and varieties in genetic, edaphic and climatic factors.

Understanding the ecology of individual species is important for conservation for cultivation purposes. Effective conservation of threatened species depends on identification of suitable habitats. Ecological niche modelling (ENM) makes it possible to prepare species distribution maps and identify areas suitable for reintroduction of threatened species (Kumar and Stohlgren, 2009). Models predict suitable potential habitats were certain species are likely to occur (Hawk, 2017).

Maxent model has been found suitable for predicted occurrence with maximum accuracy in rare species (Hernandez et al. 2006). In the present context, amongst at the six variables used precipitation of driest month and slope had the highest contribution to the model. The presented results are in consonance with earlier reports whereby the maxent model performed with high discriminatory ability due to variable related to temperature and precipitation (Bradie and Leung, 2016). It was also been found that terrain attributes such as aspect and slope are highly significant in the modelling (Lecours et al. 2016). It is important to mention here that the predicted distribution of suitable habitat does not

actually represent the true distribution of the species, but represents the prediction of distribution of suitable habitats (Setyawan et al. 2018).

The analysis of the contents of soil nutrients like organic carbon, total nitrogen and available phosphorus of the soil of *P. polyphylla* habitat showed that the organic carbon content was slightly higher in Tholung and Lachung forest areas as compared to the other sites. An earlier study reported that the study sites where the *Paris* plant grows have higher nutrient content than the soil devoid of the plant (Madhu et al. 2010). In general, the *Paris* habitats the soil was characterized by pH (4.48-5.70), high organic carbon (2.86-4.26%) and low soil temperature (10.29-15.76 $^{0}$C). Moreover, the available phosphorus was uniformly low in all the sites.

A total of thirty plant species has been identified as closely associated to *P. polyphylla* in Sikkim Himalaya. Of these species, the top three dominant associated species in terms of maximum Importance Value Index (IVI) were found to be *Fragaria nubicola*, *Pteris* sp. and *Sarcococca coriaceae*. The present observation revealed that in Uttaray the mean density of *P. polyphylla* was lowest at 0.45 (pl/m$^2$) with an IVI of 2.68. In contrast, the Tholung forest area recorded the highest density of the plant at 3.81 (pl/m$^2$) and the highest IVI (8.66). This IVI was followed by that in Barsey (6.53) which may be because both of those locations falls under protected areas. Despite showing the Medium and High 'Habitat Suitability' by the MaxEnt model run, the two lowest IVI recorded were by Uttaray followed by Pangthang. This may be because Uttaray was the first place from Sikkim where illegal trade of *P. polyphylla* started since it is near the international boundary of Nepal and Pangthang is located near to the capital city, Gangtok.

Present average population density of *P. polyphylla* from Sikkim Himalaya was found similar to that of the reported density (1.78 individual $m^{-2}$) from Nepal (Madhu et al. 2010) and higher than the population density reported from Arunachal Pradesh (1.07 individuals $m^{-2}$) (Paul et al. 2015). Since our first survey conducted during the period of 2014-15, when we revisited this area again in (2017-18) to take stock of the scenario, it was observed that the wild population of *P. polyphylla* has either declined or become completely obliterated, except a few small pockets and protected areas. All other areas where the plant was once available has significantly degraded (Lepcha et al. 2019).

Thus, the protected areas in Sikkim prove to be an important habitat for the ex-situ conservation of *P. polyphylla* germplasm. It may be safely said that just in the span of 8-10 years, the population of *P. polyphylla* in Sikkim Himalaya has drastically reduced but it could be rehabilitated as the present study suggests. The present study demonstrated that the population of *P. polyphylla* in its natural habitats in Sikkim Himalaya has declined due to developmental activities like road construction, hydel power projects, tourism development and illegal extraction of rhizomes before the plant matures to produce viable seeds. The Maxent model performance provided satisfactory result for *P. polyphylla* Smith. The model output was at accurate level with high AUC values. Field validation of the habitat vindicated similarities between habitat characteristics at predicted habitats vis-à-vis extant *P. polyphylla* population encountered during the fieldwork except in some locations heavily influenced by anthropogenic disturbances. *Fragaria nubicola, Sarcococca coriaceae* and *Pteris* sp. were the most dominant species associated with *P. polyphylla.* The model showed that the habitat suitability thresholds are the highest for Lachung and Ravangla forests. However, physical counts revealed that

the actual population is moderate in those two sites. It should be mentioned here that there is a heavy influx of tourists in these areas. On the other hand, it was found that the population of the plant was highest in Lingthem, Tholung, and Barsey which are all protected areas and least susceptible to anthropogenic disturbances. Clearly, anthropogenic activities are the main cause of their population decline. Our studies may be used for prioritizing areas for introduction of the species for cultivation and for management of the same for conservation.

Florescence analysis is one of the Pharmocognostic techniques useful in the identification of authentic samples and recognizing adulterants (Tyler et al. 1976). In the fluorescence analysis, the plant parts or crude drugs may be examined as such, or in their powered form or in solutions or extract. Although, in most cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946). The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the roots in its crude form. Further, the contents of the result could also be used as an aid to check adulteration, where the adulterated samples would show variations or difference in the emission of colours when compared with the genuine samples.

The rhizome powdered from the plants growing in Uttaray as such appeared pale greenish white under daylight. After treating with various reagents, under daylight, it showed different shades of green and grey except the treatment with Myer's regent when it appeared violet and with Dragendorff's reagent which gave black colour. Under

ultraviolet radiation of short wavelength (254 nm) the crude powdered was Creamish white which on treatment with different reagents emitted different hues of green except in case of treatment with Mayer's reagent which retained the original colour of the crude sample. However, in case of methanol treatment the powder gave grey colour. Under UV light of long wavelength (365 nm), the crude sample was light green. On treatment with different reagents, the sample gave varying colours like violet, brown, black, light brown etc. The crude rhizome powder from plants growing in Tholung appeared Creamish, light brown and brown under visible light, short UV light and long UV light respectively. The colour scheme in this plant is similar as that of the one from Uttaray. However, the colour scheme is diametrically different in cases when the rhizome powder is reacted with certain chemicals viz., sodium nitroprusade, ninhydrin, HCL (1N), methanol or NaOH (1N) in methanol. In both types of rhizomes, the characteristic violet and Creamish white colouration was observed. In general, under long UV and short UV light respectively when treated with Mayer's reagent. Similarly, long UV light and short UV light gave dark brown and light brown fluorescence respectively when the rhizome powders from the altitudes treated with $HNO_3$ (1N). Under visible light, the greyish colour on treatment with iodine and yellowish green colour on treatment with $FeCL_3$ is characteristic in both types rhizomes. The fluorescence profile could be used as an aid to check adulteration, where the adulterated samples would show variation or difference in the emission of colours when compared with the genuine samples (Selvam and Bandyopadhyay, 2005).

Preliminary phytochemical screenings have shown identical phytochemical profile in *P. polyphylla* from both Uttaray and Tholung. The main chemical groups found in both the leaves and rhizomes from plants obtained from both altitudes were alkaloids, flavonoids,

tannins, phlobatanins, steroids, saponins, glycosides, cardiac glycosides etc. However, triterpenes were found only in leaves and glycosides were found only from rhizome samples from both altitudes.

Gas chromatography -mass spectrometry (GCMS) integrates the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample (Jayapriya and Shoba, 2015). GC-MS studies on methanol extract of rhizomes revealed the presence of 47 compounds, where phenol, 2,4-bis (1,1-dimethylethyl)-phosphite constituted the major part. The constituents were identified by comparing GC-MS data with those given in library and reported in literature. The presence of 2(3H)-furanone, hexadecenoic acid, 17-hydroxy-4, 4-dimethyl estran-3-one, palmitin, undecylenic acid, phytol, vitamin E and naphthalen-l-ol are the general metabolites which occur in the rhizomes of *P. polyphylla* from different altitudinal zones of Sikkim Himalaya. These compounds are found in both the samples from Uttaray and Tholung. In addition, smaller amount of 1-methyl-4-(1-methyletnenyl) and cyclohexene were also found in both the samples. The sample from Uttaray was characterized by some major metabolites viz., cyclohexanol, (9Z)-octadec-9-enoic acid, decanoic acid ethyl ester etc. having roles as a precursor for nylons, for treating lung diseases and an inhibitor of uric acid and arachidonic acid production respectively (Payum, 2018). Similarly, the methanolic extract of rhizome from the plants growing in the relatively higher altitude of Tholung was characterized by the presence of some unique compounds viz., 1-dodecanol, diethyl phthalate, bicyclopropyl etc. having emollient, perfumery and anti-cancer activities respectively. Thus, the plants from different altitudes have both similar and dissimilar biologically active compounds. Using GC-MS Analysis, 47 compounds have

been elucidated for the first time in our study on *P. polyphylla* from Sikkim Himalaya, of which 12 compounds were effectively matched and identified. It is worth noting that the major constituents identified in the methanolic extract of *P. polyphylla* rhizome from Sikkim Himalaya with the highest composition were the peaks 27 (peak area 31.49%), 44 (peak area 27.58%) and 40 (peak area 16.39%). Biological effects of these compounds in human system are not well known and therefore needs further analysis. The same applies to the remaining compounds which have been enlisted here for them having lesser percentage composition. Identification of these compounds in the plant serves as the source of potential health enhancing properties of the plant which is fit for further biological and pharmacological studies.

**Analysis of physiological and biochemical differences**

Plant growth regulators are a wide category of compounds that can promote, inhibit or change plant physiological or morphological processes at very low concentrations. Thus, the use of PGRs has become an important component of the agro-technical producers for most cultivated species (Monselise, 1979). The most-studied PGRs include abscisic acid (ABA), indole-3-aceticacid (IAA), brassinosteriods, cytokinin, gibberellic acid, ethylene, jasmonic acid, salicylic acid etc.. These PGRs control rooting, flowering, fruiting and fruit growth, leaf or fruit abscission and senescence, and regulate some metabolic process- such as net photosynthesis or antioxidant enzyme activities- and plant resistance to biotic stresses. IAA is the major naturally occurring auxin that polar auxin transport may play a major role in growth, floral and yield-related traits and yield (Tantasawat et al. 2015). ABA is a compound associated with seed dormancy, responses to stress such as drought, extreme temperatures and excess light (Hirayama and Shinozaki, 2007;

Thompson et al. 2000) and other growth process. It has proved to be an effective tool in the modification of transplant, shoot growth and leaf abscission and in the enhancement of the drought tolerance of several plant species (Leskovar et al. 2009). Gibberellins have a particularly-interesting role in commercial farming since they lead to plant elongation and development and delay of fruits maturation and abscission of leaves (Yilmaz and Ozguyen, 2009). Application of $GA_3$ to tomato plants has been shown to induce marked stem elongation (Bukovac and Witter, 1956), increase fresh weight (Bukovac and Witter, 1956; Rappaport, 1956), accelerate flowering and produce greater numbers of flowers per plant (Witter and Bukovac, 1957).

Nowadays, several elicitors molecules like methyl jasmonate, abscisic acid, CCC, salicylic acid, phenylalanine and triazole compounds have been used to enhance the production of various medicinally important phytoconstituents (Ketchum et al. 1999). It had already been found that CCC can potentially enhance the alkaloid production of *Catharanthus roseus* in both tissue culture and filed culture and filed conditions (Choudhury and Gupta, 1996). Taxol production in *Taxus globose* shoot callus was also elevated by CCC treatment (Barrios et al. 2009). CCC, an onium compound, is a potent gibberellin biosynthesis inhibitor.

In *Stevia rebaundiana* priming of calli and micro shoots with CCC in the tissue culture media stimulates the accumulations of biologically active secondary metabolites like phenols and flavonoids. This elevated level of phenol and flavonoids in turn provides higher antioxidant potentialities without generating any cytotoxic phytochemicals (Dey et al. 2013). MH acts as an anti-auxin, anti-gibberellin or regulator of auxin metabolism and other plant growth regulators (Hoffman and Parups, 1964). MH treatments inhibits seed

germination and causes stunting of many species due to a temporary suspension of stem elongation and a localized accumulation of anthocyanins or other non-green pigments, narrowing of leaves and distinctive patterns of chlorosis and interference with water absorption (Moore, 1950). Exogenous application of PGRs play an effective role by protecting the fluidity and integrity of plant cell membranes. They properly mediate enzymatic (SOD. APX, and CAT) and nonenzymatic machinery with the result of preventing cell membrane damage by ROS (Jungklang and Songklanakarin, 2012). Hence, lower MDA content is considered an enhancement of antioxidant potential, providing improved tolerance in oxidative stress conditions (Keramat et al. 2009). The addition, cytokinin derivative (6-BAP) delays the process of senescence in plant parts. This, in turn, retards the decomposition rate of macromolecules, especially of those involved in the photosynthetic process (Stopari and Maksimovi, 2008).

Elicitors, the substance introduced in small concentrations to a living system, improves the biosynthesis of various phytochemical components, especially polyphenols (such as flavonoids, phenyl propanoids, phenolic acids, tannins) etc. These compounds exhibit a wide range of biological activities, including anticarcinogenic, anti-inflammatory, antiradical, and especially they exert antioxidative effects as free radical scavengers (Dey et al. 2013).

In the present context, plant growth promoters viz., Indole Acetic Acid (IAA), Gibberellic Acid (GA3), and Kinetin (KIN) of 100 and 200 µg/ml concentrations and plant growth retardants viz., Malic Hydrazide (MH), Chlorocholine Chloride (CCC) of 50 and 100 µg/ml concentrations as well as Abscisic Acid (ABA) of 25 and 50 µg/ml concentrations were used as a foliar spray agent on *P. polyphylla* plants and their effects

on growth parameters were determined after 120 days. Other physiological activities as well as biochemical changes were determined at three 30 day intervals i.e., after 60, 90, and 120 days of leaf emergence.

IAA is the major naturally-occurring auxin and its roles range from virtually every aspect of plant growth and development to defense responses. Auxins are believed to increase ethylene production, which is responsible for fruit maturation. Thus, auxins are used in commercial farming to control fruit drop and to improve fruit quality (Almedia et al. 2004). Gibberellins have a particularly-interested role in commercial farming since they lead to plant elongation and development and delay fruit maturation and abscission of leaves (Yilmaz and Ozguyen, 2009). The impact of exogenous $GA_3$ on growth and yield has been studied indifferent crops. In strawberry, its application increased vegetative growth although fruit size, weight and yield were reduced (Qureshi et al. 2013), while in pineapple this PGR increased fruit weight (Li et al. 2011). ABA is compound associated with seed dormancy, responses to stresses such as drought, extreme temperatures and excess light (Hirayama and Shinozaki, 2007; Thompson et al. 2000) and other growth processes. It has proved to be an effective tool in the modification of transplant, shoot growth and leaf abscission and in the enhancement of the drought tolerance of several vegetable species, including pepper, tomato, melon and artichoke (Leskovar et al. 2009).

Maleic Hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione) has been extensively used as a commercial systematic plant growth regulator and as herbicide (Schoene and Hoffman, 1949). After application to foliage, MH is freely translocated in plants to meristematic tissues, with mobility in both phloem and xylem (Meyer et al. 1987). Its mode of action in plants is not clear, although it is suggested that MH acts as an anti-auxin, anti-

gibberellin or regulator of auxin metabolism and other plant growth regulators (Hoffman and Parups, 1964).

Results from the present work showed that in comparison to control, the shoot length height was similarly influenced by both growth promoters as well as growth retardants. Thus, it was observed that $GA_3$ at 200 µg/ml had the similar effect as that of MH at 100 µg/ml or ABA at 50 µg/ml in enhancing the height of the plant i.e. each one of them was instrumental in enhancing the height by 1.8-1.9 times when compared to the control. Other PGRs like IAA, Kinetin and CCC also stimulated the height of the plant to a lesser degree in the plants from Uttaray (Table- 10). The effect is almost the same on the plants from Tholung, except that in this case IAA at 200 µg/ml also had highly stimulating effect towards the growth in shoot length (Table-11).

As regards the change in stem circumference by the application of PGRs, in general there was positive influence on the growth in diameter irrespective of the whether the treating agent was growth promoter or growth retardant. However, in the species collected from a relatively lower altitude of Uttaray IAA and $GA_3$ among the growth promoters and MH and CCC among the growth retardants had more pronounced effect (Table- 10), similarly, in the specifies from the relatively higher altitude of Tholung, more pronounced growth promoting effect was shown by higher concentration of ABA (Table-11). The effect of the experimental PGRs on the promotion of root length also followed the above trend to some extent. However, in this case more pronounced role was demonstrated by $GA_3$ and Kinetin among the growth promoters and MH and ABA in *P. polyphylla* from Uttaray while all the retardants in case of *P. polyphylla* from Tholung (Table 10, 11).

Relative Growth Index (RGI) is a measures of the dry matter content of the plant. In other words, the RGI has a positive correlation with the biosynthetic activity of the plant. During this study it was found that though the RGI was influenced by all the treatments done, maximum role in its enhancement was played by the growth retardants with exception to IAA at 100 µg/ml. Kinetin at higher concentration (200 µg/ml) gave RGI value of above 80% in PPU while $GA_3$ at higher concentration resulted in RGI value of approx. 77-78% was demonstrated by CCC treatment at 100 µg/ml while the same in case of PPT (86.15%) was demonstrated by MH at 50 µg/ml (Table-10.11).

It is apparent that the influence of PGRs were not visible at the morphological level in a short period of 120 days as exemplified by the general growth diameter or in length in either shoots or roots. However, there was a definite effect at the biosynthetic level as indirectly evidenced by the value of RGI by different PGR takes a longer time while biochemical expression is rather immediate.

In cigar tobacco it was found that the secondary metabolites like total alkaloid and nicotine was decreased by the application of MH (McEvoy and Hoffiran, 1959). Arnaud et al. (1956) stated that the chemical increased both the starch and the soluble sugar content of treated over that of untreated tobacco. The decreased alkaloid content of Maleic Hydrazide-treated tobacco is probably due to modifications in the plant's metabolism and impaired secondary root growth (Birch and Vickery, 1961). In the leaves of *P. polyphylla* grown in relatively lower altitude of Uttaray (PPU), the increase in soluble protein content was clearly visible by growth retardant treatments (MH, CCC, ABA etc.) while no such effect was seen in the leaves of the plants growing in the relatively higher altitude of Tholung except that $GA_3$ treatment slightly decreased the

soluble protein content in this case. No such trend was seen in the soluble protein content of rhizomes from both altitudes. A relatively high sugar content is correlated with quality an association of high sugar content with a depresses secondary metabolite fraction was found in the treated tobacco (Coulson, 1959). In the leaves of *P. polyphylla* from Uttaray, the soluble carbohydrate content remarkably increased due to the treatment with growth hormones, while the retardant treatment resulted in only slight increase in soluble carbohydrate content. No such visible change in soluble carbohydrate content was observed in the leaves of *P. polyphylla* from Tholung. However, the rhizome of Uttaray plant showed a relatively appreciable increase in soluble carbohydrate content on CCC and ABA treated plants. On the contrary, the Tholung plant showed appreciable increase in the soluble carbohydrate content only when it was treated with Kinetin. Insoluble carbohydrate content more or less remained unchanged over the period or treatment, especially in case of leaves from both the altitudes. However, in case of rhizomes, the content either decreased very slightly (Uttaray) or remained unchanged (Tholung).

Many factors including age, seasonal variation, nutrition, temperature and phytohormones have an influence on accumulation and metabolism of secondary products (Barz and Kastor, 1981). Here, it has been demonstrated that *P. polyphylla* accumulation of secondary metabolites is increased following treatments with plant regulators. Among the various bioregulators tested Growth retardants like CCC and ABA caused significant loss of carotenoids and CCC caused significant increase in chlorophyll-a pigments over a 4-month period in plants obtained from Uttaray. At the same time, in the plants from Tholung, the Chlorophyll-a content was significantly

increased by CCC (50 AND 100 µg/ml) while no such effect was observed in chlorophyll-b content.

Ascorbic acid (Vitamin-C) acts as potent antioxidant agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor, by donating electrons, it prevents other compounds from being oxidized (Padayatty et al. 2003). During the present studies the leaves of *P. polyphylla* showed a little increase in ascorbic acid content when treated with growth retardants tested showed remarkable enhancement in the contents of ascorbic acid irrespective of the plant cultivars from different altitudinal areas. Even among the growth retardants, CCC (100µg/ml) showed the highest increase in ascorbic acid levels in the leaves and rhizomes in case of *P. polyphylla* from both the altitudes. The levels of ascorbic acid was in conformity with that of the roots and leaves of *Hypochaeris radiata* (Senguttavan et al. 2014). In the rhizomes the increase in ascorbic acid content was gradual but less pronounced with longer duration of treatment not adding up to the increase in ascorbic acid contents. The ascorbic content in both the varieties may provide for antioxidant defense of the plants against the challenges posed by growth retardants. Ascorbic acid also helps in the absorption of iron and phenols.

**PGR induced changes in phytochemical constituents**

Polyphenolic compounds include those members of phytochemicals, which are known to exhibit antioxidant activity (Adedapo et al. 2009). The antioxidant properties of phenolics are mainly responsible for the inhibition of oxidation of low-density lipoprotein cholesterol (Paganga et al. 1999; Eberhardt et al. 2000). Therefore, consumption of plant

materials rich in phenolics can alleviate conditions related to coronary atherosclerosis (Rimm et al. 1996).

Consumption of natural edible products with suitable amount of phenolic compounds serve as primary free radical terminators leading to reduction of incidence related to cancer, heart diseases and atherosclerosis (Randhir et al. 2008; Alothman et al. 2009). Phenols and flavonoids are the most diverse and widespread secondary metabolites found in different medicinal plants and exhibited a broad spectrum of biological activities. Aqueous methanolic extracts of the leaves and rhizomes of *P. polyphylla* from two different altitudinal regions exhibited significant differences in the accumulation of total phenols. Proportionately, higher amount of total phenol production was seen in the leaves of *P. polyphylla* from lower altitude while the rhizomes was almost reduced to 50%. However, in the samples from the higher altitudes showed consistently higher content of total phenols both leaves and rhizomes. CCC at 100 µg/ml was found to give the maximum amount of total phenols.

As regards the changes of total phenol content, it was found that the plant growth promoters $GA_3$ and IAA caused no change to decrease the level of total phenols. It further noticed that with the progress in stages; treatment duration from pre-flowering to fruiting upto senile stage. The growth promotors had no influence on the level of phenols.

The polyphenol content as well as flavonoid content was slightly elevated with respect to the application of Kinetin. The foliar application of growth retardant MH had no positive effect on the lower concentration. However, the same showed increased content of total phenols at 100 µg/ml concentration. The effect in this case increased upto a 90 day period beyond which there was no possible increased in the total phenol contents.

Flavonoids belong to a group of naturally occurring low molecular weight water soluble polyphenol compounds of plant origin (Harborne, 1998). Flavonoids are also capable of exerting multi-faceted biological effects including anti-inflammatory, antioxidant, anti-allergic, antiviral and anticancer activity (Jain et al. 2012). In the present study, the highest amount of flavonoid was recorded under the influence of CCC application. Whatever be the type of tissue or the altitude from which the sample was collected. Similarly, the water extract of *Ficus cunia* showed the flavonoid content to be 151.46 and *Berberis lycinum* (141.2 mg RtE/100g) which was comparable to the flavonoid content in *D. indica* fruits (Abbasi, 2013; Chhetri et al. 2017). Like phenols, flavonoid accumulation increased steadily in leaves and rhizomes under the treatments with $GA_3$, Kinetin and CCC. Maximum production of flavonoid was observed in the leaves of the samples from both the treatments under CCC treatment. However, growth hormones like $GA_3$ and Kinetin tends to increase the flavonoids in both the leaves and the rhizomes.

*In-vivo* experiments of Ginkgo leaves indicated that externally supplied CCC and ABA could increase flavonoid contents (Cheng et al. 2011; Wang et al. 2007). However, in this case ABA had no discernable impact.

Higher content of total flavanol was obtained from the rhizomes of *P. polyphylla* obtained from the higher altitude. Results revealed that the treatments of *Paris* plants with promoters like ABA and $GA_3$ had no effect on the total flavonol content. Compared to control the increase in flavanol level with the PGR was significant especially when treated with $GA_3$, Kinetin, CCC AND ABA. However, the highest level of flavanols was found in the treatment with retardants. The most conspicuous in this case was the treatment with ABA and CCC irrespective of the type of sample or the duration of

treatments. The retardants MH did not show any effect either to the leaf or rhizome flavanols in PPH while positively effecting the same in PPT. The positive effects of CCC and ABA was more prominent at its higher concentration.

CCC was also found to promote flavanol synthesis in red cabbage and spiderwort leaves (Grebinskii and Khmil, 1980). Similar effect was also observed following CCC treatment in *Ginko biloba* leaves (Zhang et al. 2013). Exogenous ABA significantly increased the abundances of flavonoid metabolites including flavanols in tea leaves (Gai, 2020).

In grapevine leaves treated with $GA_3$ (2) had lower contents of flavanols (Murcie, 2017). Patil and Gaikwad (2017) observed foliar application of PGRs (like cytokinins, gibberellins and CCC) shows a positive influence on flavonoid in Simarouba glauca, which is corroborated in this case especially in case of PPH.

Alkaloids are derived from amino acids and contain heterocyclic nitrogen atoms in their molecular structure. Most alkaloids derived from plants have many pharmacological effects (Wink et al. 1998). Many alkaloids are in current use as drugs, such as the painkiller morphine, the antimalarial drug quinine and the cancer remedies vincristine and vinblastine (Rao et al. 1978). The enhancement of alkaloids production by cell suspension cultures has also been achieved through the use of biotic elicitor preparations from fungal plant pathogens (Cline and Coscia, 1987).

PGRs like cytokinin and benzyl adenine stimulate alkaloid synthesis in cell culture of *C. roreus* (Decendit et al. 1992). $GA_3$ application as foliar spray had little effects on alkaloid content of *Catharanthus roseus* (El-Sayeed, 2004). In the present study also $GA_3$, MH did not have any effect. Kinetin slightly increased the alkaloid content. On the other hand

CCC substantially increased alkaloid content in *P. polyphylla*. However, in in-vitro experiments, the alkaloid content increased in $GA_3$ treated plants when compared to control 90 DAP. Increased alkaloid was also reported in *Catharanthus* plants by the application of Kinetin and IAA (Amit et al. 2005).

In *Catharanthus roseus*, alkaloid content significantly increased application of CCC consistently increased the production of alkaloid at concentration of upto 500 µg/ml and 1000 µg/ml, both in leaves and roots while beyond that concentration the effect was as it became toxic (Choudhary and Gupta, 1996).

CCC, in the present study appears to be a very potential chemical from commercial view point as it increased the total alkaloid to a significant extent. However, maximum increase in alkaloid content was affected by MH at 60 µg/ml and in rhizome the content of alkaloid increased to the highest level and the rhizomes of PPT samples showed the highest level of increase in alkaloid content. A decrease in artemisinin content in *Artemisia annua* due to the treatment with kinetin have been reported (Whipkey et al. 1992). However, in *Withania somnifera,* the level of alkaloid, withanolide-A was generally higher in the roots of treated plants than that in control plants and that $GA_3$ and IAA either singly or in combination seem to be very effective treatments (Solanki and Vakharia, 2014). In *Withania somnifera* Dunal., foliar spray of CCC at 85, 115 and 145 DAS revealed that the alkaloid content was increased favorably (Barathkumar and Manivannan, 2018).

Alkaloid content has been increased in with treatment of KIN in *Papaver somniferous* (Furuya et al. 1972). In *P. polyphylla*, Kinetin slightly enhanced the alkaloid content in the levels, though Kinetin had no effect on rhizome alkaloid contents.

In general, abiotic stress improves the yield of plant secondary metabolites saponins (Zhao et al. 2005). $GA_3$ treatments has also been responsible for the increase in saponin content in *P. polyphylla*. The increased saponin accumulation in rhizomes may be explained by the longer growing phase and increased duration of environmental stress.

In Pimpinella alpine Molk., it is noted that foliar spray of the combination treatment of IAA and $GA_3$ has a significant influence on levels of leaf saponins (Fathonah and Sugiyarto, 2009). Exogenous application of gibberellins and cytokinins have been found to improve the contents of saponins in *Colubrina asiatica* (L.) (Patil et al. 2020). Cytokinin, Kinetin was able to increase the level of saponins in levels. However, the rhizome did not show such effect. In Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv.) roots showed a marginal increase in saponin content when treated with ABA over a 56 day period (Linh et al. 2019). ABA also stimulates the production of ajmalicine compounds in *Rauvolfia verticillata* roots (Chang et al. 2014). CCC at lower concentration (50 µg/ml was most effective in increasing the saponin levels both in case of PPT levels and rhizome. While higher concentration of CCC was more effective in the PPH in this respect.

Diosgenin, a steroidal sapogenin belonging to the group of tri-terpenes, plays an important role in the control of cholesterol metabolism (Marzolo and Nervi, 1989; Roman, Thewles and Coleman, 1995). Production of diosgenin have been found to be positively influenced by $GA_3$ treatment (Nandi and Chatterjee, 1978). The same trend was seen in the diosgenin contents of leaves and rhizomes of *P. polyphylla* which was positively influenced by $GA_3$ and IAA. The application of MH was found to be reducing

the total content and alkaloid yield (0.431 per cent and 0.80 kg ha-1) in roots over the control (0.452 per cent and 1.03 kg ha-1) (Barathkumar and Manivannan, 2018).

IAA treatment has been cause of an increase of about 44% in the Diosgenin levels in the roots of 15 day-old plants is observed in *Trigonella foenum gracum* (Fig. 2C). This may be to a temporary increase arising from its transport from leaves (Ortuno et al. 1999). At whole plant level, Diosgenin levels also increased 15 days after treatment by $GA_3$. The greater stimulatory effect of $GA_3$ on Diosgenin expression in this plant material compared with that observed with IAA corroborates the results found in *T. foenum gracum* by Jain and Agrawal, (1988).

Interestingly enough, ABA also stimulates the Diosgenin production in this plant especially in the rhizomes. It is quite interesting in the sense that ABA is an antagonist of $GA_3$ and $GA_3$ also promotes Diosgenin production in the plant.

CCC marginally increases the diosgenin content in the leaves. However, in the rhizomes the effect is more pronounced. MH had deleterious effect on the quantity of Diosgenin. There was a consistent decrease in the quantity of diosgenin in response to MH application which was directly proportional to the duration of treatment and the concentration of the chemical concerned. In this case the effect mimics the case of alkaloid yield in *Witharia somnifera* (Barathkumar and Manivannan, 2018).

Polyphenolic compounds are known to have an antioxidant activity and it is likely that the activity of the extracts is due to the presence of these compounds (Adedapo et al. 2009). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching single and triplet

oxygen, or decomposing peroxides. The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Phenolics are the secondary metabolic products in phenylpropanoid pathway as a result of the response of plant cells to environmental stress. Higher phenolic content is accompanied with an increase of PPO activity and the PPO activity may regulate the redox state of phenolic compounds and become involved in the phenylpropanoid pathway (Ebel and Mithorer, 1998).

Total polyphenol, flavonoid, and flavanols have been a positive relationship with the activities of PAL, CHS (chalcone synthase) and CHI chalcone isomerase) in many plants (Mato et al. 2000; Obinata et al. 2003). $GA_3$ antagonizes abscisic acid (ABA) and $GA_3$ treatment retards senescence (Yu et al. 2009). Cytokinins are also as abscisic acid (ABA) and auxin antagonists (Hameed et al. 2015). MH acts as an anti-auxin, anti-gibberellin or regulator of other plant growth regulators (Hoffman and Parups, 1964). Chlorocholine chloride is an antigibberellin growth retardant, with its mechanism based on the resistant of gibberellin biosynthesis in plant tissues (Wang and Xio, 2009). Thus, it is clear that different growth substances does not act in isolation, but there is always synergism and cross-talk among them. Any physiological or biochemical manifestation of ungums is due to the cumulative effect in time and space with respect to the plant in question. Of course, prevalent environment always plays critical role which cannot be ignored.

**Propagation of the plant: Regeneration strategy for Sikkim Himalaya**

By regeneration we mean the complex processes occurring from the time a seed is produced to the time offspring reach maturity (i.e., seed to seed). Though the dominating

regeneration method is planting, natural regeneration is a common phenomenon in forest trees (Karlsson, 2000). In the temperate forests of Sikkim Himalaya, different stages *P. polyphylla* at different stages of germination, seedling growth and maturity is a common phenomenon. However, in this thesis we use the term regeneration to mean the propagation not in the sense of true regeneration as found in forest tress but in terms of propagation strategies. While formulating the propagation strategies, care has been taken to avoid complicated lab intensive *in-vitro* methods despite several success stories (Verma et al. 2012; Raomai et al. 2015; Danu, 2016; Devi et al. 2017). In order to transmit the idea 'from lab to the land', the regeneration strategies only used simple physical manipulations so that any famer of this part of the Himalaya without any formal education could be able to replicate it.

Medicinal plants should be cultivated to save the forests and alpine regions, and at the same time meet the rising demands for herbal medicine to generate income and employment in the mountain regions. At present, the cultivation of food grains has become economically non-viable. (Nautiyal and Nautiyal, 2004). Therefore, some high value low volume cash crop may be the right alternative for the mountains.

*P. polyphylla* is a high value Himalayan medicinal herb. The population of the plant is depleting from its natural habitat due to anthropogenic activities. There is no established agro-technique for promoting its cultivation (Joshi and Dhawan, 2005). However, the massive use of this plant in different traditional as well as in the modern medicines generated a great domestic and international market (Karan et al. 1996). The plant grows in high altitude regions and the germination of the plant is negligible and it gets further reduced as the altitude of the habitat increases. Thus, Tholung shows lesser germination

as compared to Uttaray. Though natural regeneration may be a cost-effective way to increase the population of the species it cannot cope with the challenges posed by climate change and anthropogenic activities. Therefore, at first attempts were made to raise the plants in green house conditions by simple manipulation of naturally available local resources.

Germination of seeds seems negligible in the wild. Even if it germinates, it starts flowering late and takes a long time to produce good yield (Madhu et al. 2010). Therefore, at first germination was attempted through the seeds. However, *P. polyphylla* seed is characterized by a long dormancy period. Low temperature is required to break the dormancy of seeds that provides physiological mechanism to germinate during spring or early summer (Zhou et al. 2003). Seed dormancy is controlled by a balance between hormones like ABA and $GA_3$ (Taiz and Zeiger, 2002). The hormonal balance can be influenced by changes in environmental conditions, such as chilling and exposure to light (Jing and Zheng, 1999). Such conditions were obviously fulfilled in the ambient environment of Sikkim Himalayan area used for experimentation during this study. Generally, the rate of germination remained relatively low (approx. 30%) even in green house conditions.

Use of rhizome segments may be a more pragmatic idea of plant regeneration. The plant could be propagated relatively better from the segments of cut rhizomes than through the seeds. Moreover, this technique requires no hormonal usage which is suitable for the local poor farmers. However, the mortality rate of the cut rhizomes will be a bit higher if no synthetic hormones are being used (Jamir et al. 2015).

While comparing the effect of soil texture on the germination of seeds and rhizome segments under greenhouse conditions, it was found that garden soil gives the least percentage of germination or sprouting. In case of *P. polyphylla* from Uttaray, the highest rate of germination was shown by a potting mixture of garden soil, forest litter and sand (1:1:1) giving 39% and 54% germination of the seeds and rhizome segments respectively. In case of the plant from Tholung, a potting mixture of garden soil, farmyard manure and forest litter (1:1:1) gives maximum rates of germination of seeds (41%) as well as sprouting of rhizome segments (53%). In *P. polyphylla* from Uttarakhand, Danu et al. (2015) have found that a potting mixture of shown soil:loam:sand had the maximum sprouting (76.66% ) while comparison to soil alone had less sprouting (66.66% ). In contrast, in case of *Picrorhiza kurroa* Royle ex Benth highest germination (95%) was recorded in potting mix of sand and soil (1:1) (Patial et al. 2012).

In the study with rhizome cuttings, it was about 40% rhizomes showed morphogenetic responses and sprouted forming the shoot buds. The shoot buds subsequently got converted to plants in the field but most remained in vegetative condition and only about 24% and 21% (from Uttaray and Tholung respectively) of the regenerated plants attained reproductive stage in the next 6 months, Similar results were obtained in *P. polyphylla* from Nagaland but in that case, of regenerated plants of ~49%, only 7% were reproductive plants that bore the inflorescence in the next 4-5 months (Jamir et al. 2015).

Without the hormones treatment, some improvement in germination was achieved in the germination and growth of plants. However, keeping in mind such vital problems of this promising plant, an attempt was made in this investigation to invigorate the plants by chemical manipulations which include application of plant growth regulators (PGRs).

Further experiments were done with single hormone solutions with which the seeds and rhizome cutting were treated. One member each of the growth promoter, auxins, gibberellins and cytokinins were selected for the study. These PGRs have well documented literature in scientific application for the betterment of plant growth, metabolism and yield (Hernandez, 1997; Ashraf et al. 1987, 1989; Amanullah et al. 2010).

Seed priming with different hormones and growth regulators improved germination, stand establishment, growth and yield in several crops (Farooq et al. 2010). The accelerated metabolic activity of seeds due to activation of important enzymes may be the reason for fast and higher germination (Basra et al. 2005; Farooq et al. 2013).

The addition of BAP to the medium is known to have a stimulative effect on seed germination (David et al. 2010). In *Serapias vomeracea* it was found that the different types of cytokinins, the adenine-based cytokinin, BAP enhances seed germination rate as opposed to the phenylurea-type cytokinins which shows limited effects (Bektaş and Sokmen, 2016).

During germination, $GA_3$ is released from embryo which stimulates the action of α-amylase and improves germination (Taiz and Zeiger, 2002). External applications of $GA_3$ was also found to enhance seed germination (El-Barghathi and El-Bakosh, 2005). It has been reported that germination can be induced by gibberellic acid in *Salvia glutinosa*, *Lycopus europaeus*, and *Scutetlaria galericulata* (Thompson, 1969), *Rubia tinctorum L* (Sadeghi et al. 2009), *Pedicularis olympica* (Kirmizi et al. 2010), and *Amaranthus retroflexus* L. (Kepczynski and Sznigir, 2013).

Direct seed treatment with PGRs has significant effect on the rate of germination. Low germination was observed in control (28 and 39%) and highest rate of germination was in $GA_3$ treatment (66 and 74%) in the samples from Tholung and Uttaray respectively. Without the treatment, there was 39% germination of seeds in PPU while the same was 66%. However, the treatment with IBA, BAP and $GA_3$ (100 and 200 µg/ml each) positively influenced the germination rate. IBA could significantly improve the seed germination percentage (upto 62%), however, $GA_3$ (200 µg/ml) gave the best result raising the germination rate by 1.89 fold in PPU and 2.35 fold in PPT respectively. Amongst the PGRs used the least rate of germination was shown by the treatment with BAP. In *Oroxylum indicum* (L.) Vent., 0 % seed germination was observed in BAP Treatment and the rate of germination increased upto 70% by the treatment with 60 µg/ml $GA_3$ (Rami and Patel, 2014). *In Gentiana corymbifera* no germination of seed was found without $GA_3$ pre-treatment. However, on $GA_3$ (100 µg/ml) treatment, germination rate reaches 54% after 70 days (Morgan et al. 1997).

Rhizome segments were subjected under different concentrations of the same hormones whereby it was observed that the highest number of sprouting was achieved by the application of $GA_3$ (200 µg/ml) which caused upto 70% sprouting of the segments. It was followed by BAP treatment resulting in upto 55% sprouting while the control gave only about 30% sprouting.

Growth parameters like height of the plant, increased considerably with the PGR treatment, $GA_3$ in particular, seem to have a tremendous effect as compared to control. Application of PGRs enhanced plant growth and crop yield (Hernandez, 1997). $GA_3$ was

most effective on stem height, leaf area, and shoot fresh and dry weights of almond (Mobli and Baninasab, 2008).

IBA is one of the hormones included in auxin group, besides being used to stimulate rooting, it also has other benefits such as increasing germination, stimulating the growth of leaf and extending the shoots (Kusumo, 1984). IBA-derived auxin has strong roles in various aspects of root development, including regulation of root apical meristem size (Frick and Strader, 2018). In the earliest studies of auxinic compounds in rooting and propagation assays, IBA was reported to be more effective than IAA (Preece, 2003), causing speculation that IBA itself can act as a signaling molecule (Ludwig-Muller, 2000).

In *P. polyphylla* from Sikkim Himalaya, in the seed raised plants, BAP treatment had no effect on the root length while IBA caused an increase of 39% and 24% as compared to the control in the plants in PPU and PPT respectively. However, in terms of positively effecting the root length and shoot length, $GA_3$ (200µg/ml) shows the most pronounced effect increasing the shoot length by 2.26 fold in PPU and 2.11 fold in PPT while increasing the root length by 1.75 fold in PPU and 1.92 fold in PPT over the control. In contrast, in *Lilium martagon* L., seed pre-treatment with the growth hormones IBA and $GA_3$ increased the root length by 13% and 35% respectively (Guney et al. 2016).

In the plants raised from rhizome segments during the present studies, IBA gave a considerable growth of approximately 1.55 and 1.21 times more compared to the control. The higher the concentration of the given IBA solution, the higher the length of the resulting shoots length. The same trend is shown by IBA in influencing the length of the roots. Several researchers reported that IBA had positive effects on the rooting of various

medicinal and aromatic plants, such as *Origanum vulgare* L., *Mentha piperita* L. and *Melissa officinalis* L. (Kuris et al. 1980). Root length was reported to be increased after IBA treatment (Mohanty et al. 2012). Shoot growth is also dependent on the cytokinin supply and BAP is the most effective cytokinin (Romano et al. 1992). In the present research it was found that BAP slightly increases the shoot length but significantly increases the root length (upto 1.68 fold) in rhizome raised plants. Interestingly, it was reported earlier that the average effect of BAP on roots was to reduce its length (Richards and Rowe, 1977) and also that in Amanda Rose BAP had no significant effect in germination and the shoot length decreased with the increase in BAP concentration (DeVries and DuBois, 1988). As in the case of seed raised plants, $GA_3$ (200 µg/ml) caused maximum growth of shoot length increasing it by 1.44 times in PPU and 1.88 times in PPT. Similarly, the root length also got increased by 2.51 times and 2.75 times in PPU and PPT respectively.

Gibberellins are known to increase plant height (Shreve and Campbell, 1967; Taylor, 1972) in different plant species. $GA_3$ can stimulate stem and root elongation as well as seed germination (Hedden et al. 2015). The present study is in agreement with earlier studies showing that $GA_3$ pre-treatment caused highest germination percentage as well as highest radical and plumule length in black gram and horse gram (Chauhan et al. 2009).

It was found that direct PGR treatment of *P. polyphylla* effects the growth in diameter of the rhizome. In both the cases of plants regenerated from seeds and rhizomes, the maximum growth in rhizome diameter was found in $GA_3$ (200 µg.ml) treatment followed by IBA (200 µg/ml) when measured after 180 days. Of course, the rhizome

diameter obtained in case of rhizome segment raised plants were twice that of the seed raised plants during the same period.

In a study after a 120 day growth period, of the individual hormones used for rhizome segment pre-treatment, the maximum rhizome diameter was observed in IBA (100 µg/ml) treatment, the same concentration of $GA_3$ exhibited nearly equal growth. However, the maximum increase in diameter was achieved by mixed hormone pre-treatment of 100 µg/ml each of IAA and $GA_3$ (Danu et al. 2015). In this study, we did not use mixed hormone pre-treatment or any permutation-combination of it.

$GA_3$ treatment of seeds (100 µg/ml) also caused subsequent doubling of rhizome diameter. The same trend could also be seen regarding the growth in diameter in the rhizome segment raised plants. However, in this case the growth is more pronounced. Thus, an increase in diameter by 2.70 times and 3.57 times was found in the rhizome diameter of PPU and PPT respectively. The results in this thesis are in conformity with previous researches in *Alnus acuminata* (Araya et al. 2000), *Pistacia* sp. (Abou-Quad, 2007), *Capsicum annum* (García et al. 2004).

From the present study, it appears that the PGR, $GA_3$ is the clear winner as a pre-treatment medium for optimum germination, growth, biomass yield and certain chemical constituents. However, we cannot view everything in isolation. It is known that the biosynthesis of gibberellins is dependent on auxins (Ross et al. 2000) and both auxins and gibberellins are responsible for stem elongation (Haga and Iino, 1998).

## 6. CONCLUSIONS

*Paris polyphylla* is a very slow growing plant and its entire physiology including germination, growth, flowering etc are erratic. In general, seed grown plant required at least three years for complete maturation. However, underground parts may remain active beyond that period. Predictive habitat modelling revealed that the population is still somewhat maintained in the protected areas of Sikkim like Lingthem, Tholung and Barsey. Nonetheless, there was loss of population in other predicted areas like Lachung and Ravangla due to anthropogenic disturbances. Introduction of the species in protected areas may be a strategy for conservation.

Treatment with different PGRs, modulated physiological and biochemical activities of the plant. In general, plant growth regulators should have resulted in enhancement of growth of the plant. But in this slow growing plant, in a limited duration of experimental period, such growth was not pronounced. However, biochemical activities exhibited distinct changes and it was found that growth retardants in general, caused remarkable enhancement in the metabolites like total phenols, flavonoids, saponins etc. The effect of elicitors on the production of diosgenin, which could be regarded as a marker secondary metabolite in *P. polyphylla* was also studied. The positive role on the enhancement of diosgenin content by elicitor treatment was demonstrated by CCC which enhanced the level of diosgenin by upto 41% in the leaves of PPU and by 45% in the leaves of PPT. While in the rhizomes, the CCC treatment enhanced the rise in diosgenin content by upto 25% in PPU and upto 15% in PPT.

In MTT assay, significant dose-dependent inhibition of growth of human cancer cell line was observed after 72 hours of treatment especially in case of HeLa cells (more than 90%

inhibition). Moderate inhibition of growth was also seen in PC3 (more than 50%) and HepG2 cells (more than 70%). Of the two varieties, PPT was more pronounced in both antioxidant and cytotoxic activities.

The plant grows best in its natural habitat in association with the plants like *Fragaria nubicola*, *Sarcococca coriaceae* and *Pteris* sp. In the nursery condition, the plant showed good seed germination (upto 74%) from seeds with 200 µg/ml GA3 treatment which also showed a robust growth of the seedling subsequently. IBA was the next best treatment suitable for germination as well as growth. Similarly, when grown from rhizome cutting, treatment with 200 µg/ml GA3 treatment resulted in significant sprouting (approx 70%).

The use of *P.polyphylla* rhizome sections seems to be satisfactory technique for vegetative propagation. Treatment of 200µg/ml $GA_3$ produced maximum seed germination and rhizome segment sprouting under natural conditions. In terms of increase in rhizome diameter, growth regulators like $GA_3$ and IBA increases the diameter in the rhizome segment raised plants by approximately two times that of seed raised plants.

## 7. REFERENCES


Abbasi, A. M., Khan, M. A., Shah, M. H., Shah, M. M., Pervez, A. and Ahmad, M. 2013. Ethnobotanical appraisal and cultural values of medicinally important wild edible vegetables of Lesser Himalayas-Pakistan. Journal of ethnobiology and ethnomedicine. 9(1): 66.

Abbasi, T., Anuradha, J., Ganaie, S. U. and Abbasi, S.A. 2015. Gainful utilization of the highly intransigent weed ipomoea in the synthesis of gold nanoparticles. Journal of King Saud University-Science. 27(1): 15-22.

Abdal, D., Hossain, A., Lee, M. K., Kim, S. B., Saha, K., Yang, S. K. and Cho, S. G. 2017. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. International journal of molecular sciences. 18(1): 120.

Abou-Quad, H. 2007. Effect of scarification, gibberellic acid and stratification on seedgermination of three Pistacia species. An-Najah Univ. J. Res. (N. Sc.). 21:1-11.

Adedapo, A. A., Jimoh, F. O., Afolayan, A. J. and Masika, P. J. 2009. Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana.* Records of Natural Products. 3(1): 23-31.

Abdullahi, A. D., Mustapha, R. K., Yau, S. and Adam, M. S. 2018. Exploring the Nigerian Medicinal Plants with Anticancer Activities: A Pharmacological Review. Modern Chemistry. 6(2): 35.

Adedapo, A. A., Jimoh, F. O., Afolayan, A. J. and Masika, P. J. 2009. Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana*. Records of Natural Products, 3(1): 23-31.

Agarwal, N., Majee, C. and Chakraborthy, G. S. 2012. Natural herbs as anticancer drugs. International Journal of Pharm Tech Research. 4(3): 1142-1153.

Agrawal, A. D. 2011. Pharmacological activities of flavonoids: a review. International Journal of Pharmaceutical Sciences and Nanotechnology. 4(2):1394-1398.

Ahmad, V. U., Khaliq-uz-Zaman, S. M., Shameel, S., Perveen, S., Ali, Z. 1998. Steroidal saponins from *Asparagus dumosus.* Phytochemistry. 50: 481–489.

Al-Dabbagh, B., Elhaty, I. A., Al Sakkaf, R., El-Awady, R., Ashraf, S. S. and Amin, A. 2018. Antioxidant and anticancer activities of *Trigonella foenum-graecum, Cassia acutifolia* and *Rhazya stricta*. BMC complementary and alternative medicine. 18(1): 240.

Ali, S., Nasreen, S., Safeer, S., Andleeb, S., Ejaz, M., Bano, S. and Shakir, H. A. 2016. Medicinal plants as therapeutic agents for cancer treatment. Punjab Univ., J Zool. 31:305.

Almeida, I.M.L.D., Rodrigues, J. D. and Ono, E.O. 2004. Application of plant growth regulators at pre-harvest for fruit development of 'PERA' oranges. Brazilian Archives of Biology and Technology. 47(4): 511-520.

Alothman, M., Bhat, R. and Karim, A. A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry. 115(3): 785-788.

Alzoreky, N. and Nakahara, K. 2001. Antioxidant Activity of Some Edible Yemeni Plants Evaluated by Ferrylmyoglobin/ABTS+ Assay. Food science and technology research. 7(2): 141-144.

Amanullah, M. M., Sekar, S. and Muthukrishnan, P. 2010. Prospects and potential of poultry manure. Asian Journal of Plant Sciences. 9(4): 172-182.

Amit, K. J., Dubey, P. K. and Rana, R. C. 2005. *In vitro* callus induction and biomass production of *Catharanthus roseus*. Plant Arch, 5: 55-60.

Arnaud, P. 1956. The heleid genus Culicoides in Japan, Korea and Ryukyu Islands (Insecta: Diptera). Microentomology. 21: 84-207.

Araya, E., Gómez, L., Hidalgo, N. and Valverde, R. 2000. Efecto de la luz y del acido gilberalico sobre la germinacióno in vitro de (*Alnus acuminata*). Agronomía Costarricense. 24(1): 75-80.

Anderson, J. M. and Ingram, J. S. I. 1993. Tropical soil biological and fertility: a handbook of methods. Wallingford: CAB International, 2ed. pp 221.

Anonymous. 1985. Chinese Pharmacopoeia.

Anonymous. 2016. Chinese herbal medicine network of heaven and earth.

Anonymous. 2016. Global Food Antioxidants Market. Trends, Opportunities and Forecasts 2016–2021. http://www.researchandmarkets.com/research/cfm22w/global_food.

Anonymous. 2017. Medicinal and Aromatic Plants (MAP) programme, Thimphu.

AOAC. 1995. Method 991.42 and 993.19. Official methods of analysis ($16^{th}$ ed.). Washington, DC: Association of Official Analytical Chemists.

Aoshima, H., Tsunoue, H., Koda, H. and Kiso, Y. 2004. Aging of whiskey increases 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity. Journal of agricultural and food chemistry. 52(16): 5240-5244.

Ashraf, M., McNeilly, T. and Bradshaw, A. D. 1987. Selection and Heritability of Tolerance to Sodium Chloride in Four Forage Species 1. Crop Science. 27(2): 232-234.

Ashraf, M. 1989. The effect of NaCl on water relations, chlorophyll, and protein and proline contents of two cultivars of blackgram (*Vigna mungo* L.). Plant and soil. 119(2): 205-210.

Augustin, J. M., Kuzina, V., Anderson, S. B., and Bak, S. 2011. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry. 72: 435–457.

Ayoub, Z., and Mehta, A. 2018. Medicinal plants as potential source of antioxidant agents: a review. Asian J Pharm Clin Res. 11(6): 50-56.

Ajaiyeobu, E. O. 2002. Phytochemical and antimicrobial activity of *Parkia biglobosa* and *Parkia bieolor* leaf extracts. Afr. J. Biomed. Res. 5: 125-129.

Bian, H. R. and Lixn, W. H. M., 2002. Advances in studies and applieations of Rhizoma Paridis. J. Chin. Med. Mater. 25(3): 218-220.

Baldwin. E. A., Nisperos-Carriedo, M. O. and Baker, R. A. 1995. Edible coatings for lightly processed fruits and vegetables. Hort. Science. 30: 35-38.

Baral, S. R. and Kurmi, P. P. 2006. A compendium of medicinal plants in Nepal. Mrs. Rachana Sharma, 281 Maiju Bhal, Chabahil, Kathmandu, Nepal. 534.

Baranova, M. A. 1987. Historical development of the present classification of morphological types of stomates. The Botanical Review. 53(1): 53-79.

Barathkumar, T. R. and Manivannan, K. 2018. Influence of biofertilizers and plant growth regulators on growth, yield and alkaloid content of Ashwagandha (*Withania somnifera* Dunal.). Journal of Pharmacognosy and Phytochemistry. 3202-3205.

Bradie, J. and Leung, B. 2016. A quantitative synthesis of the importance of variables used in MaxEnt species distribution models, J Biog. 44(6): 1344-1361.

Barrios, H., Zhang, Y. L., Sandoval, C. and Xiao, Z.A. 2009. Increase of taxol production in *Taxus globosa* Shoot callus by Chlorocholine Chloride. The Open Natural Products Journal. 2(1):33-37.

Barz, W. and Koster, J. 1981. Turnover and degradation of secondary (Natural) products .

In : PK Stumpt and EE Conn ed. The Biochemistry of Plants, New York: Academic Press. 7: 35-84.

Basra, S. M. A., Farooq, M., Tabassam, R. and Ahmad, N. 2005. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). Seed Science and Technology. 33(3): 623-628.

Baskaran, P. and Jayabalan, N. 2008. Effect of growth regulators on rapid micropropagation and psoralen production in *Psoralea corylifolia* L. Acta Physiol Plant. 30: 345–351.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. Plant and soil. 39(1): 205-207.

Beevers, L. 1966. Effect of gibberellic acid on the senescence of leaf discs of nasturtium (*Tropaeolum majus*). Plant physiology. 41(6): 1074.

Bektaş, E. and Sökmen, A. 2016. In vitro seed germination, plantlet growth, tuberization, and synthetic seed production of *Serapias vomeracea* (Burm. f.) Briq. Turkish Journal of Botany. 40(6): 584-594.

Bensky, D., Clavey, S. and Stoger, E. 2004. Materia medica. Chinese Herbal Medicine. pp.3-6.

Bhadra, A. K. and Pattanayak, S. K. 2016. Abundance or Dominance: Which is more justified to calculate Importance Value Index (IVI) of plant species?. Asian J Sci Technol. 7: 3577-3601.

Bhat, A. A., Mayirnao, H. S. and Fayaz, M. 2017. *Paris polyphylla* Smith–A critically endangered, highly exploited medicinal plant in the Indian Himalayan region. Journal of Chemical and Pharmaceutical Sciences. 1202-1210.

Bhatia, A., Arora, S., Singh, B., Kaur, G. and Nagpal, A. 2011. Anticancer potential of Himalayan plants. Phytochemistry Reviews. 10(3): 309-323.

Bhattarai, N. K. 1992. Medical ethnobotany in the Karnali zone, Nepal. Economic Botany, 257-261.

Birch, E. C. and Vickery, L.S. 1961. The effect of maleic hydrazide on certain chemical constituents of flue-cured tobacco. Canadian Journal of Plant Science. 41(1): 170-175.

Bloor, P. A., Barker, F. S., Watts, P. C., Noyes, H. A. and Kemp, S. J. 2001. Microsatellite libraries by enrichment, Version 1.0. Website http://www. genomics. liv. ac. uk/animal/Protocol1.

Boham, A. B. and Kocipai, A. C. 1994. Flavonoid and condensed tannins from Leaves of *Hawaiian vaccininum vaticulum* and *vicalycinium.* Pacific Sci. 48: 458-463.

Braca, A., Prieto, J. M., De Tommasi, N., Tome, F. and Morelli, I. 2004. Furostanol saponins and quercetin glycosides from the leaves of *Helleborus viridis* L. Phytochemistry. 65(21): 2921-2928.

Bukovac, M. J. and Witter, S. H. 1956. Gibberellic acid and higher plants: I: general growth responses. Agric. Exp. Sta. Michi. Quart. Bull. 39: 307–320.

Cabral, H., Matsumoto, Y., Mizuno, K., Chen, Q., Murakami, M., Kimura, M., Terada, Y., Kano, M.R., Miyazono, K., Uesaka, M. and Nishiyama, N. 2011. Accumulation of sub-100nm polymeric micelles in poorly permeable tumours depends on size. Nature nanotechnology. 6(12): 815-823.

Cai, Y., Luo, Q., Sun, M. and Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74: 2157-2184.

Chan, B. C. L., Lau, C. B. S., Jolivalt, C., Lui, S. L., Ganem-Elbaz, C., Paris, J. M. and Ip, M. 2011. Chinese medicinal herbs against antibiotic-resistant bacterial pathogens. Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. 2: 773-781.

Chang, K., Chen, M., Zeng, L., Lan, X., Wang, Q. and Liao, Z. 2014. Abscisic acid enhanced ajmalicine biosynthesis in hairy roots of *Rauvolfia verticillata* by upregulating expression of the MEP pathway genes. Russian journal of plant physiology. 61(1): 136-140.

Challinor, V. L. and De Voss, J. J. 2013. Open-chain steroidal glycosides, a diverse class of plant saponins. Natural product reports. 30(3): 429-454.

Chandra, K., Purohit, V. K. and Andola, H. C. 2015. *Paris polyphylla* Smith: a medicinally important plant species is in high risk in Himalayan region, Med Plants. 7: 292-295.

Chaudhary, K., Prasad, D. N. and Sandhu, B. S. 2015. Preliminary pharmacognostic and phytochemical studies on Nerium oleander Linn. (White cultivar). Journal of Pharmacognosy and Phytochemistry. 4(1): 185-188.

Chauhan, B. S. and Johnson, D. E. 2009. Influence of tillage systems on weed seedling emergence pattern in rainfed rice. Soil and Tillage Research. 106(1): 15-21.

Cheng, H., Li, L., Cheng, S., Cao, F., Wang, Y. and Yuan, H. 2011. Molecular cloning and function assay of a chalcone isomerase gene (GbCHI) from *Ginkgo biloba*. Plant cell reports. 30(1): 49-62.

Choudhury, H. and Tandon P. 2014. Non-destructive assessment of growth performance of embryogenic suspension culture of Pinus kesiya (Royle ex. Gord.) in shake-flask and self-designed bubble bioreactor and successful regeneration of plantlets from the culture systems. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 84(3): 771-777.

Chen, F., Chan, H. Y., Wong, K. L., Wang, J., Yu, M. T., But, P. P. and Shaw P.C. 2008. Authentication of *saussurea lappa*, *an* endangered medicinal material, by ITS DNA and 5S rRNA sequencing. Planta Med. 74: 889-892.

Cheung, J. Y. N., Ong, R. C. Y., Suen, Y. K., Ooi, V., Wong, H. N. C., Mak, T. C. W., Fung, K. P., Yu, B. and Kong, S.K. 2005. Polyphyllin D is a potent apoptosis inducer in drug-resistant HepG2 cells. Cancer letters. 217(2): 203-211.

Chhetri, D. R., Parajuli, P. and Subba, G. C. 2005. Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. Journal of Ethnopharmacology. 99(2): 199-202.

Chhetri, M. S., Timilsina, Y. P., Tripathee, H. P. and Devkota, K. P. 2012. Socio-ecological status and antibacterial activity of *Paris polyphylla* from Panchase area of Kaski District. Nepal Journal of Science and Technology. 13(2): 167-174.

Chhetri, S.B.B. and Khatri, D. 2017. Phytochemical Screening, Total Phenolic and Flavonoid Content and Antioxidant Activity of Selected Nepalese Plants. World J Pharm Pharm Sci. 6(12): 951-968.

Chejara, D. R., Kondaveetia, S., Meena, R. and Siddhanta, A.K. 2014. Antioxidant activity and phytochemical analysis of a few Indian seaweed species. 43(4): 507-518.

Chen, C. X. and Zhou, J. 1987. The steroidal saponins of *Paris axialis*. Acta Bot. Yunnan. 9(2): 239-245.

Chen, C. X., Zhang, Y. T., Zhou, J. 1995a. The glycosides of aerial parts of *Paris polyphylla* var. *yunnanensis*. Acta Bot. Yunnan. 17: 473-478.

Cheng, H., Wang, W., Wang, X. 2014. Nature. 514: 14–15. https://doi.org/10.1038/nature13859.

Chib, S. S. 1977. Sikkim (Vol. 5). New Delhi: Light & Life Publishers.

Chin, T. Y. and Beevers, L. 1970. Changes in endogenous growth regulators in nasturtium leaves during senescence. Planta. 92(2): 178-188.

Choudhury, S. and Gupta, K. 1996. Effect of CCC on growth and alkaloid production in *Catharanthus roseus* (L.) G. Don. Indian Journal of Plant Physiology. 1:163-167.

Cline, S. and Coscia, C. 1987. Accumulation and compartmentalization of sanguinarine upon fungal elicitation of *Papaver bracteatum* suspension-cultures. In phytopathology Amer Phytopathological Soc. 77(12): 1739-1739.

Conrad, J., Dinchev, D., Klaiber, I., Mika, S., Kostova, I. and Kraus, W. 2004. A novel furostanol saponin from *Tribulus terrestris* of Bulgarian origin. Fitoterapia. 75(2): 117-122.

Coultas, L., Chawengsaksophak, K. and Rossant, J. 2005. Endothelial cells and VEGF in vascular development. Nature. 438(7070): 937-945.

Creelman, R. A. and Mullet, J. E. 1997. Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. The Plant Cell. 9(7): 1211-1223.

Coulson, D. A. 1959. Some effects of maleic hydrazide on flue-cured tobacco quality. Tobacco Sci. 3: 69-72.

Cunningham, A. B., Brinckmann, J. A., Bi, Y. F., Pei, S. J., Schippmann, U. and Luo, P. 2018. *Paris* in the spring: A review of the trade, conservation and opportunities in the shift from wild harvest to cultivation of *Paris polyphylla* (Trilliaceae). Journal of ethnopharmacology. 222: 208-216.

Cutter, E.G. 1971. Plant anatomy: Experiment and interpretation, part 2, Organs. Addison-Wesley Publishing Company, London, England.

Curtis, J. T. 1959. The vegetation of Wisconsin University, Wisconsin University, Wisconsin Press, Madison.

Dahal, S., Sharma, T. P., and Borthakur, S. K. 2017. Database on medicinal plants of Tamze Medicinal Plants Conservation Area (MPCA) of Sikkim Himalaya, India. NeBIO–Int J Environ Biodivers. 8: 45-56.

Dahlgren, R. M. T, Clifford, H. T and Yeo, P. F. 1985. The families of the monocotyledons: structure, evolution, and taxonomy. Berlin: Springer-Verlag.

Danu, K., Adhikari, R. S., Pande, V., Singh, M. K. and Rawal, P. 2015. Vegetative propagation of an endangered medicinal plant of Himalayan region, Paris polyphylla Smith. International Journal of Current Microbiology and Applied Sciences. 4(6): 660-665.

Darbour, N., Bayet, C., Doreau, A., Raad, I., Phung, B.H., Dumontet, C., Di Pietro, A., Dijoux-Franca, M.G. and Guilet, D. 2009. Selective modulation of P-glycoprotein activity by steroidal saponines from *Paris polyphylla*. Fitoterapia. 80(1): 39-42.

David, D., Gansau, J. A. and Abdullah, J. O. 2010. Effect of NAA and BAP on protocorm proliferation of borneo scented orchid, *Vanda helvola*. Asia–Pac J Mol Biol Biotechnol. 18: 221-224.

Deb, C. R., Jamir, S. L., and Jamir, N. S. 2015. Studies on vegetative and reproductive ecology of *Paris polyphylla* Smith: a vulnerable medicinal plant. American Journal of Plant Sciences. 6(16): 2561-2568.

Decendit, A., Liu, D., Ouelhazi, L., Doireau, P., Mérillon, J. M. and Rideau, M. 1992. Cytokinin-enhanced accumulation of indole alkaloids in *Catharanthus roseus* cell cultures the factors affecting the cytokinin response. Plant Cell Reports. 11(8): 400-403.

Demir, E., Roters, F. and Raabe, D. 2010. Bending of single crystal microcantilever beams of cube orientation: Finite element model and experiments. Journal of the Mechanics and Physics of Solids. 58(10): 1599-1612.

Deng, D. W., Lauren, D. R., Cooney, J. M., Jensen, D. J., Wurms, K. V. and Upritchard, J. E. 2008. Antifungal saponins from *Paris polyphylla* Smith. Planta Medica. 74(11): 1397-1402.

Denston, R. 1946. The stability of penicillin solutions. Quarterly journal of pharmacy and pharmacology. 19(3): 322-40.

Desai, A. G., Qazi, G. N., Ganju, R. K., El-Tamer, M., Singh, J., Saxena, A. K. and Bhat, H. K. 2008. Medicinal plants and cancer chemoprevention. Current drug metabolism. 9(7): 581-591.

Desai, S., Tatke, P. and Gabhe, S.Y. 2015. Quantification of diosgenin in extracts and formulations containing Solanum nigrum. International Journal of Pharmaceutical Sciences and Research. 6(2): pp.676.

Devi, N. B. and Yadava, P. S. 2006. Seasonal dynamics in soil microbial biomass C, N & P in a mixed-oak forest ecosystem of Manipur, North-East India, App Soil Ecol. 31: 220-227.

Devi, K. B., Lee, B., Roy, A., Kumta, P. N. and Roy, M. 2017. Effect of zinc oxide doping on *in vitro* degradation of magnesium silicate bioceramics. Materials Letters. 207: 100-103.

De Vries, D. P. and Dubois, L. A. 1988. The effect of BAP and IBA on sprouting and adventitious root formation of 'Amanda'rose single-node softwood cuttings. Scientia horticulturae. 34(1-2): 115-121.

Devkota, K., Khan, P., Ranjit, M. T. H., Meli L. R., Samreen. A. and Choudhary, I. M. 2007. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of *Paris polyphylla.* Natural product research. 21(4): 321-327.

Dey, A., Paul, S., Kundu, S., Bandyopadhyay, A. and Bhattacharjee, A. 2013. Elevated antioxidant potential of chlorocholine chloride-treated in vitro grown *Stevia rebaudiana* Bertoni. Acta physiologiae plantarum. 35(6):1775-1783.

Diekmann, M. 1996. Relationship between flowering phenology of perennial herbs and meteorological data in deciduous forests of Sweden. Canadian Journal of Botany. 74(4): 528-537.

Dingkang, W., Guifang, S., Lifang, W., Shuhua, Z. and Xiaojiang, C. 2009. A Novel Mechanism Controls Anther Opening and Closing in *Paris polyphylla* var. *yunnanensis.* Chinese Science Bulletin. 54. 244-248.

Dinis, T. C. P., Madeira, V. M. C. and Almeida, M. L. M. 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane

lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.

Dini, I., Tenore, G. C., Trimarco, E. and Dini, A. 2005. Furostanol saponins in *Allium caepa* L. Var. *tropeana* seeds. Food chemistry. 93(2): 205-214.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. and Ju, Y.H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica.* Journal of food and drug analysis. 22(3): 296-302.

Dong, W., Xu, C., Cheng, T., Lin, K. and Zhou, S. 2013a. Sequencing angiosperm plastid genomes made easy: A complete set of universal primers and a case study on the phylogeny of Saxifragales. Genome Biol Evol. 5: 989-997.

Dong, W., Xu, C., Cheng, T. and Zhou, S. 2013b. Complete chloroplast genome of Sedum sarmentosum and chloroplast genome evolution in Saxifragales. PLoS One. 8: 77965.

Dong, W., Liu, H., Xu, C., Zuo, Y., Chen, Z. and Zhou, S. 2014. A chloroplast genomic strategy for designing taxon specific DNA mini-barcodes: a case study on ginsengs. BMC Genet. 15: 138.

Duan, B. Z., Wang, Y. P., Fang, H. L., Xiong, C., Li, X. W., Wang, P. and Chen, S. L. 2018. Authenticity analyses of Rhizoma Paridis using barcoding coupled with high resolution melting (Bar-HRM) analysis to control its quality for medicinal plant product. Chinese medicine. 13(1): 8.

Dutta, I. C. 2007. Non timber forest products of Nepal: identification, classification, ethnic uses and cultivation. Hill Side Press, Kathmandu, Nepal. 484.

Dhyani, A., Nautiyal, B. P. and Nautiyal, M. C. 2010. Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. International Journal of Biodiversity Science, Ecosystem Services and Management. 6(1-2): 13-19.

Ebel, J. and Mithöfer, A. 1998. Early events in the elicitation of plant defence. Planta. 206(3): 335-348.

Eberhardt, M. V., Lee, C. Y. and Liu, R. H. 2000. Antioxidant activity of fresh apples. Nature. 405(6789): 903-904.

EL-Barghatgi, M. F. and EL-Bakkosh, A. 2005. Effect of some mechanical and chemical pre-treatments on seed germination and seedling growth.

El-Sayeed, M. and Verpoorte, R. 2004. Growth, metabolite profiling and enzyme activities of *Catharanthus roseus* seedlings treated with plant growth regulators, Plant Growth Regul. 44: 53–58.

ENVIS. 2010. Medicinal Plants of Conservation Concern. ENVIS Centre on Conservation of Medicinal Plants, FRLHT, Bangalore. http://envis.frlht.org/medicinal-plants-conservation-concernspecies.

Faegri, K. and Van der Pijl, L. 1979. The principles of pollination ecology. Oxford:Pergamon Press.

Farmer, S. B. and Schilling, E. E. 2002. Phylogenetic analyses of Trilliaceae based on morphological and molecular data. Systematic Botany. 27(4): 674-692.

Farnsworth, N. R. and Soejarto D. D. 1991. Global importance of medicinal plants. Pp. 25-51 in The Conservation of Medicinal Plants. Edited by O. Akerelev, V. Heywood & H. Synge. Cambridge University Press, Cambridge.

Farooq, U., Kozinski, J. A., Khan, M. A., Athar, M. 2010. Biosorption of heavy metal ions using wheat based biosorbents – a review of the recent literature. Bioresour. Technol. 101: 5043–5053.

Farooq, J., Khaliq, I., Akbar, M., Kashif, M. and Mahpara, S. 2013. Hybrid vigor studies for different yield contributing traits in wheat under normal and heat stress conditions. Comunicata Scientiae. 4(2): 139-152.

Farooquee, N. A., Majila, B. S. and Kala, C. P. 2004. Indigenous knowledge systems and sustainable management of natural resources in a high-altitude society in Kumaun Himalaya, India. J Hum Ecol. 16: 33-42.

Fathonah, D. and Sugiyarto, S. 2009. Effect of IAA and $GA_3$ toward the growing and saponin content of purwaceng (*Pimpinella alpina*). Nusantara Bioscience, 1(1).

Farvid, M. S., Chen, W. Y., Michels, K. B., Cho, E., Willett, W. C. and Eliassen, A. H. 2016. Fruit and vegetable consumption in adolescence and early adulthood and risk of breast cancer: population based cohort study. Bmj. 353.

Fenwick, G. R., Price, K. R., Tsukamoto, C. and Okubo, K. 1991. Toxic substances in crop plants. eds. D'Mello JPF, Duffus CM, and Duffus JH, The Royal Society of Chemistry, Cambridge. 285-327.

Fick, G. N. and Qualset, C. O. 1975. Genetic control of endosperm amylase activity and gibberellic acid responses in standard-height and short-statured wheats. Proceedings of the National Academy of Sciences. 72(3): 892-895.

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q. and Griendling, K. K. 2018. Reactive oxygen species in metabolic and inflammatory signaling. Circulation research. 122(6): 877-902.

Foyer, C. H. and Noctor, G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell and Environment. 28(8): 1056-1071.

Fraga, C. G., Galleano, M., Verstraeten, S. V. and Oteiza, P. I. 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol. Aspects Med. 31: 435–445.

Frick, E. M. and Strader, L. C. 2018. Roles for IBA-derived auxin in plant development. Journal of Experimental Botany. 69(2): 169-177.

Furuya, T., Ikuta, A. and Syōno, K. 1972. Alkaloids from callus tissue *of Papaver somniferum*. Phytochemistry. 11(10): 3041-3044.

Gai, Z., Wang, Y., Ding, Y., Qian, W., Qiu, C., Xie, H., Sun, L., Jiang, Z., Ma, Q., Wang, L. and Ding, Z. 2020. Exogenous abscisic acid induces the lipid and flavonoid metabolism of tea plants under drought stress. Scientific reports. 10(1):1-13.

Gao, L. L., Li, F. R., Jiao, P., Yao, S. T., Sang, H. and Si, Y. H. 2011. Apoptosis of human ovarian cancer cells induced by *Paris chinensis* dioscin via a Ca (2+)-mediated mitochondrion pathway. Asian Pac J Cancer Prev. 12(5): 1361-1366.

George, S. S., Wise, A. K., Shivdasani, M. N., Shepherd, R. K. and Fallon, J. B. 2014. Evaluation of focused multipolar stimulation for cochlear implants in acutely deafened cats. Journal of neural engineering. 11(6): 1-13.

Ghaedrahmati, R., Moradzadeh, A., Fathianpour, N. and Lee, S. K. 2014. Investigating 2-D MT inversion codes using real field data. Arabian Journal of Geosciences. 7(6): 2315-2328.

Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide Dismutases: I. Occurrence in higher plants. Plant physiology. 59(2): 309-314.

Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide Dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. Plant physiology. 59(2): 315-318.

García, J. A. L., Probanza, A., Ramos, B., Palomino, M. and Mañero, F. J. G. 2004. Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. Agronomie, EDP Sciences. 24 (4): 169-176

Grebinskii, S. O. and Khmil, M. V. 1980. Effect of chlorocholine chloride on biosynthesis of anthocyans in leaves of red cabbage and spiderwort. Fiziologiia i biokhimiia kul'turnykh rastenii.= Physiology and biochemistry of cultivated plants.

Guney, K., Cetin, M., Sevik, H. and Guney, K. B. 2016. Influence of germination percentage and morphological properties of some hormones practice on Lilium martagon L. seeds. Oxidation communications. 39(1): 466-474.

Guo, L., Su, J., Deng, B.W., Yu, Z.Y., Kang, L.P., Zhao, Z.H., Shan, Y.J., Chen, J.P., Ma, B.P. and Cong, Y.W. 2008. Active pharmaceutical ingredients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from *Paris polyphylla* Sm. var. *yunnanensis* used for abnormal uterine bleeding. Human Reproduction. 23(4): 964-971.

Gurfinkel, D. M. and Rao, A. V. 2003. Soyasaponins: the relationship between chemical structure and colon anticarcinogenic activity. Nutrition and cancer. 47(1): 24-33.

Gutierrez, J. B. and Salsamendi, A. L. 2001. Fundamientos de ciencia toxicologica. Diaz de Santos, Madrid.155–177.

Haga, K. and Iino, M. 1998. Auxin-growth relationships in maize coleoptiles and pea internodes and control by auxin of the tissue sensitivity to auxin. Plant Physiology. 117(4): 1473-1486.

Hajra, P. K. and Verma, D. M. 1996. Flora of Sikkim. Vol. 1 (Monocotyledons). Flora of India, series, 2.

Hameed, I. H., Ibraheam, I. A. and Kadhim, H. J. 2015. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. Journal of Pharmacognosy and Phytotherapy. 7(6): 90-106.

Han, X., Shen, T. and Lou, H. 2007. Dietary polyphenols and their biological significance. International Journal of Molecular Sciences. 8(9): 950-988.

Han, B. Y., Zhang, Y., Tian, X. Y. and Xiao, C. J. 2016. Chemical constituents from undergroud part of *Astragalus camptodontoides.* Guihaia, 36(11): 1382-1388.

Hao, D. C., Gu X. J. and Xiao P.G. 2015. 3-Taxus medicinal resources: a comprehensive study, Medicinal Plants, Woodhead Publishing. 3: 97-136.

Hara, H. 1969. Variations in *Paris polyphylla* Smith with reference to other Asiatic species. Journal of the Faculty of Science, University of Tokyo, Sect. 3, Botany. 10: 141–180.

Hara, H. 1969. Remarkable examples of speciation in Asiatic plants. American Journal of Botany. 56(7): 732-737.

Haraguchi, M., Dos Santos, A. P. Z., Young, M. C. M. and Chui, E. P. 1994. Steroidal prosapogenins from *Dioscorea olfersiana*. Phytochemistry. 36(4): 1005-1008.

Harborne, J. B. 1973. Phenolic compounds. In Phytochemical methods Springer, Dordrecht. pp. 33-88.

Harborne, J. B. and Williams, C.A. 1998. Anthocyanins and other flavonoids. Natural Product Reports, 15(6): 631-652.

Hayes, P. Y., Lehmann, R., Penman, K., Kitching, W. and De Voss, J. J. 2009. Steroidal saponins from the roots of *Trillium erectum* (Beth root). Phytochemistry. 70(1): 105-113.

Hawk, A. M. 2017. Habitat modeling of a rare endemic Trillium Species (*Trillium Simile* Gleason): a comparison of the methods maxent and domain for modeling rare species-rich habitat. Biology Department. Vol. Master of Science in Biology (Dissertation). Western Carolina University, USA.

Heath, R. L. and Packer, L. 1968. Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics. 125(1): 189-198.

Hedden, P. and Sponsel, V. 2015. A century of gibberellin research. Journal of plant growth regulation. 34(4): 740-760.

Henke, A., Huber, S., Stelzner, A. and Whitton, J. L. 1995. The role of CD8+ T lymphocytes in coxsackievirus B3-induced myocarditis. Journal of Virology, 69(11): 6720-6728.

Henke, A., Launhardt, H., Klement, K., Stelzner, A., Zell, R. and Munder, T. 2000. Apoptosis in coxsackievirus B3-caused diseases: interaction between the capsid protein VP2 and the proapoptotic protein siva. Journal of virology. 74(9): 4284-4290.

Henry, S. H., Bosch, F. X. and Bowers, J. C. 2002. Aflatoxin, hepatitis and worldwide liver cancer risks. In Mycotoxins and food safety. Springer, Boston, MA. 229-233.

Hernández, T., Garcia, C. and Reinhardt, I. 1997. Short-term effect of wildfire on the chemical, biochemical and microbiological properties of Mediterranean pine forest soils. Biology and fertility of soils. 25(2): 109-116.

Hernandez. P. A, Graham, C. H, Master, L. L. and Albert, D. L. 2006. The effect of sample size and species characteristics on performance of different species distribution modelling methods, Ecography. 29: 773-785.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A. 2005. Very high-resolution interpolated climate surfaces for global land areas. International

Journal of Climatology: A Journal of the Royal Meteorological Society. 25(15): 1965-1978.

Hirayama, T. and Shinozaki, K. 2007. Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. Trends in plant science. 12(8): 343-351.

Hipkins, V. D., Marshall, K. A., Neale, D. B., Rottmann, W. H. and Strauss, S. H. 1995. A mutation hotspot in the chloroplast genome of a conifer (Douglas-fir: Pseudotsuga) is caused by variability in the number of direct repeats derived from a partially duplicated tRNA gene. Current Genetics. 27(6): 572-579.

Hoffman, I. and Parups, E. V. 1964. Mode of action of maleic hydrazide in relation to residues in crops and soils. In Residue Reviews/Rückstands-Berichte, Springer, New York, NY. 96-113.

Hong-Xiang, Y. I. N., Hao, Z. H. A. N. G. and Dan, X. U. E. 2007. *Paris polyphylla* var. *emeiensis* HX Yin, H. Zhang & D. Xue, a new variety of Trilliaceae from Sichuan, China. Journal of Systematics and Evolution. 45(6): 822-827.

Hostettmann, K. and Marston, A. 1995. Chemistry and pharmacology of natural products Saponins. Cambridge: Cambridge University Press.

Huang, Y., Wang, Q. and Cui, L. 2005. Primary comments on chemotaxonomy of *Paris* spp. based on saponins analysis. Journal of Chinese Pharmaceutical Sciences. 14(3): p.176.

Huang, Y., Zhao, J., Zhou, L., Wang, M., Wang, J., Li, X. and Chen, Q. 2009. Antimicrobial compounds from the endophytic fungus *Fusarium* sp. Ppf4 isolated from the medicinal plant *Paris polyphylla* var. *yunnanensis.* Natural Product Communications, 4(11): 1455-1458.

Huang, X. X, Gao, W. Y., Zhao, Z. Y. and Man, S. L. 2010. Flavone and steroid chemical constituents from rhizome of *Paris axialis.* China Journal of Chinese Materia Medica. 35: 2994–2998.

Huang, Y., Li, X., Yang, Z., Yang, C., Yang, J. and Ji, Y. 2016. Analysis of complete chloroplast genome sequences improves phylogenetic resolution in *Paris* (Melanthiaceae). Frontiers in plant science. 7: 1797.

Hufford, C. D., Liu, S. and Clark, A.M. 1988. Antifungal activity of *Trillium grandiflorum* constituents. Journal of natural products, 51(1): 94-98.

Huxley, A. 1992. The New RHS Dictionary of Gardening. MacMillan Press.

Ikeda, T., Ando, J., Miyazono, A., Zhu, X. H., Tsumagari, H., Nohara, T., Yokomizo, K. and Uyeda, M. 2000. Anti-herpes virus activity of Solanum steroidal glycosides. Biological and Pharmaceutical Bulletin. 23(3): 363-364.

Iqbal, J., Abbasi, B. A., Mahmood, T., Kanwal, S., Ali, B., Shah, S. A. and Khalil, A. T. 2017. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine. 7(12): 1129-1150.

Iqbal, M. Z. and Khan, S. 2018. Progress in the performance of dye sensitized solar cells by incorporating cost effective counter electrodes. Solar Energy. 160: 130-152.

Ivanchina, N. V., Kicha, A. A. and Stonik, V. A. 2011. Steroid glycosides from marine organisms. Steroids. 76(5): .425-454.

Jain, S. C., Agrawal, M. and Sharma, R. A. 1996. The genus Trigonella–phytochemistry and biology. Ancient science of life. 16(2): 108.

Jain, P. K. and Joshi, H. 2012. Recent developments on anti-convulsants. Int J Res Dev Pharm Life Sci.1(3): 105-11.

Jogaite, V., Kleizaite, V., Stapulionyte, A. and Bjerketvedt, D. 2003. Superoxide dismutase polymorphisms in wild populations of herb *Paris* (*Paris quadrifolia* L., Trilliaceae). 59-62

Jamir, N. S. Lanusunep, and Pongener, N. 2012. Medico-herbal medicine practiced by the Naga tribes in the state of Nagaland (India). Indian J Fund Appl Life Sci. 2: 328-333.

Jamir, S. L., Jamir, N. S. and Deb, C. R. 2015. Production of clonal planting material and propagation of *Paris polyphylla* Smith var. polyphylla through rhizome splitting. European Journals of Biotechnology and Bioscience. 3(9): 43-46.

Jayapriya, G. and Shoba, F.G. 2015. GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* (Linn.). Journal of Pharmacognosy and Phytochemistry. 4(1): 113-117.

Jeong, G. T. and Park, D. H. 2005. Enhancement of growth and secondary metabolite biosynthesis: Effect of elicitors derived from plants and insects. Biotechnology and Bioprocess Engineering. 10(1): 73.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. 2011. Global cancer statistics. C A: a cancer journal for clinicians. 61(2): 69-90.

Ji, Y, Fritsch, P. W., Li, H., Xiao, T. and Zhou, Z. 2006. Phylogeny and Classification of *Paris* (Melanthiaceae) Inferred from DNA Sequence Data. Annals of Botany. 98(1): 245-256.

Ji, Y., Yang, H. and Huang, Y. 2017. A new species of *Paris* sect. *Axiparis* (Melanthiaceae) from Yunnan, China, Phytotaxa. 306 (3): 234–236.

Ji, Y. H., Li, H., and Zhou, Z. K. 2006. *Paris caobangensis* (Trilliaceae), a new species from northern Vietnam. 44(6): 700–703

Jiang, H., Zhao, P., Feng, J., Su, D. and Ma, S. 2014. Effect of paris saponin I on radio sensitivity in a gefitinib-resistant lung adenocarcinoma cell line. Oncol Lett. 7: 2059-2064.

Jin, L.Y., Lu, T. X., Qin, X. J., Ni, W., Yan, H., Chen, Y., Liu, H., He, H. P. and Liu, H. Y. 2016. Two new highly oxygenated spirostanol saponins from *Paris polyphylla* var. *stenophylla*. Natural products and bioprospecting. 6(4): 205-210.

Joshi, P. and Dhawan, V. 2005. *Swertia chirayita*–an overview. Current science. 89(4): 635-640.

Joshi, M. D and Bhatta, K. 2014. Distribution and Population Status of *Paris polyphylla* Sm. and its Conservation Challenges in Api-Nampa Conservation Area, Nepal. The Rufford Small Grants Foundation. RSG ref. 13728-1.

Joshi, N. R. 2012. A Field Manual on Nursery Management and Cultivation of Satuwa (Paris polyphylla). Asia Network for Sustainable Agriculture and Bioresources (ANSAB), Kathmandu, pp 12.

Jun, Z. 1989. Some bioactive substances from plants of West China. Pure and Applied Chemistry. 61(3): 457-460.

Jungklang, J. and Saengnil, K. 2012. Effect of paclobutrazol on Patumma cv. Chiang Mai Pink under water stress. Songklanakarin Journal of Science and Technology. 34(4): 361-366.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry. 47(10): 3954-3962.

Kan, Y., Chen, T., Wu, Y. and Wu, J. 2015. Antioxidant activity of polysaccharide extracted from *Ganoderma lucidum* using response surface methodology. International journal of biological macromolecules. 72: 151-157.

Kang, L. P., Liu, Y. X., Eichhorn, T., Dapat, E., Yu, H. S., Zhao, Y., Xiong, C. Q., Liu, C., Efferth, T. and Ma, B.P. 2012. Polyhydroxylated steroidal glycosides from *Paris polyphylla*. Journal of natural products. 75(6): 1201-1205.

Kang, L. P., Huang, Y. Y., Zhan, Z. L., Liu, D. H., Peng, H. S., Nan, T. G., Zhang, Y., Hao, Q. X., Tang, J. F., Zhu, S. D. and Yang, G. 2017. Structural characterization and discrimination of the *Paris polyphylla* var. *yunnanensis* and *Paris vietnamensis* based on metabolite profiling analysis. Journal of Pharmaceutical and Biomedical Analysis. 142: 252-261.

Kapoor, L. D., Singh, A., Kapoor, S. L. and Shrivastava, S. N. 1969. Survey of Indian medicinal plants for saponin, alkaloid and Flavonoids. Lloyd. 32: 297-302.

Kappers, I. F., Jordi, W., Maas, F. M., Stoopen, G. M. and Van Der Plas, L. H. 1998. Gibberellin and phytochrome control senescence in alstroemeria leaves independently. Physiologia Plantarum. 103(1): 91-98.

Kar, M. and Mishra, D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant physiology. 57(2): 315-319.

Karan, M., Vasist, K. and Handa, S. S. 1996. Iriods and Secoiriods of the genus Swertia. supplement to Cultivation and Utilization of Medicinal plants. 349-354.

Karlsson, C. 2000. Effects of release cutting and soil scarification on natural regeneration in *Pinus sylvestris* shelterwoods. Doctoral thesis Swedish University of Agricultural Sciences. Southern Swedish Forest research Centre, Alnarp. 137: 1401-6230.

Kazempour, O. S., Kawano, D. and Ohara, M. 1999. Trilliums, 1997. Plant Species Biology. 14(1): 75-94.

Kehie, M., Kumaria, S. and Tandon, P. 2013. In vitro plantlet regeneration from cotyledon segments of *Capsicum chinense* Jacq. cv. Naga King Chili, and determination of capsaicin content by high performance liquid chromatography. Sci Hortic. 164: 1–8.

Kępczyński, J. and Sznigir, P. 2013. Response of *Amaranthus retroflexus* L. seeds to gibberellic acid, ethylene and abscisic acid depending on duration of stratification and burial. Plant Growth Regulation. 70(1): 15-26.

Keramat, B., Kalantari, K. M. and Arvin, M. J., 2009. Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (Glycine max L.). African journal of microbiology Research. 3(5): 240-244.

Kerek, F. 1981. Boicil, a new and very efficient antialgic, spasmolytic, and blood vessel regulating drug obtained from the plant Helleborus. In: First International Conference on Chemistry and Biochemistry of Biologically Active Natural Compounds (FECS). 2: 22–37.

Ketchum, R. E., Gibson, D. M., Croteau, R. B. and Shuler, M. L. 1999. The kinetics of taxoid accumulation in cell suspension cultures of Taxus following elicitation with methyl jasmonate. Biotechnology and bioengineering. 62(1): 97-105.

Khandelwal, K. 2008. Practical pharmacognosy. Pragati Books Pvt. Ltd..

Kim, J., Lee, S. M., Srinivasan, S. and Chamberlin, C. E. 1995. Modeling of proton exchange membrane fuel cell performance with an empirical equation. Journal of the electrochemical society. 142(8): 2670.

Kim, S. Y., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. 1999. Inhibition of mouse ear edema by steroidal and triterpenoid saponins. Archives of pharmacal research. 22(3): 313-316.

Kirmizi, S., Güleryüz, G., Arslan H. and Sakar. F. S. 2010. 'Effects of moist chilling, gibberellic acid, and scarification on seed dormancy in the rare endemic *Pedicularis olympica* (Scrophulariaceae)'. Turkish Journal of Botany. 34(3): 225-232.

Klein, B. P. and Perry, A. K. 1982. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. Journal of Food Science. 47(3): 941-945.

Kong, M., Fan, J., Dong, A., Cheng, H. and Xu, R. 2010. Effects of polyphyllin I on growth inhibition of human non-small lung cancer cells and in xenograft. Acta Biochim Biophys Sin. 42(11): 827-833.

Kertadikara, A. W. S. and Prat, D. 1995. Isozyme variation among Teak (Tectona grandis L. F.) provenances. Theoretical and Applied genetics. 90(6): 803-810.

Kryston, T. B., Georgiev, A. B., Pissis, P. and Georgakilas, A. G. 2011. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res. 3(711): 0027-5107.

Kumar, S. and Stohlgren, T. J. 2009. Maxent modeling for predicting suitable habitat for threatened and endangered tree Canacomyrica monticola in New Caledonia, J Ecol Nat Env. 1: 94-98.

Kumar, U., Joshi, A. K., Kumar, S., Chand, R. and Roder. M. S. 2009. Mapping of resistance to spot blotch disease caused by Bipolaris sorokiniana in spring wheat, Theor Appl Genet. 118: 783-792.

Kumaran, A. and Joel K. R. 2006. Antioxidant Activities of the Methanol Extract of Cardiospermum halicacabum. Pharmaceutical biology. 44(2): 146-151.

Kuris, A., Altman, A. and Putievsky, E. 1980. Rooting and initial establishment of stem cuttings of oregano, peppermint and balm. Scientia Horticulturae. 13(1): 53-59.

Kusumo, S. 1984. Zat Pengatur Tumbuh Tanaman. Yasaguna. Jakarta.

Lalsangluaii, F., Chinlampianga, M. and Shukla, A. C. 2013. Efficacy and potency of *Paris polyphylla* Smith, an ethno-medicinal plant of Mizoram. Sci Technol J. 1: 36-40.

Lamien-Meda, A., Lamien, C. E., Compaore, M. M. Y., Meda, R. N. T., Kiendrebeogo, M., Zeba, B., Millogo, J. F. and Nacoulma, O. G. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules.13: 581–594.

Lange, H., Shropshire, W. and Mohr, H. 1971. An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiology. 47(5): 649-655.

Lee, M. S, Chan, J. Y, Kong, S. K, Yu, B., Eng-Choon, V. O., Nai-Ching, H. W., Chung-Wai, T. M and Fung, K. P. 2005. Effects of polyphyllin D, a steroidal saponin in *Paris polyphylla*, in growth inhibition of human breast cancer cells and in xenograft. Cancer Biology & Therapy. 4 (11): 1248–1254.

Lee, R. K., Ong, R. C., Cheung, J. Y., Li, Y. C., Chan, J. Y., Lee, M., Suen, Y. K., Fung, K. P., Ho, H. P., Yu, B. and Li, M. 2009. Polyphyllin D-a potential anti-cancer agent to kill hepatocarcinoma cells with multi-drug resistance. Current Chemical Biology. 3(1): 89-99.

Lee, R., Wong, T. Y. and Sabanayagam, C. 2015. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye and vision: 2(1): 1-25.

Lecours, V., Brown, C. J, Devillers, R., Lucieer, V. L. and Edinger, E. N. 2016. Comparing selections of environmental variables for ecological studies: A focus on terrain attributes, PLoS One. 11: 12.

Lepcha, D. L., Pradhan, A., and Chhetri, D. R. 2019. Population assessment and species distribution modeling of *Paris polyphylla* in Sikkim Himalaya, India. Biodiversitas Journal of Biological Diversity. 20(5): 1299-1305.

Lepcha, D. L., Chhetri, A. and Chhetri, D. R. 2019. Antioxidant and Cytotoxic Attributes of *Paris polyphylla* Smith from Sikkim Himalaya. Pharmacognosy Journal. 11(4): 705-711.

Leskovar, D. I., Shinohara, T., Agehara, S. and Patil, B. 2009, June. Integrated approaches for annual artichoke production in southwest Texas. In VII International Symposium on Artichoke, Cardoon and Their Wild Relatives. 942: 235-238.

Li, H., Yang, X. H., Liang, H, X., Wei, Z. X., Gu, Z. H., Yang, Y. P., Ji, B. R., Feng, W. C., Duan, J. Y., Li, C. Y., Chen, C. X. and Zhou, J. 1998. The genus Paris (Trilliaceae). Beijing: Science Press. p. 117

Li, H. 1998. The genus Paris (Trilliaceae), vol. 7, Science Press, Beijing, 182–184.

Li, H. 1998. The phylogeny of the genus *Paris* L. In: Li H, ed. The genus Paris (Trilliaceae). Beijing: Science Press. 58–65.

Li, Q., Ding, C. B., Hu and X. L. 2007. Pollen vitality and stigma receptivity in three species of *Paris* (in Chinese). China J Chin Mat Med. 10: 969-970.

Li, Q., Ge F. and Wang, L. 2003. Establishment of Sterile Culture in Primary Culture Process of Paris. Journal of Sichuan Normal University. Natural Science. 26.

Li, Z. H., Wan, J. Y., Wang, G. Q., Zhao, F. G. and Wen, J. H. 2013. Identification of compounds from *Paris polyphylla* (Chong Lou) active against Dactylogyrus intermedius, Parasitology. 140 (8): 952-958.

Li, F. R., Jiao, P., Yao, S. T., Sang, H., Qin, S. C., Zhang, W., Zhang, Y. B. and Gao, L. L. 2012. *Paris polyphylla* Smith extract induces apoptosis and activates cancer suppressor gene connexin26 expression. Asian Pac J Cancer Prev. 13(1): 205-209.

Li, P., Mou, Y., Lu, S., Sun, W., Lou, J., Yin, C. and Zhou, L. 2012. Quantitative determination of diosgenin in *Dioscorea zingiberensis* cell cultures by

microplate-spectrophotometry and high-performance liquid chromatography. African journal of pharmacy and pharmacology. 6(15): 1186-1193.

Li, Q. and Zhang, C. 2012. A Study of Antioxidant Activity about Extracts with Different Solvents from Four Chinese Herbal Medicines in Enshi. In Advanced Materials Research Trans Tech Publications Ltd. 396: 221-224.

Li, A. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical journal of the Linnean Society. 141(4): 399-436.

Li, S. P. and B. Yang. 2005. Preliminary reports on the domestic cultivation of *Paris polyphylla* var. *yunnanensis.* Yunnan Agr. Sci. Technol. 2: 22-24.

Li, W. Y., Chan, S. W., Guo, D. J. and Yu, P. H. F. 2007. Correlation between antioxidative power and anticancer activity in herbs from traditional Chinese medicine formulae with anticancer therapeutic effect. Pharmaceutical biology. 45(7): 541-546.

Li, X. L. and Zhou, A. G. 2007. Evaluation of the antioxidant effects of polysac-charides extracted from *Lycium barbarum*. Medicinal Chemistry Research. 15(9): 471–482.

Li, J., Han, Z. S. and Li, Q. W., 2008. Study on Antitumor Activity and Immunomodulating Effect of Polysaccharides Isolated from *Solanum nigrum* Linne. Journal of Anhui Agricultural Sciences. 36(33): 14589-14590.

Li, J. R., Yu, K., Wei, J. R., Ma, Q., Wang, B. Q. and Yu, D. 2010. Gibberellin retards chlorophyll degradation during senescence of *Paris polyphylla*. Biologia Plantarum. 54(2): 395-399.

Li, Y. H., Zhang, Z. and Sun, G. M. 2011. Changes in cell number and cell size during pineapple (*Ananas comosus* L.) fruit development and their relationship with fruit size. Australian Journal of Botany. 58(8): 673-678.

Li, J., Ma, W., Cheng, X., Zhang, X., Xie, Y., Ji, Z. and Wu, S. 2020. Activation of FOXO3 pathway is involved in polyphyllin I-induced apoptosis and cell cycle arrest in

human bladder cancer cells. Archives of Biochemistry and Biophysics. 687: 108363.

Liang, M. Y., Wang, Y. Z., Qiao, X., Lu, Y. W., Chen, M. H., Li, P., Wen, X. D. and Yang, J. 2019. Structural characterisation and discrimination of the aerial parts of *Paris polyphylla* var. *yunnanensis* and *Paris polyphylla* var. *chinensis* by UHPLC-QTOF-MS coupled with multivariate data analysis. Phytochemical Analysis. 30(4): 437-446.

Liang, S. Y. and Soukup, V. G. 2000. *Paris* L. Flora of China. 24: 88-95.

Lichtenthaler, H. K. and Welburn A. R. 1983. Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochemical Society Transactions. (11): 591-593.

Linh, N. T. N., Tam, H. T., Tung, H. T., Luan, V. Q., Hien, V. T., Loc, N. H. and Nhut, D. T. 2019. Improvement of bioactive saponin accumulation in adventitious root cultures of *Panax vietnamensis* via culture periods and elicitation. Plant Cell, Tissue and Organ Culture (PCTOC). 137(1): 101-113.

Lin, Z., Liu, Y., Li, F., Wu, J., Zhang, G., Wang, Y., Lu L. and Liu. Z. 2015. Anti-lung cancer effects of polyphyllin VI and VII potentially correlate with apoptosis *in vitro* and *in vivo*. Phytotherapy Research. 29: 1568–1576.

Lin, J. T., Chang, Y. Z., Lu, M. P. and Yang, D. J. 2011. Simultaneous determination of furostanol, pennogenyl, and diosgenyl glycosides in Taiwanese Rhizoma Paridis (*Paris formosana* Hayata) by high-performance liquid chromatography with evaporative light scattering detection. Journal of agricultural and food chemistry. 59(5): 1587-1593.

Lin-Wang, K., Micheletti, D., Palmer, J., Volz, R., Lozano, L. and Espley, R. 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ. 34: 1176–119.

Lin, S. Y., Johnson, S. M., Abraham, M., Vella, M. C., Pasquinelli, A., Gamberi, C., Gottlieb, E. and Slack, F. J. 2003. The C. elegans hunchback homolog, hbl-1,

controls temporal patterning and is a probable microRNA target. Developmental cell. 4(5): 639-650.

Liu, H., Yang, J., Du, Y., Bai, X. and Du, Y. 2010. Synthesis of four oligosaccharides derived from *Paris polyphylla* var. *yunnanensis* and their tobacco (*Nicotiana tabacum* L.) growth-regulatory activity. Plant growth regulation. 60(1): 69.

Liu, J. Y., Guan, Y., Zou, L., Gong, Y., Hua, H., Xu, Y., Zhang, H., Yu, Z. and Fan, W. H. 2012. Saponins with neuroprotective effects from the roots of *Pulsatilla cernua*. Molecules. 17(5): 5520-5531.

Liu, T. and Ji, Y. 2012. Molecular authentication of the medicinal plant *Paris polyphylla* Smith var. *yunnanensis* (Melanthiaceae) and its related species by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). J Med Plant Res. 6: 1181-1186.

Liu, X. Wang, X., L., Zhang, T. T. and Wang, Q. 2009. A new spirostanol saponin from the rhizomes of *Paris mairei.* Chinese Chemical Letters. 20(7): 820-822.

Liu, Y., Wang, M., Liu, K., Qiu, P., Zhang, S., Lu, Y., Tang, N. and Tang, H. 2018. New steroidal saponins from the rhizomes of *Paris vietnamensis* and their cytotoxicity. Molecules. 23(3): 588.

Liu, Z. X. and Tian, Q. J. 2014. Analysis of chemical components of volatile oil from *Paris polyphylla* and their antibacterial activities. Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials. 37(4): 612-616.

Liu, J., Man, S., Li, J., Zhang, Y., Meng, X. and Gao, W. 2016. Inhibition of diethylnitrosamine-induced liver cancer in rats by Rhizoma paridis saponin. Environmental toxicology and pharmacology. 46: 103-109.

Liu, Z., Li, N., Gao, W., Man, S., Yin, S. and Liu, C. 2012. Comparative study on hemostatic, cytotoxic and hemolytic activities of different species of *Paris* L. Journal of ethnopharmacology. 142(3): 789-794.

Lone, B. A., Chishti, M. Z., Bhat, F. A., Tak, H. and Bandh, S. A. 2017. Anthelmintic Activities of Aqueous and Methanol Extracts of Prunella vulgaris L. Nat Prod Chem Res. 5(269): 2.

Long, C. L., Li, H., Quyang, Y., Yang, X., Li Q. and Trangnal, B. 2003. Strategies for agrobiodiversity conservation and promotion; a case from Yunnan, China. Biodiversity and Conservation. 12: 1145-1156.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. Journal of biological chemistry. 193: 265-275.

Lu, W., Ogasawara M. A. and Huang P. 2007. Models of reactive oxygen species in cancer. Drug Discovery. 4: 67-73.

Ma, J. C. and Lau, F. W. 1985. Structure characterization of haemostatic diosgenin glycosides from Paris polyphylla. Phytochemistry. 24(7): 1561-1565.

Madhu, K. C., Phoboo, S. and Jha, P. K. 2010. Ecological study of *Paris polyphylla* Sm, Ecological Society, An International Journal of Ecology. 17: 87-93.

Maity, D., Pradhan, N. and Chauhan, A. S. 2004. Folk uses of some medicinal plants from North Sikkim. 3(1): 66-71

Man, S., Gao W., Zhang Y., Ma C., Yang L. and Li Y. 2010. Paridis saponins inhibiting carcinoma growth and metastasis in vitro and in vivo. Archives of Pharmacy Research. 34 (1): 43-50.

Man, S. L., Wang, Y. L., Li, Y. Y., Gao, W. Y. and Huang, X. X. 2013. Phytochemistry, pharmacology, toxicology, and structure-cytotoxicity relationship of Paridis Rhizome Saponin. Chinese Herbal Medicines. 5(1): 33-46.

Man, S., Gao, W., Zhang, Y., Huang, L. and Liu, C. 2011. Identification of chemical constituents in Rhizoma Paridis Saponins and their oral administration in rat plasma by UPLC/Q-TOF/MS. Biomedical Chromatography. 25(6): 712-719.

Man, S., Gao, W., Zhang, Y., Jin, X., Ma, C., Huang, X. and Li, Q. 2009. Characterization of steroidal saponins in saponin extract from *Paris polyphylla* by liquid chromatography tandem multi-stage mass spectrometry. Analytical and bioanalytical chemistry. 395(2): 495-505.

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, L. 2004. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 79: 727–747.

Mao, A. A. 1993. A preliminary report on the folklore Botany of Mao Naga of Manipur (India) Ibid. 5: 143:147.

Marilynn, E. 1998. Oligosaccharide signaling of plant cells. J Cell Biochem. 72: 123–128.

Marzolo, M. P. and Nervi, F. 1989. Characterization of lipoprotein catabolism in biliary cholesterol hypersecretion conditions in rats. Archivos de biologia y medicina experimentales. 22(4): 361.

Masclaux-Daubresse, C., Reisdorf-Cren, M. and Orsel, M. 2008. Leaf nitrogen remobilisation for plant development and grain filling. Plant Biology. 10: 23-36.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L. and Suzuki, A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of botany. 105(7): 1141-1157.

Mato, M., Onozaki, T., Ozeki, Y., Higeta, D., Itoh, Y., Yoshimoto, Y., Ikeda, H., Yoshida, H. and Shibata, M. 2000. Flavonoid biosynthesis in white-flowered Sim carnations (*Dianthus caryophyllus*). Scientia Horticulturae. 84(3-4): 333-347.

Matsuda, H., Pongpiriyadacha, Y., Morikawa, T., Kishi, A., Kataoka, S., Yoshikawa, M. 2003. Protective effects of steroid saponins from *Paris polyphylla* var. *yunnanensis* on ethanol- or indomethacin- induced gastric mucosal lesions in rats: Structural requirement for activity and mode of action. Bioorganic & Medicinal Chemistry Letters. 13(6): 1101-1106.

Miyamura, M., Nakano, K., Nohara, T., Tomimatsu, T. and Kawasaki, T. 1982. Steroid saponins from *Paris polyphylla* SM.-Supplement. Chemical and Pharmaceutical Bulletin, 30(2): 712-718.

Mayirnao, H. S. and Bhat, A. A. 2017. Evaluation of antioxidant and antimicrobial activity of *Paris polyphylla* Sm. Evaluation, 10(11): 315-319.

McCready, R. M., Guggolz, J., Silviera, V. and Owens, H.S. 1950. Determination of starch and amylose in vegetables. Analytical chemistry. 22(9): 1156-1158.

McEvoy, E. T. and I. Hoffman. 1959. The use of chemicals to suppress sucker growth

on cigar tobacco. Can. J, Plant Sci. 19:350-354.

Meng, F. Y., Wang, L. Y., Feng, C. Q., Han, J. P. and Yang, C. H. 2005. Research progress in domestication of *Paris polyphylla* Smith var. *yunnanensis* (Franch) Hand. Mazz. Chin. Tradit. and Herb Drugs. 36(7): 1102-1104.

Meyer, S. A., Sheets, T. J. and Seltmann, H. 1987. Maleic hydrazide residues in tobacco and their toxicological implications. In Reviews of environmental contamination and toxicology, Springer, New York, NY. pp. 43-60.

Mimaki, Y., Kuroda, M., Obata, Y., Sashida, Y., Kitahara, M., Yasuda, A., Naoi, N., Xu, Z. W., Li, M. R. and Lao, A. N. 2000. Steroidal saponins from the rhizomes of *Paris polyphylla* var. *chinensis* and their cytotoxic activity on HL-60 cells. Natural Product Letters. 14(5): 357-364.

Mir, S. A., Bosco, S. J. D., Shah, M. A. and Mir, M. M. 2016. Effect of puffing on physical and antioxidant properties of brown rice. Food Chemistry. 191: 139-146.

Mishra, R. 1968. Ecology Work Book. Oxford and IBH Publications, Co. New Delhi.

Mitchell B. 1987. Paris—Part I. Plantsman 9: 81–89.

Mitchell B. 1988. Paris—Part II. Daiswa. Plantsman 10: 167–190.

Mahato, S. B., Sahu, N. P., Ganguly, A. N., Miyahara, K. and Kawasaki, T. 1981. Steroidal glycosides of *Tribulus terrestris* Linn. Journal of the Chemical Society, Perkin Transactions. (1):2405-2410.

Mobli, M. and Baninasab, B. 2008. Effects of plant growth regulators on growth and carbohydrate accumulation in shoots and roots of two almond rootstock seedlings. Fruits. 63(6): 363-370.

Mohd, T., Belwal, T., Bhatt, I. D., Pande, V. and Nandi, S. K. 2018. Polyphenolics in leaves of *Paris polyphylla:* An important high value Himalayan medicinal herb. Industrial Crops and Products. 117: 66-74.

Mohanty, P., Paul, S., Das, M. C., Kumaria, S. and Tandon, P. 2012. A simple and efficient protocol for the mass propagation of *Cymbidium mastersii:* an ornamental orchid of Northeast India. AoB plants. 2012: 1-8.

Morgan, E. R., Butler, R. M. and Bicknell, R. A. 1997. *In vitro* propagation of *Gentiana cerina* and *Gentiana corymbifera*. New Zealand Journal of Crop and Horticultural Science. 25(1): 1-8.

Monselise, S. P. 1979. The use of growth regulators in citriculture; a review. *Scientia Horticulturae*, 11(2): 151-162.

Moore, S. and Stein, W. H. 1948. Photometric nin-hydrin method for use in the ehromatography of amino acids. Journal of biological chemistry. 176: 367-388.

Moore, R. H. 1950. Several effects of maleic hydrazide on plants. Science (Washington). 112: 52-3.

Moretti, C, Sauvain M, Lavaud C, Massiot G, Bravo J. A., Munoz V. A. 1998. J. Nat. Prod. 61(11); 1390-1393

Munne-Bosch, S. 2008. Do perennials really senesce?. Trends in plant science. 13(5): 216-220.

Murcia, G., Fontana, A., Pontin, M., Baraldi, R., Bertazza, G. and Piccoli, P.N. 2017. ABA and $GA_3$ regulate the synthesis of primary and secondary metabolites related to alleviation from biotic and abiotic stresses in grapevine. Phytochemistry. 135: 34-52.

Mushtaq, S., Hassan, Q. P., Sharma, R., Majeed, R., Dar, A. H., Sultan, P. and Ali, M. N. 2017. Evaluation of anticancer and antimicrobial activities of selected medicinal plants of Kashmir Himalayas, India. 16(1): 141-145.

Naik, P. M. and Al-Khayri, J. M. 2016. Abiotic and biotic elicitors-role in secondary metabolites production through in vitro culture of medicinal plants. Abiotic and biotic stress in plants-recent advances and future perspectives. 247-277.

Nakano, K., Murakami, K., Nohara, T., Tomimatsu, T. and Kawasaki, T. 1981. The Constituents of *Paris verticillata* M. v. BIEB. Chemical and Pharmaceutical Bulletin. 29(5): 1445-1451.

Nakamura, T., Komori, C., Lee, Y.Y., Hashimoto, F., Yahara, S., Nohara, T. and Ejima, A., 1996. Cytotoxic activities of Solanum steroidal glycosides. Biological and Pharmaceutical Bulletin. 19(4): 564-566.

Nandi, A. and Chatterjee, I. B., 1987. Scavenging of superoxide radical by ascorbic acid. Journal of Biosciences. 11(1-4): 435-441.

Nautiyal, P., Nautiyal, R., Kala, K. and Verma, J. 2004. Taxonomic richness in the diatom flora of Himalayan streams (Garhwal, India). Diatom. 20: 123-132.

Negi, S., Bisht, V., Bhandari, A., Bhatt, P., Singh, V. and Singh, N. 2014. *Paris polyphylla:* chemical and biological prospectives. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 14(6): 833-839.

Nhut, D. T, Teixeira da Silva, J. A., Le, B. V. and Van Tran, T, K. 2003. Thin cell layer studies of vegetable, leguminous and medicinal plants. In: Nhut DT, Van Tran Thanh K, Le BV, Thorpe T (eds) Thin cell layer culture system: regeneration and transformation applications. Kluwer, Dordrecht, pp 387–426.

Nohara, T., Yabuta, H., Suenobu, M., Hida, R., Miyahara K. and Kawasaki, T. 1973. Chem. Pharm. Bull. 21: 1240.

Nohara, T., Ito, Y., Seike, H., Komori, T., Moriyama, M., Gomita, Y. and Kawasaki, T. 1982. Study on the constituents of *Paris quadrifolia* L. Chem. Pharm. Bull. 30: 1851-1856.

Nolan, M. A. Craig, M. E. Lahra, M. M. Rawlinson, W. D. Prager, P. C. Williams, G. D. Bye, A. M. E. and Andrews P. I. 2003. Neurology. 60 (10): 1651-1656.

Noltie, H. J. 1994. Flora of Bhutan; Including a record of plants from Sikkim and Darjeeling. Royal Botanic Garden: Edinburg. 3(1): 21-24.

Nooden, L. D, Guiamet, J. J. and John, I. 1997. Senescence mechanism. Physiol Plant. 101: 746-53.

Obinata, N., Yamakawa, T., Takamiya, M., Tanaka, N., Ishimaru, K. and Kodama, T. 2003. Effects of salicylic acid on the production of procyanidin and anthocyanin in cultured grape cells. Plant Biotechnology. 20(2): 105-111.

Oehler, M. K., MacKenzie, I., Kehoe, S. and Rees, M. C. 2003. Assessment of abnormal bleeding in menopausal women: an update. British Menopause Society Journal. 9(3): 117-122.

Ohta, T., Kusano, K., Ido, A., Miura, C. and Miura, T. 2016. Silkrose: A novel acidic polysaccharide from the silkmoth that can stimulate the innate immune response. Carbohydrate polymers. (136): 995-1001.

Ono, M., Yanai, Y., Ikeda, T., Okawa, M. and Nohara, T. 2003. Steroids from the underground parts of *Trillium kamtschaticum*. Chemical and pharmaceutical bulletin. 51(11): 1328-1331.

Ono, M., Sugita, F., Shigematsu, S., Takamura, C., Yoshimitsu, H., Miyashita, H. and Nohara, T. 2007. Three new steroid glycosides from the underground parts of *Trillium kamtschaticum*. Chemical and pharmaceutical bulletin. 55(7): 1093-1096.

Ortuno, J., Esteban, M. A. and Meseguer, J. 1999. Effect of high dietary intake of vitamin C on non-specific immune response of gilthead seabream (*Sparus aurata* L.). Fish and Shellfish Immunology. 9(5): 429-443.

Osaloo, S. K. and Kawano, S. 1999. Molecular systematics of Trilliaceae II. Phylogenetic analyses of *Trillium* and its allies using sequences of rbcL and matK genes of cpDNA and internal transcribed spacers of 18S–26S nrDNA. Plant Species Biology. 14(1): 75-94.

Osathanunkul, M., Suwannapoom, C., Ounjai, S., Rora, J. A., Madesis, P. and De Boer, H. 2015. Refining DNA barcoding coupled high resolution melting for discrimination of 12 closely related croton species. PLoS One. 10(9): 0138888.

Osathanunkul, M., Suwannapoom, C., Osathanunkul, K., Madesis, P. and De Boer, H. 2016. Evaluation of DNA barcoding coupled high resolution melting for discrimination of closely related species in phytopharmaceuticals. Phytomedicine. 23(2): 156-165.

Paliouris, G. and Hutchinson, T. C. 1991. Arsenic, cobalt and nickel tolerances in two populations of *Silene vulgaris* (Moench) Garcke from Ontario, Canada. New Phytologist. 117(3): 449-459.

Pande, P. C., Tiwari, L. and Pande, H. C. 2007. Indian Journal of Traditional Knowledge. 6(3): 444-458.

Parekh, J. and Chanda, S. 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research. 10(2): 175 – 181.

Pervaiz, S. and Clement, M. V. 2004. Tumor intracellular redox status and drug resistance serendipity or a causal relationship? Curr. Pharm. Des. 10: 1381-6128.

Patial, V., Devi, K., Sharma, M., Bhattacharya, A. and Ahuja, P. S. 2012. Propagation of *Picrorhiza kurroa* Royle ex Benth: an important medicinal plant of Western Himalaya. Journal of Medicinal Plants Research. 6(34): 4848-4860.

Patil, M. S. and Gaikwad, D. K. 2017. Effect of PGRs on the activity of enzyme lipase in medicinally important oil yielding *Simarouba glauca* seedlings. Research paper/Article and author. 44: 35-37.

Patil, M., Desai, N., Waghmode, A. and Gaikwad D. K. 2020. Effect of exogenous application of plant growth regulators on physiology and secondary metabolite accumulation of *Colubrina asiatica* L. Studia Rosenthaliana (Journal for the Study of Research). 12(11): 103-108.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., Chen, S., Corpe, C., Dutta, A., Dutta, S. K. and Levine, M. 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American college of Nutrition. 22(1): 18-35.

Paganga, G., Miller, N. and Rice-Evans, C.A. 1999. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute?. Free radical research, 30(2): 153-162.

Pandey, V. N. 1991. Medico-Ethno-Botanical Explorations in Sikkim Himalayas. Central Council for Research in Ayurveda and Siddha, Government of India, New Delhi.

Park, S. H., Oh, S. R., Jung, K. Y., Ahn, K. S., Kim, J. G., Lee, J. J. and Lee, H. K. 1999. Anticomplement activities of oleanolic acid monodesmosides and bisdesmosides isolated from *Tiarella polyphylla.* Archives of pharmacal research. 22(4): 428-431.

Paul, A., Gajurel, P. R. and Das, A. K. 2015. Threats and conservation of *Paris polyphylla* an endangered, highly exploited medicinal plant in the Indian Himalayan Region. Biodiversitas Journal of Biological Diversity. 16(2): 295-302.

Payum, T. 2018. Phytocomposition and pharmacological importance of *Paris polyphylla* (Smith.) and needs of its conservation in Arunachal Pradesh, India. Archives of Agriculture and Environmental Science. 3(2): 143-150.

Peng W, Qin R and Li X. 2013. Botany, phytochemistry, pharmacology, and potential application of *Polygonum cuspidatum* Sieb.et Zucc.: A review. J Ethnopharmacol; 148: 729-745.

Peterson, A. T., Soberon, J., Pearson, R. G., Anderson, R. P., Martinez-Meyer, E., Nakamura, M. and Araujo, M. B. 2011. Ecological niches and geographic distributions (MPB-49) (Vol. 49). Princeton University Press.

Pfoze, N. L., Kumar, Y. and Myrboh, B. 2013. Screening of bioactive phytochemicals obtained from lesser known ethnomedicinal plants of Senapati district of Manipur, India. Pleione. 7: 489-500.

Pharmacopoeia, A. 1986. African Pharmacopoeia. General methods for Analysis. OAU/STRC Sci. Pub. Lagos. 2(2): 123-130.

Pharmacopeia, I. 1996. Ghaziaad, India: The Indian Pharmacopoeia Commission.

Phillips, S. J, Anderson, R. P and Schapire, R. E. 2006. Maximum entropy modeling of species geographic distributions, Ecol Model. 190: 231-259.

Phillips, S. J. and Dudik, M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography. 31(2): 161-175.

Pourianfar, H. R., Kirk, K. and Grollo, L. 2014. Initial evidence on differences among Enterovirus 71, Coxsackievirus A16 and Coxsackievirus B4 in binding to cell surface heparan sulphate. Virusdisease. 25(3): 277-284.

Preece, J. E. 2003. A century of progress with vegetative plant propagation.HortScience. 38: 1015-1025.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Analytical biochemistry. 269(2): 337-341.

Probert, R. J. 2000. The role of temperature in the regulation of seed dormancy and germination. Seeds: the ecology of regeneration in plant communities. 2: 261-292.

Pu, X., Ren, J., Ma, X., Liu, L., Yu, S., Li, X. and Li, H. 2015. Polyphylla saponin I has antiviral activity against influenza A virus, Int J Clin Exp Med. 8(10): 18963–18971.

Puwein, A., Thomas, S. C. and Singha, L. I. 2018. A Review on *Paris polyphylla* Smith: As an Effective andAlternative Treatment of Cancer. International Journal of Pharmaceutical Science Invention. 7(2): 2319 – 6718.

Puwein, A. and Thomas, S. C. 2020. An Overview of *Paris polyphylla,* a Highly Vulnerable Medicinal Herb of Eastern Himalayan Region for Sustainable Exploitation. 10: 3-14.

Qi, Y., Chen, X., Chan, C., Li, D., Yuan, C., Yu, F., Marie, C., David, T., Kung, H. and Lai, L. 2008. Two-dimensional differential gel electrophoresis/analysis of diethyl nitrosamine induced rat hepatocellular carcinoma. Int. J. Cancer. 122: 2682–2688.

Qi, J., Zheng, N., Zhang, B., Sun, P., Hu, S., Xu, W. and Li, X. 2013. Mining genes involved in the stratification of *Paris polyphylla* seeds using high-throughput embryo Transcriptome sequencing. BMC genomics. 14(1): 358.

Qin, X. J., Chen, C. X., Ni, W., Yan, H. and Liu, H. Y. 2013. $C_{22}$-steroidal lactone glycosides from stems and leaves of *Paris polyphylla* var. *yunnanensis*. Fitoterapia. 84: 248-251.

Qin, X. J., Ni, W., Chen, C. X. and Liu, H. Y. 2018. Seeing the light: Shifting from wild rhizomes to extraction of active ingredients from above-ground parts of *Paris polyphylla* var. *yunnanensis*. Journal of ethnopharmacology. 224: 134-139.

Qin, X. J., Sun, D. J., Ni, W., Chen, C. X., Hua, Y., He, L. and Liu, H. Y. 2012. Steroidal saponins with antimicrobial activity from stems and leaves of *Paris polyphylla* var. *yunnanensis*. Steroids. 77(12): 1242-1248.

Qin, X. J., Zhang, L. J., Zhang, Y., Ni, W., Yang, X. Z., Yu, Q., Yan, H., An, L. K. and Liu, H. Y. 2020. Polyphyllosides A–F, six new spirostanol saponins from the stems and leaves of *Paris polyphylla* var. *chinensis*. Bioorganic Chemistry. 10: 37-88.

Qureshi, K. M., Chughtai, S., Qureshi, U. S. and Abbasi, N. A. 2013. Impact of exogenous application of salt and growth regulators on growth and yield of strawberry. Pak. J. Bot. 45(4): 1179-1185.

Ram, V. J. and Nath. M. 1996. Curr. Med. Chem. 3: 303.

Rami, E. and Patel, I. 2014. Effect of growth regulators and explant types on callus induction from *Oroxylum indicum* (L.) Vent. Life Science. 1(12): 278-280.

Randhir, R., Kwon, Y. I. and Shetty, K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovative Food Science and Emerging Technologies. 9(3): 355-364.

Rao, M. K. and Koduru, P. R. K. 1978. Cytogenetics of a factor for syncyte formation and male sterility in *Pennisetum americanum*. Theoretical and Applied Genetics, 53(1): 1-7.

Raomai, S., Kumaria, S. and Tandon, P. 2014. Plant regeneration through direct somatic embryogenesis from immature zygotic embryos of the medicinal plant, *Paris polyphylla* Sm. Plant Cell Tissue Organ Cult. 118(3): 445-455.

Raomai, S., Kumaria, S., Kehie, M. and Tandon, P. 2015. Plantlet regeneration of *Paris polyphylla* Sm. via thin cell layer culture and enhancement of steroidal saponins in mini-rhizome cultures using elicitors. Plant growth regulation: 75(1): 341-353.

Romano, A., Noronha, C. and Martins-Loucao, M. A. 1992. Influence of growth regulators on shoot proliferation in Quercus suber L. Annals of botany. 70(6): 531-536.

Rappaport, L. 1956. Growth regulating metabolites. Calif. Agric. 10: 4.

Ravipati, A. S, Zhang, L., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Bartlett, J., Smith, P. T., Shanmugam, K., Münch, G., Wu, M. J., Satyanarayanan, M. and Vysetti, B. 2012. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complem Altern M. 12(1): 173.

Ray, P. D., Huang, B. W. and Tsuji, Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cellular signalling. 24(5): 981-990.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 26(9-10): 1231-1237.

Richards, D. and Rowe, R. N. 1977. Effects of root restriction, root pruning and 6-benzylaminopurine on the growth of peach seedlings. Annals of botany. 41(4): 729-740.

Ridout, C. L., Price, K. R., DuPont, M. S., Parker, M. L. and Fenwick, G. R. 1991. Quinoa saponins-Analysis and preliminary investigations into the effects of reduction by processing. J. Sci. Food Agric. 54:165-176.

Rimm, E. B., Klatsky, A., Grobbee, D. and Stampfer, M. J. 1996. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits?. Bmj. 312(7033): 731-736.

Roman, I. D., Thewles, A. and Coleman, R. 1995. Fractionation of livers following diosgenin treatment to elevate biliary cholesterol. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1255(1): 77-81.

Ross, J. J., O'neill, D. P., Smith, J. J., Kerckhoffs, L. H. J. and Elliott, R. C. 2000. Evidence that auxin promotes gibberellin A1 biosynthesis in pea. The Plant Journal. 21(6): 547-552.

Roshan, R., Ahmed, S. and Hasan, M. M. 2017. *Arisaema jacquemontii* Blume (Araceae): A review of medicinal uses, phytochemistry and pharmacology. J. Pharmacogn. Phytochem. 6: 429-432.

Rosenvasser, S., Mayak, S. and Friedman, H. 2006. Increase in reactive oxygen species (ROS) and in senescence-associated gene transcript (SAG) levels during dark-induced senescence of Pelargonium cuttings, and the effect of gibberellic acid. Plant Science. 170(4): 873-879.

Sadeghi, S., Ashrafi, Z. Y., Tabatabai, F. and Alizade, H. M. 2009. Study methods of dormancy breaking and germination of common madder (*Rubia tinctorum* L.) seed in laboratory conditions. Bot. Res. Internatio. 2: 7-10.

Samarat, A., Kuninobu, Y., & Takai, K. (2011). Synthesis of meso-substituted tetraarylalkynylporphyrins via rhenium-catalyzed formation of naphthalene units. Synlett, 2011(15), 2177-2180.

Sanchez-Salcedo, E. M., Tassotti, M., Del Rio, D., Hernandez, F., Martinez, J. J. and Mena, P. 2016. (Poly) phenolic fingerprint and chemometric analysis of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry leaves by using a non-targeted UHPLC–MS approach. Food chemistry. 212: 250-255.

Setyawan, A. D., Supriatna, J., Nisyawati, N., Sutarno, S. and Nursamsi, I. 2018. Predicting impacts of future climate change on the distribution of the widespread selaginellas (*Selaginella ciliaris* and *S. plana*) in Southeast Asia. Biodiversitas Journal of Biological Diversity. 19(5): 1960-1977.

Sautour, M., Mitaine-Offer, A. C., Miyamoto, T., Wagner, H. and Lacaille-Dubois, M. A. 2004. A new phenanthrene glycoside and other constituents from *Dioscorea opposita*. Chemical and pharmaceutical bulletin. 52(10): 1235-1237.

Schulten, H.R., Singh, S.B. and Thakur, R.S. (1984). Field desorption and fast atom bombardment mass spectrometry of spirostanol and furostanol saponins from *Paris polyphylla*. *Zeitschrift für Naturforschung C*, 39(3-4);201-211.

Schoene, D. L. and Hoffmann, O. L. 1949. Maleic hydrazide, a unique growth regulant. Science. 109(2841): 588-590.

Segelman, A.B., Farnsworth, N.R. and Quimby, M.W. 1969. Biological and phytochemical evaluation of plants. 3. False-negative saponin test results induced by the presence of tannins. Lloydia, 32(1); 52.

Selvam, A. B. D. and Bandyopadhyay, S. 2005. Fluorescence analysis on the roots of Rauvolfia serpentina (L.) Benth. Ex kurz under UV radiation. Ancient science of life. 24(4): 164.

Senguttuvan, J., Paulsamy, S. and Karthika, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pacific journal of tropical biomedicine. 4: 359-367.

Sewelam, N., Kazan, K. and Schenk, P. M. 2016. Global plant stress signaling: reactive oxygen species at the cross-road. Frontiers in plant science. 7: 187.

Shah, S. A., Mazumder, P. B. and Choudhury, M. D. 2012. Medicinal properties of *Paris polyphylla* Smith: a review. Journal of Herbal Medicine and Toxicology. 6(1): 27-33.

Shameem, N., Kamili, A. N., Parray, J. A., Hamid, R. and Bandh, S. A. 2015. Antimicrobial and antioxidant activity of methanol extracts of *Arnebia benthamii* (Wall ex. G. Don) Johnston—a critically endangered medicinal plant of North western Himalaya. Journal of Analytical Science and Technology. 6(1): 36.

Shankar, R. and Rawat, M. S. 2013. Conservation and cultivation of threatened and high valued medicinal plants in north East India. International Journal of Biodiversity and Conservation. 5: 584-591.

Sharma, A., Kalita, P. and Tag, H. 2015. Distribution and phytomedicinal aspects of *Paris polyphylla* Smith from the Eastern Himalayan Region: A review. Tang Humanitas Medicine. 5(3): 15-19.

Sharma, P. and Samant, S. S. 2014. Diversity, distribution and indigenous uses of medicinal plants in Parbati Valley of Kullu district in Himachal Pradesh, Northwestern Himalaya. Asian J. Adv. Basic Sci. 2: 77-98.

Sharma, R. C., Shrestha, S. M. and Pandey, M. P. 2007. Inheritance of blast resistance and associated microsatellite markers in rice cultivar 'Laxmi'. Journal of phytopathology. 155(11-12): 749-753.

Sharma, P., Jha, A. B., Dubey, R. S., and Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012.

Shen, S., Xu, Z., Feng, S., Wang, H., Liu, J., Zhou, L., Yuan, M., Huang, Y. and Ding, C. 2018. Structural elucidation and antiaging activity of polysaccharide from *Paris polyphylla* leaves. International journal of biological macromolecules. (107): 1613-1619.

Shetty, S. and Vijayalaxmi, K. K. 2012. Phytochemical investigation of extract/solvent fractions of *Piper nigrum* Linn. Seeds and *Piper betle* linn. Leaves. International Journal of Pharma and Bio Sciences. 3(2): 344-349.

Shino, M., Watanabe, Y. and Umezawa, K. 2001. Synthesis of N-substituted N-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. Bioorgan Med Chem. 9: 1233-1240.

Shishodia, S. and Aggarwal, B. B. 2006. Diosgenin inhibits osteoclastogenesis, invasion and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. Oncogene. 25(10): 1463-1473.

Silva, J. A. T. 2003. Thin cell layer technology in ornamental plant micropropagation and biotechnology. Afr J Biotech. 2: 683–691.

Singh, S. K., Rai, M. K., Asthana, P. and Sahoo, L. 2009. An improved micropropagation of *Spilanthes acmella* L. through transverse thin cell layer culture. Acta Physiol Plant. 31: 693–698.

Singh, R. K. I., Panmei, C., Devi, L. S., Shah, S. A., Devi, K. T. and Devi, K. S. 2008. *Paris polyphylla* Smith-The wonder Herb: In Proceeding International Conclave on

Medicinal Plants for ASEAN & BIMSTEC countries, Imphal, Manipur, India. 13: 82-86.

Singh, S. B., Thakur, R. S. and Schulten, H. R. 1980. Spirostanol saponins from *Paris polyphylla*, structures of polyphyllin C, D, E and F. Phytochemistry. 21(12): 2925-2929.

Singh, S. B., Thakur, R. S. and Schulten, H. R. 1982. Furostanol saponins from *Paris polyphylla*: Structures of polyphyllin G and H. Phytochemistry. 21(8): 2079-2082.

Singh, R. 2018. Chemotaxonomy of medicinal plants: possibilities and limitations. In Natural Products and Drug Discovery, Elsevier. pp. 119-136.

Shreve, L. W. and Campbell, R. W. 1967. Some effects of gibberellic acid on year-old pecan seedling. Combined Proceedings of the International Plant Propagators' Society. 17: 242-7.

Sirsat, A. P., Kokate, P. S. and Shirsat, R. P. 2019. Indian medicinal plants with potential antioxidant and anticancer properties: An insight. 7(1): 47-51.

Smart, R. E. and Bingham, G. E. 1974. Rapid estimates of relative water content. Plant physiology. 53(2): 258-260.

Snell, F. D. and Snell, C. T. 1971. Colometric methods of analysis Van Nostrane Reinhold. Co., New York.7-145.

Sprag, S. G., Light, M. E. and Van, S. J. 2004. Biological activities and distribution of plant saponins. J Ethnopharm. 94: 219–243

Sloan, S., Jenkins, C.N., Joppa, L.N., Gaveau, D. L. and Laurance, W.F. 2014. Remaining natural vegetation in the global biodiversity hotspots. Biological Conservation. 177: 12-24.

Solanki, H. and Vakharia, P. 2014. Study on alkaloid estimation by HPLC in *Withania somnifera* (Linn) Dunal with reference to treatment with growth regulators. Journal of Pharmaceutical Development and Technology. 4(3): 183-193.

Soltis, D. E. and Soltis, P. S. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In Molecular systematics of plants II. Springer, Boston, MA. 1-42.

Song, Y., Xu, J., Chen, N. and Li, M. 2017. The complete chloroplast genome of traditional Chinese medical plants *Paris polyphylla* var. *yunnanensis.* Mitochondrial DNA Part A. 28(2): 159-160.

Songyun, L. and Soukup, V. G. 2000. Paris Linnaeus, Sp. Pl. 1: 367. 1753. Flora of China.

2: 88-95.

Stefanowicz-Hajduk, J., Kawiak, A., Gajdus, J., Ochocka, J. R., Paszkiewicz, M., Stepnowski, P. and Lojkowska, E. 2011. Cytotoxic activity of *Paris quadrifolia* extract and isolated saponin fractions against human tumor cell lines. Acta Biologica Cracoviensia. Series Botanica. 53(2): 127-131.

Stevenson, D. W. and H. Loconte. 1995. Cladistic analysis of monocot families. pp. 543–578.

Stopari, G. and Maksimovi, I. 2008. The effect of cytokinins on the concentration of hydroxyl radicals and the intensity of lipid peroxidation in nitrogen deficient wheat. Cereal Res Communications. 36: 601–609.

Subba, S. J., and Lachungpa, D. 2016. A versatile medicinal plant species *Paris polyphylla* at Lachung Forest, Sikkim–conservation initiatives. J. Int. Acad. Res. Multidiscipl. 4(1): 283-287.

Shu-dong, Z., Hong, W. and De-Zhu, L. 2008. A new species of *Paris* (Melanthiaceae) from northeastern Yunnan, China. Novon: A Journal for Botanical Nomenclature. 18(4): 550-554.

Szakiel, A., Ruszkowski, D., Grudniak, A., Kurek, A., Wolska, K. I., Doligalska, M. and Janiszowska, W. 2008. Antibacterial and antiparasitic activity of oleanolic acid and its glycosides isolated from marigold (*Calendula officinalis*). Planta Medica. 74(14): 1709-1715.

Taiz, L. and Zeiger, E. 2002. Photosynthesis: physiological and ecological considerations. Plant Physiol. 9: 172-174.

Takhtajan, A. 1983. A revision of Daiswa (Trilliaceae), Brittonia. 35: 255–270.

Tamura, M. N. 1998. Trilliaceae. In: Kubitzki K, ed. The families and genera of vascular plants. Berlin: Springer-Verlag, 444–452.

Tan, Y., Yin, P., Tang, L., Xing, W., Huang, Q., Cao, D., Zhao, X., Wang, W., Lu, X., Xu, Z., Wang, H. and Xu, G. 2012. Metabolomics study of stepwise hepatocarcinogenesis from the model rats to patients: potential biomarkers effective for small hepatocellular carcinoma diagnosis. Mol. Cell. Proteomics. 11: 1–12.

Tandon, J. S., Srivastava, V. and Guru, P. Y. 1991. J. Nat. Prod. 54: 1102-1104.

Tandon, V. 1997. Status of collection, conservation, trade and potential for growth and sustainable use of major medicinal plant species found in the Great Himalayan National Park and its environs in Kullu District of Himachal Pradesh. Wildlife Institute of India, Dehradun.

Taiz, L. and Zeiger, E. 2002. Photosynthesis: physiological and ecological considerations. Plant Physiol. 9: 172-174.

Tantasawat, P. A., Sorntip, A. and Pornbungkerd, P. 2015. Effects of exogenous application of plant growth regulators on growth, yield, and in vitro gynogenesis in cucumber. HortScience. 50(3): 374-382.

Tariq, A., Mussarat, S. and Adnan, M. 2015. Review on ethnomedicinal, phytochemical and pharmacological evidence of Himalayan anticancer plants. Journal of ethnopharmacology. 164: 96-119.

Taylor, R. M. 1972. Influence of gibberellic acid on early patch budding of pecan seedlings. Journal of the American Society for Horticultural Science. 97: 667–79.

Teerawatsakul, Y., Dheeranupattana, S. and Sringarm, K. 2014. Effect of temperature on shoot tip culture of *Paris polyphylla* var. *chinensis* (Franch.) Hara. Planta Medica. 80(16): 2O10.

The Plant List. 2013. Version 1.1. Published on the Internet; http://www.theplantlist.org/ (accessed 1st January).

Thompson, P. A. 1969. Germination of species of Labiatae in response to gibberellins. Physiologia Plantarum. 22(3): 575-586.

Thomas, G. S. 1990. Perennial Garden Plants. J. M. Dent and Sons, London.

Thompson, L. U. 1993. Potential health benefits and problems associated with antinutrients in foods. Food Res. Int. 26:131-149.

Thompson, A. J., Jackson, A. C., Symonds, R. C., Mulholland, B. J., Dadswell, A. R., Blake, P. S., Burbidge, A. and Taylor, I. B. 2000. Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. The Plant Journal. 23(3): 363-374.

Tian, Y., Zheng L., Xu Z., Sun L. and Gao C. 1986. Clinical and pharmacological study of the hemostatic action of Rhizoma Paridis by contraction of uterus. Journal of traditional Chinese medicine. 6(3): 178-182.

Tiwari, J. K., Ballabha, R. and Tiwari, P. 2010. Ethnopaediatrics in Garhwal Himalaya, Uttarakhand, India (psychomedicine and medicine). New York Science Journal. 3: 123-126.

Tohda, C., Urano, T., Umezaki, M., Nemere, I. and Kuboyama, T. 2012. Diosgenin is an exogenous activator of 1, 25D 3-MARRS/Pdia3/ERp57 and improves Alzheimer's disease pathologies in 5XFAD mice. Scientific reports. 2(1): 1-11.

Trease, G. E. and Evans, W. C. 1983. Drugs of biological origin. Pharmacognosy, 12th ed., Balliere Tindall, United Kingdom, pp 309-540.

Tripathi, M., Shivhare, D., Tiwari, A., Ahirwar, P. and Pathak, S. 2014. Pharmacognostical evaluation of Marsdenia tenacissima Wight. & Arn. Root. Int. J. Rec. Biotech. 2(3): 18-23.

Tyler, V. E., Brady, L. R. and Robbers, J. E. 1976. “Pharmacognosy”, Lea and Febiger, Philadelphia. 24.

Van, T. T. K. 1974. Direct flower neoformation from superficial tissue of small explants of *Nicotiana tabacum* L. Planta. 115: 87–90.

Vanacker, H., Sandalio, L. M., Jimenez, A., Palma, J. M., Corpas, F. J., Meseguer, V., Gomez, M., Sevilla, F., Leterrier, M., Foyer, C. H. and Del Rio, L. A. 2006. Roles for redox regulation in leaf senescence of pea plants grown on different sources of nitrogen nutrition. Journal of Experimental Botany. 57(8): 1735-1745.

Ved, D. K., Suma, T. S., Bhutia, T. G., Ravikumar, K., Barve, V., Somashekar, B. S., Tandon, V., Goraya, G. S. Sumanth, M. V. and Soumyashree, N. 2017. Conservation Assessment and Management Prioritisation (CAMP) for the Wild Medicinal Plants of Sikkim. FRLHT-TDU, Bengaluru and SMPB-FEWMD.

Verma, P., Mathur, A. K., Jain, S. P. and Mathur, A. 2012. *In vitro* conservation of twenty-three overexploited medicinal plants belonging to the Indian sub-continent. The Scientific World Journal. 1-11.

Wang, C. W, Tai, C., Choong, C., Lin, Y., Lee, B., Shi, Y. and Tai, C. J. 2016. Aqueous extract of *Paris polyphylla* (AEPP) inhibits ovarian cancer via suppression of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha. Molecules. 21: 727.

Wang, D., Sun, G., Wang, L., Zhai, S. and Cen, X. 2009. A novel mechanism controls anther opening and closing in *Paris polyphylla* var. *Yunnanensis*. Chinese Science Bulletin. 54(2): 244-248.

Wang, B., Zhang, H., Dong, X., Lv, L., Zhao, L., Lou, Z., Chai, Y. and Zhang, G. 2013. Separation and characterization of steroidal saponins in *Paris polyphylla* by high-performance liquid chromatography coupled with time-of-flight mass spectrometry and ion trap mass spectrometry. Journal of Liquid Chromatography & Related Technologies. 36(12): 1661-1677.

Wang, G. X., Han, J., Zhao, L. W., Jiang, D. X., Liu, Y. T. and Liu, X. L. 2010. Anthelmintic activity of steroidal saponins from *Paris polyphylla*. Phytomedicine. 17: 1102-1105.

Wang, J., Li, D., Ni, W., Qin, X. J., Liu, H., Yu, L. L., Qiao, X., Ji, Y. H., He, L., Nian, S. H. and Liu, H. Y. 2020. Molecular networking uncovers steroidal saponins *of Paris tengchongensis*. Fitoterapia. 10: 46-49.

Wang, L., Yang, Y., Zhao, Y., Yang, S., Udikeri, S. and Liu, T. 2016. De novo characterization of the root transcriptome and development of EST-SSR markers in *Paris polyphylla* Smith var. *yunnanensis*, an endangered medical plant.

Wang, Y., Gao, W. Y., Zhang, T. J. and Guo, Y. Q. 2007. A novel phenylpropanoid glycosides and a new derivation of phenolic glycoside from *Paris Polyphylla* var. *yunnanensis.* Chinese Chemical Letters. 18(5): 548-550.

Wang, Y., Gao, W., Li, X., Wei, J., Jing, S. and Xiao, P. 2013. Chemotaxonomic study of the genus *Paris* based on steroidal saponins. Biochemical Systematics and Ecology. 48: 163-173.

Wang, Y., Hao, J., Gao, W., Liu, Z., Wu, S. and Jing, S. 2013. Study on hemostatic activities of the rhizome of *Paris bashanensis*. Pharmaceutical biology. 51(10): 1321-1325.

Wang, Y., Liu, E. and Li, P. 2017. Chemotaxonomic studies of nine *Paris* species from China based on ultra-high-performance liquid chromatography tandem mass spectrometry and Fourier transform infrared spectroscopy. Journal of pharmaceutical and biomedical analysis. 140: 20-30.

Wang, Y. H., Shi, M., Niu, H. M., Yang, J., Xia, M. Y., Luo, J. F., Chen, Y. J., Zhou, Y. P. and Li, H. 2018. Substituting one *Paris* for another? In vitro cytotoxic and in vivo antitumor activities of *Paris forrestii*, a substitute of *Paris polyphylla* var. *yunnanensis.* Journal of ethnopharmacology. 218: 45-50.

Wang, Z., Cai, X., Zhong, Z., Wei, Z. X., Neng, X., Hu, J., Wan, G. and Wang, Q. F. 2017. *Paris nitida* (Melanthiaceae), a new species from Hubei and Hunan, China, Phytotaxa. 314(1): 145–149.

Wang, Y. C., Yi, T. Y. and Lin, K. H. 2011. In vitro activity of *Paris polyphylla* Smith against enterovirus 71 and coxsackievirus B3 and its immune modulation. The American journal of Chinese medicine. 39(06): 1219-1234.

Wang, Y., Li, Y. and Zhang, J. 2019. Capturing the Geoherbalism Differentiation in Wild *Paris polyphylla* var. *yunnanensis* Raw Materials through the Application of Multispectral Information Fusion Combined with Chemometrics. ACS omega. 4(20): 18820-18832.

Wang, H, and Xio. Y. 2009. Expression of iNOS Protein and Change of Apoptosis in Neonatal Rats with White Matter Damage. 439-440.

Wani, P. A., Ganaie, K. A., Nawchoo, I. A. and Wafai, B. A. 2006. Phenological episodes and reproductive strategies of *Inula racemosa* (Asteraceae)-a critically endangered medicinal herb of north west Himalaya. Intl J Bot. 2(4): 388-394.

Warren, D. L, Glor, R. E and Turelli, M. 2010. Enmtools: a toolbox for comparative studies of environmental niche models, evaluation, Ecography. 31: 161-175.

Wei, S., Fukuhara, H., Chen, G., Kawada, C., Kurabayashi, A., Furihata, M., Inoue, K. and Shuin, T. 2014. Terrestrosin D, a steroidal saponin from Tribulus terrestris L., inhibits growth and angiogenesis of human prostate cancer in vitro and in vivo. Pathobiology. 81(3): 123-132.

Wen, F., Yin, H., Chen, C., Liu, X., Xue, D., Chen, T., He, J. and Zhang, H. 2012. Chemical characteristics of saponins from *Paris fargesii* var. *brevipetala* and cytotoxic activity of its main ingredient, *Paris* saponin H. Fitoterapia. 83(4): 627-635.

Whyte, P. and Luckwill, L. C. 1966. A sensitive bioassay for gibberellins based on retardation of leaf senescence in *Rumex obtusifolious.* Nature. 210(5043): 1360-1360.

Witter, S. H. and Bukovac, M. J. 1957. Some effects of gibberellin on flowering and fruit

setting. Plant Physiol. 32: 39-41.

Wu, X. M., Zhang, Q. Z. and Wang, Y. Z. 2019. Traceability the provenience of cultivated *Paris polyphylla* Smith var. *yunnanensis* using ATR-FTIR spectroscopy combined with chemometrics. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 212: 132–145.

Wu, X., Wang, L., Wang, G. C., Wang, H., Dai, Y., Yang, X. X., Ye, W.C. and Li, Y. L. 2013. Triterpenoid saponins from rhizomes of *Paris polyphylla* var. *yunnanensis*. Carbohydrate research. 368: 1-7.

Wu, Z., Zhang, J., Xu, F., Wang, Y. and Zhang, J. 2017. Rapid and simple determination of polyphyllin I, II, VI, and VII in different harvest times of cultivated *Paris*

*polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz by UPLC-MS/MS and FT-IR. Journal of natural medicines. 71(1): 139-147.

Xiao, C. M., Huang, J., Zhong, X. M., Tan, X. Y. and Deng, P. C. 2009. Two New Homo-aro-cholestane Glycosides and a New Cholestane Glycoside from the Roots and Rhizomes of *Paris polyphylla* var. *pseudothibetica.* Helvetica Chimica Acta. 92(12): 2587-2595.

Xiao, X., Bai, P., Nguyen, T.M.B., Xiao, J., Liu, S., Yang, G., Hu, L., Chen, X., Zhang, X., Liu, J. and Wang, H. 2009. The antitumoral effect of Paris Saponin I associated with the induction of apoptosis through the mitochondrial pathway. Molecular cancer therapeutics. 8(5): 1179-1188.

Xiao, Z., Wang, A., Wang, X. and Shen, S. 2013. A study on the inhibitory effect of *Solanum lyratum* thumb extract on lewis lung carcinoma lines. African Journal of Traditional, Complementary and Alternative Medicines, 10(6): 444-448.

Xiao, X. H., Yuan, Z. Q. and Li, G. K. 2014. Separation and purification of steroidal saponins from *Paris polyphylla* by microwave-assisted extraction coupled with countercurrent chromatography using evaporative light scattering detection. Journal of separation science. 37(6): 635-641.

Xu, W., Zhang, F., Luo, Y., Ma, L., Kou, X. and Huang, K. 2009. Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. Carbohydrate Research. 344(2): 217–222.

Xue, D., Yin, H., Li, J., Liu, X., Zhang, H. and Peng, C. 2009. Application of microscopy in authentication and distinguishing of 11 Paris species in West Sichuan. Microscopy Research and Technique. 72(10): 744-754.

Yan, L. L., Zhang, Y. J., Gao, W. Y., Man, S. L. and Wang, Y. 2009. *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var. *yunnanensis*. Experimental Oncology. 31(1): 27–32.

Yan, W., Ohtani, K., Kasai R. and Yamasaki, K. 1996. Steroidal saponin from fruits of *Tribulus terrestris* Phytochemistry. 42: 1417-1422.

Yan, Y. Y., Bai, J. P., Xie, Y., Yu, J. Z. and Ma, C. G. 2013. The triterpenoid pristimerin induces U87 glioma cell apoptosis through reactive oxygen species-mediated mitochondrial dysfunction. Oncology letters. 5(1): 242-248.

Yang, C, Cai, H. and Meng, X. 2016. Polyphyllin D induces apoptosis and differentiation in K562 human. International Immunopharmacology. 36: 17-22.

Yang, J., Wang, Y. H. and Li, H. 2017. *Paris qiliangiana* (Melanthiaceae), a new species from Hubei, China, Phytotaxa. 329(2): 193–196.

Yang, H., Liu, F., An, W. and Tian, M. L. 2014. Control Study on Contamination Rate of Tissue Culture of *Paris Polyphylla.* In Advanced Materials Research, Trans Tech Publications Ltd. 926: 975-980.

Yang, Y., Zhang, J., Jin, H., Zhang, J. and Wang, Y. 2016. Quantitative analysis in combination with fingerprint technology and chemometric analysis applied for evaluating six species of wild *Paris* using UHPLC-UV-MS. Journal of analytical methods in chemistry.

Yang, Y. G., Zhang, J., Zhao, Y. L., Zhang, J. Y. and Wang, Y. Z. 2017. Quantitative determination and evaluation of *Paris polyphylla* var. *yunnanensis* with different harvesting times using UPLC-UV-MS and FT-IR spectroscopy in combination with partial least squares discriminant analysis. Biomedical Chromatography. 31(7): 3913.

Yasodamma, N., Chaithra, D. and Alekhya, C. 2014. Pharmacognostic evaluation of *Curcuma neilgherrensis* WT. International Journal of Pharmacy and Pharmaceutical Sciences. 6(1): 159-168.

Ye, Y., Chen, S., Wang, L., Yang, Y. and Zhao, C. L. 2011. Effects of stratification at different temperatures on the embryo differentiation and development of *Paris polyphylla var. yunnanensis* seeds. Pak. J. Bot. 43(3): 1507-1512.

Yesil, Y. and Ozhatay, F. N. 2008. Morphological, anatomical and karyological investigations on the genus *Paris* in Turkey. 7(2): 57-69.

Yilmaz, C. and Ozguyen, A. I. 2009. The effects of some plant nutrients, gibberellic acid

and pinolene treatments on the yield, fruit quality and cracking in pomegranate. Acta Hortic. 818: 205–212.

Yin, H. X, Xue, D., Zhang, H., Wu, M., Cheng, C. and Cheng, Y. 2007a. Qualitative estimation of material medica of Rhizoma Paridis from Sichuan and Yunnan province. J. Chin. Mater. Med. 7: 771-774.

Yin, H. X., Zhang, H., Xue, D., Wu, M., Qing, L. S., Chen, C. and Chen, Y. 2007 b. Qualitative estimation of medicinal plant *Paris* L. from Sichuan and Yunnan province. China J. Chin. Mat. Med. 13: 1344-1346.

Yingming, P., Ying, L., Hengshan, W. and Min, L. 2004. Antioxidant activities of several Chinese medicine herbs. Food chemistry. 88(3): 347-350.

Yonzone, R., Bhujel, R.B. and Rai, S. 2012. Genetic Resources, Current Ecological Status and Altitude Wise Distribution of Medicinal Plants Diversity of Darjeeling Himalaya of West Bengal, India. Asian Pacific Journal of Tropical Biomedicine. 12: 439-445.

Yu, K., Wang, Y., Wei, J., Ma, Q., Yu, D. and Li, J. 2009. Improving rhizome yield and quality of *Paris polyphylla* through gibberellic acid-induced retardation of senescence of aerial parts. Plant signaling & behavior. 4(5): 413-415.

Yu, K., Wei, J., Ma, Q., Yu, D., & Li, J. 2009. Senescence of aerial parts is impeded by exogenous gibberellic acid in herbaceous perennial *Paris polyphylla.* Journal of plant physiology. 166(8): 819-830.

Yu, W., Zhao Y. and Shu, B. 2004. The radical scavenging activities of radix puerariae isoflavanoids, a chemiluminescence study. Food Chem. 86: 525-529.

Yu, Z. Y., Guo, L., Wang, B., Kang, L. P., Zhao, Z. H., Shan, Y. J., Xiao, H., Chen, J. P., Ma, B. P. and Cong, Y. W. 2010. Structural requirement of spirostanol glycosides for rat uterine contractility and mode of their synergism. Journal of Pharmacy and Pharmacology. 62(4): 521-529.

Yu, K., Fan, Q. L., Wei, J. R., Yu, D. and Li, J. R. 2012. Nitrogen remobilization in shoots of *Paris polyphylla* is altered by gibberellic acid application during senescence. Biologia plantarum. 56(4): 717-723.

Yue, G., Wei, J., Qian, X., Yu, L., Zou, Z., Guan, W., Wang, H., Shen, J. and Liu, B. 2013. Synergistic anticancer effects of polyphyllin I and evodiamine on freshly-removed human gastric tumors. PloS one. 8(6): e65164.

Yun-Heng, J. I., Heng, L. I. and Zhe-Kun, Z. H. O. U. 2006. *Paris caobangensis* YH Ji, H. Li & ZK Zhou (Trilliaceae), a new species from northern Vietnam. Journal of Systematics and Evolution. 44(6): 700-703.

Whipkey, A., Simon, J. E., Charles, D. J. and Janick, J. 1992. In vitro production of artemisinin from *Artemisia annua* L. Journal of Herbs, Spices and Medicinal Plants. 1(1-2): 15-25.

Wink, D.A. and Mitchell, J. B. 1998. Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radical Biology and Medicine. 25(4-5): 434-456.

Zakir, S. 2012. Environmental Awareness as a Tool to Promote the Wise Practices of Wild Plant Resources in the Himalayas (Sudhangali-Kashmir, Pakistan). Putaj Sciences. 19.

Zhang, W., Zhang, D., Ma, X., Liu, Z., Li F. and Wu, D. 2014. Paris saponin VII suppressed the growth of human cervical cancer Hela cells. J Med Res. 19: 41.

Zhang, H., Samadi, A. K., Rao, K. V., Cohen, M. S. and Timmermann, B. N. 2011. Cytotoxic oleanane-type saponins from Albizia inundata. Journal of natural products. 74(3): 477-482.

Zhang, J., Wang, Y., Zhang, J., Ding, Y., Yu, H. and Jin, H. 2011. Evaluation of mineral element contents in *Paris polyphylla* var. *yunnanensis* from Southwest China. African Journal of Pharmacy and Pharmacology. 5(15): 1792-1796.

Zhang, J. Y., Yu, H., Zhang, S. G. and Deng, C. X. 2004. Studies on domestication and propagation technology of *Paris polyphylla* var. *yunnanensis and Paris polyphylla* var. *chinensi*. Southwest China J. Agr. Sci. 17(3): 314-317.

Zhang, J. Y., Wang, Y. Z., Zhao, Y. L., Yang, S. B., Zuo, Z. T., Yang, M. Q., Zhang, J., Yang, W. Z., Yang, T. M. and Jin, H. 2011. Phytochemicals and bioactivities of *Paris* species. Journal of Asian Natural Products Research. 13(7): 670-681.

Zhang, M., Li, Y.W., Li, Z.Y., Huang, X., Zhu, D. and Liu, Q. 2011. Progress on studies of endangered ethno-medicine of Rhizoma *Paris.* J. Cent. Univ. Natl. Nat. Sci. Ed. (20): 65-69.

Zhang, S. Q., Zhang, J. S. and Wang, C. Z. 2007. Extraction of steroid saponins *from Paris polyphylla* Sm. var. *yunnanensis* using novel ultrahigh pressure extraction technology. Pharmaceutical Chemistry Journal. 41(8): 424-429.

Zhang, S. D., Wang, H. and Li, D. 2008. A New Species of *Paris* (Melanthiaceae) from Northeastern Yunnan, China, A Journal for Botanical Nomenclature. 18(4): 550-554.

Zhang, T., Liu, H., Liu, X. T., Chen, X. Q. and Wang, Q. 2009. Steroidal saponins from the rhizomes of *Paris delavayi.* Steroids. 74(10-11): 809-813.

Zhang, T., Liu, H., Liu, X. T., Xu, D. R., Chen, X. Q. and Wang, Q. 2010. Qualitative and quantitative analysis of steroidal saponins in crude extracts from *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* by high performance liquid chromatography coupled with mass spectrometry. Journal of pharmaceutical and biomedical analysis. 51(1): 114-124.

Zhang, T., Liu, H., Liu, X. T., Chen, X. Q. and Wang, Q. 2009. Steroidal saponins from the rhizomes of *Paris delavayi.* Steroids. 74(10-11): 809-813.

Zhang, X., Cui, Y., Huang, J., Zhang, Y., Nie, Z., Wang, L., Yan, B., Tang, Y., Liu, Y. 2007. Bioorganic and Medicinal Chemistry Letters. 17(9): 2408-2413.

Zhang, W., Feng, X. U., Cheng, H., Linling, L. I., Fuliang, C. A. O. and Cheng, S. 2013. Effect of chlorocholine chloride on chlorophyll, photosynthesis, soluble sugar and flavonoids of *Ginkgo biloba*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 41(1): 97-103.

Zhang, X. Y., Zhang, Y., Xu, X. Y. and Qi, S. H. 2013. Diverse deep-sea fungi from the South China Sea and their antimicrobial activity. Current microbiology. 67(5): 525-530.

Zhao, L. and Shi, Q. 2005. Analysis on the Therapeutic Effect on Colporrhagia due to Drug Abortion (240 cases) Treated by Gongxuening. J Pract Tradit Chin Med. 21: 455–456.

Zhao, J., Shan, T., Huang, Y., Liu, X., Gao, X., Wang, M. and Zhou, L. 2009. Chemical composition and in vitro antimicrobial activity of the volatile oils from *Gliomastix murorum* and *Pichia guilliermondii,* two endophytic fungi in *Paris polyphylla* var. *yunnanensis.* Natural product communications. 4(11): 1491-1496.

Zhao, Y., Kang, L. P., Liu, Y. X., Zhao, Y., Xiong, C. Q., Ma, B. P. and Dong, F. T. 2007. Three new steroidal saponins from the rhizome of *Paris polyphylla.* Magnetic Resonance in Chemistry. 45(9). 739-744.

Zhao, Y. 2007. Master thesis, Academy of Military Medical Sciences. 7.

Zheng, Q. A. and Yang, C. R. 2003. Dracaenoside A and B, new C-22 steroidal lactone glycosides from the stem of *Dracaena cochinchinensis.* Chinese Chemical Letters. 14(12): 1261-1264.

Zheng, J. Y., Wang, H., Chen, X. X., Wang, P., Gao, P., Li, X. N. and Zhu, G. P. 2012. Microsatellite markers for assessing genetic diversity of the medicinal plant *Paris polyphylla* var. *chinensis* (Trilliaceae). Genet. Mol. Res. 11(3): 1975-1980.

Zheng, Q., Bao, C., Guo, W., Li, S., Chen, J., Chen, B., Luo, Y., Lyu, D., Li, Y., Shi, G. and Liang, L. 2016. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nature communications. 7(1): 1-13.

Zhou, L., Cao, X., Zhang, R., Peng, Y., Zhao, S. and Wu, J. 2007. Stimulation of saponin production in *Panax ginseng* hairy roots by two oligosaccharides from *Paris polyphylla* var. *yunnanensis.* Biotechnol Lett. 29: 631–634.

Zhou, L., Wu, J. and Wang, S. 2003. Low-temperature stratification strategies and growth regulators for rapid induction of *Paris polyphylla* var. *yunnanensis* seed germination. Plant growth regulation. 41(2): 179-183.

Zhou, L., Yang, C., Li, J., Wang, S. and Wu, J. 2003. Heptasaccharide and octasaccharide isolated from *Paris polyphylla* var. *yunnanensis* and their plant growth-regulatory activity. Plant Science. 165(3): 571-575.

Zhu, Y., Chen, S. L., Yao, H., Tan, R., Song, J. Y., Luo, K. and Lu, J. 2010. DNA barcoding the medicinal plants of the genus *Paris.* Acta pharmaceutica Sinica. 45(3): 376-382.

## Appendix-A

## List of Publications

- **Lepcha DL**, Pradhan A and Chhetri DR: Population assessment and species distribution modelling of *Paris polyphylla* Smith in Sikkim Himalaya, India. Biodiversitas. 20(5): 299-1305 (2019). [UGC Sl. No. 14630].

- **Lepcha DL,** Abhijit C and Chhetri DR: Antioxidant and Cytotoxic Attributes of Paris polyphylla Smith from Sikkim Himalaya. Pharmacognosy Journal. 11(4):705-711 (2019). [UGC Sl. No. 30569].

- Chhetri DR, Acharya AK, Pradhan M, Chhetri R, Sherpa SD and **Lepcha DL** : Nutraceutical Potential of two edible wild fruits, *Bischofia javanica* Blume and *Ficus cunia* Buch.-Ham. ex Roxb. from Sikkim Himalaya. International Journal of Food Science and Nutrition. 2(6): 1-9 (2017). [UGC Sl. No. 44557].

- **Lepcha DL** and Chhetri DR: Conservation Status of *Paris polyphylla* Smith. through Micro/Macro Propagation. International Journal of Engineering Technology Science and Research. 4(10): 853 (2017) [UGC Sl. No. 44431].

- Chhetri DR and **Lepcha DL**: Simulated osmotic stress induces metabolic effects in *Rhododendron arboreum* Smith. Indian Journal of Plant Sciences. 4 (2): 67-70 (2015). [UGC Sl. No. 43710].

## Appendix-B
## List of conferences/seminars attended

- Presented paper in two (2) day International conference on "Contemporary Issues in Integrating Climate-The Emerging Areas of Agriculture, Horticulture, Biodiversity, Forestry: Engineering Technology, Fundamental/Applied Science and Business Management for Sustainable Development (AGROTECH-2017) titled - *Paris polyphylla* Smith in Sikkim Himalayan Region; Threats, Conservation and Prospects. *Organized by* Himalayan Scientific Society for Fundamental and Applied Research *in Collaboration with*: Kalimpong Science Centre, Kalimpong, UBKV, West Bengal, India held at Kalimpong Science Centre, Kalimpong, West Bengal, India from $11^{th}$ – $12^{th}$ May, 2017.

- Presented a paper in two (2) day National Conference on Science, Technology and Environment: Prospects and Limitations in the $21^{st}$ Century (NCSTEPL-2017) titled - "Developing of Propagation Protocol and Cultivation Technique to Enhance Conservation Status of *Paris polyphylla* Smith through Micro/Macro Propagation. *Organized by* Bineswar Brahma Engineering College, Chandrapara, Kokrajhar, Assam, India on $30^{th}$ and $31^{st}$ October, 2017.

- Presented poster in three(3) day National Conference on new frontiers in medicinal plant research & Special Meeting on Medicinal Plants for Livelihood Security & Community Empowerment in Eastern Himalayas titled – "Study on the Indigenous medicinal knowledge of Lepcha community in Dzongu region of North Sikkim and its status today".*Organized by* Department of Science and Technology, Department of Biotechnology, Ministry of Science and Technology, Government of India and Sikkim University held at Namgyal Institute of Tibetology, Deorali, Sikkim from $3^{rd}$ -$5^{th}$ October, 2013.

- Presented poster in two (2) day National Seminar on Value Addition of Ayurvedic Herbal Resources through Good Agricultural and Field Collection Practices' titled "Medicinal Value of *Paris polyphylla* Smith". *Organized by* the Ayurveda Regional Research Institute, Gangtok, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Government of India held at Chintan Bhawan, Gangtok, Sikkim from $20^{th}$-$21^{st}$ November, 2015.

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Original Article

# Antioxidant and Cytotoxic Attributes of *Paris polyphylla* Smith from Sikkim Himalaya

**Dawa Lhendup Lepcha, Abhijit Chhetri, Dhani Raj Chhetri***

**ABSTRACT**

**Introduction:** *Paris polyphylla* Smith is a high value medicinal plant available in Sikkim Himalaya which is well known in local traditional medicine system. Scientific study to ascertain its claimed biological activity is lacking. The objective of this work was to determine the antioxidant and anticancer activity of *Paris polyphylla* rhizomes. **Methods:** Phytochemical analysis were carried out by standard methods. Antioxidant activity of the methanolic extract was carried out by DPPH, ABTS, OH-radical and $Fe^{2+}$chelating activity assays. Cytotoxicity of the extract was determined by MTT assay on three cancer cell lines: HeLa, HepG2 and PC3. **Results:** Of the *P. polyphylla* from two altitudinal zones, *P. polyphylla* from Tholung (PPT), the one from the higher altitude showed higher total phenolic contents in methanolic extracts of rhizomes as compraed to that from the lower altitude i.e., *P. polyphylla* from Uttaray (PPU). PPT also showed a higher content of total falvonoid and total flavonols. Both types of plant were excellent scavenger of DPPH and ABTS radical and $Fe^{2+}$ chelator. A trend of a relatively greater antioxidant activity of PPT was established through these assay methods. In MTT assay, both the extract showed significant dose-dependent inhibition of HeLa cell growth after 72 hrs of treatment, while the extract had a moderately positive effect on the inhibition of PC3 and HepG2 cells growth. **Conclusion**: The study suggested a strong antioxidant activity and appreciable cytotoxic activity of *P. polyphylla* from Sikkim Himalaya. Of the two varieties, PPT was more pronounced in both type of activities.

**Key words:** *Paris polyphylla*, Sikkim Himalaya, Cytotoxicity, Antioxidant, Anticancer, Phytochemicals.

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## INTRODUCTION

The state of Sikkim is situated in the Indian Himalayan Region extending between 27° 5′ - 28° 10′ N latitude and 88° 4′ - 88° 58′ E longitude and lies sandwiched between the kingdom of Nepal in the west and the kingdom of Bhutan in the east. Sikkim is an important component of the biodiversity hot-spot of the Himalayas of India. Due to an wide array of climatic zones, this region is rich in floral diversity, many endemic elements and a number of species which have become rare, threatened or endangered.[1] Ethnomedicinal traditions are very well developed in this part of the world and herbal drugs are prescribed widely because of their effectiveness and relatively low cost.[2] Despite this, a substantially rising number cases of cancer is being reported in Sikkim.[3,4]

*Paris polyphylla* Smith (locally called 'Satuwa' in Nepali and 'Tuk-tok-bee-sungtee' in Lepcha in Sikkim Himalaya) belongs to the family Melanthiaceae. In traditional medicine, the roots are used as analgesic, antiphlogistic, antipyretic, antispasmodic, antitussive, depurative, febrifuge and narcotic.[5] Furthermore, it has been used to treat liver cancer in China for several decades.[6] In the Indian Himalayan Region, *P. polyphylla*, is used against burn, cut or injury, diarrhea, dysentery, fever, gastritis, skin diseases, stomach pain and wounds.[7-10] In Sikkim Himalaya, the dried rhizome (1.0-1.5 cm) is soaked overnight in a glass of water and the drank in the morning to cure bodyache and as a tonic by the Lepcha people. However, there has not been any report on research about anticancer and antioxidant attributes of this plant from this region.

Cancer is one of the leading causes of death worldwide and about one third of cancer deaths (64.9%) have been reported from developing nations.[11] India is likely to have over 8.8 lakh deaths due to the disease by 2020. Cancer and some of the other chronic diseases share common pathogenesis mechanisms such as DNA damage, oxidative stress and chronic inflammation.[12] Many reports suggest that cancer cells are under a continuous oxidative stress[13,14] due to the generation of ROS that is identified as one of the key molecules involved in the multistage process of carcinogenesis.[15] Research carried out with human tumor cell lines clearly indicates that cancer cells produce ROS at a much higher rate than healthy cells.[16,17] Antioxidants play an important role by inhibiting the initiation step of oxidation of bio-molecules as well as scavenging various free radicals and thereby detoxifying cells.[18] Therefore, natural antioxidants are popular for its therapeutic efficacy which makes prospecting for bioactive plant products an important area of research. Anticancer properties of herbal drugs in most cases are related to their antioxidant activities.

Medicinal plants have remained very useful in the discovery of new anticancer drugs.[19] The inhabitants

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# Population assessment and species distribution modeling of *Paris polyphylla* in Sikkim Himalaya, India

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**Abstract.** *Lepcha DL, Pradhan A, Chhetri DJ. 2019. Population assessment and species distribution modeling of* Paris polyphylla *in Sikkim Himalaya, India. Biodiversitas 20: 1299-1305.* *Paris polyphylla* Smith is an important therapeutic plant that grows in the Indian Himalayan region. In Sikkim, the survival of the species is threatened by illegal extraction of its rhizome. Therefore, immediate conservation initiative is required to conserve the existing species population in the wild. Population assessment through field survey followed by ENM revealed a positive correlation between predicted suitable habitats with the actual sites of its occurrence, except in disturbed habitats. Precipitation of driest month (Bio14) and slope were the most influential factors that contributed 41.9 % and 30 % respectively to the MaxEnt model. Field survey revealed that the density of the plants varied between 0.45 (pl/m$^2$) and 3.89 (pl/m$^2$) and the frequency varied from 36% to 76%. The IVI for *P. polyphylla* ranged between 2.68 to 8.66 based on locations. On the other hand, the IVI of associated species varied from 3.57 to 18.14 based on species. *P. polyphylla* is a vulnerable plant in Sikkim Himalaya and it is facing an imminent threat of extinction. Under this situation, it is imperative that works on predictive modeling will help conserve the species. This study identified the potential habitats for *P. polyphylla* in the higher elevations of Sikkim Himalaya where it could be reintroduced.

**Keywords:** Ecological niche modeling, habitat, Himalaya, *Paris polyphylla,* Sikkim

## INTRODUCTION

*Paris polyphylla* Smith commonly known as Love apple belongs to the Family Melanthiaceae (earlier Trilliaceae or Liliaceae) (Figure 1), and is mostly found in India, China, Bhutan, Laos, Myanmar, Nepal, Sikkim, Thailand and Vietnam (Sharma et al. 2014). In India the species have been recorded from the Himalayan states like Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Uttarakhand (Paul et al. 2015).

In traditional medicine, the roots are used as analgesic, antiphlogistic, antipyretic, antispasmodic, antitussive, depurative, febrifuge and narcotic (Duke and Ayensu 1984) Furthermore, it has been used to treat liver cancer in China for several decades (Lee et al. 2005; Shoemaker et al. 2005). In Nepal, the rhizome is indigenously used against snake bites, insect bites, alleviate narcotic effects, internal wounds, external wounds, fever, food poisoning and are fed to cattle during diarrhea and dysentery (Dutta 2007; Baral and Kurmi 2006). It is also used to treat headache, vomiting, and worms (Uprety et al. 2010). In the Indian Himalayan Region, *P. polyphylla*, used against burn, cut or injury, diarrhea, dysentery, fever, gastritis, skin diseases, stomach pain and wounds (Farooquee et al. 2004; Maity et al. 2004; Tiwari et al. 2010; Jamir et al. 2012; Lalsangluaii et al. 2013; Pfoze et al. 2013; Sharma and Samant 2014).


**Figure 1.** A. Pictorial representation *P. polyphylla* from Sikkim, Himalaya, India. A. Whole plant; B. Plant with flower; C. Mature fruits with exploded pod; D. Sprouting rhizome

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# Nutraceutical potential of two edible wild fruits, *Bischofia javanica* Blume and *Ficus cunia* Buch.-Ham. ex Roxb. from Sikkim Himalaya

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**Abstract**
**Background and Objective:** The present study encompassing nutraceutical, nutritional and antioxidant properties of two wild edible fruits *Bischofia javanica* and *Ficus cunia from Sikkim Himalaya* dwells on the study of local plants from Sikkim Himalaya that is traditionally used.
**Materials and Methods:** Proximate parameters of the selected fruits were analysed by standard methods. The content of phenolic components, ascorbic acid, tannins and antioxidant parameters were analysed. In addition, some mineral constituents were also estimated.
**Results:** The presented studies established *Bischofia javanica* as an excellent protein supplement while the other fruit, *Ficus cunia* as a good supplement for vitamin-C. Furthermore, the *Ficus cunia* fruit due to its higher content of phenolics, flavonoids and flavanols is an excellent source of antioxidant which has been proven by several anti-oxidant assays.
**Conclusion:** The antioxidant activity shown by both the fruits is due to the significant presence of ascorbic acid, tannins and phenolic components like flavonoids, flavonols and total phenols etc. These fruits may be included in the local diet together so that one will address the malnutrition due to protein deficiency while other will take care of vitamin and antioxidant deficiency of the modern diet.

**Keywords:** antioxidant activity, phytochemical analysis, nutraceuticals, proximate composition, *bischofia javanica, ficus cunia*

## 1. Introduction

Wild fruits are used for human consumption because of their assumed health benefits for which they could be categorized as medicinal foods and nutraceuticals. Knowledge of such foods is there in the cultural tradition of many ethnic communities and as such considered as traditional knowledge [1]. Low fruit and vegetable consumption is regarded as one of the main risk factor for mortality in the world [2]. However, wild edible fruits and vegetables are known to be excellent source of nutrients such as minerals, vitamins, carbohydrates etc. And they may contribute an important part of diet providing health and nutrition while also serving as an appetizer [3]. In view of this, wild foods could become useful vehicles for improved nutrition and increased food supply [4].
Studies have shown that there is a connection between the intake of fruits and vegetables and a reduced rate of heart disease, mortality, common cancers and other degenerative diseases as well as aging, and this is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds [5].
*Bischofia javanica* Blume (BJ) belongs to family Euphorbiaceae, locally known as Bishop wood (Eng), Kainjal (Nep) and Sumong-kung (Lep) is an evergreen tree, upto 30m tall with palmately 3-foliate somewhat fleshy leaves. Flowers tiny, green, without petals and fruits globose or subglobose, berrylike, fleshy, upto 10 mm in diameter, brown or blue-black in color. *Ficus cunia Buch.*-Ham. exRoxb. (FC) of family Moraceae locally known as Khasray-Khaniu (Nep) and Tungshee-kung (Lep) is a tree which grows upto 10 m in height. The Bark is thick, reddish brown and contains milky juice. Fruits are borne on special shoots arising near the base of the trunk. Fruits about 20 mm in diameter and brownish-red in color. Both these plants grow in the Sub-Himalayan tract of Sikkim Himalaya at an altitude between of 300-1400 m amsl and in both the cases the ripening of fruits takes place during April-June. In traditional medicine, the leaves and buds of BJ are used in tonsillitis and throat pain whereas infusion of ground bark is used for abortion [6] and the decoction of tree bark is used for curing diarrhoea and dysentery [7]. The major phyto-constituents isolated from *Bischofia javanica* are tannin, β amyrins, betulinic acid, friedelan-3α-ol, epifriedelinol, friedelin, luteolin, quercetin, beta-sitosterol, stigmosterol and ursolic acid [8].
Similarly, in the the local traditional medicine across the Himalaya, FC is used in various ways. The latex of the plant is used to cure boils [9], it is also drunk to cure fever [10] Raw fruits are eaten in diarrhea and the decoction of the bark is taken against dysentery and liver complaints [11]. The dried leaves of FC have shown the presence of condensed tannins (+)-catechins, flavonoids quercetin, quercitrin [12], terpenes and shikimic compounds [13].
Wild edible plants are used as supplements to the cultivated crops and as famine foods during the lean season in this Himalayan region. Although these fruits are known to be edible, the nutritional information for the fruits is not available. Poor knowledge of the nutrient composition is one of the reasons for low fruit and vegetable consumption in

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***Research Article***

# SIMULATED OSMOTIC STRESS INDUCES METABOLIC EFFECTS IN *RHODODENDRON ARBOREUM* SMITH

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## ABSTRACT
Imposition of *in-vitro* abiotic stress on *Rhododendron arboreum* (*R. arboreum)* revealed rapid disruption of physiological and biochemical activities. Simulative environmental stress conditions i.e., drought induced by polyethylene glycol-6000 (PEG) increased the production of protein, soluble carbohydrates and proline with -0.5 MPa osmotic potential having a more pronounced effect on both protein and soluble carbohydrate production. PEG induced osmotic stress also led to membrane deterioration as evidenced by electrolyte leakage and membrane lipid peroxidation.

***Keywords:*** *Abiotic Stress, Rhododendron Arboreum, Polyethylene Glycol, Water Stress, Osmotic Adjustment, Oxidative Damage*

## INTRODUCTION
*Rhododendron arboreum* (*R. arboreum*) is an important ethnomedicinal plant of the Himalaya. The dried flowers of *R. arboreum* are used by Nepalese people for curing dysentery and diarrhea. A sip of the juice of *R. arboreum* flower is believed to dissolve fish bones stuck in the throat. *Rhododendrons* are one of the very few plants can grows up to an altitude of 5000 m in the Himalayas. *R. arboreum* was confirmed the most dominant species of the Himalaya (Tiwary and Chouhan, 2006). It is an excellent model for stress research because of its diversity of tolerance and susceptibility to environmental stress and possession of abilities to acclimate. Conditions of water stress can be created in the laboratory using polyethylene glycol, a biologically inert, non toxic chemical of high molecular weight. High molecular weight of PEG induces the condition of water stress similar to that caused by dry soil (Bajracharya, 1999). The cellular water deficit can result into changes in concentration of solutes, membrane structure, disruption of water potential and denaturation of proteins (Choudhuri and Choudhur, 1993). Cells subjected to water (osmotic) stress by exposure to hyperosmotic concentration of polyethylene glycol (PEG) responded with rapid accumulation of proline. Under stressed conditions, proline acts as a mediator of osmotic adjustment, a stabilizer of sub-cellular structures, a scavenger of free radicals and a contributor of cell wall structural proteins (Nanjo *et al.,* 1999). Abiotic stresses exert their effects directly or indirectly through the production of ROS and it was suggested that under prolonged oxidative conditions, active oxygen species would cause lipid peroxidation, DNA damage and protein denaturation (Scandalios, 1993). Cellular dehydration might be under the regulatory control of several proteins viz., membrane transport proteins, molecular chaperones and dehydrins that accumulates in response to dehydration.
Understanding the responses of plants to their external environment is an attractive target for improving stress tolerance. The objective of this study is to identify biochemical markers linked to stress tolerance traits so that the information may be used for future breeding and selection programme.

## MATERIALS AND METHODS
Actively growing leafy twigs of *Rhododendron arboreum* Smith were collected from Darjeeling (87° 59′ – 88° 53′ E and 26° 31′ – 27° 13′ N). One leafy twig each was placed in Erlenmeyer flasks containing solution of PEG-6000 equivalent to -0.2 and -0.5 MPa osmotic stress (OS) levels. The flasks were covered with aluminium foil allowing only the end of the twigs to protrude. An appropriate control immersed in distilled water was also prepared. Young unopened leaves were harvested at the end of 7 and 14 days for analysis. Soluble protein was extracted from leaf tissue with 50 mM Tris-HCl buffer (pH 7.0)