"EVALUATION OF SELECTED VEGETABLES OF SIKKIM HIMALAYAS FOR SOME NUTRACEUTICAL PROPERTIES"

A Thesis Submitted
To
Sikkim University

In Partial Fulfilment of the Requirement for the
Degree of Doctor of Philosophy

By

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July, 2018

DECLARATION

I declare that the present Ph.D. thesis entitled "**Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties**" submitted by me for the award of the degree of **Doctor of Philosophy in Horticulture** to the Sikkim University under the supervision of Dr. Laxuman Sharma, Associate Professor, Department of Horticulture, Sikkim University is my original research work solely carried out by me in the Department of Horticulture, School of Life sciences, Sikkim University, Gangtok. The thesis has not been submitted for any other degree or diploma in any other university/institution.

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This is to certify that **Ms. Uzma Khatoon** [Registration No. 14/Ph.D/HOR/04] has satisfactorily prosecuted her course of research and that the Ph.D. thesis entitled **"Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties"** submitted by her to the Sikkim University, in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in **Horticulture** is the result of original research work conducted by her under my supervision and is sufficiently of high standard to warrant its presentation to the examination.

I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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"Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties"

Submitted by **Ms. Uzma Khatoon** under the supervision of **Dr. Laxuman Sharma,** Associate Professor, Department of Horticulture, Sikkim University, Gangtok, 737102, Sikkim, India.

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ABBREVIATIONS

MSL	Mean Sea Level
CVD	Cardiovascular disease
SHU	Scoville Heat Unit
FAO	Food and Agriculture Organisation
DM	Dry Matter
ATP	Adenosine triphosphate
WE	water extract
ME	methanolic extract
EE	ethanoic extract
GAE	gallic acid equivalent
RUE	rutin equivalent
ND	non detectable
FW	fresh weight
AE	Acetone extract
CE	Chloroform Extract
DPPH	2,2-Diphenyl-1-picrylhydrazyl Assay
FRAP	Ferric reducing antioxidant power assay
PMA	Phosphomolybdenum Assay
MCA	Metal chelating Activity
FRSA	Free radical Scavenging activities
HRSA	Hydroxyl Radical Scavenging Activity
TAA	Total Antioxidant activity
CUPRAC	Cupric ion antioxidant reducing capacity
USDA	United States Department of Agriculture
IU	International Unit
g	gram

mg	milligram
kcal	Kilocalorie
kg	kilogram
%	Percentage
°C	Degree Centigrade
°Brix	Degree Brix
µg	Micro gram
µl	Micro litre
µM	Micro mole
OD	Optical density
TSS	Total Soluble Solids
FSSAI	Food safety and Standards Authority of India
C18	Column number 18
CCC	Counter Current chromatography
CHD	Classical Hydro-distillation
CPC	Continuous liquid–liquid Partition Chromatographic
GC-MS	Gas Chromatography Mass Spectrometry
hr	Hour
HPLC	High Performance Liquid Chromatogarphy
mm	Milimetre
mM	Milimole
nm	Nanometer
ml	Milliliter
v/v	Volume/volume
v/w	Volume /weight
Ca	Calcium
Fe	Iron
Mg	Magnesium
P	Phosphorus

Zn	Zinc
Na	Sodium
K	Potassium
N	Nitrogen
Cu	Copper
Cd	Cadmium
Pb	Lead
Se	Selenium
Mn	Manganese
Sr	Strontium
Cr	Chromium
Ni	Nickel
Co	Cobalt

Chapter-1
Introduction

The Himalayan region is rich in biodiversity owing to varied climatic, geographical, topographical, physiological and ecological situations (Khoshoo, 1991). The north-eastern Himalayan region exhibits a high degree of plant diversity than any other region in the Indian sub-continent, and it is considered as the primary centre of origin for many plants (Vavilov, 1950). The area is one of the thirty four global biodiversity hotspots of the world (Myers *et al*., 2000; Mittermeier *et al*., 2004). The northeastern Himalayan region exhibits high plant diversity than any other region in the Indian sub-continent, and it is considered as the origin of a large number of plants (Vavilov, 1950). Sikkim, a North Eastern Himalayan state of India coordinates at 27° 04′ 46″ to 28° 07′ 48″ N latitudes and 88° 00′ 58″ and 88° 55′ 25″ E longitudes, it covers an area of just 7,096 square km with an elevation range of 300 to 8,586 m. from mean sea level (MSL). The state is inhabited by diverse ethnic communities, major being Bhutia, Lepcha and Nepali constituting more than ten hilly tribes. More than 64 percent of the population in the state depends on agriculture for their livelihood. The entire state is now organically certified land. Agricultural land in Sikkim is estimated to be around 1,09,000 hectares, i.e. 15.36 percent of the total geographical area. Sikkim has a net cultivable area of about 79,000 hectares (11.13%); with an irrigated area of 15 percent of the total operational holdings of 110000 hectares.

The food bowl of different ethnic communities of Sikkim Himalayas usually comprises of wild edible plants and local germplasm or local uses. The term wild food is used to describe all those plant resources usually found growing outside the

agricultural areas and harvested or collected for the purpose of human consumption. In the Sikkim Himalaya, several varieties of locally available vegetables (wild or cultivated) are commonly consumed and are considered as an integral part of ethno-culture. The ethnic people of Sikkim consume inflorescences, roots, tubers rhizome, leaves and fruits of wild plants (Rai *et al.,* 2005, Sundriyal *et al.,* 2004) and usually smelly plants are being attracted by them. The edible wild or local germplasms are not only consumed but also used for the therapeutic purpose as a source of nutrients. The different tribes have rich knowledge on the use of indigenous vegetables as medicine, such plant consumed as vegetables play a significant role in sustaining the livelihood of people and providing the nutrient security. Local people are often seen selling wild edibles or cultivated underutilized vegetables in the local market. The consumption pattern of these vegetables depends upon the community and locality. The vegetables known or consumed by one community are not consumed by the other. The trial and error on edibility and taste over the generation have helped in using them as vegetables. On the other hand overpopulation, increasing deforestation and overexploitation have created the danger of extinction of some of the valuable species of wild edible vegetables (Maden and Dakhal, 1998). "Food is the medicine and medicine is the food" said by Hippocrates nearly 2500 years ago (Proper diet is the medicine and there is no medicine like proper diet). Even good medicines will not cure a patient without an appropriate accompanying diet. In fact, Ayurveda says that a well-modulated or regulated diet is the best medicine. Important guidelines regarding dietetics are given in a very elaborate manner that is to be thoroughly understood and carefully practised along with the modern nutraceuticals (Gupta *et al.,* 2007, Goyal, 2007).

The increased knowledge of the relationship between nutrients and health has resulted in several new products categories, such as Nutraceuticals. The word nutraceutical is a portmanteau of the words nutrient and pharmaceutical coined by Dr DeFelice in 1989 and the product category represents a unique interaction between the pharmaceutical and food industries (Brower, 1998). Nutraceuticals are diet supplements that deliver a concentrated form of a bioactive component from a food and used for the purpose of enhancing health in dosages that sometimes exceeds the normal foods (Zeisel, 1999). The nutraceuticals can either be taken as dietary supplements or as functional foods. The dietary supplements can be in the form of liquid concentrates or capsules whereas functional foods are enriched foods which are very close to the original natural food.

The major source of biologically active substances, such as vitamins and secondary metabolites (polyphenols, carotenoids, sterols, glucosinolates, and saponins) are present in most of the vegetables (Alothman *et al*., 2009). A number of studies revealed that individuals who eat five servings daily or more of fruits and vegetables have approximately half the risk of developing a wide variety of cancer types, particularly those of the gastrointestinal tract, suggesting that consuming phenolic-rich fruits and vegetables increases the antioxidant capacity of the blood (Gescher *et al*., 1998). Vegetables are important sources of minerals, fibre and vitamins, which provide essential nutrients for human health. Increased consumption of vegetable significantly reduces the incidence of chronic diseases, such as cancer, cardiovascular diseases and other age-related disorders. Various compounds such as polyphenols, carotenoids (pro-vitamin A), vitamins C and E (tocopherol) present in vegetables have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases (Spiller, 2001; Prakash, *et al.*,

2011). Polyphenols express biological activities, such as antifungal, antibacterial, antiviral, anti-inflammatory, anticancerous and antioxidative (Harborne and Williams, 2000; Soobrattee *et al*., 2005) and therefore continue identification of vegetables with high polyphenol content is of paramount importance to the scientific community due to the potential health benefits of these compounds (Vinson *et al*., 2001).

The phenolic compounds constitute a large group of secondary metabolites derived from phenylalanine pathway and are widely distributed throughout the plant kingdom (Mann, 1987; Harborne, 2001). Although they typically comprise less than 2% of the fresh weight basis of the plant, phenolic compounds serve the diverse functions like imparting colour to leaves and fruits, attracting or repelling insects, antimicrobial action, antiviral activity, protection from harmful ultraviolet radiation and protection from herbivores (Harborne, 1967; Macheix *et al.*, 1990; Harborne and Williams, 2000). Chemically, phenolics or polyphenols are aromatic rings bearing compounds with one or more hydroxyl groups including functional derivatives (ester/methyl esters, glycosides etc.) (Harborne, 1967; Macheix *et al.*, 1990; Shahidi and Naczk, 1995). More than 8,000 phenolic compounds have been identified in plants (Wrolstad, 2005) and the major are the phenolic acids and flavonoids (Macheix *et al*., 1990; Robbins, 2003). Phenolic acids in plants are predominantly substituted derivatives of hydroxybenzoic and hydroxycinnamic acids. These derivatives differ in patterns of hydroxylation and methoxylation of their aromatic rings (Harborne, 1994). The flavonoids share a common base structure consisting of two phenolic rings connected via an oxygenated heterocyclic pyran ring (Harborne, 1967). They are divided into several groups differing in the oxidation state of the pyran ring and include five major classes: anthocyanins, flavanols, flavanones, flavones and flavonols.

Vegetables are one of the major dietary sources of various antioxidant phytocompounds for humans. Our daily diet plays a key role in healthy ageing and prevents chronic diseases including obesity, diabetes, cardiovascular ailments, cancer, and osteoporosis. Only a small percentage of the population consume the recommended intake of fruits and vegetables, to meet the requirements of vitamins, minerals, antioxidants, enzymes and nutrients. In humans, they have a beneficial effect because of the positive biological responses they elicit, often reducing the risk of chronic disease. Foods with high phytonutrient content are sometimes called "super foods" since they are known to have health benefits beyond those of most foods (Heber, 2009). A significant number of phytochemicals have been reported in fruits, vegetables and other plant foodstuffs, which is linked to the reduction of risk of disease. The anti-cancer properties of fruits and vegetables have been highly recognised recently and are also reported to fruits and vegetables not only help decrease chances of developing cancer. They also decrease the risk of developing coronary heart disease and strokes.

Increasing epidemiological data suggest that a high intake of fruits and vegetables offers a number of health benefits against degenerative diseases (Rissanen *et al*., 2003). Numerous studies have suggested that the antioxidant activity, due to the phenolic composition of a food or natural health product, contributes to their protective effects against chronic and degenerative diseases (Heinonen *et al*., 1998; Record *et al*., 2001). Several specific plant phenolic compounds have been reported to exhibit anti-inflammatory, anti-carcinogenic, vasodilatory and antimicrobial activities (Rice-Evans *et al*., 1996; Robards and Antolovich, 1997; Harborne and Williams, 2000; Wollgast and Anklam, 2000). Therefore, in assessing the potential value of a vegetable for human consumption it is pertinent to determine the phenolic

composition and potent antioxidant activity of such compounds. The phenolic composition is generally unique to the plant species and can vary with its growth and climate where they are growing (Manach *et al*., 2004). The antioxidant activities of fruits vary with the type of phenolics compounds (Rice-Evans *et al*., 1995). It has also been found that some phenolic compounds act synergistically to increase overall antioxidant activity.

Traditional vegetables are very nutritious if they are consumed fresh or cooked at a medium temperature owing to their high content such as vitamins fibres and minerals. Nutritional elements besides nutrition also play a role in contributing to human health owing to different biological properties which have been linked to disease prevention (Hollman *et al*., 1996). For instance, fruit and vegetables are documented to be high in antioxidants, which delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidising chain reactions by free radical and therefore may reduce oxidative damage to the human body (Hollman and Arts 2000, Ismail *et al*., 2004). The occurrence of such oxidative damage is believed to be a significant causative factor in the development of chronic diseases such as cancer and cardiovascular disease (CVD) (Proteggente *et al*., 2002, Arts and Hollman 2005). The risk of these diseases could be reduced by increasing daily intake of fruits and vegetables such as broccoli, spinach, shallots, potato and carrots which are rich sources of antioxidants (Hertog *et al*., 1992, Cao *et al*., 1996, Heim *et al*., 2002). Apart from having high antioxidant activities and vitamins; fruit and vegetables are the source of carotenoids, polyphenols and non-antioxidant vitamins that are responsible for protection against cancer and CVD (Heim *et al*., 2002, Tucker 2003, Rao and Rao 2007).

In recent decades, a resurgence of interest has focused on wild plant species for their possible nutritional and medicinal values to broaden the diversity of human diet (Flyman and Afolayan, 2007; Afolayan and Jimoh, 2009). This is because people today are more concern about the effects of modern agricultural technology and marketing, which only cultivate plant types that have high productivity and consequently caused massive loss of biodiversity. On the other hand, increasing research on underutilized vegetables in different regions showed that most of these wild greens have great nutritional values and antioxidant properties, which are comparable to those commercially cultivated vegetables (Afolayan and Jimoh, 2009) and there has been no document on nutraceutical potential of indigenous vegetables of Sikkim.

So, this work is envisaged for exploring these vegetables as potential nutraceuticals with the following objectives:-

1. To survey the knowledge of ethnic community of Sikkim about the medicinal properties of vegetables.
2. To characterize and quantify constituent phenolics of selected vegetables.
3. To elucidate nutritional and ionic profiling of selected vegetables.
4. To study the antioxidant activity of selected vegetables.

During the present study following five vegetables were considered to meet the above mentioned objective:-

1. *Solanum aethiopicum* L.
2. *Solanum macrocarpon* L.
3. *Capsicum annuum* var. *cerasiformae* L.
4. *Tupistra aurantiaca* Wall.

5. *Nasturtium officinale* W.T.Aiton

Solanum aethiopicum, one of the leading vegetables in tropical Africa was reported to have been domesticated from the wild *Solanum anguivi* Lam., via the semi-domesticated *Solanum distichum* Schumach. & Thonn. (Lim, 2013). *S. aethiopicum* L. is commonly known as the African eggplant or Ethiopian eggplant characterized by round shape of fruit with green strips. Its leaves are eaten as a leafy vegetable and fruits are eaten either raw or cooked, whereas berries are used as an ornament in Asia. This is perennial or annual deciduous shrub which grows up to 2 m, often heavily branched, root system extending both vertically and laterally; branches and leaves with or without prickles and stellate hairs.

Solanum macrocarpon is a small tropical perennial plant that originated from Africa, it is widely distributed in West Africa, from Sierra Leone to Nigeria, from Cameroon eastward to Ethiopia and Southern Zimbabwe, it is also found in some parts of Southeast Asia, Brazil and Southern Europe (Rubatzky and Yamaguchi, 1997). It is cultivated either for fruits, which are 3-10 cm in diameter, flat in shape, non-ribbed, with a smooth surface and white or green coloured at the commercially mature stage, or for its leaves, which are used in the same way as spinach (Nyadanu and Lowor, 2015).

Capsicum annuum var. *cerasiformae* is also one of the hottest found species in these regions are the members of *C. annuum* complex with a Scoville rating of 100,000 to 350,000 SHU (Bhutia *et al*., 2016). It is mostly grown in Sikkim and its surrounding regions like Darjeeling for its pungent fruits. *C. annuum* var. *cerasiformae* is well known for its special type of pungency. *C. annuum* var. *cerasiformae* is almost round cherry size and bright red when fully ripen. It is used to make fresh or fermented pickle and spice up curry, daal or soup. It is popular during

cold winter months as its heat keeps the body warm. It is either simply pickled in saline vinegar or with spices and jarred for several months, and consumed alongside with traditional meal by the local people.

Tupistra aurantiaca Wall commonly known as Nakima in the local language of Sikkim belongs to family Liliaceae found in Sikkim Himalaya upto 7000 ft. Its inflorescence becomes available in the market from August to October which is eaten as vegetable and pickle. It is one of the costliest wild vegetable in Sikkim and very much popular in Bhutia and Lepcha communities of Sikkim.

Nasturtium officinale W.T. Aiton (Water Cress) commonly known as Simrayo in the local language of Sikkim belongs to the family of Brassicaceae. It is found in Sikkim Himalayan area even upto an altitude of 12,000 ft. Its young twigs are used to eat as vegetable or in soup. Peoples have believed that it reduces blood pressure.

Chapter-2

Review of Literature

The inhabitants of different regions have learnt the way to use locally available domesticated and wild vegetables of their locality. In remote rural settlements where vegetable cultivation is not practiced and market supplies are not organized, the inhabitants depend on indigenous vegetables, both cultivated in kitchen gardens and those collected from wild, for enriching the diversity of food. The word *indigenous* is used to describe vegetables that have their natural habitat in a country and the ones which were introduced from other regions of the world. The introduced vegetables due to long use became part of the food culture in that country (Chweya and Eyzaguirre, 1999**).** Extensive use of the natural resources might cause a sharp decrease in the population of particular species and the species which are associated with them or depend on them. It may also cause natural imbalance and may also lead to total loss of habitat of that particular locality. So, it is our prime responsibility to conserve the resources and use them in a proper manner so that they would be available for upcoming generations. Besides this, these wild and locally available resources are found to be a good source of nutritional, phytochemicals and phenols. The knowledge of such foods is part of traditional knowledge which is largely transmitted through the participation of individuals of households. So it is imperative to investigate the nutritional values of these wild and indigenous vegetables which in turn aware the peoples to explore them in right way and use them properly for their healthy life. This types of the investigation will help local people to combat nutritive deficiency disorders and also address the food security problems in the local

communities (Bajaracharya, 1984, Bhandari, 1978) with advantages of having baseline information for further improvement and research.

In Sikkim, many food items which are used in daily basket and eaten as a vegetable comprised of many local landraces and indigenous to the Sikkim Himalaya and underutilized. Sikkim followed the conventional organic cultivation practices from decades and now it is declared as fully organic state. So far, very scanty work have been noticed for analyzing nutritional profile, bioactive contents, antioxidant activity and vitamins content of locally available crops of Sikkim. The present studies included *Solanum aethiopicum*, *Solanum macrocarpon*, *Capsicum annuum* var. *cerasiformae*, *Tupistra aurantiaca* Wall. (Nakima) and *Nasturtium officinale* (Watercress) collected from all the districts of Sikkim which are used by local people as a vegetable for their nutritional and medicinal values.

2.1 Ethnobotanical survey

Among all the civilizations, Indians were the first in utilizing plant resources for their basic inevitabilities and livelihood. Although plants have been used as a source of food, fodder, shelter, clothing, medicine and lots of other useful commodities from ancient time, but the value of wild edible vegetables in food security is not given enough consideration in India (Reddy *et al*., 2007). Despite food self-sufficiency at the national level, the country has not attained food security at a household level particularly in rural areas. A considerable proportion of rural population is still undernourished. People living in most of the rural and remote areas do not produce enough food grains to meet yearly food requirement and at times food supplies are not easily available. Therefore, a large share of the rural population meet their nutritional requirement through nonconventional means, by consuming various

wild plants and animal resources (Singh and Arora, 1978). The wide varieties of plant leaves, fruits, roots, and other parts are collected from the wild and consumed by rural masses. The primitive people, through trial and error, have selected many wild edible plants and subsequently domesticated them (Kar, 2004). However, most of the wild vegetables, which are traditionally consumed by local communities come under the underutilized category. The nutritional value of these vegetables is high in comparison to commonly cultivated vegetables (Sundriyal and Sundriyal, 2001; Orech *et al*., 2007). The wild vegetables are an important source for the supplementation of micronutrients in vegetarian diets (Agate *et al*., 2000; Odhav *et al*., 2007). Due to various natural and anthropogenic reasons, natural resources of wild vegetables and habitats from where these resources are collected are depleting rapidly (Maikhuri *et al*., 2004; Bhogaonkar *et al*., 2010). In Sikkim Himalaya, the natives consume nearly 190 such wild edible species (Sundriyal, 1999). Selection of a particular species for inclusion in the diet is location specific and influenced by the availability of plant material. Genetic resources of wild vegetables should be conserved for future use to overcome malnutrition in a vegetarian diet, food security and for crop improvement of cultivated relatives of these wild vegetables (Kala, 2007). Survey of rural and tribal areas for documentation of underutilized or wild vegetables is the first step in making suitable strategies for the conservation and sustainable utilization of these resources. Other than the conservation of diversity and availability of vegetables, the health of peoples are also an important aspect for livelihood in rural and remote settlements. Providing modern healthcare to rural people in India is still a far-reaching goal due to economic constraints (Grover *et al*., 2002). Hence, people mainly depend on the locally available plant materials to cure various health disorders (Grover and Vats, 2001). Plants possess various components, which render beneficial properties (Tanabe

et al., 2002). Of late, much attention is being drawn towards exploring plant resources for nutritional and pharmaceutical values.

Sikkim state falls in the eastern Himalayan zone of India ($27^0$4'46"–$28^0$7'48" N and $88^0$58"–$88^0$55'25" E), covers 7096 km^2 area, and is bounded by Nepal on the west, by Tibet on the north, Bhutan and Tibet on the east, and Darjeeling district of West Bengal on the south. The state forms the entire upper catchment of the river Teesta drainage system. The state is rich in cultural and biological diversity. Lepchas, Bhutias and Nepalese are three main ethnic groups of Sikkim state and they differ in their food habits, dresses, and living styles. Besides growing food grains, the ethnic communities collect large quantities of wild edible plants from natural habitats. A total 190 wild plant species have been screened from the Sikkim Himalaya that describes the botanical richness, elevation distributional and dietary use of the edible wild plant resources from the Sikkim Himalaya (Eastern Himalaya), many with promising potential by Sundriyal *et al.*, (2004). These plants belong to 143 genera and 78 families and accounting for nearly 15% of total edible wild plants resources of India. Of the total, 65% were edible for their fruits, 22% for leaves~shoots, 7% for flowers and 3% for roots/rhizomes. Nearly 91 wild edible species were recorded from low-hills, 70 from mid-hills and 28 species from high-hill areas. Within Sikkim state, the North and East districts represent the maximum diversity of edible wild plants due to the wilderness and inaccessibility to most of the habitats. An average rural family annually consumes nearly 8 types of edible wild plants, and a few species provide over five meals in a season. Selected plants also form a source of earning to a few families that sell them in local markets. It is suggested that the high diversity of edible plants needs to be conserved for future use. Watercress was reported as a leafy

vegetable consumed by 87% of people next to *Diplazium esculentum*. *Tupistra nutans* Wall was mentioned as a spice used in making traditional dishes.

The traditional knowledge about indigenous or wild vegetable is largely transmitted by oral tradition from generation to generation without any written record. Such practices are still prevalent in many parts of the world. The ethnic communities of Sikkim was surveyed by Singh *et al*., (2002) for the traditional knowledge of plants, which are used for different disease and ailments. They reported 64 species of plants belonging to 42 families and 57 genera used for traditional healing practices. Epilepsy, leprosy, paralysis, asthma, typhoid, diabetes, haemorrhage during childbirth, cholera as well as others are the main ailments for which plants are used to cure. Some of these plants are also used as food items and play a significant role in the rural economy. A dry powder of inflorescence of *Tupistra nutans* Wall was reported to cure diabetes and also as a tonic to relieve body pains. A total of 37 species of plants belonging to 28 families are used as antidiabetic agents in the folk medicinal practices in the Sikkim and Darjeeling Himalayan region and 81% of these plants are hitherto unreported as hypoglycemic agents by the local tribes (Chhetri *et al.,* 2005). They found that *Campylandra aurantiaca* was also one of them. Bantawa and Rai, (2009) conducted an ethnobotanical study among the traditional herbal practitioner of Darjeeling Himalaya. A total of 41 plant species belonging to 26 families and 41 genera were found to be used by the practitioner for treating the diseases and disorders. Many of them are consumed directly as food and vegetables, condiments and spices. *Tupistra aurantiaca* Wall roots are recorded to be consumed orally in case of food poisoning. In East Sikkim, 79 plant species were collected which are useful to cure various human ailments by Das *et al.,* (2012). The ethnobotanical survey revealed that the people of the area possessing good knowledge

of herbal drugs, however progressive exposure to modernization, their knowledge of traditional uses of plants may get lost in due course.

Besides Sikkim and adjoining region, other rural and remote regions of India were also surveyed by many authors. A total of 123 households in six villages of Nanda Devi Biosphere Reserve buffer zone surveyed using a schedule by Misra *et al.*, (2008) to assess the knowledge, availability and consumption pattern of wild leafy vegetables. The 21 species of wild leafy vegetables used by the local inhabitants belong to 14 genera and 11 families. A total of 25 species belonging to 18 families have been documented as underexploited, non-conventional and traditional indigenous vegetable from Kannauj district of Uttar Pradesh (Kumar, 2013). *Chenopodium album* was the most common and popularly used wild vegetable followed by *Ipomoea aquatica* and *Coccinea grandis* in the studied area. Seven species were reported as a wild vegetable for the first time in India. Leaves and young stem are used in the majority of the cases. The highly endangered wild vegetables in the study area were *Abrus precatorius, Centella asiatica, Dioscorea bulbifera*, and *Solanum incanum*. The Northeastern region of India of which Sikkim is a part is one of the hotspots of biodiversity and the richest reservoirs of genetic variability and diversity of different crops *i.e* fruits, vegetables, spices, ornamental plants, medicinal and aromatic plants (Asati and Yadav, 2004). Among *Solanum* species, *S. macrocarpon, S. xanthocarpum, S. indicum, S. mammosum, S. khasianum, S. torvum, S. berbisetum, S. ferox, S. spirale, S. sisymbrifolium, S. kurzi* and *S. gilo* were reported to be found grown either in wild or domesticated form. Similarly, several species of *Capsicum* are reported *i.e. C. annum, C. annum* var. *avicular, C. annum* var. *grossum, C. annum* var. *longum, C. chinense, C. eximium, C. frutescens, C. minimum* and *C.*

pubescens. Many indigenous cucurbitaceous species were also reported to be found in the Northeastern region either in cultivated or in wild form.

2.2 Proximate Analysis

According to Food and Agriculture Organization of the United Nations (FAO), about one billion people, especially in developing countries depend on edible wild plants for their diets (Bharucha and Pretty, 2010). It is also to be noted that the staple food of the developing countries like India, starch based foods like wheat, maize and rice can meet up the human energy and protein requirement, however deficient in many of the essential nutrients. The diversity in wild species offers variety in family diet and can contribute to household food security (Zamede *et al*., 2001). Locally available crops serve as alternatives to staple food during periods of the deficit and are a valuable supplement (Scoones *et al*., 1992). Analyzing local plants for nutritional and medicinal potential would enable the identification of unconventional food resources that can be used by the local inhabitants. Guerrero *et al*., (1998) compiled a comprehensive nutrient report of wild vegetables consumed by the European farmers, and nearly all the species were reported to have significant amount of several micronutrients such as copper, magnesium, zinc, iron, vitamin E, carotenoids and vitamin C. The dietary fiber is major consideration for food quality and health benefit which is largely composed of complex carbohydrates that are somewhat resistant to digestion. Insoluble fibre like cellulose, found in plant cell walls, are helpful in waste and toxin removal through several mechanisms (Weisburger *et al*., 1993). The physiological impacts of insufficient dietary fibre intake are constipation, increased risk of coronary heart disease and increased fluctuation of blood glucose and insulin levels (AACC, 2001; Jenkins *et al*., 1998).

Table 2.1. Proximate content of different vegetable crops- a review

S. No.	Crop	Author (s)	Plant parts used	Observations
1.	*Solanum melongena*	Agoreyo *et al*., (2012)	Fruit (Round)	Moisture 78.44%, Protein 5.79% DM, Lipid 1.65% DM, Fibre 1.81% DM, Ash 1.96%DM, Carbohydrate 11.77% DM
			Fruit (Oval)	Moisture 72.93%, Protein 4.58% DM, Lipid 2.13% DM, Fibre 1.78% DM, Ash 3.15%DM, Carbohydrate 15.42% DM
2.	*Solanum anguivi*	Oyeyemi *et al*., (2015)	Fruits (%)	Moisture 4.58, Ash 8.89, Crude fat 5.68, Crude protein 36.35, Crude fiber 15.50, Carbohydrate 28.98
3.	*Solanum aethiopicum*	Asaolu and Asaolu (2002)	Fruit Coat (%)	Moisture 65.7, Crude protein 16.9, Crude fat 7.7, Total ash 3.2, Carbohydrate 6.5
			Fruit Flesh (%)	Moisture 69.5, Crude protein 15.6, Crude fat 5.9, Total ash 1.0, Carbohydrate 6.5
		Chinedu *et al*., (2011)	Fruit (g/100g)	Moisture 89.27, Dry Matter 10.73, Protein 2.24, Fat 0.52, Ash 0.87, Crude fibre 2.96, Carbohydrate 4.14

		Yakeen *et al*., (2011)	Leaves (%)	Protein 29.87, Ash 10.14, Moisture 13.09, Fat 0.57, Fiber 9.23, CHO 37.10
		Eze and Kanu, (2014)	Fruit (%)	Moisture 6.69, Ash 15, Crude fibre 21.33, Lipid 37.66, Protein 4.2,
		Gbadamosi *et al*., (2014)	Leaves (%)	Moisture 9.50, Protein 17.90, Crude fat 1.85, Ash 9.95, Crude fibre 15.90, Carbohydrate 44.85
		Sodamade *et al*., (2015)	Leaves (mg/100g)	Moisture 5.90, Crude fat 2.77, Crude fibre 6.79, Crude protein 28.92, Ash 8.32, Carbohydrate 47.31
		Jose *et al*., (2016)	Fruit (g/100g)	Moisture 86.6 Proteins 1.54 Titrable acidity 1.46 pH 5.70 Carbohydrate 3.60 Total Soluble Sugar 0.42 Starch 2.46 Fibre 4.51, Total Vit C 11.6 mg/100g Ascorbic acid 4.80 mg/100g Total Phenolics 24.4 mg/100g
		Eletta *et al*., (2017)	Fruits (%)	Moisture 91.20, Crude Protein 1.07, Crude Fat 0.38, Crude Fibre 2.44, Ash 0.73, Carbohydrate 4.18, Dry Matter 8.80

		Nyadanu and Lowor, (2015)	Fruit	Energy 46.2 kcal, Carbohydrate 10.11%, Protein 4.82%, Total fat 0.70%, Dietary Fiber 2.42%
4.	*Solanum indicum*	Ali, (2012)	Fruit	Wet weight basis Crude fibre 8% total carbohydrate 40.67%, Crude Protein 23.47%, Total ash 22.66%, Crude fat 5.26%, Calorific Value 303.9, presence of alkaloids, saponins and Polyphenols 7.02 mg/g
5.	*Solanum macrocarpum*	Asaolu and Asaolu (2002)	Fruit Coat (%)	Moisture 73.9, Crude protein 8.9, Crude fat 5.5, Total ash 3.1, Carbohydrate 8.7
			Fruit flesh (%)	Moisture 84.8, Crude protein 6.1, Crude fat 1.8, Total ash 1.8, Carbohydrate 5.5
		Oboh *et al*., (2005)	Fresh leaves (%)	Protein 4.3, Fat 0.6, Crude Fibre 1.4, Ash 1.3, Moisture 89.7
		Ijarotimi *et al*., (2010)	Leaves (Dry matter %)	Ash 17.24, Protein 26.48, Fat 13.85, Fibre 10.68, Carbohydrate 34.31, Energy 359.80 kcal
		Chinedu *et al*.,	Fruit (g/100g)	Moisture 92.5, Dry Matter 7.5, Protein 1.33, Fat 0.17, Ash 0.47

		(2011)		Crude Fibre 1.11, Carbohydrate 4.42
		Dougnon *et al*., (2012)	Leaves (mg/kg)	Protein 271.6, Fat 21.670, Moisture 88.6% Ash 92.58%
			Fruit (mg/kg)	Protein 147, Fat 16.470, Moisture 92.9% Ash 90.23%
		Ajiboye *et al*., (2014)		Ascorbic acid 340, Carbohydrate 6.4, Protein 4.6, Moisture 85.6, Fibre 1.6 g/100g
		Ogbuagu *et al*., (2015)	Fruit (%)	Carbohydrate 11.43, Moisture 68.35, Protein 5.07, Fats 5.00, Crude Fiber 3.31, Ash 5.0
		Ilodibia *et al*., (2016)	Leaf (%)	Moisture 85.87, Ash 1.77, Crude fibre 1.59, Crude Fat 0.77, Crude protein 4.78
			Root (%)	Moisture 62.62, Ash 0.93, Crude fibre 0.91, Crude Fat 0.38, Crude protein 2.89
			Fruit (%)	Moisture 78.77, Ash 1.64, Crude fibre 1.50, Crude Fat 0.47, Crude protein 4.53
		Jose *et al*., (2016)	Fruit (g/100g)	Moisture 87.2 Proteins 1.33 Titrable Acidity 1.87 pH 5.61

				Carbohydrate 6.48 Total Souble Sugar 0.31 Starch 6.15, Fibre 2.86 Total Vit C 18.9 mg/100g Ascorbic Acid 6.21 mg/100g Total Phenolics 144 mg/100g
		Eletta *et al*., (2017)	Fruit (%)	Moisture 92 Crude Protein 0.52, Crude Fat 0.15, Crude Fibre 2.50, Ash 0.80, Carbohydrate 4.01, Dry Matter 8.00
		Nyadanu and Lowor, (2015)	Leaf	Energy 58.44 Kcal, Carbohydrate 15.21%, Protein 8.71%, Total Fat 0.23%, Dietary fiber 2.81
			Fruit	Energy 43.4 kcal, Carbohydrate 8.66%, Protein 4.31%, Total fat 0.57%, Dietary Fiber 2.93%
6.	*Solanum zebrina*	Asaolu and Asaolu (2002)	Fruit Coat (%)	Moisture 72.5, Crude protein 10.1, Crude fat 6.5, Total ash 1.1, Carbohydrate 9.8
			Fruit flesh (%)	Moisture 82.9, Crude protein 7.3, Crude fat 4.6, Total ash 2.0, Carbohydrate 3.2
7.	*Solanum*	Asaolu and Asaolu	Fruit Coat (%)	Moisture 60.0, Crude protein 15.8, Crude fat 8.1, Total ash 3.5,

	sessiliflorum	(2002)		Carbohydrate 12.6
			Fruit flesh (%)	Moisture 71.9, Crude protein 12.1, Crude fat 2.9, Total ash 2.1, Carbohydrate 11.0
8.	*Solanum americanum*	Ijarotimi *et al*., (2010)	Leaves (Dry matter %)	Ash 13.56, Protein 18.84, Fat 10.14, Fibre 13.79, Carbohydrate 49.38, Energy 345.72 kcal
9.	*Solanum surattense*	Mali and Harsh, (2015)	Leaves (%)	Ash 11.85, Crude fat 1.96, Crude protein 11.11, Crude fibre 33.91, Carbohydrate 75.08, Moisture 48.89
			Seeds (%)	Ash 11.24, Crude fat 4.19, Crude protein 12.83, Crude fibre 20.24, Carbohydrate 71.74, Moisture 52.22
10.	*Solanum torvum*	JothiKarurmari *et al*., (2014)	Fruits	Moisture 73%, Organic content 88.6%, Inorganic content 11.4%
		Akoto *et al*., (2015)	Fruits	Moisture 86.23%, Carbohydrate 7.033%, Protein 2.322%, Fats 0.278%, Ash 0.143%, crude Fibre 3.393%
		Nyadanu and Lowor,	Fruit	Energy 43.7 kcal, Carbohydrate 7.50%, Protein 2.20%, Total

		(2015)		fat 0.76%, Dietary Fiber 5.40%
		Otu *et al.,* (2017)	Fruit	Moisture 84.43%, Fats 1.80%, Protein 1.44%, Fibre 0.69-1.35%, Carbohydrate 10.89%, Ash 2.6%
11.	*Nasturtium officinale* (Watercress)	Rai *et al*., (2005)	Young twigs	Moisture 91.1%, Ash 1.7%, Fat 1.1%, Protein 3.1%, Carbohydrate 3.0%
		Shad *et al*., (2013)	Aerial part (%)	Moisture 80.08, Ash 3.07, Protein 3.61, Crude Fiber 9.43, Fat 1.12, Carbohydrate 2.70,
		Khan *et al.,* (2016)	Leaves (%)	Moisture 87.50, Ash 0.36, Lipid 0.81, Protein 2.85, Fiber 1.06, Carbohydrate 7.40,
		Pradhan *et al*., (2015)	Leaves	Moisture 90.6%, Ash 24.9% DM, Protein 33.8% DM, Fat 9.6% DM, Carbohydrate 31.7% DM, Crude Fibre 9.9% DM
12.	*Tupistra nutans* (Nakima)	Rai *et al*., (2005)	Inflorescence	Moisture 91.5%, Ash 1.1%, Fat 2.9%, Protein 2.4%, Carbohydrate 2.1%
13.	*Phlogacanthus*	Rai *et al*., (2005)	Inflorescence	Moisture 79.1%, Ash 2.4%, Fat 0.8%, Protein 1.5%,

	tbyrsittorus (Titay)			Carbohydrate 16.2%
14.	*Hottuynia cordata* (Hilay jhar)	Rai *et al*., (2005)	Whole plant	Moisture 88.3%, Ash 1.7%, Fat 0.8%, Protein 4.1%, Carbohydrate 5.1%
15.	*Urtica dioica* (Sishnu)	Rai *et al*., (2005)	Inflorecence and young twigs	Moisture 84.6%, Ash 2.4, Fat 0.7%, Protein 4.7%, Carbohydrate 7.6%
		Pradhan *et al*., (2015)	Leaves	Moisture 84.5%, Ash 18.9% DM, Protein 28.5% DM, Fat 5.2% DM, Carbohydrate 47.4% DM, Crude Fibre 13.2% DM
16.	*Ficus benjamina* (Kabra)	Rai *et al*., (2005)	Leaf buds	Moisture 94.5%, Ash 0.4%, Fat 1.0%, Protein 1.9%, Carbohydrate 2.1%
17.	*Aconogonum molle* (Thotnay)	Rai *et al*., (2005)	Young twigs	Moisture 93.3%, Ash 0.8%, Fat 0.5%, Protein 2.6%, Carbohydrate 2.8%
18.	*Diplazium esculentum* (Ningro)	Rai *et al*., (2005)	Young fronds	Moisture 93.1%, Ash 1.3%, Fat 2.0%, Protein 2.6%, Carbohydrate 1.0%
19.	*Basella alba*	Ijarotimi *et al*.,	Leaves (Dry	Ash 11.42, Protein 28.52, Fat 14.31, Fibre 6.62, Carbohydrate

		(2010)	matter %)	41.63, Energy 401.37 kcal
20.	*Talinum triangulare*	Yakeen *et al.*, (2011)	Leaf (%)	Protein 15.85, Ash 23.74, Moisture 17.47, Fat 0.44, Fiber 9.42, CHO 33.08
		Ajiboye *et al*., (2014)		Ascorbic acid 284, Carbohydrate 4.8, Protein 5.0, Moisture 93.0, Fibre 1.4 g/100g
21.	*Nymphaea lotus*	Gbadamosi *et al*., (2014)	Leaves (%)	Moisture 8.70, Protein 15.60, Crude fat 1.60, Ash 9.20, Crude fibre 15.40, Carbohydrate 49.45

Abbreviations: DM- Dry Matter

Inclusion of fruits and vegetables with high dietary fiber content is recommended for some cancer types (Weisburger *et al*., 1993; Harris and Ferguson, 1993). The proximate content of wild and indigenous vegetables was evaluated by several researchers which were listed in Table 2.1 with their nutritional content and part of the plant used for analysis.

2.3 Mineral Composition

All the indigenous and wild vegetables are reported to be enriched with the all essential and beneficial elements required for the balanced growth and functioning of human body. Some minerals are essential for human nutrition (e.g., Fe, Cu, Se and Zn), while others such as Cr, Cd, Ni, As and Pb were beneficial possibly through the formation of Reactive Oxygen Species (ROS) (Rojas *et al*., 1999; Linder, 2001). The trace elements essentially act as cofactors for antioxidant enzymes involved in the destruction of toxic free radicals produced in the body as a normal consequence of the metabolic processes. Three key trace elements *i.e.* Zinc, Selenium and Iron with roles in antioxidant defense are gradually gaining attention. Apparently, Fe, Cu, Zn and Se are necessary to maintain genetic stability and nutritional well-being of humans and animals (Rojas *et al*., 1999). Recent studies suggest that ultra-trace elements, such as As and Ni, may also play a role both in animal and human nutrition (Rojas *et al*., 1999). In the last 20 years, evidence has been accumulated to support the role of zinc as a cellular antioxidant (Powell, 2000). Although zinc does not react directly with ROS, a number of indirect mechanisms have been described (Powell, 2000; DiSilvestro, 2000). One of the ways in which zinc acts as an antioxidant is through the induction of the metallothioneins, a group of low-molecular-weight amino acid residues, usually produced in many tissues including the liver, gut and kidney. For

instance, cadmium is present in spinach and cauliflower, while lead is found in Brussels sprouts and Chinese beets. Many vegetables including indigenous and wild were subjected for elemental analysis across the globe. The nutrient content reported by many researchers are listed in Table 2.2.

Iron is a key element in the metabolism of almost all living organisms. In Humans, iron is an essential component of hundreds of proteins and enzymes (Wood and Ronnenberg, 2006). Haeme is an iron-containing compound found in a number of biologically important molecules like haemoglobin and myoglobin that are involved in the transport and storage of oxygen. Cytochromes are haeme-containing compounds that have important roles in mitochondrial electron transport; therefore, cytochromes are critical to cellular energy production and thus life. Non-haeme iron-containing enzymes, such as NADH dehydrogenase and succinate dehydrogenase, are also critical to energy metabolism (Yip and Dallman, 1996). Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis (Beard and Dawson, 1997; Fairbanks, 1999). Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune function.

Zinc is an essential trace element for all forms of life. Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction. On the cellular level, the function of zinc can be divided into three categories namely, catalytic, structural and regulatory (Cousins, 2006). Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc plays an important role in the structure of proteins and cell membranes. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs

Table 2.2. Mineral composition of different vegetable crops- a review

S. No.	Crop	Author (s)	Plant parts used	Mineral Composition
1.	*Solanum melongena*	Agoreyo *et al*., (2012)	Fruit (Round) (mg/100g)	Ca 3.68, Fe 2.75, Mg 2.26, P 3.72, Zn 0.25, Na 184.21, K 238.10, N 930
			Fruit (Oval) (mg/100g)	Ca 1.95, Fe 1.96, Mg 2.56, P 5.23, Zn 0.25, Na 174.93, K 245.37, N 730 mg/100g
2.	*Solanum anguivi*	Oyeyemi *et al*., (2015)	Fruits	Ca 0.49%, Na 0.03%, K 2.03%, Fe 222.25 mg/kg, Mg 255 mg/kg, Mn 27.65, Zn 28.15 mg/kg, Cu 13.80 mg/kg, P 650.86 mg/kg
3.	*Solanum aethiopicum*	Asaolu and Asaolu (2002)	Fruit Coat (mg/100g)	Na 301, K 200, Mg 20.1, Ca 9.5, P 26.1, Cu 2.7, Fe 0.9, Mn 0.2, Zn 0.3, Co 0.2
			Fruit flesh (mg/100g)	Na 230, K 160, Mg 16.8, Ca 9.8, P 35.0, Cu 2.0, Fe 1.1, Mn 0.2, Zn 0.5, Co 0.4

		Chinedu *et al*., (2011)	Fruit (mg/100g)	Ca 498.47, Mg 1.98, Fe 1.02
		Yakeen *et al*., (2011)	Leaf (ppm)	Fe 0.80, Zn 0.99, Pb 0.04, Cu 0.09, Mg 165.2
		Eze and Kanu, (2014)	Fruit (mg/g)	Ca 0.12, K 4250, P 1.14, Mg 0.56, Na 0.63, Pd 0.03, Zn 0.115, Fe 0.63, Cu 0.3, Cd 0.015, Se 0.015
		Gbadamosi *et al*., (2014)	Leaves (mg/100g)	P 60, Ca 95, Fe 6, Mn 0.08, Mg 3.50, Zn 0.05
		Sodamade *et al*., (2015)	Leaves (mg/100g)	Na 0.21, K 0.43, Ca 0.23, Mg 0.29, P 0.20, Fe 124.95, Cu 2.45, Zn 33.5, Mn 4.05, Se 0.02
		Nyadanu and Lowor, (2015)	Fruit (mg/100g)	Ca 76.6, Cu 2.76, Fe 16.28, Mg 25.66, Mn 11.21, P 31.62, Zn 2.85, K 543.18
4.	*Solanum macrocarpum*	Asaolu and Asaolu (2002)	Fruit Coat (mg/100g)	Na 362, K 215, Mg 19.8, Ca 9.5, P 25.0, Cu 1.6, Fe 1.8, Mn 0.7, Zn 0.2, Co 0.3
			Fruit flesh	Na 320, K 195, Mg 18.1, Ca 7.8, P 38.8, Cu 1.1,

			(mg/100g)	Fe 2.0, Mn 2.6, Zn 0.5, Co 0.6
		Oboh *et al*., (2005)	fresh leaves (mg/kg)	Ca 32.6, Zn 8.2
		Ijarotimi *et al*., (2010)	Leaves (mg/100g of Dry matter)	Zn 6.44, Fe 1.39, Cu 1.61, Na 1658.60, K 2857.15, Ca 3496.86, Mg 4661.74, P 2793.52,
		Chinedu *et al*., (2011)	Fruit (mg/100g)	Ca 101.56, Mg 1.01, Fe 0.70
		Dougnon *et al*., (2012)	Leaves (mg/kg)	Na 1760.33, K 45.960,Ca 19.650, Mg 5960, Fe 251, Cu 40, Zn 220, P 4300
			Fruit (mg/kg)	Na 1370.33, K 37.410, Ca 3770, Mg 2430, Fe 203, Cu 34, Zn 156, P 5100
		Ajiboye *et al*., (2014)		Ca 2.43, K 5.67, Mg 1.93, Na 4.56, Fe 0.07 mg/100g
		Ogbuagu *et al*., (2015)	Fruit (ppm)	Cu 0.158, Mn 0.696, Zn 5.239, Mg 11.304, Fe

				4.539, Co 0.123, Cr 0.073, Al 0.000, Ca 30.425, Pb 0.030, Cd 0.018, Ni 0.040
		Ojo *et al*., (2015)	Fruit (mg/100g of dry matter)	Ca 1704, K 2289, Mg 637.1, P 275.9, Fe 9.4, Mn 8.5, Zn 5.4, Cu 4.7
		Usunomena and Chinwe, (2016)	Leaves (mg/100g)	Ca 256.60, Cu 0.62, Mg 81.69, K 87.22, Na 32.51, Fe 31.41, Zn 1.41, Cr 0.75
		Nyadanu and Lowor, (2015)	Leaves (mg/100g)	Ca 252.29, Cu 1.84, Fe 22.34, Mg 355.62, Mn 5.68, P 62.79, Zn 3.21, K 326.54
			Fruit (mg/100g)	Ca 72.5, Cu 1.82, Fe 11.20, Mg 18.73, Mn 8.31, P 27.53, Zn 3.13, K 329.52
5.	*Solanum zebrina*	Asaolu and Asaolu (2002)	Fruit Coat (mg/100g)	Na 320, K 230, Mg 19.1, Ca 8.5, P 45.3, Cu 1.7, Fe 1.1, Mn 0.5, Zn 0.3, Co 0.1
			Fruit flesh (mg/100g)	Na 312, K 190, Mg 17.8, Ca 6.9, P 50.0, Cu 1.6, Fe 1.6, Mn 1.9, Zn 0.5, Co 0.3

6.	*Solanum sessiliflorum*	Asaolu and Asaolu (2002)	Fruit Coat (mg/100g)	Na 460, K 312, Mg 37.5, Ca 45.3, P 92.8, Cu 6.9, Fe 2.9, Mn 8.1, Zn 1.2, Co 1.2
			Fruit flesh (mg/100g)	Na 320, K 118, Mg 23.3, Ca 28.1, P 35.4, Cu 2.9, Fe 1.0, Mn 4.6, Zn 0.8, Co 0.7
7.	*Solanum americanum*	Ijarotimi *et al*., (2010)	Leaves (mg/100g of Dry matter)	Zn 1.02, Fe 0.47, Cu 0.83, Na 2056.15, K 941.10, Ca 1535.14, Mg 1422.73, P 1462.53
8.	*Solanum surattense*	Mali and Harsh, (2015)	Leaves (mg/100g)	Ca 1.17, K 0.19, Na 0.10, P 0.39
			Seeds (mg/100g)	Ca 1.52, K 0.22, Na 0.02, P 0.51
9.	*Solanum torvum*	Akoto *et al*., (2015)	Fruit	Fe 76.869, Mn 19.466, Ca 221.583, Cu 2.642, Zn 21.460 mg/kg
		Nyadanu and Lowor,	Fruit (mg/100g)	Ca 59, Cu 2.55, Fe 10.6, Mg 38.56, Mn 13.11, P

		(2015)		67.39, Zn 5.81, K 399.73
		Otu *et al.*, (2017)	Fruit (mg/100g)	Ca 67.35, Fe 77.60, K 695
10.	*Nasturtium officinale* (Watercress)	Rai *et al*., (2005)	Young twigs (mg/100g)	Na 72.4, K 481.7, Ca 60.1
		Shad *et* al., (2013)	Aerial part (mg/100g)	Se 0.025, Cd 0.001, Cr 0.003, Zn 0.045, Cu 1.07, Mn 0.046, Fe 4.18, Ni 0.083, Na 19.23, K 278.90, Ca 153, Mg 6.48, P 14.50
		Khan *et al.,* (2016)	Leaves (mg/100g)	Na 320.30, K 1608, Ca 870, Mg 30, Fe 25, Cu 20, Zn 40, Cr 1, Ni 2, Mn 20, Cd 0.90, Pb 0.30
		Abdul *et al*., (2014)	Leaves (ppb)	Cr 0.001, K 107.7, Na 5.451, Fe 0.219, Mn 1.835, Mg 55.23, Se 175.8
		Pradhan *et al*., (2015)	Leaves (mg/100g)	Na 68.8, K 456.2, Ca 65.6,
11.	*Tupistra nutans* (Nakima)	Rai *et al*., (2005)	Inflorescence	Na 3.1, K 292.1,Ca 200.6

			(mg/100g)	
12.	*Phlogacanthus tbyrsittorus* (Titay)	Rai *et al*., (2005)	Inflorescence (mg/100g)	Na 2.9, K 722.9, Ca 105.0
13.	*Hottuynia cordata (*Hilay jhar)	Rai *et al*., (2005)	Whole plant (mg/100g)	Na 6.9, K 801.4, Ca 11.3
14.	*Urtica dioica* (Sishnu)	Rai *et al*., (2005)	Inflorecence and young twigs (mg/100g)	Na 11.7, K 911.0, Ca 102.5
15.	*Ficus benjamina* (Kabra)	Rai *et al*., (2005)	Leaf buds (mg/100g)	Na 9.2, K 222.0, Ca 11.7
16.	*Aconogonum molle* (Thotnay)	Rai *et al*., (2005)	Young twigs (mg/100g)	Na 8.7, K 428.4, Ca 5.0
17.	*Diplazium esculentum*	Rai *et al*., (2005)	Young fronds	Na 8.1, K 927.4, Ca 200.5

	(Ningro)		(mg/100g)	
18.	*Basella alba*	Ijarotimi *et al*., (2010)	Leaves (mg/100g of Dry matter)	Zn 4.16, Fe 1.69, Cu 2.42, Na 1421.58, K 1211.44, Ca 814.13, Mg 3132.59, P 2550.55
19.	*Talinum triangulare*	Yakeen *et al.,* (2011)	Leaf (ppm)	Fe 0.37, Zn 0.05, Pb 0.05, Mg 105.4
		Ajiboye *et al*., (2014)		Ca 2.45, K 6.11, Mg 2.23, Na 0.29, Fe 0.44 mg/100g
20.	*Nymphaea lotus*	Gbadamosi *et al*., (2014)	Leaves (mg/100g)	P 120, Ca 160, Fe 8, Mn 0.01, Mg 2.00, Zn 0.01
21.	*Cyclanthera pedata*	Oliveira *et al*., (2014)	Fruits (mg/100g)	Ca 11.9, Cu 0.013, Fe 0.21, K 152, Mg 8.4, Mn 0.074, Na 0.91, P 19.4, Zn 0.13, V 0.015

their function (O'Dell, 2000). Zinc also plays a role in cell signalling and has been found to influence hormone release and nerve impulse transmission.

Calcium is the most common mineral in the human body. About 99% of the calcium in the body is found in bones and teeth, while the other 1% is found in the blood and soft tissue. Calcium levels in the blood and fluid surrounding the cells must be maintained within a very narrow concentration range for normal physiological functioning. The physiological functions of calcium are so vital for survival that the body will demineralize bone to maintain normal blood calcium levels when calcium intake is inadequate. Thus, adequate dietary calcium is a critical factor in maintaining a healthy skeleton (Weaver and Heaney, 1999). Osteoporosis may result when bone resorption chronically exceeds formation (Weaver and Heaney, 1999). Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and the secretion of hormones like insulin (Food and Nutrition Board, Institute of Medicine, 1997). Calcium is necessary to stabilize a number of proteins and enzymes, optimizing their activities. The binding of calcium ions is required for the activation of the seven vitamin K-dependent clotting factors in the coagulation cascade.

Magnesium plays important roles in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, 6% to 7% is found in other cells, and less than 1% is found outside of cells (Shils, 1997). Magnesium is involved in more than 300 essential metabolic reactions (Spencer *et al.,* 1994). The metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required

by the adenosine triphosphate (ATP)-synthesizing protein in mitochondria. ATP, the molecule that provides energy for almost all metabolic processes, exists primarily as a complex with magnesium (Rude and Shils, 2006). Magnesium is required for a number of steps during nucleic acid and protein synthesis. Several enzymes participating in the synthesis of carbohydrates and lipids require magnesium for their activity. Glutathione, an important antioxidant, requires magnesium for its synthesis (Rude and Shils, 2006). Magnesium plays a structural role in bone, cell membranes, and chromosomes. Magnesium is required for the active transport of ions like potassium and calcium across cell membranes. Through its role in ion transport systems, magnesium affects the conduction of nerve impulses, muscle contraction, and normal heart rhythm. Calcium and magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types. Such effects on cell migration may be important in wound healing (Rude and Shils, 2006).

Phosphorus is an essential mineral that is required by every cell in the body for normal function (Knochel, 2006). The majority of the phosphorus in the body is found as phosphate (PO_4). Approximately 85% of the body's phosphorus is found in bone. Phosphorus is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. Phospholipids are major structural components of cell membranes. All energy production and storage are dependent on phosphorylated compounds, such as ATP and creatine phosphate. Nucleic acids, which are responsible for the storage and transmission of genetic information, are long chains of phosphate-containing molecules. A number of enzymes, hormones, and cell-signalling molecules depend on phosphorylation for their activation. Phosphorus also helps to maintain normal acid-base balance by acting as one of the body's most important buffers. Additionally, the phosphorus-containing molecule 2,3-

diphosphoglycerate (2,3-DPG) binds to haemoglobin in red blood cells and affects oxygen delivery to the tissues of the body (Knochel, 2006).

Potassium is an essential dietary mineral and electrolyte. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells (Peterson, 1997). Potassium concentrations are about 30 times higher inside than outside cells, while sodium concentrations are more than ten times lower inside than outside cells. The concentration differences between potassium and sodium across cell membranes create an electrochemical gradient known as the membrane potential. Tight control of cell membrane potential is critical for nerve impulse transmission, muscle contraction, and heart function (Brody, 1999; Sheng, 2000). A limited number of enzymes require the presence of potassium for their activity. The activation of sodium, potassium-ATPase requires the presence of sodium and potassium. The presence of potassium is also required for the activity of pyruvate kinase, an important enzyme in carbohydrate metabolism (Sheng, 2000).

Copper (Cu) is an essential trace element for humans and animals. In the body, copper shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms, though the majority of the body's copper is in the Cu^{2+} form. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions and in scavenging free radicals (Linder and Hazegh-Azam, 1996). Copper is a critical functional component for a number of essential enzymes known as cuproenzymes *i.e.* cytochrome *c* oxidase which plays a critical role in cellular energy production. Two copper-containing enzymes, ferroxidase I and ferroxidase II have the capacity to oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation. The

myelin sheath is made of phospholipids whose synthesis depends on cytochrome *c* oxidase activity (Turnlund, 2006). The cuproenzyme, tyrosinase, is required for the formation of the pigment melanin which formed in cells called melanocytes and plays a role in the pigmentation of the hair, skin, and eyes (Turnlund, 2006). Copper-dependent transcription factors regulate transcription of specific genes. Thus, cellular copper levels may affect the synthesis of proteins by enhancing or inhibiting the transcription of specific genes. Genes regulated by copper-dependent transcription factors include genes for copper/zinc superoxide dismutase (Cu/Zn SOD), catalase, and proteins related to the cellular storage of copper (Uauy *et al.*, 1998).

2.4 Phenolic compounds

Phenolic compounds can be defined a large number of naturally occurring organic compounds (more than 8000) widely dispersed throughout the plant kingdom that possesses at least one aromatic ring with one or more hydroxyl groups attached to the ring. Most naturally occurring phenolic compounds exist as conjugates with monosaccharides and polysaccharides linked to one or more of the phenolic groups (Harbone, 1998). Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway. Phenylalanine ammonialyase (PAL) is the key enzyme catalyzing the biosynthesis of phenolics from the aromatic amino acid phenylalanine.

Phenolic acids, flavonoids and tannins are the main dietary phenolics. Phenolic acids include hydroxybenzoic and hydroxycinnamic acids. Flavonoids are a diverse group of secondary plant metabolites that include flavonols, flavanols, flavanones and flavones. The major classes of tannins in the plant kingdom are hydrolysable and condensed tannins (Harbone, 1998).

2.4.1 Importance of phenolic compounds

Phenolic compounds have received substantial attention for being potentially protective factors against cancer and heart diseases, in part because of their potent antioxidative properties and their ubiquity in a wide range of commonly consumed foods of plant origin. Plant-based foods contain significant amounts of bioactive compounds, which provide desirable health benefits beyond basic nutrition. Epidemiological evidence suggests that consumption of a diet rich in vegetables and fruits has positive implications for human health. In the last decades, special attention has been paid towards edible plants, especially those that are rich in secondary metabolites (frequently called phytochemicals) and nowadays, there is an increasing interest in the antioxidant activity of such phytochemicals present in the diet.

2.4.2 Phenolics contribution to Flavour

Phenolic compounds may contribute to the aroma and taste of numerous food products of animal and plant origin. Singleton and Nobel, (1976) related the presence of chlorogenic acid, other hydroxycinnamates and, in particular, oligomeric proanthocyanidins to the bitterness and astiry of wine and cider. Similarly, Dadic and Belleau, (1973) associated bitterness to the presence of phenolics.

2.4.3 Phenolics as Natural Food Pigments

A large and diversified group of phenolic substances known as flavonoids is responsible for the colour of fruits and vegetables. For example, anthocyanins are responsible for pink, scarlet, red, mauve, blue, and violet colours of vegetables, fruits, fruit juices, and wines (Mazza and Miniati, 1994). Other flavonoids also may contribute to the colour of food products. Some of the groups of yellow or ivory

flavonoid pigments that can be found in plants include flavonols, flavones, chalcones, aurones, flavanones, isoflavanones and biflavonyls (Mazza and Miniati, 1994).

2.4.4 Phenolics contribution to Stress Resistance

In response to stress, plants may use phenolics already present in cells, Phenolics formed after stress from compounds existing in cells as a result of hydrolysis or oxidation or initiate biosynthesis of phenolics that contribute to the healing process. The last group of phenolics that possess antimicrobial properties are phytoalexins (Macheix *et al.*, 1990). The usual response of the plant to stress is an increase in total phenolics content, especially chlorogenic acid (Rhodes and Wooltorton, 1978). Immediately after injury, there is an oxidation of existing phenolics, their subsequent degradation and decrease in phenolics content (Rhodes and Wooltorton, 1978).

2.4.5 Antimicrobial Properties

Many preservatives are added to foods either as antioxidants or as antimicrobial agents. Phenolic compounds are known to possess both of these properties. However, the lipophilic nature of phenols may reduce their antimicrobial properties (Baranowski and Nagel, 1984). Gallic acid, *p*-hydroxybenzoic acid and related phenolics have been found to retard or partially inhibit the growth and toxin production of *Clostridium botulinum* types A and B and the inhibitory activity increases with a decrease in the bacterial concentration (Pierson and Reddy, 1982). Mould growth has been found to be inhibited by naturally occurring ethyl *p*-methoxycinnamate at concentrations of 10 to 50 ppm (Gupta and Banerjee, 1976). *p*-coumaric acid at a 100 ppm level increases the lag phase of *Saccharomyces cerevisiae* and at concentrations > 250 ppm, the inhibition after 72-hour growth is proportional

to its concentration, whereas ferulic acid at 50 ppm brings about an increase in the lag phase and even complete inhibition is achieved at low concentrations of 250 ppm (Baranowski *et al.,* 1980).

2.4.6 Anti-viral Activity

A number of flavonoids present in foods of plant origin possess antiviral activity. For example, tannins from strawberries have the ability to inactivate polio, enteric, and herpes viruses (Konowalchuk and Spiers, 1976). Quercetin, a flavonol aglycone, found in a number of fruits such as apple, apricot, fig, plum, strawberry, and tomato, has shown antiviral activities against herpes simplex virus type 1, Parainfluenza virus type 3, and poliovirus type 1 both in the *in vivo* and *in vitro* studies (Middleton, 1986; Musci, 1986).

2.4.7 Anti-inflammatory Activity

Phenolic compounds extracted from bilberry juice, *Vaccinium myrtillus,* after fermentation constitute the active principal of a drug used for vascular protection. Anthocyanin's present in the extract act on capillary permeability and fragility (Wagner, 1985). In skin inflammation, tumour necrosis factor (TNF) plays an important role as a potent activator of normal human keratinocytes (NHK) and as an inducer of pro-inflammatory mediators including Vascular Endothelial Growth Factor (VEGF) and interleukin-8 (IL-8). Trompezinski *et al.,* (2003) demonstrated that green tea extract and (-) epigallocatechin gallate (EGCG) inhibited in a dose-dependent manner the upregulation of both VEGF and IL-8 in TNFα-stimulated keratinocytes. Their results confirm the anti-inflammatory activity of green tea extract and the role of its major polyphenolic constituent EGCG.

2.4.8 Anti-tumour Activity

Phenolic compounds have been reported to have antitumor activities, especially the flavonoids. The (-) epigallocatechin-3-gallate, a polyphenolic component of green tea, has been found to reduce the incidences of spontaneously and chemically induced tumours in experimental animals, as observed for tumours of liver, stomach, skin, lungs, and oesophagus (Huang *et al.,* 1992). Resveratrol has been shown to inhibit ribonucleotide reductase and certain other cellular events associated with initiation, promotion, and progression of carcinogenesis (Jang *et al.,* 1997). Administration of 25 μM of resveratrol reduced the number of skin tumours in mice by 98% and reduced the number of mice with tumours by 88% (Jang *et al.,* 1997).

2.4.9 Anti-cancer Activity

The vegetables and fruits possess ellagic and chlorogenic acids, which serve as potential chemoprevention against several carcinogens (Huang *et al.,* 1992). Quercetin and rutin have the capability of inhibiting colonic neoplasia induced by azoxymethanol (Deschner, 1992). It has been suggested that regular consumption of tannins may induce the development of a defensive mechanism by animals and human beings to lower the risk of cancer. This may include utilization of very specialized tannin-binding proteins. For example, the mammalian herbivores produced salivary proteins with a high affinity for tannins (Salunkhe *et al.,* 1989). On the other hand, some tannins may exert anti-carcinogenic effects by acting as free radical scavengers.

2.4.10 Reduction of Coronary Heart Disease Risk

In a cohort study conducted in Finland (Knekt *et al.,* 1996) with 5,133 men and women, aged 30 to 69 years, onions and apples, rich sources of dietary flavonoids

Table 2.3. Phytochemical studies in different vegetable crops- a review

S. No	Plant Species	Author (s)	Plant part used	Remarks
1.	*Solanum melongena*	Agoreyo *et al*., (2012)	Fruit (Round)	Phytate 28.19, Oxalate 41.72, Alkaloid 1.16, Tanin 12.82, Saponin 5.34 mg/100g
			Fruit (Oval)	Phytate 18.67, Oxalate 23.97, Alkaloid 0.99, Tanin 11.34, Saponin 11.63 mg/100g
2.	*Solanum macrocarpon*	Ajiboye *et al*., (2014)		Alkaloid (+), Flavonoid (+), Saponin (+), Tanin (+), Inulin (-)
		Chinedu *et al*., (2011)	Fruit	Alkaloids (+++) Cardiac Glycosides (++), Flavonoids (++), Phytosterols (+), Saponins (+++), Steroids (-), Tanins (++), Terpinoids (+)
		Ojo *et al*., (2015)	Fruit	Tanin 6.39 mg/g, Total Phenol 13.07 mg/g, Oxalates 1.8%
		Ilodibia *et al*., (2016)	Leaf (%)	Tanin 1.85, Saponin 2.30, Alkaloid 3.17, Flavonoid 0.95
			Root (%)	Tanin0.77 , Saponin 1.67, Alkaloid 0.92, Flavonoid 0.74
			Fruit (%)	Tanin 0.65, Saponin 1.59, Alkaloid 0.80, Flavonoid 0.67

		Usunomena and Chinwe, (2016)	Leaves	Falvonoid (+), Saponins (+), Alkaloids (+), Tanins (-), Phenols (+), Glycosides (+), Anthraquinones (-)
		Eletta *et al*., (2017)		Alkaloid (+++), Saponins (+++), Flavonoids (+), Tanins (++), Terpenoids (+), Steroids (-), Cardiac Glycosides (+), Anthraquinones (-)
3.	*Talinum triangulare*	Ajiboye *et al*., (2014)		Alkaloid (+), Flavonoid (+), Saponin (+), Tanin (+), Inulin (-)
4.	*Solanum indicum*	Ali, (2012)	Fruit	Alkaloid (+), Saponins (++)
		Nwanna *et al*., (2014)	Fruit (aqueous extract)	Phenols 3.76 mgGAE/g, Flavonoids 1.50 mgQE/g, Gallic acid 39.90 mg/g, Catechin 4.17 mg/g, Chlorogenic acid 10.63, Caffeic Acid 27.21 mg/g, Ellagic Acid ND, Epicatechin 8.54 mg/g, Rutin 21.78 mg/g, Quercitrin ND, Quercetin 9.15 mg/g, Kaempferol ND, Isoquercitrin 23.41 mg/g
5.	*Solanum*	Chinedu *et al*., (2011)	Fruit	Alkaloids (+++) Cardiac Glycosides (++), Flavonoids (++),

	aethiopicum			Phytosterols (+++), Saponins (+++), Steroids (++), Tanins (++), Terpinoids (+)
		Eze and Kanu, (2014)	Fruit (%)	Alkaloid 5, Flavonoid 27, Saponin 14, Tanin 3, Phenol 4.7, Cyanogenic glycosides 5.7
		Eletta *et al*., (2017)	Fruit	Alkaloid (+++), Saponins (+++), Flavonoids (+), Tanins (++), Terpenoids (+), Steroids (+), Cardiac Glycosides (+), Anthraquinones (-)
		Gbadamosi *et al*., (2014)	Leaves	Alkaloids (+), Anthraquinones (+), Carotenoids (+), Flavonoids (+), Saponins (+), Steroids (+), Tanins (+)
		Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Ac-1: Phenol 24.31 mg/100g fw, Flavonoid 5.81 mg/100fw Ac-2: Phenol 22.62 mg/100g fw, Flavonoid 6.11 mg/100fw Ac-3: Phenol 42.46 mg/100g fw, Flavonoid 7.93 mg/100fw
6.	*Nymphaea lotus*	Gbadamosi *et al*., (2014)	Leaves	Alkaloids (+), Anthraquinones (+), Carotenoids (+), Flavonoids (+), Saponins (+), Steroids (+), Tanins (+)

7.	*Solanum surattense*	Mali and Harsh, (2015)	Leaves (mg/100g)	Alkaloids 2.62, Kaempferol 0.67, Quercetin 0.79, Aminoacid 37.18, Ascorbic acid 79.18
			Seeds (mg/100g)	Alkaloids 1.07, Kaempferol 0.72, Quercetin 0.28, Amino acid 25.38, Ascorbic Acid 96.41
8.	*Solanum anguivi*	Oyeyemi *et al*., (2015)	Fruits (%)	Saponins 1.29, Alkaloids 0.05, Flavonoids 0.46, Tannins 0.17, Phenols 1.520, Steroids 1.68, Triterpenoids 0.35
9.	*Nasturtium officinale*	Shad *et al*., (2013)	Aerial part (mg/100g)	Oxalate 362.66, Phytic Acid 13.55, Tanins 59.66, Phenols 70.34
		Khan *et al*., (2016)	Leaves (mg/g)	Total phenols 27.35, Oxalate 60.12, Phytate 20.02
		Aires *et al*., (2013)	Young leaves (70% methanol water extract)	Phenols: 14000mg GAE/kg Dry wt., Flavonoids: 5600mg CAE/kg dry wt., Gallic acid: 16 mg/kg, Chlorogenic Acid 33.0 mg/kg, Caffeic Acid: 2.0 mg/kg, Quercetin: 200, dicaffeoyltartaric Acid: 55 mg/kg, isorhamutin: 193 mg/kg
		Boligon *et al*., (2013)	Leaves	Extract yield 10%, Phenols 104.41 mg GAE/g, Flavonoid

			(methanolic Extract)	71.83 mg RAE/g, Rutin 0.51 mg (1.92%), Caffeic Acid 1.37 mg (5.08%), Chlorogenic Acid 0.33 mg (1.25%)
		Abdul *et al*., (2014)	Leaves	Total Phenol (mg GAE/g extract): ME (121.4), WE (99.2), EAE (83.3), CE (34.5)
10.	*Solanum torvum*	Nithiyantham *et al*., (2012)	Fruit	Raw: Methanol extract 13.9%, Phenols 5.8, Tannins 5.3 g/100g of extract Processed: Methanol extract 10.2, Phenols 5.0, Tanins 4.5 g/100g of extract
		Ramamurthy *et al*., (2012)	Fruit	Protein content (mg/100g): WE (172.3)>ME (147.1)> EE (96.7), Total phenolic content: WE>EE>ME Total Flavonoid: EE>WE>ME Gallic acid (µg/g): WE (1394)>ME (598)> EE(2.98) Caffeic acid (µg/g): EE (0.65)>WE (0.58)>ME (0.54) Rutin (µg/g): ME (18.32)>EE (14.84)>WE (1.53)

				Ferulic acid (µg/g): WE (72.24)>EE (32.49)>ME (16.32) Quercetin (µg/g): WE (7.68)>EE (1.91)>ME (1.59)
		Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Phenol 234.46 mg/100g fw, Flavonoid 19.25 mg/100fw
		Rahman *et al*., (2013)	Fresh Fruit (70% ethanol extract)	Extract yield 4.9%, Total Phenol 358.25 mg GAE/100g
		Nwanna *et al*., (2014)	Fruit (aqueous extract)	Phenols 2.70 mgGAE/g, Flavonoids 1.60 mgQE/g, Gallic acid 50.34 mg/g, Catechin 12.67 mg/g, Chlorogenic acid 20.71, Caffeic Acid 39.46 mg/g, Ellagic Acid 25.13 mg/g, Epicatechin 14.53 mg/g, Rutin 22.18 mg/g, Quercitrin ND, Quercetin 14.92 mg/g, Kaempferol ND, Isoquercitrin 28.36 mg/g

		Khatoon *et al*., (2015)	Leaves and seed	Presence of alkaloid, flavonoid, saponins, steroids, tanins, and phenols. GCMS analysis confirmed presence of Quinic acid, Linoleic acid, Palmitic acid, Isopseudocumenol and Phytol which are anti-microbial, anti-cancer, diuretic , anti-inflammatory, anti-influenza
		Lalmuanthanga, *et al*., (2015)	Dry Leaves	Phenol: 5.34mf GAE/g of dry leaves
		Abdulkadir *et al*., (2016)		Fruit: phenol 16.15 mg GAE/g, Flavonoid 1.41 mg QAE/g Stem: Phenol 43.92 mf GAE/g, Flavonoid 16.21 mg QAE/g Leaf: Phenol 37.48 mg GAE/g, Flavonoid 40.6 mg QAE/g
11.	*S. xanthocarpum*	Nithiyantham *et al*., (2012)	Fruit	Raw: methanol extract 5.7%, Phenols 7.6, Tannins 7.0 g/100g of extract Processed: methanol extract 9.3%, Phenols 6.1, Tannins 5.6 g/100g of extract

12.	*S.violaceum*	Nithiyantham *et al*., (2012)	Fruit	Raw: methanol extract 2.9%, Phenols 6.6, Tannins 6.2 g/100g of extract Processed: methanol extract 26.7, Phenols 5.0, Tannins 4.7 g/100g of extract
13.	*S. incanum*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Phenols 3.76 mgGAE/g, Flavonoids 1.50 mgQE/g, Gallic acid 6.28 mg/g, Catechin 6.13 mg/g, Chlorogenic acid 18.65, Caffeic Acid 23.79 mg/g, Ellagic Acid 13.26 mg/g, Epicatechin 2.51 mg/g, Rutin 12.38 mg/g, Quercitrin 6.45 mg/g, Quercetin 27.05 mg/g, Kaempferol 12.68 mg/g, Isoquercitrin ND
		Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Phenol 178.33 mg/100g fw, Flavonoid 25.96 mg/100fw
14.	*S. kumba*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Phenols 3.44 mgGAE/g, Flavonoids 1.22 mgQE/g, Gallic acid 17.36 mg/g, Catechin 4.25 mg/g, Chlorogenic acid 30.81,

				Caffeic Acid 19.76 mg/g, Ellagic Acid 25.13 mg/g, Epicatechin 6.32 mg/g, Rutin 16.89 mg/g, Quercitrin 12.45 mg/g, Quercetin 26.47 mg/g, Kaempferol 7.08 mg/g, Isoquercitrin ND
15.	*S. gilo*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Phenols 2.83 mgGAE/g, Flavonoids 1.22 mgQE/g, Gallic acid 2.73 mg/g, Catechin 5.39 mg/g, Chlorogenic acid 20.67, Caffeic Acid 2.15 mg/g, Ellagic Acid 2.93 mg/g, Epicatechin 3.12 mg/g, Rutin 4.98 mg/g, Quercitrin 6.17 mg/g, Quercetin 12.58 mg/g, Kaempferol 10.92 mg/g, Isoquercitrin ND
16.	*S. sysmbrifolium*	Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Phenol 83.57 mg/100g fw, Flavonoid 7.47 mg/100fw
17.	*S. khasianum*	Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Phenol 206.37 mg/100g fw, Flavonoid 15.97 mg/100fw
18.	*S. integrifolium*	Kaur *et al*., (2014)	Fruit (80%	Phenol 36.63 mg/100g fw, Flavonoid 8.25 mg/100fw

			ethanol extract)	
19.	*Capsicum annum*	Acunha *et al*., (2017)	Fruit	Total Phenol 175.52 mgGAE/100g, Carotenoid 28.47 mg/100g
20.	*Capsicum baccatum*	Acunha *et al*., (2017)	Fruit	Total Phenol 110.67 mgGAE/100g, Carotenoid 34.97 mg/100g
21.	*Capsicum chinense*	Acunha *et al*., (2017)	Fruit	Total Phenol 144.39 mgGAE/100g, Carotenoid 23.21 mg/100g

were associated with a reduction in coronary heart diseases mortality. Individuals in the highest quartile for apple intake had an approximately 50% reduction in coronary mortality. Likewise, a similar reduction was reported for individuals in the highest quartile of onion consumption. In a prospective study of 34,492 post-menopausal women in Iowa (Yochum *et al.,* 1999), total flavonoid intake was associated with a decreased risk in the group with the highest flavonoid intake. Thus, much of the epidemiologic evidence suggests that flavonoids have a protective effect against coronary mortality. For those studies that have reported an association, putative mechanisms of action include inhibition of low-density lipoprotein (LDL) oxidation and inhibition of platelet aggregation and adhesion (Frankel *et al.,* 1993).

Keeping in view the above-discussed importance of phenols as a health benefit, a considerable amount of research work has done for identification, presence and quantification of various phenols in vegetables. Some of the studies related to phytochemicals screening and phenols identification and quantification has been listed in Table 2.3.

2.5 Antioxidants

An antioxidant is defined as a substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance (Halliwell and Guteridge, 1989). For the *in vivo* situation, the concept of antioxidants includes antioxidant enzymes, iron binding and transport proteins and other compounds affecting signal transduction and gene expression (Guteridge, 1989).

2.5.1 Classes of antioxidants

Antioxidants are divided into two major classes, namely endogenous antioxidants and exogenous antioxidants.

2.5.1 Endogenous antioxidants

Three groups of enzymes play important roles in protecting cells from oxidative stress (Becker *et al.,* 2004). Firstly, superoxide dismutases (SOD) are enzymes that catalyse the conversion of two superoxides to hydrogen peroxide and oxygen. Hydrogen peroxide is substantially less toxic than superoxide. The detoxifying reaction catalysed by SOD is ten thousand times faster than the uncatalyzed reaction (Becker *et al.,* 2004). SODs are metal-containing enzymes that depend on bound manganese, copper or zinc ion for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in the cytoplasm. SODs are inducible enzymes, with exposure to bacteria or vertebrate cells to higher concentrations of oxygen resulting in rapid increases in the concentration of SOD.

Secondly, catalase, found in peroxisomes in eukaryotic cells, degrades hydrogen peroxide to water and oxygen and hence completes the detoxification reaction started by SOD. Finally, glutathione peroxidase, a group of enzymes which are the most abundant contain selenium and like catalase, degrade hydrogen peroxide. Glutathione is the most important intracellular defence against damage by reactive oxygen species. The cysteine on the glutathione molecule provides an exposed free sulfhydryl group that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione but the reduced form is regenerated in a redox cycle that involves glutathione reductase and the electron acceptor NADPH

(Baydar *et al.,* 2007). In addition to the three enzymes above, glutathione transferase, ceruloplasmin, hemoxygenase may participate in the enzymatic control of oxygen radicals and their products.

2.5.2 Exogenous antioxidants

The three common exogenous antioxidants are vitamin E, vitamin C/ ascorbic acid and glutathione. Vitamin E is the major lipid-soluble antioxidant and plays an important role in protecting membranes from oxidative damage. The primary activity of vitamin E is to trap peroxy radicals in cellular membranes and consequently prevent lipid peroxidation of the membranes (Baydar *et al.,* 2007). Vitamin C is a water-soluble antioxidant that can reduce radicals from a variety of sources. Vitamin C participates in recycling vitamin E radicals. Vitamin C also functions as a pro-oxidant under certain circumstances and sometimes produces oxygen by-products of metabolism that can cause damage to cells (Coinu *et al.,* 2007). In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of some plant extracts have been attributed partially to their phenolic compound contents (Coinu *et al.,* 2007).

In addition to carotenoids, tocopherols, and ascorbic acid, most of the antioxidative effect related to plant food intake is mainly due to the presence of phenolic compounds, which have been associated with flavour and colour characteristics of fruits and vegetables. Among phytochemicals possessing the antioxidant capacity, phenolic compounds are one of the most important groups. It is well known that vegetables are rich in various antioxidants, including ascorbic acid, carotenoids, and phenolic and can be considered as a source of natural antioxidant. Many plants including vegetables had been categorising as sources of natural

Table 2.4. Antioxidant activities in different vegetable crops- a review

S. No.	Crops	Author (s)	Plant part used	Remarks
1.	*Solanum macrocarpon*	Eletta *et al*., (2017)	Fruit (DPPH%)	70.33 (500µg/ml)
		Usunomena and Chinwe, (2016)	Leaves	76.54% (DPPH 1mg/ml extract conc.), Reducing Power
2.	*Solanum aethiopicum*	Eletta *et al*., (2017)	Fruit (DPPH%)	69.1 (500µg/ml)
		Kaur *et al*., (2014)	Fruit (80% ethanol extract)	Ac-1 (µmol Trolox/g fw): CUPRAC 2.22, FRAP 1.13, DPPH 2.07, ABTS 2.96 Ac-2 (µmol Trolox/g fw): CUPRAC 6.45, FRAP 1.35, DPPH 2.45, ABTS 2.8 Ac-3 (µmol Trolox/g fw): CUPRAC 7.35, FRAP 1.7, DPPH 2.50, ABTS 2.70
3.	*Nasturtium officinale*	Shad *et* al., (2013)	Aerial part (DPPH assay)	37.96% (100mg/ml)

		Khan *et al.*, (2016)	Leaves	DPPH assay, Reducing Power
		Usman *et al*., (2012)	Root ethanolic extract	DPPH(0.1mg/ml):37.05%
		Aires *et al*., (2013)	Young leaves (70% methanol water extract)	For 1mg/ml: DPPH: 81.4%, FRAP 14.3µM equivalent $FeSO_4$, Reducing Power 0.473Abs (at 700nm)
		Boligon *et al*., (2013)	Leaves (Methanolic Extract)	DPPH IC50: 30.76 µg/ml
		Abdul *et al*., (2014)	Leaves	DPPH IC50 (µg/ml): ME (35.2), WE (55.5), EAE (67.3), CE (160.4) FRAP (µmol Fe^{2+}/g extract): ME (1823.3), WE (1534.5), EAE (1245.2), CE (544.5) ABTS (µmol trolox/g extract): ME (646.3), WE (604), EAE (518.4), CE (509.6)

4.	*Solanum torvum*	Loganayaki *et al*., (2010)	Leaves and Fruits	FRAP, DPPH, ABTS, Fe2+ Chelating activity and Haemolytic activity using various solvents. Extract yield obtained in order Chlroform> Acetone> Methanol. Found more antioxidant activity in *S. torvum* than *S. nigrum* and also found good antihaemolytic activity
		Nithiyantham *et al*., (2012)	Fruit	Raw: MCA 11.9 mg EDTA/g extract, PMA 56.4 µmol ascorbic acid/g extract, FRAP 7.2 µg extract/mmol, FRSA: 7.6 g extract/g DPPH, 226.1 µmol/g extract TAA, Peroxidation Inhibition 98%, HRSA 23% Processed: 29.2 mg EDTA/g extract, PMA 14.6 µmol ascorbic acid/g extract, FRAP 28.9 µg extract/mmol, FRSA: 1.9 g extract/g DPPH, 80.3 µmol/g extract TAA, Peroxidation Inhibition 98%, HRSA 52%

		Ramamurthy *et al*., (2012)	Fruit	DPPH: WE (60.48%)>ME (55.04)>EE (51.92%) β-carotene bleaching: WE (58.29%)>EE (54.64%)>ME (54.47%) Oxidative haemolysis: WE (68.95%)> EE (68.46%)>ME (56.63%) HRSA: WE (76.77%)> EE (68.46%)> ME (57.04%) Reducing Power: ME (65.04%)>EE (59.92%)>WE (55.48)
		Rahman *et al*., (2013)	Fresh Fruit (70% ethanol extract)	DPPH antioxidant activity 303 mg AEAC/100g
		Nwanna *et al*., (2014)	Fruit (aqueous extract)	Reducing Sugar 5.90 mg/g, EC50 of DPPH 4.18 mg/ml, Vit C 4.01 mg/ml
		Kaur *et al*., (2014)	Fruit (80% ethanol extract) (μmol Trolox/g fw)	CUPRAC 20.25, FRAP 8.04, DPPH 8.24, ABTS 12.94

		Lalmuanthanga, *et al*., (2015)	Dry Leaves	DPPH: 99.71mg TE/g, FRAP: 42.47mg TE/g
		Pradhan *et al*., (2015)	Leaves	DPPH: 58.40% (0.1mg/ml)
		Abdulkadir *et al*., (2016)		Fruit: DPPH 33%, FRAP 112 mM Fe(II)/g Stem: DPPH 56.3%, FRAP 540 mM Fe(II)/g Leaf: DPPH 78.7%, FRAP 438 mM Fe(II)/g
5.	*Solanum nigrum*	Loganayaki *et al*., (2010)	Leaves and Fruits	FRAP, DPPH, ABTS, Fe2+ Chelating activity and Haemolytic activity using various solvents. Extract yield obtained in order Chlroform> Acetone> Methanol. Found more antioxidant activity in *S. torvum* than *S. nigrum* and also found good antihaemolytic activity
6.	*S. xanthocarpum*	Nithiyantham *et al*., (2012)	Fruit	Raw: MCA 29.0 mg EDTA/g extract, PMA 12.4 µmol ascorbic acid/g extract, FRAP 7.0 µg extract/mmol, FRSA: 2.1 g extract/g DPPH, 144.4 µmol/g extract TAA,

				Peroxidation Inhibition 99%, HRSA 46% Processed: 24.1 mg EDTA/g extract, PMA 14.9 µmol ascorbic acid/g extract, FRAP 28.5 µg extract/mmol, FRSA: 2.5 g extract/g DPPH, 236.1 µmol/g extract TAA, Peroxidation Inhibition 98%, HRSA 23%
7.	*S. violaceum*	Nithiyantham *et al*., (2012)	Fruit	Raw: MCA 6.5 mg EDTA/g extract, PMA 9.1 µmol ascorbic acid/g extract, FRAP 39.0 µg extract/mmol, FRSA: 2.0 g extract/g DPPH, 111.9 µmol/g extract TAA, Peroxidation Inhibition 99%, HRSA 28% Processed: 6.1 mg EDTA/g extract, PMA 64.7 µmol ascorbic acid/g extract, FRAP 29.5 µg extract/mmol, FRSA: 2.7 g extract/g DPPH, 236.1 µmol/g extract TAA, Peroxidation Inhibition 98%, HRSA 17%
8.	*Urtica dioica*	Usman *et al*., (2012)	Root ethanolic extract	DPPH(0.1mg/ml):37.48%

	(Sishnu)	Pradhan *et al*., (2015)	Leaves	DPPH: 53.19% (0.1mg/ml)
9.	*S. incanum*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Reducing Sugar 6.89 mg/g, EC50 of DPPH 3.23 mg/ml, Vit C 5.82 mg/ml
		Kaur *et al*., (2014)	Fruit (80% ethanol extract) (µmol Trolox/g fw)	CUPRAC 11.98, FRAP 5.36, DPPH 5.97, ABTS 5.9
10.	*S. kumba*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Reducing Sugar 5.79 mg/g, EC50 of DPPH 3.80 mg/ml, Vit C 5.82 mg/ml
11.	*S. gilo*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Reducing Sugar 5.72 mg/g, EC50 of DPPH 4.20 mg/ml, Vit C 5.55 mg/ml
12.	*S.indicum*	Nwanna *et al*., (2014)	Fruit (aqueous extract)	Reducing Sugar 5.98 mg/g, EC50 of DPPH 3.62 mg/ml, Vit C 6.52 mg/ml
13.	*S. sysmbrifolium*	Kaur *et al*., (2014)	Fruit (80% ethanol extract) (µmol	CUPRAC 10.21, FRAP 5.4, DPPH 5.95, ABTS 5.75

			Trolox/g fw)	
14.	*S. khasianum*	Kaur *et al*., (2014)	Fruit (80% ethanol extract) (µmol Trolox/g fw)	CUPRAC 15.86, FRAP 8.11, DPPH 6.63, ABTS 15.73
15.	*S. integrifolium*	Kaur *et al*., (2014)	Fruit (80% ethanol extract) (µmol Trolox/g fw)	CUPRAC 2.83, FRAP 1.88, DPPH 1.54, ABTS 6.55
16.	*S. anguivi*	Daramola, (2015)	Fruit, Seed, Pericarp	Ethanol extract: Total phenolic content 75-196.88, Relative reducing power 165-889.29, RSA 64.0-85.0 Diethyl ether extract: Total phenolic content 2.81-45.00, Relative reducing power 18.21-25.71, RSA 14.10-40.0
17.	*Capsicum annum*	Acunha *et al*., (2017)	Fruit	DPPH Antioxidant potential 1.63 mmol TE/100g
18.	*Capsicum baccatum*	Acunha *et al*., (2017)	Fruit	DPPH Antioxidant potential 1.14 mmol TE/100g

19.	*Capsicum chinense*	Acunha *et al*., (2017)	Fruit	DPPH Antioxidant potential 1.53 mmol TE/100g

Abbreviation: ME: Methanolic extract, WE: Water extract, WE: Water extract, AE: Acetone extract, CE: Chloroform Extract, DPPH: 2,2-Diphenyl-1-picrylhydrazyl Assay, FRAP: Ferric reducing antioxidant power assay, PMA: Phosphomolybdenum Assay, MCA: Metal chelating Activity, FRSA: Free radical Scavenging activities, HRSA: Hydroxyl Radical Scavenging Activity, TAA: Total Antioxidant activity, CUPRAC: Cupric ion antioxidant reducing capacity

antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species (Dragland *et al.,* 2003; Odukoya *et al.,* 2001; Atawodi, 2005). Regular consumption of fruit and vegetables is associated with reduced risks of cancer, cardiovascular disease, stroke, Alzheimer disease, cataracts and some of the functional declines associated with ageing. Prevention is a more effective strategy than treatment of chronic diseases. In the search of sources of novel antioxidants and other important nutrients, a large number of plants have been extensively studied during last few years, some of which were tried to enlist in Table 2.4.

2.6 VITAMINS

Vitamins are a diverse group of organic compounds essential in trace amounts for the normal growth and maintenance of life. To ensure the adequate intake of vitamins the human diet can be completed with a high range of multivitamin tablets and food products enriched with vitamins, in other words, these compounds are usually administered as a nutraceutical or functional ingredient.

They are classified as either water-soluble or fat soluble. In humans, there are 13 vitamins: 4 fat-soluble (A, D, E and K) and 9 water-soluble (8 Vitamins B and vitamin C). These compounds have diverse biochemical roles. Some have hormone-like functions as regulators of mineral metabolism (e.g. Vitamin D), or regulators of cell and tissue growth and differentiation (e.g. some forms of vitamin A). Others work as antioxidants (e.g. vitamin E and sometimes vitamins B and C). The largest numbers of vitamins (e.g. B-complex vitamins) work as precursors of enzyme cofactors. The detection of vitamins is done by HPLC with several detectors as UV/Vis, VWD, PDA, FLD, MS although GC-FID (Bakowska-Barczak *et al.*, 2009; Verado *et al*., 2009) and HPTLC (Aranda and Morlock, 2006).

Vitamin C (L-ascorbic acid or L-ascorbate) is an essential nutrient for humans and other animal species. Ascorbic acid (vitamin C), a powerful, water-soluble antioxidant as a scavenger of ROS (Smirnoff, 2000). Ascorbic acid plays an important role in minimizing the damage caused by the oxidative process. This is performed by its synergistic action with other antioxidants (Smirnoff, 2005; Athar *et al*., 2008). Deficiency of this vitamin causes the disease known as scurvy in humans. This compound is also widely used as a food additive because of its antioxidant activity.

Compared to the other vitamins, the number of works about Vitamin E is by far the highest one. Vitamin E is a generic term for tocopherols and tocotrienols, and it is a fat-soluble antioxidant that blocks the production of reactive oxygen species formed during oxidation of lipids, exclusively located in the plastid or thylakoid membranes (Munne-Bosch, 2005), where they have both antioxidant and non-antioxidant functions (Kagan, 1989). They occur in four different types or isomers, namely, α, β, γ and δ-tocopherols. All four types of tocopherols structurally consist of a chromanol head group attached to the phytyl tail, which together giving vitamin E compounds amphipathic character (Kamal-Eldin and Appelqvist, 1996). Tocopherols are also known to protect lipids and other membrane components by physically quenching and chemically reacting with oxygen in chloroplasts, thus, protecting the structure and function of PSII (Igamberdiev and Hill, 2004). The main function of tocopherol lies in its fatty acyl chain-breaking activity, which scavenges Reactive Oxygen Species (ROS) resulting from photosynthesis, thus protecting polyunsaturated fatty acid chains (PUFAs) from lipid peroxidation. Increasing evidence suggests that in higher plants, vitamin E may play a protective role in cell membrane systems, thus maintaining the integrity and normal function of the photosynthetic apparatus (Havaux *et al*., 2005; Collin *et al*., 2008). The most frequently employed analytical tool for determining vitamin E has also been HPLC coupled to all possible types of detectors as FLD, UV, PDA, VWD, MS. However, it has to be noted that the main natural source of vitamin E is vegetable and vegetable oils, being HPLC the analytical tool more usually employed.

Vitamin K is a group of structurally similar, fat-soluble vitamins that the human body needs for posttranslational modification of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissue. They are 2-

methyl-1,4-naphthoquinone (3-) derivatives (Stafford, 2005). This group of vitamins includes two natural vitamers: vitamin K1 and vitamin K2. Vitamin K1, also known as phylloquinone, phytomenadione, or phytonadione, is synthesized by plants and is found in highest amounts in green leafy vegetables because it is directly involved in photosynthesis (Newman *et al*., 2008). It may be thought of as the "plant form" of vitamin K. It is active in animals and may perform the classic functions of vitamin K in animals, including its activity in the production of blood clotting proteins. Animals may also convert it to vitamin K2. Vitamin K2, the main storage form in animals, has several subtypes, which differ in isoprenoid chain length. These vitamin K2 homologs are called menaquinones and are characterized by the number of isoprenoid residues in their side chains. Vitamin K1 is found chiefly in leafy green vegetables such as dandelion greens, spinach, swiss chard and Brassica (e.g., cabbage, kale, cauliflower, broccoli and brussels sprouts) and often the absorption is greater when accompanied by fats such as butter or oils. Some vegetable oils, notably soybean, contain vitamin K, but at levels that would require relatively large calorific consumption to meet the USDA recommended levels (Weber, 2001). In the search of vitamin content in some of plants, a few studies have been carried out during last few years, some of which were tried to enlist in Table 2.5.

A new HPLC-DAD method was developed for the simultaneous detection and quantification of water- and fat-soluble vitamins in different beverages from different natural sources (orange, strawberry, apple, peach pineapple, plum and blackcurrant juices, soybean milk and beers) (Borochov *et al.*, 2008) with the additional advantage that it was not required for any previous sample preparation prior to their analysis. This fact was attributed to the use of an end-capped column, which possesses an ultralow silanol activity.

Table 2.5. Vitamins studies in different vegetable crops- a review

S.No	Plant Species	Authors (s)	Plant Part Used	Remarks
1.	*Solanum torvum*	Akoto *et al*., (2015)	Fruits	Vit A 0.078 Vit C 2.686 mg/100g
		Nyadanu and Lowor, (2015)	Fruit	Vit A 4172 IU Vit C 112.60 mg/100g Vit E 2.41 mg/100g
		Otu *et al.,* (2017)	Fruit	0.0950 mg/100ml, 0.0109 mg/100g
2.	*Solanum macrocarpon*	Chinedu *et al*., (2011)	Fruit	Vit C (++)
		Dougnon *et al*., (2012)	Leaves (mg/kg)	Vit A 3530, Vit K1 55
			Fruit (mg/kg)	Vit A 3680, Vit K1 1320
		Nyadanu and	Leaves	Vit A 2911 IU, Vit C 9.85 mg/100g, Vit E 3.47 mg/100g

		Lowor, (2015)	Fruit	Vit A 3619 IU, Vit C 124.55 mg/100g, Vit E 1.43 mg/100g
		Offor *et al*., (2015)	Fruits (mg/100g)	Retinol 39, Niacin 0.7, Ascorbic acid 2.400, Tocopherol 0.28, Thiamine 0.033, Riboflavin 0.032, Calciferol 0.001
3.	*Solanum aethiopicum*	Chinedu *et al*., (2011)	Fruit	Vit C (+++)
		Eze and Kanu, (2014)	Fruit	Vit B1 0.45 µg/g, Vit B2 10.33 µg/g , Vit B3 14.34 µg/g , Ascorbic Acid 406 mg/g, Vit E 0.53 µg/g
		Nyadanu and Lowor, (2015)	Fruit	Vit A 4518 IU, Vit C 152.34 mg/100g, Vit E 3.56 mg/100g
		Offor *et al*., (2015)	Fruit (mg/100g)	Retinol 53.55, Calciferol 0.10, Tocopherol 0.310, Thiamine 0.037, Riboflavin 0.034, Niacin 0.7, Ascorbic acid 2.2

4.	*Nasturtium officinale*	Shad *et* al., (2013)	Aerial part	β-Carotene 209.60 mg/100g, Vit C 51.85 mg/100g

Table 2.6. Pharmaceutical application of some related vegetable crops

S. No	*Plant Species*	Author (s)	Part Used	Remarks
1.	*Solanum torvum*	Nithiyantham *et al*., (2012)	Fruit	Antihaemolytic activity: Raw 35%, Processed 36%, Good Antimicrobial activity against Gram –ve bacteria
2.	*S. xanthocarpum*	Nithiyantham *et al*., (2012)	Fruit	Antihaemolytic activity: Raw 38%, Processed 28%, Good Antimicrobial activity against Gram –ve bacteria
3.	*S. violaceum*	Nithiyantham *et al*., (2012)	Fruit	Antihaemolytic activity: Raw 63%, Processed 63%, Good Antimicrobial activity against Gram –ve bacteria 9832543513
4.	*Nasturtium officinale*	Abdul *et al*., (2014)	Leaves	Methanol extract showed higher antimicrobial activity against all bacteria and fungi and chloroform extract shows no inhibitions

Chapter-3
Materials and Methods

The present investigation entitled "Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties" was carried out during 2015-2018 at Department of Horticulture, Sikkim University, Gangtok, Sikkim. The details of materials used and methods employed during the present investigation are described below:

3.1 Material:

The experiment was conducted using five select vegetables *viz. Solanum aethiopicum*, *Solanum macrocarpon, Capsicum annuum* var. *cerasiformae* (Dalle Khorsani), *Tupistra aurantiaca* Wall. (Nakima), *Nasturtium officinale* (Watercress).

Table 3.1. List of plants considered for the study

S. No.	Common Name	Local Name (Nepali vernacular)
1	Ethiopian eggplant	Sayano Bihi
2	African Eggplant	Thulo Bihi
3	Cherry Pepper	Dalle Khorsani
4	Nakima	Nakimo
5	Watercress	Simrayo

3.1.1 Survey of ethnic community for medicinal properties of vegetables

The survey was conducted to appraise the knowledge of local inhabitants about the therapeutic or medicinal uses of plants that are eaten as vegetables. The structured questionnaire was prepared for the survey. Besides, focus group discussion and personal interview was also employed to get the required information. Sikkim has four district and all the districts were considered for survey. In each district five villages were selected purposively based on the secondary information. Samples for analysis of different parameters were also collected from the surveyed village in the season of their peak availability.

3.1.2 Collection of Plant Material:

The collection of samples were carried out at different growth stage depending upon the vegetables. The fruit samples of *Solanum aethiopicum* and *Solanum macrocarpon* were collected during their fruiting season in between June September. Cherry pepper fruits were collected during the month of May- November. *Tupistra aurantiaca* Wall. was collected during its flowering time (August- October), since its economic parts is inflorescence . *Nasturtium officinale* being leafy vegetable was collected during October to May. These fruits/flowers/leaf samples were collected from four districts of Sikkim namely, East Sikkim, West Sikkim, North Sikkim and South Sikkim. The local floristic keys were used for determining the species. Approximately, 3 kg material of selected vegetable was collected. The collected material was placed in a polythene bag to prevent loss of moisture during transportation to the laboratory.

3.1.2 Sample Preparation:

The collected healthy and fresh fruits/flowers/leaves were thoroughly washed till no trace of extraneous material. They were blotted to remove the adsorbed moisture absorbed followed by air dried and weight was recorded as considered the same as fresh weight in gram. Some quantities of samples were stored at -80^{0}c for proximate analysis.

The sample to be used for mineral analysis was washed using double deionised water. Then they were cut into small pieces, the seeds were removed and placed in paper envelope and dried in the oven at 60^{0}C until constant weight was obtained. After complete drying the sample was grinded to a fine powder by using an electric grinder. The sample was packed into airtight sample bottles and used for the nutrient analysis. All analyses were conducted in triplicate by using analytical grade reagents.

3.2 Nutraceutical Analysis:

3.2.1. Proximate Analysis:

3.2.1.1 Dry matter and Moisture:

The dry matter of the sample represents the amount of material left after the complete removal of moisture from it. The moisture of the sample was lost by volatilization caused by heat. Dry matter and moisture content was determine by gravimetric method. A 2.0 g sample were placed evenly on pre-dried and cleaned sample pan. Select standard drying at 130^{0}C and close the sample chamber and press start button. After some time it will give beep sound and moisture analysis was done. The dry matter and moisture were calculated by using the following formulae:-

$$Dry\ matter\ (\%) = \frac{(Weight\ of\ dish + Weight\ of\ dried\ sample) - Weight\ of\ Dish}{weight\ of\ sample\ before\ drying} \times 100$$

$$Moisture\ content\ (\%) = \frac{Weight\ of\ fresh\ sample - weight\ of\ dry\ sample}{weight\ of\ fresh\ sample} \times 100$$

3.2.1.2. Determination of total Ash content:

Ash value was determined by following the method of AOAC (1990). For this crucible were kept in a muffle furnace at 600^0C for 1 hour. Then crucible were transferred from furnace to a desiccator and cooled to room temperature and weighed as quickly as possible to prevent moisture absorption. Two gram dry fruit powder was taken in weighed silica crucible and placed in a muffle furnace at 600^0C for 6 hours. Then crucible was transferred to a desiccator and cooled to room temperature, crucible was transferred as quickly as possible to avoid moisture absorption. The percentage of ash was calculated by using the following formula:-

$$Ash\ (\%) = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

3.2.1.3. Total soluble solids:

Total soluble solids was determined with the help of hand refractometer and represented as °Brix at 20°C.

3.2.1.4. Determination of Crude fat

The Crude fat content was determined by following the method of Sadasivam and Manikam (1992) with the help of essential oil extractor model no Socsplus-SCS 06 DLS, PELICAN. Two gram dried sample was taken in a thimble (prepared from Whatman No. 41 filter paper). The initial weight of the beaker (W1) was taken. Thimble

was fixed to the thimble holder and the thimble was placed into the beakers. 70 ml of Solvent (methyl alcohol) was taken and poured into the fat collection beakers. The beaker with solvent was inserted into the extraction system and water tap was opened for condenser. Essential oil extractor machine was operated at a temperature of 100^0C for 45 minutes. The stopper was kept in the extractor in closed position for no condensation of solvent. After completion of boiling, temperature was increased to 160^0C for 80 min to evaporate the solvent. The stopper was opened slowly when the level touches below the thimble open and the recovered solvent was allowed to flow drop by drop through the thimble and stopper was closed. Beaker was taken from extraction system and the thimble was taken out from the beakers. The beaker was kept inside hot air oven for few minutes to remove solvent vapours. Final weight of the beakers (W2) was taken to find out crude fat percentage of the sample which was calculated by the following formula:

$$Crude\ fat\ content\ (\%) = \frac{(W2 - W1)}{weight\ of\ sample} \times 100$$

3.2.1.5. Total Protein:

The sample was subjected to Lowry's method (1951) to obtain its total protein content. 1 g of sample were macerated in mortar and pestle with 5 ml of phosphate buffer and transferred to centrifuge tubes. The materials were then centrifuged at 8000 rpm for 20 min. The supernatants were collected and the procedure was repeated 4-5 times. The supernatants were mixed and volume was made up to 50 ml with phosphate buffer. 1 ml of 20% trichloro Acetic Acid (TCA) was added to 1 ml of the extract and the mixture was incubated for 30 min. The mixture was then centrifuged at 8000 rpm for 20 min. The resultant pellets were washed twice with acetone and again centrifuged.

The supernatant was then discarded. The pellet was mixed well in 5 ml of 0.1N NaOH till it had dissolved. 1 ml of the aliquot was taken in which 5 ml of freshly prepared alkaline copper sulphate reagent were added and mixed properly. After 10 min, 0.5 ml of Folin's reagent was added and mixed instantaneously and allowed to develop colour for 30 min. Absorbance at 660 nm were recorded after setting the instrument with reagent blank which contained 1 ml of 0.1 N NaOH instead of the sample aliquot.

In another set of tubes, suitable aliquot of BSA solution (in the range of 0-100µl) were taken and volume made up to 1 ml with 0.1 N NaOH and allowed to develop colour as described above. A standard curve of absorbance at 660 nm versus µg of BSA was drawn and from this standard curve, the amount of protein in the sample tube was determined as protein per gram of the sample.

3.2.1.6. Crude fibre

Crude fibre content was determined by following the method of Sadasivam and Manikam (1992) using fibre estimation system, Model no Fibra plus- FES 04 AS DLS, PELICAN. Crude fibre is the organic residue which remains after the food samples have been under standardized conditions with petroleum spirit, boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution and use of alcohol is used. The crude fibre consists largely of cellulose together with little lignin. As the recovery of cellulose using the specified procedure seldom exceeds four-fifths of that actually present, the crude fibre content does not represent a measure of a specific group of substances. Also, as the figure obtained tends to vary with the conditions employed, it is important to adopt a standardized procedure in order to obtain consistent result.

The crude fibre was extracted through Fibra plus extractor equipment. Two gram fat free samples (w) were taken weighed and the samples were transferred to the crucible. Crucible was inserted into the extraction system and tap water was opened to the condenser and 100-150ml of 1.25% of H_2SO4 was poured from the top of the extractor funnels. The power was switched on and the required temperature was set for boiling of reagent and the sample should be boiled at 500ºC for 30min and after boiling the individual value was kept in open position. Once the boiling was completed, the reagent was drained through the crucible with the help of fibre flow and the same procedure was repeated by using alkali (100-150ml) of 1.25% NaOH at 400^0C for 45min. The alkali digestion was completed after removal of the crucible from the main unit and the crucible was kept inside the hot air oven for few minutes to remove the moisture at 90^0-100^0C. The samples were cooled and the initial weight (W1) of the crucible was taken. Crucible was taken inside the muffle furnace at 500^0C for 3hrs for ashing and the crucible was taken from the muffle furnace and cooled for some time. Final weight (W2) of the crucible was taken.

Crude fibre content of the sample was calculated out by using the following formula:

$$Crude\ fibre\ content(\%) = \frac{loss\ in\ weight\ on\ ignition\ (W1-W2)}{Weight\ of\ sample\ (W)} \times 100$$

3.2.1.7. Carbohydrates

Total carbohydrate was determined by using Anthrone reagent. 100 mg of the representative sample hydrolyzed for 3 hours with 5ml of 2.5 N HCl and cooled down to normal temperature. The acid was neutralized using sodium carbonate and volume made upto 100ml. A suitable aliquot of the sample was taken and 4ml of Anthrone

reagent added. Heat for 8 minutes in a water bath and cool rapidly. Total carbohydrate content was determined by taking absorbance at 630nm (Perkin Elmer, Lamb 35 UV/VIS spectrophotometer) and calculated in percentage according to standard absorbance.

3.2.1.8. Total Starch Determination

The total starch content was determined using anthrone reagent. 500 mg of the representative sample was homogenized in hot 80% ethanol to remove sugars until it doesn't give colour with anthrone reagent. The residue was mixed with 5 ml water and 6.5 ml perchloric acid and centrifuged. A suitable volume of supernatant was taken and 4ml of anthrone reagent was added. The mixture was heated for 8 minutes in a water bath and absorbance was recorded at 630 nm (Perkin Elmer, Lamb 35 UV/VIS spectrophotometer) after cool down. The total starch content was calculated according to a standard curve in percentage.

3.2.1.9. Total Sugar

Total sugar was determined by Lane and Eynon method using Fehling solution after hydrolizing non-reducing sugars with dilute acids to reducing sugar.

3.2.1.10. Chlorophyll content

Chlorophyll A, B and total chlorophyll was determined by measuring OD of actone extract of samples at 663nm and 645 nm and calculated according to Arnon (1949). One gram of fruit was taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 –10000rpm for 5mins. The supernatant was transferred and

the procedure was repeated till the residue becomes colourless. The absorbance of the solution was red at 645nm and 663nm against the solvent (acetone) blank.

Estimation of Chlorophyll content:

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total Chlorophyll: 20.2 (A645) + 8.02 (A663)

Chlorophyll A: 12.7 (A663) – 2.69 (A645)

Chlorophyll B: 22.9 (A645) – 4.68 (A663)

3.2.2 Mineral Analysis:

Multi-elemental analysis was carried out by employing Inductively Coupled Plasma mass Spectroscopy (ICPMS) (Perkin Elmer Nex ION 300X, USA). Prior to ICPMS analysis of multi minerals in a given samples, a clear and colourless sample solution was needed. So, dried and powdered sample were digested to achieve clear and colourless sample.

i. Preparation of acid digests:

The di-acid digestion method has been followed for the analysis of inorganic constituents. Fresh sample were washed with water. Blotted to dry and then kept in oven at 60^0C till a constant weight was obtained. The oven dried plant material was randomly mixed and powdered. One hundred mg oven dried powder of fruits was transferred to 150 ml clean borosil conical flask and to that 15 ml of di-acid mixture

(Concentrated Nitric acid and Perchloric acid) in the ratio of 9 ml: 5ml was added. It was covered with watch glass and kept for an hour till the primary reactions subsided. Then, it was then heated strongly on the hot plate until the solution became colourless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day the extract was filtered through Whatman No. 44 (Ashless) filter paper. The filtrate was stored properly and used for analysis of inorganic constituents. An Inductively Coupled Plasma Mass Spectrometry(ICPMS) was used for the quantification of selected metals like K, P, S, Ca, Mg, Cd, Al, Co, Cr, Cu, Fe, Mo, Li, Mn, Na, Pb, Sr and Zn. Sodium and Potassium were estimated flame photometrically following the standard method of flame photometer . For standardization, various concentrations of sodium and Potassium were prepared by ranging from 10 to 80 ppm by diluting stock solution of NaCl (100 ppm).

3.3 Determination of Phytochemicals

3.3.1 Extraction of the Samples

The vegetable samples were rinsed under tap and deionised water, then cut into small pieces and dried at 70°C for 48 h in an electric oven. The dried samples were ground into fine powder, placed in clean bags and stored at room temperature in desiccators until further analysis. The powdered vegetable samples were put in a cellulose thimble along with thimble holder and placed in a beaker containing 150ml of HPLC grade methanol. Beakers were placed in distillation assembly and in first phase assembly was set at a temperature of 90^0 C for 3 hours and after that in recovery phase temperature was 140^0C. Further extract was concentrated in vacuum concentrator and necessary dilutions were made with HPLC grade methanol for further experiments.

3.3.2 Determination of Total Phenolic Contents

The total phenolic contents of vegetables in methanol extracts was determined according to the method reported by the Lin *et al*., (2011). Aliquots of 1.0 mL of methanol extracts was mixed with 5 mL of 10 fold diluted Folin-ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature before the absorbance was measured at 760 nm spectrophotometrically. Gallic acid was used as standard and the final results were expressed as gallic acid equivalents (GAE).

3.3.3 Determination of Total Flavonoid Contents

The flavonoid contents in methanol extract was measured using a modified colorimetric method described by Lin *et al*., (2011). A volume of 5 mL of methanol extract was transferred to the test tube, mixed with the 0.3 mL of 5% sodium nitrite for 5 minutes. Then 0.3 mL of 10% aluminium chloride was added. After 6 min, reaction was stopped by addition of 2 mL sodium hydroxide. The mixture was further diluted with distilled water up to 10 mL. The absorbance of the mixture was immediately measured at 510 nm. Rutin was used as standard and the flavonoid contents were calculated and expressed as rutin equivalents (Rt).

3.3.4 Determination of Total Flavonols

Total flavonol contents in the methanol extracts was measured using the method reported by Kumaran and Karunakaran (2006). About 2.0 mL of sample was taken and 2.0 mL of 2% AlCl3 and 3 mL sodium acetate (50 g L^{-1}) solutions were added. The absorption was measured at 440 nm after 2.5 h at 20°C. Total flavonols contents were calculated as Rutin (mg g^{-1}) which was used as standard.

3.3.5 Determination of Ascorbic Acid

Ascorbic acid was determined according to the method of Klein and Perry (1982). Methanol extracts was 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 $1000mL^{-1}$) and the absorbance was measured within 30 minutes at 515 nm. Ascorbic acid contents were calculated on the basis of calibration curve of L-ascorbic acid and results were expressed as ascorbic acid equivalents.

3.3.6 Carotene Content

Carotene content was determined by measuring OD of actone extract of samples at 663nm, 645 nm and 480 nm and calculated according to Arnon (1949).

3.4 Quantification of Phenols

High-performance liquid chromatography was performed with the HPLC system Agilent Series 1100, Agilent Technologies, U.S.A. Reversed-phase chromatographic analyses were carried out in isocratic conditions using RP-C18 column (4.6 mm × 250 mm) packed with 5-μmdiameter particles. The mobile phase was acetonitrile-water (10:90, v v^{-1}) containing 1.0% of acetic acid. The flow rate was 0.7 ml min^{-1}, injection volume was 40 μL, and detection was done at 310 nm. The mobile phase was filtered through a membrane filter (0.45 μm) and then degassed by an ultrasonic sound before use. The crude extract and the solutions of standards (gallic acid, rutin, catechol, ferulic acid and quercetin) were prepared in the same mobile phase of HPLC. All chromatographic operations were performed at room temperature and in triplicate.

3.5 Antioxidant Activities

Antioxidant activities were also measured in the methanol extracts of the vegetable samples as described in the previous section. The protocols of antioxidant activities included in the present study are described below.

3.5.1 DPPH Scavenging Activity

DPPH scavenging activity of the vegetables was determined according to Yu *et al*., (2002) and Aoshima *et al*., (2004) with slight modification**.** This method is based on the ability of an antioxidant to scavenge the DPPH cation radical. Briefly 2.0 mL of the sample extract or standards was added to the 5 mL of DPPH solution (0.1 mM in methanol) and vortexes vigorously; then incubated in dark for 30 minutes at room temperature and the decolourization of DPPH was measured against blank at 517 nm. Results were expressed as Gallic acid equivalents and % inhibition was calculated by following relationship:

$$\%\ Inhibition = \frac{\left(A_{Blank} - A_{Sample}\right)}{A_{Blank}} \times 100$$

3.5.2 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of methanol extracts was calculated as described by Yu *et al*., (2004) with slight modification. This assay is based on Fenton reaction. Briefly 2.0 mL of 0.2 M phosphate buffer (pH 7.2), 0.04 mL ferrous sulphate (0.02 M), and 2.0 mL of extract and 1 mL of 1, 10-phenanthroline (0.04 M) were delivered in to the test tube. The Fenton reaction was initiated by addition of 0.1 mL of

7 mM H_2O_2. Absorbance was measured at 560 nm after 5 minutes incubation at room temperature. The relative hydroxyl radical scavenging activity (%) was calculated as:

$$Scavenging\ Activity\ (\%) = \frac{\left(A_{Blank} - A_{Sample}\right)}{A_{Blank}} \times 100$$

3.5.3 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of the methanol extracts was determined by Aiyegoro and Okoh, (2010) method with slight modification. The extract (4 mL) was mixed with 2.4 mL of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M, pH 7.4) and incubated for 10 minutes at room temperature. The absorbance was measured at 230 nm against blank, containing the extract without H_2O_2. Scavenging activity (%) was calculated by:

$$Scavenging\ Activity\ (\%) = \frac{\left(A_{Blank} - A_{Sample}\right)}{A_{Blank}} \times 100$$

3.5.4 Fe^{2+} Chelating Activity

The extracts were assessed for their ability to compete with ferrozine for iron (II) in solution. The chelating ability of ferrous ion of various fractions was estimated by method of Dinis *et al.*, (1994). 2.0 mL of water or acetone extract was added to the 2.0 mL of ferrous sulphate (0.125 mM). The reaction was initiated by addition of 2 mL of 0.3125 mM ferrozine. The mixture was shaken vigorously and left standing at room temperature for ten minutes. Absorbance of the solution was measured at 562 nm against blank prepared in same manner using ferrous chloride and water. EDTA (0.625-5.0 mg) served as positive control and sample without extract or EDTA served as

negative control. The percentage inhibition of ferrozine-Fe (II) complex was calculated using formula:

$$Chelating\ Activity\ (\%) = \frac{\left(A_{Control} - A_{Sample}\right)}{A_{Control}} \times 100$$

3.5.5 Ferric ion Reducing Antioxidant Power (FRAP Assay)

The ferric ion reducing power of the extracts was calculated by the method of Hazra *et al*., (2008) with slight modification. Briefly 2.0 mL of sample was mixed with the 2.0 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of 0.1% potassium ferricyanide, followed by incubation at 50°C in water bath for 20 minutes. Afterward the reaction was stopped with addition of 2 mL of trichloroacetic acid (10%). The upper portion of solution (2 mL) was mixed with 2 mL of distilled water and 2 mL of 0.01% ferric chloride and left for 20 minutes at room temperature and absorbance was measured at 700 nm against blank. A higher of absorbance of reaction mixture indicated greater reducing power. Rutin, Gallic acid or ascorbic acid can be used as positive control.

3.5.6 Phosomolybdenium Complex (PM) Assay

The total antioxidant capacity of the vegetables extracts was measured by Phosomolybdenum complex assay as described by Prieto *et al*. (2006). Briefly 2.0 mL of sample solution in methanol extract was added to the 6.6 mL of reagent solution (0.6 mol L^{-1} sulphuric acid, 28 mol L^{-1} sodium phosphate and 4 mol L^{-1} ammonium molybdate), capped and incubate at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against blank containing 1 mL of reagent solution and same volume of water instead of extract solution and then

subjected to the same experimental condition. The results from three independent experiments, each run in triplicate, are expressed as the mean of relative antioxidant activity (RAA) compared to ascorbic acid (Vitamin-C).

3.6 Analysis of fat soluble vitamins

1 g of 80-mesh sample was precisely weighed and added to a 10-ml screw-capped extraction tube. 0.5 gm Ascorbic Acid was added to the tubes to prevent oxidation of vitamins. Four ml of acetone– chloroform solvent (30:70) mixture, was added to the tube and the tube was flushed with Nitrogen to protect vitamins from air exposure before sealing with the cap. The mixture was shaken on a vortex mixer for 1 min, rested for 5 min, and mixed another minute. After centrifugation at 4000 rpm for 5 min, 1 ml of supernatant was transferred to a 1.5- ml vial and evaporated under nitrogen to remove the solvent. The residue was dissolved in 1 ml methanol before injection onto the HPLC system. High-performance liquid chromatography was performed with the HPLC system Agilent Series 1100, Agilent Technologies, U.S.A. Reversed-phase chromatographic analyses were carried out in isocratic conditions using RP-C18 column (4.6 mm × 250 mm) packed with 5-μmdiameter particles. The mobile phase was acetonitrile-water with gradient flow. The flow rate with time gradient and percentage of water and acetonitrile were presented in table 3.1. The injection volume was 10 μL, and detection was done at 290 nm. The mobile phase was filtered through a membrane filter (0.45 μm) and then degassed by an ultrasonic sound before use. The crude extract and the solutions of standards were prepared in the same mobile phase of HPLC. All chromatographic operations were performed at room temperature and in triplicate.

Table 3.2. Time gradient flow of mobile phase

	Time	Flow	% A (Water)	% B (Acetonitrile)
1		0.60	90	10
2	2	0.70	90	10
3	20	0.70	10	90
4	35	0.70	10	90
5	40	0.60	90	10

3.7 Statistical Analysis

All the statistical analysis were performed with the help of JMP 11 statistical software. All the experiment was performed in completely randomized design with three replication. ANOVA was performed to know the statistical significance between the treatments. Duncan Multiple range test was also performed to determine the statistical differences between the treatments.

Chapter- 4

Experimental Results

The present investigation entitled “Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties” was undertaken to evaluate five local or underutilized vegetables of Sikkim Himalaya for different nutraceutical properties. The experimental results are presented under the following sub heads:

4.1 Survey for medicinal property of vegetables

4.2 Collection of Samples

4.3 Proximate content

4.4 Multi-elemental profile

4.5 Phytochemical content

4.6 Quantification of Phenols

4.7 Anti-oxidative Activity

4.8 Analysis of Fat soluble Vitamins

4.1 Survey for medicinal property of vegetables

A Survey was conducted as described in Chapter III to assess the knowledge of local inhabitants about the use of local indigenous or underutilized vegetables of Sikkim Himalayas. A total of 137 respondents participated in the study survey, comprising 37, 32, 41 and 27 respondents respectively from East, West, South and North district. Utmost care was taken to include local healers, farmers, local traders

Table 4.1.1. Identification of commonly used plant part of different local and indigenous vegetables

S. No.	Scientific name	Common Name	Local Name	Family	Part used
1.	*Sechium edule* Jacq.	Chow-Chow	Ishkush	Cucurbitaceae	Whole plant
2.	*Momordica subangulata var. renigera* Wall ex G. Dom	Spine gourd	Mitho Karela	Cucurbitaceae	Fruit
3.	*Cucurbita pepo* L.	Pumpkin	Pharsi	Cucurbitaceae	Fruit and Young shoot
4.	*Capsicum spp.* L.	Cherry pepper	Khorsani	Solanaceae	Fruit
5.	*Chenopodium album* L.	Bathua	Bethu	Chenopodiaceae	Whole Plant
6.	*Nasturtium officinale* W.T. Aiton	Watercress	Simrayo	Brassicaceae	Leaves with stem
7.	*Tupistra nutans* Walls	Nakima	Nakima	Asparagaceae	Inflorescence
8.	*Apium graveolens var. dulce* L.	Celery	Seleri	Apiacae	Leaf stalk and leaf
9.	*Solanum anguivi* Lam.	Poison berry	Bihi	Solanaceae	Fruit
10.	*Amaranthus viridis* L.	Amaranthus	Lalisaag	Amaranthaceae	Leaves and stem
11.	*Moringa oleifera* Lam.	Drumstick	Sajana	Moringaceae	Pod
12.	*Brassica juncea var. rugose* (L.) Czern	Mustard leaf	Rayo sag	Cruciferae	Leaves
13.	*Raphanus sativus* (L.) Domin	Radish	Mula	Cruciferae	Root
14.	*Dioscorea spp.* L.	Yams	Tarul	Araceae	Tuber
15.	*Ipomea batatas* (L.) Lam	Sweet potato	Sakhar khanda	Convolvulaceae	Root tuber and leaves
16.	*Manihot esculenta* Crantz	Tapioca	Simal tarul	Euphorbiaceae	Tuber

17.	*Amorphophallus campanulatus* (Roxb.)	Elephant's foot yam	Ool	Araceae	Tuber
18.	*Colocasia esculenta* (L.) Schott	Taro	Pindalu	Araceae	Tuber
19.	*Phaseolus vulgaris* L.	Common French bean	Simbi	Fabaceae	Tender pod and seed
20.	*Phaseolus lunatus* L.	Butter bean	Gheu simbi	Fabaceae	Tender pod
21.	*Vicia faba* L.	Broad bean	Bakuley simbi	Fabaceae	Tender pod and seed
22.	*Dolichos biflorus* Lim.	Horse gram	Gahat	Fabaceae	Tender pod
23.	*Spinacea oleracea* L.	Palak	Palak	Chenopodiaceae	Leaves
24.	*Musa sp.* L.	Banana inflorescence	Keraa ko bungoo	Musaceae	Unopened inflorescence
25.	*Cyclanthera pedata* (L.) Schrader	Sweet Gourd	Chuche Karela	Cucurbitaceae	Fruit
26.	*Solanum macrocarpon* L.	African eggplant	Thulo bihi	Solanaceae	Fruit
27.	*Urtica dioca* L.	Stinging nettle	Sisnu	Urticaceae	Plants
28.	*Diplazium esculentum* (Ritz.) Sw.	Dhekia, Dhenkir Shaak	Ningro	Athyraceae	Young leaf
29.	*Solanum betacea* Cav.	Tree tomato	Tree tomato	Solanaceae	Fruit
30.	*Allium tuberosum* Roxb.	Chinese Leek	Dundu ko Saag	Alliaceae	leaf
31.	*Basella rubra* L.	Malabar Spinach	Poi sag	Basellaceae	Stem and leaf
32.	*Fagopyrum esculentum* Moench.	Buck wheat	Phaapar ko saag	Polygonaceae	Leaf
33.	*Dendrocalamus hamiltonii* Gamble	Bamboo	Tamba	Poaceae	Shoots
34.	*Portulaca oleracea* L.	Common purslane	Dalda sag	Portulaceae	Plant
35.	*Aconogonum molle* (D.Don) H.Harce	Knotweed	Thotne	Polygonaceae	Young Shoots
36.	*Allium wallichii* Kunth	Himalaya Onion	Dung-dunge	Alliaceae	Leaves

Table 4.1.2. Knowledge of ethnic communities on medicinal property of local and indigenous vegetables

S. No.	Scientific name	Medicinal Use
1.	*Sechium edule* Jacq.	Good for thyroid health, Prevents kidney stones, anaemia, antiaging properties
2.	*Momordica subangulata var. renigera* Wall ex G. Dom	Relief fever
3.	*Cucurbita pepo* L.	Good for heart and liver health
4.	*Capsicum spp.* L.	Reduces blood cholesterol
5.	*Chenopodium album* L.	useful in curing anorexia, cough, dysentery, diarrhoea
6.	*Nasturtium officinale* W.T. Aiton	Jaundice, relief from Hypertension
7.	*Tupistra nutans* Walls	Used to treat chickenpox, Slow-healing wounds, antidiabetic
8.	*Apium graveolens var. dulce* L.	Hypertension, Dehydration, Prevent or Treat high blood pressure, gastric
9.	*Solanum anguivi* Lam.	Blood pressure, Diabetes
10.	*Amaranthus viridis* L.	Whole plant used for loose motion, dysentery, swelling
11.	*Moringa oleifera* Lam.	Antihypertensive, Antiulcer, Cholesterol lowering
12.	*Brassica juncea var. rugose* (L.) Czern	Good during the stomach upset
13.	*Raphanus sativus* (L.) Domin	No idea
14.	*Dioscorea spp.* L.	It is used in fever, leaves in rash and itch and plants in constipation
15.	*Ipomea batatas* (L.) Lam	Asthma ,arthritis
16.	*Manihot esculenta* Crantz	No idea
17.	*Amorphophallus campanulatus* (Roxb.)	Dysentery
18.	*Colocasia esculenta* (L.) Schott	Utilized for Arthritis, Diarrhoea
19.	*Phaseolus vulgaris* L.	Controls cardiovascular diseases

20.	*Phaseolus lunatus* L.	Digestion
21.	*Vicia faba* L.	No Idea
22.	*Dolichos biflorus* Lim.	Useful in kidney and Gall bladder stone
23.	*Spinacea oleracea* L.	Reducing blood pressure
24.	*Musa sp.* L.	Overcome diabetes and anaemia
25.	*Cyclanthera pedata* (L.) Schrader	The treatment of intestinal parasites, treat gastrointestinal problems
26.	*Solanum macrocarpon* L.	Diabetes
27.	*Urtica dioca* L.	Paste of roots is applied externally to bone fractures with a cotton cloth, antihypertensive property
28.	*Diplazium esculentum* (Ritz.) Sw.	No idea
29.	*Solanum betacea* Cav.	Good food for Diabetes
30.	*Allium tuberosum* Roxb.	No idea
31.	*Basella rubra* L.	No idea
32.	*Fagopyrum esculentum* Moench.	Good food for diabetic patient
33.	*Dendrocalamus hamiltonii* Gamble	No idea
34.	*Portulaca oleracea* L.	No idea
35.	*Aconogonum molle* (D.Don) H.Harce	The plants is used as an astringent and eaten relished in the hills as vegetable and pickle.
36.	*Allium wallichii* Kunth	It is used for the preparation of scented food items also helps in digestion

Table 4.2.1. GPS data of the places of collection of *Solanum aethiopicum*

East District			West District			South District			North District		
Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)
27° 18' 54"	88° 36' 35"	1282	27° 18' 53"	88° 14' 51"	1525	27° 22' 05"	88° 28' 12"	1015	27° 25' 09"	88° 35' 10"	1679
27° 18' 53"	88° 36' 34"	1309	27° 18' 55"	88° 14' 47"	1501	27° 22' 02"	88° 28' 17"	953	27° 25' 08"	88° 35' 14"	1638
27° 18' 48"	88° 36' 44"	1240	27° 18' 55"	88° 14' 49"	1499	27° 21' 58"	88° 28' 02"	1001	27° 25' 09"	88° 35' 09"	1625
27° 18' 49"	88° 36' 49"	1243	27° 18' 55"	88° 14' 50"	1500	27° 21' 57"	88° 28' 16"	926	27° 25' 14"	88° 35' 22"	1537
27° 18' 52"	88° 36' 43"	1225	27° 18' 59"	88° 14' 55"	1462	27° 22' 01"	88° 28' 21"	891	27° 25' 08"	88° 35' 23"	1553
27° 18' 56"	88° 36' 39"	1244	27° 18' 59"	88° 14' 54"	1455	27° 12' 00"	88° 21' 51"	867	27° 29' 40"	88° 31' 59"	1621
27° 18' 49"	88° 36' 44"	1229	27° 19' 02"	88° 14' 53"	1430	27° 11' 55"	88° 21' 49"	870	27° 29' 42"	88° 31' 56"	1224
27° 16' 53"	88° 36' 35"	1217	27° 17' 58"	88° 14' 42"	1818	27° 11' 56"	88° 21' 53"	895	27° 29' 42"	88° 31' 53"	1207
27° 16' 56"	88° 36' 34"	1204	27° 17' 58"	88° 14' 40"	1794	27° 11' 53"	88° 21' 49"	876	27° 29' 44"	88° 31' 52"	1186
27° 16' 55"	88° 36' 36"	1212	27° 17' 23"	88° 15' 15"	1442	27° 11' 51"	88° 22' 02"	986	27° 29' 05"	88° 32' 03"	1096
27° 16' 54"	88° 36' 39"	1231	27° 17' 21"	88° 15' 16"	1440	27° 15' 33"	88° 31' 21"	961	27° 24' 32"	88° 37' 13"	1483
27° 16' 52"	88° 36' 38"	1241	27° 17' 20"	88° 15' 08"	1370	27° 15' 30"	88° 31' 17"	988	27° 24' 31"	88° 37' 13"	1489
27° 16' 54"	88° 36' 26"	1157	27° 17' 23"	88° 14' 57"	1372	27° 15' 45"	88° 31' 28"	888	27° 24' 32"	88° 36' 58"	1475
27° 16' 51"	88° 36' 27"	1155	27° 14' 47"	88° 16' 09"	1546	27° 15' 44"	88° 31' 13"	966	27° 34' 32"	88° 37' 12"	1481
27° 11' 24"	88° 40' 30"	1442	27° 14' 41"	88° 16' 13"	1593	27° 15' 14"	88° 31' 24"	917	27° 24' 29"	88° 37' 11"	1495
27° 11' 22"	88° 40' 31"	1460	27° 14' 34"	88° 16' 09"	1583	27° 09' 57"	88° 28' 39"	1400	27° 31' 51"	88° 30' 42"	969
27° 11' 35"	88° 40' 15"	1391	27° 14' 33"	88° 16' 11"	1596	27° 09' 56"	88° 28' 25"	1472	27° 31' 50"	88° 30' 35"	1039
27° 11' 32"	88° 40' 11"	1446	27° 14' 37"	88° 16' 11"	1581	27° 09' 47"	88° 28' 19"	1409	27° 31' 48"	88° 30' 36"	1039
27° 11' 23"	88° 40' 27"	1441	27° 15' 26"	88° 16' 56"	1293	27° 09' 38"	88° 28' 30"	1308	27° 31' 46"	88° 30' 34"	1063
27° 12' 44"	88° 36' 09"	1016	27° 15' 31"	88° 16' 47"	1211	27° 09' 41"	88° 28' 35"	1284	27° 31' 42"	88° 30' 35"	1078
27° 12' 35"	88° 36' 03"	1068	27° 15' 28"	88° 16' 39"	1269	27° 05' 58"	88° 23' 40"	579	27° 26' 13"	88° 35' 30"	1835
27° 12' 30"	88° 36' 07"	1026	27° 15' 35"	88° 16' 40"	1161	27° 06' 02"	88° 23' 36"	561	27° 26' 10"	88° 35' 25"	1828
27° 12' 32"	88° 36' 10"	996	27° 15' 20"	88° 16' 29"	1404	27° 05' 53"	88° 23' 29"	523	27° 25' 54"	88° 35' 28"	1663
27° 12' 53"	88° 36' 12"	1005	27° 10' 51"	88° 12' 05"	1635	27° 06' 03"	88° 23' 49"	691	27° 25' 56"	88° 35' 40"	1663

27° 14' 41"	88° 36' 15"	1301	27° 10' 48"	88° 12' 10"	1601	27° 06' 04"	88° 23' 51"	719	27° 25' 48"	88° 35' 42"	1564
27° 14' 37"	88° 35' 58"	1335	27° 10' 58"	88° 11' 58"	1706						
27° 14' 41"	88° 36' 15"	1310	27° 10' 54"	88° 12' 00"	1682						
27° 14' 43"	88° 36' 16"	1281	27° 10' 55"	88° 12' 04"	1682						
27° 14' 48"	88° 36' 21"	1253									

Table 4.2.2. GPS data of the places of collection of *Solanum macrocarpon*

East District			West District			South District			North District		
Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)
27° 18' 54"	88° 36' 35"	1282	27° 18' 53"	88° 14' 51"	1525	27° 22' 12"	88° 27' 53"	1231	27° 25' 09"	88° 35' 09"	1625
27° 18' 53"	88° 36' 34"	1309	27° 18' 55"	88° 14' 47"	1501	27° 22' 10"	88° 27' 51"	1246	27° 25' 14"	88° 35' 22"	1537
27° 18' 48"	88° 36' 44"	1240	27° 18' 55"	88° 14' 49"	1499	27° 22' 22"	88° 28' 17"	966	27° 25' 08"	88° 35' 23"	1553
27° 18' 49"	88° 36' 49"	1243	27° 18' 55"	88° 14' 50"	1500	27° 22' 14"	88° 28' 22"	906	27° 25' 05"	88° 35' 20"	1557
27° 18' 52"	88° 36' 43"	1225	27° 18' 59"	88° 14' 55"	1462	27° 22' 05"	88° 28' 12"	1015	27° 25' 06"	88° 35' 17"	1588
27° 18' 56"	88° 36' 39"	1244	27° 18' 59"	88° 14' 54"	1455	27° 11' 56"	88° 21' 53"	895	27° 29' 42"	88° 31' 53"	1207
27° 16' 53"	88° 36' 35"	1217	27° 19' 02"	88° 14' 53"	1430	27° 11' 53"	88° 21' 49"	876	27° 29' 44"	88° 31' 52"	1186
27° 16' 56"	88° 36' 34"	1204	27° 17' 23"	88° 14' 57"	1372	27° 11' 51"	88° 22' 02"	986	27° 29' 05"	88° 32' 03"	1096
27° 16' 55"	88° 36' 36"	1212	27° 17' 19"	88° 14' 52"	1339	27° 11' 52"	88° 22' 02"	986	27° 29' 07"	88° 32' 05"	1120
27° 16' 54"	88° 36' 39"	1231	27° 17' 58"	88° 14' 40"	1794	27° 11' 52"	88° 22' 02"	973	27° 29' 05"	88° 32' 00"	1065
27° 16' 52"	88° 36' 38"	1241	27° 17' 58"	88° 14' 42"	1818	27° 15' 25"	88° 31' 22"	958	27° 24' 32"	88° 36' 58"	1475
27° 16' 54"	88° 36' 26"	1157	27° 17' 23"	88° 15' 15"	1442	27° 15' 24"	88° 31' 18"	994	27° 34' 32"	88° 37' 12"	1481
27° 16' 51"	88° 36' 27"	1155	27° 14' 37"	88° 16' 11"	1581	27° 15' 21"	88° 31' 26"	911	27° 24' 29"	88° 37' 11"	1495
27° 11' 24"	88° 40' 18"	1441	27° 14' 35"	88° 16' 08"	1571	27° 15' 17"	88° 31' 26"	906	27° 34' 26"	88° 37' 13"	1518
27° 11' 30"	88° 40' 13"	1440	27° 14' 34"	88° 16' 09"	1583	27° 15' 14"	88° 31' 24"	917	27° 24' 23"	88° 37' 04"	1500
27° 11' 23"	88° 40' 30"	1443	27° 14' 33"	88° 16' 11"	1596	27° 09' 30"	88° 28' 31"	1287	27° 31' 48"	88° 30' 36"	1039

27° 11' 24"	88° 40' 30"	1442	27° 14' 36"	88° 16' 08"	1562	27° 09' 46"	88° 28' 41"	1256	27° 31' 46"	88° 30' 34"	1063
27° 11' 22"	88° 40' 31"	1460	27° 15' 30"	88° 16' 49"	1213	27° 09' 56"	88° 28' 25"	1472	27° 31' 42"	88° 30' 35"	1078
27° 16' 46"	88° 36' 10"	1011	27° 15' 32"	88° 16' 45"	1188	27° 09' 48"	88° 28' 20"	1418	27° 31' 39"	88° 30' 34"	1093
27° 12' 35"	88° 36' 03"	1068	27° 15' 26"	88° 16' 56"	1293	27° 09' 47"	88° 28' 14"	1422	27° 26' 10"	88° 35' 25"	1828
27° 12' 32"	88° 36' 10"	996	27° 15' 31"	88° 16' 47"	1211	27° 06' 01"	88° 23' 43"	633	27° 26' 10"	88° 35' 32"	1816
27° 12' 53"	88° 36' 12"	1005	27° 15' 28"	88° 16' 39"	1269	27° 26' 02"	88° 23' 40"	613	27° 26' 07"	88° 35' 33"	1799
27° 12' 43"	88° 36' 14"	976	27° 10' 57"	88° 12' 02"	1675	27° 05' 58"	88° 23' 40"	579	27° 26' 06"	88° 35' 32"	1793
27° 14' 41"	88° 36' 15"	1301	27° 10' 55"	88° 12' 03"	1663	27° 06' 02"	88° 23' 36"	561	27° 26' 03"	88° 35' 45"	1699
27° 14' 37"	88° 35' 58"	1335	27° 10' 54"	88° 12' 05"	1644	27° 05' 53"	88° 23' 29"	523			
27° 14' 41"	88° 36' 15"	1310	27° 10' 52"	88° 12' 02"	1662						
27° 14' 51"	88° 36' 23"	1267	27° 10' 51"	88° 12' 05"	1635						
27° 14' 41"	88° 36' 15"	1294									

Table 4.2.3. GPS data of the places of collection of *Capsicum annuum var. cerasiformae*

East District			West District			South District			North District		
Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)
27° 18' 48"	88° 36' 44"	1240	27° 18' 55"	88° 14' 49"	1499	27° 22' 27"	88° 28' 00"	1081	27° 25' 01"	88° 35' 15"	1606
27° 18' 49"	88° 36' 49"	1243	27° 18' 55"	88° 14' 50"	1500	27° 22' 32"	88° 28' 10"	966	27° 25' 06"	88° 35' 11"	1664
27° 18' 52"	88° 36' 43"	1225	27° 18' 59"	88° 14' 55"	1462	27° 22' 27"	88° 28' 15"	948	27° 25' 16"	88° 35' 28"	1503
27° 18' 56"	88° 36' 39"	1244	27° 18' 59"	88° 14' 54"	1455	27° 22' 12"	88° 27' 53"	1231	27° 25' 10"	88° 35' 25"	1540
27° 18' 49"	88° 36' 44"	1229	27° 19' 06"	88° 14' 53"	1392	27° 22' 10"	88° 27' 51"	1246	27° 25' 00"	88° 35' 24"	1525

27° 18' 49"	88° 36' 49"	1167	27° 17' 58"	88° 14' 42"	1818	27° 11' 59"	88° 20' 05"	1004	27° 29' 05"	88° 32' 03"	1096
27° 16' 53"	88° 36' 35"	1217	27° 17' 23"	88° 15' 15"	1442	27° 12' 01"	88° 22' 04"	990	27° 29' 07"	88° 32' 05"	1120
27° 16' 56"	88° 36' 34"	1204	27° 17' 20"	88° 15' 11"	1397	27° 11' 59"	88° 22' 00"	928	27° 29' 05"	88° 32' 00"	1065
27° 16' 55"	88° 36' 36"	1212	27° 17' 21"	88° 15' 16"	1440	27° 11' 52"	88° 22' 02"	986	27° 29' 07"	88° 32' 02"	1093
27° 16' 54"	88° 36' 39"	1231	27° 17' 20"	88° 15' 08"	1370	27° 11' 52"	88° 22' 02"	973	27° 29' 06"	88° 32' 04"	1106
27° 16' 52"	88° 36' 38"	1241	27° 17' 23"	88° 14' 57"	1372	27° 12' 00"	88° 21' 58"	910	27° 34' 26"	88° 37' 13"	1518
27° 16' 54"	88° 36' 26"	1157	27° 14' 36"	88° 16' 08"	1562	27° 15' 24"	88° 31' 37"	775	27° 24' 23"	88° 37' 04"	1500
27° 16' 51"	88° 36' 27"	1155	27° 14' 35"	88° 16' 08"	1571	27° 15' 22"	88° 31' 43"	721	27° 24' 21"	88° 37' 01"	1494
27° 11' 32"	88° 40' 11"	1446	27° 14' 34"	88° 16' 09"	1583	27° 15' 45"	88° 31' 28"	888	27° 24' 18"	88° 37' 00"	1511
27° 11' 23"	88° 40' 27"	1441	27° 14' 33"	88° 16' 11"	1596	27° 15' 44"	88° 31' 13"	966	27° 24' 27"	88° 37' 14"	1514
27° 11' 23"	88° 40' 30"	1443	27° 14' 37"	88° 16' 11"	1581	27° 15' 41"	88° 31' 25"	918	27° 31' 51"	88° 30' 42"	969
27° 11' 24"	88° 40' 30"	1442	27° 15' 28"	88° 16' 39"	1269	27° 09' 38"	88° 28' 30"	1308	27° 31' 50"	88° 30' 35"	1039
27° 11' 22"	88° 40' 31"	1460	27° 15' 35"	88° 16' 40"	1161	27° 09' 41"	88° 28' 35"	1284	27° 31' 48"	88° 30' 36"	1039
27° 12' 44"	88° 36' 09"	1016	27° 15' 20"	88° 16' 29"	1404	27° 09' 42"	88° 28' 33"	1312	27° 31' 46"	88° 30' 34"	1063
27° 12' 35"	88° 36' 03"	1068	27° 15' 44"	88° 16' 49"	1027	27° 09' 30"	88° 28' 31"	1287	27° 26' 15"	88° 35' 21"	1875
27° 12' 30"	88° 36' 07"	1026	27° 15' 31"	88° 16' 49"	1198	27° 09' 46"	88° 28' 41"	1256	27° 26' 16"	88° 35' 25"	1878
27° 12' 32"	88° 36' 10"	996	27° 10' 48"	88° 12' 10"	1601	27° 05' 58"	88° 23' 47"	625	27° 26' 13"	88° 35' 30"	1835
27° 12' 53"	88° 36' 12"	1005	27° 10' 58"	88° 11' 58"	1706	27° 05' 59"	88° 23' 46"	628	27° 26' 10"	88° 35' 25"	1828
27° 14' 41"	88° 36' 15"	1294	27° 10' 51"	88° 12' 10"	1599	27° 05' 59"	88° 23' 45"	623	27° 26' 10"	88° 35' 32"	1816
27° 14' 41"	88° 36' 15"	1301	27° 10' 53"	88° 12' 11"	1591	27° 06' 01"	88° 23' 43"	633			
27° 14' 37"	88° 35' 58"	1335	27° 10' 57"	88° 12' 09"	1608	27° 26' 02"	88° 23' 40"	613			
27° 14' 41"	88° 36' 15"	1310									
27° 14' 43"	88° 36' 16"	1281									

Table 4.2.4. GPS data of the places of collection of *Tupistra aurantiaca*

East District			West District			South District			North District		
Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)
27° 18' 54"	88° 36' 35"	1282	27° 19' 03"	88° 15' 04"	1420	27° 22' 05"	88° 28' 12"	1015	27° 25' 16"	88° 35' 28"	1503
27° 18' 53"	88° 36' 34"	1309	27° 19' 06"	88° 14' 53"	1392	27° 22' 02"	88° 28' 17"	953	27° 25' 10"	88° 35' 25"	1540
27° 18' 48"	88° 36' 44"	1240	27° 18' 55"	88° 14' 50"	1500	27° 21' 58"	88° 28' 02"	1001	27° 25' 00"	88° 35' 24"	1525
27° 18' 49"	88° 36' 49"	1243	27° 18' 53"	88° 14' 51"	1525	27° 21' 57"	88° 28' 16"	926	27° 25' 57"	88° 35' 21"	1541
27° 18' 52"	88° 36' 43"	1225	27° 18' 55"	88° 14' 47"	1501	27° 22' 01"	88° 28' 21"	891	27° 25' 54"	88° 35' 21"	1536
27° 18' 56"	88° 36' 39"	1244	27° 18' 55"	88° 14' 49"	1499	27° 12' 01"	88° 22' 04"	990	27° 29' 07"	88° 32' 02"	1093
27° 16' 53"	88° 36' 35"	1217	27° 17' 58"	88° 14' 42"	1818	27° 11' 59"	88° 22' 00"	928	27° 29' 06"	88° 32' 04"	1106
27° 16' 55"	88° 36' 36"	1212	27° 17' 23"	88° 15' 15"	1442	27° 12' 00"	88° 21' 58"	910	27° 29' 06"	88° 32' 10"	1167
27° 16' 54"	88° 36' 39"	1231	27° 17' 20"	88° 15' 11"	1397	27° 21' 00"	88° 21' 56"	897	27° 29' 08"	88° 32' 14"	1195
27° 16' 54"	88° 36' 26"	1157	27° 17' 21"	88° 15' 16"	1440	27° 12' 00"	88° 21' 51"	867	27° 29' 05"	88° 32' 09"	1155
27° 16' 42"	88° 36' 18"	1077	27° 17' 20"	88° 15' 08"	1370	27° 15' 34"	88° 31' 18"	986	27° 24' 21"	88° 37' 01"	1494
27° 11' 24"	88° 40' 18"	1441	27° 14' 41"	88° 16' 13"	1593	27° 15' 33"	88° 31' 21"	961	27° 24' 18"	88° 37' 00"	1511
27° 11' 30"	88° 40' 13"	1440	27° 14' 34"	88° 16' 09"	1583	27° 15' 30"	88° 31' 17"	988	27° 24' 27"	88° 37' 14"	1514
27° 11' 23"	88° 40' 30"	1443	27° 14' 33"	88° 16' 11"	1596	27° 15' 29"	88° 31' 22"	961	27° 24' 11"	88° 36' 59"	1558
27° 11' 35"	88° 40' 15"	1391	27° 14' 37"	88° 16' 11"	1581	27° 15' 25"	88° 31' 22"	958	27° 24' 17"	88° 36' 59"	1507
27° 11' 35"	88° 40' 16"	1384	27° 14' 35"	88° 16' 08"	1571	27° 09' 59"	88° 28' 39"	1404	27° 31' 38"	88° 30' 40"	992
27° 16' 46"	88° 36' 10"	1011	27° 15' 32"	88° 16' 44"	1326	27° 09' 59"	88° 28' 42"	1389	27° 31' 51"	88° 30' 42"	969
27° 12' 44"	88° 36' 09"	1016	27° 15' 25"	88° 16' 46"	1299	27° 09' 57"	88° 28' 39"	1400	27° 31' 50"	88° 30' 35"	1039
27° 12' 35"	88° 36' 03"	1068	27° 15' 30"	88° 16' 49"	1213	27° 09' 56"	88° 28' 25"	1472	27° 31' 48"	88° 30' 36"	1039

27° 12' 30"	88° 36' 07"	1026	27° 15' 32"	88° 16' 45"	1188	27° 09' 47"	88° 28' 19"	1409	27° 26' 13"	88° 35' 30"	1835
27° 12' 32"	88° 36' 10"	996	27° 15' 26"	88° 16' 56"	1293	27° 06' 03"	88° 23' 49"	691	27° 26' 10"	88° 35' 25"	1828
27° 14' 41"	88° 36' 15"	1301	27° 10' 54"	88° 12' 00"	1682	27° 06' 04"	88° 23' 51"	719	27° 26' 10"	88° 35' 32"	1816
27° 14' 37"	88° 35' 58"	1335	27° 10' 55"	88° 12' 04"	1682	27° 06' 08"	88° 23' 49"	746	27° 26' 07"	88° 35' 33"	1799
27° 14' 41"	88° 36' 15"	1310	27° 10' 57"	88° 12' 02"	1675	27° 06' 11"	88° 23' 48"	750	27° 26' 06"	88° 35' 32"	1793
27° 14' 51"	88° 36' 23"	1267	27° 10' 55"	88° 12' 03"	1663	27° 06' 10"	88° 23' 53"	793			
27° 14' 48"	88° 36' 16"	1225									

Table 4.2.5. GPS data of the places of collection of *Nasturtium officinale*

East District			West District			South District			North District		
Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)	Latitude (N)	Longitude (E)	Altitude (m)
27° 18' 49"	88° 36' 49"	1167	27° 19' 03"	88° 15' 04"	1420	27° 22' 01"	88° 28' 21"	891	27° 25' 00"	88° 35' 24"	1525
27° 18' 51"	88° 36' 49"	1158	27° 19' 06"	88° 14' 53"	1392	27° 21' 57"	88° 28' 16"	926	27° 25' 57"	88° 35' 21"	1541
27° 18' 56"	88° 36' 48"	1178	27° 19' 05"	88° 15' 12"	1396	27° 22' 14"	88° 28' 22"	906	27° 25' 54"	88° 35' 21"	1536
27° 18' 55"	88° 36' 36"	1280	27° 19' 06"	88° 15' 08"	1396	27° 22' 27"	88° 28' 15"	948	27° 25' 16"	88° 35' 28"	1503
27° 18' 51"	88° 36' 37"	1301	27° 19' 16"	88° 15' 31"	1309	27° 22' 32"	88° 28' 10"	966	27° 25' 14"	88° 35' 22"	1537
27° 16' 51"	88° 36' 27"	1155	27° 17' 16"	88° 15' 04"	1311	27° 21' 00"	88° 21' 56"	897	27° 29' 05"	88° 32' 03"	1096
27° 16' 50"	88° 36' 18"	1071	27° 17' 15"	88° 14' 51"	1305	27° 12' 00"	88° 21' 51"	867	27° 29' 05"	88° 32' 00"	1065

27° 16' 41"	88° 36' 14"	1061	27° 17' 16"	88° 14' 46"	1339	27° 11' 55"	88° 21' 49"	870	27° 29' 07"	88° 32' 02"	1093
27° 16' 38"	88° 36' 13"	1060	27° 17' 20"	88° 15' 08"	1370	27° 11' 56"	88° 21' 53"	895	27° 29' 06"	88° 32' 04"	1106
27° 11' 23"	88° 40' 27"	1441	27° 17' 23"	88° 14' 57"	1372	27° 11' 53"	88° 21' 49"	876	27° 29' 06"	88° 32' 10"	1167
27° 11' 23"	88° 40' 30"	1443	27° 14' 33"	88° 16' 01"	1523	27° 15' 24"	88° 31' 37"	775	27° 24' 32"	88° 37' 13"	1483
27° 11' 35"	88° 40' 16"	1384	27° 14' 40"	88° 16' 05"	1526	27° 15' 22"	88° 31' 43"	721	27° 24' 31"	88° 37' 13"	1489
27° 11' 39"	88° 40' 13"	1382	27° 14' 41"	88° 16' 06"	1527	27° 15' 45"	88° 31' 28"	888	27° 24' 32"	88° 36' 58"	1475
27° 11' 40"	88° 40' 12"	1380	27° 14' 47"	88° 16' 09"	1546	27° 15' 21"	88° 31' 26"	911	27° 34' 32"	88° 37' 12"	1481
27° 12' 43"	88° 36' 14"	976	27° 14' 46"	88° 16' 07"	1534	27° 15' 17"	88° 31' 26"	906	27° 24' 29"	88° 37' 11"	1495
27° 12' 35"	88° 36' 21"	903	27° 15' 44"	88° 16' 49"	1027	27° 09' 30"	88° 28' 31"	1287	27° 37' 45"	88° 30' 47"	886
27° 12' 38"	88° 36' 22"	894	27° 15' 32"	88° 16' 45"	1188	27° 09' 46"	88° 28' 41"	1256	27° 31' 46"	88° 30' 47"	892
27° 12' 43"	88° 36' 25"	874	27° 15' 31"	88° 16' 47"	1211	27° 09' 41"	88° 28' 35"	1284	27° 31' 36"	88° 30' 46"	963
27° 12' 38"	88° 36' 22"	896	27° 15' 35"	88° 16' 40"	1161	27° 09' 38"	88° 28' 30"	1308	27° 31' 38"	88° 30' 40"	992
27° 14' 48"	88° 36' 21"	1253	27° 15' 30"	88° 16' 49"	1213	27° 09' 30"	88° 28' 31"	1287	27° 26' 03"	88° 35' 45"	1699
27° 14' 51"	88° 36' 23"	1267	27° 10' 51"	88° 12' 10"	1599	27° 05' 58"	88° 23' 40"	579	27° 25' 54"	88° 35' 35"	1662
27° 14' 48"	88° 36' 16"	1225	27° 10' 48"	88° 12' 10"	1601	27° 06' 02"	88° 23' 36"	561	27° 25' 54"	88° 35' 28"	1663
27° 14' 47"	88° 36' 07"	1223	27° 10' 57"	88° 12' 09"	1608	27° 05' 53"	88° 23' 29"	523	27° 25' 56"	88° 35' 40"	1663
27° 14' 43"	88° 36' 16"	1281	27° 10' 51"	88° 12' 10"	1599	27° 05' 59"	88° 23' 46"	628	27° 25' 48"	88° 35' 42"	1564
			27° 10' 51"	88° 12' 05"	1635	27° 05' 59"	88° 23' 45"	623			

and village gentries as respondent. The present study revealed the use of 36 indigenous and local vegetables with medicinal properties were commonly used by local inhabitants of Sikkim Himalayas. The vegetables belong to 21 families, among which cucurbitaceae, fabaceae, solanaceae and araceae were most commonly used by local inhabitants. The details of vegetables used by ethnic community of Sikkim with its botanical name, common name, family and plant part used are presented in Table 4.1.1. The Table 4.1.2 describes the medicinal use of the vegetables along with their botanical name. Tender shoot and leaf were most commonly used part as vegetable followed by fruits and leaves.

4.2 Collection of samples

The samples used for this study were collected from all the four districts of Sikkim along with the GPS data during their peak availability period. The GPS data of the places of collection of samples for each vegetables are presented in Table 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5.

4.3. Proximate Analysis:

The different proximate content *i.e.* moisture content, dry matter content, total soluble solids, total ash content, crude fat, crude protein, crude fibre, total carbohydrate, total starch, total sugar, Chlorophyll A, Chlorophyll B and total chlorophyll content of the five selected vegetables were analysed. The results are shown in respective graphs and Table 4.3 along with mean ± SD value.

4.3.1 Moisture (%)

The moisture content of *Solanum aethiopicum*, *Solanum macrocarpon*, *Capsicum annuum* var. *cerasiformae*, *Tupistra aurantiaca*, *Nasturtium officinale* was

Table 4.3. Mean performance of the selected vegetables for proximate content

	Moisture Content (%)	Dry Matter (%)	TSS (°Brix)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fibre (%)	Carbohydrate (%)	Total starch (%)	Total Sugar (%)	Chl A (mg $100g^{-1}$)	Chl B (mg $100g^{-1}$)	Total Chlorophyll (mg $100g^{-1}$)
S. aethiopicum	88.27^{c} ±0.06	11.73^{c} ±0.06	3.53^{c} ±0.12	0.86^{d} ±0.08	2.91^{b} ±0.19	2.10^{c} ±0.12	3.35^{b} ±0.16	7.11^{c} ±0.05	0.84^{a} ±0.03	4.93^{a} ±0.02	0.97^{d} ±0.03	6.23^{d} ±0.02	7.17^{d} ±0.03
S. macrocarpon	91.53^{b} ±0.15	8.47^{d} ±0.15	3.27^{d} ±0.12	1.37^{c} ±0.15	2.23^{c} ±0.12	1.44^{d} ±0.06	2.66^{c} ±0.21	6.59^{c} ±0.28	0.64^{b} ±0.02	5.22^{a} ±0.05	0.17^{e} ±0.02	5.99^{e} ±0.02	6.15^{e} ±0.03
C. annuum var. cerasiformae	84.39^{d} ±0.09	15.61^{b} ±0.09	6.27^{b} ±0.12	0.55^{e} ±0.13	0.89^{d} ±0.10	6.25^{a} ±0.11	1.42^{e} ±0.16	4.29^{d} ±1.61	0.17^{d} ±0.07	2.57^{b} ±0.05	9.50^{a} ±0.08	15.43^{a} ±0.04	24.89^{a} ±0.02
T. aurantiaca	80.68^{e} ±0.45	19.32^{a} ±0.45	6.93^{a} ±0.12	3.69^{a} ±0.02	22.59^{a} ±0.03	0.36^{e} ±0.05	6.56^{a} ±0.04	41.83^{a} ±0.08	0.01^{e} ±0.00	2.69^{b} ±0.49	1.40^{c} ±0.10	8.54^{c} ±0.03	9.92^{c} ±0.01
N. officinale	94.24^{a} ±0.28	5.76^{e} ±0.28	2.53^{e} ±0.12	2.38^{b} ±0.01	0.30^{e} ±0.05	4.60^{b} ±0.06	1.66^{d} ±0.02	8.73^{b} ±0.23	0.39^{c} ±0.01	5.25^{a} ±0.03	6.79^{b} ±0.03	11.00^{b} ±0.04	17.79^{b} ±0.02
CV %	0.29	2.06	2.56	5.29	1.96	2.86	4.46	5.39	8.48	5.41	1.62	0.33	0.16
SEm±	0.14	0.14	0.07	0.05	0.07	0.05	0.08	0.43	0.02	0.13	0.04	0.02	0.01
SEđ	0.20	0.20	0.09	*0.08*	0.09	0.07	0.11	0.60	0.03	0.18	0.05	0.03	0.02

found to be significantly different as depicted in Figure 4.3.1. The moisture content was recorded highest in the *Nasturtium officinale* leaves (94.24%±0.28) followed by fruits of *Solanum macrocarpon* (91.53%±0.15), *Solanum aethiopicum* (88.27±0.06) and *Capsicum annuum* var. *cerasiformae* (84.39% ±0.09) while lowest was recorded in the inflorescence of the *Tupistra aurantiaca* (80.68%±0.45).

4.3.2 Dry Matter Content (%)

The dry matter content of *Solanum aethiopicum*, *Solanum macrocarpon*, *Capsicum annuum* var. *cerasiformae*, *Tupistra aurantiaca*, *Nasturtium officinale* was found to be significantly different as shown in Figure 4.3.2. Dry matter content was highest in inflorescence of *Tupistra aurantiaca* (19.32%±0.45) followed by fruits of *Capsicum annuum* var. *cerasiformae* (15.61%±0.09), *Solanum aethiopicum* (11.73±0.06) and *Solanum macrocarpon* (8.47%±0.15) whereas lowest dry matter content was estimated in leaves of *Nasturtium officinale* (5.76%±0.28).

4.3.3 Total Soluble Solids (°Brix)

The total soluble solids (TSS) of the selected vegetables was found significantly different and data are presented in Table 4.3 and represented by Figure 4.3.3. The total soluble solids was recorded highest in inflorescence of *Tupistra aurantiaca* (6.93%±0.12) followed by fruits of *Capsicum annuum* var. *cerasiformae* (6.27%±0.12), *Solanum aethiopicum* (3.53%±0.12) and *Solanum macrocarpon* (3.27%±0.12) while it was observed lowest in leaves of *Nasturtium officinale* (2.53%±0.12).

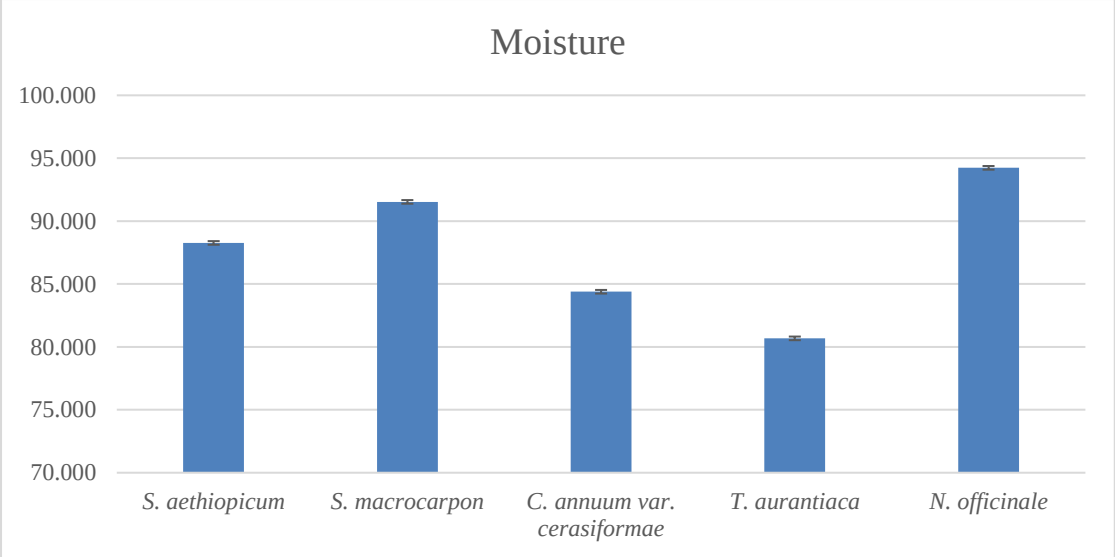

Figure 4.3.1 Moisture content (%) of vegetables

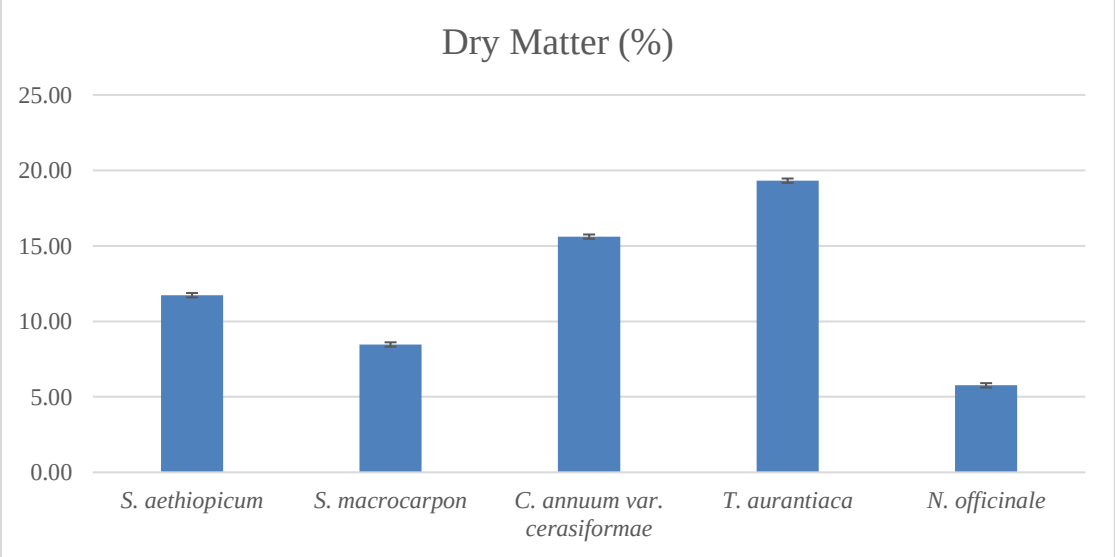

Figure 4.3.2 Dry matter content (%) of vegetables

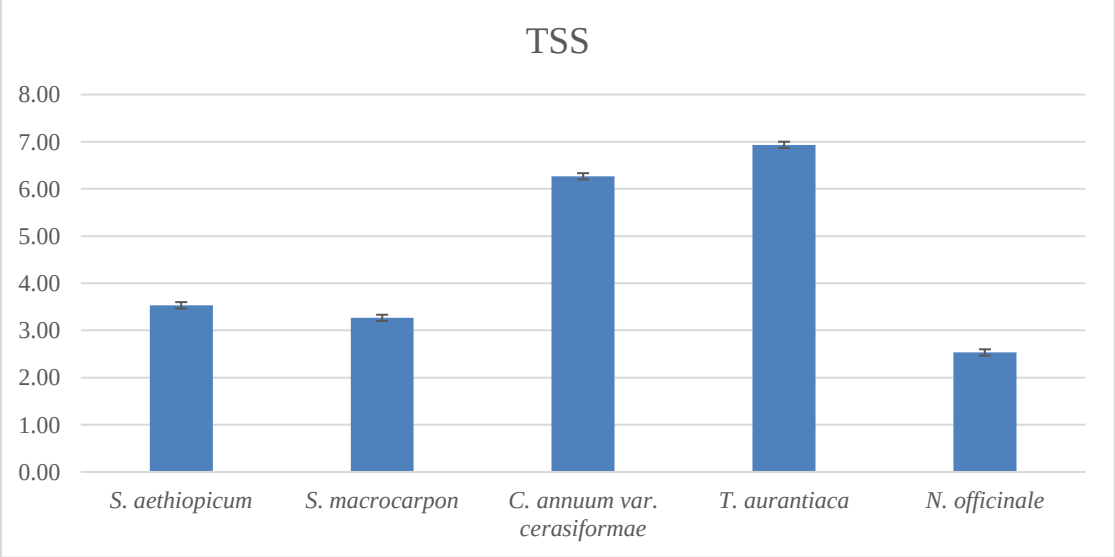

Figure 4.3.3 TSS (°Brix) of vegetables

4.3.4 Total Ash content (%)

The total ash content was found significantly different of all the studied vegetables and data are represented in Figure 4.3.4. The total ash content was found highest in inflorescence of *Tupistra aurantiaca* (3.69%±0.02) followed by leaves of *Nasturtium officinale* (2.38%±0.01), fruits of *Solanum macrocarpon* (1.37%±0.15) and *Solanum aethiopicum* (0.86%±0.08) while it is observed lowest in *Capsicum annuum* var. *cerasiformae* (0.55%±0.13).

4.3.5 Crude Fat (%)

The data regarding crude fat content of all the studied vegetables were found significantly different and represented in Figure 4.3.5. *Tupistra aurantiaca* (22.59%±0.03) was found to be contain highest crude fat content among all the selected vegetables followed by fruits of *Solanum aethiopicum* (2.91%±0.19), *Solanum macrocarpon* (2.23%±0.12) and *Capsicum annuum* var. *cerasiformae* (0.89% ±0.10). The lowest amount of crude fat content was recorded in *Nasturtium officinale* (0.30%±0.05).

4.3.6 Crude Protein (%)

The crude protein content of all the studied vegetables were found significantly different and depicted in Figure 4.3.6. *Capsicum annuum* var. *cerasiformae* (6.25%±0.11) was found to contain highest crude protein content among all the selected vegetables followed by *Nasturtium officinale* (4.60%±0.06), *Solanum aethiopicum* (2.10%±0.12) and *Solanum macrocarpon* (1.44%±0.06). The crude protein content was recorded lowest in *Tupistra aurantiaca* (0.36%±0.05).

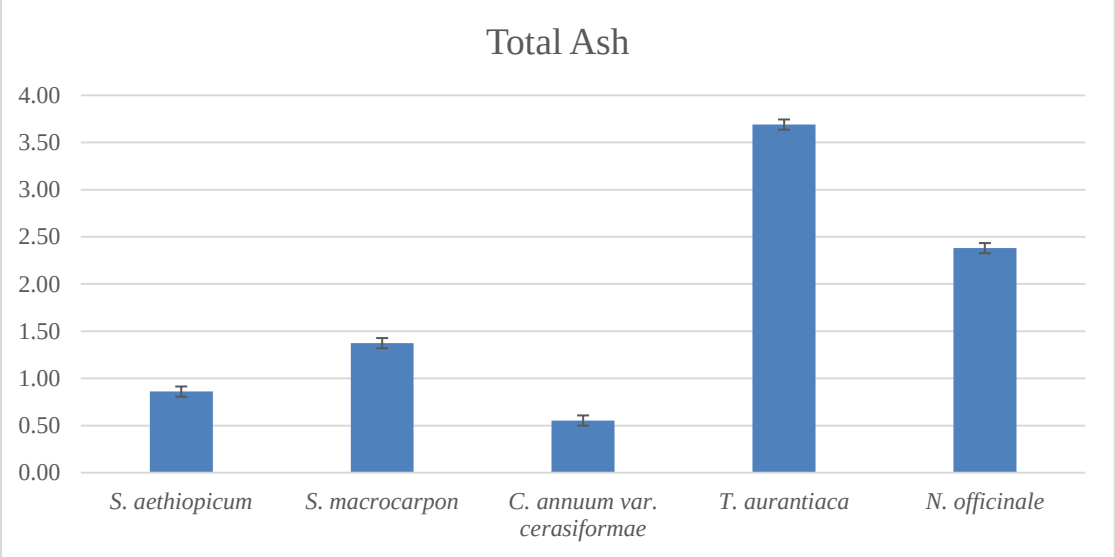

Figure 4.3.4 Total Ash content (%) of vegetables

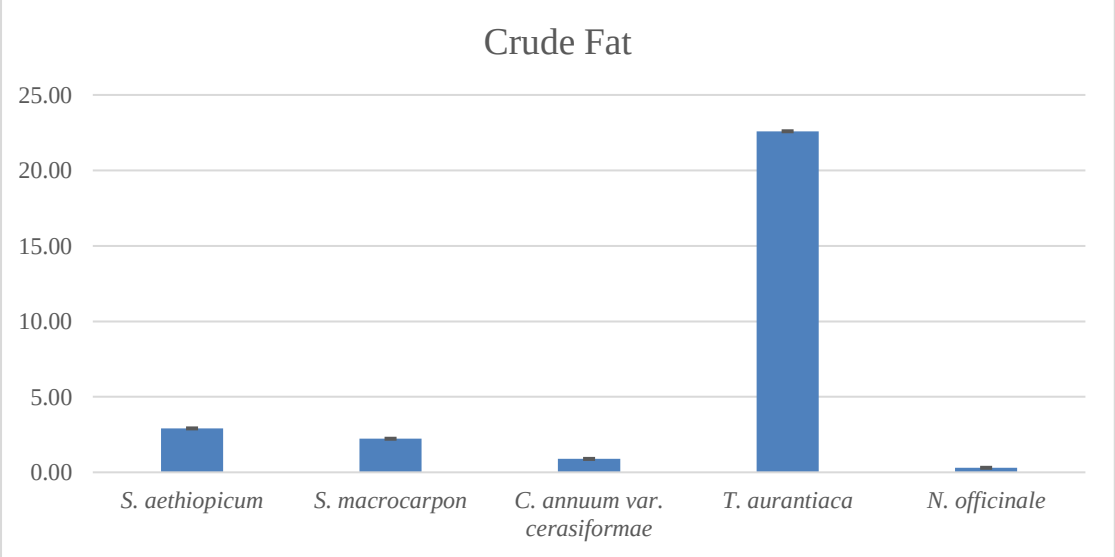

Figure 4.3.5 Crude Fat content (%) of vegetables

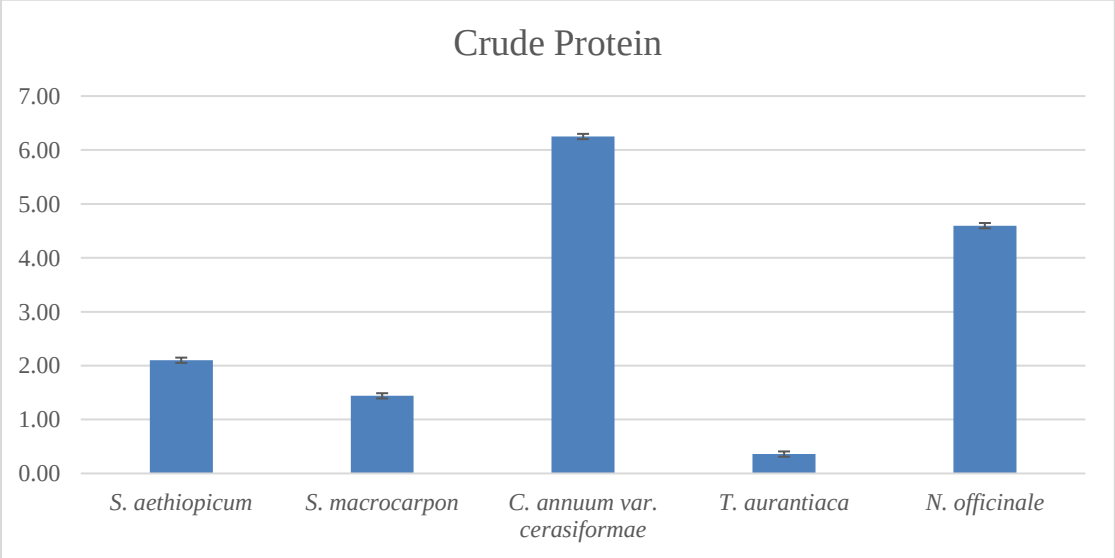

Figure 4.3.6 Crude Protein content (%) of vegetables

4.3.7 Crude Fibre (%)

The Figure 4.3.7 represents the significantly different crude fibre content of all the studied vegetables which reveals that *Tupistra aurantiaca* (6.56%±0.04) contains highest fibre content among all the studied vegetables followed by fruits of *Solanum aethiopicum* (3.35%±0.16) and *Solanum macrocarpon* (2.66% ±0.21). *Nasturtium officinale* (1.66% ±0.02) was found to be containing lowest amount of crude fibre which was statistically at par with *Capsicum annuum* var. *cerasiformae* (1.42% ±0.16).

4.3.8 Total Carbohydrate (%)

The total carbohydrate content of all the studied vegetables are represented in Figure 4.3.8 and found significantly different. *Tupistra aurantiaca* (41.83% ±0.08) had highest content of total carbohydrate among all the studied vegetables followed by *Nasturtium officinale* (8.73% ±0.23). The total carbohydrate content of *Solanum aethiopicum* (7.11%±0.05) was statistically at par with the value of *Solanum macrocarpon* (6.59% ±0.28). The lowest amount of total carbohydrate was recorded in *Capsicum annuum* var. *cerasiformae* (4.29% ±1.61).

4.3.9 Total Starch (%)

The total starch content of all the studied vegetables were found to be significantly different and depicted in Figure 4.3.9. The total starch content of fruits of *Solanum aethiopicum* (0.84%±0.03) was found highest among all the five indigenous selected vegetables followed by *Solanum macrocarpon* (0.64% ±0.02), *Nasturtium officinale* (0.39% ±0.01) and *Capsicum annuum* var. *cerasiformae* (0.17% ±0.07) while it was observed lowest in *Tupistra aurantiaca* (0.01% ±0.00).

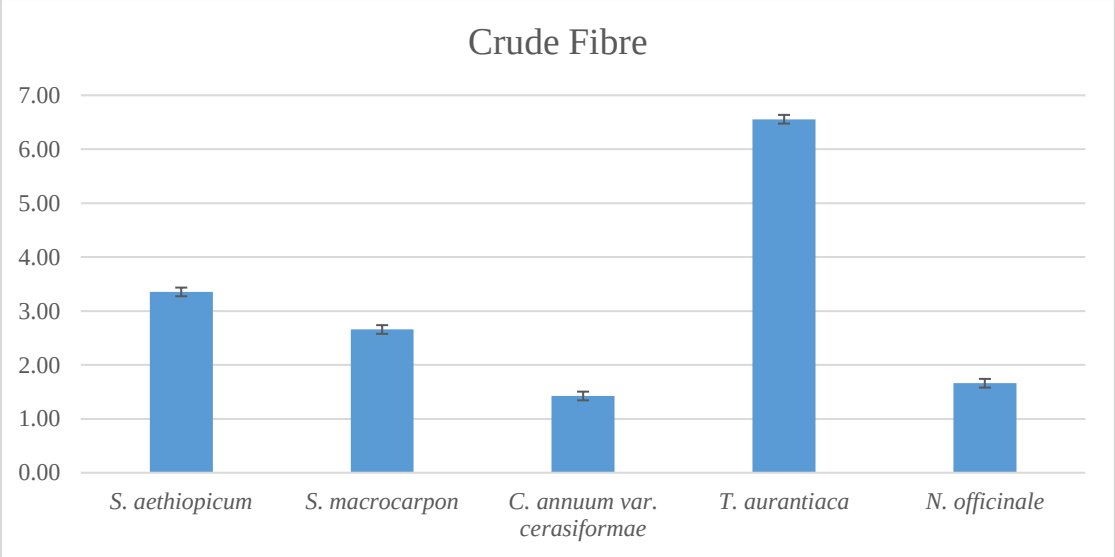

Figure 4.3.7 Crude Fibre content (%) of vegetables

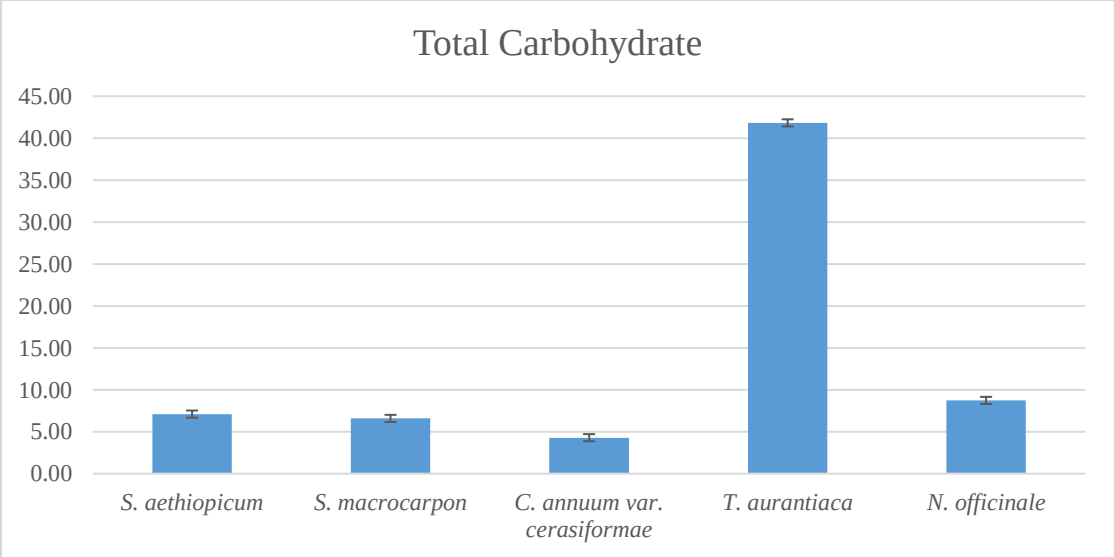

Figure 4.3.8 Total Carbohydrate content (%) of vegetables

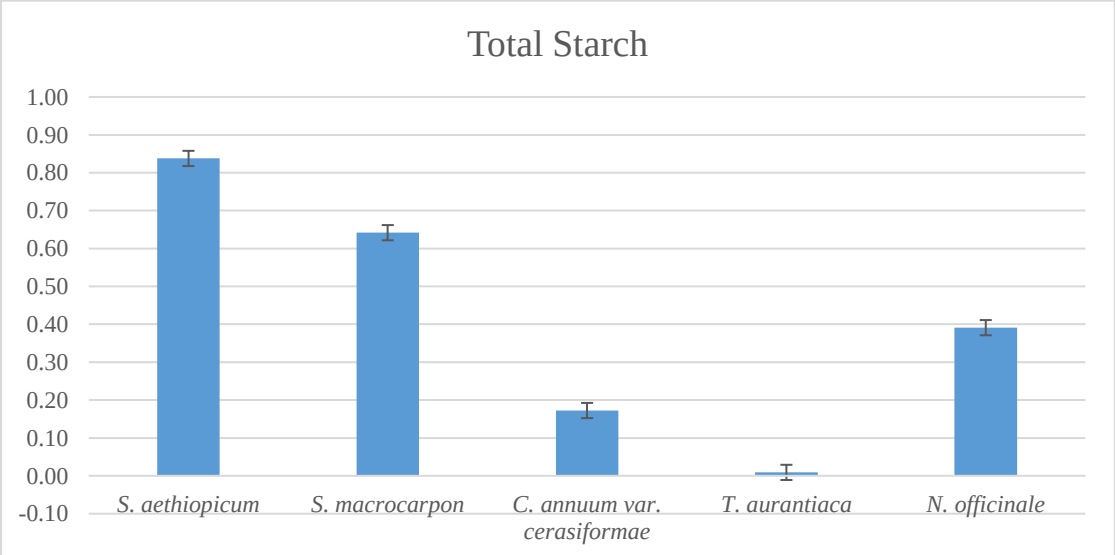

Figure 4.3.9 Total Starch content (%) of vegetables

4.3.10 Total Sugar (%)

The Figure 4.3.10 represents the total sugar content of all the studied vegetables which was found significantly different. The total sugar content was recorded maximum in the leaves of *Nasturtium officinale* (5.25% ±0.03) which was statistically at par with the total sugar content of *Solanum macrocarpon* (5.22% ±0.05) and *Solanum aethiopicum* (4.93%±0.02). The minimum sugar content was observed in *Capsicum annuum* var. *cerasiformae* (2.57% ±0.05) which was statistically at par with the total sugar content of *Tupistra aurantiaca* (2.69% ±0.49).

4.3.11 Chlorophyll A (mg $100g^{-1}$)

The chlorophyll content of all the studied vegetables were found to be significantly different and data are depicted in Figure 4.3.11. *Capsicum annuum* var. *cerasiformae* (9.50 mg $100g^{-1}$ ± 0.03) exhibited maximum amount of chlorophyll A content followed by *Nasturtium officinale* (6.79 mg $100g^{-1}$ ± 0.03), *Tupistra aurantiaca* (1.40 mg $100g^{-1}$ ± 0.10) and *Solanum aethiopicum* (0.97 mg $100g^{-1}$ ± 0.03). The minimum amount of chlorophyll A content was found in fruits of *Solanum macrocarpon* (0.17 mg $100g^{-1}$ ± 0.02).

4.3.12 Chlorophyll B (mg $100g^{-1}$)

The figure 4.3.12 depicts the significantly different chlorophyll B content of all the five studied vegetables which reveals that *Capsicum annuum* var. *cerasiformae* (15.43 mg $100g^{-1}$ ± 0.04) exhibited maximum amount of chlorophyll B followed by *Nasturtium officinale* (11.00 mg $100g^{-1}$ ± 0.04), *Tupistra aurantiaca* (8.45 mg $100g^{-1}$ ± 0.03) and *Solanum aethiopicum* (6.23 mg $100g^{-1}$ ± 0.02). While, chlorophyll B

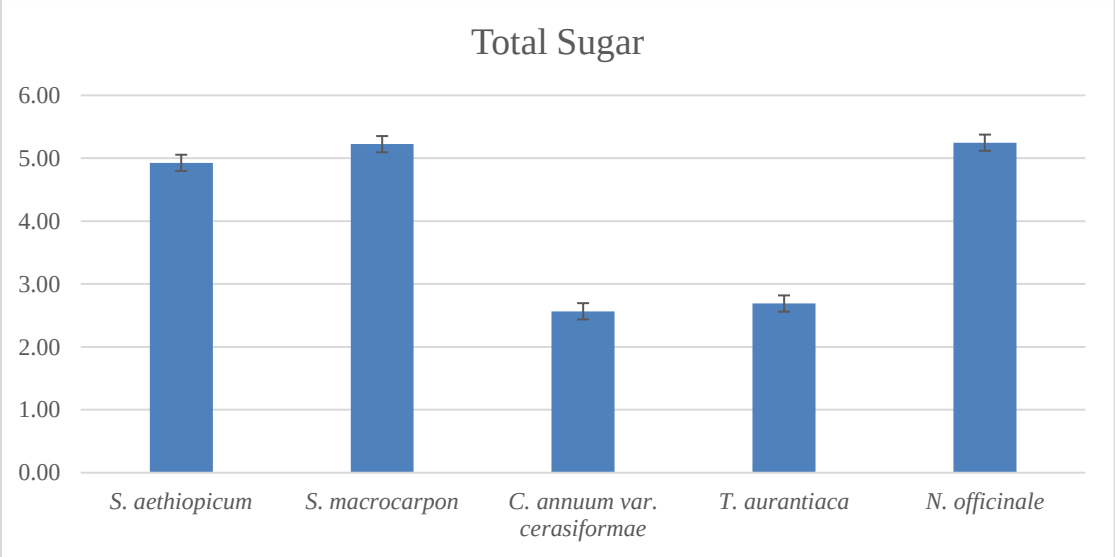

Figure 4.3.10 Total Sugar content (%) of vegetables

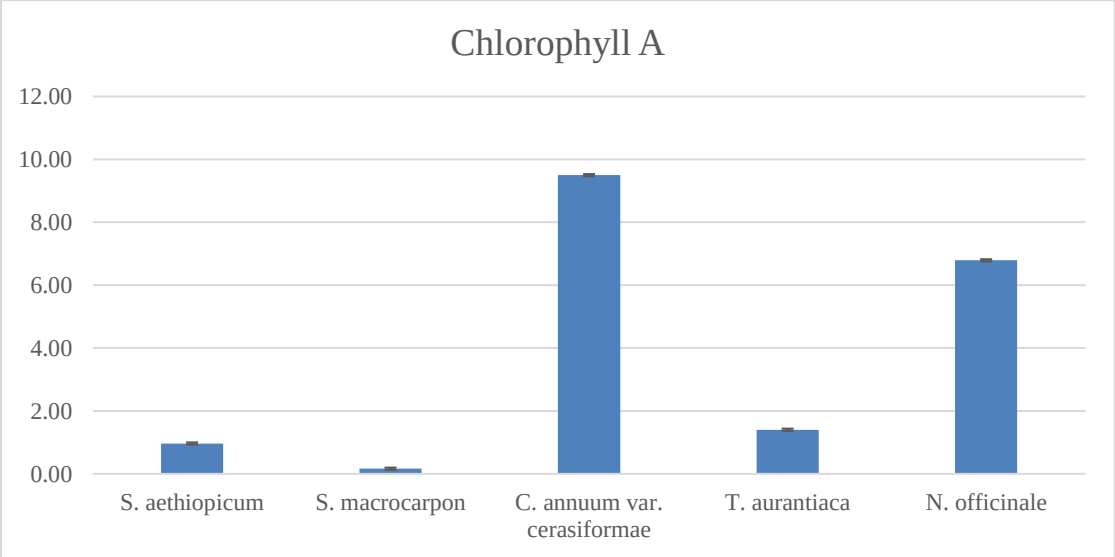

Figure 4.3.11 Chlorophyll A content (mg $100g^{-1}$) of vegetables

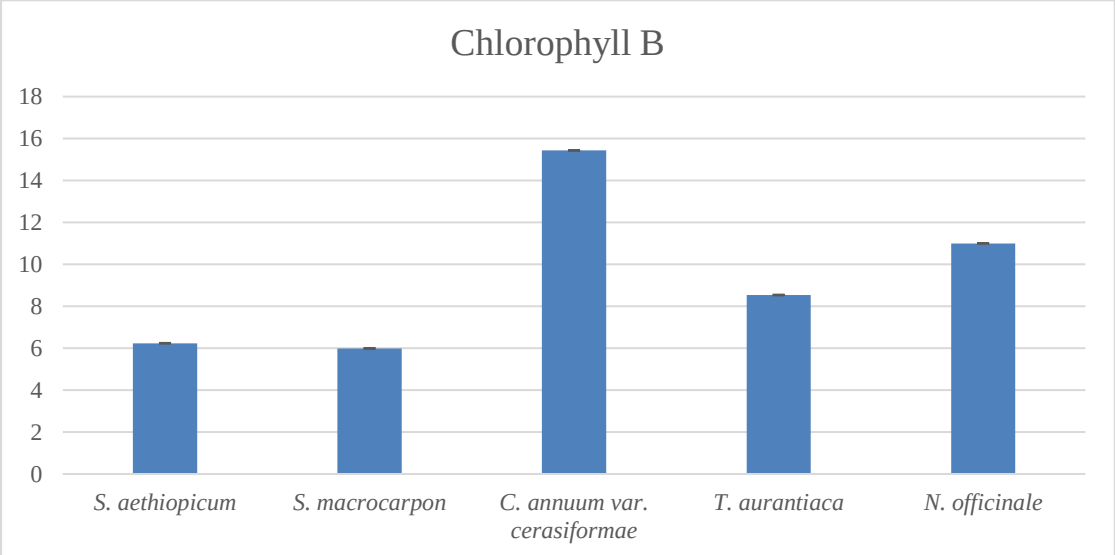

Figure 4.3.12 Chlorophyll B content (mg $100g^{-1}$) of vegetables

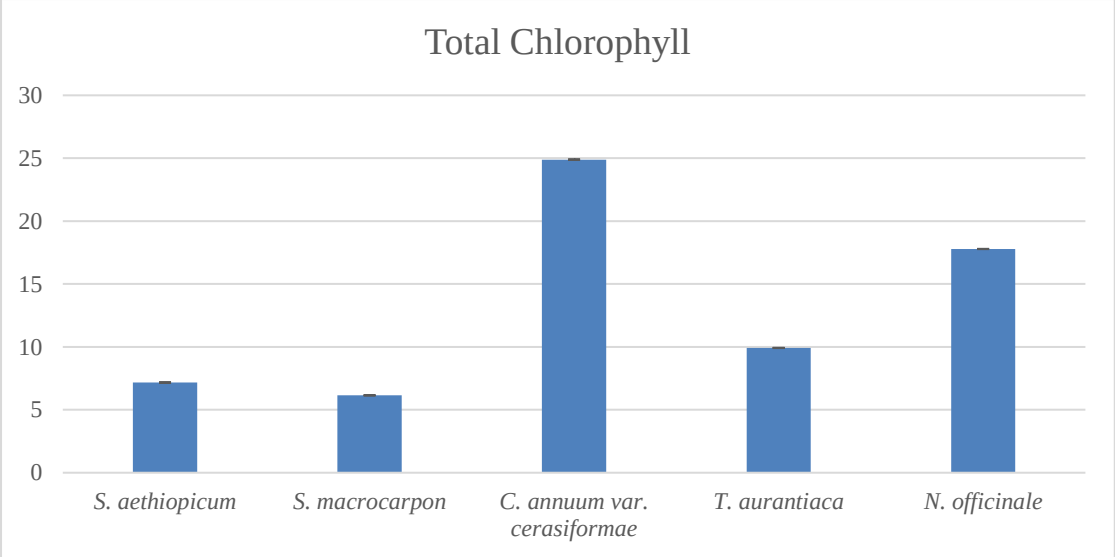

Figure 4.3.13 Total Chlorophyll content (mg $100g^{-1}$) of vegetables

content was found to be lowest in fruits of *Solanum macrocarpon* (5.99 mg $100g^{-1}$ ± 0.02).

4.3.13 Total Chlorophyll (mg $100g^{-1}$)

The total chlorophyll content of all the studied vegetables were found significantly different and presented in Figure 4.3.13. The total chlorophyll content among all the studied vegetables was found highest in *Capsicum annuum* var. *cerasiformae* (24.89 mg $100g^{-1}$ ± 0.02) followed by *Nasturtium officinale* (17.79 mg $100g^{-1}$ ± 0.02), *Tupistra aurantiaca* (9.92 mg $100g^{-1}$ ±0.01) and *Solanum aethiopicum* (7.17 mg $100g^{-1}$ ± 0.03). The fruits of *Solanum macrocarpon* (6.15 mg $100g^{-1}$ ± 0.03) was recorded to contain the lowest amount of total Chlorophyll.

4.4 Multi-elemental profiling

Essential elements including macro and micro (K, Ca, Mg, P, Fe, Mn, Zn, Cu, Mo and S)**,** trace elements (Al, Co, Li, Na and Sr) and some heavy metals (Pb, Sn, Cd and Cr) were analysed for the collected samples of the selected vegetables. Analysis was done using the ICP-MS and following the methodologies described in Chapter III.

4.4.1 Essential Elements

The concentration of essential elements of all the five studied vegetables are tabulated in Table 4.4.1 and described in following sub headings:

4.4.1.1 Potassium (K) (mg $100g^{-1}$ dry weight)

The potassium content of all the five vegetables are depicted in Figure 4.4.1. Potassium content was found highest in the fruit samples of *Solanum aethiopicum*

Table 4.4.1. Essential elements content (mg 100g^{-1} dry weight) of the selected vegetables

	K	Ca	Mg	P	Fe	Mn	Cu	Mo	S	Zn
S. aethiopicum	630.99	59.42	92.89	38.56	4.37	3.27	6.38	0.23	0.78	2.87
S. macrocarpon	467.53	65.72	55.00	31.49	5.24	8.30	7.07	0.14	3.09	6.67
C. annuum var. cerasiformae	246.15	27.41	13.64	77.43	9.82	0.74	0.16	2.18	3.98	0.51
T. aurantiaca	561.61	11.30	86.82	110.88	42.33	26.24	52.63	2.70	3.042	2.38
N. officinale	573.43	27.01	18.51	68.87	8.35	5.79	6.50	0.29	3.94	0.93
Highest RDA	4700 mg	1000 mg	420 mg	700 mg	18 mg	2.3 mg	0.9 mg	0.045 mg	NA	11 mg
Upper Tolerance limit	NE	2500 mg	350 mg	4000mg	45 mg	11 mg	10 mg	2 mg	NA	40 mg

Table 4.4.2. Trace elements content (mg 100g^{-1} dry weight) of the selected vegetables

	Al	Na	Sr	Co	Li
S. aethiopicum	1.60	2.43	1.03	0.06	0.24
S. macrocarpon	2.40	2.26	2.14	0.06	0.37
C. annuum var. cerasiformae	6.41	25.66	17.76	0.67	2.23
T. aurantiaca	14.85	19.06	14.43	0.52	1.61
N. officinale	3.81	3.18	1.38	0.08	0.31
Highest RDA	NA	1.5 g	NA	NA	NA
Upper Tolerance limit	NA	2.3 g	NA	NA	NA

(630.99 mg $100g^{-1}$ DW) followed by leaves of *Nasturtium officinale* (573.43 mg $100g^{-1}$ DW), inflorescence of *Tupistra aurantiaca* (561.61 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (467.53 mg $100g^{-1}$ DW). While, it was recorded lowest in (246.15 mg $100g^{-1}$ DW). All the mentioned data are significant different at $p < 0.05$.

4.4.1.2 Calcium (Ca) (mg $100g^{-1}$ dry weight)

The calcium content of the studied vegetables are represented by Figure 4.4.2. The calcium content was found in the wide range of 11.30 to 65.72 mg $100g^{-1}$ DW with statistically significant ($p<0.05$) variation amongst the vegetables. The calcium content was recorded highest in the fruit samples of *Solanum macrocarpon* (65.72 mg $100g^{-1}$ DW) followed by fruit samples of *Solanum aethiopicum* (59.42 mg $100g^{-1}$ DW), *Capsicum annuum* var. *cerasiformae* (27.41 mg $100g^{-1}$ DW), leaves of *Nasturtium officinale* (27.01 mg $100g^{-1}$ DW) and the least value was recorded for inflorescence of *Tupistra aurantiaca* (11.30 mg $100g^{-1}$ DW).

4.4.1.3 Magnesium (Mg) (mg $100g^{-1}$ dry weight)

The magnesium content was recorded in the range of 13.64 to 92.89 mg $100g^{-1}$ DW and represented in Figure 4.4.2. The significantly ($p<0.05$) different data of the samples for *Solanum aethiopicum, Tupistra aurantiaca, Solanum macrocarpon, Nasturtium officinale, Capsicum annuum* var. *cerasiformae* was estimated to the tune of 92.89, 86.82, 55.00, 18.51 and 13.64 mg $100g^{-1}$ DW, respectively. The content in each vegetables were tested significant ($p< 0.05$) statistically.

4.4.1.4 Phosphorus (P) (mg $100g^{1}$ dry weight)

The phosphorus content for all the samples is graphically presented in Figure 4.4.2. It is quite interesting to note that the samples of *Tupistra aurantiaca* contain

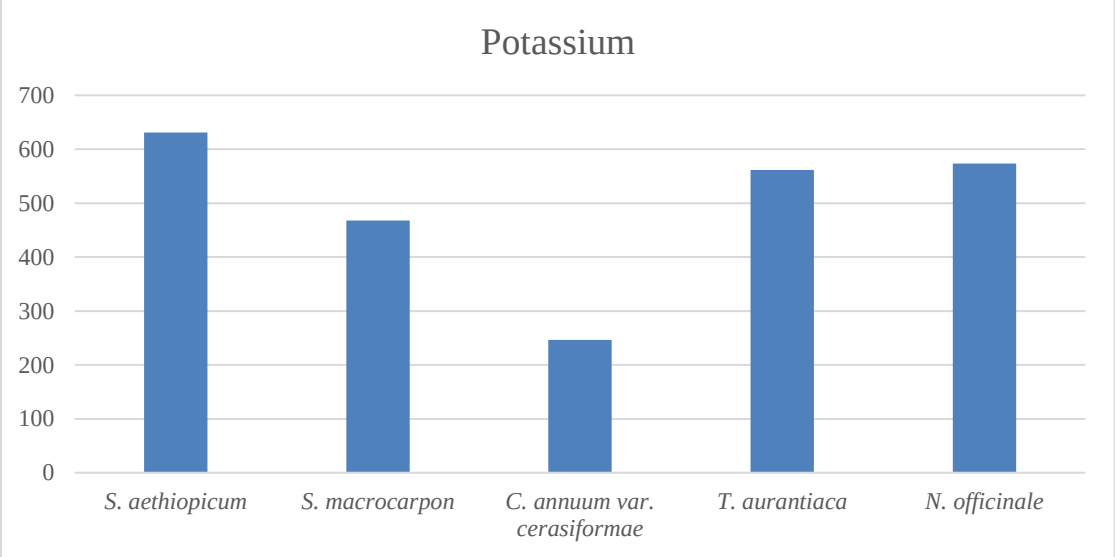

Figure 4.4.1 Potassium content (mg 100g^{-1}) of vegetables

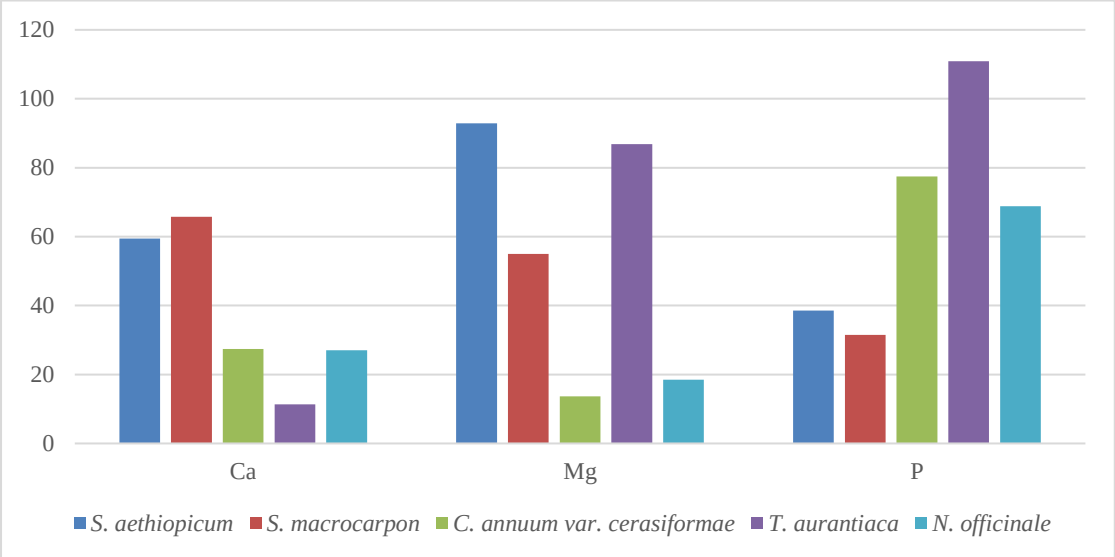

Figure 4.4.2. Calcium, magnesium & phosphorus content (mg 100g^{-1}) of vegetables

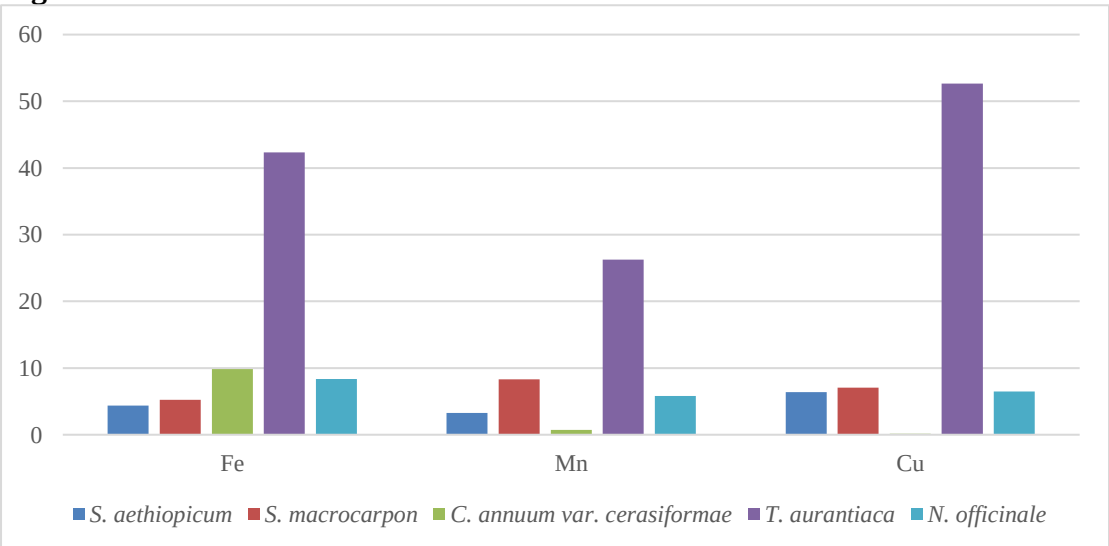

Figure 4.4.3 Iron, Manganese and Copper content (mg 100g^{-1}) of vegetables

highest phosphorus (110.88 mg $100g^{-1}$ DW) followed by followed by *Capsicum annuum* var. *cerasiformae* (77.43 mg $100g^{-1}$ DW), *Nasturtium officinale* (68.87 mg $100g^{-1}$ DW) and *Solanum aethiopicum* (38.56 mg $100g^{-1}$ DW). The lowest phosphorus content was observed in fruits of *Solanum macrocarpon* (31.49 mg $100g^{-1}$ DW).

4.4.1.5 Iron (Fe) (mg $100g^{-1}$ dry weight)

The iron content of the studied vegetables is illustrated in Figure 4.4.3. *Tupistra aurantiaca* (42.33 mg $100g^{-1}$ DW) exhibited the maximum content of iron among all the studied vegetables followed by *Capsicum annuum* var. *cerasiformae* (9.82 mg $100g^{-1}$ DW), *Nasturtium officinale* (8.35 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (5.24 mg $100g^{-1}$ DW), while minimum amount of iron content was observed in the fruits *Solanum aethiopicum* (4.37 mg $100g^{-1}$ DW). All the data were found to be significant different ($p < 0.05$).

4.4.1.6 Manganese (Mn) (mg $100g^{-1}$ dry weight)

The manganese content was more than thrice in *Tupistra aurantiaca* (26.24 mg $100g^{-1}$ DW) than other vegetables under study. Though in smaller quantity, manganese was higher in *Solanum macrocarpon* (8.30 mg $100g^{-1}$ DW) followed by *Nasturtium officinale* (5.79 mg $100g^{-1}$ DW), *Solanum aethiopicum* (3.27 mg $100g^{-1}$ DW) and the lowest in *Capsicum annuum* var. *cerasiformae* (0.74 mg $100g^{-1}$ DW). All data were analysed to be statistically different ($p < 0.05$) (Figure 4.4.3).

4.4.1.7 Copper (Cu) (mg $100g^{-1}$ dry weight)

The copper content of all the studied vegetables are shown in Figure 4.4.3. The copper content was recorded highest in inflorescence of *Tupistra aurantiaca*

(52.63 mg $100g^{-1}$ DW) which is more than seven times than that recorded for fruits of *Solanum macrocarpon* (7.07 mg $100g^{-1}$ DW), leaves of *Nasturtium officinale* (6.50 mg $100g^{-1}$ DW) and the fruits of *Solanum aethiopicum* (6.38 mg $100g^{-1}$ DW). However, the lowest and only the trace was found in *Capsicum annuum* var. *cerasiformae* (0.16 mg $100g^{-1}$ DW). All data were found to be statistically different ($p < 0.05$).

4.4.1.8 Molybdenum (Mo) (mg $100g^{-1}$ dry weight)

The figure 4.4.4 displays the molybdenum content of all the studied vegetables. *Tupistra aurantiaca* again topped the list of the studied vegetable being rich in molybdenum content (2.70 mg $100g^{-1}$ DW) followed by *Capsicum annuum* var. *cerasiformae, Nasturtium officinale, Solanum aethiopicum* and *Solanum macrocarpon* with 2.18, 0.29, 0.23 and 0.14 mg molybdenum per 100g DW of the samples, respectively.

4.4.1.9 Sulphur (S) (mg $100g^{-1}$ dry weight)

Figure 4.4.4 depicts the sulphur content in different vegetables. Higher but non-significantly different data were recorded in *Capsicum annuum* var. *cerasiformae* (3.98 mg $100g^{-1}$ DW) and *Nasturtium officinale* (3.94 mg $100g^{-1}$ DW) followed by *Solanum macrocarpon* (3.09 mg $100g^{-1}$ DW) and *Tupistra aurantiaca* (3.04 mg $100g^{-1}$ DW). While, *Solanum aethiopicum* (0.78 mg $100g^{-1}$ DW) has least content of sulphur.

4.4.1.10 Zinc (Zn) (mg $100g^{-1}$ dry weight)

The zinc content of all the studied vegetables are graphically presented in Figure 4.4.4. The maximum content of Zinc was recorded in fruits of *Solanum*

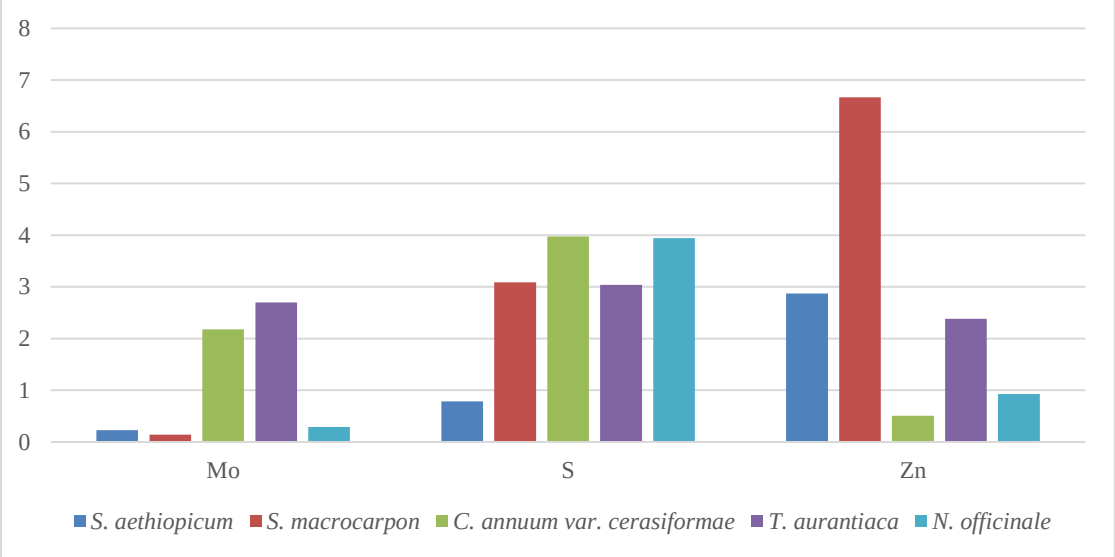

Figure 4.4.4 Molybdenum, Sulphur and Zinc content (mg 100g^{-1}) of vegetables

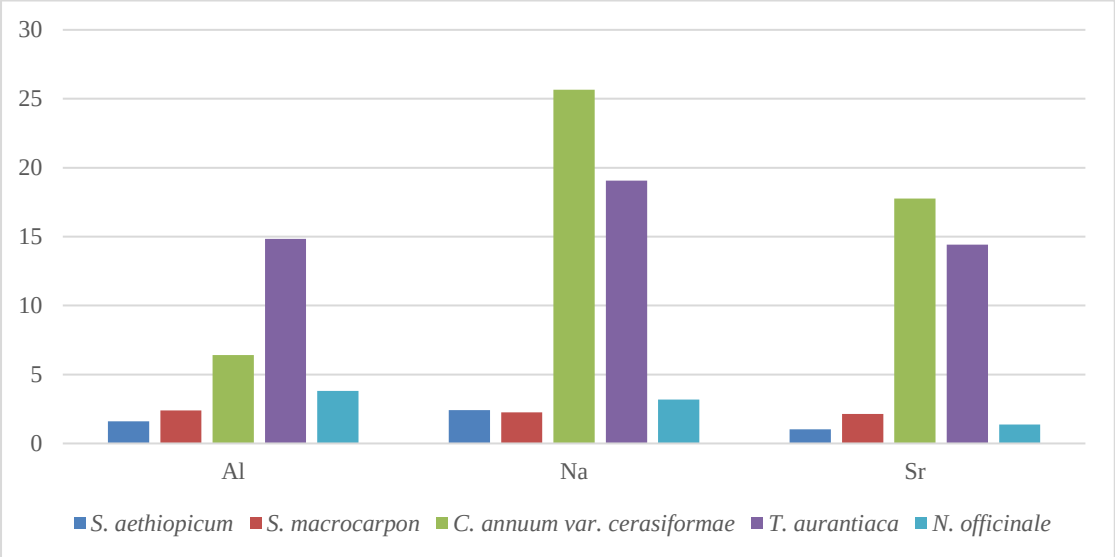

Figure 4.4.5 Aluminium, Sodium and Strontium content (mg 100g^{-1}) of vegetables

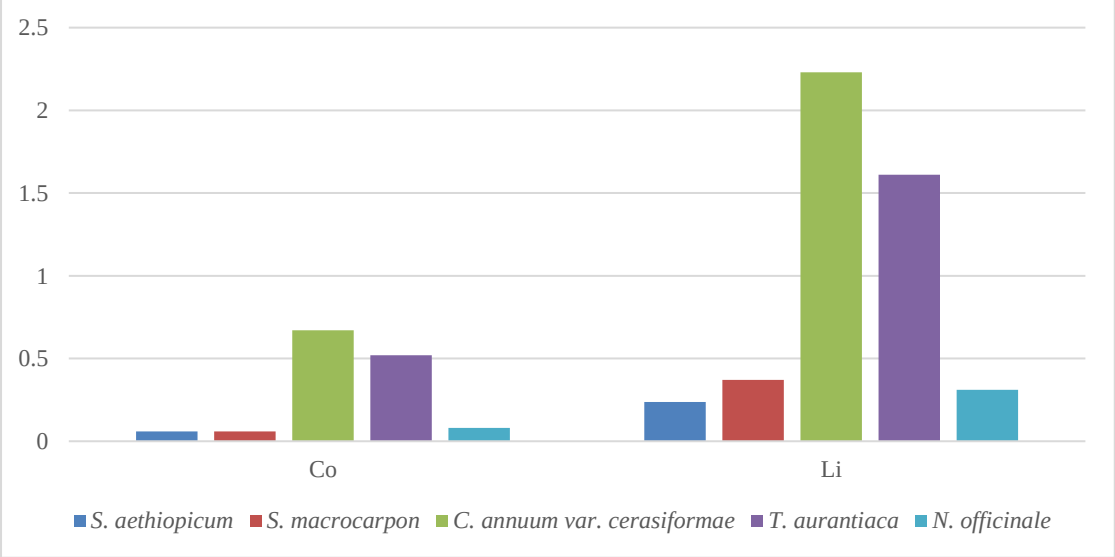

Figure 4.4.6 Cobalt and Lithium content (mg 100g^{-1}) of vegetables

macrocarpon (6.67 mg $100g^{-1}$ DW) followed by *Solanum aethiopicum* (2.87 mg $100g^{-1}$ DW) and *Tupistra aurantiaca* (2.83 mg $100g^{-1}$ DW) and *Nasturtium officinale* (0.93 mg $100g^{-1}$ DW) whereas lowest content was found in *Capsicum annuum* var. *cerasiformae* (0.51 mg $100g^{-1}$ DW).

4.4.2 Trace elements

The concentration of trace elements of all the five studied vegetables are tabulated in Table 4.4.2.

4.4.2.1 Aluminium (Al) (mg $100g^{-1}$ dry weight)

The aluminium content of all the studied vegetables are presented in Figure 4.4.5. *Tupistra aurantiaca* (14.85 mg $100g^{-1}$ DW) samples were tested to have highest content of aluminium among all the studied vegetables followed by *Capsicum annuum* var. *cerasiformae* (6.41 mg $100g^{-1}$ DW) and *Nasturtium officinale* (3.81 mg $100g^{-1}$ DW). The lowest amount of aluminium was found in fruits of *Solanum aethiopicum* (1.60 mg $100g^{-1}$ DW) followed by *Solanum macrocarpon* (2.4 mg $100g^{-1}$ DW). All the data are were significantly different (p <0.05).

4.4.2.2 Sodium (Na) (mg $100g^{-1}$ dry weight)

The sodium content of all the studied vegetables are depicted in Figure 4.4.5. *Capsicum annuum* var. *cerasiformae* (25.66 mg $100g^{-1}$ DW) was found to have rich contents of sodium. The value descends for *Tupistra aurantiaca* (19.06 mg $100g^{-1}$ DW), *Nasturtium officinale* (3.18 mg $100g^{-1}$ DW), *Solanum aethiopicum* (2.43 mg $100g^{-1}$ DW) and the lowest content of *Solanum macrocarpon* (2.26 mg $100g^{-1}$ DW).

4.4.2.3 Strontium (Sr) (mg $100g^{-1}$ dry weight)

The Figure 4.4.5 represents the strontium content of all the studied vegetables. The *Capsicum annuum* var. *cerasiformae* stands to have highest strontium content (17.76 mg $100g^{-1}$ DW) significantly higher than *Tupistra aurantiaca* (14.43 mg $100g^{-1}$ DW). Strontium content for *Solanum macrocarpon*, *Solanum aethiopicum* and *Nasturtium officinale* was 2.14, 1.38 and 1.03 mg $100g^{-1}$ DW respectively.

4.4.2.4 Cobalt (Co) (mg $100g^{-1}$ dry weight)

The cobalt content of all the studied vegetables are depicted in Figure 4.4.6. From the graph it was shown that cobalt content was highest in *Capsicum annuum* var. *cerasiformae* (0.67 mg $100g^{-1}$ DW) followed by *Tupistra aurantiaca* (0.52 mg $100g^{-1}$ DW). While it was found lowest in fruits of *Solanum aethiopicum* (0.06 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (0.06 mg $100g^{-1}$ DW) followed by *Nasturtium officinale* (0.08 mg $100g^{-1}$ DW).

4.4.2.5 Lithium (Li) (mg $100g^{-1}$ dry weight)

The lithium content of all the studied vegetables are presented in Figure 4.4.6. *Capsicum annuum* var. *cerasiformae* (2.23 mg $100g^{-1}$ DW) recorded highest amount of lithium followed by *Tupistra aurantiaca* (1.61 mg $100g^{-1}$ DW). The lithium content was found lowest in *Solanum aethiopicum* (0.24 mg $100g^{-1}$ DW) followed by *Nasturtium officinale* (0.31 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (0.37 mg $100g^{-1}$ DW).

Table 4.4.3. Heavy elements content of the selected vegetables

	Pb (ppm)	**FSSAI permissible limits**	**Sn**	**Cd (ppm)**	**FSSAI permissible limits**	**Cr**
S. aethiopicum	0.02	Fruit vegetable 0.1 ppm	ND	0.010	Fruit vegetable 0.05 ppm	ND
S. macrocarpon	0.03	Fruit vegetable 0.1 ppm	ND	0.005	Fruit vegetable 0.05 ppm	ND
C. annuum var. cerasiformae	0.08	Fruit vegetable 0.1 ppm	ND	0.003	Fruit vegetable 0.05 ppm	ND
T. aurantiaca	0.08	NA	ND	0.004	NA	ND
N. officinale	0.02	Leafy vegetable 0.3 ppm	ND	0.004	Leafy vegetable 0.2 ppm	ND

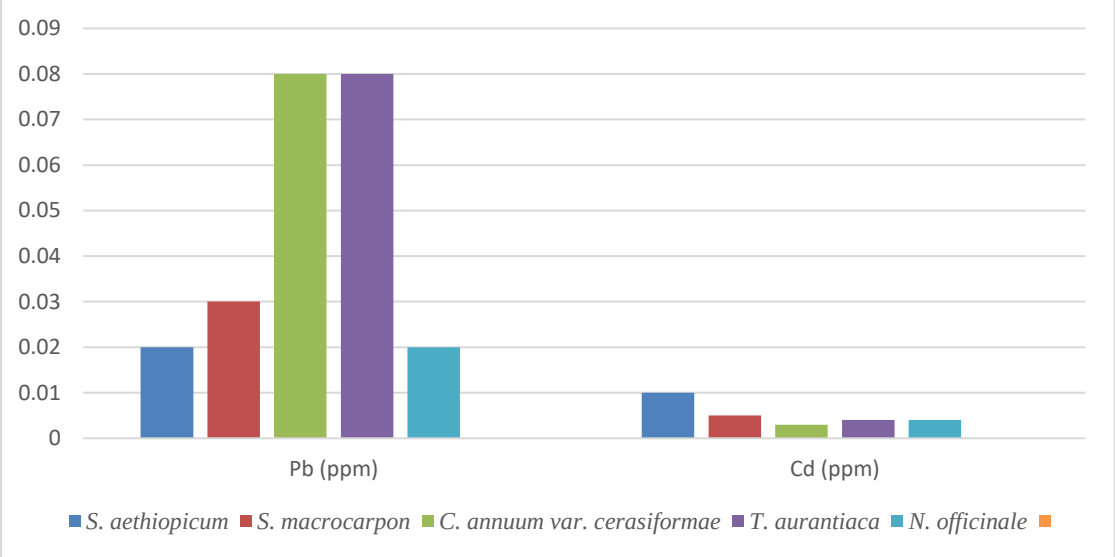

Figure 4.4.7 Lead and Cadmium content of vegetables

4.4.3 Heavy metals

All the vegetables selected for this investigation were subjected to analysis for concentration of heavy metals viz. lead, tin, cadmium and chromium. The data of heavy metal are shown in Table 4.4.3.

In all the investigated vegetables, lead was found to be in the range of 0.02 to 0.08 ppm (Figure 4.4.7) though the highest amount of lead was recorded in *Capsicum annuum* var. *cerasiformae* (0.08 ppm) and *Tupistra aurantiaca* (0.08 ppm) followed by *Solanum macrocarpon* (0.03 ppm), *Solanum aethiopicum* (0.02 ppm) and *Nasturtium officinale* (0.02 ppm).

Tin and Chromium were not detectable during analysis which means they are either not present in samples or present in very low amount that could not be detected.

The cadmium content in all the vegetables was found to be in very low quantity and presented in Figure 4.4.7. Cadmium in *Solanum aethiopicum*, *Solanum macrocarpon*, *Tupistra aurantiaca*, *Nasturtium officinale* and *Capsicum annuum* var. *cerasiformae* was 0.010 ppm, 0.005 ppm, 0.004 ppm, 0.004 ppm and 0.003ppm, respectively.

4.5 Phytochemical content

Total phenol, total flavonoid, total flavonols, ascorbic acid content and carotene content were analysed for all the studied vegetables. For total phenol, total flavonoid and total flavonols methanol (80%) extracts were used as sample. The mean data of all phytochemical content along with respective SD value are tabulated in Table 4.5.

Table 4.5. Phytochemical contents of the selected vegetables

	Total Phenol (mg g^{-1} GAE DW)	**Flavonoid (mg g^{-1} RUE DW)**	**Flavonols (mg g^{-1} RUE DW)**	**Ascorbic Acid (%)**	**Carotene (mg $100g^{-1}$)**
S. aethiopicum	3.89^{b}±0.11	27.59^{b}±1.59	9.07^{b}±0.16	0.46^{b}±0.03	0.15^{d}±0.01
S. macrocarpon	1.03^{e}±0.08	12.69^{d}±0.60	4.35^{e}±0.37	0.78^{a}±0.02	0.08^{e}±0.01
C. annuum var. cerasiformae	11.31^{a}±0.03	54.66^{a}±1.59	5.36^{d}±0.15	0.26^{c}±0.03	0.60^{a}±0.02
T. aurantiaca	3.55^{c}±0.11	26.37^{b}±0.21	6.22^{c}±0.11	0.44^{b}±0.02	0.41^{b}±0.02
N. officinale	3.07^{d}±0.12	24.61^{c}±0.82	20.44^{a}±0.12	0.12^{d}±0.02	0.38^{c}±0.01
Mean	4.57	29.18	9.09	2.05	1.63
CV %	2.09	3.79	2.24	5.74	4.88
SEm±	0.06	0.64	0.12	0.01	0.92
SEđ	0.08	0.90	0.17	0.02	0.01

4.5.1 Total Phenols (mg g^{-1} GAE DW)

The Figure 4.5.1 represents significantly different total phenol content for different studied vegetables. The methanolic extract of *Capsicum annuum* var. *cerasiformae* (11.31 mg g^{-1} GAE DW±0.03) showed highest total phenolic content which is almost thrice of the total phenol content in *Solanum aethiopicum* (3.89 mg g^{-1} GAE DW±0.11), *Tupistra aurantiaca* (3.55 mg g^{-1} GAE DW±0.11) and *Nasturtium officinale* (3.07 mg g^{-1} GAE DW ±0.12) and almost ten times than present in *Solanum macrocarpon* (1.03 mg g^{-1} GAE DW ±0.08).

4.5.2 Total Flavonoid (mg g^{-1} RUE DW)

The total flavonoid content (Figure 4.5.2) followed the similar trend as that of total phenol wherein the methanolic extract of *Capsicum annuum* var. *cerasiformae* (54.66 mg g^{-1} RUE DW±1.59) had highest content of total flavonoid, which was almost double than that of *Solanum aethiopicum* (27.59 mg g^{-1} RUE DW ±1.59) and statistically at par value of *Tupistra aurantiaca* (26.37 mg g^{-1} RUE DW ±0.21) and significantly different data of *Nasturtium officinale* (24.61 mg g^{-1} RUE DW ±0.82). The lowest amount of total flavonoid content was recorded in *Solanum macrocarpon* (12.69 mg g^{-1} RUE DW ±0.60).

4.5.3 Total Flavonols (mg g^{-1} RUE DW)

As depicted in Figure 4.5.3. the total flavonols content were found to be significantly different for all the studied vegetables. The total flavonols was very high in *Nasturtium officinale* (20.44 mg g^{-1} RUE DW ±0.12) than *Solanum aethiopicum* (9.07 mg g^{-1} RUE DW ±0.16) and *Tupistra aurantiaca* (6.22 mg g^{-1} RUE DW ±0.11)

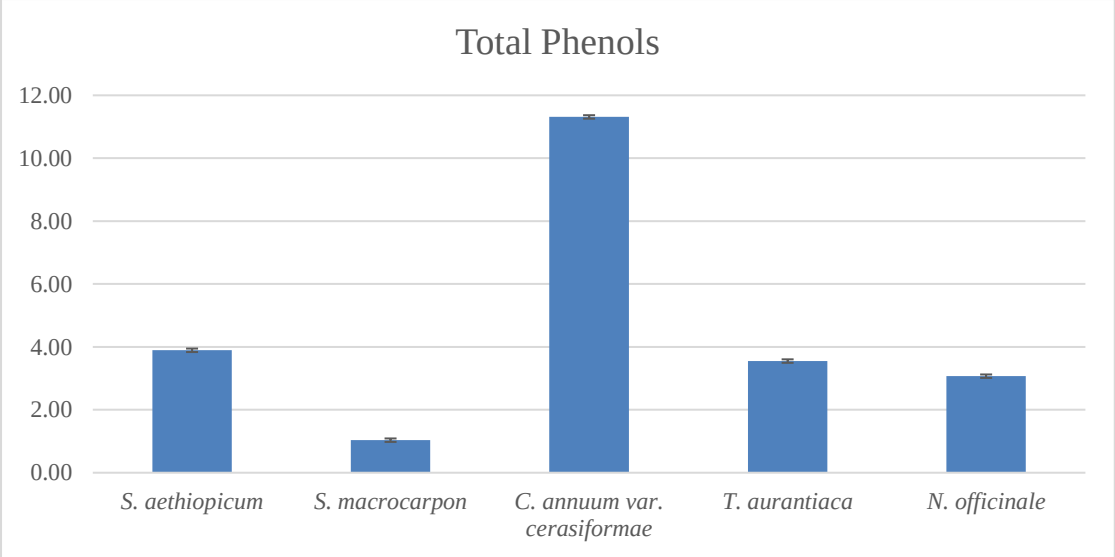

Figure 4.5.1 Total Phenol content (mg g^{-1} GAE DW) of vegetables

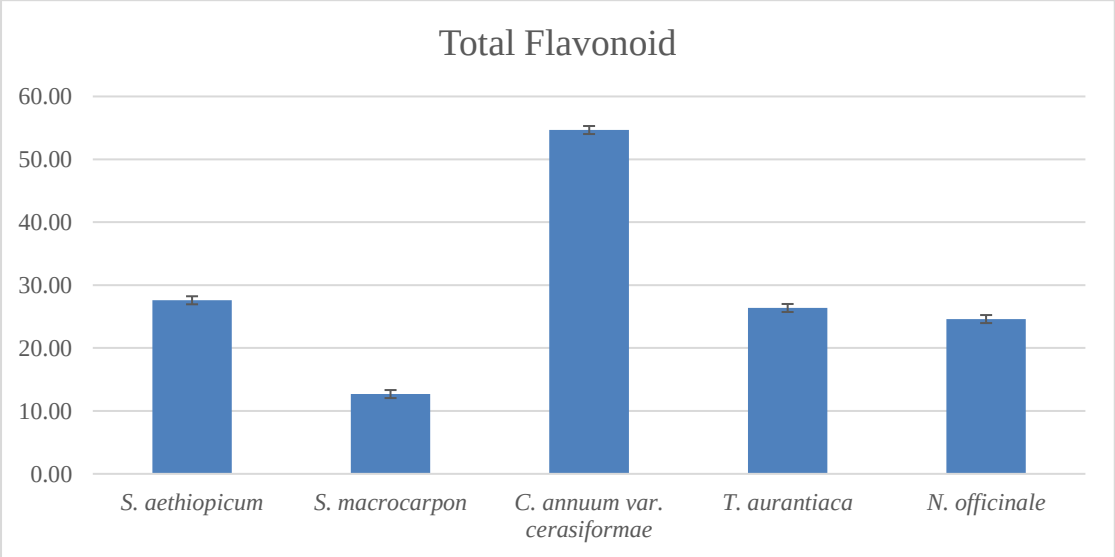

Figure 4.5.2 Total Flavonoid content (mg g^{-1} RUE DW) of vegetables

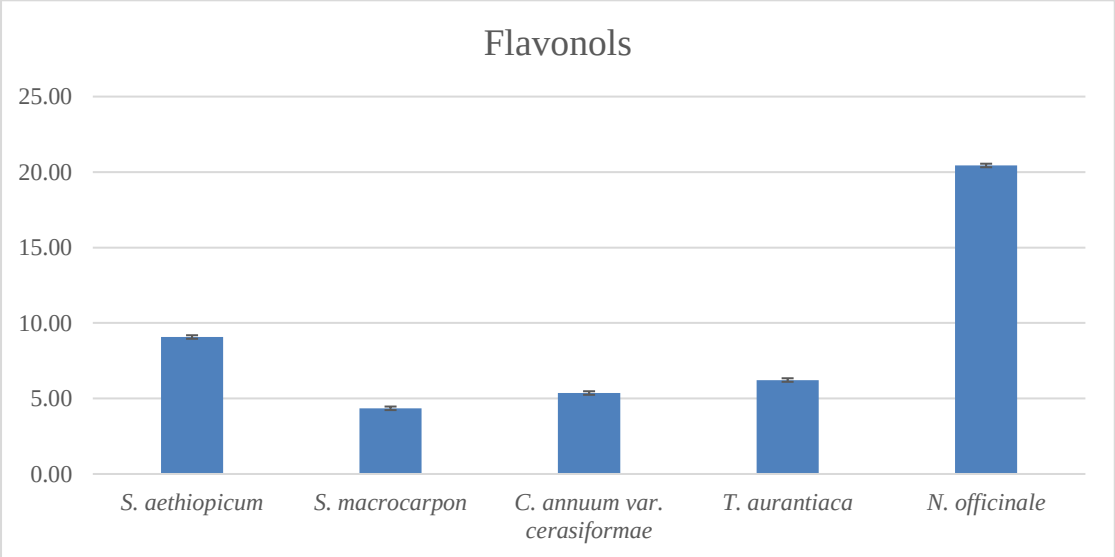

Figure 4.5.3 Total Flavonols content (mg g^{-1} RUE DW) of vegetables

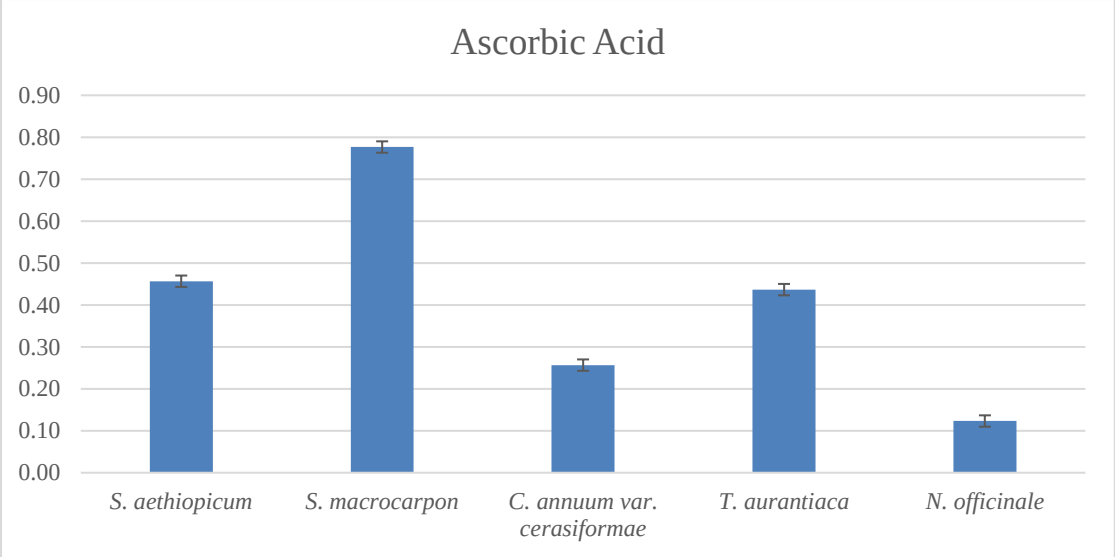

Figure 4.5.4 Ascorbic Acid content (%) of vegetables

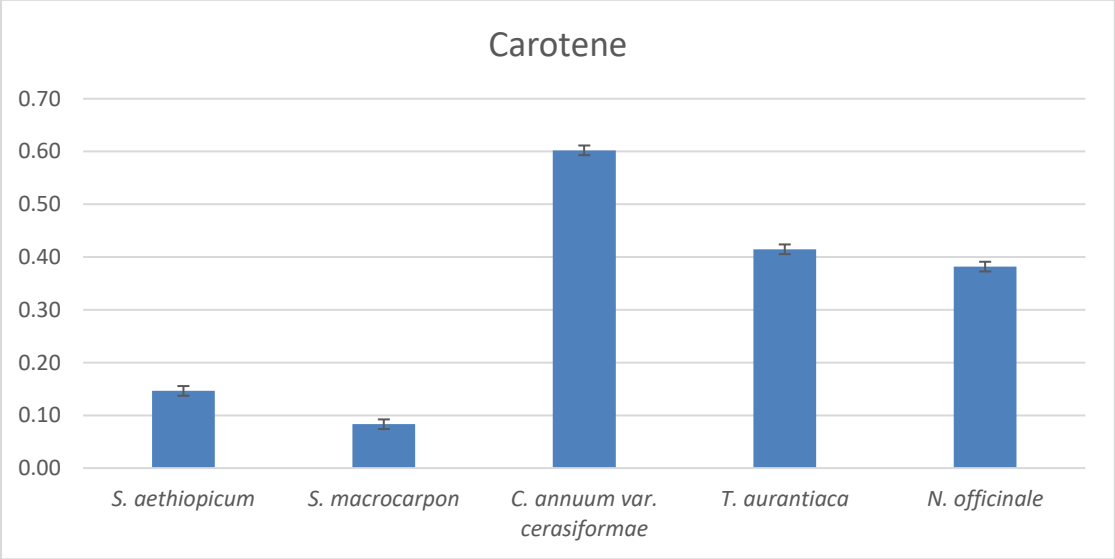

Figure 4.5.5 Carotene content (mg $100g^{-1}$) of vegetables

and *Capsicum annuum* var. *cerasiformae* (5.36 mg g^{-1} RUE DW ±0.15), while it was observed lowest in *Solanum macrocarpon* (4.35 mg g^{-1} RUE DW ±0.37).

4.5.4 Ascorbic Acid (%)

The Figure 4.5.4 represents the ascorbic acid content of all the studied vegetables which was found significantly different. The ascorbic acid content was recorded maximum in the fruits of *Solanum macrocarpon* (0.78% ±0.02) followed by *Solanum aethiopicum* (0.46%±0.03), *Tupistra aurantiaca* (0.44% ±0.02) and *Capsicum annuum* var. *cerasiformae* (0.26% ±0.03). The amount of ascorbic acid in *Solanum aethiopicum* was statistically at par with *Tupistra aurantiaca.* The minimum ascorbic acid content was observed in *Nasturtium officinale* (0.12% ±0.02).

4.5.5 Carotene (mg $100g^{-1}$)

The carotene content of all the studied vegetables were found to be significantly different and data is represented at Figure 4.5.5. *Capsicum annuum* var. *cerasiformae* (0.60 mg $100g^{-1}$±0.03) exhibited maximum amount of carotene content followed by *Tupistra aurantiaca* (0.41 mg $100g^{-1}$±0.02), *Nasturtium officinale* (0.38 mg $100g^{-1}$±0.01) and *Solanum aethiopicum* (0.15 mg $100g^{-1}$±0.01). The minimum amount of carotene content was found in fruits of *Solanum macrocarpon* (0.08 mg $100g^{-1}$±0.01).

4.6 Quantification of Phenols

Five different phenols were quantified in all the studied vegetables namely Gallic acid, Rutin, Catechol, ferulic acid and quercetin through reversed phase HPLC. The retention time of standards and samples are given in Table 4.6.1. The phenols

Table 4.6.1. Retention time of standards and vegetables for different phenols

	Standards	***S. aethiopicum***	***S. macrocarpon***	***C. annuum var. cerasiformae***	***T. aurantiaca***	***N. officinale***
Gallic Acid	5.62	5.841	5.717	5.777	4.913	5.801
Rutin	17.405	17.353	17.283	17.371	17.939	17.221
Catechol	18.703	18.808	18.800	18.851	18.725	18.735
Ferulic Acid	20.154	20.394	19.937	20.258	20.207	20.221
Quercetin	29.099	29.327	28.994	29.642	29.512	29.421

Table 4.6.2. Phenols content (mg $100g^{-1}$ DW) of the selected vegetables

	Gallic Acid	**Rutin**	**Catechol**	**Ferulic Acid**	**Quercetin**
S. aethiopicum	559.55	33.49	927.43	2.90	10.06
S. macrocarpon	468.31	33.02	238.38	6.09	51.17
C. annuum var. cerasiformae	1463.92	58.89	148.94	5.30	19.04
T. aurantiaca	161.03	27.65	187.29	3.20	12.71
N. officinale	1354.86	87.29	1759.54	7.95	10.52

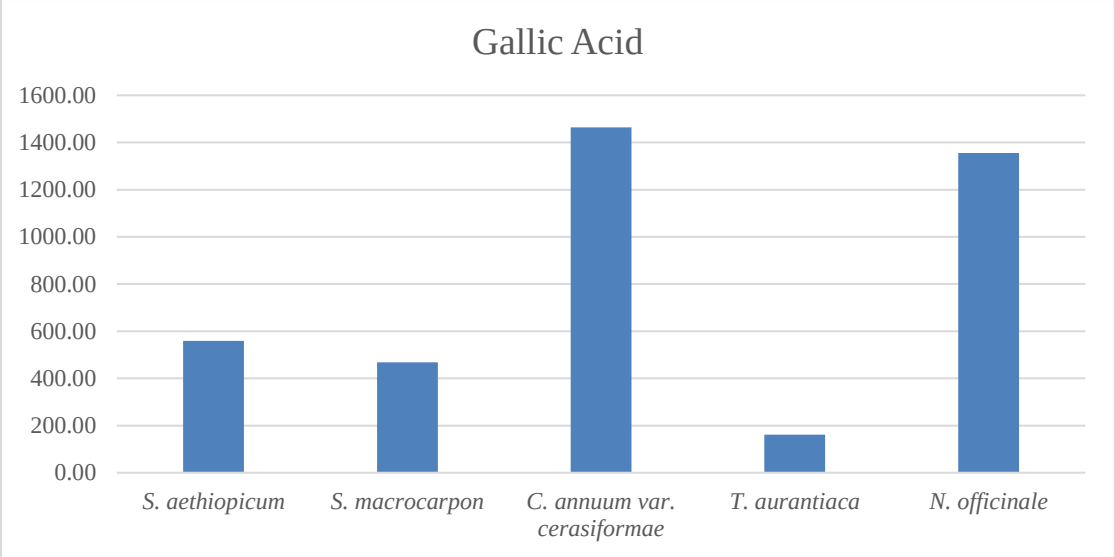

Figure 4.6.1 Gallic Acid content (mg 100g^{-1} DW) of vegetables

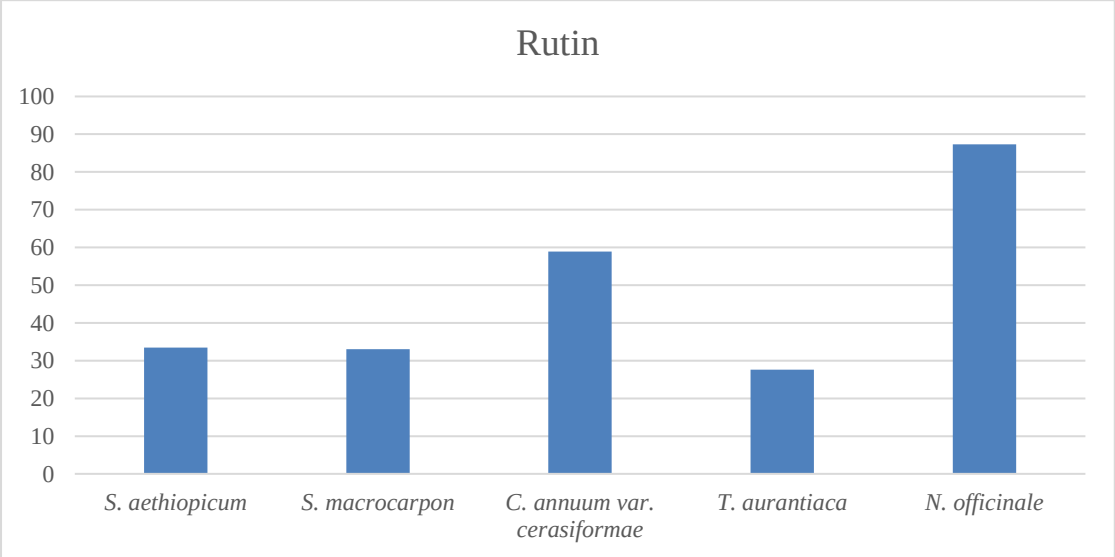

Figure 4.6.2 Rutin content (mg 100g^{-1} DW) of vegetables

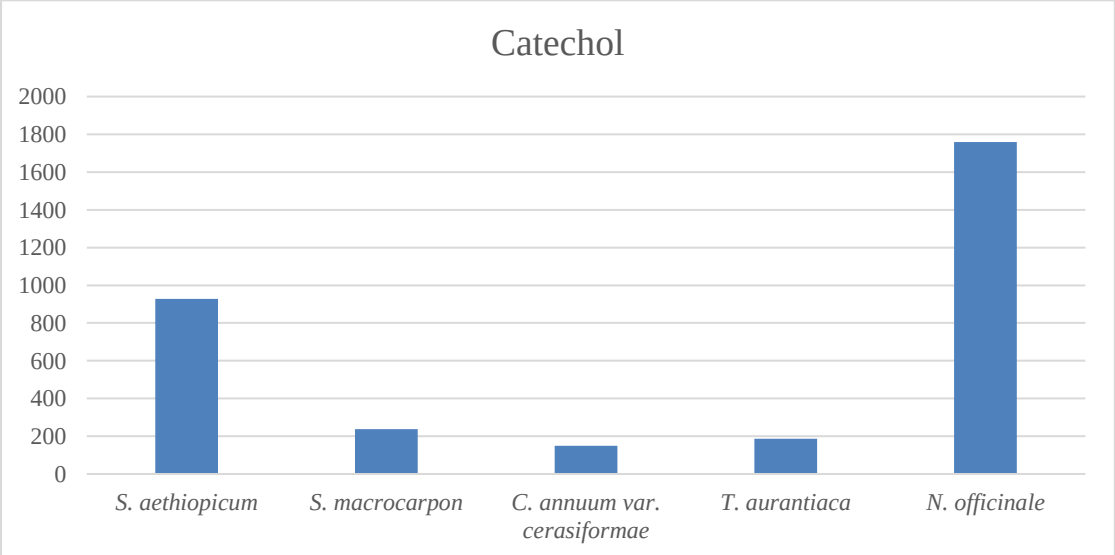

Figure 4.6.3 Catechol content (mg 100g^{-1} DW) of vegetables

content of all vegetables are tabulated in Table 4.6.2 and presented in respective figures.

4.6.1 Gallic Acid (mg $100g^{-1}$ dry weight)

The gallic acid concentration of all the studied vegetables are presented in Figure 4.6.1. *Capsicum annuum* var. *cerasiformae* (1463.92 mg $100g^{-1}$ DW) exhibited the highest content of gallic acid among all the studied vegetables followed by *Nasturtium officinale* (1354.86 mg $100g^{-1}$ DW), *Solanum aethiopicum* (559.55 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (468.31 mg $100g^{-1}$ DW). The lowest amount of gallic acid was found in fruits of *Tupistra aurantiaca* (161.03 mg $100g^{-1}$ DW).

4.6.2 Rutin (mg $100g^{-1}$ dry weight)

The rutin content is represented by Figure 4.6.2. which shows that the leaf samples of *Nasturtium officinale* (87.29 mg $100g^{-1}$ DW) was found to have highest rutin content followed by *Capsicum annuum* var. *cerasiformae* (58.89 mg $100g^{-1}$ DW) which was statistically at par value of *Solanum aethiopicum* (33.49 mg $100g^{-1}$ DW) and *Solanum macrocarpon* (33.02 mg $100g^{-1}$ DW). However, the lowest record was found in *Tupistra aurantiaca* (27.65 mg $100g^{-1}$ DW).

4.6.3 Catechol (mg $100g^{-1}$ dry weight)

. The catechol content was found to be highest in *Nasturtium officinale* (1759.54 mg $100g^{-1}$ DW), which was almost double of that recorded in samples of *Solanum aethiopicum* (927.44 mg $100g^{-1}$ DW). Low content of catechol was found in *Solanum macrocarpon* (238.38 mg $100g^{-1}$ DW), *Tupistra aurantiaca* (187.29 mg $100g^{-1}$ DW) and *Capsicum annuum* var. *cerasiformae* (148.94 mg $100g^{-1}$ DW) (Figure 4.6.3).

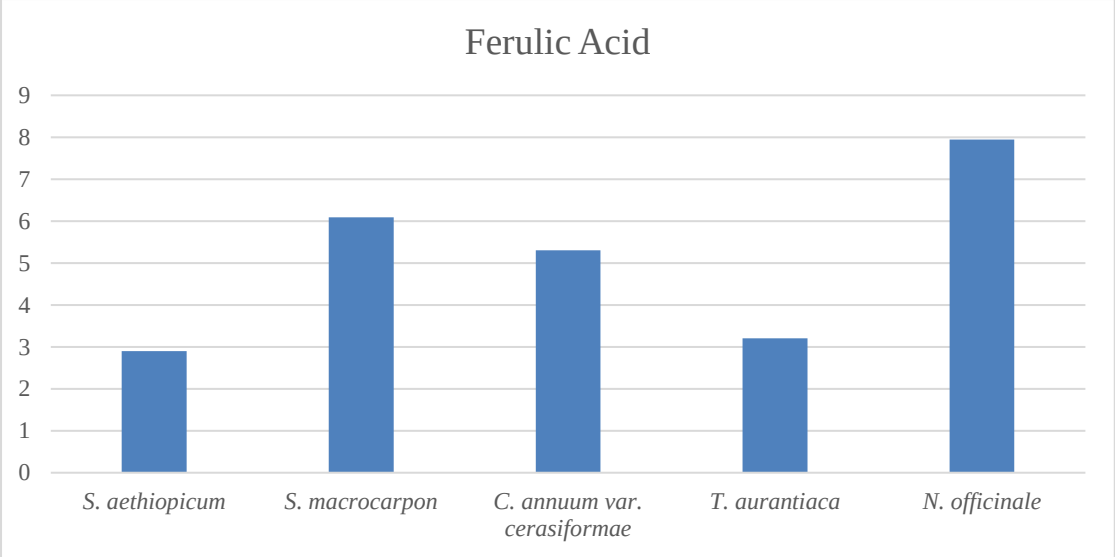

Figure 4.6.4 Ferulic Acid content (mg 100g^{-1} DW) of vegetables

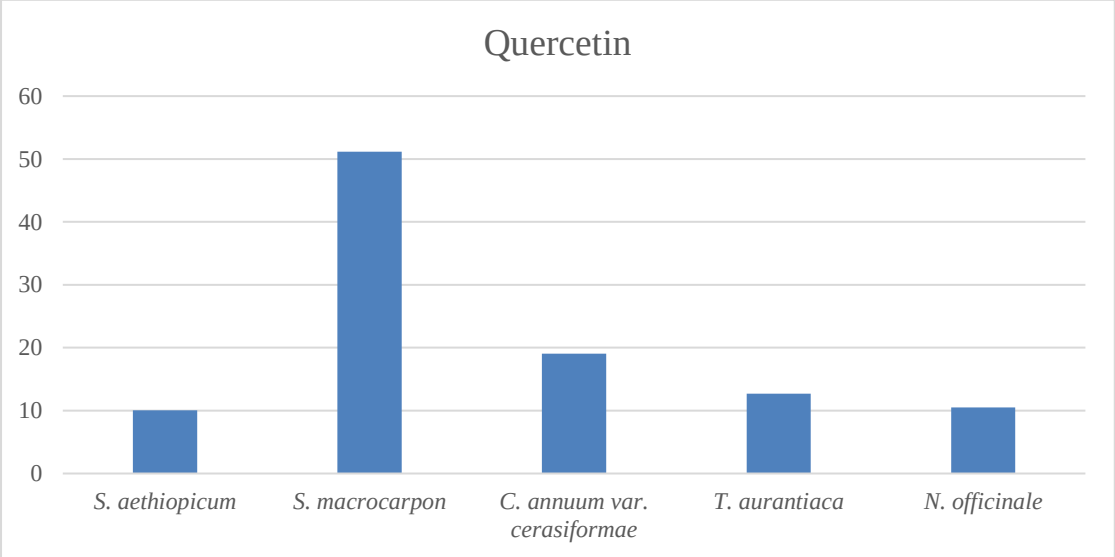

Figure 4.6.5 Quercetin content (mg 100g^{-1} DW) of vegetables

4.6.4 Ferulic Acid (mg $100g^{-1}$ dry weight)

The ferulic acid content of all the studied vegetables are depicted in Figure 4.6.4. From the graph it is understood that the ferulic acid content was highest in *Nasturtium officinale* (7.95 mg $100g^{-1}$ DW) followed by *Solanum macrocarpon* (6.09 mg $100g^{-1}$ DW), *Capsicum annuum* var. *cerasiformae* (5.30 mg $100g^{-1}$ DW) and *Solanum aethiopicum* (2.90 mg $100g^{-1}$ DW). While it was found lowest in *Tupistra aurantiaca* (3.20 mg $100g^{-1}$ DW).

4.6.5 Quercetin (mg $100g^{-1}$ dry weight)

The quercetin content of all the studied vegetables are presented in Figure 4.6.5. Highest Quercetin was found in *Solanum macrocarpon* (51.17 mg $100g^{-1}$ DW) more than double in *Capsicum annuum* var. *cerasiformae* (19.04 mg $100g^{-1}$ DW) and almost four times than that found in *Tupistra aurantiaca* (12.71 mg $100g^{-1}$ DW), *Nasturtium officinale* (10.52 mg $100g^{-1}$ DW) and *Solanum aethiopicum* (10.06 mg $100g^{-1}$ DW).

4.7 Antioxidant Activity

All the studied vegetables are analysed for their antioxidant activity using six different assays. The data of different antioxidant activity of all the vegetables were presented in respective figures and Table 4.7 along with mean data and their SD value.

4.7.1 DPPH activity (%)

All the vegetables were found significantly different for their DPPH activity and data is presented at Figure 4.7.1. The methanolic extract of *Capsicum annuum* var. *cerasiformae* (80.72%±2.29) exhibited highest activity of DPPH among all the

Table 4.7. Antioxidant activity of the selected vegetables

	DPPH (%)	FRAP (mg g^{-1} GAE DW)	Ferrous ion chelating activity (%)	PMA (mg g^{-1} GAE DW)	HRSA (%)	HPSA (%)
S. aethiopicum	74.82^{b}±1.59	20.03^{a}±0.30	39.42^{a}±1.08	41.13^{a}±1.62	67.46^{b}±2.79	61.46^{c}±1.36
S. macrocarpon	80.38^{a}±1.78	4.77^{c}±1.59	12.25^{e}±0.16	40.58^{a}±2.82	75.85^{a}±2.20	45.78^{e}±0.63
C. annuum var. cerasiformae	80.72^{a}±2.29	3.47^{c}±0.16	16.01^{d}±1.60	38.49^{ab}±1.01	78.12^{a}±1.80	66.43^{b}±3.38
T. aurantiaca	78.90^{a}±1.02	7.17^{b}±0.20	19.07^{c}±1.01	35.92^{b}±1.21	58.35^{c}±1.07	58.22^{d}±1.03
N. officinale	45.91^{c}±1.01	6.35^{b}±0.06	34.29^{b}±1.06	24.65^{c}±1.11	59.16^{c}±1.22	71.96^{a}±1.01
Mean	72.15	8.36	24.21	36.15	67.79	60.77
CV %	2.24	8.79	4.48	4.67	2.84	2.92
SEm±	0.93	0.42	0.63	0.98	1.11	1.03
SEđ	1.32	0.60	0.89	1.38	1.57	1.45

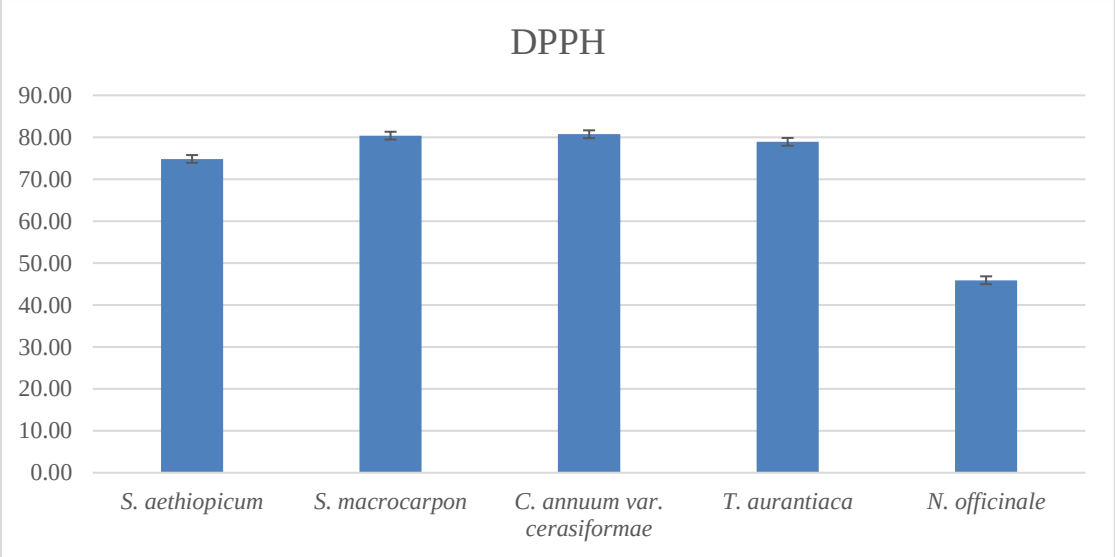

Figure 4.7.1 DPPH activity (%) of vegetables

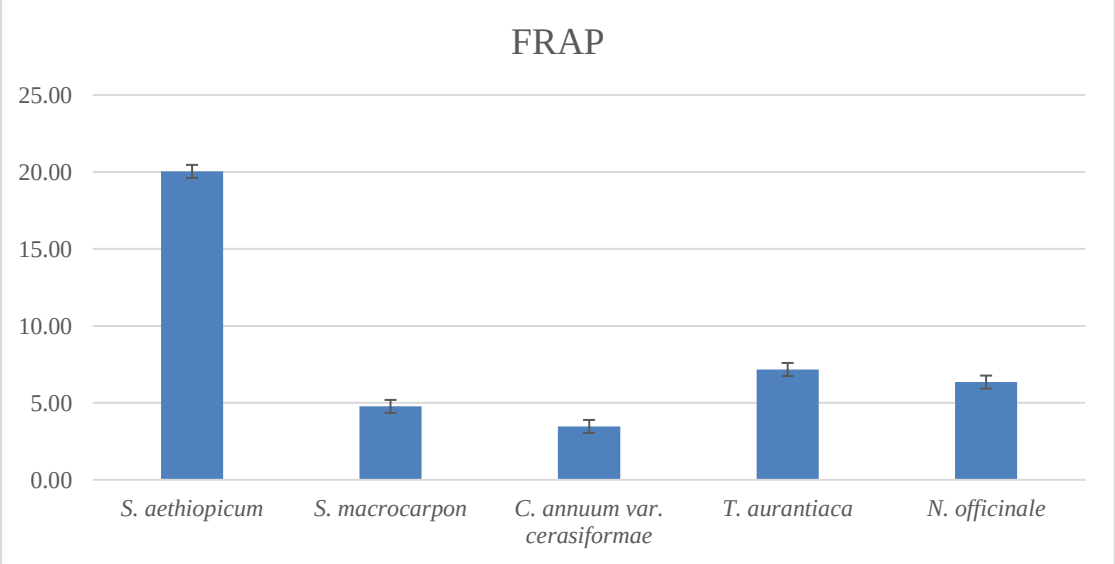

Figure 4.7.2 FRAP activity (mg g^{-1} GAE DW) of vegetables

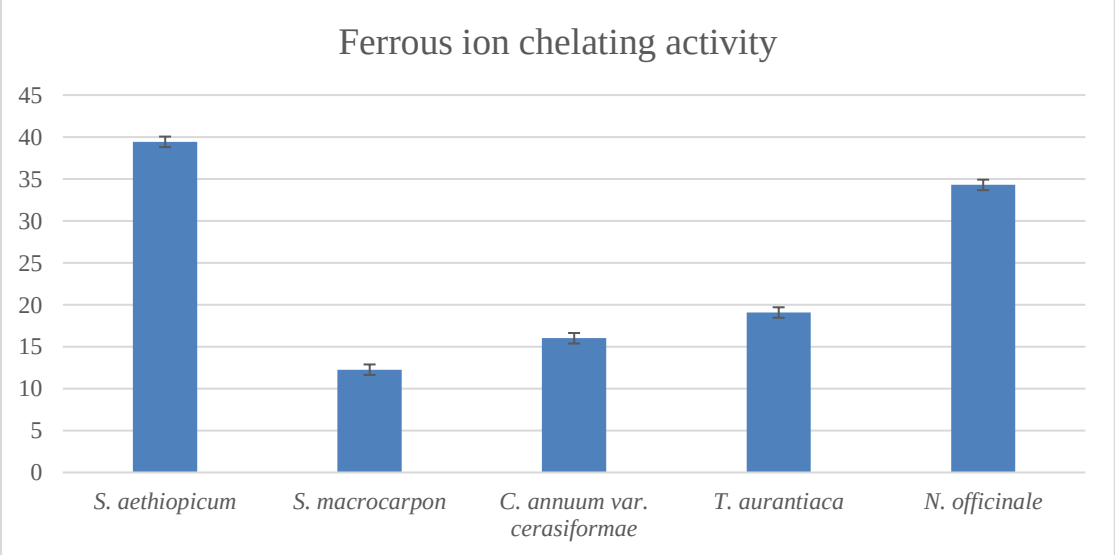

Figure 4.7.3 Ferrous ion chelating activity (%) of vegetables

vegetables extract which was statistically at par with *Solanum macrocarpon* (80.38%±1.78) and *Tupistra aurantiaca* (78.90%±1.02) followed by *Solanum aethiopicum* (74.82%±1.59). While, the minimum DPPH activity was recorded in *Nasturtium officinale* (45.91%±1.01).

4.7.2 Ferric ion reducing antioxidant power (FRAP) (mg g^{-1} GAE DW)

The ferric ion reducing antioxidant power of *Solanum aethiopicum*, *Solanum macrocarpon*, *Capsicum annuum* var *cerasiformae*, *Tupistra aurantiaca*, *Nasturtium officinale* was found to be significant and shown in Figure 4.7.2. From the graph it was revealed that FRAP was highest in *Solanum aethiopicum* (20.03 mg g^{-1} GAE DW±0.30) followed by *Tupistra aurantiaca* (7.17 mg g^{-1} GAE DW±0.20) and *Nasturtium officinale* (6.35 mg g^{-1} GAE DW±0.06). The FRAP activity of *Tupistra aurantiaca* was statistically at par with *Nasturtium officinale*. FRAP activity was recorded as lowest in *Capsicum annuum* var. *cerasiformae* (3.47 mg g^{-1} GAE DW ±0.16) followed by *Solanum macrocarpon* (4.77 mg g^{-1} GAE DW ±1.59).

4.7.3 Ferrous ion chelating activity (%)

The ferrous ion chelating activity of the five indigenous vegetables was found significantly different and data is presented in Figure 4.7.3. The ferrous ion chelating activity was recorded highest in methanolic extracts of *Solanum aethiopicum* (39.42%±1.08) followed by *Nasturtium officinale* (34.29%±1.06), *Tupistra aurantiaca* (19.07%±0.12) and *Capsicum annuum* var. *cerasiformae* (16.01%±1.60) while it was observed lowest in methanolic extracts of *Solanum macrocarpon* (12.25%±0.16).

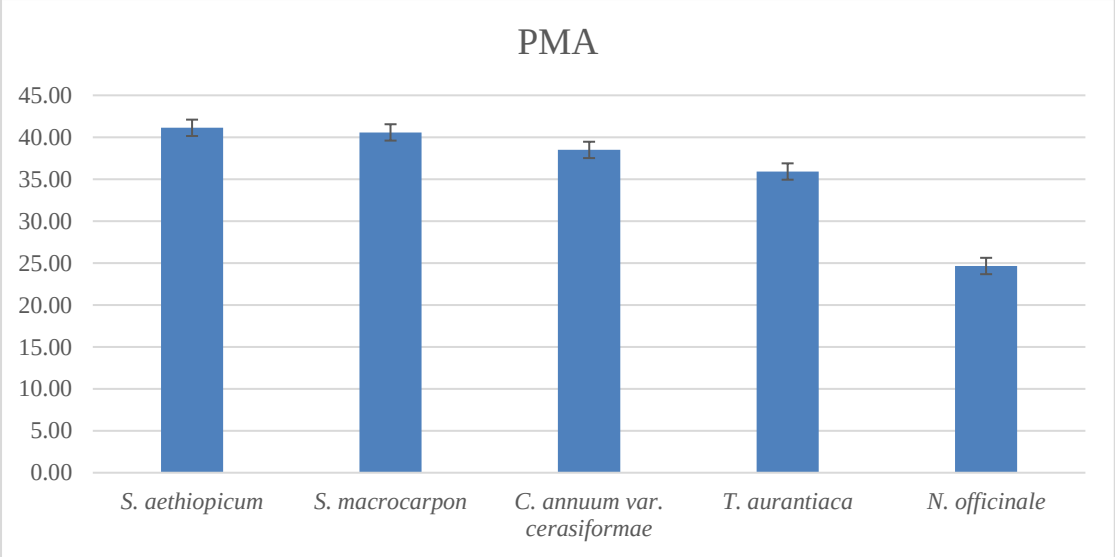

Figure 4.7.4 Phosphomolybdenum assay activity (mg g^{-1} GAE DW) of vegetables

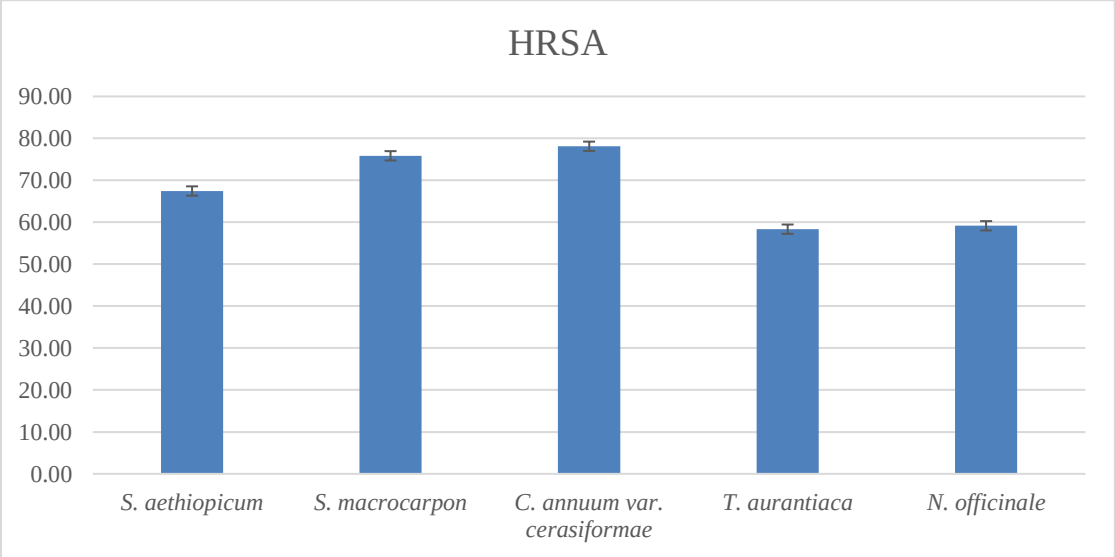

Figure 4.7.5 Hydroxyl Radical scavenging activity (%) of vegetables

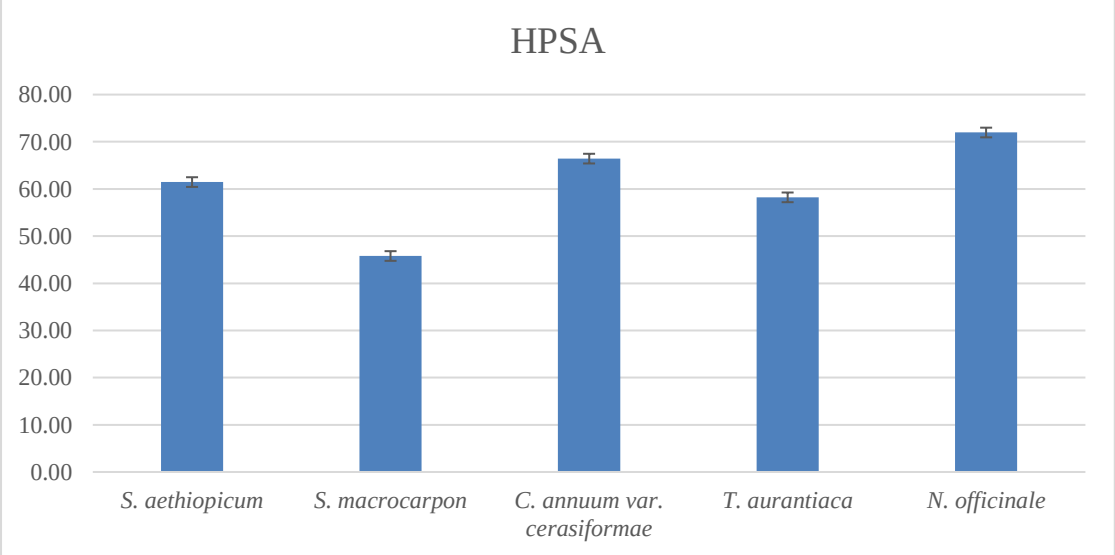

Figure 4.7.6 Hydrogen peroxide scavenging activity (%) of vegetables

4.7.4 Phosphomolybdenum Assay (mg g^{-1} GAE DW)

The phosphomolybdenum assay was found significantly different of all the studied vegetables and data is presented in Figure 4.7.4. The phosphomolybdenum assay was found highest in *Solanum aethiopicum* (41.13 mg g^{-1} GAE DW±1.62) which is statistically at par with *Solanum macrocarpon* (40.58 mg g^{-1} GAE DW ±2.82) and *Capsicum annuum* var. *cerasiformae* (38.49 mg g^{-1} GAE DW ±1.01) while it is observed lowest in *Nasturtium officinale* (24.65 mg g^{-1} GAE DW ±1.11). The value of phosphomolybdenum assay of *Capsicum annuum* var. *cerasiformae* was also statistically at par with *Tupistra aurantiaca* (35.92 mg g^{-1} GAE DW ±1.21).

4.7.5 Hydroxyl Radical Scavenging Activity (HRSA) (%)

The data regarding hydroxyl radical scavenging activity of methanolic extracts of all the studied vegetables were found significantly different and presented in Figure 4.7.5. The methanolic extract of *Capsicum annuum* var. *cerasiformae* (78.12%±1.80) was found to shows highest hydroxyl radical scavenging activity among all the studied vegetables which is statistically at par with *Solanum macrocarpon* (75.85%±2.20) followed by *Solanum aethiopicum* (67.46%±2.79). The lowest amount of hydroxyl radical scavenging activity was recorded in *Tupistra aurantiaca* (58.35%±1.07) which was statistically at par with *Nasturtium officinale* (59.16%±1.22).

4.7.6 Hydrogen Peroxide Scavenging Activity (%)

The hydrogen peroxide scavenging activity of all the studied vegetables were found significantly different and depicted in Figure 4.7.6. *Nasturtium officinale* (4.60%±0.06) exhibited maximum hydrogen peroxide scavenging activity among all

the studied vegetables followed by *Capsicum annuum* var. *cerasiformae* (66.43%±3.88), *Solanum aethiopicum* (61.46%±1.36) and *Tupistra aurantiaca* (58.22%±1.03). The hydrogen peroxide activity was recorded lowest in *Solanum macrocarpon* (45.78%±0.63).

4.8 Fat soluble Vitamins

Four fat soluble vitamins were quantified in all the studied vegetables namely Vitamin A, Vitamin D, Vitamin E and Vitamin K through reversed phase HPLC. The retention time of standards and samples are presented in Table 4.8.1. The data regarding vitamin content of all vegetables are tabulated in Table 4.8.2 and presented in respective figures.

4.8.1 Vitamin A (IU)

The vitamin A concentration of all the studied vegetables are presented in Figure 4.8.1. *Solanum aethiopicum* (1402.92 IU) exhibited the highest content of Vitamin A among all the studied vegetables followed by *Capsicum annuum* var. *cerasiformae* (999.72 IU), *Tupistra aurantiaca* (637.14 IU) and *Nasturtium officinale* (415.12 IU). The lowest amount of Vitamin A was found in fruits of *Solanum macrocarpon* (362.48 IU).

4.8.2 Vitamin D (µg g^{-1})

The vitamin D content of all the studied vegetables are depicted in Figure 4.8.2 which reveals that *Capsicum annuum* var. *cerasiformae* (66.61 µg g^{-1}) contains highest vitamin D content followed by *Tupistra aurantiaca* (52.50 µg g^{-1}) and *Solanum macrocarpon* (48.15 µg g^{-1}). The lowest content of vitamin D was found in

Table 4.8.1. Retention time of standards of vitamins and samples

	Standards	***S. macrocarpum***	***S. aethiopicum***	***C. annuum var. cerasiformae***	***T. aurantiaca***	***N. officinale***
Vitamin A	4.195	4.362	4.058	4.014	4.072	4.021
Vitamin E	5.641	5.712	5.731	5.378	5.386	5.382
Vitamin D2	19.826	19.555	-	19.793	19.781	19.698
Vitamin K	28.42	28.731	28.505	-	28.378	28.575

Table 4.8.2. Vitamin content of the selected vegetables

	Vitamin A (IU)	**Vitamin D (µg g^{-1})**	**Vitamin E (µg g^{-1})**	**Vitamin K (µg g^{-1})**
S. aethiopicum	1402.92	0.00	192.30	8555.95
S. macrocarpon	362.48	48.15	1106.01	329.03
C. annuum var. cerasiformae	999.72	66.61	140.66	0.00
T. aurantiaca	637.14	52.50	103.95	343.63
N. officinale	415.12	8.22	127.38	1001.36

Nasturtium officinale (8.22 µg g^{-1}) while it was not found in fruits of *Solanum aethiopicum*.

4.8.3 Vitamin E (µg g^{-1})

The Figure 4.8.3 presents the vitamin E content of all the studied vegetables. The vitamin E content was observed highest in *Solanum macrocarpon* (1106.01 µg g^{-1}) followed by *Solanum aethiopicum* (192.30 µg g^{-1}), *Capsicum annuum* var. *cerasiformae* (140.66 µg g^{-1}) and *Nasturtium officinale* (127.38). While, vitamin E content was found lowest in *Tupistra aurantiaca* (103.95).

4.8.4 Vitamin K (µg g^{-1})

The vitamin K content of all the studied vegetables are depicted in Figure 4.8.3. From the graph it was shown that Vitamin content was highest in *Solanum aethiopicum* (8555.95 µg g^{-1}) followed by *Nasturtium officinale* (1001.36 µg g^{-1}) and *Tupistra aurantiaca* (343.63 µg g^{-1}). While it was found lowest in *Solanum macrocarpon* (329.03 µg g^{-1}) and not found in fruits of *Capsicum annuum* var. *cerasiformae*.

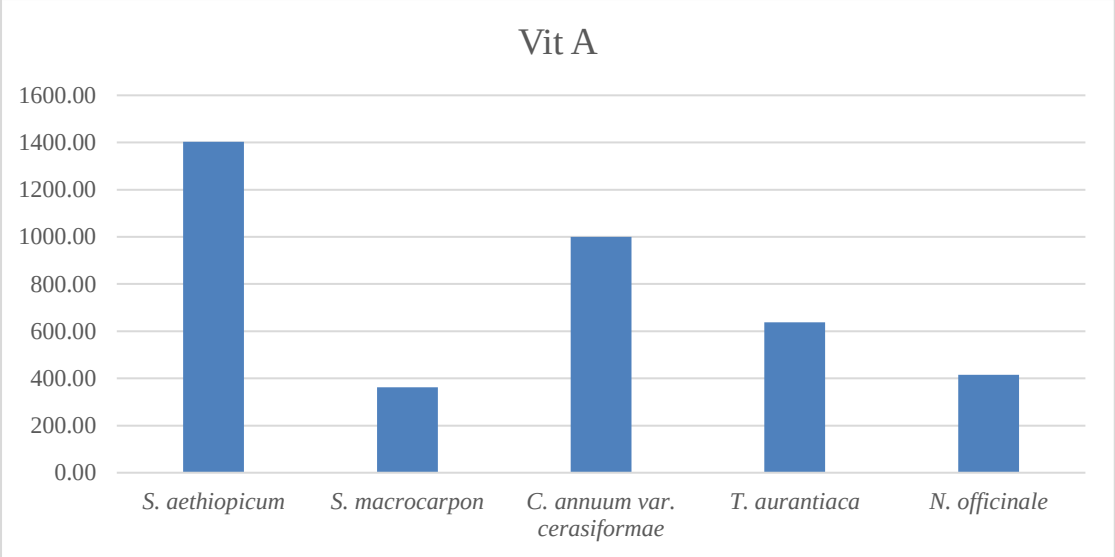

Figure 4.8.1 Vitamin A content (IU) of vegetables

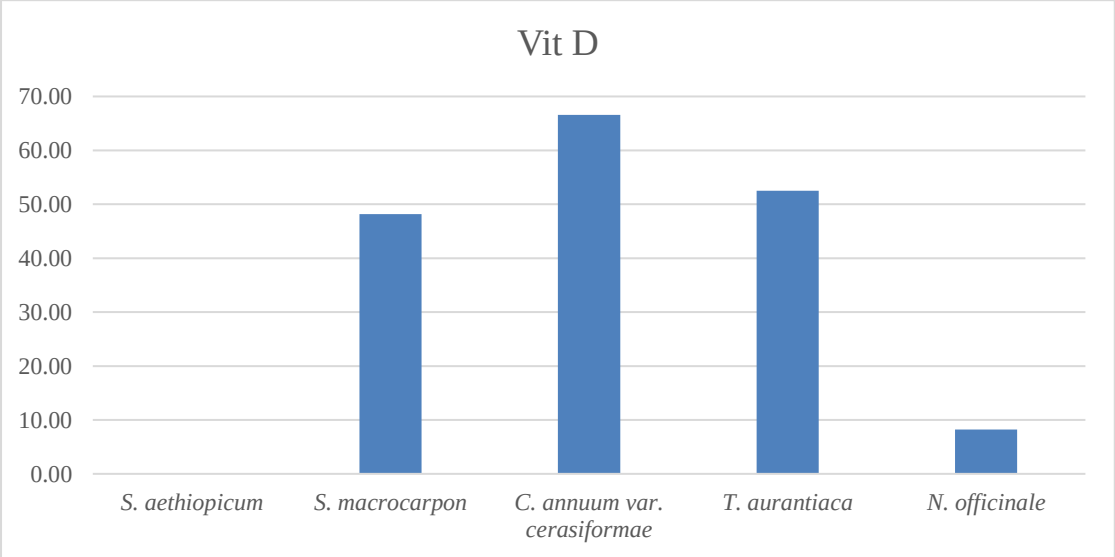

Figure 4.8.2 Vitamin D content (µg g^{-1}) of vegetables

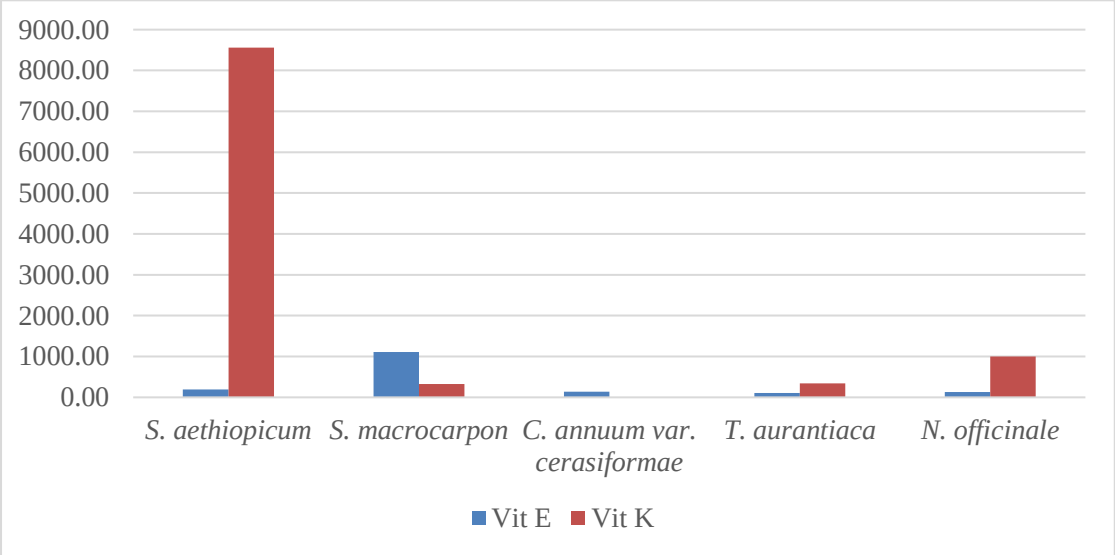

Figure 4.8.3 Vitamin E and Vitamin K (µg g^{-1}) content (IU) of vegetables

Chapter-5
Discussion

The people across the globe are well aware of nutritive values of conventional foods. National Institute of Nutrition, Hyderabad (Gopalan *et al*., 2004) has analysed 591 Indian foods, including cereals, grains, pulses, legumes, leafy vegetables, roots, tubers, nuts, oil seeds, condiments, spices, fruits, fishes, other sea foods, meat, poultry, milk, milk products, fats, edible oils, sugars, beverages (alcoholic) and (non-alcoholic). An extensive review has revealed that the proximate principles of some less familiar foods were also determined. However, hundreds of wild edibles including ethnic foods are yet to be studied and validate for their nutritive and proximate richness, which create huge gaps in knowledge on potential of underexploited or traditional vegetables as food. Unfortunately, only few species of crops were intensified and promoted for last many decades for attaining the food security. In turn, the acceptability of wild and traditional vegetables is now considerably reduced. People often have a negative perception towards these vegetables and fail to appreciate their taste. However, the health conscious consumers are ever increasing, which have widened the scope for promoting of the use of traditional vegetables with high nutritional and protective properties. Moreover, it is not to be undermined that the agrobiodiversity forms the basis for crop improvement and food security. Popularization of local germplasms is of vital importance to widened the agrobiodiversity and making the cropping system more resilient to the climate change.

Present investigation deals with the nutraceutical attributes of some indigenous and underutilized edible vegetables of Sikkim Himalayas. The five vegetables of the region, popularly accepted by the local people but less known to the scientific fraternity about their nutrition properties were chosen for the study. Main objective of this thesis was to verify in real sense the nutritionally rich vegetables amongst them. The literature

revealed that very low attention was given to verify the nutritional status of these vegetables. The present study has selected this problem with mere objective to bring these neglected crops in limelight, so that some of them, during the course of time, may emerge as commercial plant and may further contribute for economic upliftment of the farmers in the region. To attain such status for any vegetable, it is obvious to have desirable properties like medicinal, nutraceutical and phytochemical. Hence, it was considered relevant to determine some nutraceutical properties of five vegetables species (*Solanum aethiopicum* L., *Solanum macrocarpon* L., *Capsicum annuum* var. *cerasiformae* L., *Tupistra aurantiaca* Wall. and *Nasturtium officinale* W.T. Aiton) consumed by rural people and to determine the relation between their nutritional properties and phytochemical components. This research has focused on their evaluation as a source of health promoting phytochemical including major proximate components, phenol content, antioxidative activity and vitamin content of select vegetable species.

Sikkim, a Himalayan state of India is known for rich biodiversity owing to varied climatic condition across the altitudinal gradient. The state is inhabited by diverse ethnic communities with about 10 hilly tribes. In the Sikkim Himalaya, several varieties of locally available vegetables are commonly consumed and are considered an integral part of ethno-culture. These tribes have rich knowledge on use of indigenous vegetables as medicine. In recent decades, a resurgence of interest has focused on wild plant species for their possible nutritional and medicinal values to broaden the diversity of human diet (Flyman and Afolayan, 2007; Afolayan and Jimoh, 2009). This particularly ascribed to the concerns of consumers about the effects of modern agricultural technology, promotion of few crops, marketing and consequent loss of agrobiodiversity. On the other hand, increasing research on underutilized vegetables in

different regions showed that most of these wild greens have great nutritional values and antioxidant properties, which are comparable to those commercially cultivated vegetables (Afolayan and Jimoh, 2009) and there has been no document on nutraceutical potential of indigenous vegetables of Sikkim.

Nutraceutical potential of the indigenous and underutilized vegetables studied here is discussed in light of the role of nutrient components and bioactive molecules they contain. In this chapter 5, the findings of the present investigation along with the results obtained have been discussed under the following heads:

5.1 Survey for medicinal property of vegetables

The large number of local plant species in cultivated and wild form are known to be edible as vegetable in Sikkim Himalayas. Amongst them, some crops have found the special place in the food bowl of local people, owing to their various properties including the medicinal and therapeutic. Some vegetables are consumed not only to meet the nutritional requirement but also as the medicine. The local inhabitant have also inherited the knowledge of such medicinal properties of the vegetables they collected wild or cultivated in their field. The ethnic community and the consumer were surveyed of rapid appraisal on their knowledge on medicinal properties of vegetables. The survey revealed that a section of society consume the local vegetables for therapeutic uses too. (Chapter-4, Table 4.1.2). The vegetables belonging to Cucurbitaceous, solanaceous, leguminous family and tuber vegetables were found to be commonly consumed by the inhabitants as vegetables. *Tupistra nutans, Solanum anguivi, Solanum macrocarpon, Cyphomandra betacea, Fagopyrum esculentum* and *Musa spp.* were used for treatment of diabetes. For lowering of blood pressure *Apium graveolens var. dulce, Solanum anguivi* and *Spinacea oleracea* were used.

Sundriyal (1999) reported 190 wild edible species which are locally consumed by peoples of Sikkim and adjoining areas. Many plants are used for therapeutic purposes in far-flung area. The people mainly depends on the locally available plant materials to cure various health disorders, where modern health care is not adequate (Grover and Vats, 2001). Sundriyal *et al*., (2004) screened a total of 190 wild edible plant species from Sikkim Himalayas belonging to 143 genera and 78 families which accounts for nearly 15% of total wild plants resources of India. This clearly depicts the picture of botanical richness and dietary use of the edible wild plant resources from the Sikkim Himalaya (Eastern Himalaya), many with promising potential. Of the total, 65% were edible for their fruits, 22% for leaves or shoots, 7% for flowers and 3% for roots/rhizomes. Nearly 91 wild edible species were recorded from low-hills, 70 from mid-hills and 28 species from high-hill areas. Within Sikkim state, the North and East districts represent the maximum diversity of edible wild plants due to the wilderness and inaccessibility to most of the habitats. An average rural family annually consumes nearly 8 types of edible wild plants, and a few species provide over five meals in a season and some supports the livelihood. It is suggested that the high diversity of edible plants needs to be conserved for future use. Watercress was reported as a leafy vegetable consumed by 87% of people next to *Diplazium esculentum*. *Tupistra nutans* Wall was mentioned as a spice used in making traditional dishes.

The traditional knowledge of the ethnic communities on medicinal uses of plants of Sikkim was documented by Singh *et al*., (2002) and reported 64 species of plants belonging to 42 families and 57 genera used for traditional healing practices. The major ailments like epilepsy, leprosy, paralysis, asthma, typhoid, diabetes and hemorrhage during childbirth, cholera as well as others were reported to be treated using the studies plants. Some of these plants were reported to be consumed as food

items. A dry powder of inflorescence of *Tupistra nutans* Wall (Syn. *Tupistra aurantiaca)* was reported to be eaten by diabetic patients and also use as a tonic to relieve body pains. A total 37 species of plants belonging to 28 families are used as antidiabetic agents in the folk medicinal practices in the Sikkim and Darjeeling Himalayan region and 81% of these plants are hitherto unreported as hypoglycemic agents in by the local tribes (Chhetri *et al.,* 2005). They found that *Campylandra aurantiaca* was also one of them. Bantawa and Rai, (2009) conducted an ethnobotanical study among the traditional herbal practitioner of Darjeeling Himalaya. A total of 41 plant species belonging to 26 families and 41 genera were found to be used by the practitioner for treating the disease and disorder. Many of them are consumed directly as food and vegetables, condiments and spices. *Tupistra aurantiaca* Wall roots are recorded to be consumed orally in case of food poisoning. In Eastern Sikkim, 79 plant species were collected which are useful to cure various human ailments by Das *et al.,* (2012). The ethnobotanical survey of the area revealed that the people of the area possessing good knowledge of herbal drugs but as the people are in progressive exposure to modernization, their knowledge of traditional uses of plants may be lost in due course.

5.2 Proximate content

In developing countries like India, starch-based foods, including wheat, maize and rice, are the main staple foods that supply both energy and protein requirements; however, they are deficient in many other essential nutrients. The diversity in wild species offers variety in family diet and can contribute to household food security (Zamede *et al*., 2001). Locally available crops serve as alternatives to staple food during periods of the deficit and are a valuable supplement (Scoones *et al*., 1992). Usually the determination of proximate content is the first step in making a decision that a plant

species is nutritionally rich or not based on the parameters like moisture content, dry matter content, TSS, Total ash content, Crude fat, Crude protein, crude fibre, total carbohydrate, total starch, total sugar, chlorophyll A, chlorophyll B and total chlorophyll. *Tupistra aurantiaca* found best for proximate content among all the vegetables.

The moisture content of fruits is related to its dry matter content and this can be used as an index of stability and susceptibility to fungal infection. It determines quality and freshness of vegetable. The moisture content (80.68-94.24%) of all the vegetable species falls in lines with the reports of several researchers. The moisture content of *Solanum aethiopicum* (88.27%) and *Solanum macrocarpon* (91.53%) is at par with the report of Showemimo and Olarewaju (2004). Gboma fruits (*Solanum macrocarpon*) were reported to contain 89.0% moisture (Leung *et al.*, 1968) and 90.6% moisture for *Solanum aethiopicum* (Grubben and Denton 2004). Amongst the studied vegetables, *Tupistra aurantiaca* had lowest moisture content (80.68%). The level of moisture content is a determinant for perishability of the vegetables as discussed by Onyeike *et al*., (1995) in *Capsicum annum* var. *cerasiformae*. Low moisture content ascribed for long storage without spoilage than normal chilli. The spoilage is effected mainly by increased microbial action. The lower moisture content is also the desirable criteria for pickle preparation. The moisture content of *Tupistra aurantiaca* corroborates the results of Rai *et al*., (2005). The moisture content in *Nasturtium officinale* (94.24%) was in tune of previous reports (Rai *et al*., 2005; Shad *et al*., 2013; Khan *et al*., 2016; Pradhan *et al*., 2015). As expected, negative correlation of dry matter and moisture content was methodologically established during the present study, so dry matter content was found low in case of higher moisture percent in all the cases

Total soluble solids in all the studied vegetables (2.53-6.93°Brix) is enough to manifest higher nutrient potential of these vegetables. The TSS or sugar content measures includes the carbohydrates, organic acids, proteins, fats and minerals of the fruit. This may be the reason, where the higher content of carbohydrate in *Tupistra aurantiaca* also had higher total soluble solids.

Ash content is an important fruit quality because it determines the mineral composition of the fruit (Leung *et al.*, 2009). The ash content of *Solanum aethiopicum* L. (0.86%) and *Solanum macrocarpon* L. (1.37%) in present study was lower than reports of ash content of 4.06 and 5.58% of total solids respectively for round green (*Solanum aethiopicum*) and sweet white (*Solanum macrocarpon*) varieties (Showemimo and Olarewaju 2004). Ash content during the present study (0.55) was at par with than reported for green pepper. The percentage ash represents the inorganic content of the vegetable. Ash content in *Tupistra aurantiaca* was higher than that reported by Rai *et al*., (2005) with similar result in *Nasturtium officinale* by Rai *et al*., (2005) and Khan *et al*., (2016) but the ash content less than that reported by Shad *et al*., (2013).

The fat content of both *Solanum* species under study was higher than the 1.0% reported for gboma (Leung *et al.*, 1968). Similarly, the fat content of *Capsicum annuum var. cerasiformae* was higher than the green pepper and also in *Tupistra aurantiaca* than previous report of Rai *et al*., (2005). But, in case of *Nasturtium officinale* it was found lower than previous reports (Rai *et al*., 2005; Shad *et al*., 2013; Khan *et al*., 2016).

The protein content of *Solanum aethiopicum* (2.10%) and *Solanum macrocarpon* (1.44%) were higher than the previous report *viz.*, 1.5% for *S. aethiopicum* and 1.0% for *S. macrocarpon* reported by different researchers (Gbile and

Adesina 1988, Grubben and Denton 2004, Leung *et al.,* 1968). The protein content of *Dalle Khursani* (6.25%) was much higher than green chilli and other common vegetables. The protein content of Nakima (0.36%) was found to be higher than other common vegetables but lower than that reported by Rai *et al*., (2005). Simailarly, protein content of *Nasturtium officinale* was found higher than previous reports (Rai *et al*., 2005; Shad *et al*., 2013; Khan *et al*., 2016). The higher content of protein in the studied vegetables help us in propounding the includsion of these crops in daily diet as a source of protein besides many other benefits.

The crude fibre content of *Solanum aethiopicum* L. (3.35%) and *Solanum macrocarpon* L. (2.66%) was higher than 2.0% and 1.5% reported for *Solanum aethiopicum* and *Solanum macrocarpon* by Grubben and Denton (2004) and Leung *et al.,* (1968). The fibre content in *Capsicum annuum var. cerasiformae* (1.42%) and *Tupistra aurantiaca* (6.56%) was found higher than most of the common vegetables, whereas in case of *Nasturtium officinale*, the crude fibre (1.66%) was at par with that reported by Khan *et al*., (2016). The high fibre content may aids absorption of trace elements in the gut and reduces absorption of cholesterol (LeVeille and Sauberlich, 1966). Thus, fibre reduces the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Hanif *et al*., 2006, Jimoh *et al*., 2010). Moreover, low fat and high dietary fibre content of these vegetables make them an auspicious food which can be recommended as a constituent of the weight-reducing diet for obese people.

Carbohydrate content of *Solanum aethiopicum* L. (7.11%) and *Solanum macrocarpon* L. (6.59%) was higher compared to 4.0% carbohydrate content reported for *Solanum aethiopicum* (Norman 1992). The carbohydrate content of *Capsicum annuum* var. *cerasiformae* (4.29%), *Tupistra aurantiaca* (41.83%) and *Nasturtium*

officinale (8.73%) is remarkably better than many other vegetables. Interestingly, low total sugar (2.57-5.25%) and total starch content (0.01-0.84%) in all the vegetables validates their use by diabetics (Chhetri *et al*., 2005).

Chlorophyll A (0.17-9.50 mg $100g^{-1}$), Chlorophyll B (5.99-15.43 mg $100g^{-1}$) and total chlorophyll (6.15-24.89 mg $100g^{-1}$) content of all the studied vegetables were significantly higher which shows that all the studied vegetables are nutritionally rich. The quantity of chlorophyll per unit area is an indicator of photosynthetic capacity of a plant. The amount of chlorophyll in plant tissue is influenced by nutrient availability and environmental stresses such as drought, salinity, cold and heat etc. (Palta, 1990). Chlorophylls when, consumed in our daily diet as components of vegetable these photochemically active compounds are associated with potential health benefits for humans, such as antimutagenic effects, antigenotoxic properties, and potent antioxidant capacity to scavenge free radicals, preventing lipid oxidation (Roca *et al*., 2016).

5.3 Multi-elemental content

Plants accumulate a number of minerals in different parts which are also essential to human nutrition (Dushenkov *et al*., 1995). Mineral composition of a plant plays significant role in its nutritional, medicinal and therapeutic values (Rajurkar and Damame, 1998; Choudhary and Rehman, 2002 and Al-Kharusi *et al*., 2009; Higdon, 2003; Lieberman and Brunning, 2003). Living organism requires a continuous supply of large number of substances from food to complete their life cycle. This supply is called as nutrition. The mineral nutrition is an important aspect and is in dispensable for healthy growth. Plants are known to supply the needed vitamins and the minerals like Iron, calcium, magnesium, and others important for human health, and they are the most affordable source of minerals and vitamins for the local people. Mineral elements

though not provides the energy to the human body, but play important role in many activities (Malhotra, 1998). About 14 elements are essential to human health such as N, P, K, Ca, Mg, Na, Cu, Fe, Zn, Mn, Co, Si, Br and Cr etc. The deficiency of such element creates some health problems; where as their presence in excess may result in toxicity. Human bodies daily need more than 100 mg of major minerals (N, P, K, Ca, Mg, Na) and less than 100 mg of minor minerals (Cu, Fe, Zn, Mn, Co, Br, Si) (Rajangam *et al.*, 2001; Aslam *et al*., 2005).

Major and trace elements play important role in building up and restoration phenomenon and the disease management in human body. However, the direct correlation between elemental content of medicinal plant and therapeutic properties is not at all understood in terms of modern pharmacological concepts. Hence, it is quite imperative to estimate various trace element concentration in order to establish the relationship of trace element and medicinal properties of the plant.

All the five species under study were having a higher amount of essential elements compared to their counterpart vegetables. Among all the elements, potassium (246.15-630.99 mg $100g^{-1}$ DW) was the most abundant element in all the vegetables followed by magnesium (92.89 mg $100g^{-1}$ DW), calcium (59.42 mg $100g^{-1}$ DW) and phosphorus (38.56 mg $100g^{-1}$ DW) in case of *S. aethiopicum*; calcium (65.72 mg $100g^{-1}$ DW), magnesium (55.00 mg $100g^{-1}$ DW) and phosphorus (31.49 mg $100g^{-1}$ DW) in case of *S. macrocarpon*; phosphorus (77.43 and 68.87 mg $100g^{-1}$ DW), calcium (27.41 and 27.01 mg $100g^{-1}$ DW) and magnesium (13.64 and 18.51 mg $100g^{-1}$ DW) respectively in case of *Capsicum annuum var. cerasiformae* and *Nasturtium officinale* and phosphorus (110.88 mg $100g^{-1}$ DW), magnesium (86.82 mg $100g^{-1}$ DW) and calcium (11.30 mg $100g^{-1}$ DW) in case of *Tupistra aurantiaca*. All of these vegetables were found to contain lower content of potassium, calcium, magnesium and phosphorus

than their upper RDA requirement and highest tolerance limit. The higher content of potassium is associated with increased iron utilisation and is also beneficial for people suffering from hypertension (Adeyeye, 2002). Calcium is an essential element not only for children but also for lactating, pregnant and menopausal women. In human body, teeth and bones only comprises of about 99% of total calcium present in the body (Beto, 2015). Its deficiency causes osteoporosis in adults and rickets in children. In addition to the amount of calcium present in the diet. Its absorption also determines the availability of calcium for maintenance of the skeletal system. Foods enriched with calcium could be the possible way in preventing calcium related skeletal and osteoporosis-related problems. Magnesium plays important roles in the structure and the function of the human body. Magnesium plays a structural role in bone, cell membranes, and chromosomes. It is required for the active transport of ions like potassium and calcium across cell membranes. Potassium is an essential dietary mineral and electrolyte. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells (Peterson, 1997). Phosphorus is an essential mineral that is required by every cell in the body for normal function (Knochel, 2006). The majority of the phosphorus in the body is found as phosphate (PO_4). Approximately 85% of the body's phosphorus is found in bone.

About 60% of the world population suffers from Fe deficiency (below 8 mg per day) that leads to anaemia, and on another side, its excess intake (above 45mg per day) may cause cardiac and nephric malfunctions. In the present studies, considering all the species, iron content was in the range of 4.37-42.33 mg $100g^{-1}$ DW that makes them fits for consumption. Manganese is required as metalloproteins in enzymes such as pyruvate carboxylase in humans and deficiency of the metal (below 2.3 mg per day) results in severe skeletal and reproductive abnormalities in humans (Maiga *et al.*, 2005).

Manganese content in the present studies was as low as 0.74 mg $100g^{-1}$ DW in *Capsicum annuum* var. *cerasiformae* and was highest (26.24 mg $100g^{-1}$ DW) in *Tupistra aurantiaca*. According to Maiga *et al.*, (2005) copper is necessary as a cofactor in many of the important enzymes *viz.* Cytochrome P450 oxidase and Superoxide dismutase in Humans. Cu is one of the mineral elements along with Fe, Zn, Ca and Mg that is lacking in the human diet (White and Broadley 2009). Copper (0.12-52.63 mg $100g^{-1}$ DW) was present in all the species in a good amount. As a dietary mineral, molybdenum is part of sulfite oxidase. Sulfite oxidase break down sulfites that are present in many chemically preserved foods as well as specific food proteins. Molybdenum helps break down sulfites in order to reduce toxic build-up and promote overall healthy body function. Sulphur is an essential element for human body. It is an important non-metallic trace mineral and is the third most abundant (after calcium and phosphorus) based on percentage of total body weight. It is the sixth most abundant macro mineral in breast milk. Sulphur is required for building amino acids which are used to create protein for cells and tissues and for hormones, enzymes, and antibodies. In humans, among all the studied element of human diet Zinc (Zn) is the least toxic and an essential element by means of its involvement in structural, catalytic and regulatory processes of the body in immunity, brain activity and foetal growth & development. All the three species studied here were found having a higher content of Zinc.

Among trace element, aluminium is not having any beneficial role in human body, but it is found in in traces due to ingestion of food and water. The amount of aluminium in the human body ranges between 50 and 150 mg, with an average of about 65 mg. Most of this mineral is found in the lungs, brain, kidneys, liver, and thyroid. Our daily intake of aluminium may range from 10-110 mg, but the body will eliminate most of this in the faeces and urine and some in the sweat. With decreased kidney function,

more aluminium will be stored, particularly in the bones. All the studied vegetables were found to contain the safe amount of aluminium. The insufficient sodium in the body may lead to low blood pressure, muscle weakness, paralysis, mild fever, respiratory problems. The excessive amounts in the body may lead to de-hydration and hypertension (Jaworska and Kmiecik, 1999). Sodium and potassium take part in ionic balance of the human body and maintain tissue excitability, carry normal muscle contraction, help in formation of gastric juice in stomach (Brody, 1998).). Sodium (2.26-25.66 mg $100g^{-1}$ DW) was present in sufficient amount in all the studied vegetables. Strontium is a silvery metal found naturally as a non-radioactive element. About 99% of the strontium in the human body is concentrated in the bones. Several different forms of strontium are used as medicine. Scientists are testing strontium ranelate to see if it can be orally administered to treat thinning bones (osteoporosis). All the studied vegetables were found to contain safe amount of strontium. Anaemia and severe fatigue may be the result of a deficiency of Cobalt (below 0.05 mg per day) in human diet whereas its excess intake (1 mg per day) may cause angina and asthma. Here Co in all the studied species was in the range of 0.06-0.67 mg $100g^{-1}$ of dry weight. Lithium is a naturally occurring alkali metal, ingest into human body through dietary sources and usually present in trace amounts in the human body. Higher concentration of lithium is effective as a medication for mania and mood swings including manic depressive disorders (Demling *et al.,* 2001). Lithium in all the studied species was in the range of 0.24-2.23 mg $100g^{-1}$ dry weight.

All the species were found to be a rich source of all the macro and micro elements which signify that these species could be the potential sources of the minerals where malnutrition, hunger, and availability of common food is the major constraints.

Heavy metals like lead, chromium, tin and cadmium are harmful for human bodies even they are present in the very small quantity. In view of this all the food regulatory bodies set up limits for their concentration in food items for safe consumption. In India, it is regulated and decided by FSSAI. All the studied vegetable species during the present investigation were found to contain the heavy element content in safe amount in their edible parts. Potential sources of the heavy metal in agriculture system is anthropogenic and in some cases it depends on the bed rock of the particular area. Sikkim practice organic agriculture system with no contamination of soils by pesticides, neither there are any heavy industries discharging such metals. Worthington, (2001) reported the lower amount of heavy metals in organically grown crops than conventional ones

5.4 Phytochemical content

Vegetables and fruits containing high amounts of phytochemicals with the ability to scavenge free radicals in biological systems are recommended for a healthy human diet. The methanol extract of all the studied vegetables were evaluated for total phenol, flavonoid, flavonols carotene and ascorbic acid content.

A phenolic compound in *Solanum* has been recognised as major bioactive compounds which were responsible for their antioxidant effects (Kwon *et al*., 2008). Total phenol contents of *S. aethiopicum* (3.89 mg g^{-1} GAE DW) and *S. macrocarpon* (1.03 mg g^{-1} GAE DW) during the present study were in tune with the earlier experiments (Nisha *et al*., 2009; Raigón *et al*., 2010). Our result of total phenolics in *Capsicum annuum* var. *cerasiformae* (11.31 mg g^{-1} GAE DW) was comparable with the findings of Dubey, *et al.,* (2015) who reported total phenolics in *Dalle Khursani* collected from Sang of Sikkim was 13.4 mg GAE g^{-1}. High phenolics in *Dalle Khursani*

supports the facts of being good natural antioxidants. The total phenolics content in *Tupistra aurantiaca* (3.55 mg g^{-1} GAE DW) and *Nasturtium officinale* (3.07 mg g^{-1} GAE DW) are considered to be the good values. The phenol content in *Nasturtium officinale* was at par with the previous report of Aires *et al*., (2013). Phenolics have wide spectrum of biological activities like antioxidant, anticarcinogenic and antimutagenic properties and possess the ability to modify gene expression (Khan *et al*., 2016). It is a much-acquainted fact that presence of significant amount of phenolics in daily food gives health-promoting effects due to their antioxidant action. Phenolic compounds of natural origin have shown to possess antimicrobial, anti-cancerous, neuroprotective activities, helps to improve insulin secretion and helps in reducing unwanted fat in the body (Kaur *et al*., 2014). Phenolic compounds also impart peculiar taste and aroma to the foods through phenolic degradation or mechanisms of Maillard reaction (Jiang and Peterson 2010).

Flavonoids have been found powerful scavengers of singlet oxygen and various other free radicals, related to DNA damage and cancer which makes it beneficial agents for the management of a multitude of diseases states, including cancer, cardiovascular and neurodegeneration (Marchand 2002). Flavonoids also impart health benefits because of its properties like anti-inflammatory, inhibition of platelet aggregation, mitochondrial adhesion inhibitor, an antiulcer agent, an anti-arthritic agent, an anti-angiogenic agent, an anticancer agent and antimicrobial activities (Gurnani *et al*., 2016; Siddiqui *et al*., 2012). Many horticultural resources of wild origin are identified to be reservoirs of high flavonoid content. All the studied vegetable species were found to contain considerable amount of flavonoids ranging from 12.69-54.66 mg g^{-1} RUE DW and flavonols in range of 4.35-20.44 mg g^{-1} RUE DW.

The high content of Ascorbic acid in plants might link with higher free radical scavenging activity and health benefits like anti-carcinogenic and anti-atherogenic (Lui *et al*., 2008). Ascorbic acid ranges from 0.12-0.78% during the study is quiet encouraging. Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, yeast, fungi and photosynthetic bacteria. They are prominent for their distribution, structural diversity and various functions. Fruits and vegetables provide most of the carotenoids in the human diet. Carotenoids were in the ranges from 0.08-0.60 mg $100g^{-1}$ during this investigation. The results of this study suggest that phenolics, flavonoid, flavonols, ascorbic acid and carotenoids are important components of all the studied vegetables. It is well establish that the phenolics are vital for adsorbing and deactivating free radicals, quenching singlet oxygen or decomposing peroxide

5.5 Quantification of phenols

The extracts of natural origin contain several chemical components in varying concentrations, so it is important to use chromatographic methods to analyse these inherently complex mixtures. Chromatographic fingerprinting of the extracts was attempted using reverse phase HPLC. Gallic acid, rutin, catechol, ferulic acid and quercetin were identified in the studied species. The amount of Gallic acid varied from 161.03 to 1463.92, rutin 27.65 to 87.29, catechol 148.94 to 1759.54, ferulic acid 2.90 to 7.950, quercetin 10.06 to 51.17 mg per 100 g dry weight. Among the entire phenolic compound quantified here, Gallic acid was found dominant in all the studied species. Various antioxidant activities of these species could be due to the presence of ample amount of these phenolic compounds. Epidemiological studies have found consumption of foods containing these phenolics have positive association towards a reduced risk of developing several disorders such as cardiovascular diseases,

antidiabetic, antimicrobial, inflammatory and neurological activities etc. (Adefegha *et al*., 2014; Gandhi *et al*., 2011). Few reports on the phytochemical investigation of all of these species indicate the presence of these phenolics (Aires *et al*., 2013; Boligon *et al*., 2013; Chaichana, 2018; Gandhi *et al*., 2011; Nwanna *et al*., 2014; Ramamurthy *et al*., 2012; Plazas *et al*., 2014). These species may found an important place in pharmaceutical formulations due to their rich content of phenolic compounds. The presence of significant amount of phenolic in such underutilized vegetables reveals the nutraceutical potential of the crops under investigation

5.6 Antioxidant Assay

Antioxidants inhibit lipid oxidation caused by free radicals by several mechanisms like free radical scavenging activity, chelating activity and reducing activity etc. A lot of studies confirms that antioxidants derived from indigenous plant sources were very useful in preventing the damaging effects of oxidative stress and because of that there is an increasing concern in the protective biochemical functions of natural antioxidants (Zahin *et al*., 2009). A number of assays were designed to measure overall antioxidant activity or reducing potential, as an indication of a host´s total capability to endure free radical stress (Gulcin *et al*., 2010). In order to get a better estimate of antioxidant capacity multiple antioxidant assays was performed rather than a single assay. In this study, antioxidant capacity in three *Solanum* species extracts was evaluated using six in-vitro assays namely, DPPH, HRSA, HPSA, FCA, FRAP and PPMA. These antioxidant assays are very commonly used assays among researchers as they are reliable and economical and do not require any advanced instruments.

Free radicals are mainly produced as a result the oxidation process. The high potential for scavenging free radicals could inhibit the spread of oxidation. The

conversion of the ion-radical form of DPPH-H, by the antioxidants, could be assessed by monitoring the change of colour from purple to yellow. The stable free radical DPPH is commonly used to test the free radical scavenging ability of naturally available food. The degree of discolouration of DPPH points the scavenging capacity of the antioxidant extract that is due to the hydrogen donating ability (Siddiqui *et al*., 2012). All the extracts of the studied vegetables showed high free radical scavenging capacity by reducing the stable DPPH radical to the yellow coloured diphenyl picryl hydrazine (Table 4.6, Chapter 4). This result was in agreement with Boligon *et al*., (2013); Chaichana, (2018); Loganayaki *et al*., (2010), Kaur *et al*., (2014) and Nascimento *et al*., (2014).

The oxidatively induced breaks in DNA strands are produced due to hydroxyl radicals produced through Fenton reaction to yield its open circular or relaxed forms. These radicals are most reactive form among all the dioxygen reduced forms and supposed to cause cell damage *in vivo* (Rollet Labelle *et al*., 1998). They may be generated in the human body under physiological conditions, where they can react with non-selective compounds such as proteins, DNA, unsaturated fatty acids and almost all biological membrane. The hydroxyl radical scavenging activity is directly associated with its antioxidant activity (Babu *et al*., 2001). Amongst all the studied species *Capsicum annuum* var. *cerasiformae* has highest scavenging activity. The ability of extracts to reduce hydroxyl radicals is directly related to prevention of multiplication of lipid peroxidation and they may identify to be a good scavenger of active oxygen species.

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent that belongs to a non-radical form of ROS. Hydrogen peroxide can cross cell membranes quickly and form hydroxyl radical which inactivates few enzymes directly (Miller *et al*., 1993). This

result shows that all the studied species were good in hydrogen peroxide scavenging activity (45.78-71.96%).

One of the important mechanisms of antioxidant activity is the ability of chelating or deactivating the transition metals that have the capability to catalyse hydroperoxide decomposition. That's why it is important to evaluate the Fe (II) chelating ability of the extracts. The Fe (II) chelating properties of the sample extracts may be accredited to their endogenous chelating agents, mainly phenolics. The Fe^{2+}chelating activity of the extracts was measured by a decrease in absorbance of the ferrozine complex as antioxidants competes with ferrozine in chelating ferrous ion (Elmastas *et al*., 2006). Among all the studied vegetables, *Solanum aethiopicum* possesses highest metal chelating activity followed by *Nasturtium officianle*.

FRAP assay is the only assay that directly evaluate the reducing ability of antioxidants that react with ferric tripyridyl-triazine (Fe^{3+}–TPTZ) complex and produces a coloured ferrous tripyridyl-triazine (Fe^{2+}–TPTZ) (Benzie and Strain 1996), while phospho-molybdenum complex assay (PPMA), generally assist in the detection of ascorbic acid, phenolics, tocopherols and carotenoids which are used for the evaluation of total antioxidant capacity (Miladi and Damak 2008). The reducing ability of ferric ion by the extracts shown that all the studied species have optimal FRAP activity, however there was difference in the responses of the extract to different test.. The difference in the responses of the extract in different antioxidant tests could be ascribed to the transfer of electrons/hydrogen from antioxidants followed at various redox potential in different assay systems and the transfer may also be subjected to the structure of the antioxidants (Loo *et al*., 2008).

5.7 Vitamins

A vitamin is an organic molecule (or related set of molecules) which is an essential micronutrient - that is, a substance which an organism needs in small quantities for the proper functioning of its metabolism - but cannot synthesize it (either at all, or in sufficient quantities), and therefore it must be obtained through the diet. Vitamins generally classified into two main broad categories which are, water soluble vitamins (Vit B12, Vit C, folic acid, niacin Vit B3, Vit B1 and Vit B2) and fat soluble vitamins (Vit K, Vit E, Vit D and Vit A). In this study, all the select vegetables were subjected to analysis of fat soluble vitamin. Vitamin A was found to be present in all the studied vegetable with maximum values in *S. aethiopicum.* Dougnon *et al*., (2012); Nayadanu and Lowor, (2015); Offor *et al*., (2015) reported the presence of Vitamin A in both the studied *Solanum* species, while Shad *et al*., (2013) reported the presence of vitamin A in *Nasturtium officinale.* Vitamin D (8.22-66.61 µg g^{-1}) was found in ample amount in all the studied vegetable except *S. aethiopicum* where it could not be detected. Offor *et al*., (2015) reported the presence of Vitamin D in *Solanum macrocarpon.* Vitamin D, a fat soluble compound involved in maintenance of blood and bone calcium levels. Vitamin D promotes normal mineralization of bones and is needed for bone growth. Insufficient Vitamin D may lead to thinning of bones. Adequate Vitamin D and calcium intake protects adults from osteoporosis. Vitamin D is found in very few foods (Cranney *et al*., 2007). Vitamin E was found in good quantity in all the studied vegetables. In *Solanum macrocarpon* the presence of Vitamin E was reported by Nayadanu and Lowor, (2015) and Offor *et al*., (2015) while in *Solanum aethiopicum* it was reported by Eze and Kanu, (2014); Nayadanu and Lowor, (2015) and Offor *et al*., (2015). Vitamin E which comprises of tocopherols together with tocotrienols transfer hydrogen atom and scavenge singlet oxygen and other reactive

species thus protecting the peroxidation of PUFA within the biological membrane and LDL (Meydani, 2000). Vitamin E and selenium has a synergistic role against lipid peroxidation. Vitamin K was also found to be present in all the studied vegetables except *Capsicum annuum* var. *cerasiformae* where it could not be detected. Vitamin K was found to be previously reported by Dougnon *et al*., (2012) in *Solanum macrocarpon.* Vitamin K1 is known for its role in blood coagulation (clotting) and Vitamin K2 also contributes to coagulation, but more importantly, it is now recognized for its essential role in building and maintaining strong bones, as well as inhibiting calcium deposits in the arteries and blood vessels.

Chapter-6

Summary and Conclusion

The present investigation entitled “Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties” was carried out at Department of Horticulture, School of Life Sciences, Sikkim University, Gangtok, Sikkim with the objectives of evaluation of nutraceutical properties of some vegetables of Sikkim Himalayas which are indigenous and underutilized.

The study was carried out on five selected vegetables viz. *Solanum aethiopicum, Solanum macrocarpon, Capsicum annuum* var. *cerasiformae, Tupistra aurantiaca* and *Nasturtium officinale*. All the samples were collected from the villages during their peak season of availability. A survey was conducted for these vegetable to assess the knowledge of the ethnic people on the medicinal properties. Standard methods were followed for all type of analysis. The salient findings of this study are summarized below:

- *Solanum aethiopicum, Solanum macrocarpon, Capsicum annuum var. cerasiformae, Nasturtium officinale* and *Tupistra aurantiaca* are the crops, though not very commercially cultivated, consumed by the local inhabitant of the Sikkim Himalayas. All the above mentioned crops under the study were claimed to have medicinal properties as revealed from the survey. A total of 137 number of respondent participated in the survey and dominantly, vegetable belonging to cucurbitaceae, fabaceae, solanaceae and araceae were found to be used by communities of the surveyed area. Thirty Six vegetables were found to have the medicinal properties. The respondents perceived that these vegetables are the ideal food or are used for management and curing of wide range of the

diseases like hypertention, diabetics, jaundice, chickenpox, slow-healing wounds, etc.

- *Tupistra aurantiaca, Solanum anguivi, Solanum macrocarpon, Cyphomandra betacea, Fagopyrum esculentum* and *Musa spp.* were reported to be used for treatment of diabetes. For lowering of blood pressure *Apium graveolens var. dulce, Solanum anguivi* and *Spinacea oleracea* were used.
- *Tupistra aurantiaca* was found to have high proximate and mineral content than other vegetables under study. *Solanum aethiopicum, Solanum macrocarpon, Capsicum annuum var. cerasiformae* and *Nasturtium officinale* when compared to secondary data of closely related commercial species like *Solanum melongena* and *Capsicum annuum* were found to be at par for proximate and mineral content.
- Potassium and magnesium was found highest in *Solanum aethiopicum* which makes it suitable for consumption for people suffering from hypertension, high blood pressure and asthma etc. Calcium and Zinc was reported to be highest in *Solanum macrocarpon* and can be recommended for people suffering from osteoporosis, arthritis, night blindness and less immunity. Phosphorus, Iron, manganese, copper and molybdenum were found highest in *Tupistra aurantiaca* which makes it suitable for consumption for people suffering from aging problem, anaemia, muscle weakness and osteoporosis etc.
- Adequate phytochemicals and phenolics were recorded in all the vegetables under study. The higher values of total phenol, flavonoids and carotene were recorded for *Capsicum annuum var. cerasiformae*, flavonols for *Nasturtium officinale* and ascorbic acid in *Solanum macrocarpon.* Gallic acid was found

highest in *Capsicum annuum* var. *cerasiformae*, rutin, catechol and ferulic acid in *Nasturtium officinale* and quercetin in *Solanum macrocarpon*.

- Antioxidant activity were determined by six assays namely DPPH activity, FRAP value, ferrous ion chelating activity, phosphomolybdenum activity, hydrogen peroxide and hydroxyl ion scavenging activity. High DPPH and hydroxyl radical scavenging activity was found highest in *Capsicum annuum var*. *cerasiformae*, whereas FRAP value, ferrous ion chelating activity, phosphomolybdenum activity found highest in *Solanum aethiopicum* and hydrogen peroxide scavenging activity in *Nasturtium officinale*.

- Fat soluble vitamins were found in all vegetables except Vitamin D in case of *Solanum aethiopicum* and Vitamin K in case of *Capsicum annuum* var *cerasiformae* were not detected. Vitamin A and D content was found more in *Solanum aethiopicum* whereas Vitamin D in *Capsicum annuum* var *cerasiformae* and Vitamin E in *Solanum macrocarpon.*

- *Tupistra aurantiaca* and *Capsicum annuum* var. *cerasiformae* being rich in phytochemicals, phenolics, vitamins and antioxidant content can be considered as potent nutraceutical plants.

- *Solanum aethiopicum*, *Solanum macrocarpon*, *Capsicum annuum var. cerasiformae* and *Nasturtium officinale* having optimum proximate and mineral content at par with the commercial vegetables like Brinjal and tomato, are to be promoted for commercial cultivation to bring the food diversity in the population of Sikkim in particular and India as a whole. These crops being suited to the climatic condition can be more successfully grown at low input level and organic farming system.

Future line of work

1. Thorough study for finding the particular molecular responsible for medicinal properties of these plants can be undertaken.
2. The parameters with high importance as potential nutraceuticals that are not included in this study like different class of dietary polyphenols with individuals metabolites can be future thrust of study for these plants. The polyphenols putative mechanism of action responsible for protective action of these vegetables can be another facet of the study.
3. Identifying metabolites and catabolites abundantly present in these plants and testing them in cell based models at different concentration for further validating these plants as nutraceuticals.
4. None of the studied vegetables has improved varieties. Hence, breeding for high nutraceutical characters will bring new dimension to the expansion of cultivation area of the crop.
5. The cultivation practices for all the studied vegetables can be standardized, particularly under the organic farming system.

Chapter-7

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Plate No.1 Glimpses of *Solanum aethiopicum*

Plate No.2 Glimpses of *Solanum macrocarpon*

Plate No. 3 Glimpses of *Capsicum annuum* var. *cerasiformae*

Plate No. 4 Glimpses of *Tupistra aurantiaca*

Plate No. 5 Glimpses of *Nasturtium officinale*

Plate No. 6. Survey for medicinal property of vegetable in villages

Plate No. 7. Survey for medicinal property of vegetable in villages

Plate No. 8. Survey for medicinal property of vegetable in villages

Amaranthus Leaves

Diplazium esculentum

***Raphanus sativus* Pods**

***Ipomea batata* leaves**

Urtica dioca

Portulaca oleracea L.

Plate No. 9 Glimpses of local vegetables

	
Cyclanthera pedata	***Cucurbita moschata***
	
Cucumis sativus	***Phaseolus vulgaris***
	
Moringa oleifera	***Ipomea batata***

Plate No. 10 Glimpses of local vegetables

"EVALUATION OF SELECTED VEGETABLES OF SIKKIM HIMALAYAS FOR SOME NUTRACEUTICAL PROPERTIES"

A Thesis summary of Ph.D. Thesis

Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

UZMA KHATOON

Department of Horticulture

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Under the supervision of

Chairperson

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Evaluation of selected vegetables of Sikkim Himalayas for some Nutraceutical properties

The increased knowledge on the relationship between nutrients and health has resulted in several new products categories, such as Nutraceuticals. The word nutraceutical is a portmanteau of the words nutrient and pharmaceutical coined by Dr. DeFelice in 1989 and the product category represents a unique intersection of the pharmaceutical and food industries. Nutraceuticals are diet supplements that deliver a concentrated form of a bioactive component from a food and used with the purpose of enhancing health in dosages that sometimes exceeds that of the normal foods. The nutraceuticals can either be taken as dietary supplements or as functional foods. The dietary supplements can be in the form of liquid concentrates or capsules whereas functional foods are enriched foods which are very close to the original natural food.

The major source of biologically active substances, such as vitamins and secondary metabolites (polyphenols, carotenoids, sterols, glucosinolates, and saponins) are present in most of the vegetables. Number of studies revealed that individuals who eat five servings daily or more of fruits and vegetables have approximately half the risk of developing a wide variety of cancer types, particularly those of the gastrointestinal tract suggesting that consuming phenolic-rich fruits and vegetables increases the antioxidant capacity of the blood. Vegetables are important sources of minerals, fibre and vitamins, which provide essential nutrients for human health. Increased consumption of vegetable significantly reduces the incidence of chronic diseases, such as cancer, cardiovascular diseases and other age-related disorders. Various compounds such as polyphenols, carotenoids (pro-vitamin A), vitamins C and E (tocopherol) present in the vegetables have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases. Polyphenols express many biological activities, such as antifungal, antibacterial, antiviral, anti-inflammatory,

anticancerous and antioxidative and therefore continued identification of vegetables with high polyphenol content is of considerable interest and importance to the scientific community due to the potential health benefits of these compounds.

Sikkim, a Himalayan state of India is known for rich biodiversity owing to varied climatic condition across the altitudinal gradient. The state is inhabited by diverse ethnic communities with about 10 hilly tribes and ethnic communities. In the Sikkim Himalaya, several varieties of locally available vegetables are commonly consumed and are considered an integral part of ethno-culture. These tribes and the ethnic communities have rich knowledge on use of indigenous vegetables as medicine. In recent decades, a resurgence of interest has focused on wild plant species for their possible nutritional and medicinal values to broaden the diversity of human diet. This is because people today are more concern about the effects of modern agricultural technology and marketing, which only cultivate plant types that have high productivity and consequently caused massive loss of biodiversity. On the other hand, increasing research on underutilized vegetables in different regions showed that most of these wild greens have great nutritional values and antioxidant properties, which are comparable to those commercially cultivated vegetables and there has been no document on nutraceutical potential of local, indigenous or underutilized vegetables of Sikkim. So, this work envisages exploring these vegetables as potential nutraceuticals keeping in mind following objectives:-

1. To survey the knowledge of ethnic community of Sikkim about the medicinal properties of vegetables.
2. To characterize and quantify constituent phenolics of selected vegetables.
3. To elucidate nutritional and ionic profiling of selected vegetables.
4. To study the antioxidant activity of selected vegetables.

Material and methods

The present investigation entitled was carried out at Department of Horticulture, School of Life Sciences, Sikkim University, Gangtok, Sikkim during with the objective of evaluation of nutraceutical properties of some vegetables of Sikkim Himalayas which are indigenous and underutilized.

Collection and Survey:

A survey was conducted in all the four districts of Sikkim for the knowledge of traditional use of indigenous vegetables especially for their medicinal use. Collection of sample was also done during the survey for the following vegetables and samples were further subjected to different procedure depending upon the parameters to be studied.

1. *Solanum aethiopicum* L.
2. *Solanum macrocarpon* L.
3. *Capsicum annuum* var. *cerasiformae* (Dalle Khorsani)
4. *Tupistra aurantiaca* Wall. (Nakima)
5. *Nasturtium officinale* (Watercress)

Parameters for analysis:

Following parameters were estimated and analysed, as per the approved synopsis:

1. Proximate analysis

i. Moisture and Dry Matter Content

ii. Total soluble solids

iii. Ash Content

iv. Crude fat

v. Crude Protein

vi. Crude Fibre

vii. Carbohydrate

viii. Total Starch

ix. Total Sugar

x. Chlorophyll A

xi. Chlorophyll B

xii. Total Chlorophyll

2. Elemental Analysis

An Inductively Coupled Plasma Mass Spectrometry (ICPMS) will be used for the quantification of selected metals like potassium, phosphorus, sulphur, calcium, magnesium, cadmium, aluminium, cobalt, chromium, copper, iron, molybdenum, lithium, manganese, sodium, tin, strontium and zinc.

3. Phyto-chemicals

i. Determination of Total Phenols

ii. Determination of Total Flavonoid Contents

iii. Determination of Total Flavonols

iv. Determination of Ascorbic Acid

v. Determination of Carotene content

4. Phenol estimation and characterization (Gallic Acid, Rutin, Catechol, Ferulic Acid and Quercetin) through HPLC.

5. Anti-Oxidant activity

1. DPPH Scavenging Activity
2. Ferric ion Reducing Antioxidant Power (FRAP Assay)
3. Ferrous ion chelating capacity
4. Phosomolybdenium Complex (PM) Assay

5. Free radical scavenging activity
6. Hydrogen Peroxide Scavenging Activity

6. Determination of fat soluble (A, D, E and K) vitamin content through HPLC.

Statistical Analysis

All the statistical analysis were performed with the help of JMP 11 statistical software. All the experiment was performed in completely randomized design with three replication. ANOVA was performed to know the statistical significance between the treatments. Duncan Multiple range test was also performed to determine the statistical differences between the treatments.

Results and Discussion

Solanum aethiopicum, S. macrocarpon, C. annuum var. cerasiformae, N. officinale and *Tupistra aurantiaca* are the crops, though not very commercially cultivated are consumed by the local inhabitant of the Sikkim Himalayas. All the above mentioned crops under the study were claimed to have medicinal properties as revealed from the survey. A total of 137 number of respondents were interviewed during the survey. It was revealed that about 36 local vegetables belonging to 21 families are consumed for medicinal properties. Most of the vegetables were found to be belong to cucurbitaceae, fabaceae, solanaceae and araceae family. *Tupistra aurantiaca, Solanum anguivi, Solanum macrocarpon, Cyphomandra betacea, Fagopyrum esculentum* and *Musa spp.* were reported to be used for treatment of diabetes. For lowering of blood pressure *Apium graveolens var. dulce, Solanum anguivi* and *Spinacea oleracea* were used.

Tupistra aurantiaca was found to have high proximate and mineral content than other vegetables under study. *Solanum aethiopicum, Solanum macrocarpon, Capsicum*

annuum var. cerasiformae and *Nasturtium officinale* when compared to secondary data of closely related commercial species like *Solanum melongena* and *Capsicum annuum* were found to be at par for proximate and mineral content. Potassium and magnesium was found highest in *Solanum aethiopicum* which makes it suitable for consumption for people suffering from hypertension, high blood pressure and asthma etc. Calcium and Zinc was reported to be highest in *Solanum macrocarpon* and can be recommended for people suffering from osteoporosis, arthritis, night blindness and less immunity. Phosphorus, Iron, manganese, copper and molybdenum were found highest in *Tupistra aurantiaca* which makes it suitable for consumption for people suffering from aging problem, anaemia, muscle weakness and osteoporosis etc.

Adequate phytochemicals and phenolics were recorded in all the vegetables under study. The higher values of total phenol, flavonoids and carotene were recorded for *C. annuum var. cerasiformae*, flavonols for *N. officinale* and ascorbic acid in *S. macrocarpon.* Gallic acid was found highest in *C. annuum* var. *cerasiformae*, rutin, catechol, ferulic acid in *N. officinale* and quercetin in *S. macrocarpon*.

Antioxidant activity were determined by six different assays namely DPPH activity, FRAP value, ferrous ion chelating activity, phosphomolybdenum activity, hydrogen peroxide and hydroxyl ion scavenging activity. All the studied vegetables were found to be having ample antioxidant activity which makes them valuable for consumption. High DPPH and hydroxyl radical scavenging activity was found highest in *C. annuum var. cerasiformae*, whereas FRAP value, ferrous ion chelating activity, phosphomolybdenum activity was found highest in *S. aethiopicum* and hydrogen peroxide scavenging activity in *Nasturtium officinale*.

Fat soluble (A, D, E and K)vitamins were found in all vegetables except Vitamin D in case of *S. aethiopicum* and Vitamin K in case of *C. annuum* var *cerasiformae*.

Vitamin A and D content was found more in *S. aethiopicum* whereas Vitamin D in *C. annuum* var. *cerasiformae* and Vitamin E in *S. macrocarpon.*

To conclude, based on this study, *Tupistra aurantiaca* is considered rich amongst the studied vegetables for proximate and mineral content. *C. annuum* var. *cerasiformae* was found to be the rich in phytochemicals, different phenolics, vitamin and antioxidant content could be recommended to include in day to day food basket of local people and also for peoples of other than this region. Cultivation of these local vegetable not only enhance the livelihood security of the local community but also lead to the nutritional security. Growing of these vegetable at commercial scale provides ample scope for popularization of these crops and include them in food bowl locally and globally. There are much future thrust for exploring them as potential nutraceutical food.

VITA

The authoress was born on 06th October, 1993 at Shivpur, Kushinagar district (Uttar Pradesh). She passed her High School from R.V.P Inter College, Faizabad with 1st division in 2006. She passed her intermediate examination in 2008 from Faizabad (Uttar Pradesh) with 1st division.

She has completed B.Sc. (Horticulture) degree from Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad (Uttar Pradesh) in 2012 with 1st division and M.Sc (Horticulture) in Vegetable Science from Central Agricultural University, Imphal (Manipur) in 2014 with 1st division. Thereafter, she took admission in Department of Horticulture, Sikkim University, Gangtok (Sikkim) as Ph.D. (Horticulture) student in the department of Horticulture. During the present study, she was recipient of Maulana Azad National fellowship for Minority Students.

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