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). Similarly, 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 inclusions 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 (PO₄). 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 100g¹ 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 (H₂O₂) 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.