NUTRACEUTICALS POTENTIAL OF UNDERUTILIZED FRUITS OF SIKKIM

A Thesis Submitted
to

Sikkim University

In Partial Fulfillment of the Requirement for the

Degree of Doctor of Philosophy

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DECLARATION

I, Miss. **Yamuna Pandey**, hereby declare that the thesis entitled "**Nutraceuticals Potential of Underutilized Fruits of Sikkim**" was done by me and the contents of this thesis did not form basis of the award of any previous degree to me or, to the best of my knowledge, to anybody else and that the thesis has not been submitted by me for any other research degree in any other University/ institute.

The contents of this thesis have also been subjected to plagiarism check.

This is being submitted in partial fulfilment of the requirements of the degree of Doctor of philosophy in the Department of Horticulture, School of Life sciences, Sikkim University.

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"Nutraceuticals Potential of Underutilized Fruits of Sikkim"

Submitted by **Miss Yamuna Pandey** under the supervision of Dr. Sujata Upadhyay, Department of Horticulture, Sikkim University, Gangtok, 737102, India.

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All the assistance and help received during the course of the investigation have been duly acknowledged by her.

I recommend this thesis to be placed before the examiners for evaluation.

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Gangtok, Sikkim **(Yamuna Pandey)**

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ABBREVIATIONS USED

^{o}B	Degree Brix
%	Per cent
^{o}C	Degree Centigrade
µg	Micro gram
A.O.A.C	Association of Official Analytical Chemists
AAE	Ascorbic acid equivalent
CD	Critical difference
CV	Coefficient of variance
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
e.g.	For example
et al.	Co - workers
Fig.	Figure
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
G	gram
HPLC	High performance liquid chromatography
IU	International Unit
i.e.	That is
Kg	Kilogram
Mg	milligram
Ml	millilitre
mm	millimetre
nm	Nanometre
No.	Number
ND	Not Detected/Determined
O.D.	Optical Density
SD	Standard Deviation

Sikkim is one of the few places on earth with such a unique biodiversity comprising different agro climatic zones with a wide range of plant species having medicinal and nutritional properties. A large number of plants species are being used to meet food, fuel, timber and other needs. People also relied on indigenously grown wild edible plants for food and other plants from natural habitat to meet their basic need (Sundriyal, 1999 and Sundriyal *et al.,* 1998). Sikkim belongs to North Eastern region of India having small geographical area of 7096 sq. km. situated at latitude 27^{o} – 28^{o} N, and longitude of 88^{o} – 89^{o} E. The climatic range of the state varies from tropical, subtropical, temperate and alpine zones. The state is blessed with abundant natural resources which have been used by people for many years in different ways. The extreme climatic diversity of the region gives rise to wide ranging agro-ecological condition ranging from sub-tropical in the lower valleys to alpine in higher elevations. Most of the period in a year, the climate is cold and humid as rainfall is distributed round the year. The state has recently been declared as organic farming state and the farming community is also well versed with organic farming practices. Economy of Sikkim's state depends on agriculture which provides basic daily needs to improve livelihood of the number of people in the state. Due to high forest coverage the state wild edible plants are being highly consumed in the daily diet of the local people and they directly rely on wild plant species and their genetic resources for food and medicine. Human beings have relied on nature throughout the ages for their basic and household needs such as production of food stuff, shelters, transportations, clothing, fertilizers, flavours, fragrances and medicines (Fakim, 2006). In the era of 21^{st} century there has been increasing demand for health promoting food by the consumers all over the world. The consumption of plant based food has always been beneficial to human health since ages and that strongly recommends the consumption of plant origin nutraceuticals to treat or prevent the disease and for improvement of mental health and physical well-being for healthy living. Underutilized fruits and vegetables exist in abundance in the state but their potential and proper utilization has not been fully exploited yet. Significance of fruits in daily dietary index for human being is well known. Man cannot live only on cereals. Presence of vitamins and minerals in fruits act as protective food and vital source of balanced diet for better human health. Nutritionist recommends 120 g of fruits and 360 g vegetables per capita per day in addition to cereals, pulses, eggs etc. Fruits play essential role in people's health and nutrition, mainly as sources of vitamins and minerals, dietary fibre, antioxidants attributes, some of the nutrients and phytochemicals compounds also alter the metabolic function and detoxification of carcinogens and prevent the growth of tumour cell (Wargovich, 2000). Many literature revealed that, daily intake of fruits may prevent the occurrence of life threatening diseases and decrease mortality rate without which human body cannot maintain proper health and develop resistance to diseases. Plants based food such as fruits, vegetables, medicinal herbs etc. may contain versatile group of free radical scavenging molecules like phenolic compounds (e.g. phenolic acids, flavonoids, flavolons, quinones), vitamins (ascorbic acid), terpenoids (including carotenoids), peptides (glutathione) and other endogenous metabolites which are rich in antioxidant activity (Cotelle *et al*., 1996; Velioglu *et al*., 1998; Zheng and Wang, 2001).

Fruit species, particularly those currently identified as 'underutilized', can contribute significantly to improve human health and nutrition, livelihood, household food security and ecological sustainability which constitute an essential component in the diet of many ethnic population. The diversity of underutilized or lesser known plants species is very high (>250 species) in the Sikkim Himalayas. The majority of these species are considered as poor man's food (Sharma *et al.*, 2016). Sundriyal *et al.* (1998) also reported 190 fruit plant species that grow in the wild form in Sikkim. The underutilized fruit species are very well known to the rural folk of Sikkim since many generations. These species having high nutritional and therapeutic values are major source of protective food which occurs naturally and always played vital role in nutritional security of large section of rural tribe. In the present context, there is increasing concurrence that underutilized foods significantly contribute in alleviating hunger and malnutrition. In view of above, underutilized crops are receiving renewed attention with the recognition that they could become useful parents in breeding programme, convenient source of income, vehicles for improving nutrition and increased food supply (Burlingame, 2000; Olorodo, 2004). A huge diversity of indigenous plant food species has been neglected in favour of the production of exotic crops and these neglected and unexploited species have now being valued due to the recognition of their potential contribution in preventing diseases, malnutrition, obesity, diet-related disorders and hidden hunger. Many indigenous food crops constitute protein, carotenoids, vitamin C and dietary fibre. The health benefits of underutilized fruits are mainly associated with phytochemicals, antioxidant potential and other nutritional compounds (Fukumoto and Mazza, 2000). It is evident from the epidemiological studies that there is a clear significant positive connection between the consumption of fruits and decreases in rate of heart disease, mortality, cancers and other oxidative degenerative diseases. This is subjected to the fact that these plant species may provide an optimal mix of phytochemical such as natural antioxidants, polyphenols, vitamins and minerals, fibres and other biotic compounds (Kaur and

Kapoor, 2001). They also play a vital role in preventing and curing various degenerative diseases like cancer, diabetics, cardiovascular disease, ageing etc. reported by Bhatt *et al.* (2017). Underutilized fruits are vital source of an adequate diet and they serve as food supplements, appetizer and often the nutritional properties of indigenous fruits are found to be higher than other known cultivated fruit bearing species (Orech *et al.,* 2007). Regardless of agricultural practice of consuming wild or underutilized fruits plants have not completely disappeared, their nutritional role and nutraceuticals potential are being reported in many studies worldwide. However, certain underutilized edible fruit plants are consumed because they are beneficial for health and thus also called as medicinal plants (Etkin, 1994).The nutraceuticals evolved from indigenous or underutilized fruits play significant role in prevention of Gastro Intestinal Tract (GIT) infection and promote human health (Ghosh *et al.,* 2013).The term “Nutraceuticals” originally termed by Dr. Stephen De Felice in 1989, derived from combination of “nutrients” and “pharmaceuticals” is a food product that promotes health and medical benefits, including the prevention and treatment of diseases (Biesalski, 2001). Nutraceuticals can be used as an alternative to pharmaceuticals because they are becoming popular and attention is being paid by consumers because of nutritional and therapeutic potential. The nutraceuticals concept was initiated from the survey in UK, Germany and France which conclude hereditary factors for achieving good health (Mink, 1969; Brower, 1992) and its revolution began in early 80s when significant and potential benefits of calcium, fibre and fish oil were known by clinical studies published in medical journals (De Felice Stephen, 1995) and that nutritional and medical evidence has allowed nutraceuticals to emerge as being potentially effective (Dillard and German, 2000) therefore, researchers are now focussing on the examination of natural plant and animal based foods for their protective, disease preventing and curative potential (Nicoli *et al.,* 1999). Nutraceuticals is actually a broad term employed to narrate any product or substance obtained from natural plants or food sources that impart additional health advantage as supplements to the essential nutritional value found in the foods (Bhowmik *et al.,* 2013). The term nutraceutical is so vast that functional foods and dietary supplements bring forth advantages to human health that fits into the nutraceutical group (Frost and Sullivan, FICCI). Nutraceuticals are associated with the bio active substance and other major constituents which are of recognized therapeutic activity or nutritional potential which substantially contribute towards human health as a mode of therapeutic drugs. Kalia (2005) and Kokate *et al.* (2002) opined that nutraceuticals are the foods which are all derived from natural sources and can be categorised as dietary fibres, probiotics, prebiotics, polyunsaturated fatty acids, antioxidants, polyphenols, bioflavonoid etc. The concentrated sources of nutrients and other substances with beneficial nutritional effects can provide or supplement with the vitamins, minerals, botanicals and their extracts along with curative and preventive properties fall under the term nutraceuticals. Pandey *et al.* (2011) broadly classified nutraceuticals into two categories as potential nutraceuticals and established nutraceuticals. Potential nutraceuticals is one which has promising approach towards a specific health or medical benefits and a potential nutraceutical will become established nutraceuticals only when sufficient clinical studies to demonstrate its results therefore much of the nutraceutical products still lay in the potential category (Das *et al.,* 2012). Pandey *et al.* (2011) reported that the plant origin nutraceuticals are gaining popularity due to their safety and potential nutritional and therapeutic effects which have typically been positioned as natural and healthy alternatives to traditional medicine. As per the report (ASA, 2015) the world nutraceutical market value was estimated at $ 160.6 billion in

2013 and increased to $ 171.8 billion in 2014 and the market is expected to reach $ 241.1 billion by 2019. The nutraceutical industry in India is about USD 2.2 billion and expected to rise at 20 per cent USD 6.1 billion by 2019-2020 to strengthen awareness about human health and wellness and changing lifestyle. The mankind has observed increasing factual and cost effective interest in underutilized plants and products mostly due to their huge economic prospective and worldwide cultural acceptability, however < 5 per cent species have been analysed (Shinwari *et al.,* 2009). The nutraceutical industry is a dynamic, evolving industry and exploration of nutraceutical potential of underutilized fruits of Sikkim will offer the opportunities to merge scientific studies in the world of pharmaceuticals with consumer preferences in health promoting foods for the forthcoming years.The existence of alternative medicine in India and the Indian consumer's belief in them could extend a platform for the nutraceutical industry to capitalize. Moreover, India being rich in biodiversity can come up as one of the dominant countries in production of plant based nutraceuticals. The nutraceuticals of plant origin may evolve to be considered as a vital aspect of dietary disease preventive food components (Prakash *et al.,* 2012). The diversification of wild and underutilized species in the Himalayan track of India have been known to play potential function in meeting nutritional, minerals and antioxidant requirement of local communities (Maikhuri *et al.,* 2004; Rawat *et al.,* 2011). Intake of wild and underutilized fruits not only supplements with nutritional and dietary requirements of indigenous people but also have a significant role in nutraceutical development. For instance food and flavoured industries are demanding new food ingredients for developing food supplements; therefore these wild edible fruits can be a potential alternative as it contains high value bioactive compounds.The present research deals with the enumeration of nutraceutical potential of ten different underutilized fruits species wildly grown and traditionally being used by ethnic people of Sikkim Himalayas. By keeping above points in view, the present study “Nutraceuticals Potential of Underutilized fruits of Sikkim” was undertaken with the following objectives:

- To examine the proximate, phyto-chemical and antioxidant activities of selected underutilized fruits.
- To quantify the concentrations of selected elements in the underutilized fruits.
- To determine the heavy metal concentration in underutilized fruits.
- To characterize and quantify the phenols in selected underutilized fruits.

The usage of underutilized fruits belonging to natural wild flora as a supplement for healthy diet is becoming popular now days even in the most developed region of the world. Being rich in plant diversity, Sikkim behold large number of underutilized fruit species which are nutritionally rich and still remain unexplored due to lack of awareness among the farming community related to nutritional and nutraceutical properties of these crops.The present study emphasizes nutraceutical potential of some locally available fruits of Sikkim. The objectives of the literature review were to gather information regarding nutritional and nutraceuticals properties present in the following fruit species *viz. Baccaurea sapida, Diploknema butyraceae, Elaeocarpus sikkimensis, Elaeagnus latifolia, Eriolobus indica, Ficus roxburghii, Machilus edulis, Rubus ellipticus* and *Spondias axillaris*. The literature pertaining to the studies on “Nutraceutical Potential of Underutilized Fruits of Sikkim” based on research parameters *viz.* proximate, phytochemicals, antioxidant, phenols and element concentration studies in India and abroad have been reviewed and the brief account and summary of the findings are given as follows:

2.1. Proximate analysis

2.1.1. Moisture and Dry matter content

The moisture per cent is one of the important factors amongst the physical properties of edible fruits that may vary due to various factors and it is essential to know the water content of the fruits as it plays vital role in human diet and the texture, taste and appearance of any fruit is generally based on the moisture content whereas dry matter is the solid component of fruit. Dry matter is actually remaining solid matter when water is removed and mainly composed of carbohydrates which is indicator of both taste and texture. Islary *et al.* (2017) analysed the two wild fruits and observed highest moisture content in *A. bunius* i.e. 4.53 g $100g^{-1}$ of freeze dried sample and 64.46 g $100g^{-1}$ of fresh sample while the moisture content of the fruit *E. operculata* was noted 3.43 g 100 g^{-1} of freeze dried sample and 52.53 g 100 g^{-1} of fresh sample. The pulp of *Baccaurea sapida* was found to contain 81.65 per cent moisture (Mann *et al.,* 2016).

The proximate analysis of *G. sapida* revealed moisture content of 16.25 ± 0.02 g 100 g^{-1} of freeze dried sample and 81.06 ± 0.75 g 100 g^{-1} of fresh fruit and 83.75 g 100 g^{-1} of dry matter (Islary *et al.*, 2016). It was reported that 58.32 per cent of moisture was recorded in the fruits of *Gynochthodes umbellate* (Sudhakaran and Nair, 2016). While analysing the nutritional properties in eight wild fruits of Odisha, it was reported that the highest moisture content was present in *Careya arborea* (85.22 ± 0.07%) and lowest in *Melastoma malabathricum* (56.6 ± 0.71%). The other species had moisture in range from 82.84 ± 0.10 to 61.34 ± 0.77 per cent (Nayak and Basak, 2015). Khomdram *et al.* (2014a) observed the moisture content of *Spondias pinnata* (77.23 %) and *Elaeagnus pyriformis* at par with *Zizyphus mauritiana* (82%), and *Elaeagnus latifolia* species (87.3%).

Saha *et al.* (2014) observed a range of 65 to 85 per cent moisture content among the tested species (around 16 wild fruits from North East India) and highest was present in *Pyrus pashia, Prasiola crispa, Wallichia densiflora, Illicium groffithii, Houttuynia cordata, Persia frutifera* and *Persea robusta.* Bhardwaj *et al. (*2014) observed 70.8 g $100g^{-1}$ moisture content in *Annona squamosa*. Seal and Cahudhury (2014) studied the ethno botanical importance and nutritional potential of five wild edible fruits of Meghalaya state in India. *Spondias axillaris* was among them and their results indicated that fruits of *S. axillaris* was found to contain 62.29 per cent of moisture.The phytochemical properties of wild fruits *Rubus laciocarpus* from Garhwal Himalayas was studied and moisture content was recorded as 58.6 per cent (Saklani and Chandra, 2014). It is evident from the research conducted by Valvi *et al.* (2014) in which mature fruits of *Ficus racemosa* (71.6%) and ripened fruits of *Antidesma ghasembilla* (85.3%) were observed to be highest in moisture content and the dry matter content was assessed more from mature fruits of *Elaegnus conferta* (51 %) and ripened fruits of *Ficus racemosa* (28.3 %).The dry matter content of *A. digitata* pulp was detected in higher quantity among the five wild fruits of Mozambique, on average 88.5 per cent, while for the other fruits, it varied between 16.7 per cent and 34.8 per cent (Magaia *et al.,* 2013).

Purgar *et al.* (2012) reported that the amount of dry matter in *Rubus discolor* ranged from 14.11 and 15.97 per cent and 14.19–18.03 per cent in *R. idaeus. Ficus palmata* a wild fruit of Garhwal Himalayas was screened for its phytochemical properties and moisture content revealed as 48.20 per cent (Saklani and Chandra, 2012). The nutritional evaluation of *Myrica nagi*, wild edible fruits were screened and high moisture content was found as 76.60 per cent (Saklani *et al.,* 2012).The estimated value of moisture content in *Elaeagnus latifolia* and *Elaeagnus pyriformis* was 864.3 g kg^{-1} and 836.4 g kg^{-1}, respectively (Seal, 2012). Seal (2011) reported highest moisture content in the fruit of *M. indica* (90.36 %) and least in *T. bellirica* (56.30 %) which was similar to commercial fruits like apple (84.6 %) and mango (81 %). Fresh fruit of *A. hygromatricus* contains 83.87 per cent moisture.

Desai *et al.* (2010) analyzed the proximate composition of *Morinda* fruit pulp and revealed that the moisture content was very high in *Morinda citrifolia* (63%) compared to that of *M. pubescens* (22%).While studying the nutritional composition of lesser known fruits of Kerala, Nazarudeen (2010) found maximum moisture present in the fruit of *Aporusa lindleyana* (92.43 %) and minimum moisture was observed in *Palaquium ellipticum* (78.8%). Many literature revealed moisture present in any food can critically affect the growth rates of microbes as well as affect the biochemical reactions and texture quality (Ovando Martinez *et al*., 2009; Bhat and Sridhar, 2008). Charoensiddhi and Anprung (2008) results depict 67.74 per cent moisture in Thai bael fruits.

Rai *et al.* (2005) studied the food value of edible wild plants of Sikkim and reported varied range of moisture content in seven different underutilized fruits *viz. Castanopsis hystrix* (55.2 %), *Choerospondias axillaries* (84.8 %), *Docynia indica* (85.1%), *Machilus fructifera* (69.0 %), *Fragaria nubicola* (93.1 %), *Ficus hookeriana* (89.0 %) and *Elaeagnus conferta* (91.7 %). Parmar and Kaushal, (1982) also reported moisture in the fruit of *Ficus roxburghii* (87.1 %).

2.1.2. Crude protein, Crude Fat, Crude Fibre and Ash content

Man needs wide range of nutrients to perform various metabolic activities in the body and to lead a healthy life they include protein which is a key to good health, it is involved in immune functions, oxygen transport and maintaining strong muscles tissues which act as a source of nutraceuticals in the form of food supplements, beverage or any other product. Fibre rich diets are essential for effective removal of waste and percentage of ash represents the mineral content that is present in the fruits. Islary *et al.* (2017) results revealed crude protein content in two wild fruits i.e. *A.*

bunius (1.231±0.050 g) and *E. operculata* (1.323±0.035 g 100 g^{-1}). The crude fat content of the wild fruits investigated were found to be 0.97 ± 0.026 g in *A. bunius* and 1.86 ± 0.02 g in *E. operculata.* Higher value of crude fibre was found in *E. operculata* fruit (17.566 ± 0.351 g 100 g^{-1}) than *A. bunius* fruit (9.433 ± 0.305 g 100 g^{-1}) and ash content was found as 0.516±0.003 g in *A. bunius* and 0.343±0.004 g in *E. operculata.* Rajalakshmi *et al.* (2017) reported the protein content in *S. jambos* (24.40 g $100g^{-1}$), *T. orientalis* (22.47 g $100g^{-1}$) and *R. ellipticus* (21.06 g $100g^{-1}$), fat content in *S. jambos* (0.026 g 100 g^{-1}), *T. orientalis* (2.39 g 100 g^{-1}) and *R. ellipticus* (0.031 g 100 g^{-1}), the fibre content in *S. jambos* (3.88 g 100 g^{-1}), *T. orientalis* (13.57 g 100 g^{-1}) and *R. ellipticus* (2.98 g 100 g^{-1}) and the total ash content in the fruits of *S. jambos* (2.46 %), *T. orientalis* (3.18 %) and *R. ellipticus* (4.02 %).

The fruit of *Grewia sapida* studied for its proximate composition was found to contain 0.78 g $100g^{-1}$ of crude protein, 2.5 g 100 g^{-1} of crude fat, 1.71g $100g^{-1}$ crude fibre and 0.29g $100g^{-1}$ of ash (Islary *et al.*, 2016). Mann *et al*. (2016) evaluated the nutritional and phytochemical properties of *Baccaurea sapida* fruits and content of pulp and peel was observed as 9.31 g $100g^{-1}$ and 3.96 g 100 g^{-1} respectively.The concentration of fat in pulp and peel was 0.11 g $100g^{-1}$ and 0.05 g 100 g^{-1}, fibre content of pulp *was* noted as 3.6 g $100g^{-1}$ and the ash content of pulp and peel of *Baccaurea sapida* was 5.42 g $100g^{-1}$ and 5.31 g $100g^{-1}$, respectively. Misra and Misra (2016) conducted the research on nutritional properties of some edible fruits of southern Odisha and among 23 edible fruits under study, the fruits of *Flacourtia indica* contained the highest lipid content (0.075 mg g^{-1}) followed by fruit of *Carissa carandas* (0.029 mg g–^{1}). Lowest fat content was noted in the fruits of *Diospyrous melanoxylon and Ziziphus mauritiana* (ripe). The observed value of fat content in the fruit of *Ficus benghalensis*, *Ficus hispida* and *Ficus racemosa* are 0.018 mg 100 g^{-1}, 0.017 mg 100 g^{-1} and 0.007 mg 100 g^{-1} respectively.

Sudhakaran and Nair (2016) reported high fibre content (32.58%) in dried fruit of *G. umbellata* (an underutilized species). Kumar *et al.* (2015) studied the important aspects of underutilized fruits crops and their wild relatives for food and nutritional security in Darjeeling Himalayas and their results depict maximum protein content in *Prunus cerasoides* while minimum was estimated for *Duchesnia indica, Terminalia chebula and Eriolobus indica* ranged from 2 to 10 per cent.Total protein content was highest in *Melastoma malabathricum* (5.48 ± 0.58 %) and lowest in *Dillenia pentagyna* (0.32 ± 0.11%) as per the results of Nayak and Basak, (2015).

Seal *et al.* (2014) studied the ethno botanical importance and nutritional potential of five wild edible fruits of Meghalaya state in India. *Spondias axillaris* was among them and their results depict fruits of *S. axillaris* to contain appreciable amount of protein (1.88 %), fat (7.39%), fibre (9.35%) and ash (3.53 %). Saha *et al.* (2014) observed highest protein content in *Pleurotus sajor-caju* (37.92 %) and *Prasiola crispa* (37.88 %) while estimated fat content varied from 0.51 to 22.48 per cent for different species and a range of 2.75 to 30.10 per cent of fibre content as observed among the tested species. Seal *et al.* (2014) in another study the crude protein ranged from 20.83 per cent in the fruits of *G. cochinchinense* to 6.94 per cent in the fruits of *Baccaurea sapida* while highest fat content (12.54 % and 9.67 %) was found in the fruits of *Z. armatum* and *A. gomeziana*, respectively, the highest amount of fibre (17.06%) was noted in *Z. armatum* and lowest (0.80 %) in *B. sapida* and lowest ash content in *A. gomeziana* (3.13%) and highest in *G. cissiformis* (11.03 %).

Bhardwaj *et al.* (2014) results showed 5.44 g $100g^{-1}$ of protein, 0.067g $100g^{-1}$ fat, 2.78 g $100g^{-1}$ dietary fibre and 0.57g $100g^{-1}$ ash content in *Annona squamosa*. The protein content was found satisfactory in the fruit of *Spondias pinnata* i.e. 18.92 mg $100g^{-1}$ and the fruit of *Elaeagnus pyriformis* contain 5.28 mg 100 g^{-1} of protein (Khomdram *et al.*, 2014a).The protein content of 2.82 per cent and crude fibre (10.45 %) was reported in the fruit of *a cowa* (Sarma *et al.*, 2014).The phytochemical screening of wild edible fruits *Rubus laciocarpus* from Garhwal Himalayas was revealed protein (3.87 %), fat (1.20 %) fibre (3.25 %) and ash (1.80 %) (Saklani and Chandra, 2014). *Ficus palmate* could be a good source of fibre with exceptionally high crude fibre content (17.81 g/100 g^{-1} dry weight) among all the studied fruits. *Morus* species also have a considerable content of dietary fibre i.e. 11.0 g/100 g^{-1} dry weight (Sadia *et al.*, 2014).

A. digitata kernels were found to contain 39.3 per cent of protein content and *S. birrea* contained 32.6 per cent in kernel (Magaia *et al.*, 2013). Bhutia (2013) worked on nutritional aspects of underutilized fruits of Sikkim and results of the study depict the estimated values of protein varied from 2 to 10 per cent and maximum was observed in *Prunus cerasoides* followed by *Morus alba* (8.9 %), *R. ellipticus* (7.6 %) *E. sikkimensis* (6.5 %), *E. latifolia* (6.2 %) while minimum was noted in *Duchesnia indica* (1.8 %), *Terminalia chebula* (2.2 %) and *Eriolobus indica* (2.1 %) while estimated values of fat content of underutilized fruits varied from 0.2 to 21.5 % and highest was observed in *M. edulis* (21.5 %) while lowest in *P. edulis* (0.2 %). The values of fat content of other fruits are *P. cerasoides* (9.7 %), *R. ellipticus* (5.9 %), *C. hystrix* (3.4 %), *T. chebula* (1.5 %), *S. axillaris* (1.4 %), *D. butyraceae* (1.1 %), *E. latifolia* (0.8 %), *F. roxburghii* (0.8 %), *E. sikkimensis* (0.3 %) and *E. indica* (0.4 %).

Ibrahim *et al.* (2013) reported 6.3 per cent of total dietary fibre in the freeze dried fruits of *Baccaure angulata. Ficus palmata* a wild fruit of Garhwal Himalayas was screened for its phytochemical properties in which 4.06 per cent protein, 17.65 per cent crude fibre, as 4.71 per cent crude fat and 4.06 per cent of ash content was observed (Saklani and Chandra, 2012). The estimated value of crude fibre content in *Elaeagnus latifolia* and *Elaeagnus pyriformis* was 7.0g and 7.8 g kg^{-1} respectively whereas protein content was 148.2 g and 231.8 g kg^{-1} respectively, fat content was 15.1 g and 14.9 g kg^{-1} respectively the estimated value of ash content in *Elaeagnus latifolia* and *Elaeagnus pyriformis* was 86.16 g and 54.03 g kg^{-1} respectively (Seal, 2012). The nutritional evaluation of *Myrica nagi* fruits were screened and 1.30 per cent of total protein, crude fibre (3.40 %), fat (0.02 %) and ash (1.25 %) was found (Saklani *et al.* 2012). Fresh fruits of *A. hygromatricus* contains 2.5 per cent ash (Singh, 2011).

The nutritional composition of wild fruits in Meghalaya was analyzed by Seal (2011) and reported crude protein varied from 1.04 % to 19.75 % in *M. indica* and *P. roxburghii*, respectively and that was almost similar to commercial fruits like almond (20.80 %) and cashew nut (21 %) and an appreciable amount of crude protein was also noted in the fruits of *P. napalensis* further he observed the ash content ranged from 1.91 % (in *M. nagi*) and 8.73 % (in *M. indica*) varied amount of crude fat content ranged from 1.36 per cent (in *P. nepalensis*) to 5.07 per cent (in the fruits of *M. indica*) and the fruit of *M. nagi* contained the highest amount of crude fibre (7.53 %) and *M. indica* (1.10 %) contained lowest.

Protein content of 15- 18 per cent was recorded for *Capparis zeylanica* fruit and 0.4 per cent for *Carissa congesta* while in other wild fruit species like *Elaeagnus* *conferta* and *Limonia acidissma* (2 and 8 %, respectively) was observed, whereas fat content of 5 per cent was reported for *Capparis zeylanica* fruit while for other wild fruit species like *Elaeagnus conferta* and *Limonia acidissma* (2.0 and 2.8%, respectively) was observed by Deshmukh and Waghmode (2011). *A. hygromatricus* fruits are good source of protein (11.71 %) in outer part and in inner part (4.66%) of the fruit bodies and in the fruit of *R. dumetorum* (9.14 %) crude protein was observed by Singh (2011).Vunchi *et al*. (2011) obtained the value of crude protein (8.24 %), fat (34.62 %), ash (11.50 %) and the value of crude fibre (0.58 %) in the fruit of *Vitex doniana.*

Hui (2010) has quoted in his book "Fruit has been a part of human diet and is an important nutritional source, with high water content (70-85%) and a relatively high amount of carbohydrates but low contents of fat (less than 0.5%) and protein (<3.5%)". The crude protein content was found double in *M. citrifolia* fruits (8.32-9.13 mg g^{-1} DW) as compared to *M. pubescens* (4.87-5.09 mg g^{-1} DW) and the fibre content was found low in *M. citrifolia* (33%) and high in *M. pubescens* fruits (48%) (Desai *et al.*, 2010). The total, soluble, and insoluble dietary fibre contents (19.84, 11.22, and 8.62 g 100 g^{-1} DW, respectively) was observed in fully ripe Thai bael fruit pulps (Charoensiddhi and Anprung, 2008).

Aberoumand (2008) worked on the comparative studies of the protein values of the seven edible plants from Iran. They studied *Arum maculatum*, *Portulaca oleracia, Semicarpus anacardium, Carissa karandus, Cordia myxa, Solanum indicum and Chlorophytum comosum.* They estimated the protein values in *A. maculatum,* (57.0), *Portulaca oleracia* (44.8), *C. comosum* (28.4), *C. karandus* (22.6), *C. myxa* (20.2), *S. indicum* (17.5) and *Semicarpus anacardium* (7.93). Among all the plants, *C.*

myxa syn. *dichotoma* is one of them. Owolarafe *et al*. (2006) found protein (0.93 %) in the fruits of *Spondias pinnata.* Persons who consume huge amounts of fibre are in low risk of gastric disorders, high blood pressure, obesity, stroke, diabetes, (Whelton *et al.,* 2005; Petruzziello *et al.,* 2006; Lairon *et al.,* 2005; Steffen *et al.,* 2003; Montonen *et al.*, 2003). Some evidence from the laboratory analysis regards to protein content found in wild and underutilized fruits show efficient value like *Elaeagnus umbellata* berry, content of 5.1 per cent protein (Sabir and Riaz, 2005).

Rai *et al.* (2005) studied the food value of edible wild plants of Sikkim and reported the varied range of fat content in seven different underutilized fruits as *Castanopsis hystrix* (1.0 %), *Choerospondias axillaries* (0.9 %), *Docynia indica* (1.0 %), *Machilus fructifera* (1.5 %), *Fragaria nubicola* (1.1 %), *Ficus hookeriana* (0.9 %) and *Elaeagnus conferta* (0.8 %). While,the varied range of protein content in seven different underutilized fruits showed as *Castanopsis hystrix* (3.6 %), *Choerospondias axillaries* (2.2 %), *Docynia indica* (2.1 %), *Machilus fructifera* (3.1 %), *Fragaria nubicola* (1.5 %), *Ficus hookeriana* (2.7 %) and *Elaeagnus conferta* (1.4 %) and range of ash content in seven different underutilized fruits as *Castanopsis hystrix* (0.9 %), *Choerospondias axillaries* (0.7 %), *Docynia indica* (0.6%), *Machilus fructifera* (0.8 %), *Fragaria nubicola* (0.6 %), *Ficus hookeriana* (1.3%) and *Elaeagnus conferta* (0.7 %).

Sundriyal and Sundriyal (2003) have given the value of protein content in six different underutilized fruits of Sikkim as *B. Sapida* (5.58 %), *D. butyraceae* (3.81 %), *E. latifolia* (7.80 %), *E. indica* (1.75 %), *S. axillaris* (4.11 %) and *M. edulis* (4.54 %) and the reported the value of ash content in those underutilized fruits of Sikkim are *B. Sapida* (3.85 %), *D. butyraceae* (3.20 %), *E. latifolia* (3.16 %), *E. indica*

(3.03%), *S. axillaris* (2.75 %) and *M. edulis* (2.65 %). Similarly, fat content in the fruits of various wild species like *Castanopsis* species (30.50 %), *Machilus edulis* (25.50 %), *Prunus cerasoides* (9.33 %), *Rubus ellipticus* (7.10 %), *Diploknema butyraceae* (1.57 %), *Terminalia chebula* (1.25 %), *Elaegnus latifolia* (0.51 %), *Eriolobus indica* (0.35 %), *Spondias axillaris* (0.05 %), *Elaeocarpus sikkimensis* (0.21 %) and least in *Passiflora indica* with 0.10 % was reported by Sundriyal and Sundriyal (2001).

Fibre helps to prevent risk of some of the world's most prevalent disease like obesity, diabetes, high blood cholesterol, cardiovascular disease, and numerous gastrointestinal disorders (Venn and Mann, 2004; Tungland and Meyer, 2002).The intake of high fibre food lowers high blood pressure (Keenan *et al.,* 2002), improves serum lipoprotein values (Brown *et al.,* 1999) and CHR (Liu *et al.,* 1999). Agarwal and Chauhan (1988) reported variation in diatery fibre varied from 38.5 to 55.7 per cent, while studying on the composition of dietary fibre from some plants like *Prosopsis cinceria, Ficus religiosa, Ficus bengalensis, Ficus glomerata* and *Capparis deciduas.*

2.1.3. Total Carbohydrate and Sugar (Total, Reducing and Non -reducing).

Carbohydrates are one of the major components of fruits. Islary *et al.* (2017) analyzed the nutritional value of two wild fruits of Assam and reported total carbohydrate content was found highest in *A. bunius* (92.743 ± 0.428 g) and *E. operculata* (93.123 ± 0.084 g). Rajalakshmi *et al.* (2017) worked on the underutilized derived nutraceuticals and observed the total carbohydrate content of following fruits *viz. S. jambos* (32 g 100 g^{-1}), *T. orientalis* (32.18g 100 g^{-1}) and *R. ellipticus* (20.80 g

100 g^{-1}).The fruit of *Grewia sapida* was found to contain 80.18 g 100^{-1} carbohydrate (Islary *et al.*, 2016).

The carbohydrate content of pulp and peel of *Baccaurea sapida* was reported as 3.51g $100g^{-1}$ and 10.39 g $100g^{-1}$, respectively (Mann *et al.,* 2016). Fruits of *Gynochthodes umbellate* was found to contain 6.98 g $100g^{-1}$ of carbohydrate in fresh fruit (Sudhakaran and Nair, 2016). Misra and Misra (2016) conducted the research on nutritional evaluation of some edible fruits of southern Odisha and observed out of 23 different fruits studied, fruits of *Phoenix sylvestris* (339.17 mg g^{-1}, 33.9%) showed the highest total sugar content and the lowest value was exhibited by fruits of *Carissa carandas* (29.0 mg g^{-1}) and the calculated value of total sugar found in the fruits of *Ficus bengalensis* , *Ficus hispida* and *Ficus racemosa* are 119.17 mg 100 g^{-1}, 148.50 mg 100 g^{-1} and 76.50 mg.$100g^{-1}$, respectively.

Nayak and Basak (2015) recorded the highest carbohydrate content in *Dillenia pentagyna* (18.5 ± 0.99 %) and lowest in *Ficus hispida* (1.75 ± 1%). Total carbohydrate content in other fruits varied from 11.8 ± 0.50 to 4.33 ± 1.00 per cent whereas total sugar content was found highest in *Dillenia pentagyna* (16.78 ± 0.005 %) and lowest in *Ficus hispida* (0.63 ± 0.005 %). In other fruits, they observed total sugar content ranged from 12.7 ± 0.99 to 0.78 ± 0.98 per cent. As per results of Kumar *et al.* (2015) highest total sugar was recorded in fruits of *Eriobolus indica* (13.04 %) while *Machilus edulis*, *Spondias axillaris*, *Terminalia chebula*, *Castanopsis hystrix* and *Elaegnus latifolia* showed least total sugar content (3 %). Non-reducing sugar per cent (0.1 to 6.0) varied in fresh fruits amongst the species. *Diploknema butyraceae* contained highest per cent of non-reducing sugar whereas *E. indica* and *C.hystrix* fruits possessed minimum. Valvi *et al*. (2014) conducted research on phytochemical assessment of five wild edible fruits and total sugar and reducing sugar was recorded higher in ripened fruits of *Ficus racemosa* i.e. 29.43 g 100 g^{-1} DW and 10.07 g $100g^{-1}$ DW, respectively.

Khomdram *et al.* (2014a) studied on nutritional profiling of two underutilized fruits *Elaeagnus pyriformis* and *Spondias pinnata* and reported total soluble sugar, reducing sugar and non-reducing sugar contents to be 5.28 ± 0.10 mg $100g^{-1}$, 33.93 ± 1.8 mg $100g^{-1}$, 17.83 ± 0.99 mg $100g^{-1}$ and 16.1 ± 2.16 mg $100g^{-1}$ respectively in the fruits of *E. pyriformis* which indicates fruit is a good source of sugar, while the fruit of *S. pinnata* contains 4.35 mg 100 g^{-1}, 1.51 mg $100g^{-1}$ and 2.83 mg $100g^{-1}$ of total, reducing and non reducing sugar, respectively. Panja *et al.* (2014) observed 10.96 mg 100 g^{-1} in the fruit of *Elaeagnus latifolia.*

Saha *et al.* (2014) determined total carbohydrate of some wild edible plants and found highest for *Wallichia densiflora* (51. 17 %) and it significantly varied between 21.7 to 33.72 per cent for *Cornus capitata, Holboellia latifolia, Houttuynia cordata, Malus sikkiminensis and Laetiporus sulphureus.* Seal *et al.* (2014) analyzed the proximate composition of the fruits collected from Meghalaya state and reported the highest values of carbohydrate content in *B. sapida* (85.83 %) followed by *G. cissiformis* (67.05 %). Bhardwaj *et al.* (2014) results showed 20.41 g $100g^{-1}$ carbohydrate content in *Annona squamosa*, an underutilized fruits of India.The phytochemical analysis of wild fruits of *Rubus laciocarpus* from Garhwal Himalayawas was done and 31.28 per cent of carbohydrate content was recorded (Saklani and Chandra, 2014). The carbohydrates were found to be most abundant macronutrient (75.58 g/100 g–1) in *M. nigra*. The content of total available carbohydrates in mulberries and figs was three-fold than in other wild fruits, *viz*. and strawberry (23.55 %) (Sadia *et al.,* 2014). Sarma *et al.* (2014) found 15.68 per cent carbohydrate in the fruits of *Garcinia cowa.* Ibrahim *et al.* (2013) reported 74.14 per cent of carbohydrate content in the freeze dried fruits of *Baccaurea angulata.*

Bhutia (2013) analyzed the chemical composition of different underutilized fruits of Sikkim and the respective value of total, reducing and non reducing sugar of various fruits were recorded as *C. hystrix* (3.33 %, 3.17 and 0.14 %), *D. butyraceae* (12.21 %, 5.98 % and 5.91%), *D. indica* (7.52 %, 3.55 %, and 3.76 %), *E. indica* (13.04 %, 12.90%, and 0.92%), *E. latifolia* (3.03 %, 2.72 %, 4.99 %), *E. sikkimensis* (7.26 %, 5.10 %, 2.05 %), *F. roxburghii* (8.53%, 8.38 %, 0.13 %), *M. edulis* (3.03 %, 2.31 % and 0.68 %), *R. ellipticus* (8.73 %, 5.66% and 2.90 %) and *S. axillaris* (3.16 %, 2.01 %, 1.11 %). Deb and Bhawmik, (2013) carried out the research on physico-chemical properties of Burmese Grape (*Baccaurea sapida* Muell. Arg.)- An underutilized fruits of West Bengal and their results showed the total sugar content in a range of 4.01-4.29 per cent among different accessions.

Nutrient analysis of some selected wild fruits of deciduous forests of India was done by (Mahapatra *et al.,* 2012) and observed highest carbohydrate percentage in *Eugenia rothii* and lowest in *Terminalia citrina* (3.10 %) and their results also depicted that the total sugar content was found to be abundant in *Ziziphus rugosa* (20.7 %) followed by *Eugenia rothii* (18 %) and *Mimusops elengi* (15.9 %). Least total sugar content was observed in *Glycosmis pentaphylla* and *Streblus taxoides* (1.35 %) Similarly the amount of non-reducing sugar ranged from 0.17 per cent in *Carissa spinarum* to 19.13 per cent in *Ziziphus rugosa.*

Seal (2012) estimated the carbohydrate content in five different wild plants and reported highest in fruits of *Elaeagnus latifolia* (743.40 g kg^{-1}) followed by

Elaeagnus pyriformis (691.36 g kg^{-1}). *Ficus palmata* a wild fruit of Garhwal Himalayas was screened for its phytochemical properties and carbohydrate content was recorded as 20.78 per cent (Saklani and Chandra, 2012). The nutritional evaluation of *Myrica nagi* fruits were screened and carbohydrate content was observed as 16.13 per cent (Saklani *et al.*, 2012). Carbohydrate content was estimated to be highest in *M. glabra* (548 mg/100 g) (Singh *et al.*, 2012).Vunchi *et al.* (2011) obtained the value of carbohydrate (28.40 %) in the fruit of *Vitex doniana*. Physico-chemical and nutritional evaluation of Kasmal, *Berberis lycium* was carried out and the value of total carbohydrate was reported as 15.45 per cent and the value of total, reducing and non-reducing sugar were reported (15.45 %, 9.61% and 5.53 %, respectively) (Sood *et al.*, 2010).

The fruits rich in carbohydrates are nutritious for health and responsible for high calorific value (Ozcan and Haciseferogullari, 2007). Rai *et al.* (2005) studied the food value of edible wild plants of Sikkim and reported the varied range of carbohydrate content in seven different underutilized fruits as Castanopsis hystrix (39.3 %), *Choerospondias axillaries* (11.4 %), *Docynia indica* (11.2 %), *Machilus fructifera* (25.6 %), *Fragaria nubicola* (3.7 %), *Ficus hookeriana* (6.2 %) and *Elaeagnus conferta* (5.3 %). Similarly, carbohydrate content of various fruits of Sikkim was analysed by Sundriyal and Sundriyal (2001) and reported highest in *Diploknema butyraceae* (81.63 %) followed by *Elaegnus latifolia* (74.06 %), *Eriolobus indica* (71.73 %), *Spondias axillaris* (52.28 %) and *Machilus edulis* (51.50 %).

2.1.4. Total Soluble Solids and Acidity

Total soluble solid is an important attribute giving information about sugar content of fruits and is a main source of energy found high in many wild fruits. Titrable acidity indication for presence of total or vital acidity which includes both of the protonated and unprotonated total acid molecules. Determination of TSS is not only important for organoleptic quality of fruits but it also possesses the commercial and economic value of fruits. Rymbai *et al.* (2016) carried out the chemical analysis of fruits and revealed that TSS (8.2–14.1 %), acidity (1.93 %) in the fruit of *Baccaurea sapida*, *Elaeagnus latifolia* (TSS 11.9 % and acidity (2.8 %), *M. esculenta* (TSS 5.7–6.5 % and acidity 2.5–4.8 %). Kumar *et al.* (2015) recorded maximum TSS in fruit of *Diploknema butyraceae* (18 ^{o}Brix) and minimum was recorded in *Machilus edulis* (3 ^{o}B). Physico-chemical characteristics of five wild edible fruits grown in Manipur were studied by Sharma *et al.* (2015) and TSS and titrable acidity were recorded to be highest in *Dyconia indica* (8 ^{o}Brix) and *Rhus semialata* (16.51%), respectively.

Deb and Bhawmik (2013) carried out the research on physico-chemical properties of Burmese grape (*Baccaurea sapida* Muell. Arg.)- an underutilized fruit of West Bengal and results showed the total soluble solids in a range of 11.52 ^{o} Brix - 13.12 ^{o} Brix and acidity in a range (1.99 % to 2.236 %) amongst different accessions of Burmese Grape. Bhutia (2013) reported estimated TSS for various fruits of Sikkim and they were significantly different among the species as *D. butyraceae* (18.50 ^{o}Brix), *E. sikkimensis* (17.03 ^{o}Brix), *E. indica* (11.53 ^{o}Brix), *F. roxburghii* (7.60^{o} Brix), *E. latifolia* (6.92^{o} Brix), *F. roxburghii* (7.60 ^{o}Brix), *R. ellipticus* (6.14 ^{o} Brix), *S.*

axillaris (6.86 ^{o} Brix), *and M. edulis* (3.55 ^{o} Brix). The fruit of *Elaeagnus angustifolia* was reported to have highest TSS as 13.14 ^{o} Brix (Ersoy *et al.,* 2013).

TSS ranged from 5.10^{o} to 7.40^{o} Brix in *R. discolor* to 9.40^{o}–11.50 ^{o}Brix in *R. idaeus* was reported by Purgar *et al.* (2012).Total soluble solids of 8.32^{o} B and titrable acidity (0.14 %) in *Morinda citrifolia* pulp and 4.98° Brix and titrable acidity (0.09 %) in *M. pubescens* pulp were recorded (Desai *et al.*, 2010). Physico- chemical and nutritional evaluation of Kasmal (*Berberis lyceum)* was carried out and the value of TSS and titrable acidity were reported to be 18.28 ^{o}Brix and 1.32 per cent, respectively (Sood *et al.*, 2010). The pulp of Thai bael fruit contains 39.5^{o} Brix of TSS and acidity (0.94 %) (Charoensiddhi and Anprung, 2008). TSS of mulberry white (*Morus alba*), mulberry red (*M. rubra*) and mulberry black (*M. nigra)* in the East Anatolia region of Turkey varied from 15.9 to 20.4 per cent (Ercisli and Orhan, 2007). Mean total solids and soluble solids contents of chokeberry reported to be 35.2 per cent and 18.3° B, respectively (Zatylny *et al*., 2005). Similarly, TSS of native black mulberry genotypes ranged between 13.11 and 16.23 per cent (Koyuncu *et al*., 2004). TSS determination of fruits *Machilus edulis*, *Baccaurea*, *Passiflora*, *Eriolobus*, *Elaeocarpus*, *Bassia sapida*, *Elaeagnus latifolia*, *Spondias axillaris* and *Diploknema butyracea* wasreported to range from 4.66 and 21.0 per cent (Sundriyal and Sundriyal, 2001, Sundriyal *et al*., 1998). TSS of *Rubus ellipticus* juice was 16.6 per cent (Parmar and Kaushal, 1982).

2.2. Phyto chemicals

2.2.1. Vitamins

Rajalakshmi *et al.* (2017) presented the physicochemical properties of some wild fruits in which vitamin C was noted maximum as 19.1 mg 100 g^{-1} in the fruit of *S. jambose* followed by 12.35 mg 100 g^{-1} in *T. orientalis* and Vitamin A was observed maximum in the fruit of *T. orientalis* i.e. 87.18 mg 100 g^{-1}. The fresh fruits of *Antidesma bunius* and *Eugenia operculata* were found to contain vitamin C of 7.30 ± 1.452 mg $100g^{-1}$ and 6.60 ± 1.123 mg $100g^{-1}$ respectively (Islary *et al.,* 2017).The Vitamin content of the fresh fruit of *G. umbellata* reveals high content of Vitamin C (25 mg $100g^{-1}$). Vitamin A (1.29 mg $100g^{-1}$), Vitamin B_1 (0.023 mg $100g^{-1}$) and Vitamin B_{12} (0.014 mg $100g^{-1}$) were detected in fresh fruit of *G. umbellata* (Sudhakaran and Nair, 2016).

The result of vitamin analysis of fruits showed that fruits of *Semecarpus anacardium* (0.0024 mg g^{-1}) showed the highest vitamin B^1 content and the lowest value was shown by fruits of *Phyllanthus emblica*, *Syzygium cumini* (each 0.0003 mg g^{-1}) (Misra and Misra, 2016). The vitamin C estimated in fresh fruit of *Grewia sapida* was 8.6 ± 0.30 mg $100g^{-1}$ (Islary *et al.,* 2016). Hazali *et al.* (2015) determined the phyto-chemicals and vitamin content of underutilized *Baccaurea angulate* fruit and the amount of Vitamin A in whole fruit, skin and pulp were recorded in the unit of microgram per 100 gram (μg $100g^{-1}$) as 25.2±2.83, 0.00±0.00 and 28.3±12.45 respectively and amount of Vitamin C in whole fruit, skin and pulp were recorded as 2.55 ± 0.00 mg $100g^{-1}$, 1.29±0.00 mg $100g^{-1}$and 2.57± 0.00 mg $100g^{-1}$, respectively.

While Vitamin E analysis shows there is no reading in the part of whole fruit and pulp compared with skin that recorded average amount of 7.79 ± 0.28 µg/100 g. Analyses revealed that soursop (potential underutilized fruits) are rich in Vitamin C (48 mg $100g^{-1}$) (Singh *et al.*, 2014).While evaluating the nutritional potential of five unexplored wild edible plants Kalita *et al.* (2014) reported Vitamin C contents of *S. spirale* fruits had higher value (53.4 mg $100g^{-1}$), which was followed by *S. spirale* (leave) and *P. pedicellatum, G. hirta, M. roxburghii* and *C. spinulosa* had similarity in possessing nearly the same value. *S. spirale* (leaf) was characterized as possessing highest value of α -tocopherol content (55.7mg $100g^{-1}$) and the second highest was possessed by *G. hirta* (43.2 mg 100 g^{-1}) and *P. pedicellatum* (32.4 mg 100 g^{-1}) which was slightly more than the other three plants, whereas *M. roxburghii* had highest amount of Vitamin A content (66.1 mg 100 g^{-1}). Olayiwola *et al.* (2013) investigated for phytochemical constituents of some wild edible fruits from South West Nigeria and they found that Vitamin C content ranged from 15.87 mg 100 g^{-1} in *C. albidum* to as high as 204.86 mg $100g^{-1}$ in *S.mombin*. Vunchi *et al.* (2011) obtained the vitamin A content as 0.27mg $100g^{-1}$ in *Vitex doniana* fruits.

2.2.2. Total phenols

Islary *et al.* (2017) estimated total phenolic content in *E. operculata* and *A. bunius* fruits which was found as 226.741 ± 2.099 mg GAE g^{-1}DE and 119.356 ± 1.395 mg GAE g^{-1} DE respectively. Rajalakshmi *et al.* (2017) had noticed that the fruit of *T.orientalis* was rich in vitamin –A. while, vitamin-C was found highest in *Syzygium jambos* fruits.The total phenolic content of *Baccaurea sapida* fruit pulp was found to be highest as 32.71 ± 0.12 mg GAE/g of methanol extract (Mann *et al.*, 2016). The total phenolic in *G. sapida* fruit was found to be 294.353 ± 4.696 mg GAE g^{-1} of dried extract (Islary *et al.*, 2016).

Sharma *et al.* (2015) found the highest total phenol content in the order of *R. semialata* (172.84 mg GAE g^{-1}), *Dyconia indica* (49.26 mg GAE g^{-1}) *A. carambola* (43.11 mg GAE g^{-1}), *G. xanthochymus* (31.31 mg GAE g^{-1}) and lowest in *G. pedunculata* (9.44 mg GAE g^{-1}). Singh *et al.* (2014) found (117.71 mg $100g^{-1}$) polyphenols in the fruit of Soursop (Underutilized fruits). Korekar *et al.* (2014) carried out research on variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Seabuckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya and mentioned that the fruits were found to be rich in TPC ranging from 964 to 10,704 mg gallic acid equivalent 100 g^{-1}. At population level, the highest TPC (6863 mg GAE 100 g^{-1} FW) was observed in HUN while the lowest was observed in CHU population (2023 mg GAE 100 g^{-1} FW). The overall mean TPC was 2986 mg GAE 100 g^{-1} FW. In general, fruits were found with high amount of TPC. In the fruit of *Garcinia cowa*, the total phenol content was found at amount of 74.18±0.64 μg GAE mg^{-1} (Sarma *et al.*, 2014).

Singh *et al.* (2014) studied the phenolic content and antioxidant activity of some underutilized wild edible fruits of Sikkim Himalaya and value of total phenolic content of different fruits were reported as highest in Spondias axillaris (69. 4 mg GAE g^{-1}) followed by *Baccaurea sapida* (51. 4 mg GAE g^{-1}), *Diploknema butyraceae* (37.1 mg GAE g^{-1}), *Elaeagnus latifolia* (24.2 mg GAE g^{-1}), *Elaeocarpus Sikkimensis* (18.2 mgGAE/g), *Machilus edulis* (12.7 mg GAE g^{-1}) and *Eriolobus indica* (10 mg GAE g^{-1}). Singh *et al.* (2014) carried out estimation of phytochemicals and determination of β carotene in *Haematocarpus validus* an underutilized fruits of

Andaman and Nicobar Islands and maximum total polyphenol (400 GAE mg 100g^{-1}) was observed in pulp, pericarp (341.04) and seed (249.55). Bhardwaj *et al.* (2014) found the highest phenolic content in the fresh pulp followed by dried pulp and then seeds of *Annonna squamosa*.

Qualitative and quantitative phytochemical analysis of 70% methanolic extract of *Elaeagnus latifolia* Linn. possessed 7.04 ± 0.27 mgGAE 100 g^{-1} of total phenolic content (Panja *et al.,* 2014). Hassan *et al.* (2013) revealed that the total phenolic content was relatively higher in the flesh (7.2 mg GAE g^{-1}) as compared to the peel (5.3 mg GAE g^{-1}) of *Garcinia parvifolia* fruit in the 80% methanol extracts. It is also suspected that the phenolic content in *G. parvifolia* has the same compound as *G. mangostana* since they belong to the same family. Pal *et al.* (2013) found the higher total phenolic content (30.47 mg g^{-1} extract) in the fruit of *Berberis asiatica* as compared to *Pyracantha crenulata* (7.43 mg g^{-1} extract).

Olayiwala *et al.* (2013) carried out some phyto-chemical analysis of some wild fruits in South West Nigeria and total phenolics was observed maximum 398.23 mg 100 g^{-1} in (*C. albidum*) and lowest 121.39 mg 100g^{-1} in (*C. millenii*). Antioxidant profiling of wine preparedfrom *Baccaurea ramiflora* was carried out and total phenolic content was found in a range of 141.27 to 313.78 mg GAE L^{-1} (Goyal *et al.,* 2013). Prakash *et al.* (2012) found the total phenolic contents (TPC) in wide variation from 7.3 (*Ficus hookeri*, fruits) to 119.2 mg g^{-1} GAE (*Emblica officinalis*, fruits). Fruit pericarp of *Castanopsis tribuloides* (46.8 mg/g), fruits of *Spondias axillaris* (69.4 mg g^{-1}) and *Baccaurea sapida* (51.4 mg/g), fruits (119.2 mg g^{-1}) and seeds (81.5 mg g^{-1} GAE) of *Emblica officinalis* were found to have good amounts of TPC. Purgar *et al.* (2012) observed the total phenol content within a range of 382–586 mg kg^{-1} in the fruit of native blackberry (*R. discolor*) genotypes. Among native blackberries (*R. discolor*) investigated in this study, the highest amounts of total phenolics were registered in genotype 1 (586 mg kg^{-1}). The total phenolic content of the 39 fruits tested which were ranged from 604.80 to 35.10 mg GAE 100 g^{-1} FW. Thingbaijam *et al.* (2012) reported 21.404 mg GAE mg^{-1} dw total phenolic content in the leaves of *Ficus auriculata*.

The largest concentration of polyphenols were found in *Ziziphus mauritiana*, followed in order by *Hibiscus sabdariffa*, *Annona cherimola, Chrysophyllum cainito, Flacourtia jangomas*, and *Annona muricata* (more than 400 mg GAE 100^{-1} g FW). Whereas, *Genipa americana, Musa balbisiana, Anona purpurea, Persea Americana* and *Bactris gasipaes* was reported to have lowest polyphenol content (Murillo *et al.,* 2012). Ercisli *et al.,* 2012 results revealed that the total phenolic contents of the fruits of medlar genotypes ranged from 114 mg GAE 100 g^{-1} FW in M11 genotype to 293 mg GAE 100 g^{-1} in M5 genotype. The average total phenolic content of genotypes was 194 mg GAE 100^{-1} g FW.

Among the few indigenous fruits of Manipur studied, the total phenolic content was found to be maximum in *Elaeocarpus serratus* Linn (chorphon) i.e. 400 mg 100 g^{-1} while minimum is *Ficus palmata* Forsk (Heibam) i.e. 160 mg 100 g^{-1} (Swapana *et al.,* 2012). Saini *et al.* (2012) carried out the research on comparative study of three wild edible fruits of Uttarakhand for antioxidant, antiproliferative activities and polyphenolic composition and they observed that the total phenolic contents of methanol extracts varied from 58 to 490.5 mg GAE 100 g^{-1} FW and that of acetone extracts were 74.51 to 494.7 mg GAE 100 g^{-1} of FW using gallic acid as standard (R2 = 0.9928). The highest amount of phenolic content was found in the acetone extract of *Ficus palmata* (494.7 mg GAE 100 g^{-1} FW) while least amount was observed in methanol extract of *Ficus auriculata* (58 mg GAE/100 g^{-1} FW). Karuppusamy *et al.* (2011) experimented on antioxidant activity of selected six lesser known edible fruits from Western Ghats of India their results showed phenolic content in order of higher to lower in following fruits *Mahonia leschenaultii* (86.8 ± 0.30 GAE 100g^{-1}), *Gaultheria fragrantissima* (80.4 ± 3.18 GAE 100g^{-1}), *Rubus ellipticus* (72.0 ± 1.25 GAE 100g^{-1}), *Grewia tiliaefolia* (44.1 ± 1.81 GAE 100g^{-1}), *Ziziphus rugosa* (41.8 ± 0.20 GAE 100g^{-1}) and *Flueggea leucopyrus* (31.7 ± 4.92 GAE 100g^{-1}). Ashraf *et al.* (2011) found 690.62–998.29 mg L^{-1} of total phenolic constituent from durian fruits extract. Aberoumand (2011) had evaluated the total phenolic contents in eight plant foods which were used as traditional vegetables and fruits. Their phenolic contents varied from 0.87 to 7.02 mg gallic acid g^{-1}. *Cordia myxa* Roxb contained 402 mg 100g^{-1} of phenolics. Their result suggested that the plant based foods contains potential antioxidant properties and valued for culinary and possible nutritive use. Seal (2011) found huge variation in amount of total phenols which ranged from 6.42 ± 0.34 to 28.56 ± 0.78 mg GAE g^{-1} dry matter. The acetone extract of *M. esculanta* (2.56) ± 0.78 mg GAE g^{-1}) was reported to be highest followed by *M. nagi* (19.31 ± 0.51 GAE g^{-1}) while lower amount of phenol was observed in acetone extract of *Elaeagnus pyriformis* (6.42 ± 0.34 mg GAE g^{-1}).

Prakash *et al.* (2011) investigated the antioxidant potential of some under-utilized fruits and they reported varied amount of TPC from 10.5 mg GAE g^{-1} (in *Carissa carandus*) to 343.2 mg GAE g^{-1} (*Caesalpinia mexicana*). Appreciable amount of TPC was also noted in the fruit of *Eriolobus indica* (23.7 mg GAE g^{-1}), *Ficus hookeri* (20.7 mg GAE g^{-1}) and *Elaeocarpus sikkiminensis* (18.2 mg GAE g^{-1}).

Milivojević *et al*. (2010) reported the total phenol content for raspberry cultivars within the range 1.02–2.22 mg g^{-1}, and 1.10 mg g^{-1} for native raspberry.

Fu *et al.* (2010) estimated the total phenolic contents of 56 wild fruits using the Folin–Ciocalteu method the total phenolic contents varied from 0.06 to 14.1 mg GAE g^{-1} wet weight with a 235-fold difference for the water-soluble fractions and eight wild fruits, *Eucalyptus robusta* (14.1 ± 0.56 mg GAE g^{-1}), *Melastoma sanguineum* (9.68 ± 0.10 mg GAE g^{-1}), *Melaleuca leucadendron* (9.28 ± 0.37 mg GAE g^{-1}, *Melastoma candidum* (8.56 ± 0.30 mg GAE g^{-1}), *Gordonia axillaris* (7.88 ± 0.44 mg GAE g^{-1}), *Diplospora dubia* (7.49 ± 0.42 mg GAE g^{-1}), *Rosa laevigata* (7.45 ± 0.28 mg GAE g^{-1}) and *Eurya nitida* (6.27 ± 0.36 mg GAE g^{-1}) showed the highest total phenolic contents while *Areca triandra* had the lowest total phenolic content with 0.06 ± 0.01 mg GAE g^{-1} wet weight. For the fat-soluble fractions, the total phenolic contents varied from 0.38 ± 0.02 to 40.7 ± 2.49 mg GAE g^{-1} wet weight with a 107–fold difference and eight wild fruits, *Eucalyptus robusta* (40.7 ± 2.49 mg GAE g^{-1}), *Eurya nitida* (28.8 ± 0.60 mg GAE g^{-1}), *Caryota mitis* (17.5 ± 0.71 mg GAE g^{-1}), *Gordonia axillaris* (16.7 ± 0.64 mg GAE g^{-1}), *Melaleuca leucadendron* (16.4 ± 1.22 mg GAE g^{-1}), *Lagerstroemia speciosa* (14.9 ± 0.32 mg GAE g^{-1}), *Viburnum sempervirens* (13.9 ± 0.12 mg g^{-1}) and *Melastoma sanguineum* (13.6 ± 0.39 mg GAE g^{-1}) showed the highest total phenolic contents. Meanwhile, *Diospyros kaki* had the lowest total phenolic content with 0.38 ± 0.02 mg GAE g^{-1} wet weight. For the combined value of the water-soluble and fat-soluble fractions, the total phenolic contents varied from0.49 ± 0.04 to 54.8 ± 3.05 mg GAE g^{-1} wet weight with a 112–fold difference, and eight wild fruits,*Eucalyptus robusta* (54.8 ± 3.05 mg GAE g^{-1}), *Eurya nitida* (35.0 ± 0.96 mg GAE g^{-1}), *Melaleuca leucadendron* (25.6 ± 1.59 mg GAE g^{-1}), *Gordonia axillaris* (24.6 ± 1.08 mg GAE g^{-1}), *Melastoma sanguineum*

(23.3 ± 0.49 mg GAE g^{-1}), *Caryota mitis* (21.4 ± 0.85 mg GAE g^{-1}), *Lagerstroemia speciosa* (20.1 ± 0.35 mg GAE g^{-1}) and *Viburnum sempervirens* (18.5 ± 0.40 mg GAE g^{-1}), showed the highest total phenolic contents.

Ikram *et al.* (2009) worked on the underutilized fruits and analyzed for AC and TPC and their findings showed the underutilized fruits, *Sandoricum* and *Phyllanthus* fruits contained the highest TPC (>2000 mg 100 g^{-1} edible portion). Phenolic compounds contribute to fruit quality and nutritional value in terms of modifying colour, taste, aroma, and flavour. Total phenolic content of wild materials from Turkey was found in a range between 610 mg and 1,455 mg 100 g^{-1} fresh weight (Yilmaz *et al.*, 2009). Charoensiddhi and Anprung (2008) reported that his research results revealed that bael fruit pulps had total phenolic content of 87.34 mg GAE g^{-1} dw and which were in the range of traditional Chinese medicinal plants associated with anticancer (2.2-503 mg GAE g^{-1} dw). Rimpapa *et al.* (2007) studied the total phenolic content of edible fruits bonsai Bonsia and TPC was found in a range of 0.2 to 12.7 mg g^{-1} fresh weight. Highest TPC was observed in the fruits of *Sambucus nigra L* (12.7 mg g^{-1}) followed by *Vaccinium myrtillus* L. (10.4 mg g^{-1}) and *Rubus fruticosus* (9.8 mg g^{-1}) while, the lowest TPC was observed in *Cucumis melo* (0.2 mg g^{-1}). Pantelidis *et al*. (2007) estimated phenolic constituents of raspberries cultivars that varied from 657 to 2494 mg kg^{-1} of GAE.

2.2.3. Total Flavonoid and flavonol contents

Islary *et al.* (2017) noted that the total flavonoid content (TFC) in the fruits (*Eugenia operculata* Roxb. and *Antidesma bunius* L.) were 108.761±7.015 mg QE g^{-1} DE and 64.323±8.828 mg QE g^{-1} DE, respectively. The total flavonoid content in *G. sapida* fruit was found to be 116.95 ± 10.71 mg QE g^{-1} of dried extract (Islary *et al.*, 2016).The maximum flavonoid and flavonol content as 71.67 µg RE and 85.58 µg RE per mg of methanol extract, respectively were obtained from the fruit of *Baccaurea sapida* (Mann *et al.,* 2016).

Sharma *et al.* (2015) reported the highest total flavonoid in the fruit of *G. xanthochymus* (5.313 mg QE g^{-1}) among the five wild fruits from Manipur whereas lowest flavonoid content was observed as 0.379 mg QE g^{-1} in the fruit of *Averrhoea carambola.* As per the results of Hazali *et al.* (2015) phytochemical screening of *Baccaurea angulata* revealed that whole fruit part contains strong flavonoid.While screening of nutraceutical potential of *Annona squamosa* an underutilized exotic fruits of India, Bhardwaj *et al.* (2014) observed highest flavonoid content (TFC) was found to be present in seeds followed by dried and fresh pulp. The seeds were found to have a high flavonol content followed by dried and fresh pulp. Singh *et al.* (2014) investigated phytochemicals and β- carotene in *Haematocarpus validus* an underutilized fruits of Andaman and Nicobar Islands and total flavonoids found were 542 RE mg $100g^{-1}$ in pulp, whereas less amount from pericarp and seed (290.45 & 285.66), respectively.

Qualitative and quantitative phytochemical analysis of 70% methanolic extract of *Elaeagnus latifolia* Linn. possessed 5.44 ± 0.16 mg QE 100 g^{-1} of total flavonoid content (Panja *et al.*, 2014). The highest total flavonoid content was shown in the 80%methanolic extract of the flesh of *G. parvifolia* with the value of 5.9 ± 0.1mg RE g^{-1} followed by the peel (80% methanol), flesh (aqueous extract) and peel (aqueous extract), with the values of 3.6 ± 0.3, 2.2 ± 0.1, and 1.2 ± 0.0 RE g^{-1}, respectively (Hassan *et al.,* 2013). Antioxidant profiling of wine prepared from *Baccaurea ramiflora* was carried out and total flavonoid content was found in a range of 149.22

to 531.2 mg QE L^{-1} while, flavonol content ranged from 103.2 – 179.2 QE L^{-1} (Goyal *et al.,* 2013).The total flavonoid content among the few indigenous fruits of Manipur was studied and it was found to be maximum in methanolic extract of *Brucea javanica* Linni.e. 610 mg $100g^{-1}$ while minimum is *Ficus palmata* Forsk (Heibam) i.e. 430 mg $100g^{-1}$ (Swapana *et al.,* 2012).

Total flavonoid contents were found to be 50.83 mg GAE mg^{-1}dw in the leaves of *Ficus auriculata* (Thingbaijam *et al.* 2012).The total flavonoid content was determined using catechin as standard (R2 = 0.9994) and it varied between 96.45 to 201 mg GAE 100 g^{-1} FW in methanol extracts while total flavonoid contents in acetone extracts were 240 to 298 mg GAE 100 g^{-1} FW. The highest amount of total flavonoids was observed in the acetone extract of *Ficus palmata* (298 mg GAE 100 g^{-1} FW) while least amount was found in methanol extract of *Ficus auriculata* (96.45 mg GAE 100 g^{-1} FW) (Saini *et al.,* 2012).The flavonoid content found in several varieties of durian was observed in the range of 211.36-220.34 mg L^{-1} as CE (Ashraf *et al.,* 2011).

Seal (2011) estimated the flavonoid content of extract associated with quercetin equivalent to range between 1.49 ± 0.05 to 9.67 ± 0.08 mg g^{-1} dry matter. The flavonoid content in the acetone extract of *Ealeagnus latifolia* (9.67 ± 0.08 mg g^{-1}) and *M. nagi* (3.79 ± 0.05 mg g^{-1}) was found to be higher than the aq methanol and acetone extract of *Eleagnus pyriformis* and *M. esculanta*. In case of flavonol the highest amount was observed in the acetone extract of *Elaeagnus latifolia* (16. 58 ± 0.14 mg g^{-1}) followed by *M. nagi* (11.17 ± 0.05 mg g^{-1}). Appreciable amount of flavonols were also found in the acetone extract of *M. esculanta* (8.77 ± 0.06 mg g^{-1}). Karuppusamy *et al.* (2011) evaluated the antioxidant activity of selected six lesser known edible fruits from Western Ghats of India and their results revealed total flavonoid content found in order of higher to lower in following fruits *Mahonia leschenaultii* (95.5 ± 1.76 QE $100g^{-1}$), *Gaultheria fragrantissima* (94.3 ± 1.35 QE $100g^{-1}$), *Rubus ellipticus* (86.4 ± 2.04 QE $100g^{-1}$), *Grewia tiliaefolia* (47.1 ± 0.92 QE $100g^{-1}$), *Flueggea leucopyrus* (42.7 ± 0.46 QE $100g^{-1}$) and *Ziziphus rugosa* (41.8 ± 0120 QE $100g^{-1}$). Charoensiddhi and Anprung (2008) had found relatively high amount of total flavonoids (15.20 mg CE g^{-1}dw) in the pulp of bael fruits which was higher than other commercial fruits and vegetables.

2.2.4. Ascorbic acid, anthocyanin and total carotenoids

Kumar *et al.* (2015) estimated maximum ascorbic acid in the fruit of *Spondias axillaris* (32.0 mg $100g^{-1}$ fw) and minimum 1 mg 100 $g^{-1}$of fw in *Castanopsis hystrix*. Total carotenoid of the fruits was found ranging between 0.2 to 63.0 mg $100g^{-1}$ of fresh weight. *Machilus edulis* was estimated with highest carotenoid while *Rubus ellipticus* was found to be least. Anthocyanin ranged from 0.1 to 3.8 mg $100g^{-1}$ fresh weight and was found highest in fruits of *Rubus ellipticus* while *Castanopsis hystrix* and *Duchesnia indica* showed lowest.

Korekar *et al.* (2014) mentioned that the ascorbic acid content ranged from 56 to 3909 mg 100 g^{-1} FW in seventeen natural genotypes of seabuckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya. The highest content 2318 mg 100 g^{-1} was in KHA and lowest value 621 mg 100 g^{-1} was observed in PHY and the carotenoids content was found to range between 0.1 to 14.4 mg 100 g^{-1} FW. Singh *et al.* (2014) had found significant amount of carotenoids (33.65 µg 100 g^{-1}) and ascorbic acid content of 145.23 mg 100 g^{-1} in the fruit of soursop. Study revealed that the total concentration of anthocyanin in the investigated fruits of *Haematocarpus validus* was found to be 203.77 C3GE mg $100g^{-1}$ in pulp followed by pericarp (164.41) and seed (102.17) (Singh *et al.,* 2014). Sarma *et al.* (2014) reported 68.03 mg 100 g^{-1} ascorbic acid from the fruit of *Garcinia cowa.*Mature fruits of *Elaegnus conferta* (8.4mg $100g^{-1}$ FW) and ripened fruits of *Schleichera oleosa* (3.4mg $100g^{-1}$ FW) showed highest carotenoid content (Valvi *et al.,* 2014). Bhutia (2013) studied on physicochemical characteristics of some indigenous minor fruits of Sikkim and it was revealed that the concentration of ascorbic acid varied in the range of 0.7 – 32. 0 mg 100 g^{-1} FW).

It was noted maximum in the fruit of *Spondias axillaris* and minimum in the fruit of *C. hystrix*. Appreciable amount of ascorbic acid was found in *Prunus edulis* (27.7 mg $100g^{-1}$), *Diploknema butyraceae* (24.3 mg 100 g^{-1}), *Morus alba* (15.7 mg 100 g^{-1}), *Elaeagnus latifolia* (11.2 mg 100 g^{-1}), *Eleaocarpus sikkimensis* (10.0 mg 100 g^{-1}), *Eriolobus indica* (7.5 mg 100 g^{-1}), *Rubus ellipticus* (5.9 mg 100 g^{-1}) *Machilus edulis* (5.0 mg 100 g^{-1}) and *Ficus roxburghii* (3.1 mg $100g^{-1}$). Total carotenoid content was found in a range of 0.1 mg 100 g^{-1} (*M. alba*) to 62.9 mg 100 g^{-1} (*Machilus edulis*). It was also noted appreciable amount in other fruits like *Eleaocarpus sikkiminensis* (59.7 mg 100 g^{-1}). Anthocyanin content ranged from 0.1 to 3.8 mg $100g^{-1}$ fresh weight. Highest concentration of anthocyanin was found in fruits of *Rubus ellipticus* followed by *Prunus cerasoides* (3.0 mg $100g^{-1}$ fresh weight), *Elaegnus latifolia* (1.8 mg $100g^{-1}$ fresh weight), *Elaeocarpus sikkimensis* (1.5 mg $100g^{-1}$ fresh weight) and *Passiflora edulis* (1.3 mg $100g^{-1}$ fresh weight) while least was estimated for *Castanopsis hystrix* and *Duchesnia indica*. In remaining estimated fruits anthocyanin ranged from 0.4 to 0.9 mg $100g^{-1}$ fresh weight. Hassan *et al.* (2013) stated that the highest total anthocyanin content was found in the 80% methanolic extract of the peel of *G. parvifolia* i.e. 4.4 ± 0.2 mg C-3-GE 100 g^{-1} while total carotenoid was noted maximum (17.0 ± 0.3 mg BC 100 g^{-1}) in peel and lowest in flesh (3.0 ± 0 mg BC 100 g^{-1}) of *G. parvifolia* fruits. It was reported that the total proanthocyanin content of Latkan (*Baccaurea ramiflora*) wine and juice ranged from 1.46 – 8.45 µg Catechin L^{-1} (Goyal *et al.*, 2013).

The two wild edible fruits *Pyracantha crenulata* and *Berberis asiatica* from North western Indian Himalaya were evaluated and noted *P. crenulata* possessed the comparatively higher levels of β-carotene and lycopene (5.08±0.253 µg g^{-1} extract and 16.86±0.841 µg g^{-1} extract) than *B. asiatica* (4.53± 0.216 µg g^{-1} and 10.62 ± 0.484 µg g^{-1}), respectively. However ascorbic acid (ASA) content was recorded significantly higher in *B. asiatica* (31.96 ± 1.524 mg g^{-1} extract) as compared *P. crenulata* (18.68±0.891mg g^{-1} extract) (Pal *et al.*, 2013). Olayiwola *et al.* (2013) obtained the values for total carotenoid in a range of 172.77 µg 100g^{-1} in *C. millenii* to 1380.17 µg 100 g^{-1} in *C. albidum* and total anthocyanin content in a range of 27.78 mg 100 g^{-1} to 57.42 mg 100 g^{-1} in *I. gobonensis* and *S. mombin,* respectively while investigating of some wild fruits from SW Nigeria.

Singh *et al.* (2012) reported the fruit of *Malpighia glabra* an underutilized fruits of Andaman Island was found to be rich in total carotenoids (precursor of Vitamin A) i.e., 109.16 mg 100g^{-1}. Purgar *et al.* (2012) observed the value of ascorbic acid in the fruit of *R. discolor* genotypes which varied from 30.64 to 33.09 mg 100 g^{-1} and in *R. idaeus* genotypes ranged between 22.34 and 45.00. They also reported that *R. discolor* demonstrated the most abundant quantity of anthocyanin (2367 mg kg^{-1}). Karuppusamy *et al.* (2011) evaluated the antioxidant activity of selected six lesser known edible fruits from Western Ghats of India and their results revealed that the anthocyanin content was recorded in a range of 0.34 to 8.58 CGE 100g^{-1}, whereas it was noted highest in *Mahonia leschenaultia* (8.58 CGE 100g^{-1}) followed by *Grewia tiliaefolia* (2.60 CCG 100g^{-1}) and lowest was observed in *Ziziphus rugosa* i.e. 0.34 ± 0.02 CCG 100 g^{-1}. As far as ascorbic acid is concerned it was found maximum in *Grewia tiliaefolia* (70.5 AAE 100 g^{-1}) followed by *Mahonia leschenaultia* (69.9 AAE 100g^{-1}) and *Gaultheria fragrantissima* (67.6 AAE 100g^{-1}) while lowest ascorbic acid was observed in the fruit of *Ziziphus rugosa* i.e. 35.0 AAE 100 g^{-1}.

The investigated research related to physicochemical and nutritional properties of kasmal (*Berberis Lycium* Royle.) revealed that the fruit of kasmal possessed 10.83 mg 100 g^{-1} ascorbic acid, 343.0 µg 100 g^{-1} β carotene and 82.47 mg 100^{-1} ml anthocyanin (Sood *et al.*, 2010). Charoensiddhi and Anprung (2008) also reported the total carotenoid content of 32.98 µg and ascorbic acid content of (26.17 mg 100 g^{-1} dw) in the pulp of bael fruits. Rimpapa *et al.* (2007) estimated the total anthocyanin content of edible fruits from Bonsia and it was found in a range of 0.6 to 6.8 mg g^{-1} fresh weight as highest was noted in the fruits of *Prunus avium* (6.8 mg g^{-1}).

Anthocyanin content in the pulp of various indigenous minor fruits like *Rubus ellipticus* contained 3.81 mg 100 g^{-1}, *Diploknema butyracea* (0.615 mg 100 g^{-1}), *Elaegnus latifolia* (1.58 mg 100g^{-1}), *Eriolobus indica* (0.435 mg 100 g^{-1}), *Spondias axillaris* (0.59 mg 100 g^{-1}), *Machilus edulis* (0.391 mg 100 g^{-1}), *Elaeocarpus sikkimensis* (2.98 mg 100 g^{-1}), *Passiflora edulis* (1.31 mg 100 g^{-1}) and 2.43 mg 100g^{-1} in *Prunus cerasoides* and fruits of *Morus sp* was reported to have high ascorbic acid (286 mg/100 g) followed by *Baccaurea sapida* (273.33 mg 100 g^{-1}) and *Hippophae rhamnoides* with 263 mg 100 g^{-1} (Sundriyal and Sundriyal, 2001).

2.3. Antioxidant activities

The previous studies and evidence associated with different antioxidant activities like DPPH Scavenging Activity, Hydroxyl Radical Scavenging Activity, Hydrogen Peroxide Scavenging Activity, Fe^2+ Chelating Activity, Ferric ion Reducing Antioxidant Power (FRAP Assay), Phospomolybdenium Complex (PM) Assay related to underutilized or wild indigenous fruits will be discussed below. Islary *et al.* (2016) reported that the DPPH and ABTS assays were used to evaluate the antioxidant activities in methanolic extract of *G. sapida* fruit and both the methods showed antioxidant activities. The IC50 value found in DPPH assay was 257.666 ± 2.516 μg mL^{-1} and the study also revealed that ABTS free radical scavenging assay showed higher antioxidant capacities with IC50 value 134.33 ± 4.041 μg mL^{-1} than DPPH assay (IC50 = 257.666 ± 2.516 μg mL^{-1}).

The antioxidant activities of crude extracts from *Baccaurea sapida* (pulp and peel) were determined as trolox equivalent antioxidant capacity (TEAC) calculated from DPPH, ABTS and FRAP assay showed the wide range of antioxidant capacities (Mann *et al*., 2016). The antioxidant properties of the Lapsi (*Cherospondias axillaris*) fruit extracts was quantified by using DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) and the results depicted that lapsi fruit contains a strong antioxidant power with higher percentage of inhibition of DPPH radical recorded in ethanolic extracts (98%) (Labh *et al.*, 2015).

Bhardwaj *et al.* (2014) found the results depending on the capacity of antioxidant to decolorize the ABTS+ cation radical and it was reported that the highest antioxidant potential was found in *A. squamosa* pulp (5 mg/ml); followed by seeds (9.06 mg ml^{-1}). They also investigated the ability of the plant extracts to reduce ferric ions into ferrous ions under low pH which was determined using the FRAP reagent and noted the highest antioxidant activity found in dried pulp (62.88 μg 100 mg^{-1}), followed by fresh pulp (45.58 μg 100 mg^{-1}) and seeds (23.07 μg 100 mg^{-1}).

Singh *et al.* (2014) carried out the research on phenolic content and antioxidant activity of some underutilized wild edible fruits of the Sikkim Himalaya. The antioxidant activity of wild edible fruits showed wide variation ranging from 8.6 % (*Eriolobus indica)* to 73.9% (*Spondias axillaris).* Among different fruits analysed, *Spondias axillaris* extract found to possess the highest antioxidant activity (73.9 %) followed by *Baccaurea sapida* (64.7 %), *Cyphomandra betacea* (50.3 %), *Machilus edulis* (38.1 %), *Elaeagnus latifoila* (32.1 %), *Elaeocarpus sikkimensis* (31.8 %), *Ficus hookeri* (21.2 %), *Diplokenema butyracea* (19.6 %) and *Eriolobus indica* (8.6 %).

They also reported that the extract of *Baccaurea sapida*, *Elaeagnus latifoila, Spondias axillaris*, *Eleocarpus sikkimensis* and *Cyphomandra betaceae* exhibited remarkable reducing capacity as evident by their low ASE/ml values. The fruits of *Baccaurea sapida* showed highest reducing power that is in close proximity to standard, quercetin. Further, it was noticed that *Elaeagnus latifolia* fruits exhibited low ARP (38.44) but good reducing power (0.81 ASE ml^{-1}). Khomdram *et al.* (2014a) reported that the antioxidant activity of two wild edible fruits, *Spondias pinnata* and *Elaeagnus pyriformis* were found to be of lower IC50 518.8 μg ml^{-1} and IC50867.8 μg ml^{-1} respectively were exhibited.

In another study Khomdram *et al*. (2014 b) reported the DPPH assay IC50 value of some wild endemic fruits of the Manipur region of India that ranged from 181.21 ± 2.0 μg mL^{-1} to 2717.46 ± 363.6 μg mL^{-1}. Hassan *et al.* (2013) investigated the antioxidant potential of indigenous *Garcinia parvifolia* fruit and their results revealed that the scavenging activity of 80% methanol extract of different parts of the fruit of *G. parvifolia* assayed by DPPH free radical scavenging method showed that the flesh displayed slightly higher scavenging effect as compared to the peel.

The EC50 value was determined to quantify the radical scavenging activity in the samples, the lower EC50 values indicate the stronger antioxidant potential therefore they observed that for 80 % methanolic extract, the flesh showed the lower EC50 of the two fruit parts with values of 58.0 ± 2.0 μg mL^{-1} and 72.7 ± 2.5 μg mL^{-1} they also observed that the reducing ability of the 80% methanol extracts of *G. parvifolia* was higher in flesh followed by the peel. *In-vitro* antioxidant activities of wine and juice of *Baccaurea ramiflora* fruit sample was determined on the basis of DPPH radical scavenging assay for five months of aging process and inhibition of the sample was recorded in the range of 5.85 to 37 % (Goyal *et al.,* 2013).The percent scavenging activities of methanolic extracts of *P. crenulata* and *B. asiatica* on DPPH radical ranged from 13.00 to 32.98 and 23.52 to 79.45 respectively.

While the scavenging activities of methanolic extract of *P. crenulata* and *B.asiatica* on ABTS$^{\bullet+}$ radical ranged from 12.95 to 30.75 % and 30.38 to 88.90 % respectively, (Pal *et al.,* 2013). Prakash *et al.* (2012) determined the antioxidant and free radical scavenging activities of some promising wild edible fruits and antioxidant activities showed a wide variation ranging from 8.6 % (*Citrullus colocynthus*, seeds) to 80.3 % (*Emblica officinalis* fruits). The fruit pericarp (51.6 %) of *Castanopsis tribuloides* fruits (80.3 %) and seeds (62.4 %) of *Emblica officinalis*, fruits of *Spondias axillaries* (73.9 %), *Baccaurea sapida* (64.7 %) and *Cyphomandra betaceae* (50.3 %) were found with reasonably good AOA.

The fruits and seeds of *Emblica officinalis* and fruits of *Baccaurea sapida*, *Eleocarpus sikkimese*, *Spondias axillaries*, *Cyphomandra betaceae* showed low IC50 ranging from 0.016 to 0.044 mg mg^{-1}, low EC50 from 0.70 to 1.91 mg mg^{-1} DPPH, reasonably high values (52.36 to 142.17) of ARP. They also showed high reducing power as evident by their low 0.47 to 1.52 ASE ml^{-1} values. Further, it was noticed that *Elaeagnus latifolia* fruits exhibited low ARP (38.44) but good reducing power (0.81 ASE ml^{-1}).

Promising fruit samples were further subjected to concentration-dependent FRSA using different methods and expressed in terms of IC50 values. The IC50 values for inhibition of lipid per oxidation (LPO) measured by ammonium thiocyanate assay ranged from 0.50 to 4.30 mg ml^{-1}; fruits (0.50 mg ml^{-1}) and seeds (0.92 mg ml^{-1}) of *Emblica officinalis.* Fruits of *Spondias axillaries* (0.66 mg ml^{-1}) and *Baccaurea sapida* (0.84 mg ml^{-1}) showed better inhibition of peroxide formation compared to reference standard quercetin (1.27 mg ml^{-1}). The ferrous ion-chelating capacity with respect to IC50 values ranged from 0.28 (*Emblica officinalis* fruits) to 2.83 mg ml^{-1} (*Spondias axillaris* seeds). The fruits of *Baccaurea sapida* (0.47 mg ml^{-1}) and *Emblica officinalis* (0.15 mg ml^{-1}) were observed to be better compared to standard quercetin (0.66 mg ml^{-1}). The results of non enzymatic SRSA of the extracts showed that fruit of *Emblica officinalis* (1.56 mg ml^{-1}), *Baccaurea sapida* (1.09 mg ml^{-1}) and *Spondias axillaries* (1.24 mg ml^{-1}) were found to be potent superoxide radical scavengers. Fruits of *Emblica officinalis* and *Baccaurea sapida* reported to have better inhibition of hydroxyl radical induced deoxyribose degradation as compared to other extracts. The inhibition of hydroxyl radical noted in non site specific assay exhibited IC50 values of 0.45 (*Emblica officianilis* fruits) to 4.01 mg ml^{-1} (*Cyphomandra betaceae* seeds).

Murillo *et al.* (2012) used the DPPH radical-scavenging assay to assess and compare the scavenging activity of extracts of 39 tropical fresh fruits harvested in Panama, out of them guinda (*Ziziphus mauritania*), soursop (*Annona muricata*), and coffee plum (*Flacourtia jangomas*) exhibited the highest antioxidant capacity (1083.33, 975.00, 928.57, and 928.27 mg TEAC 100 g^{-1} FW respectively). On the other hand, avocado (*Persea americana*) and toreta (*Anona purpurea*) presented very low antioxidant activity (17.00 and 16.00 mg TEAC 100 g^{-1} FW). In this study undomesticated fruits showed a moderate radical scavenging capacity, yet *Chrysophyllum cainito* showed a remarkable high antioxidant capacity (886.36 mgTEC 100 g^{-1} FW).

Haripyaree and Guneshwor (2012) investigated the antioxidant activity of twenty fruits, *Baccaurea sapida, Eleocarpus floribundus, Rubus ellipticus and Spondias pinnata* were among them. Inhibition of DPPH (%) activity of methanolic extracts of these fruits and their IC50 values were found as *Baccaurea sapida* (21.5±0.72 µg ml^{-1} & 293.0 IC50 values), *Eleocarpus floribundus* (20.2±0.40 µg ml^{-1} & 294.2 IC50 Values), *Rubus ellipticus* (28.1±0.22 µg ml^{-1} & 348.9 IC50 values) and *Spondias pinnata* (45.9±0.40 µg ml^{-1} & 238.2 IC50 values).The reducing powers of the four wild fruits were evaluated as mg AAE g^{-1} dry materials and the highest reducing power was exhibited by the acetone extract of *M. esculenta*, (19.33±0.06 mg g^{-1} AAE) which is also high in phenolic content (28.56±0.78 mg GAE g^{-1} dry material) and aq methanol extract of *E. pyriformis* showed lowest activity in terms of ascorbic acid equivalent. The evaluation of anti-radical properties of four wild edible fruits was also performed by DPPH radical scavenging assay and the maximum radical scavenging activity was shown by the acetone extract of *M. esculenta* (IC50= 0.16±0.001 mg dry material), whereas the aq methanol extract of *E. latifola* showed lowest activity (IC50= 0.58±0.01 mg dry material). Strong inhibition was also observed for the aq methanol extract of *M. esculenta* (IC = 0.19±0.0008 mg dry material and *M. nagi* (IC = 0.17±0.0007 mg dry material) (Seal, 2011).

Karuppusamy *et al.* (2011) determined the antioxidant activities using DPPH scavenging methods in some lesser known fruits from Western Ghats if India and demonstrate highest antioxidant activity for two fruit species of *M. leschenaultia* (361.2 ± 3.69 µg ml^{-1}) and *G. fragrantissima* (240.2 ± 1.28 µg ml^{-1}) which are 2.5 times higher than the antioxidant activity of the *Z. rugosa* (116.9 ± 1.02 µg ml^{-1}) and *F. leucopyrus* (76.8 ± 1.22 µg ml^{-1}) extracts. The fruit extract of *R. ellipticus* and *G. tiliaefolia* were found as medium in antioxidant activity i.e. 196.4 ± 1.84 µg ml^{-1} and 120.2 ± 1.86 µg ml^{-1}, respectively.

Fu *et al.* (2010) conducted an investigation on antioxidant capacities of 56 wild fruits from south China and found that the FRAP values ranged from 0.67 ± 0.04 to 143 ± 2.00 µ mol Fe (II)/g wet weight with a 213-fold difference for the water-soluble fractions, and eight wild fruits, namely *Melastoma sanguineum* (143 ± 2.00 µ mol Fe(II)/g), *Eucalyptus robusta* (125 ± 2.49 µ mol Fe(II)/g), *Lagerstroemia indica* (118 ± 1.49 µ mol Fe(II)/g), *Melastoma candidum* (95.2 ± 5.89 µ mol Fe(II)/g), *Rosa laevigata* (86.9 ± 2.18 µ mol Fe(II)/g), *Eurya nitida* (83.3 ± 2.63 µ mol Fe(II)/g), *Viburnum fordiae* (75.7 ± 2.06 µ mol Fe(II)/g) and *Gordonia axillaris* (74.4 ± 3.25 µ mol Fe(II)/g), had the highest antioxidant capacities, while *Areca triandra* had the lowest antioxidant capacity among the 56 wild fruits at 0.67 ± 0.04 µ mol Fe(II)/g wet weight. For the fat-soluble fractions the FRAP values varied from 0.61 ± 0.04 to 377 ± 3.92 µ mol Fe(II)/g wet weight with a 618-fold difference and eight wild fruits, *Eucalyptus robusta* (377 ± 3.92 µ mol Fe(II)/g), *Eurya nitida* (345 ± 9.39 µ mol

Fe(II)/g)), *Lagerstroemia speciosa* (182 ± 7.89 μ mol Fe(II)/g), *Melaleuca leucadendron* (146 ± 5.38 μ mol Fe(II)/g), *Melastoma sanguineum* (145 ± 8.44 μ mol Fe(II)/g), *Caryota mitis* (138 ± 2.50 μ mol Fe(II)/g), *Lagerstroemia indica* (137 ± 4.26 μ mol Fe(II)/g) and *Diplospora dubia* (117 ± 6.56 μ mol Fe(II)/g) had the highest antioxidant capacities, while *Areca triandra* again had the lowest antioxidant capacity among the 56 tested wild fruits with a value of 0.61 ± 0.04 μ mol Fe(II)/g wet weight.

For the combined value of water-soluble and fat-soluble fractions, the FRAP values varied from 1.28 ± 0.08 to 502 ± 6.41 μ mol Fe(II)/g wet weight with a 392-fold difference and eight wild fruits, *Eucalyptus robusta* (502 ± 6.41 μ mol Fe(II)/g) *Eurya nitida* (428 ± 12.0 μ mol Fe(II)/g), *Melastoma sanguineum* (288 ± 10.4 μ mol Fe(II)/g), *Lagerstroemia indica* (254 ± 5.75 μ mol Fe(II)/g), *Lagerstroemia speciosa* (225 ± 9.68 μ mol Fe(II)/g), *Melaleuca leucadendron* (214 ± 8.86 μ mol Fe(II)/g), *Gordonia axillaris* (180 ± 11.2 μ mol Fe (II)/g) and *Caryota mitis* (174 ± 4.00 μ mol Fe(II)/g), had the highest antioxidant capacities.

Bioactive compounds and volatile compounds of Thai bael fruit (*Aegle marmelos* (L.) Correa) was studied by Charoensiddhi and Anprung (2008) they mentioned that the antioxidant activities were measured in ethanol extract obtained using DPPH and FRAP assays. The DPPH assay was selected to evaluate the antioxidant activities of bael fruit extracts in terms of EC50 and the antioxidant activities obtained from this assay was (6.21μg dw/ μg DPPH).It was also noted that 102.74 μM TE/g dw antioxidant activities was determined by FRAP assay of bael fruit.

2.4. Elements

Rajalakshmi *et al.* (2017) presented the data on mineral composition of some underutilized fruit samples which revealed that calcium (1100mg $100g^{-1}$) was found as the major element which was noted maximum in *T. orientalis* fruits. The potassium (410mg $100g^{-1}$) content was observed highest in *R. ellipticus* fruits as compared to the other samples. Among the three different fruit samples, the *T.orientalis* fruits were noted to have highest value of phosphorus (210mg $100g^{-1}$) content.

Islary *et al.* (2017) studied the mineral constituent in two wild fruits indicated that sodium content of *E. operculata* and *A. bunius* as 640±0.046 mg and 5.377±0.032 mg per 100 g respectively. High content of potassium was found in *A. bunius* (3043.852 ± 6.088 mg 100 g^{-1}) and *E. operculata* (2219.736±6.659 mg 100 g^{-1}). Calcium was found to be 714.820 ± 8.578 mg in *E. operculata* and in *A. bunius* (787.900 ± 14.182 mg). The significant amount of magnesium in *E. operculata* (172.387 ± 0.517 mg 100 g^{-1}) and 250.703 ± 0.251 mg 100 g^{-1} in *A. bunius* was also observed.The iron detected in *E. operculata* (8.279 ± 0.033 mg) and *A. bunius* (7.579 ± 0.015 mg) were noticed.

The concentration of copper, zinc and manganese were 1.774 ± 0.060 mg, 2.903 ± 0.012 mg and 7.616 ± 0.023 mg, respectively in *A. bunius* and in *E. operculata* concentrations were 1.493 ± 0.051 mg, 1.828 ± 0.011 mg and 2.817 ± 0.020 mg, respectively. The amount of cobalt was 0.352 ± 0.050 mg 100 g^{-1} and 0.390 ± 0.019 g 100 g^{-1} respectively in *E. operculata* and *A. bunius* fruits. Cobalt is a part of vitamin B_{12} and also known as cyanocobalamin and its deficiency can cause pernicious anemia. As human body is not capable to synthesize vitamins, the consumption of diets containing these compounds be beneficial.

The fruit of *Grewia sapida* in relation to minerals was found rich in potassium (1243.788 ± 8.707 mg 100 g^{-1}), calcium (472.555 ± 0.945 mg 100 g^{-1}) and magnesium (122.004 ± 0.244 mg 100 g^{-1}). Sodium content of the fruit was found to be 3.873 ± 0.019 mg 100 g^{-1}. The concentration of iron and zinc detected in *G. sapida* fruit were 7.574 ± 0.015 mg 100 g^{-1} and 1.318 ± 0.004 mg 100 g^{-1} respectively. The amount of copper, manganese and cobalt detected in the fruit were 0.905 ± 0.047 mg, 3.208 ± 0.003 mg and 0.299 ± 0.017 mg per 100 g respectively. It was stated that iron is essential for synthesis of haemoglobin in red blood cells which is required for oxygen transportation to the body. Iron deficiency causes anemia and immune system dysfunction (Islary *et al*., 2016).

Mann *et al.* (2016) reported that the fruit peel and pulp of *Baccaurea sapida* was found to be a good source of calcium (39.7 ±0.29 and 42.5 ± 0.31 mg g^{-1}), potassium (1195 ± 0.71 and 738 ± 0.67 mg g^{-1}), magnessium (103.4 ± 0.32 and 109.3 ± 0.21 mg g^{-1}), sodium (75.8 ± 0.91 mg g^{-1}) and iron (10.9 ± 0.22 and 13.2 ± 0.25 mg g^{-1}) respectively. Sudhakaran and Nair (2016) showed the results of mineral analysis which are depicted as potassium (956 mg $100g^{-1}$ dw) was found to be higher followed by calcium (450 mg $100g^{-1}$ dw) in the fruit of *G. umbellate.*In addition the fruit was also having substantial amount of magnesium (245 mg $100g^{-1}$ dw), iron (3.4 mg 100 g^{-1}), (14.1 mg $100g^{-1}$) and zinc (1 mg $100g^{-1}$).

Magnesium is very essential element for various reactions related to enzymes and magnesium deficiency leads to various disorders including asthma, high blood pressure, angina pectoris, cardiac arrhythmias, coronary artery disease, all types of musculoskeletal disorders, mitral valve prolapse, panic disorder, epilepsy, anxiety, chronic fatigue syndrome and psychiatric conditions (Nayak and Basak, 2015). The highest iron content was also recorded in *P. americana* fruit (40mg $100g^{-1}$) (Rajalakshmi *et al.,* 2017).

Kalita *et al.* (2014) evaluate the nutritional potential of five unexplored wild plants from eastern himalayan biodiversity hotspot region (India) and significant variation among the mineral compositions was noticed as potassium and phosphorus were the most abundant elements considered followed by calcium and sodium. Among these wild plants, *M. roxburghii* and *S. spirale* (leaves) were shown to possess the highest amount of potassium (23.8g % and 23.3g %), followed by *P.pedicellatum* and *G. hirta*. The lowest level of potassium was noted in *S. spirale* (fruit) and *C. spinulosa* (0.64g % and 0.05g %). The sodium content was identical in all the five wild food plant samples but calcium level was found to be very low in *C. spinulosa* (0.08g %) in comparison to the other four plants which were very much identical in their calcium content. *S. spirale* (fruits) was characterized and was found to have the highest amount of phosphorus (1.02 g %), which was followed by *S. spirale* (leaves) (0.99g %), *G. hirta* (0.89g %) and *M. roxburghii* (0.77g %); *P. pedicellatum* and *C. spinulosa* had comparatively less phosphorus content (0.22- 0.41g %).

Seal *et al.* (2014) studied the ethnobotanical importance and nutritional potential of five wild edible fruits of Meghalaya state in India. *Spondias axillaris* was among them and their results indicate *S. axillaris* fruit was found to contain varying concentration of minerals *viz*. sodium (0.81 mg g^{-1}), potassium (10.81 mg g^{-1}), calcium (6.05 mg g^{-1}), manganese (0.05 mg g^{-1}), magnesium (0.85 mg g^{-1}), iron (0.37 mg g^{-1}), zinc (0.30 mg g^{-1}) and copper (0.052 mg g^{-1}).The concentration of potassium was reported to be highest among other minerals.

Seal *et al.* (2014) reported the highest concentration of sodium (Na) ranged from 0.17 ± 0.004 mg g^{-1} (*G. cissiformis*) to 0.66 ± 0.005 mg g^{-1} (*Baccaurea sapida*). Highest amount of potassium was noted in the fruit of *G. cochinchinense* (57.22 ± 0.84 mg g^{-1}) and least in the fruit of *Elaegnus latifolia* (6.16 ± 0.16 mg/g). Calcium concentration was found to be maximum in the fruit of *G. cochinchinense* (10.74 ± 0.13 mg g^{-1}) followed by *G. cissiformis* (7.27 ± 0.10 mg g^{-1}) and *Z.armatum* (6.58 ± 0.10 mg g^{-1}). Significant amount of copper was detected in *G. cochinchinense* (0.014 ± 0.0002 mg g^{-1}) and *A. gomezione* (0.012 ±0.0001 mg g^{-1}). An appreciable amount of zinc was found in the fruit of 0.099 ± 0.0008 mg g^{-1} (*B. sapida*) to 0.310 ± 0.001 mg g^{-1} (*Z. armatum*). The manganese concentration of the plants varied from 0.003 ± 0.0001 mg g^{-1} to 0.076 ± 0.001 mg g^{-1} (*Z. armatum*). The highest concentration of iron was noted in fruits of *G. cissiformis* (0.247 ± 0.0008 mg^{-1}) and *Z. armatum* (0.218 ± 0.001 mg g^{-1}).

Ersoy *et al.* (2013) had found around 28 minerals in the fruit of *Elaeagnus angustifolia* in detectable level. Among them potassium (10296.906ppm) was present in the highest concentration, followed by Mg (762.314 ppm), P (609.694 ppm), Ca (547.647 ppm), Na (222.749 ppm), S (190.444 ppm) and B (30.51 ppm) were present in higher amounts and traces of Zn, Li, Se, Al, Ni, V, Cr were also detected. Seal (2012) evaluated the mineral composition of five wild edible plants, traditionally used by local tribes of Meghalaya. Among the two underutilized fruits species *E. latifolia and E. Pyriformis* showed appreciable amount of following minerals like sodium (965 ± 80 and 950 ±10 mg Kg^{-1}), potassium (13580 ± 80 and 14410 ± 80 mg Kg^{-1}), calcium (5860 ±40 and 6260 ±100 mg Kg^{-1}), manganese (21 ± 0.20 and 36 ± 0.20 mg Kg^{-1}), iron (172 ± 0.20 and 159 ± 0.10 mg Kg^{-1}) and zinc (248 ±1.3 and 76 ± 1.5mg Kg^{-1}).

Mineral analyses of some wild edible fruits of Kolhapur district have been carried out by Valvi and Rathod (2011). Among the fruits studied by them, the maximum amount of N, P and Mg were observed in *Grewia tiliifolia* fruits, Ca, Na and K in *Ficus racemosa* fruits respectively and microelement found like iron is higher in *Meyna laxiflora* fruits, zinc in *Elaeagnus conferta* fruits, while copper and manganese are present abundantly in *Flacourtia indica* fruits. Among all minerals, in mature fruits potassium is higher and copper is lower. Hussain (2011) reported mineral elements of *Elaeagnus umbellate* L. fruits showed that calcium, magnesium and phosphorus were 6.34mg, 15.10 mg and 18.90, respectively.

Adepoju (2009) determined elemental composition of three wild fruits *viz. S. mombim, D. guineese* and *M. whytii.* In all fruits Mg was higher and *S. mombin* fruit contains the maximum value of Mg (465.0 ± 21.21), Na (400.0 ± 12.43) and Cu (1.0 ± 0.14) whereas *M. whytii* is high in potassium (410.0 ± 12.20) calcium (300.0 ± 12.20), phosphorus (170.0 ± 7.50), zinc (2.2 ± 0.12), manganese (6.2 ± 0.15). Potassium is major nutrients. Foods rich in K are generally used for the treatment of rheumatoid arthritis and heart disease (Borah *et al.*, 2009). Leterme *et al.* (2006) reported 991 mg 100 g^{-1} of calcium content in the fruit of *Annona squamosa* L.

Rai *et al.* (2005) reported the appreciable amount of mineral concentration from the wild edible fruits of Sikkim. Sodium was observed in a range of 2.9 to 22.3 mg 100 g^{-1}. It was noted maximum in the fruits of *Machilus fructifera* (22.3 mg 100 g^{-1}) and lowest in the fruits of *Castanopsis hystrix* (2.9 mg 100 g^{-1}) and content of other fruits species are as *Dyconia indica* (15. 3 mg 100 g^{-1}), *Ficus hookeriana* (12.8 mg 100 g^{-1}), *Elaeagnus conferta* (6.6 mg 100 g^{-1}) and *Cherospondias axillaris* (5.0 mg 100g^{-1}). The potassium content was found in a range of 160.5 to 911.6 mg 100

g^{-1}. It was noted maximum in *Castanopsis hystrix* (911.6 mg $100g^{-1}$) and least was observed in *Fragaria nubicola* (160.5 mg $100g^{-1}$). It was also observed appreciable amount in the fruits of *Ficus hookeriana* (736.1 mg 100 g^{-1}), *Cherospondias axillaris* (639.3 mg 100 g^{-1}, *Machilus fructifera* (415.3 mg 100 g^{-1}) and *Eleagnus conferta* (233.5 mg 100 g^{-1}) and calcium content was found maximum in *Chorospondias axillaris* (202.1 mg 100 g^{-1}) followed by *Dyconia indica* (200. 5 mg 100 g^{-1}) and *Castanopsis hystrix* (199.0 mg 100 g^{-1}).

Agrahar-Murugkar and Subbulakshmi (2005) analysed 8 fruits and reported *Solanum indicum* was found to be highest in Ca, *Solanum gilo* was rich in P and Mg, Fe was more in *Prunus nepalensis*, Mn in *Viburnum corylifolia, Solanum xanthocarpum* contains higher amount of Na and Cu. *Vangeria spinosa* was higher in zinc. *Gomphogyne cissiformis* was rich in potassium. Sundriyal and Sundriyal (2001) evaluated the mineral composition of 23 wild edible species. The appreciable amount of mineral concentration *viz*. N, P, Na, K, Ca, Fe, Zn, Mg and Cu in per cent was detected in the following underutilized fruits of Sikkim like *Baccaurea sapida* (0.780, 0.132, 0.035, 0.730, 0.158, 0.075, 600 µg g^{-1}, 0.504 and 76.67 µg g^{-1} respectively), *Diploknema butyraceae* (0.546, 0.090, 0.065, 0.816, 0.817, 0.178, 860 µg g^{-1}, 0.611 and 35.66 µg g^{-1} respectively), *Elaeocarpus sikkimensis* (0.860, 0.0683, 0.042, 1.010, 0.631, 0.151, 638 µg g^{-1}, 0.353, 80.0 µg g^{-1} respectively), *Elaeagnus latifolia* (1.250, 0.096, 0.051, 0.910, 1,470, 0.181, 1186.66 µg g^{-1}, 0.544 and 46.66 µg g^{-1} respectively). *Eriolobus indica* (0.280, 0.142, 0.033, 0.431, 0.124, 0.110, 816. 67 µg g^{-1}, 0.446 and 33.33 µg g^{-1} respectively), *Machilus edulis* (0.726, 0.118, 0.024, 0.610, 0.150, 0.253, 392. 50 µg g^{-1}, 0.339 and 100.66 µg $^{-1}$g respectively) and *Spondias axillaris* (0.353, 0.156, 0.039, 0.673, 1.583, 0.109, 831.25 µg g^{-1}, 0.675 and 60.00 µg g^{-1} respectively).

2.5. Characterization and quantification of phenols

The characterization and quantification of phenolic compounds from different fruit species is necessary because these compounds are associated with a various health properties. Identification and characterization of chemical composition of *Rhus coriaria* L. fruit from Hamadan, Western Iran was carried out by Ardalani *et al.* (2016) in which they reported total 191 compounds were identified in *R. coriaria* fruits including 78 hydrolysable tannins (gallic acid), 59 flavonoid such as Apigenin, 40 other compounds such as Butein, 9 anthocyanin such as cyanidin. Seal and Chaudhury (2016) conducted an experiment on identification and quantification of phenolic compounds (aesculin, catechin, rutin, naringin, myrecetin, coumarin, luteolin, quercetin, naringenin, apigenin and kaempferol) using two different solvent extracts (methanol and 80 % aq. ethanol) in the leaves of *V. foetidum* and *P. ocimoides* from NE region of India. They performed this experiment using a reversed-phase high-performance liquid chromatography with photodiode array detector. The experimental results showed high amount of myricetin (36.19 ± 0.020 mg g^{-1} dry extract) and luteolin (30.242 ± 0.033 mg g^{-1} dry extract) present in the 80 % aq. ethanol extract of *P. ocimoides* while a good amount of naringin (18.304 ± 0.022 mg g^{-1} dry extract) was detected in the methanol extract of *V. foetidum*. The HPLC chromatogram of methanol extract of the fruits of *V. foetidum* revealed the presence of gallic acid (0.38 ± 0.007 mg g^{-1} dry extract DE), gentisic acid (5.09 ± 0.03 mg g^{-1} DE), chlorogenic acid (0.83 ± 0.0004 mg g^{-1} DE), syringic acid (3.19 ± 0.008 mg g^{-1} DE), *p*-coumaric acid (0.29 ± 0.003 mg gm^{-1} DE), ferulic acid (0.27 ± 0.001 mg gm^{-1}DE), salicylic acid (0.21±0.006 mg/gm DE) and ellagic acid (12.83±0.004 mg gm^{-1} DE) (Seal *et al.*, 2016).

Seal (2016) investigated and revealed that the HPLC chromatogram of chloroform extract of fruit *S. arvensis* were subjected to presence of quercetin and kaempferol whereas *O. lineari* contained caffeic acid, quercetin and apigenin. Gallic acid, catechin, caffeic acid, syringic acid, quercetin and kaempferol was detected in the leaves of *O. linearis* while only quercetin was found in *S. arvensis*. Rutin, p-coumaric acid, ferulic acid and quercetin were also found in *O. linearis* in small amount. The amount of gallic acid varied in *O. linearis* (0.201 ± 0.002 mg g^{-1}) and *S. arvensis* (0.05 ± 0.0003 mg g^{-1}) using 80 % methanol extract. *O. linearis* showed highest concentration of catechin (0.379 ± 0.003 mg g^{-1}) in the extracts of 1 % aq. acetic acid. Appreciable amount of catechin was also detected in both 80 % aq. ethanol extract (0.204±0.001 mg/g) and methanol extract of the plant (0.113 ± 0.03mg g^{-1}). However, catechin was found only in the 80 % ethanol extract of *S. arvensis* (0.136 ± 0.004 mg g^{-1}). The HPLC analyses of the two plants under investigation revealed that ferulic acid (0.023 ± 0.002 mg g^{-1}) and p-coumaric acid (0.013 ± 0.003) were present only in the 80 % ethanol extract of *O. linearis*. The rutin content in the extract of 1% aq. acetic acid (0.073 ± 0.005 mg g^{-1}) and in 80% aq. ethanol (0.070 ± 0.002 mg g^{-1}) of *O. linearis* was also appreciable. Good amounts of quercetin were found in chloroform extract of *S. arvensis* (0.058 ± 0.0004 mg g^{-1}) and *O. linearis* (0.065 ± 0.003 mg g^{-1}).

Shende *et al.* (2016) conducted an experiment on phytochemical characterization and biological activities of *Docynia indica* fruit extracts and their results revealed that methanolic extract of fresh matured fruits showed highest phenolic concentration at various maturity stages. Phenolic compound were identified using HPLC by comparing with retention times of different standards. Gallic acid was detected only in fresh. Catechin (82.85 mg g^{-1} of extract) was most predominant phenolic acid observed in fresh fruit extract, followed by rutin (5.89 mg g^{-1} of extract), ferulic acid (4.71mg g^{-1} of extract) and gallic acid (3.36 mg g^{-1} of extract).

Seal *et al.* (2016) worked on identification and quantification of flavonoids in *Houttuynia cordata*, *Solanum gilo* and *Solanum kurzii* of North-Eastern region of India and revealed that the HPLC chromatogram of methanol extract of the roots of *H. cordata* showed the presence of rutin whereas 80 % aq.ethanol extract of this plant found to contain good amount of catechin along with small amount of naringin and kaempferol. The methanol extract of the fruits of *S. gilo* revealed the presence of naringenin whereas catechin, rutin and naringenin were detected in 80% aq. ethanol extract of the fruits of this plant as depicted in the HPLC chromatogram. The present study indicated the occurrence of large amount of catechin (6.632 ± 0.046 mg g^{-1} dry extract) and minoramount of naringin (0.172 ± 0.004 mg g^{-1} dry extract) in the 80 % aq. ethanol extract of *H. cordata.* A very small amount of rutin (0.227 ± 0.004 mg g^{-1} dry extract) was also detected in the methanol extract of this plant. The HPLC analysis also revealed the presence of varying amounts of naringenin in the methanol and 80% aq. ethanol extract of *S.gilo* and *S. kurzii*. A moderate amount of catechin (2.70 ± 0.041 mg g^{-1} dry extract) and small amount of rutin were also detected in the 80 % aq. ethanol extract of *S. gilo.*

Leouifoudi *et al.* (2014) worked on “Identification and characterization of phenolic compounds extracted from Moroccan olive mill wastewater” and they revealed that HPLC provided the separation of the major biophenols in the OMWW extracts for detection at 279 nm where the differences between mountainous and plain areas Olive Mill Waste water extracts are observed. Mountainous areas extracts had higher levels of biophenols classes (68.01 %; 68.81 %) than plain areas' extracts (52.59 %; 58.98 %).

Abadio *et al.* (2012) detected 10 polyphenols compound from Bacaba (*Oenocarpus bacaba* Mart.) and characterized compounds were quercetin and rhamnetin derivatives in addition to a range of flavonoids.The black sapota (*Diospyros digyna* Jacq.) fruits were studied by Yahia *et al.* (2011) to identify and quantify phenolics, carotenoids and tocopherols. Total phenolic content was 247.8 mg GAE 100 g^{-1} fw (fresh weight). Important phenolics identified were sinapic acid, myricetin, ferulic acid and catechin. Although low levels of ferulic acid have been reported in fruits, they found that it was almost 75% of the main phenolic sinapic acid. Catechin and epicatechin was also found maximum in black sapota.

The present study entitled "Nutraceuticals Potential of Underutilized Fruits of Sikkim" was carried out at Laboratory of Department of Horticulture, Sikkim University, Gangtok, Sikkim during the year 2014-2017. Sikkim University is located at 6^{th} mile, Samdur, Tadong, Gangtok. The details of materials used and procedures followed during the course of investigation are being described in this chapter.

3.1. Location of the Experimental Site

The different underutilized fruits were directly collected from their natural vegetation located in Sikkim. The experiment on nutraceuticals potential of underutilized fruits of Sikkim was carried out at PG laboratory of department of horticulture and Chemistry Lab, Dept. of Chemistry, Sikkim University, Gangtok.

3.2 Climatic Conditions

The climate of the state has been roughly divided into the tropical, subtropical, temperate and alpine zones and the state is blessed with abundant natural resources. The extreme climatic diversity of the region gives rise to wide ranging agro-ecological situations ranging from sub-tropical in the lower valleys to alpine in higher elevations. For most of the period in a year, the climate is cold and humid as rainfall occurs in each month. The maximum temperature is recorded usually during July and August, and minimum during December and January. Rainfall is heavy and well distributed during the months from May to early October. July is the wettest month in most of the places. Fog is a common feature in the state from May to September.

3.3 Experimental Materials

The present studies were carried out on ten (10) underutilized fruits grown naturally at forest vegetation of Sikkim Himalayas.

Table no. 1. The lists of underutilized fruits taken as experimental materials are given below:

Sl. No.	Botanical Name	Local Name	Geographical location		
			Collection site	Latitude	Longitude
1.	*B. sapida*	Kusum	Rongli	27^o13.418'N	88^o35.421E
2.	*C. erectus*	Phyakrey	Lingmoo	27^o20.471'N	88o28.136E
3.	*D. butyraceae*	Cheuree	Namphing	27^o21.518'N	88^o 25.421E
4.	*E. latifolia*	Mallero	Thamidara	27^o21.124'N	88^o35.327'E
5.	*E. sikkimensis*	Bhadrasey	Thamidara	27^o21.220'N	88^o35.130'E
6.	*E. indica*	Mael	Khamdong	27^o23.471'N	88^o27.136'E
7.	*F. roxburghii*	Nebara	Thamidara	27^o20.994'N	88^o53.436'E
8.	*M. edulis*	Pumsi	Pangthang	27^o22.080'N	88^o36.879'E
9.	*S. axillaris*	Lapsi	Thamidara	27^o21.095'N	88^o35.385'E
10.	*R. ellipticus*	Aiselu	Thamidara	27^o21.233'N	88^o35.070'E

Details of underutilized fruits taken as experimental materials are given below:

1. ***Baccaurea sapida* (Phyllanthaceae)**

The species is originated Southeast Asia and found naturally growing as wild in Nepal, India, Myanmar, South China, Indo-China, Thailand, the Andaman Islands, and Peninsular Malaysia. The fruit of *Baccaurea sapida* was collected from Rongli, East Sikkim. It is an underutilized fruit species locally known as Kusum. The different species of *Baccaurea* is found in Sikkim, Darjeeling, Arunachal Pradesh, Assam, Meghalaya, Tripura, Mizoram and Manipur. It is a semi deciduous tree small to medium in size, approximately 8-10 m in height. Generally ripe fruits are edible and the colour of the fruit is yellowish to pinkish pulpy aril uses as table fruit. The fruits ripen during June –July which are sold in the local market usually at Rs. 40/kg. The fruit is used to cure the skin diseases.

2. *Calamus erectus*

It belongs to the Arecanut family (Arecaceae). *Calamus erectus* distributed throughout the North eastern states like Sikkim, Assam, Meghalaya, Manipur etc and West Bengal where this plant grows naturally in lower hills up to 1400 MSL. It is wild non climbing habit with spiny leaves. In Sikkim the fruit is locally known as Phyakrey, plants are available near the Teesta valleys. Fruits are eaten raw by local people as an anti diabetic agent.

3. ***Diploknema butyraceae* (Sapotaceae)**

This plant is known as Indian butter tree and locally known as chiuree in Sikkim. Tree is medium-size tree 15-30 m tall at maturity. The plants flowers during winter and the fruit ripen in June- July. The fruits are oval or ellipsoid drupe (0.8-1.8 cm) with a fleshy outer covering the single seed. Pulps of the fruits are sweet and juicy has low keeping quality (Bhutia, 2013).The species has been used to cure human ailments as they hold components of therapeutic values. Butter extracted from the fruits is used in treating rheumatism.

***4. Elaeagnus latifolia* (Elaeagnaceae)**

The fruit is locally known as mallero or musleri in Sikkim. The plant is distributed over NE region. These species are naturally grown at an elevation of 1500 meters in the Himalayan region. It is a large evergreen woody shrub with rusty-shiny thorny scales. The fruit of *E. latifolia* is blossoms during September-December and the fruits are harvested in March-April. The fruits are eaten raw with salt and utilized to make pickles and also used for making processed product like jam and refreshing drink. The fruits are rich in vitamins and minerals, particularly vitamins A, C and E, flavanoids and other bio-active compound (De, 2017). It is a fruit that is powerful to reduce the cancer cells (Matthews, 1994).

***5. Eleaocarpus sikkimensis* (Elaeocarpaceae)**

It is woody evergreen large tree grow up to 40 m high. Leaves are clustered spirally arranged near the end of the branches. Fruit is ellipsoidal from broad base, greenish in colour. Ripe fruits are available during November- December. Edible fruits are used to make pickles and chutneys. Different species of Eleaocarpus are very important in the field of medical science due to the presence of their phytochemicals with their high medicinal values. Mainly these chemicals are alkaloids, flavonoids, tannins, glycosides, and ellagic acid derivatives (Dadhich *et al.*, 2013).

***6. Eriolobus indica* (Rosaceae)**

This is the Indian crab apple and this belongs to the family Rosaceae, locally known as Mehl in Nepali (Sikkim) is commonly found in the region particularly in Sikkim, Darjeeling and Meghalaya. It is a tree of the lower temperate zone which grows between 900 to 1900 msl. Tree attains a height of up to 9-12 m. The fruits are round, pear shaped and pale green colour when ripe. They are eaten either fresh or processed into pickles as well as used in jelly preparation also. The fruit is sold in the local markets is handsome price (Rs. 50/kg) in Sikkim. Extracts of E. indica fruits are being used by folk to cure blood dysentery and bark used for piles.

7. Ficus roxburghii **(Moraceae)**

This is a small, spreading type evergreen tree species grows up to 12 m tall. The tree is harvested from the wild for its edible fruit, medicinal uses and its leaves, which are used as plates. The average yield of a tree of *Ficus roxburghii* Wall. was recorded to be 32.4 kg. The plant is grown in India and from Myanmar to Vietnam and SW China and Brazil for its edible fruits. The fruit is available almost round the year usually eaten raw or cooked. Unripe fruits can be used as salads. The fruits claimed to have anti diabetic properties but no proper scientific evidence have found yet.

8. ***Machilus edulis*** **(Lauraceae)**

This species belongs to the Avocado family Lauraceae, is an evergreen tree of about 15-20 m height, with a straight bowl and spreading branches. It is mainly found in entire NE region particularly in Sikkim and Arunachal Pradesh. It is considered to be one of the forms of avocado (*Persea americana*) in the region and locally known as pumsi. Fruits are commonly available during November - March. Fruit yield varies 5-75 kg/tree and fruit weight is 31.72 g and diameter is 1.4-3.7 cm. The fruits are highly nutritious, rich in fat and carbohydrate content (De, 2017).

***9. Spondias axillaris* (Roxb.) (Anacardiaceae)**

S. axillaris is a wild large deciduous fruit tree belonging to Anacaediaceae family. Plant height is 25 to 30 m approximately and has a diameter up to 50 cm. The fruit is fleshy, ovoid drupe usually one per inflorescence. Fruit is green in colour, turning yellow when ripe. Skin ripens to yellow with white flesh that has an acidic flavor. The tree is largely known for its delicious fruit in Sikkim, Darjeeling and neighbouring country Nepal, for timber in China and for medicine in Vietnam. Fruits of *S. axilla*ries consists of several properties for treatment of myocardial ischemia, calming nerves, ameliorating blood circulation and improving microcirculation in Mongolia.

10. Rubus ellipticus (Rosaceae).

R. ellipticus is a thorny shrub of 1–3 m tall. *R. ellipticus* bear aggregate, etaerios of drupes on a nipple shaped thalamus of around 444 mg, yellow colour fruits which can be detached easily from the thalamus and fall down at maturity (Pandey and Bhatt, 2016). Flowering time March–April and fruiting time is April–May.

3.4 Observations recorded and their procedure

3.4.1. Proximate composition

3.4.1.1. Moisture and Dry matter content.

Moisture and dry matter content were determined by following the method given by AOAC (1990), weighed sample (5.0 g) of each fresh fruit was taken in a sterilize weighed Petridis and kept in the hot air oven at 105^{o} C for 12 hours and

Petridis were then allowed to cool and weighed. The moisture and dry matter content were calculated by using the following formulae:-

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

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

3.4.1.2. Crude protein

The protein estimation done by Lowry's method (Lowry *et al.*, 1951) by using UV/VIS Spectrophotometer, Perkin Elmer, Lambda 35 UV/VIS spectrometer.

Reagents used:

- Reagent A - 2.0 % Na_2Co_3 mixed in 0.1N NaOH (20 g of sodium carbonate and 4 g of sodium hydroxide in distilled water and volume was made up to 1 litre).
- Reagent B -1 % $CuSO_4.5H_2O$ in 2% sodium potassium tartarate (1 g of copper sulphate was dissolved in 100 ml distilled water and 2 g of sodium potassium tartarate is dissolved in 100 ml distilled water then the working solution of reagent B was prepared fresh by mixing the solution of 1% CuSo4.5H2O in 2% sodium potassium tartrate).
- Reagent C- Alkaline Copper solution: prepared by mixing 50 ml of reagent A and 1 ml of reagent B prior to use.
- Reagent D- Folin Ciocalteu Reagent

- Protein Stock Solution- BSA mg/ml (100 mg of Bovine serum albumin was weighed accurately and dissolved 100 ml water).
- Working standard- Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 µg protein.

Procedure:-

a) Extraction of fruit sample: Fresh fruit sample (0.5 g) was weighed and ground well with pestle and mortar in 5 ml of phosphate buffer. It was centrifuged for 20 minutes at 5000 rpm and supernatant was collected for protein estimation.
b) Preparation of standard curve and estimation of protein:
    - From the BSA stock solution 0.1, 0.2, 0.4, 0.6, 0.8 was pipette out and 1 ml of working standard and poured into the series of test tubes.
    - Sample extract (0.5 ml) was pippetted out in other set of test tubes.
    - Volume made up to 1 ml in all the test tubes with distilled water. A tube with 1 ml of water served as a blank.
    - Reagent C (5 ml) was added in each tube and was mixed well and waited for 10 minutes.
    - FCR (Reagent D) at the rate of 0.5 ml was added in each tube and mixed well then incubated at room temperature for 30 minutes and reading was taken at 660 nm.
    - Standard curve was drawn and protein per cent was calculated and the amount of protein was expressed in percentage.

3.4.1.3. Crude fat

Crude fat content was determined by method described by AOAC, (1990) using soxhlet. The fat from the oven dried fruit sample was extracted in essential oil extractor (model no. Socsplus-SCS 06 DLS, PELICAN) using petroleum ether as solvent then ether is evaporated and determined the weight of the fat recovered. The following steps were followed during extraction as the initial weight of the beaker (W_1) and 2.0 g ground sample (w) were taken and the samples were transferred to the thimble. The thimble was placed into the beakers which contain 100 ml solvent (Petroleum ether). Beaker with solvent was inserted into the extraction system and water tap was open for condenser. Essential oil extractor machine was operated and temperature was adjusted at 90^0C for 6 hrs. The stopper was kept in the extractor in closed position for no condensation of solvent. After completion of boiling, temperature was increased to 150^0C for 45min to evaporate the solvent. 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 evaporate the solvent. Final weight of the beakers (W_2) was taken to find out crude fat percentage of the sample which was calculated by the following formula:

$$\text{Crude Fat (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

3.4.1.4. Crude fibre

Crude fibre was analyzed using fibre estimation system, model no Fibra plus-FES 04 AS DLS, PELICAN. Two gram of moisture and fat free sample were taken in the crucibles then they were loaded in the instrument. Sulphuric acid (150 ml of 1.25

%) was added from the top and boiled at 500^o C for 30 minutes. Once the boiling was completed the reagents were drained out with the help of fibra flow then 150 ml of 1.25 % NaOH was added from the top and the sample was heated at 400^o C for 45 minutes which led to digestion of sample. After completion of digestion reagents were drained out and residue was dried in hot air oven at 90 -100^o C and cooled and weighed the dried residue (W_1) then the residue was kept in pre-weighed porcelain crucible and put in the muffle furnace for and ashes at 600^o C in 3 hours then it was cooled and weighed (W_2). Crude fibre was expressed as per cent loss in weight on ignition (AOAC, 1990) and calculated using following formula:

$$\text{Crude Fibre (\%)} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

3.4.1.5. Ash content

Ash content was determined by following the method of AOAC (1990). For this crucible were kept in a muffle furnace at 600^0C for 1h. Then they were transferred from furnace and cooled to room temperature and weighed (W_1) as quickly as possible to prevent moisture absorption. Two gram dried fruit sample was taken in crucible and placed in a muffle furnace at 600 0C for 6h. Then crucible was transferred to cooled at room temperature and weighed (W_2), Then the percentage of ash was calculated by using the following formula:-

$$\text{Ash (\%)} = \frac{W_2 - W_1 \text{ (weight of ash)}}{\text{Weight of sample}} \times 100$$

3.4.1.6. Available carbohydrate

The per cent of available total carbohydrate content was computed by: 100- (Percentage of ash+ Percentage of fat + Percentage of fibre + Percentage of protein) (AOAC, 1990).

3.4.1.7. Total Soluble Solid (T.S.S.)

The total soluble solids of the fruits were determined with the help of hand refractometer and it was expressed in (°B) degree brix.

3.4.1.8. Titratable acidity

The total titratable acidity was determined by titrating 10 ml aliquot against 0.1N sodium hydroxide using phenolphthalein as indicator by method as suggested by AOAC (1980). Generally citric acid is added in most fruit, Thus results expressed as per cent of citric acid (Equivalent wt. 64.04). Fresh fruit sample (10 g) was crushed in pestle and mortar and mixed thoroughly to obtain pulp by adding some water boiled for 1hr to replace the water lost in evaporation. Cooled and transferred to a volumetric flask, volume made up to 100 ml. Diluted on aliquot of sample to 10 ml and placed in titration flask then few drops of 1% phenolphthalein as an indicator and titrated with N/10 NaOH to light pink colour using burette or pipette. Titre value was noted and per cent titrable acidity as citric acid was calculated using following formula:

$$T.\ A\ (\%) = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made} \times \text{equivalent wt. of acid}}{\text{Weight of sample} \times \text{Volume of aliquot} \times 1000} \times 100$$

3.4.1.9. Total sugar:

Total sugar was calculated method narrated by AOAC (1980). The sugar aliquot was hydrolysed with concentrated HCl and titrated against 10 ml of mixed Fehling solution (5 ml Fehling solution A + 5 ml Fehling solution B) using indicator (methylene blue). The percentage of total sugar was computed by using the formula given below and the results were expressed as per cent total sugar.

$$\text{Total sugar (\%)} = \frac{\text{Factor x Dilution}}{\text{Weight of sample x Titre reading}} \times 100$$

3.4.1.10. Reducing Sugar (RS):

Reducing sugar per cent was analysed by the method of AOAC (1980). The extract was titrated against mixed Fehling solution A and B using indicator (Methyl blue). The results were given as percentage of reducing sugar and computed using following formula:-

$$\text{Reducing sugar (\%)} = \frac{\text{Factor x Dilution}}{\text{Weight of sample x Titre reading}} \times 100$$

3.4.1.11. Non Reducing Sugar (NRS):

NRS was calculated by subtracting reducing sugar amount from that of total sugars then multiplied by 0.95.

3.4.2. Phytochemicals

3.4.2.1. Vitamins A

The vitamin A was analysed by method narrated by Nield and Pearson (1963).

Reagents

1. 2N KOH
2. 90% alcohol
3. Petroleum ether
4. Trifluro acetic acid
5. Chloroform
6. TFA reagent: Mixed 1.0 ml of TFA and 2 ml of chloroform (Prepared fresh).
7. Vitamin A stock standard (160µg/ml): Transferred 16 mg retinyl acetate in standard flask (100 ml) and volume made up with anhydrous chloroform.
8. Vitamin A working solution: Pipetted out 0.2-1.0 ml of stock standard and volume made up (100 ml) with anhydrous chloroform with corresponding concentration of 3-15 µg respectively.
9. β-carotene stock standard (200µg/ml): Transferred 20 mg of β-carotene to 100 ml standard flask. Dissolved in approximately 4 ml chloroform and diluted with petroleum ether.
10. Carotene working standard: Pipette out 0.05-0.2 ml of β-carotene stock and volume made (1.0 ml) by adding petroleum ether. It will have a concentration corresponding to 1-4 µg respectively.

Procedure

To 1.0 ml of 10% homogenate 1.0 ml of saponification mixture (2N/KOH in 90% alcohol) was added and heated for 20 min at 60°C. Twenty five milliliter water was poured to the mixture after cooling at room temperature and the solution was transferred to a separating funnel then extracted thrice (25, 15 and 10 ml) using petroleum ether (40-60°C). The extracts was then thoroughly washed and made free of alkali then dried by adding anhydrous Na_2SO_4. Three milliliter of phase petroleum ether was transferred in a cuvette and reading was taken at 420 nm against blank with no delaying to prevent evaporation of the solvent and destruction of carotenoids by light (Marked as A1). The β-carotene working standards reading was taken at 450 nm. The aliquots were dried at 60°C in water bath. The residue then immediately taken and TFA reagent (2.0 ml) were added. The mixture was transferred in a cuvette and absorbance was taken at 620 nm after addition of TFA reagent (as A2). The vitamin A working standard reading was noted at 620 nm.

A3 = A2-A1

A1 = Absorbance of carotene at 450 nm

A2 = Absorbance at 620 nm due to both carotene and vitamin A

A3 = Absorbance at 620 nm of vit A.

$$\text{Sample} = \frac{\text{A3 x µg retinol calibrator/ cuvette x 3 total volume}}{\text{A620 retinol calibrator x 2 x gram}}$$

3 = Vol of petroleum ether from 1.0 ml extract

2 = Aliquot of the petroleum ether used for the assay

1 = 10% extract from initial sample.

The results are expressed as µg/g tissue.

3.4.2.2. Vitamin E

Vitamin E was determined by the method given by Baker *et al.,* (1980) with some modification. The method implies the depletion of ferric ions to ferrous ions by α-tocopherol and the development of red coloured complex with 2,2-dipyridyl.

Absorbance of the chromophore was measured at 520 nm using UV- Vis spectrophotometer.

Reagents

1. Petroleum ether 60-80°C
2. Ethanol
3. 2,2-Dipyridyl solution: 0.2 % in ethanol
4. Ferric chloride solution: 0.5 % in ethanol
5. Stock standard: 10 mg of Vitamin E in 100 ml ethanol
6. Working standard: The stock solution was diluted in ethanol to a concentration of 10 μg/mL.

Procedure

Two gram of sample was extracted using 5.0 ml petroleum ether and ethanol (2 ml). The tubes were centrifuged and the supernatant was blended with 2, 2-dipyridyl and ferric chloride (0.5 ml each). The tubes were stored in the dark room for five minutes for development of intense red colour. In all tubes 4.0 ml water was added and shake well. Standard Vit- E in the range of 10-100 μg were taken and treated similarly along with a blank containing reagent. The colour in the aqueous layer was noted at 520 nm. The values were expressed as mg gm^{-1} of dried sample.

3.4.2.3. Total phenols

Extraction of sample: The various underutilized fruits congregate from various places of Sikkim were cleaned thoroughly in running water. Fruits were the cut into small pieces and dried at 105^{o} C for 48 hours in hot air oven. Dried sample were then ground into fine powder using willey mill and 5 gram of each sample was extracted using 50 ml solvent (80 % methanol) for 12 hours at 60^o C temperature in soxhlet apparatus (essential oil extractor: model no. Socsplus-SCS 06 DLS, PELICAN). Temperature was raised to 150^0 C for 45min to evaporate the solvent just after completion of boiling. The extract were concentrated and dried and weighed. The extracts were then diluted using methanol of known volume (mg ml^{-1}) in air tight container and stored under fridge at 4 ^{o} C until analysis.

The total phenols of different underutilized fruits were detected in UV/VIS Spectrophotometer (by the method given by Singlaton, *et al.* (1999) with some modification involving Folin-Ciocalteau Reagent as oxidizing agent and gallic acid as standard.

Reagent used:

- Folin Ciocalteau Reagent (Diluted 10 times): 5ml of FCR was diluted 10 folds. (by adding 45 ml DW)
- Sodium Carbonate solution (7.5%) prepared by dissolving 7.5 g of Na_2CO_3 with distilled water and volume made up to 100 ml.
- Methanol 80 %
- Gallic acid: Stock solution of gallic acid (1mg/ml) was prepared by dissolving 1 mg of gallic acid in 1 ml methanol and volume made up to 10 ml with 80 % methanol. The stock solution was further diluted into 1, 2, 3, 4, and 5 ml in 10 ml methanol to give final concentration of 100, 200, 300, 400, and 500µg/ml.

Procedure: Aliquots of 1 ml of the test sample and each sample of the standard solution were taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionized water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubated at ambient temperature for 2 hours to complete the reaction. The absorbance was taken at 765 nm against the blank using UV/VIS Spectrophotometer; the total phenol was determined with the help of standard curve prepared from pure phenolic standard (gallic acid). The data for total phenolic contents of underutilized fruit extracts were expressed as mg of weight (GAE)/ g of dry mass.

3.4.2.4. Total Flavonoid

The aluminum chloride assay was used estimation of total flavonoid of different fruits as per the method explained by Kumaran and Karunakaran (2007) with slight modifications in UV/VIS Spectrophotometer.

Reagents required:

- Aluminum Chloride ($AlCl_3$)- (Prepared by dissolving 10 g of aluminum chloride in 100 ml volumetric flask with distilled water and volume made up to 100 ml).
- Potassium acetate (1N) was prepared by dissolving 9.8 gram potassium acetate in 300 ml volumetric flask and dissolved with distilled water and final volume made up to the mark by adding required amount of distilled water.
- Quercetin standard solution prepared using 10 mg quercetin in 10 ml methanol so the concentration of solution was 1mg/ml. The stock solution was further diluted into 0.2, 0.4, 0.6, 0.8, and 1.0 ml in 10 ml methanol to give final concentration of 20µg, 40µg, 60µg, 80µg, and 100 µg/ ml.
- Blank was prepared by adding the entire reagent except fruit extract and standard solution.

Experimental procedure:

Total flavonoids content was determined by using 1 ml of each extract stock solution (1mg/ml) and each dilution of standard quercetin (10 µg- 100 µg/ml) was taken in a series of test tubes. To each test tube 2 ml methanol, 0.2 ml of 10 % aluminium chloride, 0.2 ml 1M potassium acetate solution and 5.6 ml of distilled water were added and mixed well then the test tubes were allowed to incubate at room temperature for 1 hour to complete the reaction. The absorbance of the solution was noted at 420 nm against the blank in UV/VIS Spectrophotometer; all the samples were analyzed in three replications. The total flavonoid was determined with the help of standard curve prepared from pure quercetin standard. The data for total flavonoids contents of underutilized fruit extracts were calculated using the following equation C=(c x v)/m and expressed as mg of quercetin equivalent weight (QE)/ g of dry mass.

3.4.2.5. Flavonols content

The flavonol content in different underutilized fruits were determined by aluminium chlorometric method followed by Pattanayak, *et al*. (2011) with some modification using 0.5 ml of each extract stock solution (1mg/ml) and each dilution of standard quercetin (10 µg- 100 µg/ml) was taken in a series of test tubes. To each test tube 2 ml methanol, 0.2 ml of 10 % aluminium chloride, 0.2 ml sodium acetate solution and 5.6 ml of distilled water were added and mixed well then the test tubes were allowed to incubate at room temperature for 2.5 hour to complete the reaction. The absorbance was measured at 440 nm against the blank in UV/VIS Spectrophotometer; all the samples were analyzed in three replications. The total flavonol was determined with the help of standard curve prepared from pure quercetin standard. The data for total flavonol contents of underutilized fruit extracts were computed using the given equation C=(c x v)/m and expressed as mg of quercetin equivalent weight (QE)/ 100 g of dry mass.

3.4.2.6. Ascorbic acid

The ascorbic acid was determined by reduction of 2, 6-dichlorophenol indophenols dye by ascorbic acid as procedure given by AOAC (1980). Ten (10) ml of juice was taken and blended with 0.4% HPO_3 and finally volume was made up to 100 ml with 0.4% HPO_3 and then 10 ml aliquot was titrated against standardized dye to develop a pink colour which last for 15 seconds. Ascorbic acid was expressed in terms of mg per 100 gm pulp by using formula:

$$\text{Ascorbic acid (mg/100 g pulp)} = \frac{\text{Dye factor x titre reading x dilution}}{\text{Weight of sample}} \text{x} 100$$

3.4.2.7. Anthocyanin content

Sample was extracted by blending 10 g of finely ground sample with 10 ml of 95 % ethanolic HCl and centrifuged at 10000 rpm for 20 minutes then supernatant was collected and transferred into 100 ml volumetric flask and volume was made up to the mark and solution was stored in the refrigerator at 4^{o} C until analysis. The optical density of the aliquot was determined at 530 nm in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer). The value of total Anthocyanin content was expressed as mg/100 gram. Calculation was done by using the following formula.

$$\text{Total O.D/ 100 g} = \frac{\text{O.D. X volume made up X 100}}{\text{Weight of sample}}$$

$$\text{Total Anthocyanin (mg/ 100g)} = \frac{\text{Total O.D. }/100}{98.2}$$

3.4.2.8. Total carotenoids

One gram of sample was weighed and ground with acetone using acid and alkali washed sand in mortar and pestle. The extract is decanted into a conical flask. Continue the extraction was continued till the residue was colourless. The acetone extract was transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. About 25 ml of 5% sodium sulphate solution was added. It was shaked and kept for some time and yellow colour pigment was transferred into the petroleum ether later. The layer was collected in a volumetric flask and acetone layer containing 5 % sodium sulphate was separated, 15 ml petroleum ether was kept on added to the acetone layer containing Na_2SO_4 until the colour gets transferred into the petroleum ether and measured the colour intensity at 452 nm in a spectrophotometer and the total carotenoids content was calculated using the following formula:

$$\text{Total carotenoids (mg/100 g)} = \frac{3.857 \times \text{O.D} \times \text{Volume made up} \times 100}{\text{Weight of the sample} \times 1000}$$

3.4.3. Antioxidant Activities

Sample extraction: The fruits were cleaned thoroughly in running water and then cut into small pieces and dried at 105 ^{o} C for 48 hours in hot air oven. Dried sample were then ground into fine powder and 5g of each sample was extracted using 50 ml solvent (80 % methanol) for 12 hours at 60^{o} C temperature in soxhlet apparatus. The extracts was diluted with known volume (mg/ml) of methanol in air tight small container and kept under fridge at 4 ^{o} C until analysis.

3.4.3.1. DPPH Scavenging Activity

Reagents and apparatus used:

- DPPH (1,1-Diphenyl-2-picrylhydrazyl)
- Methanol (Merck Millipore)
- Ascorbic acid
- Pipettes, test tubes, beakers and flasks
- UV-Vis spectrophotometer

Preparation of reagents:

- DPPH Solution (0.004 w/v): in order to prepare 0.004 % w/v DPPH solution 4 mg of DPPH was dissolved in 100 ml of methanol (80%) in dark room.
- Preparation of Ascorbic acid stock solution: 2 mg ascorbic acid dissolved in 2.5 ml distilled water so the concentration of the solution is 800 µg/ ml.
- Serial dilution of Ascorbic acid stock solution was performed in order to prepare different concentrated solution (25µg/ml, 50 µg/ml, 100µg/ ml, 200 µg/ml and 400 µg/ml).
- Preparation of sample extract solution 4 mg of extract was dissolved in 10 ml of methanol in order to prepare 400 µg/ml solutions then the serial dilution was performed to prepare required concentrated solution.
- Control was prepared by adding 3ml of DPPH solution and methanol was used as a blank.

Estimation procedure:

- Two ml of methanol solution of plant extract of different concentration was taken in test tubes.
- Three ml of methanol solution of DPPH was added into each test tube then incubated for 50 minutes in dark place at ambient temperature to complete the reaction (discoloration of DPPH from purple to yellow will occur- more discoloration into purple to yellow indicates that there will be more anti oxidant activity).
- Then the absorbance of the solution was measured at 517 nm using a UV-Vis Spectrophotometer against the blank (A typical blank solution contained the all reagent except plant extract and standard solution).
- Then the percent scavenging activity was calculated from the following equation:-

$$\% \text{ scavenging activity} = \frac{\{(A0 - A1)\}}{A0} \times 100$$

Where, A0 = Absorbance of control

A1= Absorbance of sample extract/ Standard

3.4.3.2. Hydroxyl Radical Scavenging Activity

The scavenging activity for Hydroxyl Radical Scavenging activity in different underutilized fruits were determined using deoxyribose assay in an aqueous medium following the method of Halliwell *et al.* (1987) with some modification. UV/VIS Spectrophotometer was used to determine the absorbance.

Reagents Used:

- Iron (III) chloride ($FeCl_3$)- 1mM.
- EDTA (1 mM)
- Ascorbic acid (1mM)
- Hydrogen peroxide (H_2O_2) (10 mM)
- Deoxyribose (10 mM)

Estimation procedure:

Stock solution of each EDTA (1mM), $FeCl_3$ (10 mM), ascorbic acid (1mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in double distilled water and the assay was prepared by mixture of EDTA (0.2 ml), $FeCl_3$ (0.02 ml), H_2O_2 (0.2 ml) and deoxyribose (0.35 ml) with 0.1 ml of extract of different concentration (1 – 1000µg/ml) in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9) and 0.1 ml ascorbic acid in series of test tubes in a sequence. The mixture was then incubated at ambient temperature for 2 hours to complete the reaction. After the completion of reaction the incubated mixture was mixed with 0.5 ml TCA (Trichloro acitic acid) and 0.5 ml TBA (Thiobarbituric acid) to develop pink colour and the absorbance was measured at 532 nm in UV-Vis spectrophotometer. All the absorbance was triplicate and the results was averaged then hydroxyl radical scavenging activity of the fruit extract was expressed as % scavenging of deoxyribose degradation is calculated by following equation:

% Hydroxyl Radical Scavenging Activity = $\{ \frac{(A0-A1)}{A0} \text{ x } 100\}$

Where, A0 = Absorbance of control

A1= Absorbance of sample extract/ Standard

3.4.3.3. Hydrogen Peroxide Scavenging Activity

Reagents used:

- Hydrogen peroxide
- Gallic acid as standard
- Phosphate buffer (0.5 M 7.4 pH)

Estimation procedure:

The hydrogen peroxide scavenging activity of methanolic extract of some underutilized fruits of Sikkim were determined by using the method followed by Ruch *et al.* (1989) with some modification. A solution of (42mM) hydrogen peroxide was prepared in phosphate buffer (0.1 M 7.4 pH. Different concentration of fruit extract and standard Gallic acid in equal volume was prepared in series of test tube separately (40, 80, 120, 160 and 200 µg/ml in methanol). Hydrogen peroxide solution (1 ml) was added to each tube and incubated for 15 minutes then the absorbance was taken at 230 nm against the blank solution containing phosphate buffer without hydrogen peroxide using UV-Vis Spectrophotometer. The experiment was performed in triplicate and averaged.

The percentage of scavenging of different fruit extract and standards were calculated using following equation:-

H_2O_2 scavenging activity (%) = [A0 – A1/ A0] x 100.

3.4.3.4. Fe^2+ Chelating Activity

Iron chelating capacity was investigated using the method of Denis *et al.* (1994) with minor modification. The methanolic extract of underutilized fruits were assessed in UV-Vis Spectrophotometer for their ability to react with ferrozine for iron (II) in the solution.

Reagents used

- Ferrous chloride ($FeCl_2$)
- Ferrozine (II)
- Methanol
- EDTA as Standard

Estimation procedure: the different concentration of sample extract and standard were prepared in methanol as 0.2, 0.4, 0.6, 0.8 and 1 mg/ml and mixed with 0.5 ml of ferrous chloride (4 mM) and 1 ml of Ferrozine (III) 5 mM and equal volume was made up with distilled water. After 15 minutes of incubation at room temperature the optical density (Absorbance) ferrous ion ferrozine complex was taken at 562 nM using UV-vis spectrophotometer against the blank solution. Blank was prepared using ferrous chloride and water without standard and fruit extract. EDTA was used as standard and all the measurement was taken in triplicate and averaged. The percentage of iron chelating activity (Inhibition of Ferrozine-Fe^{2+} complex) = ([A0-A1)/ A0] x 100

3.4.3.5. Ferric ion Reducing Antioxidant Power (FRAP Assay)

Chemicals used: Potassium ferricyanide, Sodium chloride, Potassium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, HCL, Trichloroacetic acid, Ferric chloride.

Reagent preparation:

- phosphate buffer (0.2M ; pH 6.6): Sodium chloride (8 g), potassium chloride (0.2 g), disodium hydrogen phosphate (1.44 g), potassium dihydrogen phosphate (0.24 g) was taken in a 1,000 mL standard flask and add 800 mL of distilled water and adjust the pH was adjusted to 6.6 using hydrochloric acid and volume was adjusted with deionized water.
- Potassium ferricyanide (1%): 1 g of potassium ferricyanide was dissolved in 100 mL of deionized water.
- Trichloroacetic acid (10%): 10 g of trichloroacetic acid was dissolved in 100 mL of deionized water.
- Ferric chloride (0.1%): 100 mg of ferric chloride was dissolved in 100 mL of double distilled water.
- Ascorbic acid (0.1%): 1 mg of ascorbic acid was dissolved in 1 mL of water.

Estimation procedure: FRAP assay was performed according to the methods of Benzie and Strain (1999) with slightly modification.

- Different concentrations of the methanolic extract of *M. serratulum* and its various fractions (10-50 μg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K3Fe(CN)_6$] solution.

- The reaction mixture was mixed well and then incubated at 40 °C for 30 min using vortex shaker.
- At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 15 min.
- The supernatant (2.5 mL) was mixed with 2.5 mL of double distilled water and 0.5 mL of 0.1% ferric chloride.
- The optical density of colored solution was read at 593 nm against the blank with reference to standard using UV- vis Spectrophotometer where ascorbic acid was used as a standard, the reducing power of the samples were comparable with that standard.
- FRAP inhibition % = ([A0-A1)/ A0] x 100

3.4.3.6. Phospho-molybdenum Complex (PM) Assay

The total antioxidant activity was determined by Phospho-molybdenum method, it is based on the reduction of M_O (VI) to M_O (V) by the sample and subsequence formation of a green Phosphate/ M_O (V) complex at acidic pH. The absorbance is measured at 695nm using an UV/Vis spectrophotometer. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

Reagents required:

i. Standard solution:- 50mg of Ascorbic acid was dissolved in 50ml standard flask using distilled water.(conc., 1mg/ml)

ii. Extract solution:- Methanolic dried extract (50 mg) was dissolved in 50ml standard flask using distilled water. (conc., 1mg/ml)

iii. Phosphomolybdenum Reagent:- prepared by mixing 0.6M H_2S0_4, 28mm sodium phosphate and 4mm ammonium molybdate.

Estimation procedure

- Standard and extract solution (100-1000 µg) was prepared from that take 0.3ml of each sample was taken respectively.
- To all the tubes 3.0 ml of Phosphomolybdenum reagent was added.
- Water (0.3 ml) and 3.0 ml of reagent alone served as blank.
- All the tubes were incubated at room temperature for 2 hours
- The absorbance was measured at 695nm using UV/Vis spectrophotometer against the blank. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

3.4.4. Elemental profiling

ICP-MS was used for element estimation. Digested samples were analysed for the ionic constitution using multi elements. Standards solution 1, 3 and 5 solution were used as a standards. The micro wave digestion system (Anton par microwave 3000) was used for sample digestion as 0.5 gm. sample were along 9ml of 69% nitric acid and 2ml HCl were added into the digestion tube and run the instrument was run for 40 minutes. The digested samples were then transferred into 50ml volumetric flask when the temperature of the sample was reduced and distilled water was added for making the volume of 50 ml. The liquid sample was transferred into narrow mouth bottle until the minerals were determined in ICP-MS. The value of the elements were expressed as µg/L.

3.4.5. Identification and quantification of phenols

Extraction of sample: Two gram of each coarsely powdered fruit sample were extracted using HPLC grade methanol in soxhlet apparatus. Sample extract was filtered through 0.45 µm PVDF syringe filter and volume was made up to 10 ml using same solvent and stored at 4^{o} C till further investigation.

Chemical standard: The chemicals from sigma Aldrich like gallic acid, ferulic acid, rutin, catechol and quercetin were used as phenolic standard and HPLC grade methanol was used as solvent. The stock solution of each standard was prepared by dissolving 1 mg standard in 1 ml methanol likewise the working solution of sample were prepared by further dilution of standard solution with methanol and the standard.

HPLC analysis: the HPLC analysis was performed with Agilent Series 1100 (Agilent Technology, USA). The mobile phase consisted of (A) water and (B) acetonytrile (10:90 v/v) with flow rate of 1ml. Min^{-1}. The column was C_{18} which was thermostatically controlled at 28^{o} C. The injection volume was 20 µl. The total analysis duration per sample was 50 minutes. The HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (380, 280 and 320 nm). Each phenolic compound was identified by its retention time and by spiking with standard under same peak. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak areas against concentration of respective standard sample. The data were reported as means ± Standard error mean of three independent variables.

3.5. Statistical analysis

One way ANOVA was performed for all parameters except ionome analysis which was analysed in triplicate and recorded as mean ± standard deviation (SD). Mean difference were tested by ‘F’ test at 5 per cent level of significance (LOS). Critical difference (CD) at 5 per cent level of probability was used for comparison among fruit species. The results have been presented by way of tables and figures.

Plate No. 1: Glimpse of *Baccaurea sapida* fruits.

Plate No. 2: Glimpse of *Calamus erectus* fruits

Plate No. 3: Glimpse of *Diploknema butyraceae* fruits.

Plate No. 4: Glimpse of *Elaeagnus latifolia* fruits.

Plate No. 5: Glimpse of *Eleaocarpus sikkimensis* fruits.

Plate No. 6: Glimpse of *Eriolobus indica* fruits

Plate No. 7: Glimpse of *Ficus roxburghii* fruits.

Plate No. 8: Glimpse of *Machilus edulis* fruits

Plate No. 9: Glimpse of *Rubus ellipticus* fruits

Plate no. 10: Glimpse of *Spondias axillaris* fruits.

The present investigation 'Nutraceuticals Potential of Underutilized Fruits of Sikkim' was carried out in the laboratory of Department of Horticulture, School of Life Sciences, Sikkim University, 6th mile Samdur, Gangtok, during 2014-2017. The results obtained from the present study in respect to proximate content, phytochemical constituents, antioxidant activities, concentrated elements and phenolic contents of the underutilized fruits have been presented in this chapter.

4.1. Proximate composition

The perusal of data pertaining to proximate composition of different underutilized fruits of Sikkim were analysed on different fruit species of Sikkim *viz. Baccaurea sapida, Calamus erectus, Diploknema butyraceae, Elaeagnus latifolia, Eleaocarpus sikkimensis, Eriolobus indica, Ficus roxburghii, Machilus edulis, Spondias axillaris* and *Rubus ellipticus* and the results have been described under the following heads:

4.1.1. Moisture content

Moisture content of different underutilized fruits of Sikkim revealed significant difference among various species (Table no. 2 and fig. 1). Moisture content represents significant character for fruit quality and was found in a range of 35.12 ± 0.04 per cent to 90.05 ± 0.13 per cent among different fruits species. Fruits of *E. latifolia* were found to have maximum moisture per cent (90.05 ± 0.13%) followed by *R. ellipticus* (87.92 ± 0.33 %) and *F. roxburghii* (87.70 ± 0.20%) while minimum moisture content was recorded in the fruit of *Calamus erectus* (35.12 ± 0.04 %).

Table no. 2. Moisture and dry matter content of underutilized fruits of Sikkim

Sl. no	Scientific name of fruits	Moisture content (%)	Dry matter (%)
1.	*Baccaurea sapida*	81.17 ± 0.65	18.83 ±0.65
2	*Calamus erectus*	35.12 ± 0.04	64.88 ±0.04
3	*Diploknema butyraceae*	76.26 ± 0.12	23.74 ±0.12
4	*Elaeagnus latifolia*	90.05 ± 0.13	9.95 ±0.13
5	*Eleaocarpus sikkimensis*	68.67 ± 0.40	31.33± 0.40
6	*Eriolobus indica*	86.03 ± 0.06	13.91 ±0.22
7	*Ficus roxburghii*	87.70 ± 0.20	12.30 ±0.20
8	*Machilus edulis*	68.81 ± 0.09	31.19 ±0.09
9	*Rubus ellipticus*	87.92 ± 0.33	12.08± 0.33
10	*Spondias axillaris*	78.70 ± 0.48	21.40 ± 0.43
C.V.		0.417	1.300
S.E.M.		0.183	0.180
C.D. 5%		0.540	0.530

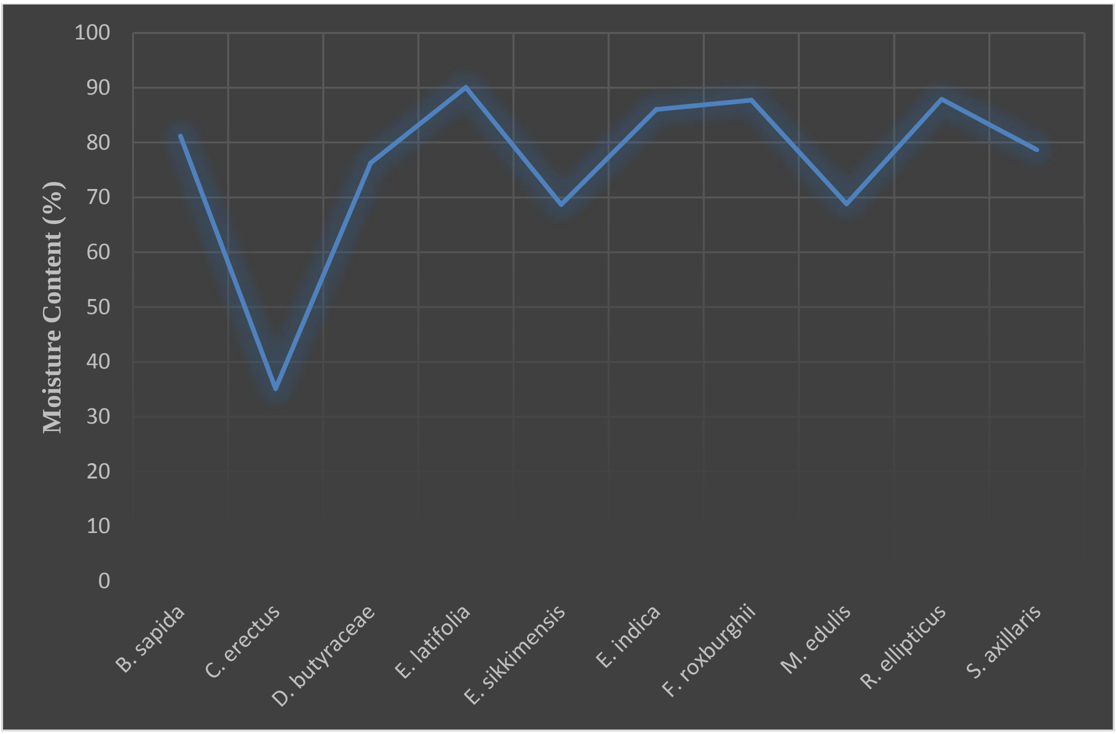

Fig. 1. Moisture content of different underutilized fruits of Sikkim

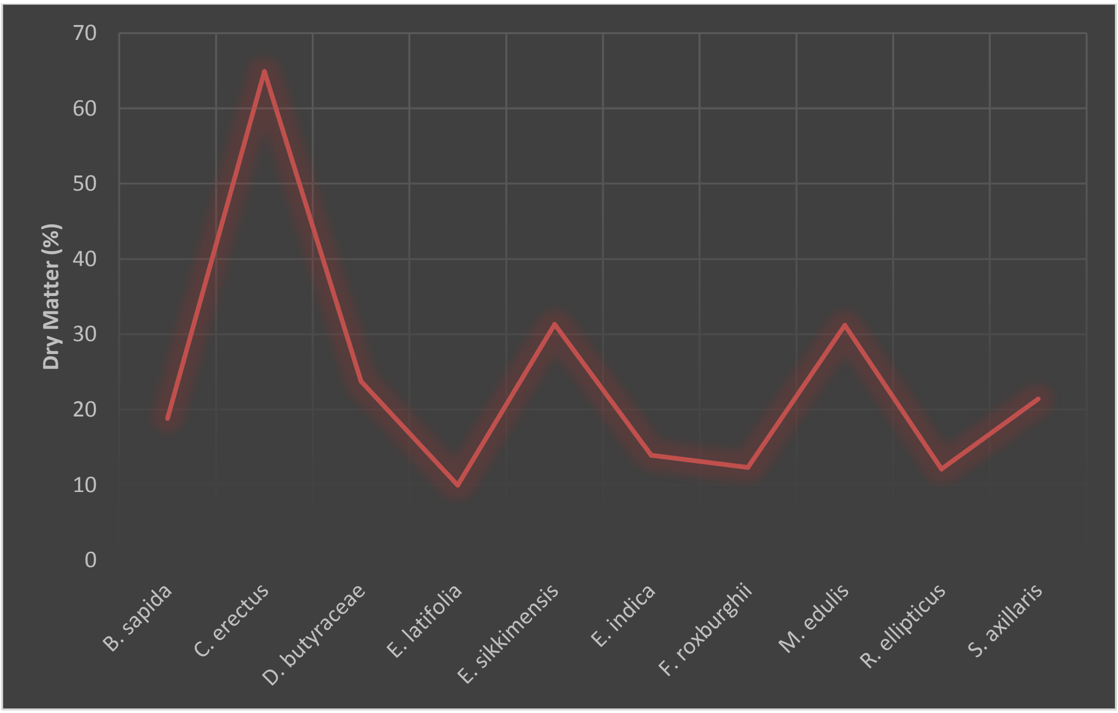

Fig. 2. Dry matter content of different underutilized fruits of Sikkim.

Moreover, the fruit of *E. indica* (86.03 ± 0.06 %), *B. sapida* (81.17 ± 0.65 %), *S. axillaris* (78.70 ± 0.48%) and *D. butyraceae* (76.26 ± 0.12 %) were also found to have significantly higher moisture content than the fruit of *C. erectus. Eleaocarpus sikkimensis* and *Machilus edulis* were found *at par* with each other with respect to moisture content.

4.1.2. Dry matter content.

Table no. 2 and fig. 2 pertains data related to dry matter content of different underutilized fruits of Sikkim. The dry matter content was found within a range of 9.95 ± 0.13 per cent to 64.88 ± 0.04 per cent. The dry matter content was observed maximum in the fruit of *C. erectus* (64.88 ± 0.04 %) followed by *E. sikkimensis* (31.33 ± 0.40 %) and *M. edulis* (31.19 ± 0.09%). *Ficus roxburghii, Rubus ellipticus, Eleaocarpus sikkimensis* and *Machilus edulis* were found to be *at par* with each other. Lowest dry matter content was observed in *E. latifolia* (9.95 ± 0.13 %) followed by *R. ellipticus* (12.08 ± 0.33 %), *F. roxburghii* (12.30 ± 0.20 %) and *E. indica* (13.91 ± 0.22 %).

4.1.3. Crude protein

The investigation on crude protein content from the underutilized fruits of Sikkim showed significant variation (Table no. 3 & fig. 3). The crude protein content of underutilized fruits under study was recorded in a range of 2.61 ± 0.58 per cent to 7.94 ± 0.03 per cent. The highest amount of crude protein content was recorded in the fruit of *Rubus ellipticus* (7.94 ± 0.03 %) followed by *Eleaocarpus sikkimensis* (6.93 ± 0.03 %), *Spondias axillaris* (6.31± 0.58 %) and *Elaeagnus latifolia* (6.16 ± 0.59 %) whereas, the lowest amount of crude protein content was observed in the fruit of

Table no. 3: Crude protein, fat and carbohydrate per cent present in underutilized fruits of Sikkim.

Sl. No	Scientific name of fruits	Crude protein (%)	Fat (%)	Total carbohydrate (%)
1	*Baccaurea sapida*	5.43 0.87	1.14 ± 0.09	86.14 ± 1.49
2	*Calamus erectus*	3.74 0.56	1.24 ± 0.06	87.69 ± 0.70
3	*Diploknema butyraceae*	4.36 0.64	1.02 ± 0.24	87.04 ± 0.37
4	*Elaeagnus latifolia*	6.16 ± 0.59	0.94± 0.05	89.60 ± 0.93
5	*Eleaocarpus sikkimensis*	6.93 ± 0.03	1.02± 0.03	88.17 ± 0.80
6	*Eriolobus indica*	2.61 ± 0.58	0.87± 0.02	89.06 ± 0.52
7	*Ficus roxburghii*	3.00 ± 0.06	0.13± 0.04	90.81 ± 0.44
8	*Machilus edulis*	5.73 ± 0.06	20.07 ± 0.31	72.32 ± 0.72
9	*Rubus ellipticus*	7.94 ± 0.03	0.08 ± 0.02	89.87 ± 0.69
10	*Spondias axillaris*	6.31± 0.58	2.19 ± 0.28	87.07 ± 1.01
C.V.		9.621	5.361	0.951
S.E.M.		0.290	0.090	0.477
C.D. 5%		0.856	0.265	1.406

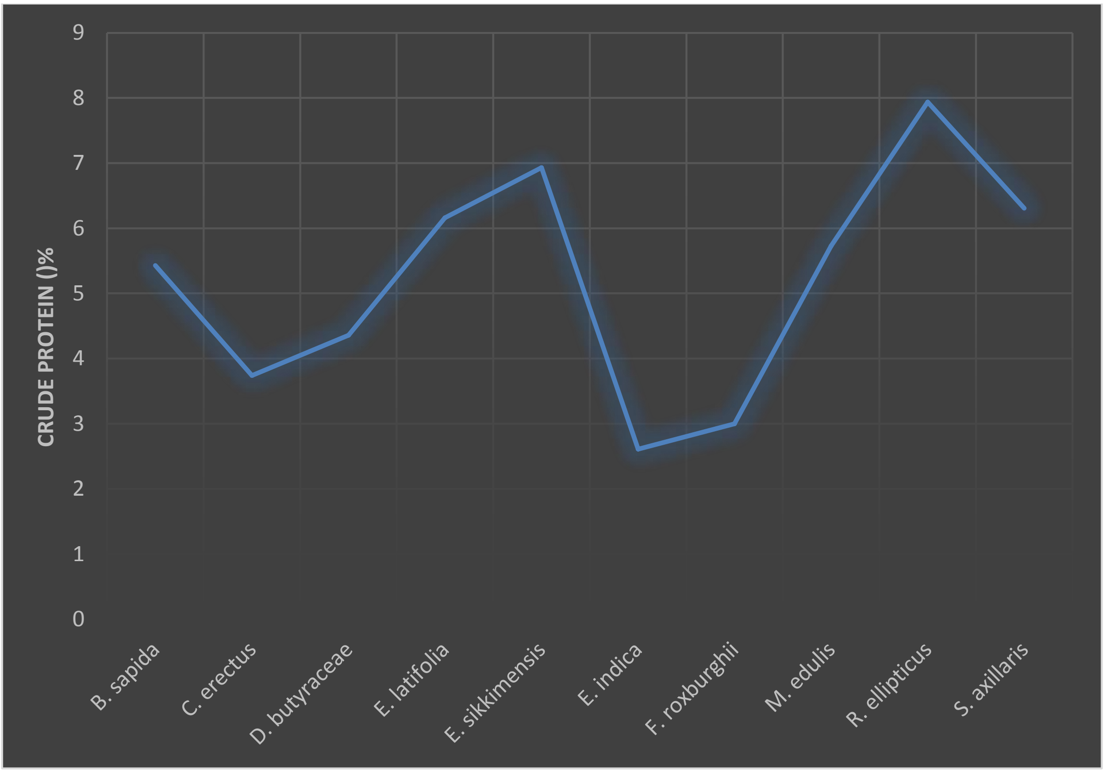

Fig. 3. Per cent crude protein content in different underutilized fruits of Sikkim

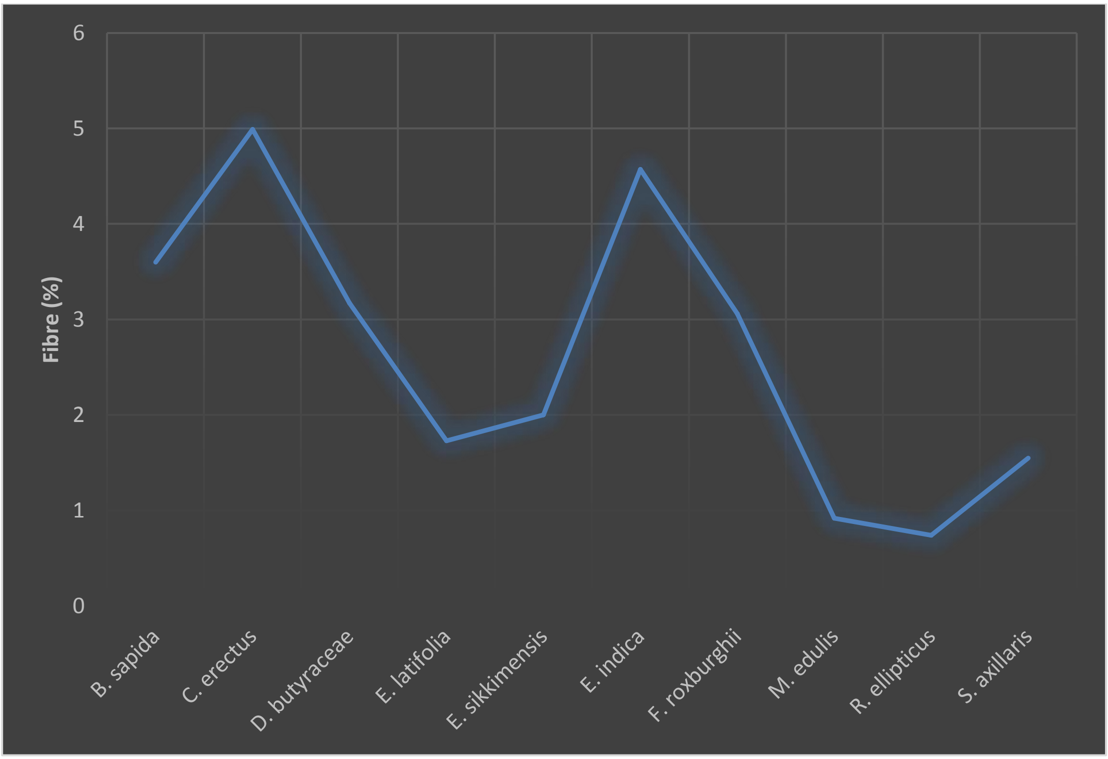

Fig. 4. Per cent fibre content in underutilized fruits of Sikkim.

Eriolobus indica (2.61 ± 0.58 %) followed by *Ficus roxburghii* and *Calamus erectus* (3.00 ± 0.06 % and 3.74 ± 0.56 %, respectively). *Baccaurea sapida* and *Machilus edulis*, *Elaeagnus latifolia* and *Eleaocarpus sikkimensis* were found to be *at par* with each other.

4.1.4. Crude fat

The crude fat content of different underutilized fruits revealed that the amount of crude fat content among ten different underutilized fruit species varied between 0.08 ± 0.02 per cent to 20.07 ± 0.31 per cent (Table 3). Crude fat per cent for *Baccaurea sapida*, *Calamus erectus* and *Diploknema butyraceae* were found to be *at par* with each other. The highest crude fat content was recorded in the fruits of *Machilus edulis* (20.07 ± 0.31 %) followed by *Spondias axillaris* (2.19 ± 0.28 %) whereas lowest crude fat content was noted in the fruit of *Rubus ellipticus* (0.08 ± 0.02 %) followed by *Ficus roxburghii* (0.13 ± 0.04 %) and *Eriolobus indica* (0.87 ± 0.02 %).

4.1.5. Total Carbohydrate

Total carbohydrate content of underutilized fruits of Sikkim varied between 72.32 ± 0.72 per cent to 90.81 ± 0.44 per cent (Table no. 3). The total carbohydrate content of *F. roxburghii* (90.81 ± 0.44 %) was found to be highest followed by *R. ellipticus* (89.87 ± 0.69 %), *E. latifolia* (89.60 ± 0.93 %) and *E. indica* (89.06 ± 0.52 %) whereas, lowest amount of carbohydrate was noted in the fruits of *M. edulis* (72.32 ± 0.72 %). All species were found at par with each other except *Machilus edulis* in respect to total carbohydrate content.

Table no. 4: Fibre and ash percent present in underutilized fruits of Sikkim

Sl. no	Scientific name of fruits	Fibre %	Ash %
1	*Baccaurea sapida*	3.60 ± 0.03	3.59 ± 0.72
2	*Calamus erectus*	4.99 ± 0.02	2.32 ± 0.29
3	*Diploknema butyraceae*	3.17 ± 0.11	4.18 ± 0.95
4	*Elaeagnus latifolia*	1.73 ± 0.67	1.76 ± 0.23
5	*Eleaocarpus sikkimensis*	2.00 ± 0.05	1.88 ± 0.78
6	*Eriolobus indica*	4.57 ± 0.06	2.90 ± 0.49
7	*Ficus roxburghii*	3.06 ± 0.02	2.99 ± 0.41
8	*Machilus edulis*	0.92 ± 0.06	0.97 ± 0.80
9	*Rubus ellipticus*	0.74 ± 0.08	1.37 ± 0.65
10	*Spondias axillaris*	1.55 ± 0.30	2.89 ± 0.28
C.V.		9.087	24.509
S.E.M.		0.138	0.351
C.D. 5%		0.408	1.037

4.1.6. Fibre

Table no. 4 and fig. 4 indicates fibre per cent observed in different fruit species found wildly under Sikkim conditions. The amount of fibre in all the fruits species under study was recorded in the range of 0.74 ± 0.08 per cent to 4.99 ± 0.02 per cent. The fruits of *Calamus erectus* showed maximum fibre per cent (4.99 ± 0.02 %) followed by *Eriolobus indica* (4.57 ± 0.06 %) and *Baccaurea sapida* (3.60 ± 0.03 %) whereas, the fruits of *Rubus ellipticus* quantified minimum fibre content (0.74 ± 0.08 %) followed by *Machilus edulis* (0.92 ± 0.06 %) and *Spondias axillaris* (1.55 ± 0.30 5). *Calamus erectus* with *Eriolobus indica* and *Machilus edulis* with *Rubus ellipticus* were found to be *at par* with each other while examining fibre percent.

4.1.7. Ash

The perusal of data presented in table 4 pertaining to ash content among ten underutilized fruits varied between 0.97 ± 0.80 per cent to 4.18 ± 0.95 per cent. The highest ash content was reported in the fruits of *Diploknema butyraceae* (4.18 ± 0.95 %) followed by *Baccaurea sapida* (3.59 ± 0.72 %), *Ficus roxburghii* (2.99 ± 0.41 %) and *Eriolobus indica* (2.90 ± 0.49 %) while, the lowest amount of ash was noted in the fruits of *Machilus edulis* (0.97 ± 0.80 %) followed by *Rubus ellipticus* and *Elaeagnus latifolia* (1.37 ± 0.65 % and 1.76 ± 0.23 %, respectively). *Elaeagnus latifolia*, *Eleaocarpus sikkimensis*, *Eriolobus indica*, *Ficus roxburghii* and *Spondias axillaris* were *at par* with each other implying the nutritional magnitude of underutilized fruit species.

4.1.8. Total soluble solids (TSS)

Total soluble solid of different underutilized fruits ranged from 2.38 ± 0.30^{o} Brix to 17.46 ± 0.45^{o} Brix (Table 5 & fig. 5). Highest TSS content was recorded in the fruit of *D. butyraceae i.e.* 17.46 ± 0.45^{o} Brix followed by *R. ellipticus* (16.11 ± 0.08 ^{o} Brix), *E. sikkimensis* (14.03 ± 0.15 ^{o} Brix) and *Elaeagnus latifolia* (13.20 ± 0.20 ^{o} Brix) whereas, lowest TSS content was observed in *C. erectus* (2.38 ± 0.30^{o} Brix) followed by *M. edulis* (3.63 ± 0.15^{o} Brix) and *Ficus roxburghii* (5.87 ± 0.42 ^{o} Brix) Other species such as *B. Sapida* (12.57 ± 0.15^{o} Brix) and *S. axillaris* (12.17 ± 0.15 ^{o} Brix) were also found to contain significant amount of TSS. *Baccaurea sapida*, *Elaeagnus latifolia* and *Ficus roxburghii* were found to be *at par* with each other.

4.1.9. Titrable Acidity

Titrable acidity of different underutilized fruits was found in varying concentration ranged from 0.02 ± 0.01 % to 3.88 ± 0.17 % which has been presented in Table no.5. Minimum titrable acidity was recorded in the fruit of *F. roxburghii* (0.02 ± 0.01%) followed by *C. erectus* (0.03 ± 0.06 %), *D. butyraceae* (0.04 ± 0.06 %) and *M. edulis* (0.12 ± 0.02 %) whereas, maximum titrable acidity was noted in the fruit of *E. latifolia* (3.88 ± 0.17 %) followed by *S. axillaris* (3.47 ± 0.24 %) and *Eriolobus indica* (2.94 ± 0.07 %). *Eleaocarpus sikkimensis* and *Eriolobus indica* were *at par* with each other with respect to titrable acidity.

4.1.10. Total Sugar

Ten different species were subjected to phytochemical analysis of Sikkim of which data revealed that total sugar per cent in all underutilized fruits varied

Table no. 5. Total Soluble Solid (TSS) and titrable acidity present in underutilized fruits of Sikkim.

Sl. no	Scientific name of fruits	TSS (^{o} Brix)	Titrable acidity
1	*Baccaurea sapida*	12.57 ± 0.15	1.95 ± 0.04
2	*Calamus erectus*	2.38 ± 0.30	0.03 ± 0.06
3	*Diploknema butyraceae*	17.46 ± 0.45	0.04 ± 0.06
4	*Elaeagnus latifolia*	13.20 ± 0.20	3.88 ± 0.17
5	*Eleaocarpus sikkimensis*	14.03 ± 0.15	2.03 ± 0.05
6	*Eriolobus indica*	10.17 ± 0.25	2.94 ± 0.07
7	*Ficus roxburghii*	5.87 ± 0.42	0.02 ± 0.01
8	*Machilus edulis*	3.63 ± 0.15	0.12 ± 0.02
9	*Rubus ellipticus*	16.11 ± 0.08	1.97 ± 0.04
10	*Spondias axillaris*	12.17 ± 0.15	3.47 ± 0.24
C.V.		24.509	6.140
S.E.M.		0.351	0.058
C.D. 5%		0.440	0.172

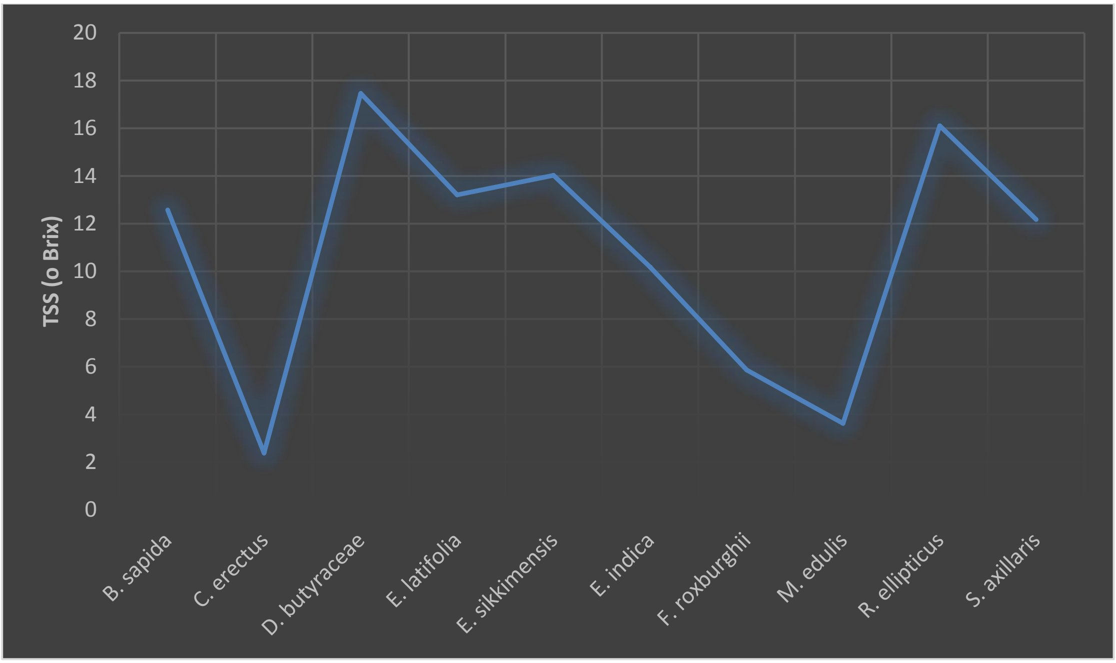

Fig. 5. Total soluble solid present in underutilized fruits of Sikkim

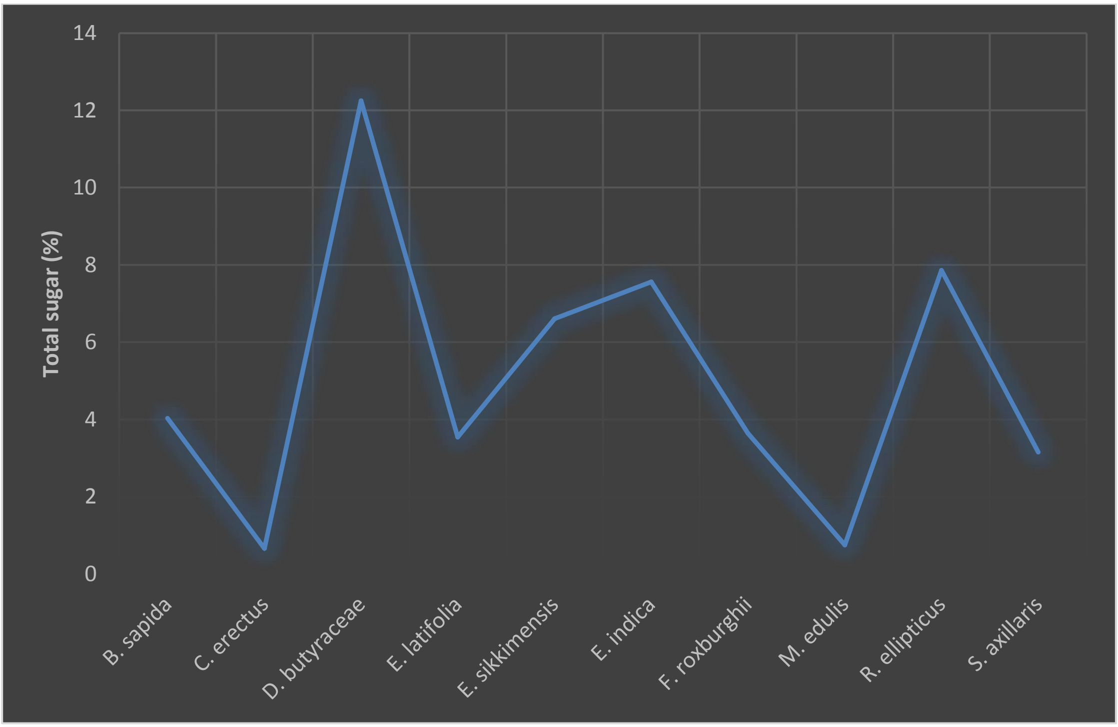

Fig. 6. Total sugar per cent present in underutilized fruits of Sikkim.

significantly and found in a range of 0.66 ± 0.95 per cent to 12.25 ± 0.04 percent (Table no. 6 & fig. 6). The highest total sugar per cent was noted in the fruits of *D. butyraceae* (12.25 ± 0.04 %) followed by *R. ellipticus* and *E. indica* (7.86 ± 0.16 % and 7.56 ± 0.02) respectively whereas, the lowest amount of total sugar was recorded in the fruit of *C. erectus* (0.66 ± 0.95 %) followed by *M. edulis* (0.75 ± 0.21 %) and *Spondias axillaris* (3.15 ± 0.03 %). None of the species were found to be *at par* with each other revealing differential total sugar content in different underutilised species.

4.1.11. Reducing sugar

The reducing sugar content of different underutilized fruit species revealed that reducing sugar content of ten different underutilized fruits varied significantly and recorded in a range of 0.40 ± 0.59 per cent to 5.57 ± 1.20 per cent. The maximum reducing sugar content was observed in the fruit of *R. ellipticus* (5.57 ± 1.20 %) followed by *D. butyraceae* (5.22 ± 1.36 %) and *E. sikkimensis* (5.04 ± 0.44%) whereas minimum reducing sugar per cent was noted in the fruit of *C. erectus* (0.40 ± 0.59 %) followed by *M. edulis* (0.61± 0.33 %) and *Ficus roxburghii* (1.91± 0.55 %). *Baccaurea sapida, Elaeagnus latifolia* and *Ficus roxburghii* were *at par* with each other (Table no. 6).

4.1.12. Non Reducing sugar

The per cent non reducing sugar of different underutilized fruits species presented in Table no. 6 revealed that reducing sugar per cent of all the fruits under study were recorded in a range of 0.13 ± 0.11 per cent to 6.68 ± 1.26 per cent. The highest non reducing sugar per cent (6.68 ± 1.26 %) was found in the fruit of *D. butyraceae* followed by *E. indica (*3.62 %) and *Rubus ellipticus* (2.18 ± 1.24 %). The

Table no. 6: Total sugar, reducing sugar and non-reducing sugar per cent present in underutilized fruits of Sikkim

Sl. no	Scientific name of fruits	Total sugar (%)	Reducing sugar (%)	Non- reducing sugar (%)
1	*Baccaurea sapida*	4.03 ± 0.05	2.39 ± 0.62	1.56 ± 0.60
2	*Calamus erectus*	0.66 ± 0.95	0.40 ± 0.59	0.25 ± 0.34
3	*Diploknema butyraceae*	12.25 ± 0.04	5.22 ± 1.36	6.68 ± 1.26
4	*Elaeagnus latifolia*	3.54 ± 0.64	2.08 ± 0.57	1.22 ± 0.55
5	*Eleaocarpus sikkimensis*	6.61 ± 1.15	5.04 ± 0.44	1.36 ± 0.70
6	*Eriolobus indica*	7.56 ± 0.02	3.75 ± 0.71	3.62 ± 0.70
7	*Ficus roxburghii*	3.64 ± 0.45	1.91± 0.55	1.65 ± 0.71
8	*Machilus edulis*	0.75 ± 0.21	0.61± 0.33	0.13 ± 0.11
9	*Rubus ellipticus*	7.86 ± 0.16	5.57 ± 1.20	2.18 ± 1.24
10	*Spondias axillaris*	3.15 ± 0.03	2.22 ± 0.69	0.91 ± 0.67
C.V.		10.809	26.369	39.022
S.E.M.		0.312	0.444	0.441
C.D. 5%		0.922	1.311	1.300

lowest per cent of non-reducing sugar was noted in the fruit of *M. edulis* (0.13 ± 0.11 %) followed by *C. erectus* (0.25 ± 0.34 %) and *S. axillaris* (0.91 ± 0.67%). *Baccaurea sapida, Elaeagnus latifolia, Eleaocarpus sikkimensis* and *Rubus ellipticus* were *at par* with each other.

4.2. Phytochemical composition

4.2.1. Vitamin A

The Vitamin A content of different underutilized fruits of Sikkim was determined and is given in the Table no. 7 and fig. 7. The results revealed that vitamin A content of various underutilized fruits was found in a range of 43.44 ± 2.52 IU to 385.33 ± 2.03 IU. The highest vitamin- A content was recorded in the fruit of *D. butyraceae* (385.33 ± 2.03 IU) followed by *E. latifolia* (345.33 ± 2.73 IU) and *E. sikkimensis* (294.68 ± 3.61 IU) while lowest vitamin A content was noted in the fruit of *C. erectus* i.e. 43.44 ± 2.52 IU followed by *Baccaurea sapida* (80.33 ± 4.80 IU). Significant amount of vitamin A was also detected from the fruit of *Eriolobus indica* (223.22 ± 0.69 IU) and *Rubus ellipticus* (182.24 ± 1.40 IU). *Calamus erectus, Diploknema butyraceae* and *Ficus roxburghii* were *at par* with each other.

4.2.2. Vitamin E

The vitamin E content of different underutilized fruit species were investigated and results are presented in Table No. 8. The data in respect to Vitamin E content was found in a range of 2.10 ± 0.14 IU to 47.40 ± 1.10 IU. Higher amount of Vitamin E content was recorded in the fruit of *M. edulis* (47.40 ± 1.10 IU) followed by *E. sikkimensis* (38.37 ± 1.04 IU), *S. axillaris* (24.52 ± 0.96 IU) and *Baccaurea sapida*

Table no. 7: Vitamin A and Vitamin E content present in underutilized fruits of Sikkim.

Sl. no	Scientific name of fruits	Vitamin A (IU)	Vitamin E (IU)
1	*Baccaurea sapida*	80.33 ± 4.80	23.86 ± 0.17
2	*Calamus erectus*	43.44 ± 2.52	2.88 ± 0.10
3	*Diploknema butyraceae*	385.33 ± 2.03	12.44 ± 0.69
4	*Elaeagnus latifolia*	345.33 ± 2.73	22.56 ± 0.21
5	*Eleaocarpus sikkimensis*	294.68 ± 3.61	38.37 ± 1.04
6	*Eriolobus indica*	223.22 ± 0.69	7.02 ± 0.07
7	*Ficus roxburghii*	140.66 ± 11.84	2.10 ± 0.14
8	*Machilus edulis*	129.66 ± 2.35	47.40 ± 1.10
9	*Rubus ellipticus*	182.24 ± 1.40	18.64 ± 1.21
10	*Spondias axillaris*	124.99 ± 2.00	24.52 ± 0.96
C.V.		5.65	4.85
S.E.M.		2.65	2.48
C.D. 5%		3.45	3.65

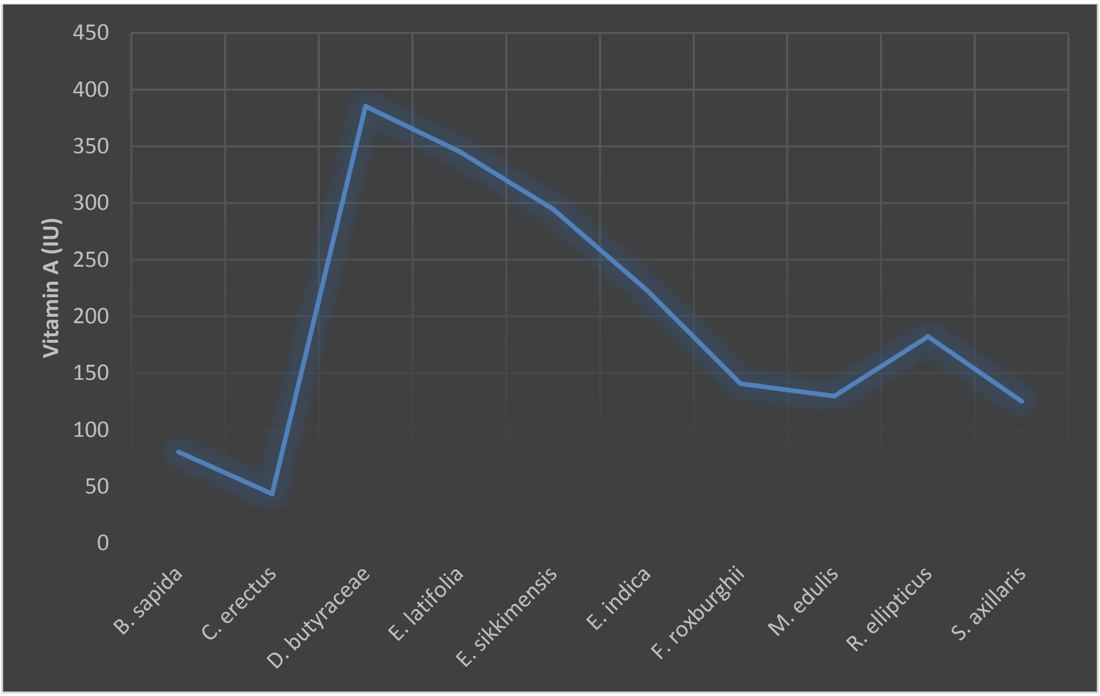

Fig. 7. Vitamin A content present in underutilized fruits of Sikkim.

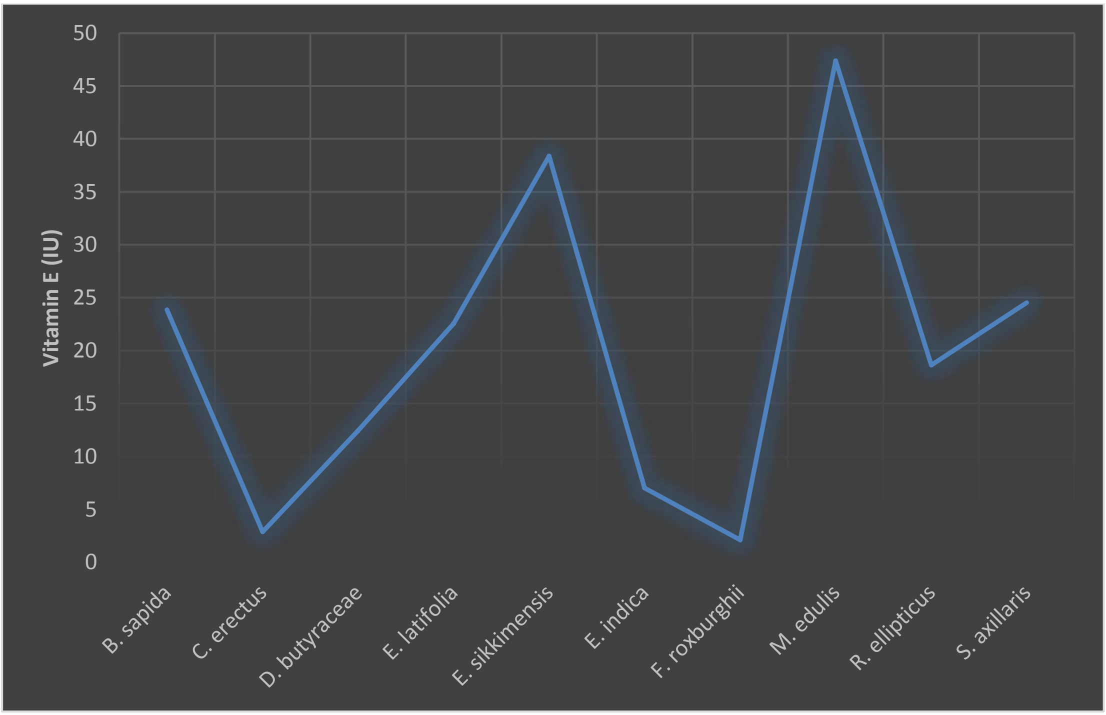

Fig. 8. Vitamin E present in underutilized fruits of Sikkim.

(23.86 ± 0.17 IU) whereas lower amount of vitamin E content was recorded from the fruit of *F. roxburghii* (2.10 ± 0.14 IU) followed by *C. erectus* (2.88 ± 0.10 IU) and *E. indica* (7.02 ± 0.07 IU). *Baccaurea sapida*, *Elaeagnus latifolia* and *Ficus roxburghii* were found to be *at par* among different underutilized fruits.

4.2.3. Total phenols

The screening of the methanol extracts of ten different wild edible fruit species of Sikkim revealed that there was a wide variation in the amount of total phenols ranging from 11.17 ± 0.06 mg GAE g^{-1} DM to 71.83 ± 0.42 mg GAE g^{-1} DM (Table no. 8 & fig 9). The fruit of *S. axillaris* (71.83 ± 0.42 mg GAE g^{-1}) was found to contain maximum amount of total phenol among all other species followed by *R. ellipticus* (54.27 ± 0.31mg GAE g^{-1}) and *B. Sapida* (51.53 ± 0.50 mg GAE g^{-1}) and minimum amount of total phenols among other species was recorded in the fruit of *M. edulis* i.e. 11.17 ± 0.06 mg GAE g^{-1} followed by *E. indica* (12.53 ± 0.50 mg GAE g^{-1}) and *E. sikkimensis* (17.53 ± 0.83 mg GAE g^{-1}).

4.2.4. Total flavonoid

The result of total flavonoid content have been presented in Table no. 8 and fig.10. The flavonoid content of the methanol extract of different underutilized fruit in terms of quercetin equivalent was varying significantly and found within range of 4.83 ± 0.67 mg QE g^{-1} to 70.13 ± 0.25 mg QE g^{-1}. The highest amount of total flavonoid content was observed in the fruit of *B. sapida* (70.13 ± 0.25 mg QE g^{-1}) followed by *R. ellipticus* (32.41 ± 0.80 mg QE g^{-1}) and *C. erectus* (22.72 ± 0.21mg QE g^{-1}) whereas, lowest amount of total flavonoid was observed in the fruit of *F.*

roxburghii (4.83 ± 0.67mg QE g^{-1}) which was followed by *E. sikkimensis* (5.16 ± 0.55 mg QE g^{-1}) and *S. axillaris* (7.83 ± 0.50 mg QE g^{-1}).

4.2.5. Flavonols content

The results of flavonols content of different underutilized fruit species (Table no. 8) indicated that the amount of flavonols among different species was recorded in a range of 6.57 ± 0.47 mg QE g^{-1} to 21.65 ± 1.45 mg QE g^{-1}. The fruit of *B. sapida* possessed higher amount of flavonols content i.e. 21.65 ± 1.45 mg QE g^{-1} followed by *R. ellipticus* (21.51 ± 0.33 mg QE g^{-1}) and *S. axillaris* (18.98 ± 0.36 mg QE g^{-1}) whereas, lower amount of flavonols was observed in the fruit of *D. butyraceae* (6.57 ± 0.47 mg QE g^{-1}) followed by *C. erectus* (8.55 ± 0.88 mg QE g^{-1})

4.2.6. Ascorbic acid

The critical examination of the data indicates the presence of significantly varied quantities of ascorbic acid content among all the underutilized fruit species (Table no. 9 and fig. 11). The mean value of ascorbic acid content ranged from 2.20 ± 0.11 mg 100 g^{-1} to 51.10 ± 1.40 mg 100 g^{-1}. Among all the species under study, the fruit of *B. sapida* was detected with maximum ascorbic acid i.e. 51.10 ± 1.40 mg 100 g^{-1} followed by *S. axillaris* (34.54 ± 0.99 mg 100^{-1}) and *D. butyraceae* (22.72 ± 0.65 mg 100 g^{-1}) while, minimum ascorbic acid was detected in the fruit of *C. erectus* (2.20 ± 0.11 mg 100 g^{-1}) followed by *F. roxburghii* (3.36 ± 0.27 mg 100^{-1}), *M. edulis* (5.19 ± 0.37 mg 100 g^{-1}) and *R. ellipticus* (5.67 ± 0.06 mg 100^{-1}). *Machilus edulis* and *Rubus ellipticus* were only found *at par* with each other among all fruit species.

Table no. 8: Total phenols, flavonoid and flovonols content present in underutilized fruits of Sikkim

Sl. no	Scientific name of fruits	Total phenol (mg GAE/ G)	Total flavonoid (mg QE/g)	Flavonols mg QE/g
1	*Baccaurea sapida*	51.53 ± 0.50	70.13 ± 0.25	21.65 ± 1.45
2	*Calamus erectus*	39.00 ± 1.32	22.72 ± 0.21	8.55 ± 0.88
3	*Diploknema butyraceae*	34.33 ± 0.74	18.23 ± 0.31	6.57 ± 0.47
4	*Elaeagnus latifolia*	26.90 ± 0.75	11.06 ± 0.49	17.92 ± 0.11
5	*Eleaocarpus sikkimensis*	17.53 ± 0.83	5.16 ± 0.55	12.14 ± 0.05
6	*Eriolobus indica*	12.53 ± 0.50	17.48 ± 0.31	9.51 ± 0.29
7	*Ficus roxburghii*	48.80 ± 0.53	4.83 ± 0.67	17.34 ± 0.03
8	*Machilus edulis*	11.17 ± 0.06	15.19 ± 0.34	11.30 ± 0.21
9	*Rubus ellipticus*	54.27 ± 0.31	32.41 ± 0.80	21.51 ± 0.33
10	*Spondias axillaris*	71.83 ± 0.42	7.83 ± 0.50	18.98 ± 0.36
C.V.		4.30	5.20	3.50
S.E.M.		3.65	2.02	1.52
C.D. 5%		1.30	1.25	1.05

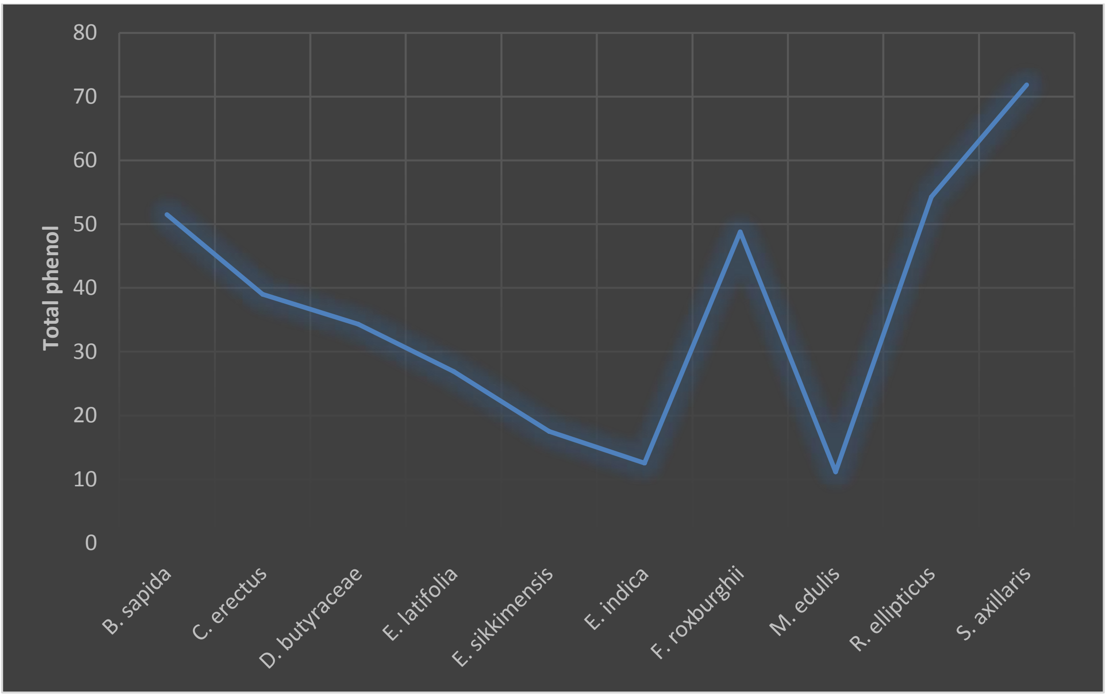

Fig. 9. Total phenols content present in underutilized fruits of Sikkim

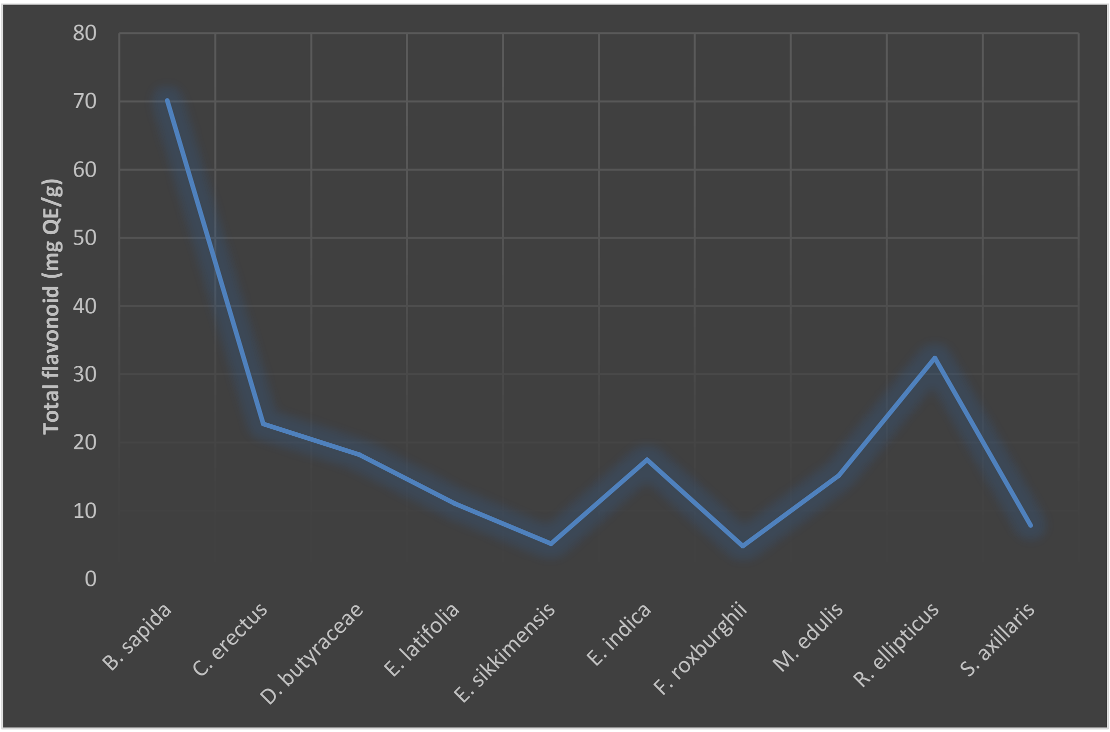

Fig. 10. Flavonoid content present in underutilized fruits of Sikkim

4.2.7. Anthocyanin content

The anthocyanin content of selected underutilized fruits were determined and presented in Table no. 9. The significant variation were observed for anthocyanin content among all the species under investigation and they were found in a ranged between 0.47 ± 0.02 % to 3.06 ± 0.20 %. The fruit of *R. ellipticus* was noted with highest per cent of anthocyanin content i.e. 3.06 ± 0.20 % followed by *E. latifolia* (1.82 ± 0.02 %) and *B. sapida* (1.71 ± 0.02 %) while lowest anthocyanin content was reported from the fruit species *M. edulis* (0.47 ± 0.02 %) followed by *D. butyraceae* (0.64 ± 0.05 %) and *S. axillaris* (0.74 ± 0.02 %). *Baccaurea sapida, Calamus erectus, Elaeagnus latifolia*, *Eleaocarpus sikkimensis,* and *Eriolobus indica* were *at par* with each other.

4.2.8. Total carotenoids

The observation on total carotenoids contents of different underutilized fruits species from Sikkim showed significant variation (Table no. 9 and fig 12). The mean value of total carotenoids exhibited a range of 0.68 ± 0.10% to 63.80 ± 1.66 %. In the present investigation maximum carotenoids was recorded in the fruit of *M. edulis* (63.80 ± 1.66 %) followed by *E. sikkimensis* 58.13 ± 0.26 %) while, lowest amount of total carotenoids was noted in the fruit of *F. roxburghii* (0.68 ± 0.10 %) followed by *S. axillaris* (0.90 ± 0.30 %) and *E. indica* (0.99 ± 0.09 %). *Eriolobus indica* and *Spondias axillaris* were quantified *at par* with each other among different fruit species.

Table no. 9: Ascorbic acid, anthocyanin and total carotenoids content present in underutilized fruits of Sikkim

Sl. no	Scientific name of fruits	Ascorbic acid (mg/100 g)	Anthocyanin (%)	Total carotenoids (%)
1	*Baccaurea sapida*	51.10 ± 1.40	1.71 ± 0.02	6.62 ± 0.30
2	*Calamus erectus*	2.20 ± 0.11	1.55 ± 0.04	4.55 ± 0.23
3	*Diploknema butyraceae*	22.72 ± 0.65	0.64 ± 0.05	8.31 ± 0.07
4	*Elaeagnus latifolia*	14.13 ± 1.50	1.82 ± 0.02	9.84 ± 0.48
5	*Eleaocarpus sikkimensis*	11.43 ± 1.12	1.38 ± 0.16	58.13 ± 0.26
6	*Eriolobus indica*	9.70 ± 0.59	1.33 ± 0.74	0.99 ± 0.09
7	*Ficus roxburghii*	3.36 ± 0.27	1.13 ± 0.15	0.68 ± 0.10
8	*Machilus edulis*	5.19 ± 0.37	0.47 ± 0.02	63.80 ± 1.66
9	*Rubus ellipticus*	5.67 ± 0.06	3.06 ± 0.20	1.79 ± 0.17
10	*Spondias axillaris*	34.54 ± 0.99	0.74 ± 0.02	0.90 ± 0.30
C.V.		5.391	18.220	3.720
S.E.M.		0.498	0.146	0.334
C.D. 5%		1.470	0.429	0.986

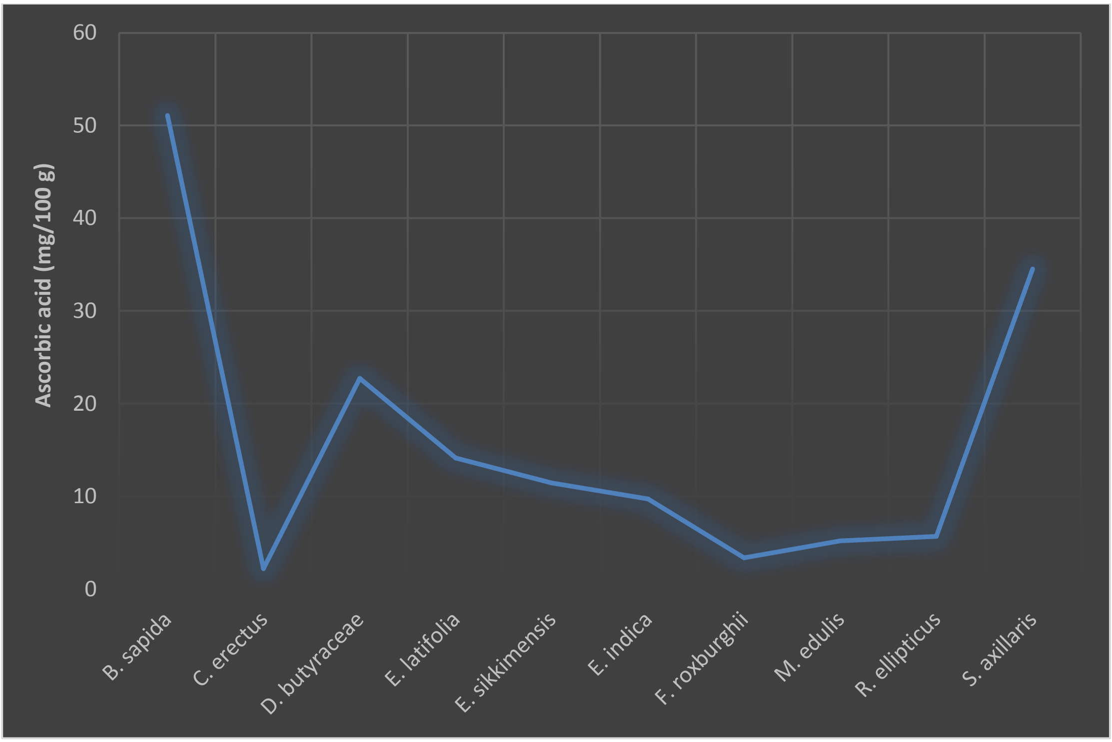

Fig. 11. Total ascorbic acid present in underutilized fruits of Sikkim

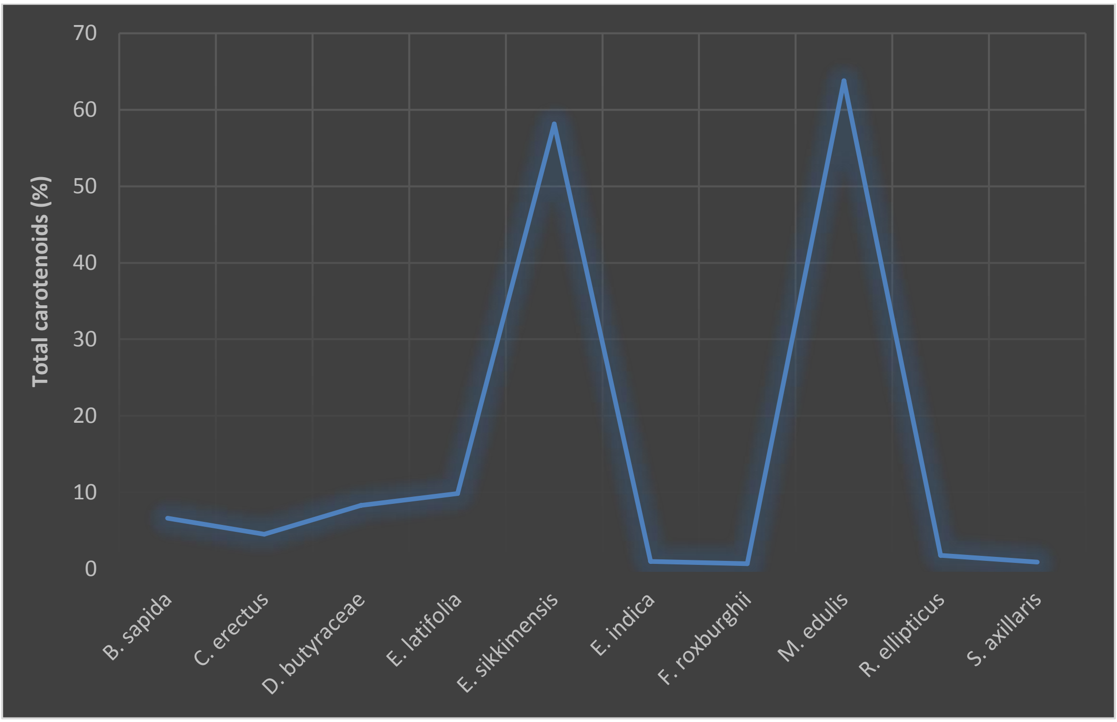

Fig. 12. Total carotenoids content present in underutilized fruits of Sikkim.

4.3. Antioxidant activities

4.3.1 DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activities of different underutilized fruits have been shown in Table no. 10.The selected underutilized fruits were having varying levels of DPPH radical scavenging activity and were ranged from 42.09 ± 0.56 % to 72.26 ± 0.06 %. *Ficus roxburghii* showed highest DPPH radical scavenging activity and lowest in *Eleaocarpus sikkimensis*. Significant levels of DPPH radical scavenging activity were also observed in *Spondias axillaris* (67.63 ± 0.27 %), *Rubus ellipticus* (64.82 ± 0.27 %), *Elaeagnus latifolia* (63.76 ± 0.51 %) *and Baccaurea sapida* (61.89 ± 0.29 %). *Diploknema butyraceae* and *Elaeagnus latifolia*, *Calamus erectus* and *Eleaocarpus sikkimensis* were found to be *at par* with each other.

4.3.2. Hydroxyl Radical Scavenging Activity

Table no. 10 pertains data related to hydroxyl radical scavenging activity of different underutilized fruits of Sikkim. All the tested samples possessed radical scavenging capacity but in varying degree ranged from 33.59 ± 0.02 % to 61.46 ± 0.04 %. Methanol extract of *Rubus ellipticus* fruits shows maximum hydroxyl radical scavenging activity i.e. 61.46 ± 0.04 % followed by *Spondias axillaris* (60.35 ± 0.03 %), *Diploknema butyraceae* (59.14 ± 0.03 %), *Eriolobus indica* (56.14 ± 0.08 %) and *M. edulis* (53.73 ± 0.03 %) whereas, lowest hydroxyl radical scavenging capacity was observed in the fruit of *Ficus roxburghii* (33.59 ± 0.02 %). *Calamus erectus* and *Eleaocarpus sikkimensis* were observed to be *at par* with each other.

4.3.3. Hydrogen Peroxide scavenging activity

Hydrogen peroxide scavenging activity of different underutilized fruits have been given in Table no. 10. The ability of all the underutilized fruits extracted in methanol to scavenge hydrogen peroxide was found in a range of 45.70 ± 0.52 % to 71.85 ± 0.16 %. Highest scavenging capacity was recorded in the fruit of *Eriolobus indica* and lowest in the fruit of *Ficus roxburghii* (71.85 ± 0.16 % and 45.70 ± 0.52 %, respectively). The fruits of *Spondias axillaris, Baccaurea sapida, Eleaocarpus sikkimensis, Rubus ellipticus and Diploknema butyraceae* also showed significant amount of scavenging capacity (68.88 ± 0.07 %, 65.73 ± 0.61 %, 63.23 ± 0.15 %, 61.63 ± 0.24 % and 60.13 ± 0.06 %, respectively). *Eleaocarpus sikkimensis* and *Diploknema butyraceae* were found to be *at par* with each other.

4.3.4. Ferrous ion chelating activity

Ferrous ion chelating activity of different underutilized fruits have been shown in Table no. 11. It was found in a range of 17.62 ± 0.6 % to 42.04 ± 0.08 %. Highest ferrous ion chelating activity was observed in the fruit of *Eriolobus indica* (42.04 ± 0.08 %) which was followed by *Baccaurea sapida* (39.24 ± 0.4 %), *Spondias axillaris* (38.09 ± 0.2 %) and *Rubus ellipticus* (35.55 ± 0.7 %) whereas, lowest was observed in the fruit of *Diploknema butyraceae* i.e. 17.62 ± 0.6 % followed by *Ficus roxburghii* (19.34 ± 0.04 %).

Table no. 10: ***DPPH % inhibition, hydroxyl radical % inhibition and hydrogen peroxide scavenging activity*** **present in underutilized fruits of Sikkim**

Sl. No	**Scientific Name of fruits**	***DPPH Assay (%)***	***Hydroxyl Radical Scavenging Assay (%)***	**Hydrogen Peroxide Scavenging activity**
1.	*Baccaurea sapida*	61.89 ± 0.29	53.24 ± 0.04	65.73 ± 0.61
2.	*Calamus erectus*	45.12 ± 0.15	47.05 ± 0.04	57.30 ± 0.10
3.	*Diploknema butyraceae*	53.81 ± 0.42	59.14 ± 0.03	60.13 ± 0.06
4.	*Elaeagnus latifolia*	63.76 ± 0.51	42.10 ± 0.03	51.47 ± 0.25
5.	*Eleaocarpus sikkimensis*	42.09 ± 0.56	45.18 ± 0.03	63.23 ± 0.15
6.	*Eriolobus indica*	59.32 ± 0.43	56.14 ± 0.08	71.85 ± 0.16
7.	*Ficus roxburghii*	72.26 ± 0.06	33.59 ± 0.02	45.70 ± 0.52
8.	*Machilus edulis*	47.58 ± 0.47	53.73 ± 0.03	54.56 ± 0.33
9.	*Rubus ellipticus*	64.82 ± 0.27	61.46 ± 0.04	61.63 ± 0.24
10.	*Spondias axillaris*	67.63 ± 0.27	60.35 ± 0.03	68.88 ± 0.07
C.V.		0.650	0.075	0.510
S.E.M.		0.217	0.022	0.177
C.D. 5%		0.640	0.066	0.522

Table no. 11: Ferrous ion chelating assay (%), FRAP Assay (mg GAE/g DW) and Phosphomolybdenum assay mg AAE/g DW) present in underutilized fruits of Sikkim.

Sl. No	Scientific Name of fruits	Ferrous ion chelating assay (%)	FRAP Assay (mg GAE/g DW)	Phospho-molybdenum assay mg AAE/g DW)
1.	*Baccaurea sapida*	39.24 ± 0.4	26.26 ± 02	51.11 ± 0.7
2.	*Calamus erectus*	21.02 ± 0.03	14.71 ± 0.4	44.09 ± 0.2
3.	*Diploknema butyraceae*	17.62 ± 0.6	13.67 ± 0.08	39.03± 0.4
4.	*Elaeagnus latifolia*	32.03 ± 0.02	7.75 ± 0.6	49.98 ± 0.11
5.	*Eleaocarpus sikkimensis*	28.12 ± 0.1	14.09 ± 0.03	45.62 ± 0.06
6.	*Eriolobus indica*	42.04 ± 0.08	17.01 ± 0.3	57.09 ± 0.22
7.	*Ficus roxburghii*	19.34 ± 0.04	11.02 ± 0.6	33.01 ± 0.5
8.	*Machilus edulis*	31.09 ± 0.02	9.05 ± 0.08	29.03 ± 0.08
9.	*Rubus ellipticus*	35.55 ± 0.7	25.01 ± 0.2	54.40 ± 0.4
10.	*Spondias axillaris*	38.09 ± 0.2	31.04 ± 0.2	59.99 ± 0.6
C.V.		5.40	2.06	1.54
S.E.M.		3.02	1.75	46.21
C.D. 5%		0.52	0.12	1.25

4.3.5. Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was performed and antioxidant activity is based on the capacity of antioxidant to reduce ferric (III) ions to ferrous (II) ions. The data shown in Table no. 11 depicted that the all the tested samples possessed antioxidant properties which ranged from 7.75 ± 0.6 mg GAE g^{-1} to 31.04 ± 0.2 mg GAE g^{-1}. Highest antioxidant properties was noted in the fruit of *Spondias axillaris* (31.04 ± 0.2 mg GAE g^{-1}) followed by *Baccaurea sapida* and *Rubus ellipticus* **(**26.26 ± 02 mg GAE g^{-1} and 25.01 ± 0.2 mg GAE g^{-1}, respectively**)** while lowest antioxidant activity was observed in the fruit extract of *E. latifolia* (7.75 ± 0.6 mg GAE g^{-1}) followed by *M. edulis* (9.05 ± 0.08 mg GAE g^{-1}). *Baccaurea sapida* and *Spondias axillaris* were found to be *at par* with each other.

4.3.6. Total antioxidant capacity (TAC) by Phospho-molybdenum assay

The total antioxidant activity of different underutilized fruit extracts was estimated, using ascorbic acid as standard and is expressed as the number of equivalents of the ascorbic acids and the value was expressed as mg g^{-1} Ascorbic acid equivalent. Total antioxidant activities were found in a range between 29.03 ± 0.08 mg AAE g^{-1} to 59.99 ± 0.6 mg AAE g^{-1} (Table no. 11). It was recorded highest in the fruit extract of *S. axillaris* whereas lowest in the fruit of *M. edulis.* Fruit of *E. indica, R. ellipticus, B. sapida and E. latifolia* also showed significant amount of total antioxidant activity (57.09 ± 0.22 mg AAE g^{-1}, 54.40 ± 0.4 mg AAE g^{-1}, 51.11 ± 0.7 mg AAE g^{-1} and 49.98 ± 0.11 mg AAE g^{-1}, respectively).

4.4. Quantification of selected elements

4.4.1. Macro elements

4.4.1.1. Potassium

The perusal of data presented in Table no. 12 pertaining to potassium content among ten underutilized fruits varied between 98.75 ± 3.9 µg kg^{-1} to 819.64 ± 2.54 µg kg^{-1}. Maximum Potassium content was recorded in the fruit of *F. roxburghii* (819.64 ± 2.54 µg kg^{-1}) followed by *R. ellipticus* (421 ± 1.9 µg kg^{-1}), *B. Sapida* (375.37 ± 1.68 µg kg^{-1}), *E. latifolia* (293.51 ± 1.01 µg kg^{-1}) and *S. axillaris* (280.12 ± 1.58 µg kg^{-1}) whereas, minimum potassium content was observed in the fruit of *D. butyraceae i.e.* 98.75 ± 3.9 µg kg^{-1}.

4.4.1.2. Magnesium (Mg)

The magnesium content of different underutilized fruits have been presented in the Table no. 12. The range of magnesium content recorded was 6.88 ± 0.8 µg kg^{-1} to 73.09 ± 1.42 µg kg^{-1}. The highest amount of magnesium content was observed in the fruit of *F. roxburghii* (73.09 ± 1.42 µg kg^{-1}) followed by *S. axillaris* (40.16 ± 1.9 µg kg^{-1}), *R. ellipticus* (35.17 ± 1.9 µg kg^{-1}), *E. sikkimensis* (21.67 ± 1.4 µg kg^{-1}) and *B. Sapida* (21.67 ± 1.2 µg kg^{-1}) while, the magnesium content was recorded lowest in the fruit of *E. indica* (6.88 ± 0.8 µg kg^{-1}) followed by *C. erectus* (7.04 ± 0.18 µg kg^{-1}) and *D. butyraceae* (7.81 ± 2.1 µg kg^{-1}).

Table no. 12. Quantification of macro elements in selected underutilized fruits of Sikkim

Sl. No	Scientific Name of fruits	Macro elements (µg kg^{-1})				
		Potassium (K)	Magnesium (Mg)	Calcium (Ca)	Sodium (Na)	Sulphur (S)
1.	*Baccaurea sapida*	375.37 ± 1.68	21.67 ± 1.2	23.77 ± 2.0	7.99 ± 1.8	5.03 ± 1.3
2.	*Calamus erectus*	160.94 ± 1.56	7.04 ± 0.18	2.08 ± 0.11	2.46 ± 1.0	0.086 ± 0.06
3.	*Diploknema butyraceae*	98.75 ± 0.99	7.81 ± 2.1	1.98 ± 1.0	2.87 ± 1.1	3.17 ± 1.9
4.	*Elaeagnus latifolia*	293.51 ± 1.01	17.35 ± 0.65	2.21 ± 0.14	9.91 ±0.30	4.89 ± 0.5
5.	*Eleaocarpus sikkimensis*	223.59 ± 2.21	21.67 ± 1.4	2.89 ± 0.32	8.09 ± 1.6	4.70 ± 1.0
6.	*Eriolobus indica*	111.37 ± 1.38	6.88 ± 0.8	1.98 ± 0.02	7.05 ± 1.4	4.28 ± 1.2
7.	*Ficus roxburghii*	819.64 ± 2.54	73.09 ± 1.42	23.69 ± 1.43	6.73 ± 1.6	5.45 ± 0.72
8.	*Machilus edulis*	126.09 ± 1.56	19.52 ± 1.0	0.72 ± 0.11	6.4 ± 1.0	5.74 ± 1.8
9.	*Rubus ellipticus*	421 ± 1.9	35.17 ± 1.9	18 ± 09	8.21 ± 2.0	5.26 ± 1.8
10.	*Spondias axillaris*	280.12 ± 1.58	40.16 ± 1.9	12.37 ± 1.9	6.96 ± 1.3	5.19 ± 1.6

4.4.1.3. Calcium (Ca)

The calcium content of ten different underutilized fruits have been shown in the Table no. 12 and the data revealed calcium content was found in a range of 0.72 ± 0.11 µg kg^{-1} to 23.77 ± 2.0 µg kg^{-1}. Among ten underutilized fruits studied, highest calcium content was observed in the fruit of *B. sapida* (23.77 ± 2.0 µg kg^{-1}) followed by *F. roxburghii* (23.69 ± 1.43 µg kg^{-1}), *R. ellipticus* (18 ± 09 µg kg^{-1}) and *S. axillaris* (12.37 ± 1.9 µg kg^{-1}) whereas, the lowest calcium content was detected in the fruit of *M. edulis* (0.72 ± 0.11 µg kg^{-1}) followed by *E. indica* (1.98 ± 0.02 µg kg^{-1}) *and D. butyraceae* (1.98 ± 1.0 µg kg^{-1}).

4.4.1.4. Sodium (Na)

The sodium content of different underutilized fruits is given the Table no.12. The range of sodium content in ten different underutilized fruit species was found between 2.46 ± 1.0 µg kg^{-1} to 9.91 ± 0.30 µg kg^{-1}. The maximum amount of sodium was observed in the fruit of *E. latifolia* (9.91 ± 0.30 µg kg^{-1}) followed by *R. ellipticus* (8.21 ± 2.0 µg kg^{-1}), *E. sikkimensis* (8.09 ± 1.6 µg kg^{-1}) and *B. Sapida* (7.99 ± 1.8 µg kg^{-1}) whereas, the minimum amount of sodium content was recorded from the fruit of *C. erectus* (2.46 ± 1.0 µg kg^{-1}) followed by *D. butyraceae* (2.87 ± 1.1 µg kg^{-1}).

4.4.1.5. Sulphur (S)

The sulphur content of selected underutilized fruits has been presented in a Table No. 12. The range of sulphur content found in different underutilized fruit species was 0.086 ± 0.06 µg kg^{-1} to 5.74 ± 1.8 µg kg^{-1}. The highest amount of sulphur was detected from the fruit of *M. edulis* (5.74 ± 1.8 µg kg^{-1}) followed by *F. roxburghii* (5.45 ± 0.72 µg kg^{-1}) *R. ellipticus* (5.26 ± 1.8 µg kg^{-1}) and *S. axillaris* (5.19

± 1.6 μg kg^{-1}) and the lowest amount of sulphur content among different underutilized fruits was noted in the fruit of *C. erectus* (0.086 ± 0.06 μg kg^{-1}) followed by *D. butyraceae* (3.17 ± 1.9 μg kg^{-1}).

4.4.2. Micro Elements

4.4.2.1. Iron (Fe)

The iron content of different underutilized fruit species have been given in the Table no. 13. The data revealed for iron content was observed in a range of 1.28 ± 0.16 μg kg^{-1} to 31.40 ± 0.51 μg kg^{-1}. The presence of maximum iron content was recorded from the fruit of *E. latifolia* (31.40 ± 0.51 μg kg^{-1}) followed by *B. Sapida* (29.55 ± 2.6 μg kg^{-1}), *F. roxburghii* (26.55 ± 2.8 μg kg^{-1}), *S. axillaris* (26.47 ± 2.4 μg kg^{-1}) and *E. sikkimensis* (24.29 ± 2.3 μg kg^{-1}) whereas the presence of minimum iron content was observed in the fruit of *C. erectus* (1.28 ± 0.16 μg kg^{-1}) followed by *D. butyraceae* (2.4 ± 0.69 μg kg^{-1}).

4.4.2.2. Zinc (Zn)

The investigation of micro elements from the different underutilized fruit varied significantly and found in a range of 0.11 ± 0.08 μg kg^{-1} to 0.97 ± 0.18 μg kg^{-1} and the data regarding zinc content of different underutilized fruits have been shown in the Table no. 13. The fruit of *B. Sapida* was found to contain highest amount of zinc i.e. 0.97 ± 0.18 μg kg^{-1} followed by *D. Butyraceae, R. ellipticus and C. erectus* (0.65 ± 0.26 μg kg^{-1}, 0.54 ± 0.9 μg kg^{-1}*and* 0.45 ± 0.5 μg kg^{-1}, respectively) while, the fruit of *S. axillaris* was found to contain lowest amount of zinc i.e. of 0.11 ± 0.08 μg kg^{-1} followed by *E. indica* (0.16 ± 0.2 μg kg^{-1}) and *M. edulis* (0.16 ± 0.3 μg kg^{-1}).

4.4.2.3. Copper (Cu)

The current research on quantification of selected micro elements from ten different underutilized fruits species (Table no. 13) revealed element copper found in the fruits species varied significantly and recorded in the range of 1.14 ± 0.09 μg kg^{-1} to 14.33 ± 1.2 μg kg^{-1}. The fruit of *B. sapida* (14.33 ± 1.2 μg kg^{-1}) was observed to contain highest amount of copper amongst other fruits under present investigation followed by *S. axillaris* (9.06 ± 1.8 μg kg^{-1}), *E. latifolia* (8.70 ± 0.08 μg kg^{-1}), *Rubus ellipticus* (7.21 ± 2.0 μg kg^{-1}) and *M. edulis* (7.18 ± 2.09 μg kg^{-1}) whereas lowest copper content was detected in the fruit of *C. erectus i.e.* 1.14 ± 0.09 μg kg^{-1}followed by *Diploknema butyraceae* (2.10 ± 0.87 μg kg^{-1}).

4.4.2.4. Manganese (Mn)

The manganese content of selected underutilized fruits of Sikkim has been depicted in Table No. 13. The result of the present investigation showed that the manganese content was detected in a range of 1.1 ± 0.02 μg kg^{-1} to 37.29 ± 3.8 μg kg^{-1}. The highest manganese content was detected in the fruit of *B. sapida i.e.* 37.29 ± 3.8 μg kg^{-1} followed by *E. latifolia* (29.90 ± 0.41 μg kg^{-1}), *S. axillaris* (11.57 ± 1.2 μg kg^{-1}) and *E. sikkimensis* (11.46 ± 1.1 μg kg^{-1}) whereas, the lowest amount of manganese content was recorded in the fruit of *C. erectus i.e.* 1.1 ± 0.02 μg kg^{-1} followed by *D. butyraceae* (1.90 ± .08 μg kg^{-1}).

Table no. 13. Quantification of micro elements in selected underutilized fruits of Sikkim.

Name of fruits	Micro elements (µg kg^{-1})				
	Iron (Fe)	**Zinc (Zn)**	**Copper (Cu)**	**Manganese (Mn)**	**Molybdenum (Mo)**
B. sapida	29.55 ± 2.6	0.97 ± 0.18	14.33 ± 1.2	37.29 ± 3.8	1.03 ± 0.12
C. erectus	1.28 ± 0.16	0.45 ± 0.5	1.14 ± 0.09	1.1 ± 0.02	0.035 ± 0.21
D. Butyraceae	2.4 ± 0.69	0.65 ± 0.26	2.10 ± 0.87	1.90 ± .08	0.056 ± 0.08
E. latifolia	31.40 ± 0.51	0.32 ± 0.05	8.70 ± 0.08	29.90 ± 0.41	0.76 ± 0.009
E. sikkimensis	24.29 ± 2.3	0.33 ± 0.8	4.27 ± 0.96	11.46 ± 1.1	1.99 ± 0.009
E. indica	23.29 ± 2.1	0.16 ± 0.2	4.16 ± 0.9	3.09 ± 0.18	0.79 ± 0.05
F. roxburghii	26.55 ± 2.8	0.34 ± 0.10	4.22 ± 0.20	7.11 ± 0.11	0.58 ± 0.06
M. edulis	15.64 ± 1.8	0.16 ± 0.3	7.18 ± 2.09	4.84 ± 1.8	0.55 ± 0.08
R. ellipticus	21.20 ± 2.1	0.54 ± 0.9	7.21 ± 2.0	9.45 ± 2.7	1.67 ± .098
S. axillaris	26.47 ± 2.4	0.11 ± 0.08	9.06 ± 1.8	11.57 ± 1.2	2.63 ± 0.8

4.4.2.5. Molybdenum (Mo)

The molybdenum content of underutilized fruit of Sikkim has been shown in the Table no. 13. The molybdenum content of all the fruits under investigation was observed in varied range of 0.035 ± 0.21 μg kg^{-1} to 2.63 ± 0.8 μg kg^{-1}. The maximum amount of molybdenum was detected in the fruit of *S. axillaris* i.e. 2.63 ± 0.8 μg kg^{-1} followed by *E. sikkimensis, R. ellipticus and B. sapida* (1.99 ± 0.009 μg kg^{-1}, 1.67 ± .098 μg kg^{-1} and 1.03 ± 0.12 μg kg^{-1}, respectively) while, the minimum molybdenum content was recorded in the fruit of *C. erectus i.e.* 0.035 ± 0.21 μg kg^{-1} followed by *D. Butyraceae* (0.056 ± 0.08 μg kg^{-1}).

4.4.3. Quantification of heavy metals from selected underutilized fruits of Sikkim.

The current investigation of underutilized fruits of Sikkim subjected to heavy metals content showed some traces of heavy metals such as Cd, Ba, Hg, Pb, Al, Cr that are presented in Table no. 14.

4.4.3.1. Cadmium (Cd)

The cadmium content among ten different fruits was detected in a minute quantities which ranged from 0.005 ± 0.002 μg kg^{-1} to 0.021 ± 0.11 μg kg^{-1}. Cadmium was not detected in the fruit of *D. butyraceae, E. sikkimensis, E. indica, F. roxburghii* and *R. ellipticus*. The lowest concentration of cadmium was detected in the fruit of *C. erectus* (0.005 ± 0.002 μg kg^{-1}) and *M. edulis* (0.0060 ± 0.18 μg kg^{-1}), whereas maximum cadmium content was noted in the fruit of *B. sapida* i.e. 0.021 ± 0.11 μg kg^{-1}.

Table no. 14. Presence of heavy metals in selected underutilized fruits of Sikkim

Name of fruits	Heavy metals (µg kg^{-1})					
	Cadmium (Cd)	**Barium (Ba)**	**Mercury (Hg)**	**Lead (Pb)**	**Aluminum (Al)**	**Chromium (Cr)**
B. sapida	0.021 ± 0.11	0.099 ± 1.29	0.018 ± 0.01	0.011 ± 0.09	0.098 ± 0.1	0.081 ± 0.27
C. erectus	0.005 ± 0.002	0.033 ± 0.12	0.002 ± 0.001	nd	0.68 ± 0.46	0.0086 ± 0.38
D. Butyraceae	Nd	0.21 ± 0.03	nd	0.013 ± 0.09	0.01 ± 0.6	0.021 ± 0.50
E. latifolia	0.013 ± 0.006	0.062 ± 1.33	0.021 ± 0.01	0.048 ± 0.19	0.54 ± 0.70	0.093 ± 0.29
E. sikkimensis	Nd	0.048 ± 1.18	0.040 ± 0.018	nd	0.82 ± 0.14	0.013 ± 0.08
E. indica	Nd	nd	0.026 ± 0.002	nd	0.15 ± 0.06	0.042 ± 0.09
F. roxburghii	Nd	0.083 ± 0.31	0.025 ± 0.021	nd	0.066 ± 0.24	0.054 ± 0.41
M. edulis	0.006 ± 0.18	0.046 ± 1.08	nd	0.022 ± 0.8	0.057 ± 0.12	0.081 ± 0.28
R. ellipticus	Nd	0.032 ± 2.5	nd	nd	0.14 ± 0.24	0.045 ± 0.06
S. axillaris	0.010 ± 0.5	0.59 ± 0.06	0.055 ± 0.11	0.04 ± 0.54	0.61 ± 1.01	0.038 ± 0.41

4.4.3.2. Barium (Ba)

The Barium content of different underutilized fruits is presented in the Table no.14. The barium content was detected in a range of 0.032 ± 0.5 µg kg^{-1} to 0.59 ± 0.06 µg kg^{-1}. The lowest barium content was noted in *R. ellipticus i.e.* 0.032 ± 0.5 µg kg^{-1} followed by *C. erectus* (0.033 ± 0.12 µg kg^{-1}) and *M. edulis* (0.046 ± 1.08 µg kg^{-1}) while, highest barium content was observed in the fruit of *S. axillaris* (0.59 ± 0.06 µg kg^{-1}) followed by *D. butyraceae* (0.21 ± 0.03 µg kg^{-1}). Barium was not detected in the fruit of *E. indica.*

4.4.3.3. Mercury (Hg)

The mercury content of underutilized fruits has been depicted in Table no. 14 and the traces of mercury were detected in a range of 0.002 ± 0.001 µg kg^{-1} to 0.055 ± 0.11 µg kg^{-1}. The mercury content in the fruit of *D. Butyraceae, M. edulis and R. ellipticus* was not detected. The least amount of mercury was detected in the fruit of *C. erectus i.e.* 0.002 ± 0.001 µg kg^{-1} followed by *B. sapida* (0.018 ± 0.01 µg kg^{-1}) and *E. latifolia* (0.021 ± 0.01 µg kg^{-1}) and the highest mercury content was detected in the fruit of *S. axillaris i.e.* 0.055 ± 0.11 µg kg^{-1}.

4.4.3.4. Lead (Pb)

The lead content of different underutilized fruits has been presented in Table no. 14. The traces of lead were observed in a range of 0.011 ± 0.09 µg kg^{-1} to 0.048 ± 0.19 µg kg^{-1}. The lead content in the fruit of *C. erectus, R. ellipticus, E. sikkimensis, E. indica and Ficus roxburghii* was not detected. The least amount of lead was detected in the fruit of *B. sapida i.e.* 0.011 ± 0.09 µg kg^{-1} followed by and *D. Butyraceae* (0.013

± 0.09 µg kg^{-1}) whereas, highest lead content was detected in the fruit of *E. latifolia i.e.* 0.048 ± 0.19 µg kg^{-1} followed by *M. edulis* (0.022 ± 0.8 µg kg^{-1}).

4.4.3.5. Aluminium (Al)

The aluminium content of selected underutilized fruits has been shown in Table no. 14. The traces of aluminium were observed in a range of 0.01 ± 0.6 µg kg^{-1} to 0.82 ± 0.14 µg kg^{-1}. The lowest amount of aluminium was observed in the fruit of *D. Butyraceae* i.e. 0.01 ± 0.6 µg kg^{-1} followed by *M. edulis* (0.057 ± 0.12 µg kg^{-1}) and *F. roxburghii* (0.066 ± 0.24 µg kg^{-1}), whereas the highest aluminium content was detected in the fruits of *E. sikkimensis followed by C. erectus and S. axillaris* (0.82 ± 0.14 µg kg^{-1}, 0.68 ± 0.46 µg kg^{-1} and 0.61 ± 1.01 µg kg^{-1}, respectively).

4.4.3.6. Chromium (Cr)

The amounts of chromium present in underutilized fruits of Sikkim has been given in the Table no. 14. The traces of chromium content was found to range between 0.0086 ± 0.38 µg kg^{-1} to 0.093 ± 0.29 µg kg^{-1}. The lowest amount of chromium content was present in the fruit of *C. erectus i.e.* 0.0086 ± 0.38 µg kg^{-1} followed by *E. sikkimensis* (0.013 ± 0.08 µg kg^{-1}) and *D. Butyraceae* (0.021 ± 0.50 µg kg^{-1}) whereas, the highest chromium content was noted in the fruit of *E. latifolia* (0.093 ± 0.29 µg kg^{-1}) followed by *B. Sapida* and *M. edulis* (0.081 ± 0.27 µg kg^{-1} and 0.081 ± 0.28 µg kg^{-1}, respectively).

4.5. Quantification of phenols

Chromatographic finger printing of the methanol extracts of different fruits by using reversed phase HPLC method with diode array detection was developed for the quantitative estimation of phenolic acids *viz*. Gallic acid, Rutin, Ferulic acid, Catechol and Quercetin. The results of the quantification of phenols are given below in Table no. 15.

4.5.1. Gallic acid

Gallic acid was identified in all the fruits which was found in a range of 0.79 ± 0.00 mg g^{-1} DW to 5.96 ± 0.00 mg g^{-1} DW. Maximum Gallic acid was observed in *Eriolobus indica* (5.96 mg g^{-1} DW) while minimum was observed in *Machilus edulis* (0.79 mg g^{-1} DW). Notable amount of gallic acid was also found in *Baccaurea sapida, Rubus ellipticus, Spondias axillaris and Eleaocarpus sikkimensis* (5.75 mg g^{-1} DW, 4.19 mg g^{-1} DW, 3.47 mg g^{-1} DW and 3.19 mg g^{-1} DW, respectively).

4.5.2. Rutin

The phenolic compound rutin was quantified and was found maximum in *Eleaocarpus sikkimensis i.e.* 2.59 ± 0.02 mg g^{-1} DW followed by *Baccaurea sapida* (1.45 ± 0.02 mg g^{-1} DW), *Spondias axillaris* (1.29 ± 0.03 mg g^{-1} DW) and *Ficus roxburghii* (1.20 ± 0.02 mg g^{-1} DW), whereas, minimum amount of rutin was noted in *Elaeagnus latifolia* (0.14 ± 0.01 mg g^{-1} DW) followed by *Machilus edulis* (0.15 ± 0.04 mg g^{-1} DW) and *Calamus erectus* (0.23 ± 0.02 mg g^{-1} DW).

Table no. 15. Characterization and quantification of phenols in selected underutilized fruits

Sl. No	Scientific Name of fruits	Characterized Phenolic standards				
		Gallic acid (mg/g DW)	**Rutin (mg/g DW)**	**Ferulic acid (mg/g DW)**	**Catechol (mg/g DW)**	**Quercetin (mg/g DW)**
1.	*Baccaurea sapida*	5.75 ± 0.02	1.45 ± 0.02	6.29 ± 0.05	3.74 ± 0.13	0.97 ± 0.06
2.	*Calamus erectus*	1.77 ± 0.02	0.23 ± 0.02	6.36 ± 0.02	0.47 ± 0.03	0.088 ± 0.03
3.	*Diploknema butyraceae*	1.35 ± 0.03	1.04 ± 0.06	0.84 ± 0.02	8.42 ± 0.02	0.049 ± 0.06
4.	*Elaeagnus latifolia*	2.45 ± 0.00	0.14 ± 0.01	0.07 ± 0.02	2.07 ± 0.02	0.53 ± 0.02
5.	*Eleaocarpus sikkimensis*	3.19 ± 0.00	2.59 ± 0.02	1.89 ± 0.01	5.49 ± 0.02	0.56 ± 0.05
6.	*Eriolobus indica*	5.96 ± 0.00	1.09 ± 0.01	1.71 ± 0.00	10.54 ± 0.06	0.36 ± 0.01
7.	*Ficus roxburghii*	1.89 ± 0.00	1.20 ± 0.02	0.46 ± 0.01	3.39 ± 0.33	0.63 ± 0.06
8.	*Machilus edulis*	0.79 ± 0.00	0.15 ± 0.04	0.52 ± 0.01	3.59 ± 0.02	0.21 ± 0.06
9.	*Rubus ellipticus*	4.19 ± 0.00	1.09 ± 0.03	0.98 ± 0.01	2.73 ± 0.04	0.26 ± 1.18
10.	*Spondias axillaris*	3.47 ± 0.00	1.29 ± 0.03	0.28 ± 0.03	4.46 ± 0.07	0.06 ± 0.02
C.V.		2.01	5.20	4.025	2.15	4.19
S.E.M.		2.05	3.50	0.87	2.21	0.45
C.D. 5%		0.42	0.25	0.99	3.05	1.05

4.5. 3. Ferulic acid

The amount of ferulic acid was quantified and noted in varying amount in different fruit extracts. Highest amount of ferulic acid was detected in the fruit of *Calamus erectus* (6.36 ± 0.02 mg g^{-1} DW) which was followed by *Baccaurea sapida* (6.29 ± 0.05 mg g^{-1} DW) while, least amount of ferulic acid was found in the fruit of *Elaeagnus latifolia* (0.07 ± 0.02 mg g^{-1} DW) followed by *Spondias axillaris* (0.28 ± 0.03 mg g^{-1} DW), *Ficus roxburghii* (0.46 ± 0.01 mg g^{-1} DW), *Machilus edulis* (0.52 ± 0.01 mg g^{-1} DW) and *Diploknema butyraceae* (0.84 ± 0.02 mg g^{-1} DW).

4.5.4. Catechol

The phenolic compound catechol was quantified and recorded in varying amount which ranged from 0.47 ± 0.03 mg g^{-1} DW to 10.54 ± 0.06 mg g^{-1} DW. Highest amount of catechol *i.e.* 10.54 ± 0.06 mg g^{-1} DW was observed in the fruit extract of *Eriolobus indica,* whereas, lowest amount of catechol was recorded in *Calamus erectus i.e.* 0.47 ± 0.03 mg g^{-1} DW. The significant amount of catechol was also detected in the fruits of *Diploknema butyraceae, Eleaocarpus sikkimensis* and *Spondias axillaris* (8.42 ± 0.02 mg g^{-1} DW, 5.49 ± 0.02 mg g^{-1} DW and 4.46 ± 0.07mg g^{-1} DW, respectively).

4.5.5. Quercetin

The significant amount of quercetin content was recorded in different underutilized fruits under study. It ranged from 0.06 ± 0.02 mg g^{-1} DW to 0.97 ± 0.06 mg g^{-1} DW. Maximum quercetin content was found in the fruit of *Baccaurea sapida* (0.97 ± 0.06 mg g^{-1} DW) followed by *Ficus roxburghii* (0.63 ± 0.06 mg g^{-1} DW) while lowest quercetin content was found in *Spondias axillaris* (0.06 ± 0.02 mg g^{-1} DW) followed by *Diploknema butyraceae* (0.049 ± 0.06 mg g^{-1} DW) and *Calamus erectus* (0.088 ± 0.03 mg g^{-1} DW).

The results obtained from the present study on 'Nutraceutical potential of underutilized fruits of Sikkim' are discussed in this chapter and are justified with possible scientific causes and support of available literature under below:

5.1. Proximate composition

The data pertaining to proximate composition of different underutilized fruits of Sikkim analysed and presented (Chapter 4) are discussed under the following heads:

5.1.1. Moisture content

The moisture content is important factors amongst the physical parameters of edible fruits that may vary among different fruits. It is essential to determine the water content of the fruits as it is one of the measured properties of human diet as the texture, taste and appearance of any fruits influenced by the amount of water present. In the present study moisture content was recorded in a range of 35.12 ± 0.04 per cent to 90.05 ± 0.13 per cent (Table no. 2). Highest was observed in the fruits of *E. latifolia* whereas, lowest was noted in the fruits of *Calamus erectus.* Mann *et al.* (2016) also investigated moisture content in different fruit crops and measured 81.65 per cent moisture in the fruits of *B. sapida* which was in close proximity to our findings (81.17 %). The variable amount of moisture content in different fruits is due to physiological characteristics of different species and also depends on humidity, temperature and harvest time of the species (Islary *et al.,* 2016). On the contrary, Seal *et al.* (2014) reported 62.29 per cent moisture in the fruits of *S. axillaris* which was lower than our current findings i.e. 78.70 %. The possible cause of the variation may be due to growing condition, harvesting time, humidity and time of evaluation. This range of moisture content among different underutilized fruits under study are comparable with major commercial fruits like apple (84 %), Apricot (86 %), Avocado (73 %), Banana (74 %), Mango (84 %), Kiwifruit (87 %) etc. (Gebhardt *et al.*, 2002).

5.1.2. Dry matter content

Dry matter is the solid component of fruits, essentially, what is left when all the water dries up and mainly composed of carbohydrates in terms of quality which is indicator of both taste and texture. Data regarding dry matter content showed the maximum value for *Calamus erectus* (64.88 %) followed by *Eleaocarpus sikkimensis* (31.33 %) whereas the minimum value for dry matter content was found in *Elaeagnus latifolia* (9.95 %) and *Ficus roxburghii* (12. 30 %). The value of dry matter content found in the species *Rubus ellipticus* (12.08 %) is almost in close agreement with Purgar *et al.* (2012) in *Rubus discolor* ranging from 14.11-15.97 per cent and 14.19–18.03 per cent in *R. idaeus.* The average dry matter content varies between species to species and cultivars to cultivars. Many factors are associated for the variability which include crop load, canopy cover, period of fruits on the tree and fruit maturity, harvesting time etc.

5.1.3. Crude protein

Protein is a key to good health, it is involved in immune functions, oxygen transport and maintaining strong muscles tissues which act as a source of nutraceuticals in the form of food supplements or beverage or any other product. In the present study per cent protein content were found in a range of 2.61 ± 0.58 per cent to 7.94 ± 0.03 per cent. The highest in *Rubus ellipticus i.e.* (7.94 ± 0.03) whereas, the lowest in *Eriolobus indica* (2.61 ± 0.58 %). The value obtained under study are similar with Bhutia (2013), Rai *et al.* (2005) and Sundriyal and Sundriyal (2003). Any differences or variation in range of protein may be due to agro climatic conditions or any other biotic and abiotic factors. Previous studies of underutilized fruits or wild edibles of different areas were also reported to have significant amount of protein content (Mahapatra *et al.*, 2012; Murugkar & Subbulakshmi, 2005; Sabir & Riaz, 2005; Aberoumand, 2008; Desai *et al.* 2010; Deshmukh & Waghmode, 2011; Saklani *et al.* 2012; Magaia *et al.*, 2013). The amount of protein content recorded in the present study are equivalent to some of the major cultivated fruits which clearly indicate that underutilized fruits have immense potential to serve as a protective food supplement.

5.1.4. Crude fat

The crude fat content of different underutilized fruits are presented (Table no. 3). Crude fat content was found to vary from 0.08 ± 0.02 per cent to 20.07 ± 0.31 per cent. The highest crude fat content was recorded in the fruit of *Machilus edulis* (20.07 ± 0.31 %), whereas lowest in the fruits of *Rubus ellipticus* (0.08 ± 0.02 %). The amount of crude fat recorded in the present study amongst underutilized fruits was closely associated with the investigations of Bhutia (2013), Rai *et al.* (2005) and Sundriyal and Sundriyal (2003). The highest amount of fat noted in *Machilus edulis* (20.07 %) is closely associated with its related species i.e. avocado and it is notable fact that avocado contains highest amount of fat among the fruits i.e. 26.4 %. Previous studies on many indigenous fruits from different agro-climatic condition of India and abroad were also reported to have significant amount of crude fat (Mahapatra *et al.*, 2012; Murugkar & Subbulakshmi, 2005; Sabir & Riaz, 2005; Aberoumand, 2008; Desai *et al.* 2010; Deshmukh &Waghmode, 2011; Saklani *et al.* 2012; Magaia *et al.,* 2013). The fruit of *S. axillaris* studied in Meghalaya was reported to contain appreciable amount of crude fat i.e. 7.39 per cent (Seal *et al.,* 2014) which is higher than our finding (2.19 %). This may be due to variation caused by agro climatic condition, age of the plant, varietal differences and harvesting time.

5.1.5. Fibre

Fibre rich diets are essential for effective removal of waste and it is one of the important nutritional constituent of human diet. Data pertaining to fibre present in different underutilized fruit under study are given in table no. 4. The results ranged from 0.74 ± 0.08 per cent to 4.99 ± 0.02 per cent. The maximum fibre content was noted in the fruit of the fruits of *Calamus erectus* and lowest in *Rubus ellipticus.* The amount of crude fibre found in the present study amongst underutilized fruits were closely related with the results of Bhutia (2013), Rai *et al.* (2005) and Sundriyal and Sundriyal (2003). The dietary fibre of pulp of *Baccaurea sapida* was noted as 3.6 g $100g^{-1}$ by Mann *et al*. (2016) which is in agreement with the present findings. Ibrahim *et al.,* (2013) also reported higher (6.3 per cent) content of total dietary fibre in the freeze dried fruits of *B. angulate* which may be due to varietal and genetic factors. High fibre content of fruits and vegetables may aid to absorption of trace elements in the gut and reduces the absorption of cholesterol (Le Ville and Suberlich, 1966). Dietary fibre is known as pivotal aspect of healthy nutrition. Intake of fibre transform the viscosity, water content and microbial mass of the intestine resulting in proper bowel function and ease of passage through intestine (Elleuch *et al.,* 2011). Intake of fibre rich food helps in reducing body weight and prevents from several diseases.

Dietary fibre improves glucose tolerance and eliminates the risk of heart diseases and improves bowel movement and prevents constipation and piles (Anderson et al. 1994). Present investigated data can contributes knowledge about dietary fibre content in underutilized fruits of Sikkim. The result of present finding pertaining to fibre content indicate that these minor fruits can also be the part of human diet which can supplement the basic nutrient as essentially as other major fruits like apple, banana, fig etc.

5.1.6. Ash

Ash is one of the important aspects of human diet as its presence in any food indicates the availability of essential minerals. The ash content of different underutilized fruits are presented in the Table no. 4. The data pertaining to ash content noted in a range of 0.97 ± 0.80 per cent to 4.18 ± 0.95 per cent. The highest ash content was reported in the fruits of *Diploknema butyraceae* while, lowest in the fruits of *Machilus edulis* (0.97 ± 0.80 %). The values of the ash content in most of the fruits are closer side when compared with the result of Seal *et al.* (2014) and Mann *et al*. (2016). Since the ash content is an index to evaluate the grade of nutritive quality of any food product (Pearson, 1976) the results of present findings indicate that underutilized fruits of Sikkim are likely to have very high quality of essential minerals and can be the source of protective food for human diet.

5.1.7. Total Carbohydrate

The fruits containing good amount of carbohydrate are nutritious for health (Ozcan and Haciseferogullari, 2007). The highest total carbohydrate per cent was examined in *F. roxburghii* (90.81 ± 0.44 %) followed by *R. ellipticus* (89.87 ± 0.69

%), *E. latifolia* (89.60 ± 0.93 %) and *E. indica* (89.06 ± 0.52 %) whereas, lowest amount of total carbohydrate per cent was noted in the fruit of *M. edulis* (72.32 ± 0.72 %). In the current study the fruit of *Baccaurea sapida* was found to contain 86.14 per cent of total carbohydrate which is in close conformity with previous examination reported by Seal *et al.* (2014). Previous studies on many wild fruits from different regions of India and abroad were also reported to have significant amount of crude fat (Rajalakshmi *et al. (*2017); Sudhakaran & Nair, 2016; Nayak & Basak, 2015 and Saha *et al.* 2014). The differences may be due to varietal and climatic factors. The presence of appreciable amount of total carbohydrate in these underutilized fruits indicates that these fruits can be consumed as an important source of healthy food supplements.

5.1.8. Total Soluble Solid (TSS)

Total soluble solid is an important characteristic gives information about sugar content of fruits and is a main source of energy found high in many wild fruits. Determination of TSS is not only important for organoleptic quality of fruits but it also possesses the commercial and economic value of fruits. Total soluble solid of different underutilized fruits ranged from 2.38 ± 0.30^{o} B to 17.46 ± 0.45^{o} B and the results were in close conformity with the results reported by Bhutia, (2013); Sundriyal & Sundriyal, 2001a and Sundriyal *et al*., 1998). Almost similar findings have been revealed by Sharma *et al.* (2015) and Deb and Bhawmik (2013) with slight differences that may be due to climatic and varietal factors. The present findings also indicate that the fruit of *Diploknema butyraceae* possesses 17.46 ± 0.45^{o} B which is as equivalent to TSS present in some varieties of mango, litchi, guava, apple and other commercial fruits, therefore there is immense possibility to diversify the horticulture and food processing industry.

5.1.9. Titrable Acidity

Titrable acidity indicates the total or potential acidity. Titrable acidity of different underutilized fruits in present studies was found in varying concentration ranging from 0.02 ± 0.01 per cent to 3.88 ± 0.17 per cent. Earlier reports also indicated the similar values pertaining to titrable acidity in these fruits (Bhutia, 2013). Sugar acid blend in fruit crops make the fruit more palatable and increase the consumer acceptance.

5.1.10. Sugar (total sugar, reducing sugar and non -reducing sugar)

Sugar is a source of carbohydrate and energy. The data revealed that the per cent sugar (total, reducing and non-reducing) in all the underutilized fruits varied significantly and found in a range of 0.66 ± 0.95 - 12.25 ± 0.04 per cent, 0.40 ± 0.59 - 5.57 ± 1.20 per cent and 0.13 ± 0.11 - 6.68 ± 1.26 per cent, respectively. The highest total sugar per cent was noted in the fruit of *D. butyraceae* (12.25 ± 0.04 %) followed by *R. ellipticus* and *E. indica* (7.86 ± 0.16 % and 7.56 ± 0.02, respectively) whereas, the lowest amount of total sugar was recorded in the fruit of *C. erectus* (0.66 ± 0.95 %). The maximum reducing sugar content was observed in the fruit of *R. ellipticus* (5.57 ± 1.20 %) followed by *D. butyraceae* (5.22 ± 1.36 %) and *E. sikkimensis* (5.04 ± 0.44%) whereas, minimum reducing sugar per cent was noted in the fruit of *C. erectus* (0.40 ± 0.59 %). The highest non reducing sugar per cent (6.68 ± 1.26 %) was found in the fruit of *D. butyraceae* followed by *E. indica* (3.62 %) and *Rubus ellipticus* (2.18 ± 1.24 %) whereas, lowest non reducing sugar was noted in the fruit of *M. edulis* (0.13 ± 0.11 %). Similar observations were also investigated by Bhutia (2013) while working on underutilized fruit crops. Normally, sugar content in any fresh fruit ranges from 2-30 % (Norman, 1976). Previous studies on many wild fruits from different regions of India and abroad were also reported to have significant amount of sugars (Misra & Misra, 2016; Kumar *et al.* 2015; Nayak & Basak, 2015; Valvi *et al*., 2014 and Deb & Bhawmik, 2013). The results of the present findings indicate that some of underutilized fruits of Sikkim have the potential to serve the nutrient in the form of sugar. It is also noted that sugar per cent present in some of these fruits can be the alternative source for poor people who cannot afford expensive fruits during peak season.

5.2. Phyto-chemicals

5.2.1. Vitamin A

Vitamin A is categorised under fat soluble vitamin and its deficiency is prevalent in developing countries like India. Vitamin A plays an important role in human health by reducing the risk of many lifestyle diseases. Identification of food rich in Vitamin A actively improves its deficiency if the foods are locally or economically accessible. According to the phytochemical analysis of underutilized fruits of Sikkim (Table no. 7) revealed vitamin A content in a range of 43.44 ± 2.52 IU to 385.33 ± 2.03 IU. The highest vitamin- A was recorded in the fruit of *D. butyraceae*, while lowest in *C. erectus*. Previous record on these crops from Sikkim regarding vitamin A are not available but many other earlier reports suggest that underutilized indigenous fruits of different parts of India were reported to have ample amount of vitamin A (Rajalakshmi *et al.* 2017; Islary *et al.* 2017; Sudhakaran and Nair, 2016 and Hazali *et al.* (2015). Vitamin A promote cell division, promote bone growth, tooth development, healthy skin and hair. The results of present findings indicate that these underutilized fruits of Sikkim can be recommended to add these fruits to supplement Vitamin A for all individuals suffering from low vision, weak immune system, inflammatory diseases etc.

5.2.2. Vitamin E

Vitamin E is also a fat soluble vitamin and one of the important antioxidant. Data pertaining to vitamin E content of different underutilized fruit species was analysed (Table no. 7) and was found in a range of 2.10 ± 0.14 IU to 47.40 ± 1.10 IU. Higher amount of Vitamin E was recorded in the fruit of *M. edulis* whereas, lower amount of vitamin E was recorded from the fruit of *F. roxburghii.* Significant amount of vitamin E was also recorded in *Eleaocarpus sikkimensis, Spondias axillaris and Baccaurea sapida.* No previous record was found regarding vitamin E in underutilized fruits. Current research indicates that underutilized wild edible fruits are equally rich in Vitamin E and as potential as other cultivated crops like avocado, banana, nuts, cherry, strawberry etc. These fruits can also serve the people to curb the many deficiency diseases related to vitamin E however, Vitamin E is naturally occurring antioxidant and helps in reducing various heart and blood related ailments (Lako *et al.*, 2007).

5.2.3. Total phenols

Availability of polyphenols in fruits determine quality parameters such as colour, taste and nutritional value (Cheynier, 2005). Epidemiological studies suggest a close connection of polyphenols and reduce risk of cancerous cell (Potter, 2005; Fuhrman, 1995). Total phenols of ten different underutilized fruits of Sikkim were investigated and analysed (Table 8). A wide variation in the amount of total phenols ranging from 11.17 ± 0.06 mg GAE g^{-1} to 71.83 ± 0.42 mg GAE g^{-1} was observed.

Total phenols of methanol extracts showed that *S. axillaris* possessed significantly higher amount of phenolic than other fruits with the value 71.83 ± 0.42 mg of gallic acid equivalent and the minimum amount of total phenols among other species was recorded in the fruit of *M. edulis* 11.17 ± 0.06 mg GAE g^{-1}. The varying concentration of total phenols in fruits is influenced by many factors, including soil, irrigation, stress and other agro climatic conditions. The result of current investigation is in close agreement with the results reported by Singh *et al.* (2014). Earlier investigation also indicated that underutilized fruits of different parts of India and abroad are potential source of phenolic compound reported by Islary *et al.* (2017); Rajalakshmi *et al.* (2017); Mann *et al.*, (2016); Sharma *et al.* (2015); Korekar *et al.* (2014); Pal *et al.* (2013); Prakash *et al.* (2012); Karuppusamy *et al.* (2011); Fu *et al.* (2010); Ikram *et al.* (2009); Charoensiddhi & Anprung (2008) and Rimpapa *et al.* (2007). Polyphenol content in the fruits are greatly correlated with antioxidant potential which indicates the vital characteristics of these compounds for reducing risk of many life threatening diseases such as cancer, neurological ailments, cardiovascular diseases through diverse antioxidant mechanisms (Murillo *et al.*, 2012).

5.2.4. Total flavonoid

Flavonoid compound generally constitute a major group of compound, which plays a primary role of antioxidant (Adesegun *et al.*, 2009) and are known to react with hydroxyl radicals (Husain *et al.*, 1987), lipid peroxy radicals (Torel *et al.*, 1986) and superoxide anion radicals (Afanas'ev *et al.*, 1989). The interest in flavonoid compound derived from underexploited fruit crops and their roles in human health and nutrition are increasing. In the study flavonoid content of ten different underutilized fruits of Sikkim differed among each other and was found in a range between 4.83 ± 0.67 mg QE g^{-1} to 70.13 ± 0.25 mg QE g^{-1}. The highest amount of total flavonoid content was observed in the fruit of *B. sapida* whereas, lowest amount of total flavonoid was observed in the fruit of *F. roxburghii*. Variation may be due to varietal and climatic factors. Previous investigation also indicated that indigenous wild edible fruits of different parts of India and abroad are potential source of antioxidant in the form of flavonoid (Islary *et al.* 2017; Mann *et al.,* 2016; Hazali *et al.* 2015; Bhardwaj *et al.* 2014; Hassan *et al.,* 2013; Swapana *et al.,* 2012; Ashraf *et al.,* 2011 and Charoensiddhi & Anprung, 2008). The results showed that the investigated fruits are suitable source of medicinal properties. Flavonoid compounds have long been recognized to contain anti –allergic, anti- inflammatory, anti- viral, anti-proliferative and anti- carcinogenic properties (Middleton and Kandaswami, 1993). Due to the notable pharmacological properties these underutilized species can be popularized to increase the health benefit of local people and to reduce many life threatening ailments.

5.2.5. Flavonols content

Flavonoid including flavones, flavonols and condensed tannins, are plant secondary metabolites, the antioxidant activities depends on the presence of free OH groups, especially 3- OH (Baba and Malik, 2015). Secondary metabolites from plant origin foods have potential biological and therapeutic values such as anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogenic (Borneo *et al.,* 2008; Katalinic *et al.,* 2004; Mulabagal & Tsay, 2004). Data pertaining to flavonols content of ten different underutilized fruit species from Sikkim ranged between 6.57 ± 0.47 mg QE g^{-1} to 21.65 ± 1.45 mg QE g^{-1}. The fruit of *B. sapida* contained higher amount of flavonols followed by *R. ellipticus* (21.51 ± 0.33 mg QE g^{-1}) and *S. axillaris* (18.98

± 0.36 mg QE g^{-1}) whereas, lower amount of flavonols was observed in the fruit of *D. butyraceae*. The variation in flavonoid contents among the fruits species may be attributed to morphological as well as physico chemical properties of fruits. Previous record related to these fruits on flavonols were not found but many reported on different species which claimed to have pharmacological effect due to presence of flavonoid compounds (Islary *et al.,* 2016; Mann *et al.,* 2016; Bhardwaj *et al.* 2014; Hassan *et al.*, 2013; Goyal *et al.,* 2013 and Seal 2011). The results of present findings also indicate that these underutilized fruits can be the potential source of nutraceutical and improve the nutritional and medicinal securities for the rural people.

5.2.6. Ascorbic acid

Ascorbic acid (Vitamin C) is one of the important aspects of nutrition in fruits and vegetables. The ascorbic acid was determined by reduction of 2, 6-dichlorophenol indophenols dye by ascorbic acid as procedure given by AOAC (1980). The results of critical examination of ten underutilized fruits shows significant amount of ascorbic acid. It is observed from the Table no. 9 that the ascorbic acid of each fruit species is different and varies from 2.20 ± 0.11 mg 100 g^{-1} to 51.10 ± 1.40 mg 100 g^{-1}. Among all the species under study, the fruit of *B. sapida* was detected with maximum ascorbic acid (51.10 ± 1.40 mg 100 g^{-1}) followed by *S. axillaris* (34.54 ± 0.99 mg 100^{-1}) and *D. butyraceae* (22.72 ± 0.65 mg 100 g^{-1}) while, minimum ascorbic acid was detected in the fruit of *C. erectus* (2.20 ± 0.11 mg 100 g^{-1}).

Kumar *et al.* (2015) also estimated maximum ascorbic acid in the fruit of *Spondias axillaris* (32.0 mg 100g^{-1}) which is in close agreement with the present findings. Bhutia (2013**)** revealed that the concentration of ascorbic acid varied in the range of 0.7 – 32. 0 mg 100 g^{-1} (FW) in different underutilized fruits species of

Sikkim which is closely associated with the present findings. Determination of ascorbic acid content from many wild fruit species was carried out to identify the species with high ascorbic acid and many reported that number of species having appreciable amount of ascorbic acid (Korekar *et al.* 2014; Singh *et al.* 2014; Sarma *et al.* 2014; Pal *et al.,* 2013; Purgar *et al.* 2012; Karuppusamy *et al.* 2011; Sood *et al.,* 2010 and Charoensiddhi and Anprung, 2008). The present and previous report on underutilized fruits pertaining to ascorbic acid content in underutilized fruit species clearly indicate that these fruits can be recommend to the local people to supplement the vitamin C/ ascorbic acid to prevent the disease related to deficiency of Vitamin C such as scurvy. Ascorbic acid also act as an antioxidant against oxidative stress (Padayatty *et al.,* 2003) and it is widely used as food additive, to prevent oxidative stress. Intake of fruits rich in ascorbic acid will help the people to fight against many ailments such a cardiovascular disease, stroke, cancer (Willett, 2002).

5.2.7. Anthocyanin content

Anthocyanins are type of flavonoid, a group water soluble pigment or class of natural compound with antioxidant effect, which are widely distributed in plant origin food especially in fruits and vegetables. Anthocyanins are pigment that, depending on their pH may appear as red purple and blue colour (Rommel *et al.,* 1992). The significant variation were observed for anthocyanin content among all the species under investigation and they were found in a range between 0.47 ± 0.02 per cent to 3.06 ± 0.20 per cent. Present results are in close conformity with the results reported by Bhutia, (2013) and Sundriyal and Sundriyal, (2001a) with slight differences. The variation of anthocyanin content among the species may be due to climatic or other biotic and abiotic stress. Results suggest that maximum anthocyanin was observed in the fruit of *Rubus ellipticus* with the value 3.06 ± 0.20 per cent which indicates that anthocyanin pigment present in fruits represents the quality trait of fruits and make the fruits more acceptable to consumer. Earlier reports also suggested that the wild fruit species were found to contain appreciable amount of anthocyanin (Kumar *et al.* 2015; Singh *et al.* 2014; Hassan *et al.*, 2013; Goyal *et al.*, 2013; Olayiwola *et al.* 2013; Purgar *et al.* 2012; Karuppusamy *et al.* 2011; Sood *et al.*, 2010 and Rimpapa *et al.* 2007). The current investigation clearly indicate that, presence of anthocyanin in the fruits can play effective role in promoting various aspects of health benefits. Anthocyanin is beneficial for fighting against free radicals, anti-inflammatory, anti - viral and anti- cancer benefits. In therapeutic medicine, anthocyanin rich plant species have been used to prevent or cure many ailments such as high blood pressure, colds, heart diseases etc. It is observed that the underutilized fruits of Sikkim possesses ample amount of anthocyanin which can be further recommended for the local people to harness the beneficial effect of locally available fruits.

5.2.8. Total carotenoids

Carotenoids is the precursor of vitamin A, which has numerous health benefits in decreasing risk of diseases, particularly certain cancers and eye diseases and the beneficial effect of carotenoids is due to their antioxidant properties (Johnson, 2002). In current investigation total carotenoids was determined in ten different fruit species of Sikkim by spectrophotometric method. The mean value of total carotenoids exhibited a range of 0.68 ± 0.10 per cent to 63.80 ± 1.66 per cent. Present results are in close conformity with the results reported by Bhutia (2013). From the present study it is suggested that these underutilized fruits have appreciable amount of carotenoids that are potential source of antioxidants.

5.3. Antioxidant activities

5.3.1 DPPH Free Radical Scavenging Activity

A number of assays are designed to determine the radical scavenging effect of antioxidant. The DPPH method is most preferred method because it is easy, fast and reliable. The free radical scavenging properties of any extracts usually depend on the capacity of antioxidant compounds to lose hydrogen and structural conformation of these compounds (Shimada *et al.*, 1992 and Fukumoto and Mazza, 2000).

In the current study, it is observed that the antioxidant activities of methanol extracts of ten different underutilized fruits shows significant free radical scavenging capacity i.e. ranged from 42.09 ± 0.56 per cent to 72.26 ± 0.06 per cent. *Ficus roxburghii* showed highest DPPH radical scavenging activity and lowest was observed in *E. sikkimensis.* Significant levels of radical scavenging activity were also observed in *Spondias axillaris*, *Rubus ellipticus*, *Elaeagnus latifolia and Baccaurea sapida.* Various studies have confirmed that the antioxidants derived from indigenous plant sources are very much potential in preventing the damaging effects of oxidative stress (Zahin *et al.*, 2009).

The results of our findings are in close conformity with the results reported by Singh *et al.*, (2014) and Prakash *et al.* (2012). The antioxidant properties of the Lapsi (*Cherospondias axillaris*) fruit extracts was quantified by using DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) and the results depicted that lapsi fruit contains a strong antioxidant power with higher percentage of inhibition of DPPH radical recorded in ethanolic extracts (98%) (Labh *et al.*, 2015). Antioxidant activities of fruits and other plant species may be attributed to genetic as well as climatic factors. A lot of investigation on underutilized fruit species have conducted across the world in the past reported that these plant species have very high potential for scavenging free radicals and could inhibit the spread of oxidation (Khomdram *et al*. (2014b; Hassan *et al.* 2013; Goyal *et al.,* 2013; Pal *et al.,* 2013; Murillo *et al.* 2012; Haripyaree and Guneshwor, 2012; Seal, 2011; Karuppusamy *et al.* 2011). According to current investigation it is clear that the extracts of underutilized fruits have significant antioxidant capacity and these fruits species are locally available and can be the source of natural antioxidant and can be utilized to develop nutraceuticals product in various forms.

5.3.2. Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are major active oxygen species which affects lipid peroxidation and biological damage (Aurand *et al.,* 1977) leading to various degenerative diseases. All the tested sample in methanol extract of different underutilized fruits possessed radical scavenging capacity but in varying degree ranging from 33.59 ± 0.02 per cent to 61.46 ± 0.04 per cent. According to current findings it can be concluded that most of the wild edible fruits under study were found to have ability to trap hydroxyl radicals. The results indicates that the underutilized fruit species had better scavenging capacity. The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity. There has be trend to use synthetic drugs which protects against oxidative damage but they have adverse side effects. Since it is proved that these wild fruits are having natural antioxidant which can be recommended to be consumed in the form of food supplements as an alternative source to curb many oxidative diseases.

5.3.3. Hydrogen Peroxide scavenging activity

Hydrogen peroxide is considered as one of the free radicals which is injurious for the living cells when present in excess (Halliwell and Gutteridge, 1999) also it can be scavenge by antioxidant compound such as phenolic acids (Sroka and Cisowski, 2003). All the tested sample in the experiment possessed antioxidant properties, but to varying degrees found in a range of 45.70 ± 0.52 per cent to 71.85 ± 0.16 per cent. Highest scavenging capacity was recorded in the fruit of *Eriolobus indica* and lowest in the fruit of *Ficus roxburghii* (71.85 ± 0.16 % and 45.70 ± 0.52 %, respectively). The fruits of *Spondias axillaris, Baccaurea sapida, Eleaocarpus sikkimensis, Rubus ellipticus and Diploknema butyraceae* also showed significant amount of scavenging capacity. The present results are is close agreement with the results reported by Singh *et al.,* (2014) and Prakash *et al.* (2012). Any living or biological system can produce hydrogen peroxide. It is useful in the respiratory burst of activated phagocytes according to MacDonald-Wicks *et al.,* (2006). Dietary consumption of these underutilized species can be useful in the management of oxidative stress and could be used in pharmaceutical and nutraceutical industries as an alternative against synthetic drugs.

5.3.4. Ferrous ion chelating activity

Data pertaining to ferrous ion chelating activity, the formation of Fe^{2+} - ferrozine complex is inhibited in the presence of antioxidant. The antioxidants that are able to inhibit the formation of Fe^{2+} - ferrozine complex are expressed as Fe^{2+} chelation (Aloqbi *et al.,* 2016). Ferrous ion chelating activity of different underutilized fruits was found in a range of 17.62 ± 0.6 per cent to 42.04 ± 0.08 per cent. Highest ferrous ion chelating activity was observed in the fruit of *Eriolobus* *indica* (42.04 ± 0.08 %) whereas, lowest was observed in the fruit of *Diploknema butyraceae.* Current findings underline the importance of fruit of *E. indica, B. sapida, S. axillaris* and *Rubus ellipticus* as chelating agent and an effective secondary antioxidant. In addition the antioxidant activities of these fruits could be the good source of medicine for many diseases as well as good dietary supplements to the local people. Significant results of ferrous ion chelating activity or antioxidant activity of many underutilized fruits had previously been reported (Saha *et al.,* 2016; Tan *et al.,* 2013). Previously reported epidemiological studies indicated that fruits and vegetables have complimentary effect on human health due to their antioxidant properties, that can scavenge free radicals, stimulate immune system, regulate cell proliferation, etc. (Etherton *et al*., 2002).

5.3.5. Ferric reducing antioxidant power (FRAP) assay

Free radicals released during oxidative stress leads to major threat to biological function. This type of problem often associated with degenerative diseases and disorders for instance cancer, cardiovascular diseases, and weak immune function. In the present study, there was a significant difference amongst fruits sample for FRAP values, that all the tested samples possessed antioxidant properties ranging from 7.75 ± 0.6 mg GAE g^{-1} to 31.04 ± 0.2 mg GAE g^{-1}. The free radicals scavenging power of different fruits increased with an increasing amount of extract. Fruits of *Spondias axillaris* possessed highest FRAP values followed by *Baccaurea sapida* and *Rubus ellipticus***,** while lowest antioxidant activity was observed in the fruit extract of *E. latifolia*. Mann *et al*., 2016 also determined FRAP values of *Baccaurea sapida* which is in close proximity with their results. Higher the FRAP values higher will be antioxidant activity because FRAP value is dependent on reducing ferric ion, where antioxidant are the reducing agent.

5.3.6. Total antioxidant capacity (TAC) by Phospho-molybdenum assay

The result on total antioxidant capacity based on Phosphomolybdenum assay used in this study revealed that methanol extract of different underutilized fruits showed variable values of total antioxidant ranging between 29.03 ± 0.08 mg AAE g^{-1} to 59.99 ± 0.6 mg AAE g^{-1}. The fruit extract of *S. axillaris* contained maximum antioxidant properties whereas, lowest was noted in the fruit of *M. edulis*. Fruit of *E. indica*, *R. ellipticus*, *B. sapida and E. latifolia* also showed significant amount of total antioxidant activity (57.09 ± 0.22 mg AAE g^{-1}, 54.40 ± 0.4 mg AAE g^{-1}, 51.11 ± 0.7 mg AAE g^{-1} and 49.98 ± 0.11 mg AAE g^{-1}, respectively). The result of total antioxidant capacity is based on the reduction of Mo (IV) to Mo (V) by the effect of methanol extract and formation of green phosphate complex at acidic pH. The result indicates that the methanol extract of *S. axillaris, E. indica, R. ellipticus, B. sapida and E. latifolia* had excellent quantity of antioxidant compounds as equivalents of ascorbic acid that can effectively reduce the oxidant in the reaction matrix. The antioxidant capacity of the fruits may be derived from phenolic and flavonoid compound in it. Report from previous study also support the present study regarding wild edible fruits and presence of antioxidant compounds (Karuppusamy *et al.* 2011; Fu *et al.* 2010; Charoensiddhi and Anprung (2008).

5.4. Quantification of selected elements

5.4.1. Macro elements

5.4.1.1. Potassium

Macro elements are generally required in amount greater than 100 mg/ day which further represents 1 per cent of body weight (Insel *et al*. 2011; Imelouane *et al.* 2011). Potassium is one of the essential macro elements required for human body. The amount of potassium present in different underutilized fruits are given in Table no. 12. Presence of potassium in various fruits under study were observed in a concentration range of 98.75 ± 3.9 µg kg^{-1} to 819.64 ± 2.54 µg kg^{-1}. From the current investigation, it was observed that the fruit of *F. roxburghii* possessed highest concentration of potassium (819.64 ± 2.54 µg kg^{-1}) followed by *R. elipticus* (421 ± 1.9 µg kg^{-1}), *B. Sapida* (375.37 ± 1.68 µg kg^{-1}), *E. latifolia* (293.51 ± 1.01 µg kg^{-1}) and *S. axillaris* (280.12 ± 1.58 µg kg^{-1}) whereas, minimum potassium content was observed in the fruit of *D. butyraceae* (98.75 ± 3.9 µg kg^{-1}).

Rai *et al.* (2005) determined the mineral content from different indigenous fruits of Sikkim. They reported potassium content in a range of 160.5 to 911.6 mg 100 g^{-1}. In their study amount of potassium reported in the fruit of *Ficus hookeriana* (736.1 mg 100 g^{-1}), *Cherospondias axillaris* (639.3 mg 100 g^{-1}, *Machilus fructifera* (415.3 mg 100 g^{-1}) and *Elaeagnus conferta* (233.5 mg 100 g^{-1}) which stands in close agreement with our current findings. Slight variation in the amount may be due to agro climatic factors. Sundriyal and Sundriyal (2003) reported 0.910 per cent potassium in the fruit of *Elaeagnus latifolia.* The values of potassium analysed by candidate are according to recommended dietary allowances. Rajalakshmi *et al.*

(2017) reported 410mg 100g^{-1} of potassium in the fruit of *R. ellipticus* which is quite less than our finding, which may be further due to various abiotic factors. Various studies reported that wild edible fruits are major source of potassium (Islary *et al.* 2017; Mann *et al.* 2016; Sudhakaran & Nair 2016; Nayak & Basak, 2015; Rajalakshmi *et al.*, 2017; Kalita *et al.* 2014; Seal *et al.* 2014; Ersoy *et al.* 2013; Seal, 2012; Valvi and Rathod, 2011and Adepoju, 2009).

Fruits are often referred as protective food due to presence of vitamins and minerals. Potassium is one of the major mineral which plays an important role in human nutrition. Suitable amount of potassium helps to relieve high blood pressure, stroke, heart and kidney disorder, anxiety and stress. It also helps in enhancing muscles strengthening, metabolisms, water balance and the nervous system. Potassium present in these underutilized fruits according to current experiment clearly indicate that these fruits possessed highest potential to serve the potassium defiency as much as other commercial fruits like citrus, papaya, mango, banana etc. further it can be recommended for the people to add these fruits in their daily diet to supplement with natural source of potassium.

5.4.1.2. Magnesium (Mg)

The magnesium content of different underutilized fruits are presented in the Table no.12 ranged from 6.88 ± 0.8 µg kg^{-1} to 73.09 ± 1.42 µg kg^{-1}. The highest amount of magnesium content was recorded in the fruits of *F. roxburghii* whereas, lowest concentration was observed in the fruit of *E. indica*. While conducting experiment on different wild fruits Islary *et al.* (2017) reported satisfactory amount of magnesium as 172.387±0.517 mg 100 g^{-1} in *E. operculata* and 250.703±0.251 mg 100 g^{-1} in *A. bunius*. The pulp of *Baccaurea sapida* was reported to contain 109.3 ± 0.21

mg/g of magnesium (Mann *et al.,* 2016). In present investigation 21.67 ± 1.2 µg Kg^{-1} was found in the same fruit. Seal *et al.* (2014) detected 0.85 mg g^{-1} of magnesium in the fruit of *Spondias axillaris* from the hills of Meghalaya whereas, 40.16 ± 1.9 µg kg^{-1} was quantified in the same species from Sikkim. The obtained values of these minerals is somewhat similar to present study. The variation in concentration of minerals among the same species may be due to various biotic and abiotic factors.

Magnesium is a vital mineral for various enzymatic reactions. Magnesium deficiency leads to various health disorders including asthma, high blood pressure, angina pectoris, cardiac arrhythmias, coronary artery disease, all types of musculoskeletal disorders, mitral valve prolapse, panic disorder, epilepsy, anxiety, chronic fatigue syndrome and psychiatric conditions (Nayak and Basak, 2015). From the experiment it is observed that most of the underutilized fruits such as *F. roxburghii, S. axillaris, R. ellipticus, E. sikkimensis* and *Baccaurea sapida* were found to be rich in magnesium content.

The present finding clearly indicate that these underutilized fruits contain appreciable amount of magnesium and further it can be recommended for the people to add in their diet as is one of the essential mineral. Intake of right amount of magnesium is beneficial for human health and taking these fruits in season helps the people to fight with many nutritional disorders. From the current experiment we can also conclude that these fruits are equivalent to many commercial fruits like banana, bael etc.

5.4.1.3. Calcium (Ca)

The calcium content of ten different underutilized fruits have been shown in the Table no.12. The experiment data revealed that the calcium content ranged from 0.72 ± 0.11 µg kg^{-1} to 23.77 ± 2.0 µg g^{-1}. The highest calcium content was observed in the fruit of *B. Sapida* (23.77 ± 2.0 µg kg^{-1}) followed by *F. roxburghii* (23.69 ± 1.43 µg kg^{-1}), *R. ellipticus* (18 ± 09 µg g^{-1}) and *S. axillaris* (12.37 ± 1.9 g kg^{-1}) whereas, the lowest calcium content was detected in the fruit of *M. edulis* (0.72 ± 0.11 µg g^{-1}) followed by *E. indica* (1.98 ± 0.02 µg g^{-1}) *and D. butyraceae* (1.98 ± 1.0 µg g^{-1}). The values of calcium concentration observed are according to recommended dietary allowances. Rai *et al.* (2005) also reported appreciable amount of mineral concentration from the wild edible fruits of Sikkim. Regarding calcium content, they was found maximum in *Spondias axillaris* (202.1 mg 100 g^{-1}) and *Eriolobus indica* (200. 5 mg 100 g^{-1}). The observed variation may be due to climactic and other physiological factors. The fruit peel and pulp of *Baccaurea sapida* was found to be a good source of calcium (39.7 ±0.29 and 42.5 ± 0.31 mg g^{-1}) (Mann *et al.* (2016) with almost similar result obtained in current experiment while determining minerals from fruit pulp. Seal *et al.* (2014) observed 6.05 mg g^{-1} of calcium from the fruit of *Spondias axillaris* which was on the contrary i.e. less than our finding. The observed variation may be due to climatic differences.

Seal (2012) evaluated the mineral composition of five wild edible plants, traditionally used by local tribes of Meghalaya and reported the suitable concentration of calcium (5860 ±40 and 6260 ±100 mg Kg^{-1}) from the fruit of *Elaeagnus latifolia*. Sundriyal and Sundriyal (2001) also determined the mineral concentration of different underutilized fruits of Sikkim and reported appreciable amount of calcium. Calcium is major minerals required by human body for maintaining the good healthy bones and teeth, muscles contraction and secretion of certain hormones and enzymes. A deficiency of calcium leads to numbness in fingers and toes, muscles cramps, lethargy, loss of appetite and abnormal heart rhythm. Finding calcium in the fruits as a potential source is matter of concern therefore it is advisable to consume the locally available indigenous seasonal fruits because it is an important mineral for development and maintenance of body. Considering the, different calcium excess/ deficient issues, WHO/ FAO recommends the daily intake of 400- 500 mg of calcium for adults and 1200 mg/ day until the age of 24 years (WHO/FAO, 1973). In this context, the fruits which are found to be significant source of calcium, if intake 100-150 g/day can satisfy the recommended daily allowance of calcium.

5.4.1.4. Sodium (Na)

Sodium is one of the essential macronutrients which constitutes 2 per cent of total mineral content of the body (Christian and Ukhun, 2006). The result of sodium concentration present in different underutilized fruits has been given the Table no. 12. The range of sodium concentration was found varying in a range of 2.46 ± 1.0 µg kg^{-1} to 9.91 ±0.30 µg kg^{-1}. It is apparent that *E. latifolia* fruit was highest source of sodium followed by *R. ellipticus*, *E. sikkimensis,* and *B. Sapida* whereas, fruit of *C. erectus* was found to contain minimum concentration of sodium in the present study. Mann *et al.* (2016) reported 75.8 ± 0.91 mg g^{-1} of sodium while Seal *et al.* (2014) observed concentration of 0.66 mg/g in the fruit of *B. sapida*. *Spondias axillaris* was reported to have 0.81 mg/g of sodium (Seal *et al.* (2014) which is in close agreement with the current investigation. The variations in the concentration of sodium is mainly due to differences of agro-climatic condition, age of the tree and growing conditions like weather and climate.

Recommended Dietary allowances (RDA) of sodium is 300 mg (NRC, 1989). Sodium is a vital element for human body to function properly as it maintains body fluid volume, osmotic equilibrium, acid base balance, and nerve and muscle contraction. (Christian and Ukhun, 2006). Deficiency of sodium generally occurs in the body during hot weather and leads to muscles weakness, respiratory problems, mild fever, low blood pressure, paralysis etc. High dietary intake of sodium has also been associated with hypertension and dehydration. Consumption of right amount of sodium is essential for the proper body function, in this context from the present finding it can be recommended that daily intake of fruits like *E. latifolia*, *R. ellipticus*, *E. sikkimensis,* and *B. Sapida* will provide the RDA of sodium as (9.91 µg g^{-1}, 8.21 µg kg^{-1}, 8.09 µg kg^{-1} and 7.99 µg kg^{-1}, respectively) as an aid to daily dietary intake without any threat to consumer.

5.4.1.5. Sulphur (S)

Sulphur is an essential macro element which plays vital role in the diet of inhabitants around the world. Most of the literature consider SAA as the important source of sulphur in diet (Parcell, 2002). In the investigated sample the range of sulphur content varied from 0.086 ± 0.06 µg kg^{-1} to 5.74 ± 1.8 µg g^{-1}. The highest amount of sulphur was detected from the fruit of *M. edulis* (5.74 ± 1.8 µg kg^{-1}) followed by *F. roxburghii* (5.45 ± 0.72 µg kg $^{-1}$) *R. elipticus* (5.26 ± 1.8 µg kg^{-1}) and *S. axillaris* (5.19 ± 1.6 µg kg^{-1}) and the lowest amount of sulphur content among different underutilized fruits was noted in the fruit of *C. erectus* (0.086 ± 0.06 µg kg^{-1}) followed by *D. butyraceae* (3.17 ± 1.9 µg kg^{-1}). Sulphur is usually taken in the form of sulphur containing amino acids as cysteine, cysteine, homocysteine, homocystine and methionine by human beings and lack of sulphur leads to arthritis, muscles and joint stiffness, spondylitis.

Utilization of sulphur is for many dermatological disorders, ingredient in acne ointments and as remedy for skin rashes has been recorded in the literature (Tarimci *et al.,* 1997). The recommended dietary allowance for sulphur containing amino acids for human is 14 mg kg^{-1} of body weight (Prasad and Shivay, 2018). In this regard the fruit of *M. edulis, F. roxburghii*, *R. ellipticus,* and *S. axillaris* may contribute in providing basic needs of sulphur for local inhabitants. The role of element sulphur in human nutrition has not been extensively studied. From the current investigation it may be noted that most of these fruits can be the source of sulphur containing amino acids. Further research on these aspects may be beneficial.

5.4.2. Micro Elements

5.4.2.1. Iron (Fe)

Trace elements are essentially important in right amount, as excess or deficiency of these elements may pose threat to human health. Essential trace elements like iron, zinc, copper, manganese, selenium, iodine and molybdenum are usually required in amount less than 100 mg per day representing 0.01 per cent of the body weight (Imelouane *et al.* 2011).The iron content of different underutilized fruit species have been given in Table no 13. The data revealed from the current investigation regarding iron ranged between 1.28 ± 0.16 µg kg^{-1} to 31.40 ± 0.51 µg kg^{-1}. Islary *et al.* (2017) also reported 8.279 mg and 7.579 mg per 100 g from the two wild fruit *E. operculata* and *A. bunius*, respectively. The pulp of *Baccaurea sapida* was observed to contain 13.2 mg g^{-1} of iron in previous study reported by Mann *et al.* (2016) whereas, it was measured as 29.55 µg kg^{-1} in the present study. The variation in the result of iron concentration may be associated with different factors such soil condition, climate, age of the plant, varieties etc. The RDA for iron is 10 mg /day for adults (NRC, 1989). The iron concentration in the fruits *E. latifolia, B. sapida, F. roxburghii, S. axillaris,* and *E. sikkimensis* was much higher than that of the other fruits analysed in current findings and fulfil the needs of iron as per RDA for adults.

These findings support the possible use of these fruits as a source of iron in diet. Iron (Fe) is an essential micro element vital for human health or body metabolism which act as catalyst and is present in abundance than any other trace element. It is stated in many literature that iron is required for haemoglobin synthesis in red blood cells which is needed for oxygen transportation to all parts of the body. Deficiency of iron leads to most prevalent disease *i.e.* anaemia and immune system dysfunction (Islary *et al*., 2016), reduced work efficiency, impaired body temperature and regulation, impairment in behaviour and intellectual performance and susceptible to infections.

It is essential to know that there is possibility that low intake of iron may increase sensitivity to toxic elements like cadmium, chromium, mercury etc. because low dietary iron conc. may enhance the absorption and retention of toxic elements (Kostlal *et al.,* 1980). The concentration of iron obtained from present investigation were not exceeding the limits and also similar and comparable to other commercially cultivated fruits and also indicates that these fruits can be further used in food fortification.

5.4.2.2. Zinc (Zn)

Zinc is an essential micro element vital for human growth and development as well as immune functions. It plays catalytic, structural and regulatory roles as an integral part of many enzymes in human body. It is estimated that 20 per cent population of around the world are reported to be at risk of zinc deficiency. Studies on different fruits in the context of micro elements shows varied results ranging from 0.11 ± 0.08 µg kg^{-1} to 0.97 ± 0.18 µg kg $^{-1}$ (Table no.13). The fruit of *B. sapida* was found to contain highest amount of zinc i.e. 0.97 ± 0.18 µg kg^{-1} followed by *D. butyraceae, R. ellipticus and C. erectus* while, the fruit of *S. axillaris* was found to contain lowest amount of zinc.

Many literature support the present findings and many reported significant amount of zinc from the various underutilized fruits from India and abroad (Sundriyal and Sundriyal 2001; Rai *et al.* 2005; Adepoju 2009; Valvi and Rathod, 2011; Ersoy *et al.*, 2013; Seal *et al.*, 2014; Kalita *et al.* 2014; Sudhakaran and Nair, 2016; Mann *et al.* 2016; Islary *et al*., 2016; Islary *et al.* 2017; Rajalakshmi *et al.* 2017). Present results are in close agreement with Rai *et al.* 2005 and the observed variation might have resulted from geographic, climatic and seasonal variation. The RDA for zinc is 10 mg per day for an adults (NRC, 1989). Excess of zinc can be toxic to organism (Rajkovic *et al.*, 2008) Current investigation clearly shows that underutilized fruits studied lower amount of zinc content but not in excess therefore, intake of 200 to 300 g of these fruits will satisfy the requirement of RDA factors.

Zinc is known to play pivotal role in the immune system, and zinc deficiency leads to increased susceptibility to the diversity of pathogens. Presence of zinc in the body acts as a co factor for enzymes. It plays role in synthesis of DNA, insulin and protein. Excess consumption of zinc has long term effect while, deficiency leads to growth retardation, anorexia, skin rashes, etc. In this context estimated concentration of zinc recorded in underutilized fruits do not cause health hazards to consumers.

5.4.2.3. Copper (Cu)

Copper is important essential micro element widely distributed in plants and animal. It is necessary for normal biological functions. The current research on quantification of selected micro elements from ten different underutilized fruits species revealed that the element copper found in the fruits species varied significantly and recorded in the range of 1.14 ± 0.09 µg kg^{-1} to 14.33 ± 1.2 µg kg^{-1}. The fruit of *B. sapida* was observed to contain highest amount copper followed by *S. axillaris, E. latifolia, Rubus ellipticus* and *M. edulis* whereas, lowest copper content was detected in the fruit of *C. erectus*. Seal *et al.* (2014) reported that *Spondias axillaris* contained 0.052 mg kg^{-1} of copper whereas, it was noted 9.06 ± 1.8 µg kg^{-1} in the present study which is quite similar to previous finding**.** Sundriyal and Sundriyal (2001) also experimented on underutilized fruits of Sikkim and reported significant amount of copper which is in close conformity with our results. Many literature from earlier investigation also reported that underutilized fruits are the potential source of many essential trace elements and which can satisfy the daily need of copper for consumption (Ersoy *et al.* 2013; Seal, 2012; Valvi and Rathod 2011; Hussain 2011; Adepoju 2009; Murugkar and Subbulakshmi 2005; Leterme *et al.* 2006).

Copper can contributes in haemoglobin formation and has a role in iron and energy metabolism (FAO, 2001). Copper plays a significant role in protein ceruloplasmin, an enzyme that catalyse oxidation of iron ion (Saupi *et al.* 2009). A recommended dietary allowance of 2- 3 mg daily intake of copper is recommended for human adults (Dara, 1993). Fruits and vegetables are important source of vitamins and minerals and a plant based food which have ample concentration of copper could be useful in preventing a deficiency of copper which normally results in anaemia and bone problems. In this context it is a clear indication that present work on underutilized fruits gives satisfactory knowledge regarding requirement of copper for daily intake as per the NRC.

5.4.2.4. Manganese (Mn)

Manganese is an essential element can be derive from plant and animal food sources. During the present investigation the concentration of manganese content in underutilized fruits was detected in a range of 1.1 ± 0.02 µg kg^{-1} to 37.29 ± 3.8 µg kg^{-1}. The mineral analysis carried out of ten fruits sample showed highest manganese content in the fruit of *B. sapida i.e.* 37.29 ± 3.8 µg kg^{-1} followed by *E. latifolia* (29.90 ± 0.41 µg kg^{-1}), *S. axillaris* (11.57 ± 1.2 µg kg^{-1}) and *E. sikkimensis* (11.46 ± 1.1 µg kg^{-1}). In present study, manganese concentration in the wild fruits was found to be similar to the concentration reported by (Sudhakaran and Nair, 2016; Rajalakshmi *et al.,* 2017; Seal *et al.* 2014; Seal, 2012; Valvi and Rathod, 2011; Hussain, 2011; Adepoju, 2009)**.** Fruits of *Calamus guruba* was reported to have 50 mg/100 g of manganese as reported by Nayak and Basak, 2015, whereas, in current experiment it was found to be 1.1 ± 0.02 µg kg^{-1} in *Calamus erectus* which is lesser than the previous study. Plants derive manganese from the soil therefore deficiency of nutrient in soil, growing condition, farming practices may results in variation in manganese concentration of fruits.

Manganese plays an essential role in haemoglobin formation (Indrayan *et al.*, 2005). Manganese is widely known as co-factor for many enzymes that plays vital role in glucose and amino acid metabolism (FAO, 2001). The RDA for manganese is 7 mg per day for adults (NRC, 1989). Manganese in the studied sample was relatively low in concentration. The consumption of these fruits may supplement the manganese content in the nutrient index and also satisfy the need of RDA in daily diet.

Micro elements are required in small concentration, excess and deficiency of such minerals leads to various disorders that pose threat to human health**.** Deficiency of manganese would affect the development of bone, diabetics, arthritis, and many more serious disorder such as neurologic symptoms, impaired mitochondrial oxidation, impaired blood clotting and possible alterations in glucose metabolism (Utter, 1976). Consuming right and suitable amount of Mn is harmless so the use of Mn rich fruits and vegetables locally available would be beneficial as these investigated fruits can supplement the diet and fulfil the recommended dietary allowance. In this regard underutilized fruits which are potential source of minerals can further be domesticated and be properly utilized.

5.4.2.5. Molybdenum (Mo)

The molybdenum is one of an essential trace element required by human body in lower concentration. An element is considered essential when its reduction below certain limit affects the physiological function of the body and the ‘trace’ is applied to the concentration of element not more than 250 µg g^{-1} (WHO, 1996). Molybdenum concentration detected from selected underutilized fruits of Sikkim during investigation have been given in Table no 13.

The molybdenum concentration in present study ranged from 0.035 ± 0.21 µg kg^{-1} to 2.63 ± 0.8 µg kg^{-1}. The fruit of *S. axillaris* measured highest molybdenum content than other fruits under study i.e. 2.63 ± 0.8 µg kg^{-1}, followed by *E. sikkimensis, R. ellipticus and B. sapida* while, the minimum molybdenum content was recorded in the fruit of *C. erectus i.e.* 0.035 ± 0.21 µg kg^{-1}. There is not sufficient data available regarding molybdenum concentration in wild fruit species, the relevance of this mineral has not been studied particularly in underutilized fruits of Sikkim Himalaya.

Deficiency of molybdenum in dietary intake gives rise to pathological symptoms including irritability leading to coma, tachycardia and night blindness (Abumrad *et al.,* 1981). Molybdenum intoxication also leads to copper depletion in the body and gives rise to skeletal and joint deformities and mandibular exostoses (Ostrom *et al.,* 1961). Studies claim that molybdenum exerts an anti-carcinogenic effect in human has reviewed by Hadjimarkos, (1973). RDA for molybdenum is 10-15 mg /day for an adult (NRC, 1989). In this context, fruits of *S. axillaris, E. sikkimensis, R. ellipticus and B. sapida* could be the potential source of molybdenum for human population. As these fruit contain relatively lower amount of molybdenum, consuming right amount of molybdenum could contribute in RDA per day.

5.4.3. Quantification of heavy metals from underutilized fruits of Sikkim.

Fruits are important source of human nutrition and intake of heavy metal contaminated fruits pose threat to human health hence the effect of heavy metals in it cannot be underestimated. The current investigation of underutilized fruits of Sikkim with respect to heavy metals content showed some traces of heavy metals such as Cd, Ba, Hg, Pb, Al and Cr as presented in Table no. 14.

5.4.3.1. Cadmium (Cd)

It is well known fact that cadmium toxicity is recognized as major environmental health hazard all over the world. It was reported from the International Agency for Research on Cancer that cadmium has carcinogen which affects human body. The cadmium content was detected in minute quantities ranging from of 0.005 ± 0.002 µg kg^{-1} to 0. 021 ± 0.11 µg kg^{-1}. The minimum cadmium was reported from the fruit of *C. erectus* (0.005 ± 0.002µg kg^{-1}) and *M. edulis* (0.0060 ± 0.18 µg kg^{-1}) whereas maximum cadmium content was noted in the fruits of *B. sapida* i.e. 0.021 ± 0.11 µg g^{-1}.

Cadmium was not detected in the fruit of *D. butyraceae, E. sikkimensis, E. indica, F. roxburghii* and *R. ellipticus* and hence these fruits are safer for consumption. The presence of cadmium in the fruit species *B. sapida, C. erectus and M. edulis* was observed in negligible concentration or below toxic level. Jisha Chand and Azeez (2018) studied underutilized fruits of Tamil Nadu and reported that most of the trace elements were in minute quantity but cadmium was not detected from any of the fruit studied. The certain level of cadmium found in some fruits species under study may be due to polluted irrigation water and contaminated soil. As these underutilized fruits of Sikkim are having permissible limit of cadmium and are good source of multi elements which are sufficient to satisfy the daily requirement of micro nutrients and for regular consumption.

5.4.3.2. Barium (Ba)

Barium occurs abundantly in nature. In the present study, barium content among fruits varied from 0.032 ± 0.5 μg kg^{-1} to 0.59 ± 0.06 μg kg^{-1}. The lowest barium content was noted in *R. ellipticus* (0.032 ± 0.5 μg kg^{-1}) followed by *C. erectus* (0.033 ± 0.12 μg g^{-1}) and *M. edulis* (0.046 ± 1.08 μg g^{-1}) while, highest barium content was observed in the fruit of *S. axillaris* (0.59 ± 0.06 μg g^{-1}) followed by *E. indica* (0.44 ± 0.32 μg g^{-1}) and *D. butyraceae* (0.21 ± 0.03 μg g^{-1}).Very few data on barium (Ba) has been reported from previous research on different wild fruit species. The level of barium concentration in these fruits are based on various factors like species and ecology and soil characteristics. Exposure in barium for short term may cause vomiting, abdominal cramps, diarrhoea, difficulties in breathing, fluctuation in blood pressure, numbness around the face and muscles weakness well it is not reported as carcinogen but excessive intake of barium leads to high blood pressure, paralysis or even death (Martin and Griswold, 2009). In the present findings, the concentration of this element was observed in minute quantities and that may not pose any threat to human health.

5.4.3.3. Mercury (Hg)

The traces of mercury were detected in a range of 0.002 ± 0.001 μg kg^{-1} to 0.055 ± 0.11 μg kg^{-1} in current investigation. Mercury was not observed in the fruit of *D. butyraceae, M. edulis and R. ellipticus.* The least amount of mercury was detected in the fruits of *C. erectus*, *B. sapida* and *E. latifolia,* whereas the highest mercury content was detected in the fruit of *S. axillaris* i.e. 0.055 ± 0.11 μg kg^{-1}. Data on mercury are not reported in these fruit species previously. Nervous system is very sensitive to all forms of mercury, The FAO/WHO (1972) jointly recommended 5μg kg^{-1} of body weight per week as total mercury intake. It is clear that fruits contain a very few concentration of mercury which may be due to soil health and water condition of fruit growing area. Intake of these fruits may not pose any problem to human health rather they are good source of other essential minerals which can be added in daily diet. Further research on heavy metal concentration in these fruits may contribute to food industries.

5.4.3.4. Lead (Pb)

The lead content of different underutilized fruits has been presented in Table no. 14. The traces of lead were observed in a range of 0.04 ±0.02 µg kg $^{-1}$ to 0.048 ± 0.19 µg kg^{-1}. The lead in the fruit of *C. erectus, R. ellipticus, E. sikkimensis, E. indica and Ficus roxburghii* was not detected. The least amount of lead was detected in the fruit of *F. roxburghii i.e.* 0.04 ±0.02 µg kg^{-1} followed by *B. sapida* (0.011 ± 0.09 µg kg^{-1}) and *D. butyraceae* (0.013 ± 0.09 µg kg^{-1}) whereas, highest lead content was detected in the fruit of *E. latifolia i.e.* 0.48 ± 0.19 µg kg^{-1}followed by *M. edulis* (0.022 ± 0.8 µg kg^{-1}). In earlier study Muhammad *et al.* (2011) reported 0.37± 0.06 mg 100g $^{-1}$ from the fruit of *Gardenia aqualla* and recommended for consumption. The presence of lead in the fruit sample may be connected with the soil and water condition and ecology of growing area because plants uptake lead from erosion of lead containing rock (Lin-Fu, 1976). Acceptable range of lead in plant material is 0.02 mg 100 g^{-1} to 2 mg 100 g^{-1}**.** In the present study concentration of lead found in different fruit sample was observed within an acceptable range. Plants containing higher concentration of lead should be avoided because excessive intake of lead leads to nervous damage, brain, kidney in both adult and children (Lin-Fu, 1976).

5.4.3.5. Aluminium (Al)

There is a main concern with respect to aluminium and its effect on human health. Aluminium is an element found abundantly on earth's crust and plants take it from the soil surface. In the present study the concentration of aluminium was detected and ranged from 0.01 ± 0.6 µg kg^{-1} to 0.82 ± 0.14 µg $^{-1}$. The lowest amount of aluminium was observed in the fruit of *D. butyraceae* i.e. 0.01 ± 0.6 µg kg^{-1} followed by *M. edulis* (0.057 ± 0.12 µg kg^{-1}) and *F. roxburghii* (0.066 ± 0.24 µg kg^{-1}) whereas, the highest aluminium content was detected in the fruits of *E. sikkimensis* followed by *C. erectus* and *S. axillaris* (0.82 ± 0.14 µg kg^{-1}, 0.68 ± 0.46 µg $kg^{-1}$and 0.61 ± 1.01 µg kg^{-1}, respectively). The level of aluminium present in this fruits might be connected with the soil water environment and ecology. Data with regard to aluminium concentration in these fruits are not available but many research on other plant based food, mushroom, vegetables were reported to contain aluminium in acceptable range. The acceptable range of aluminium intake is approx. 7mg kg^{-1} body weight (FAO/WHO, 1989; WHO, 1989). It is clear that fruits under present study are safer for consumption.

5.4.3.6. Chromium (Cr)

The amount of chromium concentration in underutilized wild fruits may be due to nature of the soil in which they grow because plant uptake minerals from the soil. The traces of chromium ranged from 0.0086 ± 0.38 µg kg^{-1} to 0.093 ± 0.29 µg kg^{-1}. The lowest amount of chromium content was present in the fruit of *C. erectus i.e.* 0.0086 ± 0.38 µg kg^{-1} followed by *E. sikkimensis* (0.013 ± 0.08 µg kg^{-1}) and *D. butyraceae* (0.021 ± 0.50 µg kg^{-1}) whereas, the highest chromium content was noted in the fruit of *E. latifolia* (0.093 ± 0.29 µg kg^{-1}) followed by *B. sapida* and *M. edulis* (0.081 ± 0.27 µg kg^{-1} and 0.081 ± 0.28 µg kg^{-1}, respectively).

Data with regard to chromium concentration in these fruits are not available but many research on other plant based food, mushroom, vegetables were reported to contain chromium in acceptable range. NRC (1989) report says the estimated safe intake of chromium for adults is 0.2 mg 100g $^{-1}$. In earlier study Muhammad *et al.* (2011) reported 1.07± 0.06 mg 100g $^{-1}$ from the fruit of *Gardenia aqualla* and recommended for safe consumption. Zahin *et al.* (2009) stated the importance of chromium in human body for glucose tolerance, they also reported the minimum and maximum concentration of chromium present in different fruit species 2.864 to 4.343 µg g^{-1}, respectively. In this context present findings clearly indicate that the concentration of chromium present in different fruit sample was in acceptable range and safer for consumption.

5.4.4. Quantification of phenols

Chromatographic finger printing of the methanol extracts of different fruits by using reversed phase HPLC method with diode array detection was developed for the quantitative estimation of phenolic acids *viz*. gallic acid, rutin, ferulic acid, catechol and quercetin. Many research has shown that presence of phenolic compound in fruits and vegetables are directly correlated with their antioxidant capacity and consumption of these fruits and vegetables can prevent the disease caused by oxidative stress such as risk of cardiovascular disease, stroke, diabetes, cancers, neurological disorders, inflammation, microbial infections and many more. The possible reason of present findings in the context of phenolic compounds are discussed in detail below.

5.4.4.1. Gallic acid

Gallic acid is a secondary metabolites of plant origin which act as a powerful antioxidant in the context of human health**.** In the current investigation the amount of gallic acid varied from 0.79 ± 0.00 mg g^{-1} DW to 5.96 ± 0.00 mg g^{-1} DW among the ten different fruits. Maximum gallic acid was observed in *Eriolobus indica* while minimum was observed in *Machilus edulis.* Notable amount of gallic acid was also found in *Baccaurea sapida, Rubus elipticus, Spondias axillaris and Eleaocarpus sikkimensis.* The concentration of gallic acid present in these fruits are similar to previous findings such as in fruits of *V. foetidum* (0.38±0.007 mg/g), *O. linearis* (0.201±0.002 mg g^{-1}**)** and *S. arvensis* (0.05±0.0003 mg g^{-1}**)** as reported by Seal *et al.,* 2016. Gallic acid content of lemon (2.03 mg g^{-1}), onion bulb (1.55 mg g^{-1}), chilli pepper (3.33 mg g^{-1}) and spinach (1.82 mg g^{-1}) was reported by Romaric *et al.* (2011) and are in agreement with present findings. It is clear from the present finding that the methanol extracts of different underutilized fruits shows presence of good amount of gallic acid which is widely distributed in edible plants. Gallic acid has various nutraceutical activities due to their higher antioxidant activities and disease preventing ability, therefore these fruits can further be utilized in many pharmacological industries.

5.4.4.2. Rutin

Rutin is a bioflavonoid or secondary metabolite of plant origin which has powerful antioxidant property and generally found abundantly in certain fruits and vegetables. In the present study rutin concentration was detected in a range of 0.14 ± 0.01 mg $g^{-1}{}_{DW}$ to 2.59 ± 0.02 mg g^{-1} DW. *Eleaocarpus sikkimensis* measured maximum concentration whereas, *Elaeagnus latifolia* showed minimum concentration. The rutin content in the extract of 1% aq. acetic acid (0.073±0.005 mg g^{-1}) and in 80% aq. ethanol (0.070±0.002 mg g^{-1}) in the fruit species of *O. linearis* was also observed by Seal (2016). Variation in rutin concentration in different species might be due to agro ecological conditions, edaphic factors etc. Underutilized fruits are naturally grown in degraded soil which can tolerate stress condition and it is also a well-known fact that plant grown in stress are generally rich in phenolic compounds. Rutin has been utilizing in traditional medicine since ages to aid circulation. Some literature says rutin helps in improving overall health including ease varicose veins, strengthen flexibility of blood vessels and can treat haemorrhoids etc. To avail the benefits of this phenolic compound people can eat these fruits add rutin in their diet or they can be utilized in the form of supplements.

5.4.4.3. Ferulic acid

Ferulic acid is one of the important phenolic compound and also known as secondary plant metabolites with higher antioxidant activity along with therapeutic values like anti- carcinogenic properties, anti-inflammatory activity **(**Imaida *et al.*, 1990), anti-diabetic effect (Balasubashini et al., 2004), anti-aging, neuroprotective, antiviral, anti-inflammatory, anti-microbial, properties etc. In the present study the fruit of *Calamus erectus* showed highest amount of ferulic acid followed by *Baccaurea sapida* while, least amount of ferulic acid was found in the fruit of *Elaeagnus latifolia*. Seal *et al.* (2016) also reported 0.27 mg g^{-1} of ferulic acid in the fruit of *V. foetidum,* a fruit. Ferulic acid has a phenolic compound ferulic acid which is important component of human diet and its presence in wild edible fruits is widely known. However, only few studies have been undertaken concerning phenolic acids of underutilized fruits of Sikkim. Many literature reported presence of ferulic acid at the concentration of 0.5 to 2 % in plant food such as pineapple, banana, spinach, beetroot etc. mostly in trans-isomeric form (Harris and Hartley, 1980; Hartley and Ford, 1989; Hartley and Harris, 1981). In the context of present finding, it can be suggested that fruits like *Calamus erectus* and *Baccaurea sapida* can be used as functional food and nutraceuticals. The presence of good amount of ferulic acid in these fruits clearly indicate their biomedical properties like antioxidant, anti-allergic, ant diabetic, anti-inflammatory, antiviral etc. which can be used for betterment of human health.

5.4.4.4. Catechol

Catechol or catechin comes under the category of flavonoid compound and found abundantly in plant based food. In the current investigation the phenolic compound catechol ranged from 0.47 ± 0.03 mg/g DW to 10.54 ± 0.06 mg/g DW. Highest amount of catechol was observed in the fruit extract of *Eriolobus indica,* whereas, lowest amount of catechol was recorded in *Calamus erectus*. The significant amount of catechol was also detected in the fruits of *Diploknema butyraceae, Eleaocarpus sikkimensis* and *Spondias axillaris.* Amount of catechol content was highly variable among the species however, the presence of catechol content in these fruits indicate its potent antioxidant properties and may contribute in pharmaceutical industry as well as intake of these fruits may improve human health and prevent from oxidative diseases.

The variation may be attributed to environmental condition, soil status, age of the plant, species etc. In previous investigation Seal (2016) reported *O. linearis* with highest concentration of catechin (0.379 ±0.003 mg g^{-1}) in the extracts of 1 % aq. acetic acid and appreciable amount of catechin was also detected in both 80 % aq.

ethanol extract (0.204±0.001 mg/g) and methanol extract of the plant (0.113±0.03mg/g). *H. cordata* an underutilized fruits were also observed to contain higher amount of catechin i.e. 6.632 ± 0.046 mg/g (Seal *et al.*, 2016). Intake of fruits having phenolic compound like catechol might help people to fight against various oxidative stress.

5.4.4.5. Quercetin

Quercetin is one of the vital flavonoid compound present abundantly in fruits and vegetables and its antioxidant properties have been used to improve human health since ages. The significant amount of quercetin content was recorded in different underutilized fruits under study. It ranged from 0.06 ± 0.02 mg g^{-1} DW to 0.97 ± 0.06 mg g^{-1} DW. Appreciable amount of quercetin was also detected in other underutilized fruits under study and were found to be comparable to apple (0.021 mg g^{-1}) and tomato (0.055 mg g^{-1}) DW. Maximum quercetin content was found in the fruit of *Baccaurea sapida* while, lowest quercetin content was found in *Spondias axillaris* followed by *Diploknema butyraceae* and *Calamus erectus.* Quantative analysis showed variable amount of quercetin content and this variation might be due to soil and environmental condition where they have grown naturally. Many literature reported similar findings (Seal *et al.* 2016; Shende *et al.* 2016 & Ardalani *et al.,* 2016). Onion is important source of quercetin (2.60 mg g^{-1}). In present study fruits of *Baccaurea sapida, Ficus roxburghii* and *E. sikkimensis* were found to be good source of quercetin. Wach *et al.* (2007) worked on different fruits and vegetables such as tomato and found appreciative amount of quercetin. Intake of underutilized fruits in season may help in preventing many diseases as it possess antioxidants, anti-inflammatory, ant diabetic properties etc.

The present investigation 'Nutraceuticals Potential of Underutilized Fruits of Sikkim' was carried out in the laboratory of Department of Horticulture, School of Life Science, Sikkim University, 6th mile Samdur, Gangtok, Sikkim during 2014-2017. The observations were recorded with respect to proximate content, phytochemical constituents, antioxidant activities, phenolic contents, macro and micro elements and heavy metals content. The experimental findings of this investigation are being summarized below:

5.1. Proximate composition

- Among the different underutilized fruit species of Sikkim which showed significant data pertaining to moisture content, the fruits of *E. latifolia* were found to contain maximum moisture per cent (90.05 ± 0.13%) while, the minimum moisture content was recorded in the fruits of *Calamus erectus* (35.12 ± 0.04 %).
- Various underutilized fruit crops were investigated under the present study among which the dry matter content was observed maximum in the fruits of *C. erectus* (64.88 ± 0.04 %) followed by *E. sikkimensis* (31.33 ± 0.40 %) and *M. edulis* (31.19 ± 0.09%). Lowest dry matter content was recorded in *E. latifolia* (9.95 ± 0.13 %) followed by *R. ellipticus* (12.08 ± 0.33 %).
- Crude protein content was significantly affected among different underutilized fruit crops of Sikkim. The highest amount of crude protein content was recorded in the fruits of *Rubus ellipticus i.e.* 7.94 ± 0.03 per cent followed by *Eleaocarpus sikkimensis* (6.93 ± 0.03 %), *Spondias axillaris* (6.31± 0.58 %) and *Elaeagnus*

latifolia (6.16 ± 0.59 %) whereas, the lowest amount of crude protein content was noted in the fruits of *Eriolobus indica* (2.61 ± 0.58 %).

- Different underutilized fruit crops of Sikkim recorded differential and significant crude fat content. The highest crude fat content was noted in the fruits of *Machilus edulis* (20.07 ± 0.31 %) followed by *Spondias axillaris* (2.19 ± 0.28 %) whereas lowest crude fat content was noted in the fruits of *Rubus ellipticus* (0.08 ± 0.02 %) followed by *Ficus roxburghii* (0.13 ± 0.04 %).
- The total carbohydrate content was found to be significantly differed among different underutilized fruit crops of which *F. roxburghii* (90.81 ± 0.44 %) was found to measure highest followed by *R. ellipticus* (89.87 ± 0.69 %) whereas, lowest amount of carbohydrate was noted in the fruits of *M. edulis* (72.32 ± 0.72 %).
- Studies pertaining to maximum fibre content showed significant values among different underutilized fruit crops. The fruit of *Calamus erectus* measured maximum fibre per cent i.e. 4.99 ± 0.02 % followed by *Eriolobus indica* (4.57 ± 0.06 %) and *Baccaurea sapida* (3.60 ± 0.03 %) whereas, the fruits of *Rubus ellipticus* (0.74 ± 0.08 %) showed minimum fibre content followed by *Machilus edulis* (0.92 ± 0.06 %).
- The highest ash content was reported in the fruit of *Diploknema butyraceae* (4.18 ± 0.95 %) followed by *Baccaurea sapida* (3.59 ± 0.72 %), *Ficus roxburghii* (2.99 ± 0.41 %) and *Eriolobus indica* (2.90 ± 0.49 %) while, lowest amount of ash was noted in the fruits of *Machilus edulis* (0.97 ± 0.80 %).
- Highest T.S.S content was recorded in the fruit of *D. butyraceae i.e.* 17.46 ± 0.45^{o} B followed by *R. ellipticus* (16.11 ± 0.08 ^{o} B), *E. sikkimensis* (14.03 ± 0.15^{o} B) and *Elaeagnus latifolia* (13.20 ± 0.20^{o} B) whereas, lowest TSS content was observed in *C. erectus* (2.38 ± 0.30^{o} B).

- Investigation related to titrable acidity demonstrated that minimum titrable acidity was recorded in the fruits of *F. roxburghii* (0.02 ± 0.01%) followed by *C. erectus* (0.03 ± 0.06 %), *D. butyraceae* (0.04 ± 0.06 %) and *M. edulis* (0.12 ± 0.02 %) whereas, maximum titrable acidity was noted in the fruits of *E. latifolia* (3.88 ± 0.17 %) followed by *S. axillaris* (3.47 ± 0.24 %).
- Estimation of total sugar per cent revealed that maximum total sugar content was noted in the fruits of *D. butyraceae* (12.25 ± 0.04 %) followed by *R. ellipticus* and *E. indica* (7.86 ± 0.16 % and 7.56 ± 0.02, respectively) whereas, the lowest amount of total sugar was recorded in the fruits of *C. erectus* (0.66 ± 0.95 %).
- Among different underutilized fruits crops of Sikkim the maximum reducing sugar content was observed in the fruits of *R. ellipticus* (5.57 ± 1.20 %) followed by *D. butyraceae* (5.22 ± 1.36 %) and *E. sikkimensis* (5.04 ± 0.44%) whereas, minimum reducing sugar per cent was estimated in the fruits of *C. erectus* (0.40 ± 0.59 %).
- Estimation of non-reducing sugar in different underutilized fruit crops revealed highest non reducing sugar per cent (6.68 ± 1.26 %) was found in the fruits of *D. butyraceae* followed by *E. indica* (3.62 %) and *Rubus ellipticus* (2.18 ± 1.24 %). The lowest per cent of non-reducing sugar was noted in the fruits of *M. edulis* (0.13 ± 0.11 %).

5.2. Phytochemical composition

- Different phytochemicals were estimated in different underutilized fruit crops of Sikkim. The highest vitamin-A was recorded in the fruits of *D. butyraceae* (385.33 ± 2.03 IU) followed by *E. latifolia* (345.33 ± 2.73 IU) and *E. sikkimensis* (294.68 ± 3.61) while, lowest vitamin A content was noted in the fruits of *C. erectus* (43.44 ± 2.52 IU).

- Estimation of Vitamin E showed that higher amount of Vitamin E content recorded in the fruits of *M. edulis* (47.40 ± 1.10 IU) followed by *E. sikkimensis* (38.37 ± 1.04 IU), *S. axillaris* (24.52 ± 0.96 IU) and *Baccaurea sapida* (23.86 ± 0.17 IU) whereas, lower amount of vitamin E content was recorded from the fruits of *F. roxburghii* (2.10 ± 0.14 IU).
- Studies pertaining to total phenol content showed that the fruits of *S. axillaris* (71.83 ± 0.42 mg GAE g^{-1}) was found to contain maximum amount of total phenol among all other species followed by *R. ellipticus* (54.27 ± 0.31mg GAE g^{-1}) and *B. Sapida* (51.53 ± 0.50 mg GAE g^{-1}), while the minimum amount of total phenols among other species was recorded in the fruits of *M. edulis* (11.17 ± 0.06 mg GAE g^{-1}).
- Highest amount of total flavonoid content was observed in the fruits of *B. sapida* i.e. 70.13 ± 0.25 mg QE g^{-1} followed by *R. ellipticus* (32.41 ± 0.80 mg QE g^{-1}) and *C. erectus* (22.72 ± 0.21mg QE g^{-1}) whereas, lowest amount of total flavonoid was observed in the fruits of *F. roxburghii* (4.83 ± 0.67mg QE g^{-1}) among different underutilized fruit species.
- The fruits of *B. sapida* possessed higher amount of flavonol content i.e. 21.65 ± 1.45 mg QE g^{-1} followed by *R. ellipticus* (21.51 ± 0.33 mg QE g^{-1}) and *S. axillaris* (18.98 ± 0.36 mg QE g^{-1}) whereas, lower amount of flavonol was observed in the fruits of *D. butyraceae* (6.57 ± 0.47 mg QE g^{-1}).
- Among all the species under study, the fruits of *B. sapida* detected maximum ascorbic acid (51.10 ± 1.40 mg 100 g^{-1}) followed by *S. axillaris* (34.54 ± 0.99 mg 100 g^{-1}) and *D. butyraceae* (22.72 ± 0.65 mg 100 g^{-1}) while, minimum ascorbic acid was detected in the fruits of *C. erectus* (2.20 ± 0.11 mg 100 g^{-1}).
- Anthocyanin content varied among all fruit species under study. The fruits of *R. ellipticus* contained highest per cent of anthocyanin content (3.06 ± 0.20 %) followed

by *E. latifolia* (1.82 ± 0.02 %) and *B. sapida* (1.71 ± 0.02 %) while, lowest anthocyanin content was reported in the species of *M. edulis* (0.47 ± 0.02 %).

- In the present investigation maximum carotenoids was recorded in the fruits of *M. edulis* (63.80 ± 1.66 %) followed by *E. sikkimensis* 58.13 ± 0.26 %) while, lowest amount of total carotenoids was noted in the fruit of *F. roxburghii* (0.68 ± 0.10 %).

5.3. Antioxidant activities

- Studies pertaining to antioxidant activity revealed variable data among different species of Sikkim. *Ficus roxburghii* (72.26 ± 0.06 %) showed highest DPPH radical scavenging activity and lowest in *Eleaocarpus sikkimensis* (42.09 ± 0.56 %).
- Methanol extract of *Rubus ellipticus* fruits showed maximum hydroxyl radical scavenging activity (61.46 ± 0.04 %) followed by *Spondias axillaris* (60.35 ± 0.03 %) whereas, lowest hydroxyl radical scavenging capacity was observed in the fruit of *Ficus roxburghii* (33.59 ± 0.02 %).
- Highest scavenging capacity of hydrogen peroxide was recorded in the fruits of *Eriolobus indica* and lowest in the fruits of *Ficus roxburghii* (71.85 ± 0.16 % and 45.70 ± 0.52 %, respectively).
- Investigation on ferrous chelating activity revealed that highest ferrous ion chelating activity was observed in the fruits of *Eriolobus indica* (42.04 ± 0.08 %) which was followed by *Baccaurea sapida* (39.24 ± 0.4 whereas, lowest was observed in the fruits of *Diploknema butyraceae* (17.62 ± 0.6 %).
- Perusal of ferric reducing antioxidant power data was also investigated in different species of Sikkim. Highest antioxidant properties was noted in the fruits of *Spondias axillaris* (31.04 ± 0.2 mg GAE g^{-1}) followed by *Baccaurea sapida* and *Rubus*

ellipticus (26.26 ± 02 mg GAE g^{-1}), while lowest antioxidant activity was observed in the fruit extract of *E. latifolia* (7.75 ± 0.6 mg GAE g^{-1}).

- The total antioxidant activity of different underutilized fruit extracts was estimated, by Phospho-molybdenum assay. Total antioxidant activities was recorded highest in the fruits extract of *S. axillaris* whereas, lowest in the fruits of *M. edulis* (59.99 ± 0.6 mg AAE g^{-1} and 29.03 ± 0.08 mg AAE g^{-1}).

5.4. Quantification of selected elements

5.4.1. Macro elements

- Quantification of different elements was done in different species of Sikkim. Maximum potassium content was recorded in the fruits of *F. roxburghii* (819.64 ± 2.54 µg kg^{-1}) followed by *R. ellipticus* (421 ± 1.9 µg kg^{-1}), whereas, minimum potassium content was observed in the fruits of *D. butyraceae* (98.75 ± 3.9 µg kg^{-1}).

- Highest amount of magnesium content was recorded in the fruits of *F. roxburghii* (73.09 ± 1.42 µg kg^{-1}) followed by *S. axillaris* (40.16 ± 1.9 µg kg^{-1}), *R. ellipticus* (35.17 ± 1.9 µg kg^{-1}), *E. sikkimensis* (21.67 ± 1.4 µg kg^{-1}) and *B. sapida* (21.67 ± 1.2 µg kg^{-1}) while, the magnesium content was recorded lowest in the fruits of *E. indica* (6.88 ± 0.8 µg kg^{-1}).
- Amongst the ten underutilized fruits studied the highest calcium content was observed in the fruits of *B. sapida* (23.77 ± 2.0 µg kg^{-1}) followed by *F. roxburghii* (23.69 ± 1.43 µg kg^{-1}), whereas, the lowest calcium content was detected in the fruits of *M. edulis* (0.72 ± 0.11 µg kg^{-1}).
- The maximum amount of sodium was observed in the fruits of *E. latifolia* (9.91 ±0.30 µg kg^{-1}) followed by *R. ellipticus* (8.21 ± 2.0 µg kg^{-1}), *E. sikkimensis* (8.09 ± 1.6 µg

kg^{-1}) and *B. sapida* (7.99 ± 1.8 µg kg^{-1}) whereas, the minimum amount of sodium content was recorded from the fruits of *C. erectus* (2.46 ± 1.0 µg kg^{-1}).

- Sulphur was found to be insignificant among all species with less variable amount. The highest amount of sulphur was detected from the fruit of *M. edulis* (5.74 ± 1.8 µg kg^{-1}) followed by *F. roxburghii* (5.45 ± 0.72 µg kg^{-1}) *R. ellipticus* (5.26 ± 1.8 µg kg^{-1}) and *S. axillaris* (5.19 ± 1.6 µg kg^{-1}) and the lowest sulphur content among different underutilized fruits was observed in the fruits of *C. erectus* (0.086 ± 0.06 µg kg^{-1}).

5.4.2. Micro Elements

- Apart from macro elements, quantification of micro elemente was also done among different fruit species of Sikkim. The presence of maximum iron content was recorded from the fruits of *E. latifolia* (31.40 ± 0.51 µg kg^{-1}) followed by *B. sapida* (29.55 ± 2.6 µg kg^{-1}), *F. roxburghii* (26.55 ± 2.8 µg kg^{-1}), *S. axillaris* (26.47 ± 2.4 µg kg^{-1}) and *E. sikkimensis* (24.29 ± 2.3 µg kg^{-1}) whereas the presence of minimum iron content was observed in the fruits of *C. erectus* (1.28 ± 0.16 µg kg^{-1}).
- The fruit of *B. sapida* was found to contain highest amount of zinc i.e. 0.97 ± 0.18 µg kg^{-1} followed by *D. butyraceae, R. ellipticus and C. erectus* (0.65 ± 0.26 µg kg^{-1}, 0.54 ± 0.9 µg kg^{-1} *and* 0.45 ± 0.5 µg kg^{-1}, respectively) while, the fruits of *S. axillaris* was found to contain lowest amount of zinc i.e. of 0.11 ± 0.08 µg kg^{-1}.
- Copper content was found to be highly variable among different fruit species. The fruits of *B. sapida* (14.33 ± 1.2 µg kg^{-1}) were observed to contain highest amount of copper amongst all fruits under present investigation followed by *S. axillaris* (9.06 ± 1.8 µg kg^{-1}), whereas lowest copper content was detected in the fruits of *C. erectus* *i.e.* 1.14 ± 0.09 µg kg^{-1}.

- Maximum manganese content was detected in the fruits of *B. sapida i.e.* 37.29 ± 3.8 µg kg^{-1} followed by *E. latifolia* (29.90 ± 0.41 µg kg^{-1}), *S. axillaris* (11.57 ± 1.2 µg kg^{-1}) and *E. sikkimensis* (11.46 ± 1.1 µg kg^{-1}) whereas, the lowest amount of manganese content was recorded in the fruits of *C. erectus i.e.* 1.1 ± 0.02 µg kg^{-1}.
- Molybdenum was detected highest in the fruit of *S. axillaris* i.e. 2.63 ± 0.8 µg kg^{-1}while, the minimum molybdenum content was recorded in the fruit of *C. erectus* (0.035 ± 0.21 µg kg^{-1}) followed by *D. butyraceae* (0.056 ± 0.08 µg kg^{-1}).

5.4.3. Quantification of heavy metals

- Studies pertaining to heavy metal in fruit species of Sikkim revealed that the minimum cadmium was reported from the fruit of *C. erectus* (0.005 ± 0.002 µg kg^{-1}) and *M. edulis* (0.0060 ± 0.18 µg kg^{-1}) whereas, maximum cadmium content was noted in the fruit of *B. sapida* i.e. 0.021 ± 0.11 µg kg^{-1}.
- Least barium content was noted in *R. ellipticus i.e.* 0.032 ± 0.5 µg kg^{-1} followed by *C. erectus* (0.033 ± 0.12 µg kg^{-1}) and *M. edulis* (0.046 ± 1.08 µg kg^{-1}) while, highest barium content was observed in the fruit of *S. axillaris* (0.59 ± 0.06 µg kg^{-1}).
- Mercury was detected lowest in the fruits of *C. erectus* (0.002 ± 0.001 µg kg^{-1}) followed by *B. sapida* (0.018 ± 0.01 µg kg^{-1}) and *E. latifolia* (0.021 ± 0.01 µg kg^{-1}) and the highest mercury content was detected in the fruits of *S. axillaris* (0.055 ± 0.11 µg kg^{-1}).
- The least amount of lead was detected in the fruits of *B. sapida i.e.* 0.011 ± 0.09 µg kg^{-1} followed by *D. butyraceae* (0.013 ± 0.09 µg kg^{-1}) whereas, highest lead content was detected in the fruits of *E. latifolia i.e.* 0.048 ± 0.19 µg kg^{-1} followed by *M. edulis* (0.022 ± 0.8 µg kg^{-1}).

- The lowest amount of aluminum was observed in the fruits of *D. butyraceae* i.e. 0.01 ± 0.6 µg kg^{-1} followed by *M. edulis* (0.057 ± 0.12 µg kg^{-1}) and *F. roxburghii* (0.066 ± 0.24 µg kg^{-1}) whereas, the highest aluminum content was detected in the fruits of *E. sikkimensis* followed by *C. erectus* (0.82 ± 0.14 µg kg^{-1} and 0.68 ± 0.46 µg kg^{-1}).
- Quantification of heavy metals also revealed lowest chromium content in the fruits of *C. erectus i.e.* 0.0086 ± 0.38 µg kg^{-1} followed by *E. sikkimensis* (0.013 ± 0.08 µg kg^{-1}) and *D. butyraceae* (0.021 ± 0.50 µg kg^{-1}) whereas, the highest chromium content was noted in the fruits of *E. latifolia* (0.093 ± 0.29 µg kg^{-1}).

5.4.4. Quantification of phenols

- Gallic acid was identified in all fruits under study. Maximum Gallic acid was observed in *Eriolobus indica* (5.96 mg g^{-1} DW) while minimum was observed in *Machilus edulis* (0.79 mg g^{-1} DW).
- The phenolic compound Rutin was found maximum in *Eleaocarpus sikkimensis i.e.* 2.59 ± 0.02 mg g^{-1} DW followed by *Baccaurea sapida* (1.45 ± 0.02 mg g^{-1}DW), whereas minimum amount of Rutin was noted in *Elaeagnus latifolia* (0.14 ± 0.01 mg g^{-1}DW) followed by *Machilus edulis* (0.15 ± 0.04 mg g^{-1}DW).
- Highest amount of ferulic acid was detected in the fruit of *Calamus erectus* (6.36 ± 0.02 mg g^{-1} DW) which was followed by *Baccaurea sapida* (6.29 ± 0.05 mg g^{-1} DW) while, least amount of ferulic acid was found in the fruits of *Elaeagnus latifolia* (0.07 ± 0.02 mg g^{-1} DW) followed by *Spondias axillaris* (0.28 ± 0.03 mg g^{-1} DW).
- Highest amount of catechol *i.e.* 10.54 ± 0.06 mg g^{-1} DW was observed in the fruit extract of *Eriolobus indica,* whereas, lowest amount of catechol was recorded in *Calamus erectus i.e.* 0.47 ± 0.03 mg g^{-1} DW.

- Maximum quercetin content was found in the fruits of *Baccaurea sapida* (0.97 ± 0.06 mg g^{-1} DW) followed by *Ficus roxburghii* (0.63 ± 0.06 mg g^{-1} DW) while, lowest quercetin content was found in *Spondias axillaris* (0.06 ± 0.02 mg g^{-1} DW) followed by *Diploknema butyraceae* (0.049 ± 0.06 mg g^{-1} DW) and *Calamus erectus* (0.088 ± 0.03 mg g^{-1} DW).

Conclusion

Based on the present findings it can be concluded that underutilized fruit species viz. *B. sapida* was found to be superior in terms of total flavonoid, flavonol, ascorbic acid, quercetin content and minerals like Ca, Zn, Cu and Mn was higher however heavy metal like lead was least. *C. erectus* was superior in terms of fibre, dry matter and ferulic acid content whereas, in this species heavy metals like Cd, Hg, Cr was lowest. *D. butyraceae* was found to contain high ash content, TSS, total sugar and Vitamin A. Fruits of *E. latifolia* was good in terms of element Fe and Na and also possessed highest moisture content. *E. indica* was good in terms of phenolic compound like gallic acid and catechol content and also has highest hydrogen peroxide scavenging activity and Fe^{++} chelating activity. *F. roxburghii* was found good in total carbohydrate, K, Mg and DPPH scavenging activity. *M. edulis* was superior in terms of crude fat, Vit. E, carotenoid and sulphur content. *R. ellipticus* was excellent in terms of crude protein and anthocyanin content. *S. axillaris* was superior in terms of total antioxidant activity, total phenols and Mo content. The study marked the presence of nutraceutical compounds in different species which can be harnessed by their cultivation. The results indicate that the nutritional, phytochemical, antioxidant activities and presence of phenols indicate that these underutilized fruits of Sikkim are *at par* with other commercial fruits and could be the potential source for nutraceuticals.

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