STUDIES ON THE NUTRITIONAL AND ANTIOXIDANT POTENTIALS OF THE MINOR FRUITS OF ARUNACHAL PRADESH

A Thesis Submitted to
Department of Botany, Rajiv Gandhi University for the fulfilment of the Degree of Doctor of Philosophy in BOTANY

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-2019-

Declaration

I do hereby declare that the present research entitled "**Studies on the Nutritional and Antioxidant Potentials of the Minor fruits of Arunachal Pradesh**" was carried out by me for the degree of Doctor of Philosophy in Botany under the guidance and supervision of Dr. Ayam Victor Singh, Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, India.

The elucidations of data are based on my experimental condition and understanding of the literature reports. The original works of the authors or sources of information have been acknowledged at the end of chapters.

No degree or diploma has been conferred earlier for this thesis to anyone by the University or any other University.

Place: Doimukh

Date: 28th August 2019.

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(Research Scholar)

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TO WHOM IT MAY CONCERN

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I further certify that the entire thesis represents the independent work of Ms. Hage Asha and all the research work was undertaken by the candidate under my supervision and guidance. Miss Asha is a sincere and hardworking researcher with good moral character, I wish her every success in life. She is not related to me.

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PRE-SUBMISSION SEMINAR

This is to certify that Ms. Hage Asha, a Ph.D Scholar under the guidance of Dr. Ayam Victor Singh, presented and defended her Doctoral research work entitled "**Studies on the Nutritional and Antioxidant Potentials of the Minor Fruits of Arunachal Pradesh**" in a pre-Ph.D submission seminar held on 6th June 2019 at 12.30 pm in the Department of Botany, Rajiv Gandhi University, Doimukh under the chairmanship of Dr. Hui Tag, HoD, Department of Botany.

I wish her a successful career ahead.

Rksingh

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Abstract

Minor fruits are the lesser-known fruits found in a region. Recently they have been of much interest in the quest for finding an alternative food source in the ever-growing food shortage around the world. Thus this study was undertaken to document the lesser-known fruits found in Arunachal Pradesh with objectives to analyse their antioxidant and nutritional potential. DPPH, ABTS-TEAC, FRAP, TAC, TPC and TFC assays were used for the antioxidant assay. Nutritional parameters such as carbohydrate, starch, total sugar, protein, free amino acid, fat and fiber were analyzed. A total of 69 minor fruit samples were collected from 36 families with ethnobotanical data for 33 samples. Rosaceae family accounted for the highest number of collections. Tawang, West Kameng, Lower Dibang Valley, East Siang, West Siang, Kra Daadi, Kurung Kumey, Upper Subansiri, Lower Subansiri, Tirap, Anjaw, Papumpare, Changlang districts were covered during the field survey from 2015-18. IC_{50} value of *Livistona jenkinsiana* was found to be lowest (6.387±0.314µg/ml) in DPPH assay. TEAC value for *Quercus semecarpifolia* was highest (2.525±0.014µMTE/gm dry extract). *Livistona jenkinsiana* also had lowest EC_1 value (0.193±0.007µg/mL) in FRAP assay and the highest value (50.392±1.142 mg AAE/gm) in TAC assay. Total phenol content (388.602±32.878 mg GAE/gm dry extract) and flavonoid content (921.485±43.419 mg RE/gm dry extract) also were highest in *Livistona jenkinsiana*. Minor fruits *Docynia indica, Elaeagnus latifolia, Embelia ribes, Ficus semicordata, Livistona jenkinsiana, Macrosolen cochinchinensis, Quercus semecarpifolia, Rhus chinensis, Rosa sericea, Spondias axillaries, Spondias pinnata and Terminalia bellerica* were found to show high antioxidant activity. Carbohydrate content in *Cotoneaster microphyllus* was found to be highest (74.04±4.49%DW). *Docynia indica* had the highest (3.81±0.21%DW) starch content. Highest (28.51±2.53%) total soluble sugar content was found in *Annona squamosa*. *Diospyros kaki* had the highest protein content (29.86±8.99% DW). Highest (3.73±0.05%DW) free amino acid content was recorded for *Terminalia bellerica*. Fat content was highest in *Livistona jenkinsiana* at 37.6% DW. *Rhus chinensis* had the highest fiber content (68% DW). The nutritive value for *Cotoneaster microphyllus* (431.92Cal/100gm), *Livistona jenkinsiana* (382.60Cal/100gm) and *Spondias pinnata* (240.52Cal/100gm) were found to be quite high. *Livistona jenkinsiana, Spondias pinnata* and *Spondias axillaries* were found to be promising fruits with both high antioxidant activity and nutritive values.

Keywords: Minor fruits; Arunachal Pradesh; antioxidant; nutritional parameters.

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Hage Asha

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General Introduction

Introduction

Fruits have been consumed by mankind since time immemorial. They supply hydration and instantaneous energy. They are rich source of vitamins and other nutritional components and possess potent antioxidant property. Their irresistible savor and vibrant color is a complete feast to one's eye. With all those properties, fruits have carved a niche in our diet routine. They may be consumed raw or processed for later consumption.

In botanical term, a fruit may be defined as the ovary which gets matured after fertilization and bear seeds for future propagation. Sometimes the whole of the inflorescence may turn into fruit. They may or may not include accessory parts (Mitra *et al.*, 2009) When a fruit is developed from ovary (mango), it is known as 'True fruit' and if is developed from any other part of flower such as peduncle (cashew nut) or thalamus (apple and cucumber) then it is known as 'False fruit'. A fruit basically consists of the seed(s), which is developed from ovule(s) and pericarp which is developed from wall of the ovary. If the pericarp is thick, it is further divided into outermost epicarp, middle mesocarp and innermost endocarp.The skin denotes epicarp while the pulpy part the mesocarp. Endocarp may be membranous or stony. Fruits can be classified into three broad categories (Dutta, 1964):

1) Simple type

This type occurs when only one fruit is developed from a matured ovary of a simple flower. It is further divided into

a) Dry type

- Dehiscent type- pericarp of fruit bursts upon ripening.

  Example: Follice in *Alstonia scholaris*, Legume in *Pisum sativum*, Capsule in *Rhododendron* sp., Silique in *Brassica* sp.

- Indehiscent- pericarp of fruit does not open up on maturing and resort to decaying for seed dispersal.

  Example: Cypsella in *Helianthus annuus*, Caryopsis in *Zea mays*, Achene in *Mirabilisjalapa*, Utricle in *Chenopodium* sp., Nut in *Quercus* sp.

- Schizocarpic- Fruits which split into indehiscent individual carpellary components (mericarp) upon maturing. Example: Cremocarp of *Coriandrum sativum*, Samara in *Dioscorea* sp., Regma in *Ricinus communis*, Lomentum in *Mimosa* sp., Carcerule in *Ocimum* sp.

b) *Fleshy type* (always indehiscent).

Example: Drupe in *Prunus domestica*, Berry in *Phoenix sylvestris*, Hesperidium in *Citrus* sp., Pepo in *Cucurbit* sp., Pome in *Malus sylvestris*.

2) Aggregate type

It comprises of cluster of fruitlets which are formed from respective free carpels. For example: aggregate of berries (*Annona squamosa*), Achenes (*Clematis* sp), Follicles (*Michelia champaca*), Drupes (*Fragaria vesca*).

3) Multiple or Composite type

Fruits formed from the whole of inflorescence. For example:Syconus of *Ficus* sp. and Sorosis of *Ananas comosus*.

Based on the climate type of a region a fruit may be broadly classified as tropical type, sub-tropical type, temperate type or arctic type (Joy *et al*., 2016). Tropical types grow in high temperature climatic zone and evergreen in nature (Joy *et al*., 2016) and cannot withstand low temperatures (Yahia, 2006) such as are mango, banana, papaya. About 300 tropical fruit species have been reported from the Indian subcontinent (Paull and

Duarte, 2011). Subtropical types need comparatively milder temperature to grow and can survive slight cold temperature and may be evergreen or deciduous in nature (Joy *et al*., 2016; Encyclopedia of Food and Culture, 2018). For example, oranges, grapes, lemons and limes etc. The temperate fruit trees can survive very low temperatures and are deciduous in nature (Joy *et al*., 2016) such as apples, peaches, pears, cherries etc. Arctic types are prone to extremely low temperature throughout the year (Joy *et al*., 2016). However, it may be noted that there is no hard and fast rule for growing them in those particular climatic zone. For example, banana and avocado can be grown in both tropical and subtropical regions (Encyclopedia of Food and Culture, 2018).

When it comes to layman, there is often confusion differentiating between fruits and vegetables. The International Agency for Research on Cancer (IARC, WHO, 2003) has designated vegetable as any edible part of a plant. In such case fruits automatically fall under the vegetable category. But they, IARC, had also defined fruit as the pulpy part of plant containing seed(s) with particular taste which can be consumed as meals. So this thesis will focus on fruits based on the second context.

Fruits serve as functional food and keep diseases at bay (Joy *et al*., 2016). The antioxidants present in them have been known to fight against various diseases such as cancer, cardiovascular disorder, type 2 diabetes, blood pressure disorder and other chronic aging related diseases (Doll, 1990; Ames *et al*., 1993; Dragsted *et al*., 1993; Willett, 1994; Seal, 2011). Consuming fruits also help in rebuilding of tissues and fetal development (ChooseMyPlate.gov, United States Department of Agriculture, 2015). Such beneficial characteristic feature come from their antioxidant property which is generally composed of polyphenolics, flavonoids, anthocyanin, vitamin C, E and beta carotenoids such lutein and beta carotene (Steinberg *et al*., 1989, 1991;Gey, 1993; Olsson *et al*., 2004; Gulcin, 2010; Harput, 2011; Singh *et al*., 2014).

An antioxidant is a molecule capable of inhibiting oxidation process. During oxidation reaction, reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), hypochlorous acid (HOCL) and free radicals such as the hydroxyl radical (*OH) and the superoxide anion (O^{2-}) are produced (Valko *et al*., 2007) which is quite normal and indispensable for important physiological function. But an imbalance between these radicals and antioxidants leads to a degenerative condition. These unstable molecules can easily react rapidly and initiate a chain reaction resulting into oxidative stress. Antioxidants can terminate such chain reactions by removing free radical intermediates and inhibiting further oxidation by oxidizing themselves. This property of antioxidant has been a crucial factor in Research and Development Area. Thus they have been of great significance to mankind both in health and cosmetic sector (Fukumoto & Mazza, 2000). Catechins, anthocyanins, ellagic acid, beta carotene, vitamin E, resveratrol, lycopene, vitamin C are few examples of popular antioxidants found in fruits.

There has been rapid increase in fruit consumption due to increasing awareness about its beneficial effects. The market thus tries to maintain the availability of fruits in market whole year round (Retamales, 2011). However, cost and accessibility have been identified as key barriers to fruit consumption (International Food Information Council Foundation, 2018). In such situation the locally available minor fruits could be used in place of conventional fruits. About 50% of the world's fruit production is fulfilled by 5 countries and India is one among them, the other four are China, Brazil, USA and European Union (Retamales, 2011). According to Agricultural and Processed Food Products Export Development Authority, Ministry of Commerce and Industry, Government of India, (APEDA, 2015) India is the largest supplier of fruits in the world with Maharashtra, Andhra Pradesh, Tamil Nadu, Gujarat, Karnataka, Uttar Pradesh, Bihar, Madhya Pradesh, West Bengal, Kerala, Jammu & Kashmir, Orissa and Assam

categorized as the key producer states while USA, UK, Germany, Holland and Russia as active importers of fruits (Retamales, 2011).

Apart from some major fruits of the North East Region such as apple, orange, kiwi, peach and pear, it is also a home to many minor fruits as well (Mitra and Roy, 2014). Arunachal Pradesh is biodiversity hotspot region and is a storehouse to different kinds of fruits of which many are lesser known or underutilized. Fruits such as Kiwi, mandarin Orange, Pine apple are few examples of major fruits of Arunachal Pradesh (APEDA, 2015). There is a need for more exploration among the lesser known fruits in the state to study about their potency in both antioxidant as well as nutritional dimension. Such way people will have more ideas about those lesser known fruits and could commercialize at larger scale and Arunachal Pradesh could be one among the key producers of fruits in India.

OBJECTIVES

1. Documentation on the wild minor fruit plants of Arunachal Pradesh and their ethnobotanical accounts.
2. Preliminary qualitative phytochemical screening for nutritional and antioxidant components. Selection of the maximum possible number of minor fruits based on the preliminary test and also by taking into account the ethnobotanical and literature reports.
3. Quantitative estimation of nutrients like total Protein, aminoacids, carbohydrate, starch, total sugar, fat, fiber etc. of the selected fruit.
4. Quantitative estimation of antioxidant activity of the selected fruits from their solvent and essential oil extracts and comparison among them.

Hypothesis of the Proposed Research

Minor fruits of Arunachal Pradesh, consumed by the local people could possess high nutritive values and antioxidant agents. If any minor fruit with such values is discovered it could be cultivated for commercial purpose and be converted into major fruit crop. This study therefore would help us understand the nutritive parameters and antioxidant potential of the various minor fruits found here.

Chapter 2

Literature Review

According to Fuleky (2009) about 80,000 plant species on Earth are fit for human consumption. They are endowed with rich nutrients essential for our health (Liu *et al*., 2000; Duyn & Pivonka, 2000; Agudo, 2004). As per Hoejskov (2014), sufficient fruits and vegetables consumption help boost immunity, safeguard eyes, cardiovascular system and give protection against cancer, obesity, and stroke and could save about 2.7 million deaths around the world. Edible fruits comprise about 3000 species as reported by Mohapatra and Panda (2009). They can be consumed directly in raw form or processed for later consumption with value addition. Consuming fruits is related with decreased rates of kidney stones, bone density loss and enhancement of brain cells, bowel movement etc (Ridgewell, 1998; Mintah *et al*., 2012; Urology Care Foundation (UCF), 2016).

Plant based foods generally contain phytochemicals. These are naturally occurring chemicals within plants and are produced by primary or secondary metabolism in plants (Harborne *et al.*, 1999; Molyneux *et al*., 2007). Primary metabolites are essential for growth and development of plant while secondary metabolites protect them from harsh environmental stresses such as predation and pathogen attack etc (Keeling, 2006). Patra (2012) has reported about 200,000 phytochemicals discovered by researchers of which 20,000 are known to have been derived from fruits, vegetables and grains. They are generally reported to be bioactive and have influence human health in outstanding ways (Schreiner, 2006).

Huang *et al*., (2016) has grouped phytochemicals into 5 categories:

- *Carbohydrates*: comprising of monosaccharides, disaccharides, polysaccharides, oliogosaccharides and sugar alcohols
- *Lipids*: comprising of monosturated fats, saturated fats, polysaturated fats andfatty acids.

- *Terpenoids*: comprising of carotenoids, monoterpenoids, diterpenoids, sesquiterpenoids, triterpenes, triterpenoidsaponins, sesquiterpene lactones, polyterpenoids.
- *Phenolic compounds*: comprising of flavonoids, phenolic acids, stillbenoids, tannins, lignans, xanthones, quinines, coumarins, phenylpropanoids, benzofurans.
- *Alkaloids and other nitrogen containing metabolites*: comprising of glucosinolates, betalain, indole, peptide, amino acids, ateroidal, cyanogenic glycosides, proteins, quinoline, purine, pyrimidine etc.

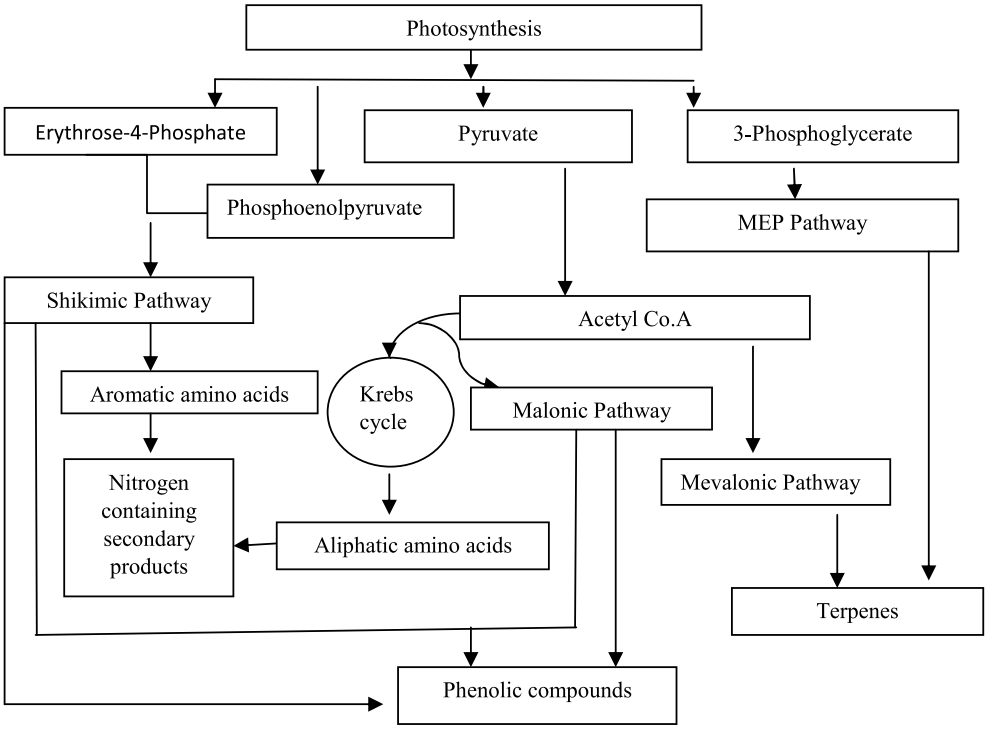


Fig 2.1: Diagrammatic representation of production of secondary metabolites

These phytochemicals have substantial role in pharmacognosy, perfumery, cosmetics, agro-industries and food industries (Hill, 1993; Newman *et al.*, 2000; Tucci, 2010; Patra, 2012; Varma *et al*., 2017; Saleem *et al*., 2018). Bioactivities reported from phytochemicals comprise antimicrobial, anti-inflammatory, anti-tumor, anticancer, anti-

diarrhoeal, anti-HIV, anti-diabetic, anti-viral, hepatoprotection, cholesterol-lowering, cardiovascular protection, and various non-communicable diseases etc (Selway, 1986; Gould *et al*., 1997; Kren & Martinkove, 2001; Schreiner, 2006; Ee *et al*., 2010; Sasidharan *et al*., 2011; Godlaski, 2011; Xia *et al*., 2011; Tanwar and Modgil, 2012). Among all these bioactivities antioxidant activity has gained tremendous interest in scientific community (Delaquis *et al*., 2002; Rufino *et al*., 2011; Basumatary *et al*., 2015; Santos and Goncalves, 2016). Studies have reported that antioxidant content in fruits and vegetables can lessen the chances of oxidative stress which leads to cardiovascular diseases, degenerative diseases such as cancer and other aging related diseases and some chronic diseases (Ames *et al*., 1993; Cao *et al*., 1996; Wang *et al*., 1996; Dragsted *et al*., 2003). According to Larson (1988) ascorbic acid, carotenoids such as carotenes and xanthophylls, vitamin E such as tocopherols and tocotrienols, phenolic compounds such as phenolic acids, flavonoids, stilbenes, coumarins, lignans and lignin and other sulphur derived antioxidants are the chief components of antioxidant property in fruits and vegetables. Studies made by other workers (Gulcin, 2010; Harput, 2011; Singh *et al.,* 2014) also support the role played by phenolics, anthocyanins and flavonoids as antioxidants in various fruits. Weiss (2005) has reported the role of vitamins and carotenoids in prevention of fatty acid peroxidation chain reactions. According to Sandmann (2001) the conjugated double bonds are the reason for antioxidant activity of carotenoids. In plants, polyphenolics have been reported to have significant role in protecting the integrity of plants by providing resistance against pathogen attacks, extreme temperature conditions, radiations etc (Haslam, 1996; Lira *et al.,* 2007). Many biological activities such as antifungal, anti-histamine, antibacterial, antiviral, anti-inflammatory, anticancer, antioxidant activities and lessening the chances of diseases such as diabetes, osteoporosis and neurodegenerative diseases have been

associated with phenolic content in fruits and vegetables (Kim *et al.,* 1998; Carb *et al.,*1999; Wang *et al.,* 1999; Harborne & Williams 2000; Graf *et al.,* 2005; Soobrattee *et al.,* 2005; Nitta *et al.,* 2007)

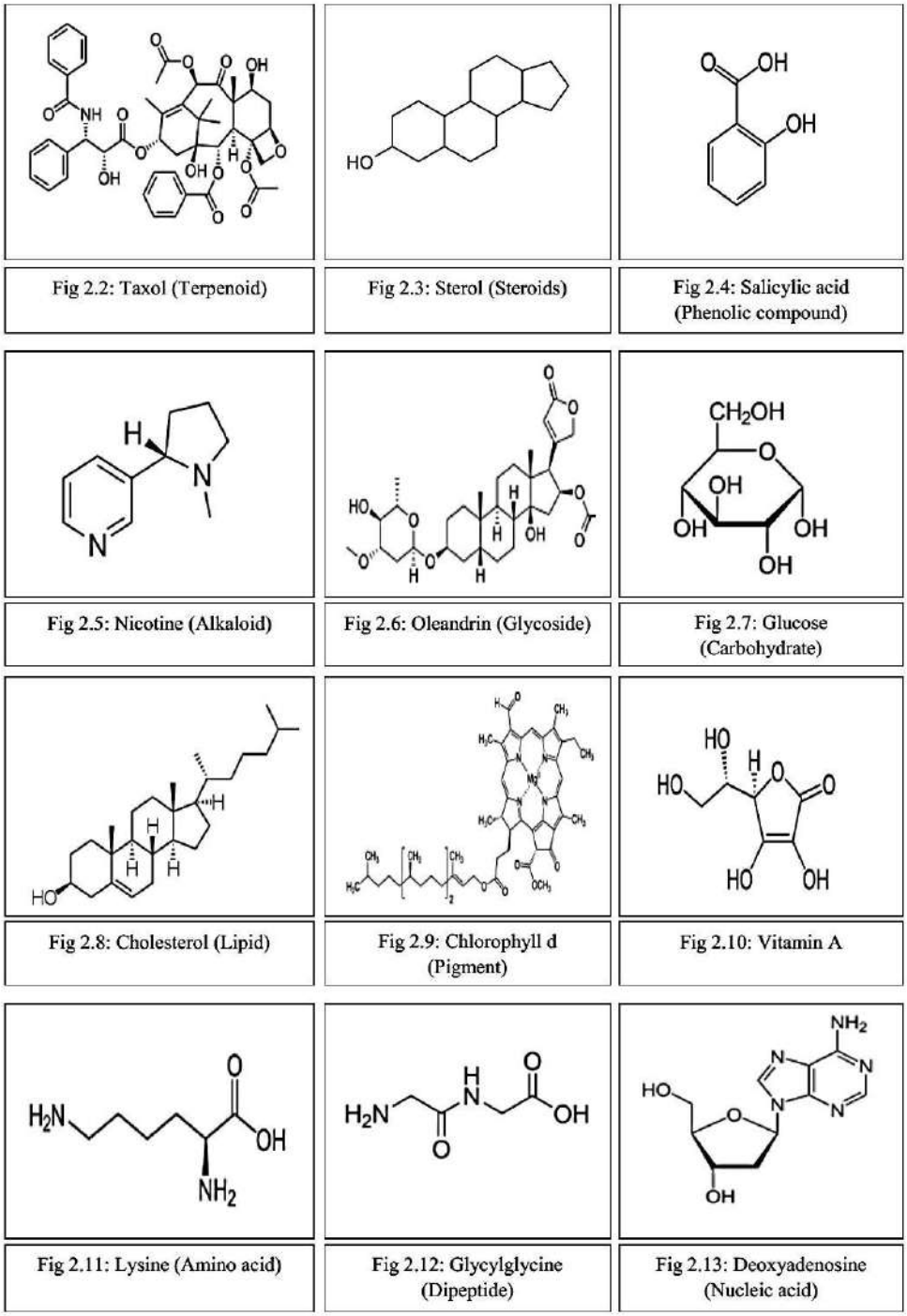


Fig 2.2: Taxol (Terpenoid)

Fig 2.3: Sterol (Steroids)

Fig 2.4: Salicylic acid (Phenolic compound)

Fig 2.5: Nicotine (Alkaloid)

Fig 2.6: Oleandrin (Glycoside)

Fig 2.7: Glucose (Carbohydrate)

Fig 2.8: Cholesterol (Lipid)

Fig 2.9: Chlorophyll d (Pigment)

Fig 2.10: Vitamin A

Fig 2.11: Lysine (Amino acid)

Fig 2.12: Glycylglycine (Dipeptide)

Fig 2.13: Deoxyadenosine (Nucleic acid)

Fig: (2.2) -(2. l3): Examples of phytochemicals as categorized by Raaman (2006); Chemical structure (image source- Wikipedia)

It is reported that 3.4 million deaths worldwide could be due to low fruit consumption (Lancet, 2015) where non-communicable diseases (NCDs) alone accounts for 70% of deaths according to World Health Organization (WHO, 2017). To keep diseases at bay and keep one's body hale and hearty (WHO, 2019) recommends daily intake of at least 400 grams of fruits and vegetables. But with about 7.7 billion (World Bank, 2019) people around the world, there is an ever-growing need of fruits. Mitra (2014) has reported that awareness about the commercial and nutritional values has led to global demand of fruits. In such state of affairs minor fruits become excellent alternatives (Patel *et al*., 2010; Paul, 2013; Mahato and Chhetri, 2015; Srivastava *et al*., 2017).

Minor fruits are those fruits which are lesser known than conventional popular fruits. According to Mazumdar (2004) minor fruit may be defined as those fruits which can be consumed but are less palatable in comparison to other fruits available in a region. They are also known as "lesser known" or "less exploited" fruits and sometimes as "wild fruit". Roy (2014) states that a minor fruit is defined by factors such as use value related geographical availability, adaptation to agro-ecological niches, scarcity in scientific knowledge and current economic importance.

But it is to be noted that there is a thin line between a fruit being major type or minor type. For example, a popular fruit at temperate region may be a minor fruit in a tropical region. Thus no sharp demarcation can be drawn between them. Use value, popularity among people, low market value, dearth in scientific knowledge, climatic and edaphic factors may be some of the contributing factors for unpopularity of these fruits among the people (Mazumdar, 2004; Beluhan and Ranogajee, 2010; Roy, 2014). It is also reported that people have hardly any idea about their nutritional value. Being short of such knowledge people may domesticate them but not necessarily cultivate them (Beluhan and Ranogajee, 2010; Mahato and Chhetri, 2015). Many of them are thus

found in feral state and are stigmatised as “Food of the Poor” (Dandin and Kumar, 2016). Such ignorance leads to less demand in market automatically. They might, however, sometimes have economic values at regional market only (Anang and Chan, 1999) or may have an important part in socio-cultural life of the indigenous people (Arora, 1998). Inconvenience in eating such as unpleasant taste or too many seeds or storing problem are considered some of the common problems associated with their negligence. It is reported that poor documentation on their distribution, cultivation, needs, uses, poor commercial status, processing status, under estimation of their potentiality along with vanishing ecosystem etc., are some of the major setbacks of these minor fruits (Gajanan, 2010; Roy, 2014; Dandin and Kumar, 2016).

The above limitations are hindrance to the fact that minor fruits can be a popular potent dietary supplement. Such limitations conceal some exceptional qualities in them such as hardiness, defiance to pathogen attack and their ability to utilize the naturally available nutrients in nature without much effort (Mazumdar, 2004).

Despite such hindrances their potentiality as medicinal, therapeutic and industrial value has been identified by the indigenous communities (Rai *et al.,* 2005). At present world their relevance has been noted by the researchers around the world due to their climate pliant nature, natural adaptation to stress, neutraceutical and medicinal significance etc (Dandin and Kumar, 2016). Reports suggest that these fruits have the possibility of balancing the growing food and nutritional insecurity around the world (Kunkel, 1984; Bhag *et al.,* 1997; Paul, 2013; Dandi and Kumar, 2016; Srivastava *et al.,* 2017).

Antioxidant activity and nutritional information has been reported for some of the minor fruits (Guinand & Dechasa*,* 2001; Mitra *et al.,* 2008; Chalise *et al.,* 2010; Seal, 2012; Paul, 2013; Diengngan and Hasan, 2015; Dasgupta *et al*., 2017; De, 2017; Basumatary and Narzary, 2017). It is also reported that nutritional value of these minor

fruits show discrepancy from species to species depending on the climatic and edaphic factors (Mitra *et al.,* 2008; Seal, 2012; Paul, 2013).

International Perspective

Dandin and Kumar (2016) has mentioned 1750 edible underutilized edible fruits and nuts in 11 regions of the world, namely- Chinese- Japanese (222), Indochinese-Indonesia (226), Australian (57), Hindustani-Indian (344), Central Asian and Near Eastern (38), Mediterranean (30), African (131), European-Siberian (62), South American (263), Central American and Mexican (122) and North American (255). Minor fruits have been identified as a major food supplement for developing countries (Cook *et al.,* 2000; Lockeett *et al.,* 2000; Ogle *et al.,* 2001). Minor fruits such as *Ziziphus spina-christi, Balanites aegyptiaca, Grewia flavescens* have been studied to be important food supplement in the Semiarid Parts of East Shewa Zone, Ethiopia (Feyssa, 2011).*Aegle marmelos*, *Arbutus unedo, Asimina triloba***,** *Berberis aristata*, *Castanea sativa,Choerospondias axillaris*,*Cornus kousa var.chinensis***,***Cipadema bacifera*, *Crataegus monogyna*, *Diospyros embryopteris*, *Diospyros kaki,Diospyros virginiana, Diplokemna butyracea*, *Eryobotria japonica,Lithocarpus pachylepis*, *Melastoma malabathricum*, *Morus spp*, *Phyllanthus emblica, Prunusmartima, Prunus persica*, *Prunus spinosa, Pyrularia edulis, Rubus ulmifolius*, *Sorbus domestica*, *Sambucus canadensis, Spondias pinnata, Terminalia bellirica, Terminalia chebula*, *Ziziphus incurve, Ziziphus mauritiana,* are some of the minor fruits reported from various countries such as Nepal, America, Ethiopia, China, Iberian peninsula and Mediterranean regions etc. (Bellini & Giordani, 1998; Chalise *et al.,* 2010; Feyssa, 2011). Ikram *et al.,* (2009) has reported on the antioxidant capacity and total phenol content on 58 underutilized fruits from Malaysia with higher antioxidant capacity for *Averrhoa bilimbi, Phyllantus emblica, Pomentia, Salacca conferta, Syzyzium jambos,*

Sandoricum macropodum, Maipighia punicirolia and *Garcinia atroviridis* among the collections. Fu *et al*.,(2010) has reported on antioxidant and total phenolic content of 56 wild fruits from South China with highest antioxidant activity and potentiality as functional food recorded for *Eucalyptus robusta, Eurya nitida, Melastoma sanguineum, Melaleuca leucadendron, Lagerstroemia indica, Caryota mitis, Lagerstroemia speciosa* and *Gordonia axillaris*.

National Perspective

In India About 27% of the fruit production have been reported to be contributed by minor fruits (Srivastava *et al.,* 2017). Mazumder (2004) has reported about 150 palatable minor fruit species in India. *Aegel marmelos, Anacardium occidentale, Annona reticulata, Averrhoa carambola, Borassus flabellifer, Cassiac aranda, Chrysophyllum cainito, Dillenia sp.*, *Feronia limonea*, *Grewia subinaequals etc* have been reported to be some of the minor fruits of India (Mazumdar, 2004).Protein, crude fibre, carbohydrate, Ca., K, Na., P, Vit, C, Vit A have been reported from some of the wild minor fruits viz, *Aegle marmelos*, *Averrhoa bilimbi*, *Baccaurea sapida,Cordia* myxa, *Elaeangnaus latifolia*, *Eriobotrya japonica*, *Grewia asiatica*, *Myrica esculenta, Syzygium cumini*, *Zizyphus mauritiana* (Mitra *et al.,* 2008; Seal, 2012; Paul, 2013). Antioxidant activity of *Ficus palmata, Pyrus pashia* and *Ficus auriculata* have been reported from Uttarkhand and were reported to be potential tonic for reducing oxidative stress and prevention of cancer development (Saini *et al.,* 2012). *Flueggea leucopyrus willd., Grewia tiliaefolia Vahl., Mahonia leschenaultia,Gaultheria fragrantissima, Zizyphus rugosa* and *Rubus ellipticus* have been reported for the same from Western ghats (Karuppusamy *et al.,* 2011). Shiva *et al*., (2017) has reported on the usage and nutritional value of some wild fruits from Telangana area. Paul (2013) in the2nd International Symposium on Minor Fruits and Medicinal Plants for better lives, has

reported study on minor fruits from the Eastern region such as Assam (Shadeque, 1989), Eastern Ghat region (Reddy *et al.,* 2006), Meghalaya (Jasmine *et al.,* 2007), Odisha (Sinha & Lakra, 2005), Sikkim (Sundriyal & Sundriyal, 2011), Tripura (Mazumder & Dutta, 2009) and West Bengal (Roy, 2014).

Regional Perspective

Some of the wild minor fruits from the North eastern region of India whose antioxidant potential has been studied (Seal, 2011; Prakash *et al.,* 2012; Singh *et al.,* 2014) are- *Baccaurea sapida*, *Castanopsis tribuloides, Citrullus colocynthus, Cyphomandra betacea, Diplokenema butyracea*, *Elaeagnus latifoila*, *Elaeocarpus Sikkimensis, Emblica officinalis,Eriolobus indica*, *Ficus hookeri, Machilus edulis*, *Myrica esculenta,Spondias axillaris* etc. Mahato and Chhetri (2015) has documented on 10 minor fruits from Darjeeling Himalayas and discussed on their morphological as well as nutritional aspect along with utilization and value addition. Patel *et al*., (2010) has discussed the distribution and processing of minor fruits from North Eastern India. Some of the recorded (Roy *et al.,* 1998; Chadha, 2001; Peter, 2007; Malik *et al.,* 2010; Singh *et al.,* 2014, Mahato and Chhetri, 2015) minor and uncultivated fruits of Eastern India are: *Aegel marmelos, Annona reticulata, Annona squamosa, Artocarpaus altilis, Artocarpus lakoocha*, *Averrohoe carrmbole*, *Baccaruea sapida, Baccaurea sapida*, *Borassus flabellifer*, *Castanopsis hystrix*, *Cassia caranda, Citrus grandis, Cordia myxa, Cyphomandrabetacea*, *Diospyros blancoi*, *Diospyrus melanoxylon*, *Diospyros peregrine, Diospyros malabarica*, *Diploknemabutyracea*, *Duchesnia indica*, *Duraize bethinus, Elacocarpus floribunda, Elaeagnuslatifolia*, *ElaeocarpusSikkimensis*, *Elaegnus latifolia*, *Eriobotrya japonica*, *Eriolobus indica*, *Euphoria longan, Ficus hookeri*, *Ficus racemosa, Feronia limonea, Flaocortia indica, Flacortia jangomas, Garcinia mangostene, Garcinia xanthochymus*, *Grewia asiatica, Machilus edulis*,

Morus alba, Malphigia punicifolia, Mimusops elengi, Mangifera sylvatica, Dillenia india, Phyllanththus embelica, Inga dulcis, Madhuca latifolia, Madhucaindica, Nephelium lappaceum, *Nipa fruticans*, *Phoenix sylvestris*, *Phyllanthus acidus*, *Physalis peruviana*, *Plassiflora edulis, Prunus cerasoides*, *Rubus ellipticus*, *Semeocarpusanacradium*, *Sonneratia acida*, *Spondias axillaris*, *Spondius cythera, Spondius pinnata*, *Sterculia foetida, Syzygium cuminii*, *Syzgium jambos ,Syzygiumsamarengense*, *Tamarindus indica*, *Terminalia chebula*, *Trapa bispinosa, Zyzyphus mauritiana* etc.

Arunachal Pradesh

Hazarika and Lalruatsangi (2016) has reported the role of minor fruits such as *Nephelium lappaceum, Emblica officinalis, Zizyphus maurutiana, Dimocarpus longan, Syzygium cumini, Aegle marmelos, Grewia subinaequalis, Averrhoa carambola, Dillenia indica, Durio zibethinus, Carissa carandas, Diospyrous kaki, Passiflora* spp*, Tamarindus indica, Ficus carica* in ethno-medicine. Angami *et al.,* (2006) has reported the use of wild fruits for sustaining food security among local population of Arunachal Pradesh. Documentation has been done on wild fruits of the State by Singh and Asha (2017). De (2017) has also reported on physico-chemical properties as well as other beneficial use of some of the underutilized fruits of Arunachal Pradesh.

Chapter 3

Study site: Arunachal Pradesh

STUDY SITE: Arunachal Pradesh

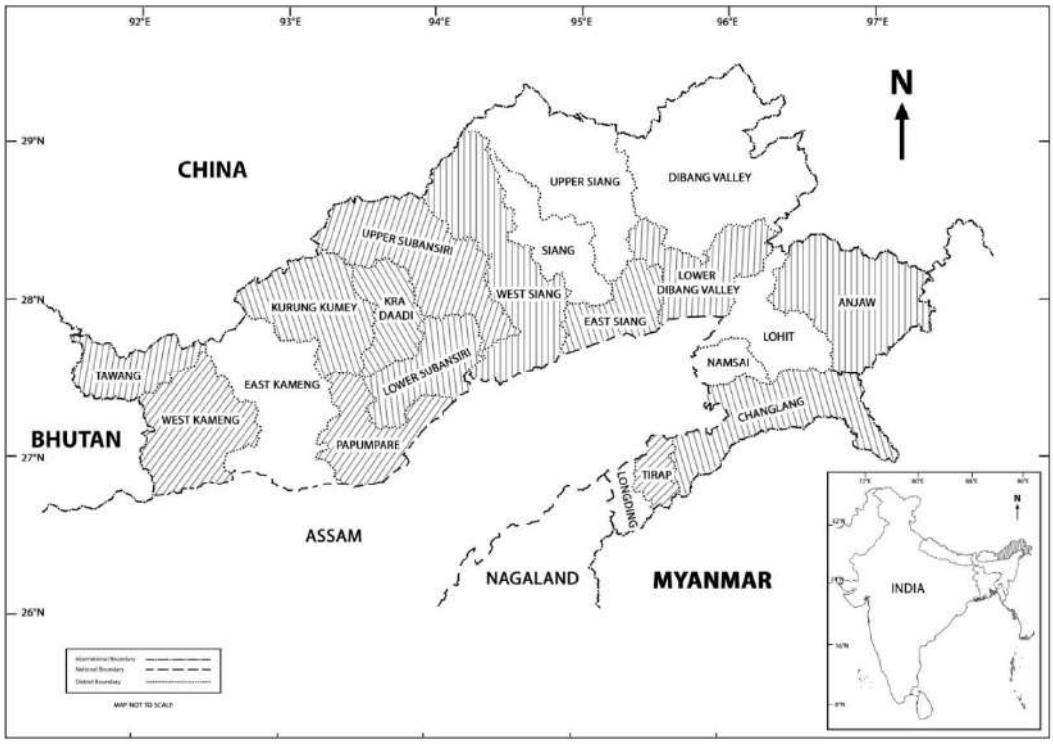


Fig 3.1: Map of Arunachal Pradesh; shaded regions denote areas visited (Source: District Administration Department; Credit Hage Konya Perme and Hage Yato)

Arunachal Pradesh, also known as the 'Land of Dawn Lit Mountain' is located on the North Eastern region of India in between 26° 28'N to 29° 30'N latitude and 91°30'E to 97°30'E longitude. The State is largest (covering 2.39% of India's total area) among the north-eastern States with its total area of 83,743 sq. km and popularly known as the 'Orchid State of India' or the 'Paradise of the Botanist' (Dept. of Environment and Forest, Arunachal Pradesh, 2014). The State is divided into 25 administrative districts at present with Itanagar as the capital. The State shares its boundary with China in the north, Bhutan in the west, Myanmar in the east, Assam and Nagaland in the south.

Climate type varies from Humid Subtropical to Subtropical Highland type to Alpine climatic type owing to changing elevation at each point. The State ranks second (area wise) in forest cover in whole of India. 79.96% of its area is under forest cover (Forest

Survey of India, 2017) and accounts for one third of the habitat area within the Eastern Himalayan Biodiversity Region. It is the 12th mega biodiversity hotspot of the world (Agarwal,1999) and regarded as the Ecological hotspot with about 5000 plant species of which 238 are endemic to the state (ENVIS: Arunachal Pradesh, 2016). The vegetations found here are divided as:

- Tropical Forest: up to the altitude of 900m from MSL.
- Sub-Tropical: up to the altitude of 1800m from MSL.
- Pine Forest: between 1000m to 1800m from the MSL.
- Temperate Forest: between 1800m to 3500 from MSL.
- Alpine Forest: at the altitude above 3000m.
- Secondary Forest: e.g. - Degraded forests, Bamboo forest and Grasslands.

Forest play significant role in the economic sector of the State as the inhabiting populations are mostly rural, which is about 79.6% of the population of the State.

The State has 28 major tribes with 110 sub-tribes (Tag *et al*., 2005). Adi, Apatani, Aka, Galo, Khampti, Mishmi, Monpa, Nocte, Nyishi, Tagin, Tangsa, Shingpo, Sherdukpen etc are some of the major tribes of Arunachal Pradesh.

Chapter 4
Documentation of Minor Fruit Plants of Arunachal Pradesh and their Ethnobotanical accounts.

4.1 Introduction

Written records of uses of plants dates back from ancient times as can be seen from renowned works of Theophrastus, Ibn al-Baitar and Burkill, to name a few (Nirmalkumar, 2014). Such documentation has helped us trace back the forgotten values of plants around us. As per WHO plants have procured special place in health sector especially in developing countries where most of the population are economically weaker and backward (Awoyemi *et al*., 2012). The indigenous communities have always deemed plants as mystic on its own ways and still believe to consider them as natural healers (Abbink, 1995). Their ecological as well as economic importance has been well understood by them (Leonti *et al*., 2003). Today's traditional health care system is stated to be resultant of oral transcendence of primeval knowledge system around the world (Gurib-Fakim, 2006). As such ethnobotany and traditional knowledge system go hand-in- hand. Traditional knowledge system is passing-on of any kind of knowledge/information that is practiced by an indigenous community in pursuit of keeping a balanced ecology and maintaining a sustainable biodiversity (Dennis *et al*., 1995; Amend, 2008). Ethnobotanical studies deal with the uses of plants in traditional and cultural facet of the human civilization. Voeks (2017) describes ethnobotany as "the study of the dynamic relationship between plants and people". Such studies have led to many drug discoveries around the world (Newman *et al*., 2000; Samuelsson, 2004; Butler, 2004). Plants are always looked upon for new remedies, be it from ancient records or a new venture into scientific world. They are inevitable part of our life and time and again the importance of ethnobotany will surface yet again.

Various ethnobotanical surveys have been done in Arunachal Pradesh. According to Mao and Roy (2016) 14 publications have been reported under ethnobotany category from the State. Tangjang *et al*., (2011) has recorded ethnobotanical data from Adis of

Lower Dibang Valley, Noctes of Tirap district and Nyishis of Papumpare district with special emphasis on medicinal plants. Ethnobotanical data for Hill Miri tribe has been reported by Tag and Das (2004). Namsa *et al*., (2009) has reported on the anti-inflammatory plants used by the Khamptis of Lohit area. Other such ethnobotanical survey has been done by Namsa *et al*., (2011) on Monpa community, Bharali *et al*., (2017) on spices and condiments used by Adi, Apatani and Galo tribes and Murtem and Chaudhry (2016) has reported on the medicinal plants used by Tagin, Nyishi and Galo tribe of Upper Subansiri district of the State. Most of these records have taken into consideration any part of the plant useful for any valuable ethnobotanical parameter. However, the present record is based only on the ethnobotanical use value with respect to only minor fruits of Arunachal Pradesh and not on the context of any community or any particular specification as done by earlier authors.

Documentation and ethnobotanical data collection of minor fruits of Arunachal Pradesh were done during field surveys from 2015-2018. The surveys were carried out either on fruiting or flowering season for proper identification of samples. The geographical points of views were kept at forefront rather than administrative division for the same. Efforts were made to cover maximum places but only Tawang, West Kameng, Lower Dibang Valley, East Siang, West Siang, Kra Daadi, Kurung Kumey, Upper Subansiri, Lower Subansiri, Tirap, Anjaw, Papumpare, Changlang districts could be visited. To keep track of the ethnobotanical uses of minor fruits collected, data were recorded simultaneous during the field visit.

4.2 Materials and method

4.2(a) Preparation of herbarium: Healthy sample specimens were collected for preparation of herbarium according to Jain and Rao (1977). The sample specimens were first dipped in 4% formalin for few minutes to keep the tissues intact followed by

application of 2% mercuric chloride in 95% ethanol (Arnoldia, 1968) for protection against insect pests and finally pressed under papers for drying with heavy weight on the top for complete compression of the specimen. Voucher number and brief descriptions of the plant were noted down on the herbarium sheets.

4.2(b) Identification: Plant identification was done with the help of scientists of BSI-Itanagar, BSI-Shillong and Taxonomists of Department of Botany, Rajiv Gandhi University.Herbaria with the Official University Accession number were deposited at Rajiv Gandhi University Herbarium Repository under Dr. Hui Tag, Department of Botany, RGU.

4.2(c) Ethnobotanical records: Ethnobotanical data were collected from elderly indigenous people through "questionnaire based interview responses" (Schultes, 1960, 1962; Jain,1987).

4.2 (d) Data analysis: Data was arranged and analysed with the help of MS Word and MS Excel.

4.3 Result

4.3.1 Documentation of Minor Fruits of Arunachal Pradesh

Table 4.3.1.1: List of Minor fruits collected from the districts of Arunachal Pradesh

Sl. No.	Scientific name	Family	Habit	Edible part	Local name
1	*Actinidia arguta* (Siebold & Zucc.) Planch. Ex Miq.	Actinidiaceae	Climber	Mesocarp	Harkhu (Ap)
2	*Actinidia callosa* Lindl.	Actinidiaceae	Climber	Mesocarp	Anti tari (Ap)
3	*Aegle marmelos* (L.) Correa	Rutaceae	Tree	Mesocarp	Bael (As)
4	*Alpiniamalaccensis* (Burm.f.) Roscoe	Zingiberaceae	Herb	Mesocarp	Gumbabara (A)
5	*Annona squamosa* L.	Annonaceae	Small tree	Mesocarp	Shyam roma (M)
6	*Averrhoa carambola* L.	Oxalidaceae	Small tree	Whole fruit	Kurangi (Kh)
7	*Baccaurea ramiflora* Lour.	Euphorbiaceae	Small tree	Mesocarp	Khiju (T)
8	*Bischofia javanica* Blume	Phyllantaceae	Large tree	Whole fruit	Sitil aye (A)
9	*Calamus floribundus* Griff.	Arecaceae	Small tree	Whole fruit	Geying (A)
10	*Canarium strictum* Roxb.	Burseraceae	Tree	Pericarp and mesocarp	Konker (A)
11	*Cardamomum subulatum (*Roxb.) Kuntze	Zingiberaceae	Small plant	Mesocarp	Lachung paku (N)
12	*Citrus maxima* (Burm.) Osbeck.	Rutaceae	Small tree	Mesocarp	Rubup ape (N)
13	*Coix lacryma-jobi* L.	Poaceae	Shrub	Mesocarp	Tajariyang (Ap)
14	*Cotoneaster microphyllus* Wall.ex Lindl.	Rosaceae	Shrub	Whole fruit	Mhapek (MM)
15	*Dillenia indica* L.	Dilleneaceae	Tree	Pericarp	Champa (As)

16	*Diospyros kaki* L.f.	Ebenaceae	Small tree	Whole frnespouit	Jenggong (M)
17	*Diospyros lotus* L.	Ebenaceae	Small tree		Moremiji (Ap)
18	*Docynia indica* (Wall.) Decne.	Rosaceae	Tall tree	Whole fruit	Pecha (Ap)
19	*Elaeagnus latifolia* L.	Elaeagnaceae	Shrub	Pericarp and mesocarp	Dam mrep (M)
20	*Elaeocarpus floribundus* Blume.	Elaeocarpaceae	Tree	Pericarp and mesocarp	Jolpai (As)
21	*Embelia ribes* Burm.f.	Primulaceae	Small tree	Whole fruit	Mumbran (MM)
22	*Ficus auriculata* Lour.	Moraceae	Tree	Mesocarp	Taking (A)
23	*Ficus hispida* L.f.	Moraceae	Small tree	Mesocarp	Taku (A)
24	*Ficus semicordata* Buch.-Ham. ex Sm.	Moraceae	Tree	Mesocarp	Takop (Tg)
25	*Ficus subulata* Blume	Moraceae	Semi epiphyte	Mesocarp	Siireh maloh (Ap)
26	*Fragaria indica* Andrews	Rosaceae	Herb	Whole fruit	Aki tayin (Ap)
27	*Fragaria vesca* L.	Rosaceae	Herb	Whole fruit	Sah mrep (M)
28	*Garcinia pedunculata*Roxb. ex Buch.-Ham.	Clusiaceae	Large tree	endocarp	Miibya (N)
29	*Garcinia anomala* Planch. & Triana	Clusiaceae	Small tree	Pericarp and mesocarp	Taktar (Tg)
30	*Gaultheria fragrantissima* Wall.	Ericaceae	Shrub	Pericarp and mesocarp	Shegshing mrep (M)
31	*Holboelllia latifolia* Wall.	Lardizabalaceae	Climber	Mesocarp	Shulumba (M)
32	*Juglans regia* L.	Juglandaceae	Large tall	Endocarp	Ruuh (Nc)
33	*Lithocarpuselegans* (Blume) Hatus.ex Soepadmo	Fagaceae	Large tree	Endocarp	Tibeh (Ap)
34	*Lithocarpus fenestratum* (Roxb.) Rehder	Fagaceae	Tree	Endocarp	Kra ahi (Ap)

35	*Livistona jenkinsiana* Griff.	Arecaceae	Tall tree	Mesocarp	Tokopatta gutti (N)
36	*Macrosolen cochinchinensis* (Lour.) Tiegh.	Loranthaceae	Parasitic small tree	Whole fruit	Ngiiongi (MM)
37	*Magnolia champaca* (L.) Baill. ex Pierre	Magnoliaceae	Large tree	Whole part	Salyo (Ap)
38	*Mahonia nepaulensis* DC.ex Dippel	Berberidaceae	Small tree	Whole fruit	Thaming (Ap)
39	*Melastoma malabathricum* L.	Melastomataceae	Shrub	Endocarp	Dai dasa (N)
40	*Morus indica* L.	Moraceae	Small tree	Whole part	Hinska (A)
41	*Myrica esculenta* Buch.-Ham. ex D. Don	Myricaceae	Tree	Mesocarp	Baching (Ap)
41	*Nephelium lappaceum* L.	Sapindaceae	Tree	Mesocarp	Wild lichi
43	*Passiflora edulis* Sims	Passifloraceae	Climber	Endocarp	Bhel rhi (Nc)
44	*Pegia nitida* Colebr.	Anacardiaceae	Shrub	Whole fruit	Eyi dorge (A)
45	*Pinanga gracilis* Blume	Arecaceae	Small tree	Pericarp and mesocarp	Tachar (N)
46	*Prunus cerasoides* Buch.-Ham. ex D.Don	Rosaceae	Tree	Pericarp and mesocarp	Sembo (Ap)
47	*Prunus nepalensis* Koch.	Rosaceae	Tree	Pericarp and mesocarp	Chod rhi (Nc)
48	*Pyrus pashia* Buch.-Ham. ex D.Don	Rosaceae	Tree	Pericarp and mesocarp	Jaatoh (M)
49	*Quercus lamellosa* Sm.	Fagaceae	Tree	Endocarp	Santih (Ap)
50	*Quercusoxyodon* Miq.	Fagaceae	Tree	Mesocarp	Sankhe (Ap)
51	*Quercus semecarpifolia* Sm.	Fagaceae	Tree	Endocarp	Pah sheng grabo (M)
52	*Rhus chinensis* Mill.	Anacardiaceae	Tree	Whole fruit	Taam ahi (N)
53	*Rosa sericea* Wall, ex Lindl.	Rosaceae	Shrub	Thalamus	Jamkhuyu (M)

54	*Rubus calycinus* Wall. ex D.Don	Rosaceae	Herb	Whole fruit	Subu tutey (Ap)
55	*Rubus ellipticus* Sm.	Rosaceae	Shrub	Whole fruit	Mipya jilyung (Ap)
56	*Rubus fairholmianus* Gardn.	Rosaceae	Shrub	Whole fruit	Mipya yoyu(Ap)
57	*Rubus niveus* Thunb.	Rosaceae	Shrub	Whole fruit	Yikhe jilyung (Ap)
58	*Rubus wardii* Merr.	Rosaceae	Shrub	Whole fruit	Thuglong(M)
59	*Saurauia armata* Kurz.	Actinidiaceae	Tree	Whole fruit	Pupururu (N)
60	*Saurauia nepaulensis* DC.	Actinidiaceae	Tree	Endosperm	Hinchi (N)
61	*Spondias axillaries*. Roxb.	Anacardiaceae	Large Tree	Mesocarp	Biiling (Ap)
62	*Spondias pinnata* (L.f.) Kurz.	Anacardiaceae	Large tree	Pericarp and mesocarp	Sudumpona (N)
63	*Sterculia lanceifolia* Roxb.	Sterculiaceae	Small tree	Endocarp	Taklam (G)
64	*Stixis suaveolens* (Roxb.) Pierre	Resedaceae	Small tree	Mesocarp	Rokpu tong (N)
65	*Syzygium jambos* (L.)Alston	Myrtaceae	Small tree	Mesocarp	Adi jamun (A)
66	*Terminalia bellerica* (Gaertn.) Roxb.	Combretaceae	Tree	Pericarp and mesocarp	Barbah (M)
67	*Tetrastigma rumicispernum*	Vitaceae	Liana	Whole fruit	Riich (N)
68	*Viburnum erubescens* Wall.	Adoxaceae	Shrub	Whole fruit	Yoyu (Ap)
69	*Zizyphus mauritiana* Lam.	Rhamnaceae	Tree	Pericarp and mesocarp	Bogori(As)

Note: A= Adi; Ap= Apatani; As= Assamese; G= Galo; N= Nyishi; Nc= Nocte; T= Tangsa; Tg= Tagin; MM= Miju Mishmi; Kh= Khampti

Table 4.3.1.2: Number of Collections Made Per Family

Sl.No.	Family	Number of collection
1	Actinidiaceae	4
2	Adoxaceae	1
3	Anacardiaceae	4
4	Annonaceae	1
5	Arecaceae	3
6	Berberidaceae	1
7	Burseraceae	1
8	Clusiaceae	2
9	Combretaceae	1
10	Dilleneaceae	1
11	Ebenaceae	2
12	Elaeagnaceae	1
13	Ericaceae	1
14	Euphorbiaceae	1
15	Fagaceae	5
16	Juglandaceae	1
17	Lardizabalaceae	1
18	Loranthaceae	1
19	Magnoliaceae	1
20	Melastomataceae	1
21	Moraceae	5
22	Myricaceae	1
23	Myrtaceae	1
24	Oxalidaceae	1
25	Passifloraceae	1
26	Phyllantaceae	1
27	Poaceae	1
28	Primulaceae	1
29	Resedaceae	1
30	Rhamnaceae	1
31	Rosaceae	13
32	Rutaceae	2
33	Sapindaceae	1
34	Sterculiaceae	1
35	Vitaceae	2
36	Zingiberaceae	2

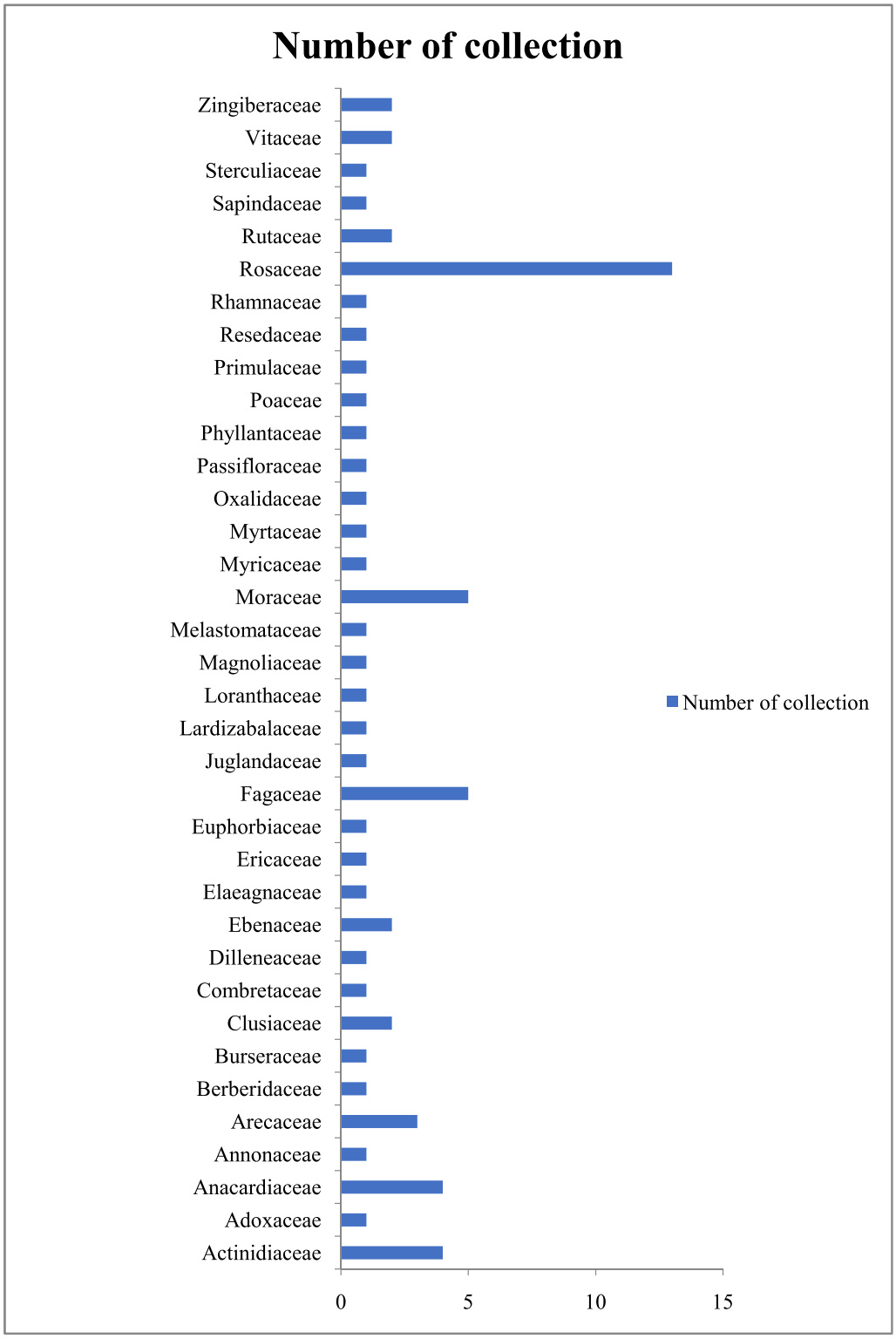


Fig 4.3(A): Graphical representation of total Number of Collection vs. Families collected

4.3.2Ethnobotanical data of Minor Fruits of Arunachal Pradesh.

Table 4.3.2.1: Ethnobotanical uses of Minor fruits collected from the district of Arunachal Pradesh

Sl. No.	Scientific name	Family	Local name	Uses
1	*Actinidia callosa* Lindl.	Actinidiaceae	Anti tari (Ap)	Beverage
2	*Cardamomum subulatum (*Roxb.) Kuntze	Zingiberaceae	Lachung paku (N)	Appetizer
3	*Annona squamosa* L.	Annonaceae	Shyam roma (M)	Spiritual value of whole plant, indigestion
4	*Averrhoa carambola* L.	Oxalidaceae	Kurangi (Kh)	Jaundice
5	*Baccaurea ramiflora* Lour.	Euphorbiaceae	Khiju (T)	Constipation
6	*Coix lacryma-jobi* L	Poaceae	Anayat (A)	Urinay trouble, diarrhoea
7	*Cotoneaster microphyllus* Wall.ex Lindl.	Rosaceae	Mhapek (MM)	Ornamental
8	*Dillenia indica* L.	Dilleneaceae	Champa	Anti-dandruff
9	*Diospyros kaki* L.f.	Ebenaceae	Jenggong (M)	Spiritual value of fruits
10	*Diospyros lotus* L.	Ebenaceae	Moremiji (Ap)	Fever
11	*Docynia indica* (Wall.) Decne.	Rosaceae	Pecha (Ap)	Loose motion
12	*Elaeagnus latifolia* L.	Eleagnaceae	Dam mrep (M)	Beverage
13	*Embelia ribes* Burm.f.	Primulaceae	Mumbran (MM)	Herbal preparation
14	*Ficus auriculata* Lour.	Moraceae	Taking (A)	Laxative
15	*Ficus hispida* L.f.	Moraceae	Taku (A)	Herbal preparation
16	*Ficus semicordata* Buch.-Ham ex Sm.	Moraceae	Takop (Tg)	Menstruation

17	*Fragaria vesca* L.	Rosaceae	Sah mrep (M)	Tonic
18	*Garcinia anomala* Planch. & Triana	Clusieaceae	Taktar (G)	Inflammation
19	*Gaultheria fragrantissima* Wall.	Ericaceae	Shegshing mrep (M)	Stomach problems
20	*Macrosolen cochichinensis* (Lour.) Tiegh	Loranthaceae	Ngiiongi (MM)	Source of natural gum
21	*Mahonia nepalensis* DC.ex Dippel	Berberidaceae	Thaming (Ap)	Diuretic
22	*Melastoma malabathricum* L.	Melastomataceae	Dai dasa (N)	Dye yielding
23	*Myrica esculenta* Buch-Ham.ex D. Don	Myricaceae	Baching (Ap)	Stomachic
24	*Nephelium lappaceum* L.	Sapindaceae	Wild lichi	Fever
25	*Passiflora edulis* Sims	Passifloraceae	Bhel rhi (Nc)	Tonic
26	*Pinanga gracilis* Blume	Arecaceae	Tachar (N)	consumed in place of betel nut
27	*Rhus chinensis* Mill.	Anacardiaceae	Taam ahi (N)	Blood dysentrry
28	*Saurauia nepaulensis* Roxb.	Actinidiaceae	Hinchi (N)	Spiritual value of whole plant
29	*Spondias pinnata* (L.f.) Kurz.	Anacardiaceae	Sudumpona (N)	Asthma , throat pain
30	*Sterculia lanceifolia* Roxb.	Sterculiaceae	Taklam (G)	As seasoning
31	*Syzygium jambos* (L.) Alston	Myrtaceae	Adi jamun (A)	Anaemia
32	*Terminalia bellerica* (Gaertn.) Roxb.	Combretaceae	Barbah (M)	head ache, chest pain , flu
33	*Viburnum erubescens* Wall.	Adoxaceae	Yoyu (Ap)	Cough tonic

Note: A= Adi; Ap= Apatani; As= Assamese; G= Galo; N= Nyishi; Nc= Nocte; T= Tangsa; Tg=Tagin; MM= Miju Mishmi; Kh= Khampti

4.4 Discussion

Total collection of 69 minor fruits was made in 36 Families from 13 districts. Rosaceae family had the highest collection with 13 species followed by Fagaceae and Moraceae at 5 species each. *Ficus carica*, *Carissa carandas*, *Durio zibethinus*, *Tamarindus indica*, *Grewia subinaequalis*,*Aegle marmelos*, *Dimocarpus longan*are some of the other minor fruits of Arunachal Pradesh reported by Hazarika and Lalruatsangi (2016). Ethnobotanical records were obtained for only 33 numbers of species. It was observed that people had no much idea about other uses of those fruits other than just consuming them. Ethnobotanical uses of some of these minor fruits have been reported earlier as well. *Pyrus pashia* has been reported as latent beverage plant while *Quercus semecarpifolia* has been reported to lessen appetite (Tsering *et al.,* 2017) *Nephelium lappaceum* has been reported to have anti-carminative property. *Syzygium jambos* has been reported to cure diarrhoea and dysentery. *Dillenia indica* has been reported to be useful against rheumatism. *Diospyros kaki*fruits have been reported to cure sore throats (Hazarika and Lalruatsangi, 2016). *Baccaurea ramiflora* has been reported to be anti inflammatory (Hossain, 2017).*Prunus nepaulensis* fruits have been reported as functional food and helpful for treating hepatic problems (Chaudhuri *et al*., 2015).Some of these fruits or fruit trees had strong cultural and belief system associated with them (Singh and Asha, 2017). According a responder in Tawang *Annona squamosa* trees are planted in the compound of one's house for spiritual well being and prosperity of the family. *Prunus cerasoides* tree is also considered sacred in Hindu belief (Joseph *et al*, 2018). *Melastoma malabathricum* fruits are used as holy offering to Diety for healthy harvest of paddy by Adi community (Srivastava and Adi, 2008). During the ethnobotanical data collection, it was learned that*Averrhoa carambola, Dillenia indica, Docynia indica, Myrica esculenta, Elaeocarpus floribundus, Zizyphus mauritiana*

weremade into pickles or jams or candies for later consumption. The same has been reported for*Eleagnus latifolia, Dillenia indica, Prunus nepalensis* (Patel *et al*., 2008a and 2008b; Gandhi and Mehta, 2013;Makdoh *et al*., 2014) and their juices as instant energy booster. *Rosa sericea* has been reported to be a digestive by Tsering *et al*.(2017). Literature survey of these collected fruits showed that all of them have chief role in maintaining healthy lifestyle or had important pharmacological property. *Dillenia indica* has been reported to be laxative in nature and possess anti-leukemic property against the affected lines of cell (Kumar *et al*., 2010). They also alleviate dandruff issues, flatulence, boils on skin, fever, dysentery, diarrhoea, diabetes, constipation and as hepatoprotective in nature (Pradhan and Badola, 2008; Gandhi and Mehta, 2013;Bhagyasri *et al*., 2017). They are also used freshly in culinary to enhance the taste of dishes (Kumar *et al*., 2011). *Rhus chinensis* has been reported as important traditional medicine in Sikkim area (Sharma *et al*., 2019). They are known to treat diarrhoea and dysentery and have the potential to act as antibacterial and anti-HIV effect (Shim *et al*., 2003; Bose *et al*., 2008). Value added products such as *Rhus chinensis* tablets and candy has been reported by Heirangkhongjam & Ngaseppam (2019). *Livistona jenkinsiana* has been reported to have anticancer property and described as important bio-cultural plant of Adi community of Arunachal Pradesh by Payum (2018). *Szyzium jambos* fruits have been reported as hepatoprotective and diuretic in function (Morton, 1987) and also beneficial against problems of constipation, muscle cramp, skin problems and helpful for developing immunity (Kuiate *et al*., 2006; Rx foundation, 2019). The vibrant anthocyanins from *Prunus nepalensis* fruits are used as astringent (Chaudhuri *et al*., 2015) and has been reported to be potential candidate as colouring agent that can replace artificial agents used in food industry (Swer *et al*., 2016). The fruits of *Myrica esculenta* are important part of

Ayurvedic medicinal system and are reported to have antimicrobial and anti-inflammatory property (Joshi *et al*., 2013). Apart from those therapeutic properties, this plant has been recognized for improving the soil quality by harvesting nitrogen from the atmosphere into the soil (Haridasan and Rao, 1987). *Ficus semicordata* have been reported to be useful in treating upset stomach and relieve headaches (Manandhar, 1992; Dhami, 2008). *Cotoneaster microphyllus* plant was recorded to have ornamental value during the study. The berries of this genus has been reported to contain cyanogenic glycosides and categorised under Level 4 toxic plants by the California Poison Control Centre. The level of these glycosides was reported to be affected by seasonal variation as well as age of plant (Swati *et al*., 2018). *Embelia ribes* usage has been in herbal medicinal system since ages. The bioactive compound 'embelin' present in this fruit has been of multiple therapeutic actions such as anti-inflammatory, anti-astringent, anti-fertility, antimicrobial, anti-carminative and useful for curing ulcers, respiratory malfunction, stomach problems, obesity, liver problems, mental illness and many more (Stasiuk and Kozubek, 2011;Nazish *et al*., 2012;Lal and Mishra, 2013). Fruits of *Pyrus pashia* are known to be useful for quenching thirst and act against stomach problems, sore throat, eye infections, urinary problems and constipation (Lama *et al.*, 2001; Chettri *et al*., 2005;Khandelwal *et al*., 2008;Abbasi *et al*., 2013;Siddiqui *et al*., 2015; Rawat *et al*., 2015). Fruits of *Docynia indica* are popularly used for preparation of alcohol, syrup and vinegar (Lua *et al*., 2013). They are known to have hypoglycaemic, anti-microbial, anti-inflammatory property and reduce hypertension (Do, 2004; Nguyen *et al*., 2011; Vo, 2012;Hoang *et al*., 2018). Shende *et al*., (2016) has recognized this fruit as potent food preservative. It is a known fact *Terminalia bellerica* is one of the important ingredients of popular "Triphala" available in Ayurvedic herbal practice. It is useful against cough, piles, bone problems, diabetes, leprosy, HIV,

hypertension, liver cirrhosis, microbial infections etc (Valsaraj *et al*., 1997; Aqil *et al*., 2007; Saraswathi *et al*., 2012; Chauhan *et al*., 2013; Tanaka *et al*., 2016; Dharmaratne *et al*., 2018). Ripe fruits of *Prunus cerasoides* are reported to produce brandy (Tanaka and Nakao, 1976). *Cardamomum subulatum* have been reported to cure respiratory problems, nausea and skin irritation (Verma *et al*., 2012).*Gaultheria* species have been reported to cure cold, rheumatism, inflammation and prostatitis etc (Liu *et al*., 2013). Methyl salicylate compound in this species is considered to be responsible for the medicinal property of this plant (Pandey *et al*., 2017). *Spondias pinnata* fruits are reported to be appetizer, enhance blood circulation, ease rheumatism and act as aphrodisiac (Vijetha *et al.*, 2018). *Actinidia arguta* is considered as "superfood" with efficient mineral content, nutritional and antioxidant property along side medicinal value such as anti-allergic, anti-inflammatory, help maintaining blood glucose level and enhancing cardiovascular health. (Ferguson and Ferguson, 2003; Lim *et al.,* 2006; Liu and Liu, 2016; Leontowicz *et al*., 2016;Latocha, 2017). *Annona squamosa* fruit is also reported to quench thirst, act as stomachic, antihelminthic and prevent ulcers and bones problems (Yoganarsimhan, 2000; Nadkarni, 2000;Park *et al*., 2011). Fruits of *Eleagnus latifolia* have been reported to contain essential fatty acid (Patel *et al*., 2008a). *Diospyros lotus* fruit are reported to act as stomachic, febrifuge, sedative (Kuroanagi *et al.,* 1971; Gao *et al*., 2014; Gul *et al.,* 2014). Among the collections made, *Embelia ribes, Rhus chinensis, Livistona jenkinsiana* have been reported to be endangered, vulnerable or near threatened species (Rolla and Joseph, 1962; Anonymous, 1987; Srivastava, 2006; ENVIS, 2017). These species need special care to help them survive and thrive in the changing environment. Activities such as practise of shifting cultivation, deforestation for mega projects such as highway, ring-road, dams and increasing human settlement towards jungle were found to be causative of losing these

minor fruits. Forest survey of India (2017) has identified jhuming and developmental activities as the basis for decrease in forest cover in the State. With the decreasing forest cover there is lesser and lesser interaction with nature and natural products. As a result, there is a diminishing interest in consuming these fruits yet alone the traditional knowledge system. It was noted that urbanization too played a key role in diminishing these interests. Taking on modern culture by discarding indigenous cultural practise also has been reported to attribute to the loss of traditional knowledge system (Kong *et al*., 2003; Berhanemeskel, 2009). As such, passing of indigenous knowledge system is observed to be at a vulnerable stage.

4.5 Conclusion

The State of Arunachal Pradesh has good number of fruits with beneficial bioactivities. Most of them are underutilized due to lack of scientific knowledge among the indigenous population. As per the ethnobotanical data collected and literature review one can say that consuming these fruits according to its time of seasonal availability would keep one hale and hearty. Proper documentation and ethnobotanical records may help recognize these fruits to larger community. Practise of passing on of knowledge system from a generation to another should be practised more often to save the extinguishing flame of traditional knowledge system. Proper compilation of scientific data may be used as a bridge between transient generations. Also such data could help save the germplasm of biodiversity.

Chapter 5

Sample selection and Preliminary tests

5.1 Introduction

Phytochemicals are plant-derived chemicals, produced through primary and secondary metabolism in plants(Koche *et al*., 2016). Sugars, Proteins, Nucleic acids, Chlorophylls etc are some examples of primary metabolites while Terpenoids, Phenolic acids, Saponoins, Alkaloids etc are some examples of secondary metabolites. They are liable for the scent, savour and color of a plant along with protection against diseases, pests etc. Preliminary detection of these phytochemicals gives us some cue about the property of plants of which we are interested into (Senguttuvan *et al*., 2014; Watal*et al.*, 2014). Thus the samples were examined for qualitative property before quantification of antioxidant and nutritional parameters. The selections of fruits were made on the basis of already mentioned objective no. 2 of proposed research work. These samples were then subjected to preliminary assay for detection of photochemical associated with the objective no.3 and 4.

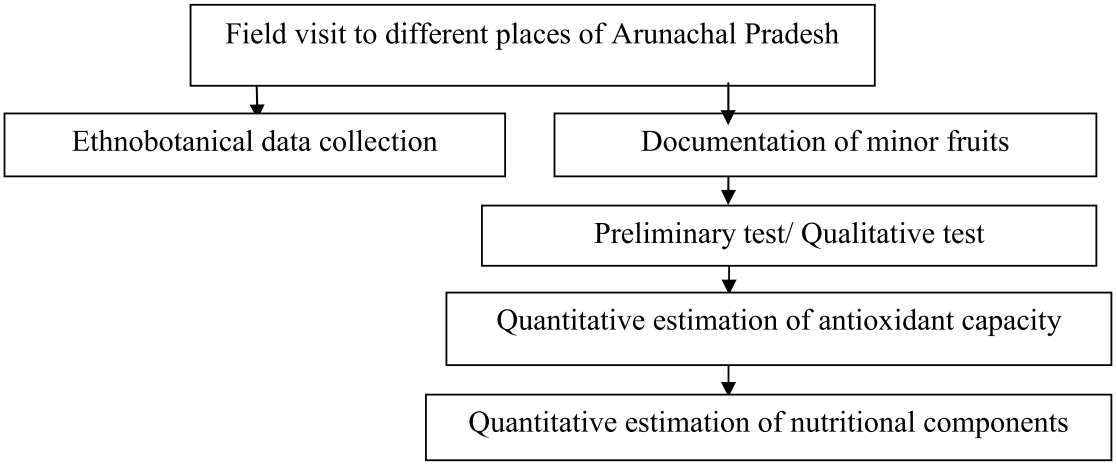


Fig 5(A): Systematic approach for qualitative and quantitative analysis.

5.2) Materials and method

(*a) Sample selection*:

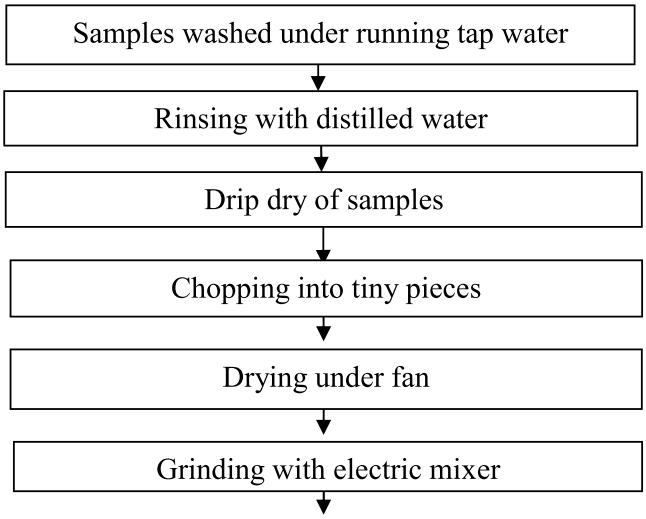
Sample selection was done by Random sampling technique (Taherdoost, 2016) due to limitation of time period. Matured healthy fruits were chosen for the experiments.

(b) Preparation of plant samples:

Sample preparation was done according to Hebber and Nalini (2014). Samples first washed under running tap water followed by rinsing with distilled water. They were then chopped into tiny pieces and shade dried under fan at room temperature for few days until fully dried. They were then grinded into powder with the help of electronic blender. Such grinding of sample increases their surface area of contact with solvent (Bandiola, 2018). The powdered samples were then stored in an airtight container for future use.

(c) Preparation of plant extracts:

Sample extracts were prepared by maceration (Bandiola, 2018). 20 grams of powdered samples were dissolved in 100mL of methanol in a conical flask and covered well with aluminium foil. The setup was kept for a week with daily intermittent shaking. Owing to the polar nature of the solvent, during this period, phytoconstituents get absorbed out by the solvent. After the completion of a week, the filtrate was collected using Whatman® Filter Paper number 1. The filtrate was then concentrated with the help of Buchi® Rotary Evaporator until a sticky mass of extract was obtained. The extracts were then transferred into 2mL centrifuge tubes and kept at 0°C until further use (Bandiola, 2018).



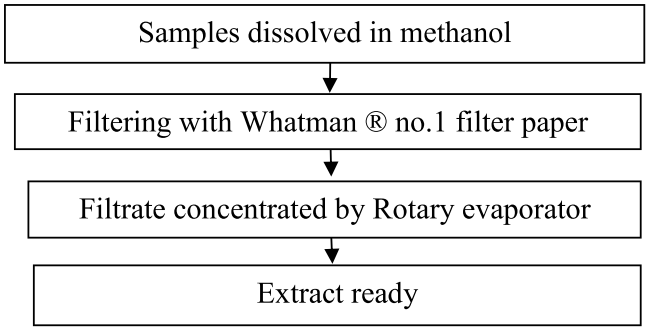


Fig: 5 (B): Systematic flow chart of extract preparation

The yield % of samples were calculated using the formula-

$$\% \text{ Yield} = \frac{\text{Crude yield extract (gm)}}{\text{Amount of dried sample Used for extraction (gm)}} \times 100$$

(d) Preliminary Analysis/Qualitative assay

Preliminary tests were conducted according to standard methods of various authors (Raaman, 2006; Bhandary*et al*., 2012; Gupta *et al*., 2013; Sheel *et al*., 2014):

(d.1) Preparation of reagents:

i. *Lead Acetate solution*:10% lead acetate in distilled water
ii. *Gelatin solution*:1% gelatin solution containing 10% sodium chloride in distilled water
iii. *Ferric chloride solution*: 5% ferric chloride in distilled water
iv. *Alkaline solution:* 10% sodium hydroxide in distilled water.
v. *Biuret reagent:* 2% copper sulphate solution; 95% Ethanol; Ammonium hydroxide pellets.
vi. *Ninhydrin reagent solution:* 0.2gm Ninhydrin dissolved in 10mL ethanol
vii. *Molisch reagent:* 5% alpha naphthol dissolved in 95% Ethanol.

viii. *Benedict's reagent*: Copper sulphate17.3gm, sodium carbonate100 gm, sodium citrate 173gm mixed together to form a total volume of 1 Liter.

ix. *Wagner's regent*: 1.27gm Iodine and2gm potassium iodide dissolved in 100 mL of distilled water.

(d.2) Experimental procedures:

For detection of phenolic compounds, flavonoids, tannin, protein, free amino acid, carbohydrate, sugar and saponin the solvent-free extracts were dissolved in distilled water while for alkaloids they were dissolved in diluted hydrochloric acid and for steroids in chloroform. They were then filtered using Whatman® filter paper no.1 and filtrates were examined for following tests:

i. *Detection of Phenolic compounds*: By Lead Acetate test. 2mL of extract filtrate was treated with Lead Acetate reagent. Bulky white precipitate was indicative of positive result.

ii. *Detection of Tannin*: By Gelatin test. 2mL of extract filtrate was treated with 0.5 mL Gelatin reagent. White precipitate was indicative of positive result

iii. *Detection of Phenols*: By Ferric chloride test. 2mL of extract filtrate was treated with 3-5 drops of Ferric chloride solution. Formation of dark green color was indicative of positive result.

iv. *Detection of Flavonoids*: By Alkaline reagent test. 2mL of extract filtrate was treated with 0.5mL of alkaline reagent. Yellow fluorescence indicated a positive result.

v. *Detection of Protein*: By Biuret test. 2mL of extract filtrate was treated with 2 drops of Copper sulphate solution, followed by addition of 1mL ethanol and excess of Ammonium hydroxide pellets. Formation of Pink color on the ethanolic layer indicated positive result.

vi. *Detection of Free Amino acids*: 2 mL of extract filtrate was treated with 3 drops of Ninhydrin. Purple color denoted positive result.

vii. *Detection of Carbohydrate*: 2 mL of extract filtrate was treated with Molisch reagent. Followed by addition of 0.5 ml Conc. Sulphuric acid by the side of test tube. Formation of violet ring indicated positive result.

viii. *Detection of Sugars:* By Benedict's test. 2mL of extract filtrate was treated with 0.5 mL Benedict's reagent and heated for 2 minutes on boiling water bath. Formation of reddish-orange colored precipitate indicated positive result.

ix. *Detection of Fats*: By Saponification test. 2ml of filtrate containing 4-5 drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein was heated on water bath for 2 hours. Soap formation indicated a positive result.

x. *Detection of Alkaloids*: By Wagner's test. 2mL of extract filtrate was treated with Wagner's reagent. Formation of reddish brown precipitate indicated a positive result.

xi. *Detection of Steroids and Terpenoids*: By Salkowski's test. 2mL of extract filtrate mixed with 2mL of concentrated Sulphuric acid and shaken vigorously. Red color on chloroform layer and yellowish green color on acid layer gave positive inference.

xii. *Detection of Saponin*: By Foam test. 5mL of extract filtrate was shaken for 15 in vortex shaker. Formation of thick layer of foam indicted their presence.

5.3 Result

Table 5.3.1: Yield % of samples dissolved in Methanol

Sl.No.	Samples	Amount of dried sample used (gm)	Amount of Crude extract obtained (gm)	Yield %
1	*Actinidia arguta*	20	2.13	10.65
2	*Annona squamosa*	20	4.12	20.6
3	*Canarium strictum*	20	2.63	13.15
4	*Cardamomum subulatum*	20	2.58	12.90
5	*Cotoneaster microphyllus*	20	4.45	22.25
6	*Dillenia indica*	20	4.74	23.70
7	*Diospyros kaki*	20	4.58	22.90
8	*Diospyros lotus*	20	4.04	20.20
9	*Docynia indica*	20	6.11	30.55
10	*Elaeagnus latifolia*	20	4.22	21.10
11	*Embelia ribes*	20	0.96	4.80
12	*Ficus semicordata*	20	0.89	4.45
13	*Gaultheria fragrantissima*	20	4.61	23.05
14	*Holboelllia latifolia*	20	2.53	12.65
15	*Lithocarpus fenestratum*	20	0.65	3.25
16	*Livistona jenkinsiana*	20	2.14	10.70
17	*Macrosolen cochinchinensis*	20	1.04	5.20
18	*Magnolia champaca*	20	3.00	15.00
19	*Mahonia nepaulensis*	20	5.04	25.20
20	*Myrica esculenta*	20	2.00	10.00
21	*Prunus cerasoides*	20	5.25	26.25
22	*Prunus nepalensis*	20	4.60	23.00
23	*Pyrus pashia*	20	5.30	26.50
24	*Quercus semecarpifolia*	20	0.25	1.25
25	*Rhus chinensis*	20	4.54	22.70
26	*Rosa sericea*	20	2.38	11.90
27	*Saurauia armata*	20	0.81	4.05
28	*Spondias axillaries*	20	5.85	29.25
29	*Spondias pinnata*	20	3.78	18.90
30	*Syzygium jambos*	20	4.28	21.40
31	*Terminalia bellerica*	20	4.96	24.80
32	*Viburnum erubescens*	20	1.86	9.30

Table 5.3.2: Result of Qualitative Phytochemical Screening.

Sl. No.	Samples	Qualitative Tests for nutritional and antioxidant component											
		Lead Acetate test (Phenolic compounds)	Gelatin test (Tannin)	Ferric Chloride test (Phenol)	Alkaline reagent test (Flavonoid)	Biuret test (Protein)	Ninhydrin test (Free amino acid)	Molisch test (Carbohydrate)	Benedict's test (Sugar)	Saponification test (Fats)	Wagner's test (Alkaloids)	Salkovski's test (Steroids and terpenoids)	Foam test (Saponin)
1	*Actinidia arguta*	+-	+-	+-	-	+	+-	++	+	+	-	+	+
2	*Annona squamosa*	-	-	+-	+-	++	+-	++	+	-	-	-	-
3	*Canarium strictum*	+	+	+-	+	+	+	++	+	+	-	++	+-
4	*Cardamomum subulatum*	+-	-	+	+	+	-	++	++	-	-	+-	+
5	*Cotoneaster microphyllus*	+	+	+	+	+	-	++	+	-	-	-	-
6	*Dillenia indica*	++	++	++	++	++	+-	++	+	-	-	+-	+
7	*Diospyros kaki*	+	+	+	+	++	-	+	+	-	-	+-	+-
8	*Diospyros lotus*	+	+	+	+	+	-	+	+	-	-	+-	+-
9	*Docynia indica*	++	++	++	++	++	+	++	+	-	-	-	-
10	*Elaeagnus latifolia*	+-	-	+-	+	+-	-	++	+	-	-	+-	+-
11	*Embelia ribes*	++	++	++	++	+	+	+	+-	-	-	+-	-
12	*Ficus semicordata*	+	+	+	+	+-	-	+	+	-	-	+	+-
13	*Gaultheria fragrantissima*	+-	-	+	+	+	-	+	+	-	-	+	+

14	*Holboelllia latifolia*	+-	-	+-	+-	-	-	++	++	-	-	-	+-
15	*Lithocarpus fenestratum*	+-	-	+	+-	-	-	++	+	+	-	+	-
16	*Livistona jenkinsiana*	++	++	++	+	+	+	+	+	+	-	+-	-
17	*Macrosolen cochinchinensis*	++	+-	++	+	+-	-	+	+	-	-	+	+
18	*Magnolia champaca*	+	+-	+-	+	+-	-	+	-	-	+	+	+
19	*Mahonia nepaulensis*	+	-	+-	+		+-	+	+	-	+	+-	-
20	*Myrica esculenta*	++	++	++	+	+-	+	+	+	-	-	+	+-
21	*Prunus cerasoides*	+-	-	+-	+	+	-	++	++	-	-	+-	-
22	*Prunus nepalensis*	++	-	+	+	+	-	++	++	-	-	+-	-
23	*Pyrus pashia*	+-	-	+-	+	+	-	++	++	-	-	+	-
24	*Quercus semecarpifolia*	++	++	++	++	++	+-	++	++	-	-	+-	-
25	*Rhus chinensis*	++	+	++	+	+	-	+	+	-	-	+-	+
26	*Rosa sericea*	++	++	++	++	+	+-	+	+	-	-	+-	-
27	*Saurauia armata*	++	+-	+	+	+-	-	+	+	-	-	+	-
28	*Spondias axillaries*	++	++	++	+	+	+-	++	++	-	-	+-	-
29	*Spondias pinnata*	++	++	++	+	+	+-	++	++	-	-	+	-
30	*Syzygium jambos*	+	-	++	++	+	+	++	+	-	-	+-	-
31	*Terminalia bellerica*	++	++	++	+	+	+-	++	+	-	-	-	-
32	*Viburnum erubescens*	-	-	+	+	+	+	+	+	-	-	+	-

Note: + = Present; ++ = Present in high amount; +- = present in negligible amount; - = absent.

5.4 Discussion

The yield % obtained (Table 5.3.1) as well as detection of various preliminary tests (Table 5.3.2)is affirmative of methanol as a suitable solvent for the samples in the present study. The polar nature of methanol could be the reason for extraction of components from the sample.

Based on the preliminary tests it is observed that on the nutritional aspect, all of the samples were detected positive for carbohydrate content, making them an efficient source of energy. From the positive inference of Benedict's test, it is observed that these samples contain reducing sugar which can act as a reducing agent in our system. All except *Magnolia champaca* gave positive Benedicts test. Presence of protein in these samples indicates that fruits can also be a reliable source of protein and help in body's growth and development. *Annona squamosa, Dillenia indica, Diospyros kaki, Docynia indica* and *Quercus semecarpifolia*were detected to have comparatively more protein content than rests of the other detectable samples' amount. Free amino acid content was detected only *Canarium strictum, Embelia ribes, Livistona jenkinsiana,Syzyzium jambos* and *Terminalia bellerica*. It could be because the rest samples had too little free amino acid content making them difficult to detect. Fat content was detected in *Actinidia arguta, Canarium strictum, Lithocarpus fenestratum* and *Livistona jenkinsiana*. The presence of fats along with carbohydrate makes them an enormous energy source.

Secondary metabolites such as phenols, flavonoids and other phenolic compounds are well recognized for antioxidant activity. Their preliminary detection in almost all samples is indicative of the samples' potential antioxidant capabilities. In case of detection of alkaloids only 2 samplesviz.*Magnolia champaca* and *Mahonia nepaulensis* gave positive test. Saponin content was detected in*Actinidia arguta, Cardamoum*

subulatum, Dillenia indica, Gaultheria fragrantissima, Lithocarpus fenestratum, Macrosolen cochinchinensis, Magnolia champaca and *Rhus chinensis.* Steroids were either not detected or were present in negligible amount. From the literature study it is learned that secondary metabolites such as flavonoids terpenoids, alkaloids, saponins, glycosides are bioactive in nature and act as analgesic, antimicrobial, anti-inflammatory, antimalarial,diuretic, cytotoxicity, anticancer, antihelmintic, antiadiarrhaeal, anti-mutagenic, antioxidant substance besides serving as febrifuge, hepato-protector and cardioprotector (Wadood *et al.,* 2013; Dua *et al*., 2013; Thite *et al*.,2013; Ajiboye *et al*., 2013; Rajurkar& Gaikwad, 2012;Bhandary *et al*., 2012;Souto *et al*., 2011; Bachaya *et al.*, 2009; Mukhtar *et al*., 1988; Gali *et al*., 1992).

5.5 Conclusion

From the preliminary analysis it can be concluded that these tested minor fruits, in general, were tested positive for antioxidant and nutrition parameters.A quantitative approach towards these parameters using the standard test methods on the samples will give a comprehensive view about their actual potentiality on antioxidant and nutritional scale and add essence to their property in totality.

Chapter 6

Quantitative Estimation of Antioxidant activity

6.1 Introduction

All living beings undergo oxidation and reduction in their system to keep themselves alive. During such processes, free radicals or molecular species are formed. These species have unpaired electron(s) and are highly reactive despite their short life-span. They are collectively known as Reactive Oxygen Species (ROS) and have the capacity to oxidize other atoms/molecules and cause cellular damage by reacting with nucleic acids, lipids, proteins and enzymes etc. (Kunwar and Priyadarsini, 2011; Kumar, 2014). Hydroxyl radicals, hydrogen peroxide, superoxide anion, singlet oxygen, hypochlorite radical and nitric oxide radical are to name a few. They have been known to be part of some of the important metabolic functions which include oxidative phosphorylation for production of energy, xenobiotics detoxification, cell death (apoptosis), phagocytosis by cytotoxic lymphocytes, gene expression, embryonic developments, thyroxine synthesis, production of prostaglandins and leukotrienes, regulation of neuro-transmittance, cell signalling etc (Schreck and Baeuerle, 1991; Droge, 2002; Devasagayam *et al.,* 2004; Kunwar and Priyadarsini, 2011). Under the normal healthy condition, the body can cope up such reactive species by maintaining a balanced state between their production and confiscation. But as one ages, the body's ability to defend themselves downgrade overtime. Other factors which catalyse formation of ROS comprise smoking (Devasagayam and Kamar, 2002), consumption of tobacco (Wijk*et al*., 2008), exposure to ionizing radiations such as X-rays and γ-rays, UV-radiations (Devasagayam*et al.,* 2004), heavy metals (Wijk*et al*., 2008), pollutants, infection, intense exercise (Kunwar and Priyadarsini, 2011) and antibodies as well (Wentworth *et al*., 2002). As an unstable species tries to stabilize itself by process of reduction, there is complementary oxidation on the reducing agent which again tries to stabilize itself by the same process which finally results into a never-ending chain of reactions. This situation is known as

"oxidative stress". With oxidative stress, the body becomes susceptible to many diseases leading to physiological transformations. Impairment of DNA, Alzheimer's disease, Parkinsons's disease, arthritis, cancer, diabetes, atherosclerosis, ageing etc. are some of the major outcomes of oxidative stress (Satyavati *et al*., 1976; Beckman and Ames, 1997; Kunwar and Priyadarsini, 2011; Kumar, 2014). However, nature has endowed these living beings with inherent property to keep in check these "pro-oxidants" by creating "anti-oxidants". Antioxidants are those substances which can neutralize an oxidative environment. They are capable to act even in a minute concentration (Halliwell, 1990, 1997). They do so by donating electron(s)/hydrogen(s) or scavenging them thereby mitigating the stressful oxidative condition. They are broadly classified into enzymatic antioxidants and non-enzymatic antioxidants. Superoxide dismutase (SOD) and glutathione peroxidase are categorized under primary enzymatic type while gluthathione reductase, glucose-6-phosphate comes under secondary enzymatic antioxidants. Vitamins, carotenoids, polyphenols, minerals etc comes under non-enzymatic type. These antioxidants help in maintaining a healthy life form by preventing or at least postponing the dangers of oxidative stress which were mentioned above.

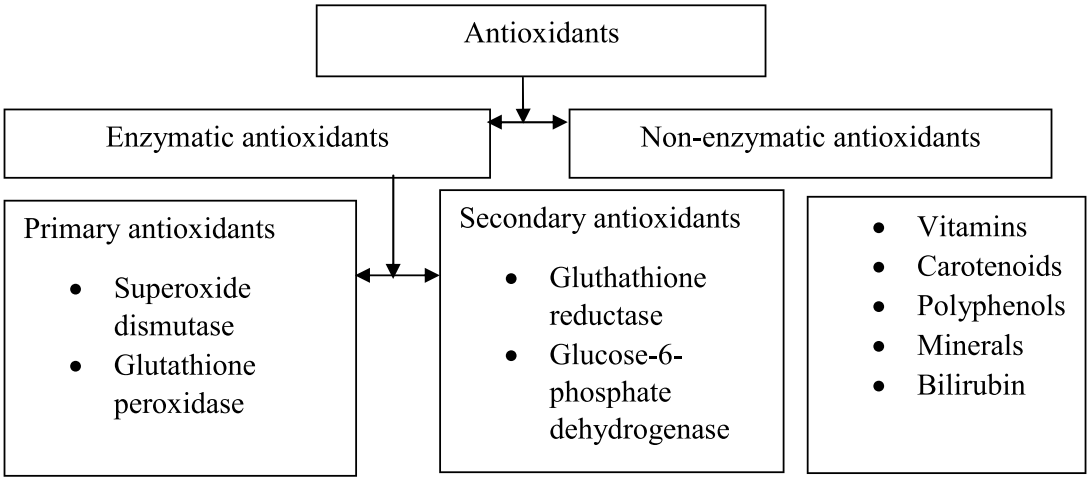


Fig 6.1(A): Diagrammatic representation of the classification of antioxidants (Mates, 2000; Kohen and Nyska, 2002; Kattapagari*et al*., 2019)

Sources of antioxidants may be synthetic or dietary. Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), tert-butylhydroxyquinone (TBHQ), propyl gallate (PG), dodecyl gallate (DG), octylgallate (OG) and ethylene diaminetetraacetic acid (EDTA) etc are some of the popular synthetic antioxidants (Atta *et al*., 2017). They are usually used in food industries as preservatives (Sherwin, 1976). Fruits and vegetables form the dietary source of antioxidants. Being a plant source, they are usually rich in secondary metabolites such as phenolic compounds, vitamins, carotenoids, nitrogen compounds etc which are known to have high antioxidant activity (Cotelle *et al*., 1996; Velioghu *et al*., 1998; Zheng *et al*., 2001; Cai *et al*., 2003). Some studies have shown that synthetic antioxidant BHT may have carcinogenic activity if taken in high concentration (Witschi, 1981; Kaczmarski *et al*., 1999). To overcome such issues of a synthetic product, finding natural sources of antioxidants has paved a new route for researchers around the world.

Fruits and vegetables have become essential sources of dietary nutrition as well as an antioxidant. Their consumption has been related to decreased chances of cancer, cataract, coronary diseases, diabetes, hypertension and obesity (Halvorsen*et al*., 2002). Antioxidant activity of commonly used fruits has been studied around the world (Proteggente *et al*., 2002; Karadeniz*et al*., 2005; Almeida *et al*., 2011; Arshiya, 2013). Antioxidant activity of minor fruits had been overlooked for a long time. Investigation of their antioxidant property has become a trend presently. Bellini and Giordani, (1998), Chalise*et al.,* (2010) and Feyssa (2011) have reported some minor fruits from various regions of the world. Data on different perspectives of minor fruit in India have been reported by various authors (Reddy *et al.,* 2006; Mitra *et al.,* 2008; Seal, 2011; Saini *et al.,* 2012; Prakash *et al.,* 2012; Seal, 2012; Paul, 2013; Singh *et al.,* 2014; Srivastava *et*

al., 2017). So in this research, an attempt was made to study the antioxidant activity of minor fruits found in Arunachal Pradesh.

Accordingly, after testing of qualitative parameters, quantitative analysis was performed. Quantitative estimations are expressed in a variable form to explore the possibilities of preconceived hypothesis. Various methods of techniques for quantification of antioxidant activity such as spectrometry, biosensoring, electrochemistry and chromatography are given below (Su *et al*., 2007; Giardi *et al*., 2010; Pisoschi *et al*., 2016):

- DPPH, ABTS, FRAP, PFRAP, CUPRAC ORAC, HORAC, TRAP, DMPD, PPR, iodimetry, Phosphomolybdenum method, Super Radical scavenging activity, Beta Carotene bleaching assay, Xanthin oxidase inhibition assay, Catalase activity method, Super Oxide dismutase assay, Lipid Peroxidation Inhibition and Fluorimetric assays etc. are detected by spectroscopy.
- Biosensoring involves monitoring of superoxide, nitric oxides, glutathione, phenolic compounds and enzyme-based biosensing etc.
- Cyclic voltammetry, Differential pulse voltammetry,Square-wave voltammetry, Potentiometry,Amperometry and Biamperometry are the electrochemical techniques.
- Gas chromatography, Thin Layer Chromatography, Liquid chromatography, High-Performance Thin Layer Chromatography and High-Performance Liquid Chromatography are some chromatographic techniques.

Phyto-constituents such as Flavonoids and phenolic compounds are acclaimed for the antioxidant property (Jahangir *et al*., 2009; Kasote *et al*., 2019; Park *et al*., 2019;). Thus quantification of flavonoid and phenolic compounds is an essential part of quantitative estimation of antioxidant activity.

6.2 Materials and methods

6.2.1 Sample collection and identification:

Sample collection and identification were done according to 4.2 (b)

6.2.2 Extract preparation:

Extract preparation of samples were done according to 5.2 (c)

6.2.3 Experiments performed:

a) DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

b) ABTS-TEAC (Trolox Equivalent Antioxidant Assay)

c) FRAP (Ferric Reducing Antioxidant Potential) assay

d) TAC (Total Antioxidant Capacity)

e) TPC (Total Phenol Content)

f) TFC (Total Flavonoid Content)

All of the above (6.2.3) mentioned assays are colorimetric assay and were detected by Thermo® Scientific Multiscanner with SkanIt software 4.1 for Microplate Readers. DPPH, ABTS-TEAC, FRAP and TAC are techniques for measuring antioxidant activity while TPC and TFC are for quantification of phytoconstituents phenolic compounds and flavonoids respectively.

For the preparation of concentration ranges of a sample or standard reference Beer-Lambert Law was maintained (Hardesty and Attili, 2010) which is defined by the formula-

$$\text{Absorbance} = \varepsilon \times C \times L$$

Where,

ε=molar extinction coefficient

c = concentration of samples (Moles/liter)

L= path length of light through the solution (cm)

For the determination of IC_{50} values, the concentrations were prepared such that at least twovalues each lied above and below 50% inhibition (Sebaugh, 2010)

6.2.3(a):DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH• is a dark-purplecoloured, stable free radical. The delocalized electron (•) gives it the distinctive violet colouration which is visible between 515-518 nm wavelengths (Pisoschi and Negulescu, 2011; Pisoschi *et al*., 2016). It can be dissolved in either methanol or ethanol (Apak*et al*., 2013). This method is one of the most common, easy and relatively fast assays for determination of antioxidant activity (Raghavendra *et al*., 2013; Wojdylo *et al*., 2007; Brand-Williams *et al*., 1995).

Principle:

When DPPH• radical comes in contact with an antioxidant (A-OH) or a radical species (R•), it gets reduced to molecular DPPH by the gain of a hydrogen atom from an antioxidant sample. It is observed by the disappearance of dark-purple colour. Depending upon the intensity of activity the colour may change from dark purple to yellow to pale yellow colour. Thus, more the discolouration, moreis the antioxidant activity of sample made to interact with DPPH• radical.

$$DPPH\bullet + A\text{-}OH \rightarrow DPPH\text{-}H + AO\bullet$$

$$DPPH\bullet + R\bullet \rightarrow DPPH - R$$

Chemicals required:

- 0.1mM DPPH
- Methanol
- Ascorbic acid
- Butylated hydroxyl toluene (BHT)
- Trolox
- distilled water

Chemical preparations:

(i) ***Preparation of 0.1mM DPPH solution***: 1.97mg DPPH was dissolved in 50mL methanol

Experimental procedure:

DPPH assay was performed according to Doley*et al.*, (2016). Samples were dissolved in methanol at various concentrations ranging from 3.125µg/mL to 1000µg/mL. Standard concentrations were prepared from 1.5625µg/mL to 100µg/mL. 100µL of the prepared sample and standard concentrations were reacted with 200µL of 0.1mM DPPH solution and allowed to stand in a dark condition at room temperature for half an hour. The control consisted of the solvent and DPPH solution. Blank constituted only the solvent. Radical scavenging power of samples was calculated using the formula-

$$\text{Inhibition \%} = \frac{\text{(O.D of Control-O.D of Sample)}}{\text{O.D of Control}} \times 100$$

Where, OD= Optical density

Inhibition % of each of the samples, as well as standards, was determined. Results were expressed as IC_{50} (µg/mL) values in comparison to standard references.

6.2.3(b): ABTS assay-TEAC (Trolox Equivalent Antioxidant Assay)

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) is a light green coloured compound. It is converted into its radical cation form($ABTS^{\bullet+}$)by reacting with peroxyl radicals (viz, MnO_2, $K_2S_2O_8$) or enzymes such as metmyoglobin or haemoglobin(Pellegrini *et al*., 2003;Thaipong *et al*., 2006;Su *et al*., 2007)to study about phenolic compounds, thiols and vitamin C and antioxidant activity of samples of interest (Sharma and Singh, 2013).

Principle:

ABTS radical cation ($ABTS^{\bullet+}$) is first produced by removing an electron from nitrogen atom of ABTS by reacting with potassium persulfate (oxidizing agent). During this transition, the colour of this reagent changes from light green to dark greenish–blue colour which can be detected at 734nm.This radical cation is then tested with samples with potential antioxidant activity. As the nitrogen atom quenches back hydrogen atom from the antioxidant sample, the colour changes from dark greenish-blue colour to light green to light pink colour. Like DPPH assay, here also the intensity of antioxidant activity is directly proportional to the discolouration of ABTS reagent.

ABTS +potassium persulfate → $ABTS^{\bullet+}$

$ABTS^{\bullet+}$+A-OH → ABTS + AO• + H^+

Chemicals required:

- 0.007 M ABTS
- distilled water
- ethanol
- methanol
- 0.00245 M potassium persulfate
- Trolox.

Chemical preparations:

(i) ***Preparation of 0.007M ABTS solution***: 7.68mg ABTS was dissolved in 2mL distilled water

(ii) ***Preparation of 0.00245M potassium persulfate solution***: 13.25mg potassium persulfate was dissolved in 20mL distilled water.

(iii)***Preparation of stock reagent solution***: ABTS reagent was prepared by mixing together 0.007M ABTS with 0.00245 M Potassium persulfate in 1:1 ratio (v/v).

The mixture was allowed to react for 16-24 hours at room temperature in dark condition. During this period an intense dark green coloured stable free radical cation ($ABTS^{\bullet+}$)is developed. This constitutes the 'Stock solution'.

(iv) ***Preparation of working reagent solution***: Few amounts from the stock solution were mixed with ethanol till an absorbance of 0.7 ± 0.50 was obtained at 734nm.

Experimental procedure:

ABTS assay was performed according to Suriyatem*et al*., (2017) with bit modifications.Samples were prepared as 1mg/mL or dilution of 1mg/mL as required following the Beer-Lambert Law.Troloxwas used as a standard reference. Control constituted ethanol and ABTS reagent while ethanol constituted the blank. 30µL of sample or standard was reacted with 170µL of working ABTS reagent solution. After incubation for 10 minutes' absorbance was noted at 734nm. Inhibitory % of samples was calculated using the same formula-

$$\text{Inhibition \%} = \frac{\text{(O.D of Control-O.D of Sample)}}{\text{O.D of Control}} \times 100$$

Where, OD= Optical density

The result obtained was compared with the standard reference curve by Trolox (4.7µM to 300µM). Results were then expressed as µMTrolox equivalents per gram dried sample.

6.2.3(c): FRAP (Ferric Reducing Antioxidant Potential) assay

It is a redox – linked colorimetric assay. It depends on the reducing ability of antioxidants fromferric ion-TPTZ (2, 4, 6-tri(2-pyridyl)-1,3,5-triazine) complex to

ferrous ion-TPTZ complex. FRAP assay has been known to measure the total reducing capability ofantioxidants (Gupta, 2015; C. Guo*et al*., 2003)

Principle:

At low pH(3.6), Fe(III)-TPTZ complex gets reduced to Fe(II)- TPTZ complex by gaining electron from antioxidants. As such, there is a change in colouration from light blue to dark blue colour which can be detected at 593nm. The intensity of blue colour development is directly proportional to the reducing ability of antioxidants (Lee *et al*., 2012)

$$Fe(TPTZ)_2^{3+} + ArOH \rightarrow Fe(TPTZ)_2^{2+} + ArO\bullet + H^+$$

Chemicals required:

- 300 mM Acetate buffer (pH 3.6)
- Sodium acetate trihydrate
- Glacial acetic acid
- Hydrochloric acid
- 10 mM TPTZ
- 20 mM Ferric chloride

Chemical preparations:

i. ***Preparation of 300mM Acetate buffer (pH 3.6)***: 0.31gm sodium acetate trihydrate was dissolved in 1.6ml glacial acetic acid and total volume was adjusted to 100ml with distilled water. pH was adjusted to 3.6 using HCl or NaOH before final volume was reached.

ii. ***Preparation of 10mM TPTZ solution***: 0.031gm TPTZ was dissolved in 10ml of 40mMHCl.

iii. ***Preparation of 40mM HCl***: 0.36ml HCl was mixed with distilled water to make a final volume of 100ml.

iv. ***Preparation of 20mM Ferric chloride solution***: 0.54gm ferric chloride was dissolved in 100ml distilled water.

v. ***Preparation of working FRAP reagent***: Working solution of FRAP reagent was prepared freshly by adding 300 mM Acetate buffer, 10 mM TPTZ and 20 mM Ferric chloride in 10:1:1 ratio.

Experimental Procedure:

The assay was performed according to Fidrianny*et al*., (2016) with a little modification. The sample preparation was done similar to the DPPH assay. Ferrous sulphate (1-100 µg/mL) used as the standard reference. 10µL of the sample was added to 300µL of working FRAP solution and incubated at 37°C for half an hour.

The result obtained was expressed as EC_1. EC_1 is defined as the concentration of an antioxidant having a ferric reducing ability equivalent to that of a 1mM ferrous salt. The smaller, the value of EC_1 the greater is the reducing capacity.

6.2.3(d): Total Antioxidant Capacity by phosphomolybdate method

Principle:

In the presence of antioxidants, at acidic pH and high temperature, the hexavalent form of molybdenum [Mo(VI)] is reduced to the pentavalent form [Mo(V)]. The phosphate/Mo(V) complex so formed is green coloured which can be detected by spectrophotometer at 695nm.

Chemicals required:

- 0.6 Molar sulphuric acid
- 28mM Sodium Phosphate
- 4mM Ammonium molybdate

Chemical preparations:

i. ***Preparation of 0.6M sulphuric acid***: 6.117ml of sulphuric was mixed with distilled water making a final volume of 1000ml.

ii. ***Preparation of 28mM sodium phosphate***: 0.0397gm sodium phosphate was dissolved in 10ml distilled water.

iii. ***Preparation of 4mM ammonium molybdate***: 0.0494gm ammonium molybdate was dissolved in 10ml distilled water.

iv. ***Preparation of working phosphomolybdate reagent***: 1 mL from each (i), (ii) and (iii) was mixed in 20mL of distilled water and final volume was made to 50mL with distilled water. Fresh working solution was prepared each time.

Experimental procedure:

The assay was done according to Olugbami*et al*., (2015). Sample concentration was prepared at 1mg/mL which was diluted if required for different samples. 100µL of the sample solutions were mixed with 1mL of the working reagent and incubated at 95ºC for 90 minutes in a water bath. On cooling down their absorbance was checked at 695nm against reagent blank. Blank consisted of 100µL of the solvent and 1mL of the working reagent. Ascorbic acid (1-100µg/mL) was used as the standard reference. Total antioxidant activity was calculated using the formula

$$A = (c \times V)/m$$

Where,

A= total content of antioxidant compounds (mg/gm plant extract) in ascorbic acid equivalent.

c= the concentration of ascorbic acid established from the calibration curve (mg/mL)

V=the volume of extract (mL)

m= the weight of crude plant extract (gm)

6.2.3(e): Total Phenol Content

Principle:

In alkaline medium, Folin-Ciocalteau gets reduced by gaining electron(s) from phenolic compounds. The blue chromophore so formed by this reaction can be detected between 750-765nm.

Chemicals required:

- 10% Folin-Ciocalteau
- 7% sodium carbonate

Chemical preparations:

i. ***Preparation of 10% Folin-Ciocalteau reagent***: 1ml Folin Ciocalteau reagent was mixed with 9ml distilled water.

ii. ***Preparation of 7% sodium carbonate solution***: 7 gm sodium carbonate dissolved in 100ml distilled water.

Experimental procedure:

Total phenol content was determined by the Folin-Ciocalteau method modified according to Pradip *et al*., (2016). Samples were prepared at a concentration of 1mg/mL which was diluted as per required Beer-Lambert Law for different samples.500µL of Folin-Ciocalteau was introduced into100 µL of sample, blank and gallic acid (1-100 µg/mL) and allowed to stand for 10 minutes. Subsequently, 400µL of sodium carbonate was added to the reaction mixture, vortexed briefly and incubated at 40°C for half an hour. On completion of the set time period, the absorbance was noted at 760nm against blank. Total phenol content was expressed as mg/g (Gallic acid equivalent) and calculated using the formula

$$TPC=(c \times V)/m$$

Where,

TPC= total phenol content (mg/g plant extract) in Gallic acid equivalent.

c= the concentration of gallic acid established from the calibration curve (mg/ml)

V= the volume of extract (ml)

m= the weight of crude plant extract(gm)

6.2.3(f): Total Flavonoid Content

Principle:

The carbonyl and hydroxyl groups of flavones and flavonols formed a yellow coloured complex with aluminium ion, Al(III) which can be detected at 510nm.

Chemicals required:

- 10% aluminium chloride
- 5% sodium nitrite
- 1M sodium hydroxide

Chemical preparation:

i. ***Preparation of 10% aluminium chloride***: 10gm aluminium chloride was dissolved in 100ml distilled water.

ii. ***Preparation of 5% sodium nitrite***: 5gm sodium nitrite was dissolved in 100ml distilled water.

iii. ***Preparation of 1M sodium hydroxide***: 8gm sodium hydroxide was dissolved in 200ml distilled water.

Experimental procedure:

Total flavonoid content was determined by Aluminium chloride method as described by Pradip *et al*., (2016). Samples were prepared at similar concentrations as in TPC assay (6.2.3.d). Rutin (1-100µg/mL) was used as the standard reference. 100µL of the sample, blank and standard (at different concentrations) was mixed with 400µL of distilled

water. 30µL of sodium nitrite was then added and allowed to stand for 5 minutes. Then 30µL of aluminium chloride was added and allowed to stand for 6 minutes. It was then followed by the addition of 200µL of 1M sodium hydroxide solution and 240µL of distilled water making a final volume of 1mL. The aliquots were vortexed briefly and incubated for 15 minutes at room temperature. The yellow chromophore at the end of the reaction was detected at 510nm. Total flavonoid content was expressed as rutin equivalent (RE) and calculated using the formula-

$$TFC=(c \times V)/m$$

Where,

TPC= total flavonoid content (mg/g plant extract) in Rutin equivalent.

c= the concentration of rutin established from the calibration curve (mg/mL)

V=the volume of extract (mL)

m= the weight of crude plant extract (gm)

6.2.4 Statistical Analysis

All the experiments were performed in independent triplicates. Results were expressed as Mean ± Standard Deviation. Graphpad Prism® software version 5.03 was used for deducing IC_{50}, EC_1 and unknown values of the samples. For finding IC_{50} values non-linear regression curve: log (inhibitor) vs. normalised response- variable slope was performed. EC_1 values were deduced from non-linear regression by log (agonist) vs. response (find EC anything) under Dose-response special. Linear regression model was used for interpolating unknown value. Pearsons Correlation analysis and ANOVA was performed accordingly

6.3 Result

Table 6.3.1: Antioxidant activity of sample extracts

Sl. No.	Samples	DPPH (IC_{50}µg/ml)	ABTS (mg TE/gm)	FRAP (EC_1µg/ml)	TAC (mg AAE/gm)
1	*Actinidia arguta*	251.700±19.152	0.018±0.005	33.777±20.955	2.955±0.542
2	*Annona squamosa*	435.800±46.969	0.018±0.001	121.800±19.789	1.541±0.458
3	*Canarium strictum*	281.067±12.258	0.040±0.007	69.260±0.276	3.201±0.693
4	*Cardamomum subulatum*	192.767± 2.914	0.184±0.016	19.963±2.501	12.674±1.163
5	*Cotoneaster microphyllus*	259.513±10.495	0.089±0.012	92.156±7.254	3.957±0.693
6	*Dillenia indica*	91.433±4.621	0.858±0.061	22.413±11.337	11.822±1.310
7	*Diospyros kaki*	159.933±45.059	0.007±0.005	40.323 ±5.173	2.142±0.262
8	*Diospyros lotus*	178.300±3.124	0.071±0.027	50.683±7.660	6.982±0.454
9	*Docynia indica*	23.413±1.625	0.565±0.075	5.627±2.730	11.822±2.284
10	*Elaeagnus latifolia*	14.18 ±0.240	0.916±0.018	1.770±1.332	4.259±0.454
11	*Embelia ribes*	10.155±0.525	2.125±0.056	9.751±0.562	27.704±0.524
12	*Ficus semicordata*	41.097 ± 1.796	0.056 ±0.023	15.302± 5.656	10.612±1.636
13	*Gaultheria fragrantissima*	92.109±1.800	0.091±0.057	49.897±18.137	8.797±0.454
14	*Holboelllia latifolia*	384.233±41.791	0.012±0.003	164.750± 3.889	3.352±0.454
15	*Lithocarpus fenestratum*	195.467± 6.047	0.160±0.015	92.651±2.163	5.016±0.262
16	*Livistona jenkinsiana*	**6.387±0.314**	2.500±0.014	**0.193 ±0.007**	**50.392±1.142**
17	*Macrosolen cochinchinensis*	22.110±0.609	1.916±0.514	3.378±2.435	12.881±1.361
18	*Magnolia champaca*	347.800±9.938	0.209±0.017	115.256 ±9.695	9.100±1.142
19	*Mahonia nepaulensis*	239.500 ±18.543	0.198±0.006	57.110±5.061	11.368±2.284
20	*Myrica esculenta*	102.673±3.898	0.250±0.003	12.173 ±1.680	11.477±0.791
21	*Prunus cerasoides*	313.000	0.052±0.005	69.503±3.854	1.9110±0.791

		±21.443			
22	*Prunus nepalensis*	332.733±8.631	0.125±0.012	72.380±7.953	5.167±0.454
23	*Pyrus pashia*	272.705±14.885	0.063±0.011	57.280 ±12.882	2.747±0.262
24	*Quercus semecarpifolia*	9.983±0.867	**2.525±0.014**	9.510±0.481	11.066±1.361
25	*Rhus chinensis*	9.738±0.089	2.484±0.034	8.433±1.698	8.797±0.454
26	*Rosa sericea*	24.473±1.524	2.347±0.026	5.982±1.023	13.335±0.786
27	*Saurauia armata*	86.680±2.635	0.207±0.047	30.143±3.851	4.562±0.262
28	*Spondias axillaries*	14.976±0.094	2.424±0.050	1.842±0.252	9.553±2.284
29	*Spondias pinnata*	13.180±0.117	2.281±0.104	2.027±0.776	9.856±0.693
30	*Syzygium jambos*	160.144±2.63	0.205±0.011	84.143±10.994	14.545±3.859
31	*Terminalia bellerica*	11.440±0.458	2.521±0.020	2.565±0.345	7.284±0.524
32	*Viburnum erubescens*	116.733±4.077	0.215±0.008	37.433±4.844	20.721±7.811

The values obtained are Mean±SD; n = 3 independent replicates; p value <0.0001 were considered significant.

Table 6.3.2: Quantitative Determination of Phenolic compounds and Flavonoids

Sl.no.	Samples	TPC (mg GAE/gm)	TFC (mg RE/gm)
1	*Actinidia arguta*	10.201±0.778	109.383 ±12.952
2	*Annona squamosa*	11.986±1.224	89.149±7.732
3	*Canarium strictum*	13.828±1.306	69.794±9.546
4	*Cardamomum subulatum*	126.627±7.121	177.122±6.983
5	*Cotoneaster microphyllus*	36.298±1.191	13.491±4.571
6	*Dillenia indica*	115.453±7.378	297.647±15.908
7	*Diospyros kaki*	24.096±1.675	2.145±1.312
8	*Diospyros lotus*	56.732±1.966	24.928±3.321
9	*Docynia indica*	148.054±5.201	322.279±19.528
10	*Elaeagnus latifolia*	31.832±0.718	8.213±1.320
11	*Embelia ribes*	292.687±9.074	261.577±5.752
12	*Ficus semicordata*	155.421±5.228	192.078±4.242
13	*Gaultheria fragrantissima*	73.412±1.266	46.041±2.016
14	*Holboelllia latifolia*	18.237±1.091	3.588±0.573
15	*Lithocarpus fenestratum*	24.752±0.938	2.934±1.320
16	*Livistona jenkinsiana*	**388.602± 32.878**	**921.485 ±43.419**
17	*Macrosolen cochinchinensis*	127.563±1.697	184.160±5.494
18	*Magnolia champaca*	52.229±1.912	176.243±6.772
19	*Mahonia nepaulensis*	5.845±1.798	152.490 ±11.529
20	*Myrica esculenta*	57.640±1.207	130.936 ±9.237
21	*Prunus cerasoides*	24.440±1.152	5.573 ±4.758
22	*Prunus nepalensis*	36.897±1.284	108.943±9.546
23	*Pyrus pashia*	21.471±1.488	108.063 ±8.786
24	*Quercus semecarpifolia*	353.699±3.186	92.228±2.747
25	*Rhus chinensis*	295.956±9.457	218.470 ±5.950
26	*Rosa sericea*	117.157±3.373	146.332 ±4.634
27	*Saurauia armata*	102.191±2.846	55.279±3.321
28	*Spondias axillaries*	209.375±1.153	60.997±2.639
29	*Spondias pinnata*	253.381±5.721	80.791±4.758
30	*Syzygium jambos*	82.345±4.989	165.246±5.752
31	*Terminalia bellerica*	461.126±2.912	107.000±2.016
32	*Viburnum erubescens*	104.151±3.047	148.091±8.241

The values obtained are Mean±SD; n = 3 independent replicates; p value <0.0001 were considered significant.

6.3(a) DPPH Assay:

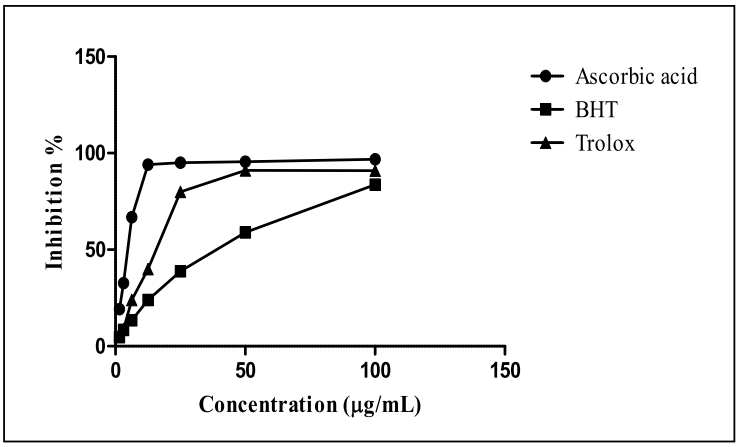


Fig 6(a-i): Graph of Concentration vs. Inhibition % of Standards (DPPH assay)

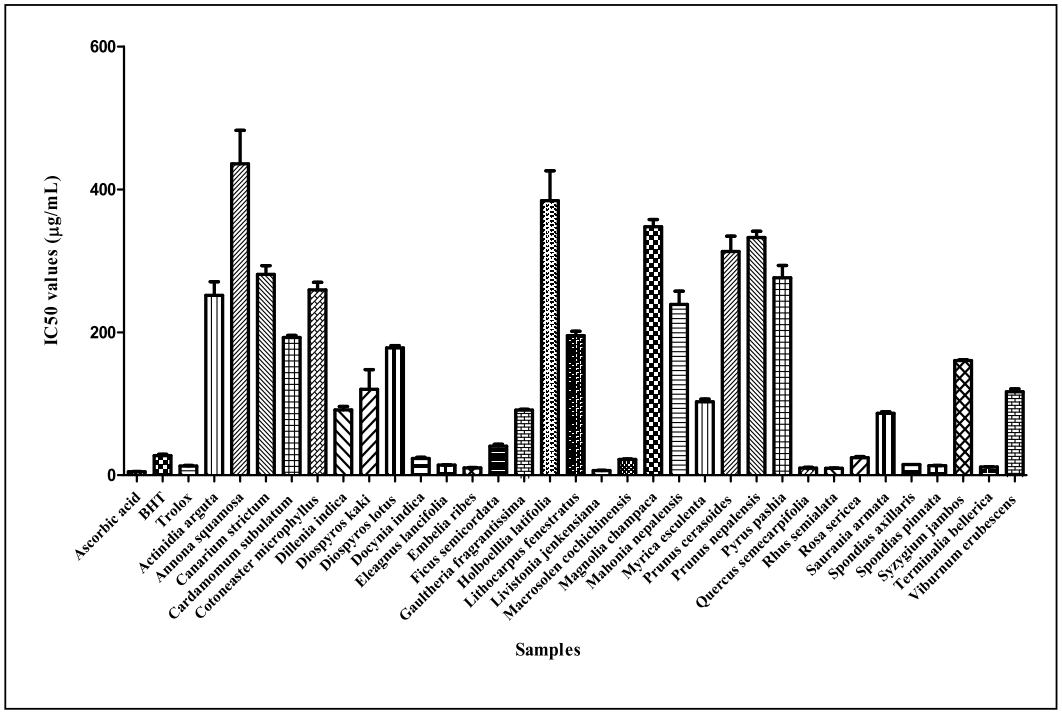


Figure 6 (a-ii): Graphical representation of IC_{50} values of standard and samples

6.3(b) ABTS Assay:

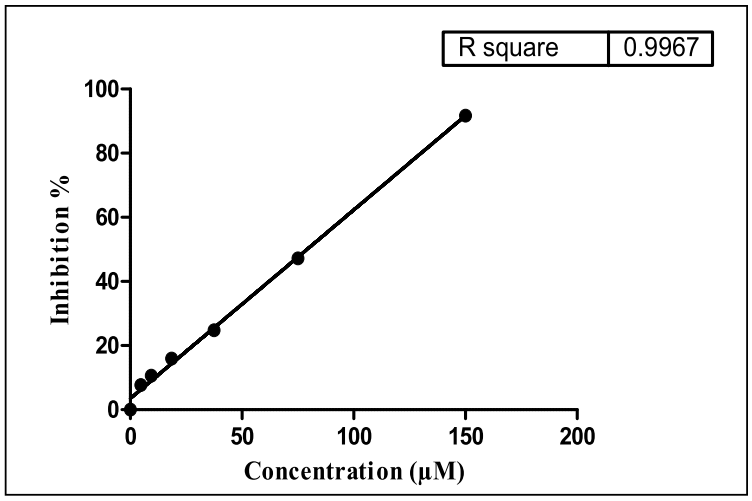


Fig 6(b-i): Graph of Concentration vs. inhibition % of Trolox (ABTS assay)

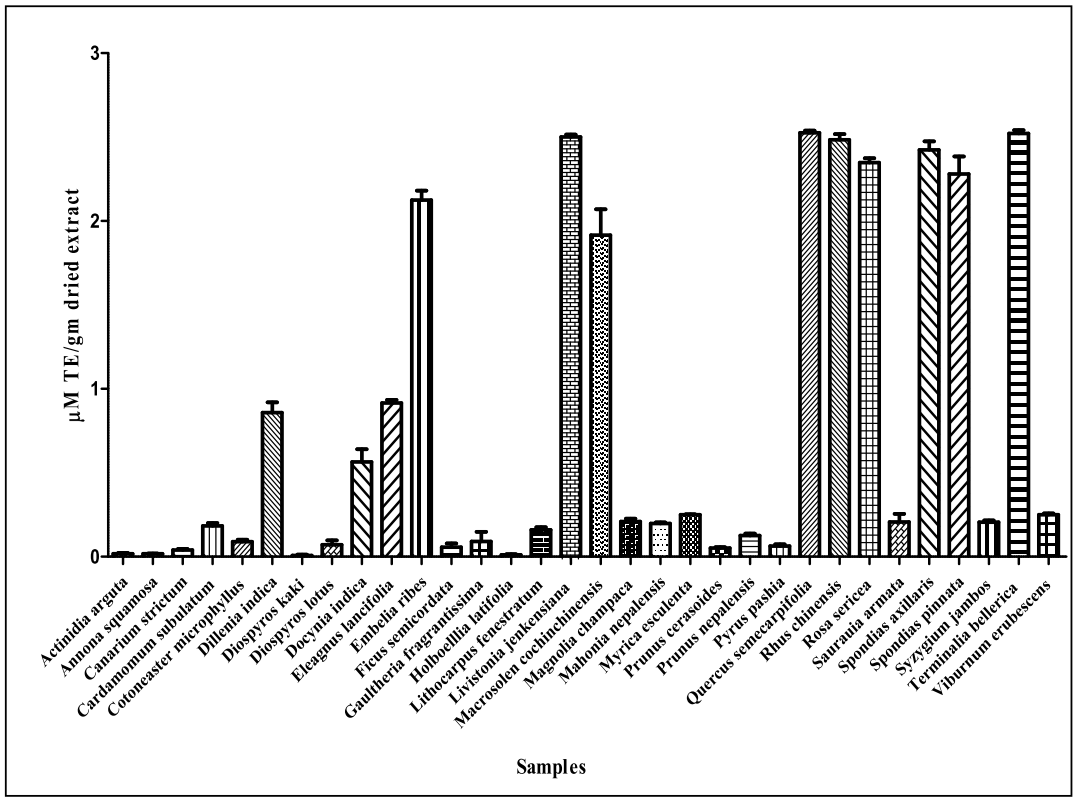


Fig 6(b-ii): Graphical representation of TEAC values of samples for ABTS assay

6.3 (c) FRAP Assay:

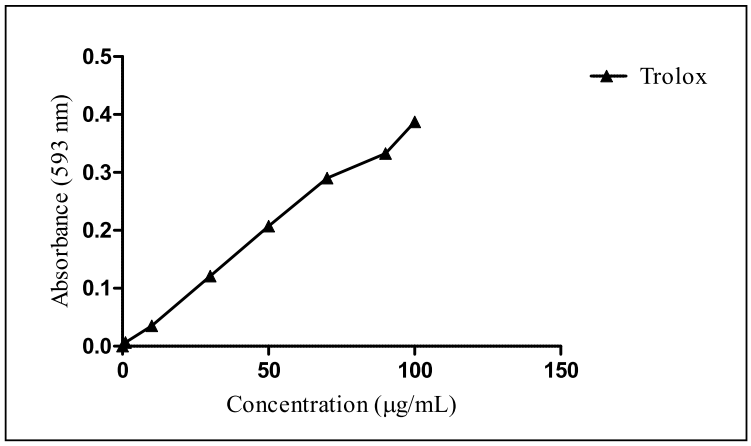


Fig 6(c-i): Graph of Concentration vs. Absorbance of Trolox (FRAP assay)

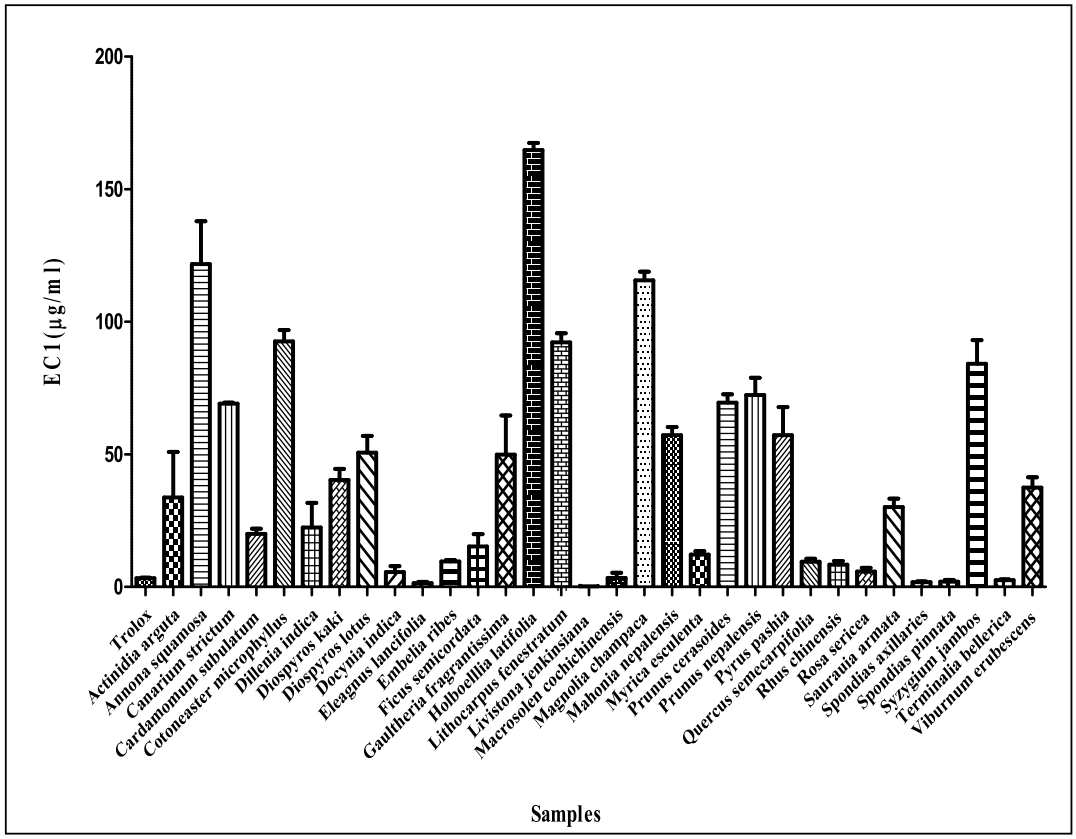


Fig6(c-ii): Graphical representation of EC1 values of standard and samples

6.3 (d) Total Antioxidant Activity:

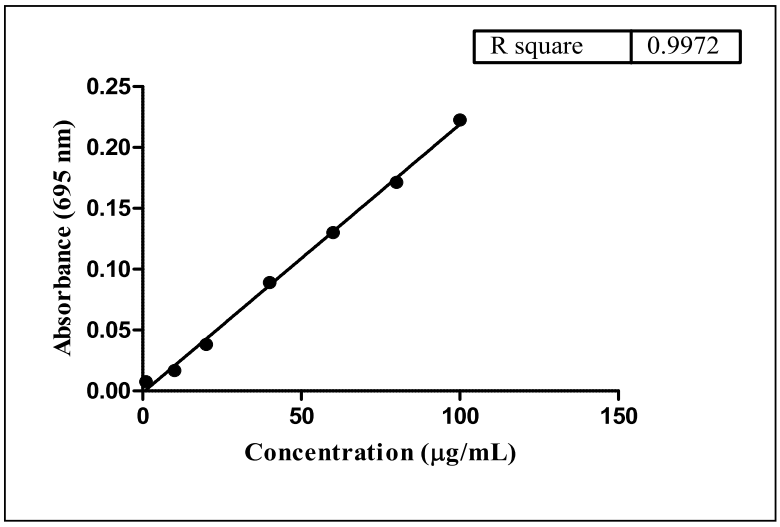


Fig 6(d-i): Graph of Concentration vs. Absorbance of Ascorbic acid (TAC assay)

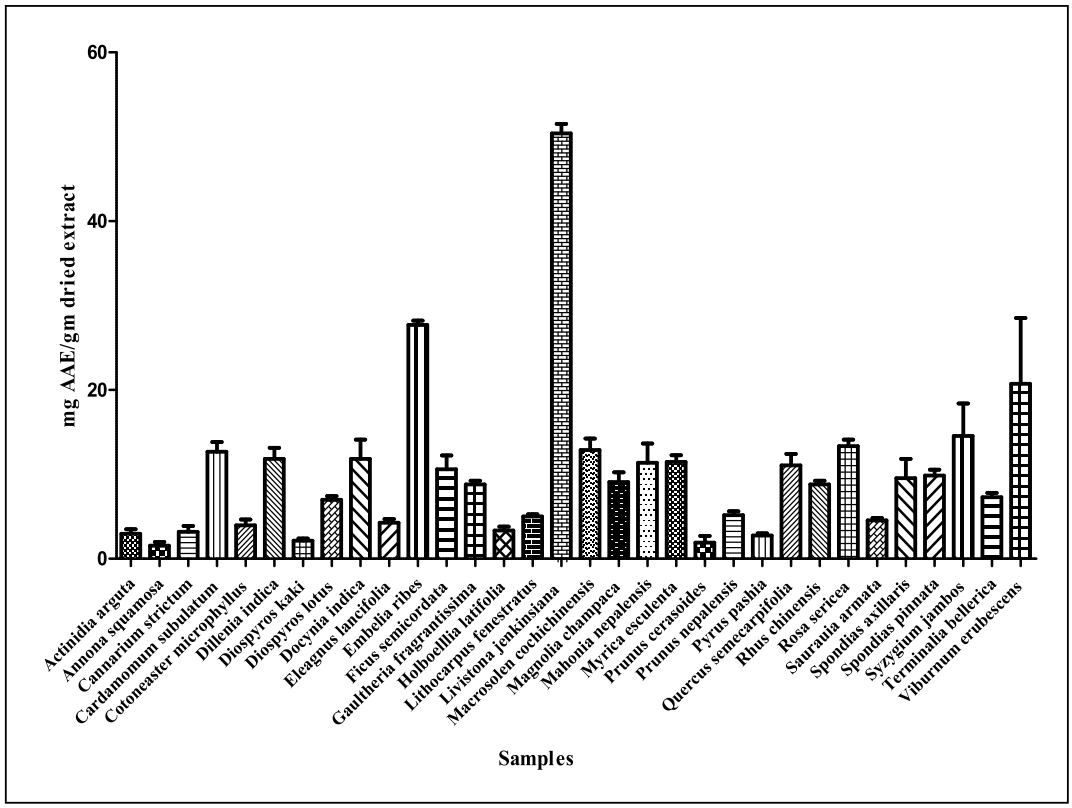


Fig 6(d-ii): Graphical representation of Total Antioxidant Activity of samples

6.3 (e) Total Phenol Content Determination:

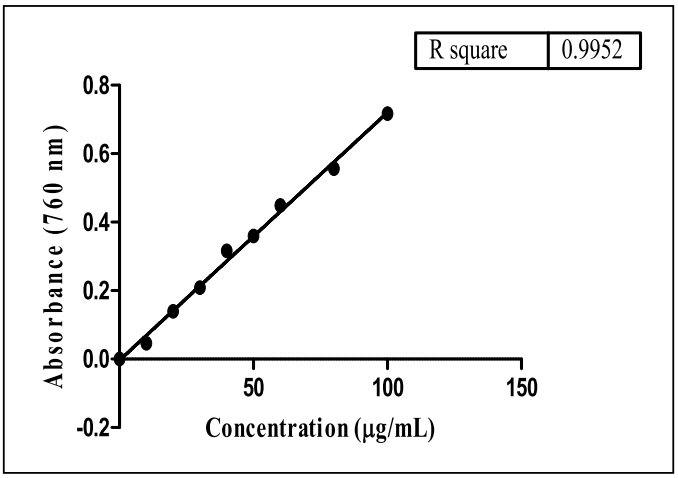


Fig 6(e-i): Graph of Concentration vs. Absorbance of gallic acid (TPC assay)

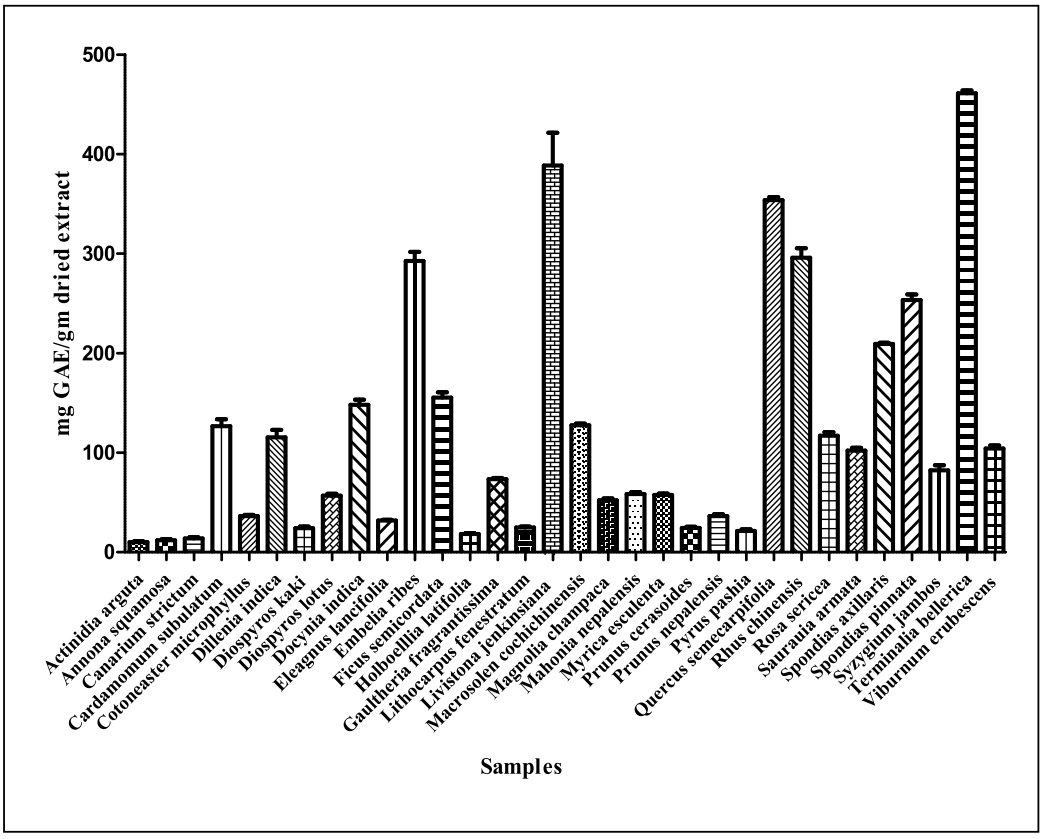


Fig 6(e-ii): Graphical representation of Total Phenol Content in samples

6.3(f) Total Flavonoid Content Determination:

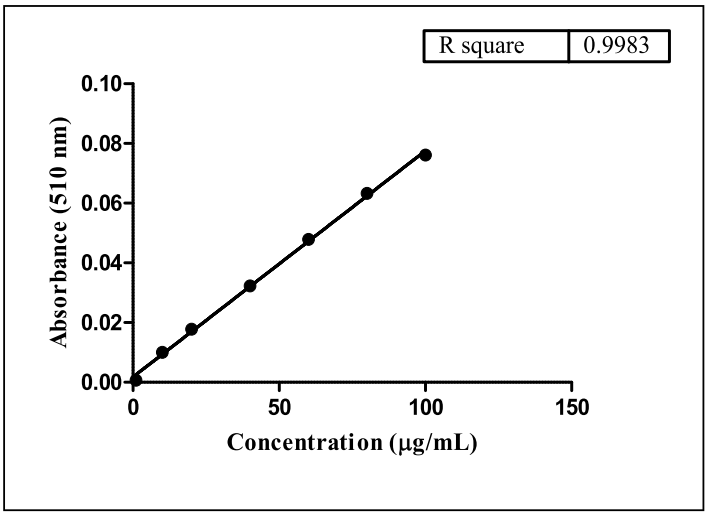


Fig 6(f-i): Graph of concentration vs. Absorbance of rutin (TFC assay)

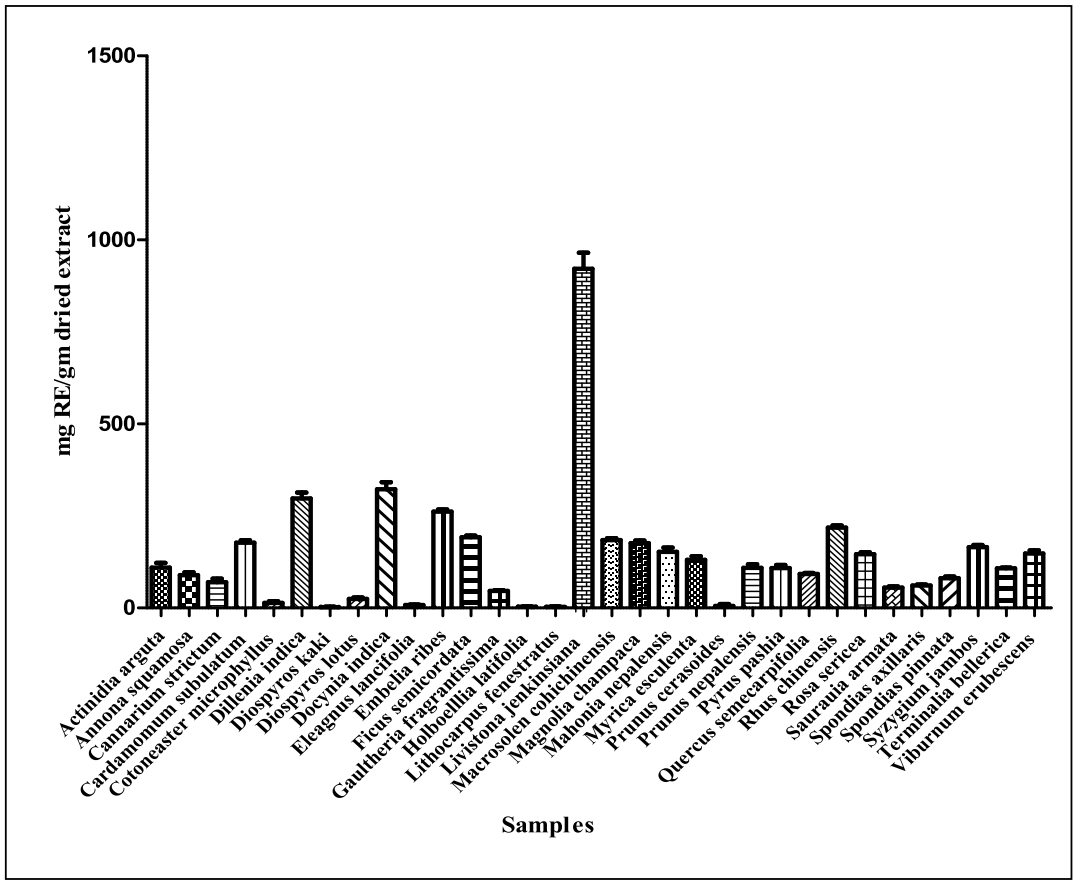


Fig 6(f-ii) Graphical representation of Total Flavonoid Content in samples

6.3.3Correlation test among antioxidant activity and Phytoconstituents

Table 6.3.3.1: Linear Correlation summary of Total Phenol Content and antioxidant assays

Parameter	DPPH	ABTS	FRAP	TAC
Number of XY Pairs	32	32	32	32
Pearson r	-0.7015	0.8519	-0.6070	0.5687
95% confidence interval	-0.8438 to -0.4669	0.7159 to 0.9257	-0.7888 to -0.3276	0.2743 to 0.7656
P value (two-tailed)	< 0.0001	< 0.0001	0.0002	0.0007
P value summary	***	***	***	***
Is the correlation significant? (alpha=0.05)	**Yes**	**Yes**	**Yes**	**Yes**
R square	0.4921	0.7258	0.3685	0.3234

Table 6.3.3.2: Linear Correlation summary of Total Flavonoid content and antioxidant assays.

Parameter	DPPH	ABTS	FRAP	TAC
Number of XY Pairs	32	32	32	32
Pearson r	-0.3300	0.3836	-0.3431	0.8820
95% confidence interval	-0.6087 to 0.02127	0.04024 to 0.6460	-0.6179 to 0.006501	0.7702 to 0.9412
P value (two-tailed)	0.0651	0.0302	0.0546	< 0.0001
P value summary	**ns**	*	**ns**	***
Is the correlation significant? (alpha=0.05)	**No**	**Yes**	**No**	**Yes**
R square	0.1089	0.1472	0.1177	0.7780

Table 6.3.3.3: Summary of Correlation Matrix among all the tests

	DPPH	ABTS	FRAP	TAC	TPC	TFC
DPPH		-0.7211***	0.8859	-0.4730*	-0.7014***	-0.3299
ABTS			-0.6367***	0.4774*	0.8519	0.3836*
FRAP				-0.4010*	-0.6070**	-0.3430*
TAC					0.5686**	0.8820
TPC						0.4995*
TFC						

Pearson correlation coefficients (R^2) for Correlation Matrix of all antioxidant test performed in the present study; negative sign on the values denote Negative Correlation; $p<0.05$ =*; $p<0.001$ = **; $p< 0.0001$ = ***

6.4) Discussion

(a) DPPH Assay

The inhibitory capacity of samples was expressed as IC_{50} value. IC_{50} is defined as the concentration of the sample required to inhibit 50% of free radicals in a given oxidative environment. Thus, lower the IC_{50} value, the higher will be antioxidant activity as both of them are inversely related. The values were calculated by calibrating non-linear dose-response curve using logarithmic scales. Fig 6(a-i) shows a typical dose-response curve obtained for standard references. Table 6.3.1 shows the IC_{50} values in µg/mL unit for all the samples tested. From the table, it is observed that 12 samples have IC_{50} lower than 50µg/mL, 3 samples under 100µg/mL and 18 samples above 100µg/mL. According to Phongpaichit (2007), IC_{50} values ranging from 10-50µg/mL is reported to have strong antioxidant activity while those between 50-100µg/mL has intermediate antioxidant activity. Weak antioxidant activity is defined by IC_{50} values higher than 100µg/mL. The highest IC_{50} is depicted by *Livistonajenkinsiana*at 6.387±0.314µg/mL which is quite near to Ascorbic acid (4.900 0.233µg/mL) and surpasses the IC_{50} values of Trolox (12.733±0.940µg/mL) and BHT (27.273±2.029 µg/mL). From the result table (Table 6.3.A) it is observed that *Docynia indica, Elaeagnus latifolia, Embelia ribes, Ficus semicordata, Livistona jenkinsiana, Macrosolen cochinchinensis, Quercus semecarpifolia, Rhus chinensis, Rosa sericea, Spondias axillaries, Spondias pinnata and Terminalia bellerica* are some of the minor fruits having high antioxidant activity. *Dilleniaindica, Gaultheria fragrantissima* and *Saurauiaarmata* are categorized under intermediate antioxidant activity. *Annona squamosa* has the weakest antioxidant activity from all the samples at 435.800±46.969µg/mL. The IC_{50} value ofmethanolic extract of *Dillenia indica*has been reported from Assam as 31.250µg/mL (Das *et al*., 2012), 62.17±0.620µg/ml (Gogoi *et al*., 2012) which indicates higher antioxidant

activity than the present study (91.433±4.621µg/ml). IC_{50} value for *Elaeagnus latifolia* from North Lakhimpur (Dutta *et al*., 2018) and Garo Hills (Panja *et al*., 2014) of Assam are around similar having the values 144.640±0.250µg/ml and 134.310±8.390µg/ml (70% methanol extract) respectively, but are comparatively lower in antioxidant activity than the present analysis value (14.180±0.24µg/ml). The IC_{50} value for *Embelia ribes* was found to be 10.155±0.525µg/ml which denotes higher activity than the reported value (78.077µg/ml) by Thyloor (2018). *Diospyros lotus* from China has lower antioxidant activity with higher IC_{50} value 201.000±1.02µg/ml (Gao *et al*., 2014) when compared with the present value which is 178.300±3.124µg/ml. Antioxidant activity of *Diospyros lotus* from Iran has been reported to be very high with IC50 value as low as 1.45±0.03µg/mL (Nabavi *et al*., 2009). IC_{50} value of *Docynia indica* from Manipur for 80% methanol extract has been reported as 80.68±2.16µg/ml by Shende *et al.*, (2016). Khomdram *et al*., (2014) have reported IC_{50} value 1657.28±867.1µg/mL for the same sample but Sharma *et al*., (2015) has reported IC_{50} value 33.89±0.89µg/ml which has more likely antioxidant activity with the present study as can be deduced from IC_{50} value 23.413±1.625µg/ml. *Rhus chinensis* antioxidant activity (IC_{50} value 5.31±0.07µg/ml) from Manipur (Sharma *et al*., 2015) is slightly higher than the given result (9.738±0.089µg/ml). *Spondias pinnata* had lower IC_{50} value (13.180±0.117µg/ml) in comparison to those reported by Khomdram *et al*., (2014) from Manipur as 518.77±6.9µg/ml. IC50 value (435.800±46.969µg/ml) of *Annona squamosa* was higher than 135.2µg/ml reported from Tamil Nadu by Nandhakumar and Indumathi (2013). IC_{50} value (251.700±19.152µg/ml) of *Actinidia arguta*was lower than the IC_{50} value of the fresh weight of the same from China reported (9.890±0.75mg/ml) by Zou*et al*., (2012). *Pyrus pashia* from Azad Jammu Kashmir (Siddiqui *et al*., 2015) has much lower IC50 value (37.160±0.10µg/ml) than the present study (272.705±14.884µg/ml).

The reaction between these antioxidants and DPPH are mostly by electron transfer or hydrogen atom transfer sometimes (Ou *et al*., 2005).Due to steric hindrance, it is reported that only small molecules can have better contact with the radical site (Prior *et al*., 2005). According to literature reports it is considered that establishing stoichiometry for the quenching reaction would give a clearer idea about antioxidant activity associated with substrate molecule structure (Molyneux, 2003). Apart from all of that, it is still one of the most popular techniques to deduct antioxidant activity due to its simplicity in procedure and rapid result inference (Apak *et al*., 2013).

(b) ABTS-TEAC Assay

The radical cation scavenging activity of samples was determined as Trolox equivalent antioxidant activity derived from linear curve calibrated as Fig6(b-i). The more the TEAC value, the more will be radical scavenging activity. Here *Quercus semecarpifolia* has highest TEAC value at 2.525±0.014µMTE/gm dry extract, followed by *Elaeagnus latifolia, Embelia ribes, Livistona jenkinsiana, Macrosolen cochinchinensis, Rhus chinensis, Rosa sericea, Spondias axillaries, Spondias pinnata and Terminalia bellerica.* 0.916±0.018 TEAC value for *Eleagnus latifolia* is reported from Garo hills, Assam by Panja *et al*. (2014) as 0.070±0.003mgTE/gm from 70% methanolic extract.Li *et al*., (2018) had reported TEAC value 55.81±0.71µmol/gm for *Actinidia arguta* from China which is higher than the present value (0.018±0.005µM/gm).

The samples, more or less, which had low IC50 value in DPPH assay, had higher TEAC value. Such similarity in activity further supports the results of antioxidant assays for samples of interest. It is reported that phenolic compounds or any compound having redox potential lower than ABTS (0.68V) can reduce $ABTS^{\bullet+}$(Prior *et al*., 2005). For those samples showing low TEAC value, it may be assumed that the reaction might be

of a slow type and thus required a longer time to arrive at its TEAC equivalent(Prior *et al*., 2005).

(c) FRAP Assay

The reducing ability of samples was expressed as EC_1. EC_1 is defined as the concentration of an antioxidant which has the equivalent power of 1mM ferrous salt required for reducing ferric–TPTZ complex to ferrous-TPTZ complex. Thus lesser the EC_1 value, the more will be reducing ability. *Livistona jenkinsiana* depicted the highest EC_1 value at 0.193±0.007µg/mLwhich is much higher than EC_1 of Trolox (3.308±0.291µg/mL). *Docynia indica, Elaeagnus latifolia, Embelia ribes, Macrosolen cochinchinensis, Rhus chinensis, Rosa sericea, Spondias axillaries, Spondias pinnata and Terminalia bellerica* are other samples having low EC_1 value. FRAP assay follows single electron transfer mechanism. Compounds having redox potential lower than the redox potential of $Fe3^{+}$-TPTZ (0.7V) are reported to be detected in this assay. The similarity in the antioxidant activity of samplesby FRAP and TEAC assay (Section 6.4.b) may be due to proximity in the redox potential of FRAP (0.7 V) and TEAC (0.68 V). Due to such proximity, compounds alike are known to be detected by these two assays (Prior *et al*., 2005).

(d) TAC Assay

The results of Total Antioxidant Capacity by Phosphomolybdate assay were expressed in mg Ascorbic Acid Equivalent per gram. This assay takes into account the thermodynamic efficiency related to the number of reactive species scavenged, concerning the time. It is much of a collaborative property present in a sample (Apak*et al*., 2016). TAC value for *Livistona jenkinsiana* was obtained highest at 50.392±1.142 mg AAE per gram dry extract followed by *Embelia ribes* (27.704±0.524 mg AAE/gm)

and *Viburnum erubescens* (20.721±7.811 mg AAE/gm). The more the TAC value, the more will be the antioxidant capacity of samples.

(e) TPC Determination

Phenolic compounds are phytoconstituent containing hydroxyl group with aromatic benzene rings. They are extensively present in the plant kingdom (Walton *et al*., 2003) and are major contributors of antioxidant capacity and various biological activities (Xu and Chang 2007; Dudonne*et al*., 2009; Koche *et al*., 2016). Total phenol content of samples was expressed as mg Gallic acid equivalent per gram dry extract as per the linear calibration curve by Gallic acid given as Fig 6 (e-i). The highest total phenol content was observed for *Terminalia bellerica*(461.126±2.912mgGAE/gm) followed by *Livistona jenkinsiana* (388.602±32.878GAE/gm) and *Quercus semecarpifolia* (353.699±3.186 GAE/gm). 3 samples had phenol content more than 200mg GAE/gm and 8 samples above 100mg GAE/gm. Total phenol content of methanolic extract of *Dilleniaindica* has been reported as 49.80±0.40mg GAE/gm (Gogoi *et al*., 2012) which is lower than the present study (115.453±7.378mgGAE/gm). *Elaeagnuslatifolia* from North Lakhimpur, Assam has been reported to have 8.18±0.182 µg/ml GAE total phenol content (Dutta *et al*., 2018). TPC in *Myrica esculenta* was found to be 57.649±1.207mg GAE/gm which is lower than the report (321.68±0.06 mg/gm GAE) from Meghalaya by Goyal *et al*. (2013) but higher than that (2.12mg GAE/gm) from Uttarkhand as reported by Rawat *et al*. (2011). *Diospyros lotus* had higher TPC content (56.732±1.966mg GAE/gm) than that reported by Gao *et al*. (2014) as 3.3±0.18 mg GAE/gm from China and those from Iran as 10.2±0.9 mg GAE/gm (Nabavi *et al*., 2009). Karuppusamy *et al*. (2011) have reported TPC for *Gaultheria fragrantissima* from Palni Hills of Western Ghats, India as 80.400±3.18mg GAE/gm which is slightly higher than the present value 73.412±1.266mg GAE/gm. *Prunus nepalensis* from

Meghalaya (Seal *et al*., 2011) has lower TPC (10.49±0.14mg GAE/gm) than the present study (36.897±1.284mg GAE/gm). The same was observed for *Terminalia bellerica* from Meghalaya as 95.40±0.74mg GAE/gm in comparison to 461.126±2.912mg GAE/gm found in the present study. According to Shende *et al*. (2016), *Docynia indica* from Manipur has 22.62±0.21mg GAE/gm TPC in 80% methanol extract. Sharma *et al*. (2015) have reported TPC of *Docynia indica* as 49.26±4.21mg GAE/gm and 172.84±15.33mg GAE/gm for *Rhus chinensis* from Manipur which is lesser than the present values 148.054±5.201mg GAE/gm and 295.956±9.457mg GAE/gm respectively. TPC of *Actinidia arguta* in the present study (10.201±0.778mg GAE/gm) was higher than that reported (4.71±0.18mg GAE/gm) by Li *et al*. (2018) from China but both values are lower than the reported (Zou *et al*., 2012) value (362.18±19.87mg GAE/gm) from fresh weight of the same sample. *Spondias pinnata* had higher TPC (253.381±5.721mg GAE/gm) than earlier reported (71.83±0.76mg GAE/gm) from Sikkim by Pandey *et al*. (2018). *Pyrus pashia* from Azad Jammu Kashmir had 141.02±0.34mg GAE/gm (Siddiqui *et al*., 2015.) which is much higher than the present value (21.471±1.488 mg GAE/gm).

Overall, the phenolic content was quite affable in all 32 samples. This assay is reported to be non-specific in nature and so the reagent can be reduced by other components such as reducing sugar or ascorbic acid (Hidalgo and Almajano, 2017). This possibly explains the high amount of phenolic content in the samples, as fruits are a natural source of sugar and vitamin C. It is reported that hydroxylation and conjugation by polyphenols are related with the reducing potential of antioxidants (Pulido *et al*., 2000). It is also reported that the exact chemical interaction and redox potential of Folin reagent remains ambiguous but because it is sensitive, simple and independent of the

degree of polymerization of phenols, it is still used for determining the quantity of phenolic content (Prior, 2005; Gupta, 2015).

(f) TFC Determination

Flavonoids are phenolic compounds of low molecular weight having benzo-g-pyrone derivatives and are known to have the antioxidant property (Hertog*et al*.,1992; Pal and Verma, 2013;Koche*et al*., 2016). Rutin standard linear curve was calibrated as Fig 6(f-i). Results were expressed as mg Rutintrihydrate equivalent per gram dry extract. The highest flavonoid content was observed for *Livistona jenkinsiana* (921.485±43.419mg RE/gm) followed by *Dillenia indica* at 297.647±15.908mg RE/gm. Total flavonoid content for *Elaeagnus latifolia* has been reported as 6.375±0.128µg/mL RE by Dutta *et al*. (2018). TFC in *Myrica esculenta* from Meghalaya has been reported to have 187.2±0.04 mg QE/gm (Goyal *et al*.,2013) and between 1.31mg-1.59mg QE/gm as reported by Rawat *et al*. (2011) while in the present study it was 130.936±9.237mg RE/gm. 2.8±0.10mg RE/gm TFC is reported for *Diospyros lotus* from China by Gao *et al*. (2014) which is lower than the present value 24.928±3.321 mg RE/gm. TFC value for this fruit from Iran has been reported to be 2.1±0.05mg QE/gm (Nabavi *et al*., 2009). TFC for *Gaultheria fragrantissima* from Palni Hills of Western Ghats, India has been reported to be 94.3±1.35mg QE/gm (Karuppusamy *et al*., 2011). TFC for *Prunus nepalensis* and *Terminalia bellerica* has been reported to be 2.14±0.02mg QE/gm and 7.07±0.01mg QE/gm respectively from Meghalaya by Seal *et al*. (2011). *Docynia indica* TFC (322.279±19.528mg RE/gm) is found to be much higher than 80% methanol extract value (49.05±0.22mg RE/gm) reported from Manipur by Shende *et al.* (2016).TFC for *Docynia indica* and *Rhus chinensis* also has been reported from Manipur by Sharma *et al*.(2015) as 0.504±0.074mg QE/gm and 2.775±0.275mg QE/gm respectively. TFC in fresh samples of *Actinida arguta* has been reported as

188.43±3.65mg CE/gm by Zou *et al*. (2012) from China. *Spondias pinnata*TFC from Sikkim has been reported as 7.83±0.17mg QE/gm by Pandey *et al*, 2018.

Similar to total phenol content in the samples, total flavonoid content too was quite appreciable and is evident from the preliminary phytochemical test result given asTable 5.3.B in Chapter 5. It is reported that structure and substitution pattern of the hydroxyl group has a significant role in determining the antioxidant activity of flavonoids (Sharififar, 2008).Flavonoids have been known to have many biological activities and have been in the medicinal system since ancient times(Pal and Verma, 2013; Koche*et al*., 2016).

Correlations among Antioxidant activity and phytochemical constituents

From Table 6.3.3.1 it is quite evident that phenolic compounds and antioxidant activity assays are correlated. The value of p at < 0.0001 shows a strong significant relation among antioxidant assays and phenolic contents. The negative sign on DPPH and FRAP assay stand for the significant negative correlation further supporting the above sections 6.4(a) and (c). The relation among DPPH, TEAC and FRAP assays with phenolic content have been well established (Pulido *et al*., 2000;Bors and Michel, 2002;Prior *et al*., 2005;Olajire and Azeez, 2011;Shahriar*et al*., 2013).

However, the case is not the same when it comes to the relation between total flavonoid content and antioxidant assays. Table 6.3.3.2 shows that flavonoids have some significant relation with ABTS assay with p-value < 0.05 and the relation between total antioxidant capacity and flavonoid content were highly significant at p-value < 0.0001. But for DPPH and FRAP assay there was no significant relationship for the same. It could be stated that flavonoid content in sample would not always define the antioxidant activity.

The inter-relation among all the assays performed is summarized as Correlation matrix in Table 6.3.3.3. Of the total 15 correlations, 11 were found to be significant and 4 non-significant. For those having significant correlation, it may be concluded that the result obtained through one assay may show a similar result pattern in another assay. For those having no significant correlation, the resulting pattern would be independent of each experiment.

6.5 Conclusion

The 32 samples collected showed a mixture of high, intermediate and low antioxidant activity. *Docynia indica, Elaeagnus latifolia, Embelia ribes, Ficus semicordata, Livistona jenkinsiana, Macrosolen cochinchinensis, Quercus semecarpifolia, Rhus chinensis, Rosa sericea, Spondias axillaries, Spondias pinnata* and *Terminalia bellerica*have been identified as an excellent source of antioxidant freely available in nature without burning a hole in the pocket. The vast literature reports about the role of phenolic compounds in antioxidant property of plant have been proved once again. It should be noted that apart from the mode of chemical interaction between antioxidant substance and assay reagents, environmental factors where plants are exposed to, as well sample preparation methods could also be a determining factor in measuring the antioxidant property of plants. It is observed that these minor fruits have substantial antioxidant property. Consuming these fruits can protect one from so many oxidation-related diseases.

Chapter 7

Antioxidant activity of Essential oil

7.1 Introduction

Oil obtained from the oil glands of aromatic plants are known as “Essential oil”. They are also called as ethereal oil, fragrant oil or simply aromatic oil. They share vital space in cosmetic, pharmaceutical and food industries. They can be extracted from any part of the aromatic plant such as leaves, flowers, fruits, seeds, bark or root. However, the most efficient source would be only one among them. They are produced by plants as secondary metabolite to fight against environmental stress and pathogen attacks (Rassem *et al*., 2016; Duarte *et al*., 2018). It is reported that the coloration of essential oils is imparted by indigenous pigment of the plant (Tongnuanchan and Benjakul, 2014). Terpenes (monoterpenes and sesquiterpenes), terpenoids (isoprenoids) and aromatic compounds (alcohol, phenol, aldehyde etc) are reported to be major chemical constituent of an essential oil (Bakkali *et al*., 2008; Mohamed *et al*., 2010). Essential oils can be extracted by various means broadly categorized (Tongnuanchan and Benjakul, 2014) as:

a) Distillation technique
    - Steam distillation: By heating the sample with water vapour
    - Hydrodistillation: By directly boiling the sample in water
    - Hydrodiffusion: By heating the sample with steam which is supplied from top of the chamber unlike steam distillation where steams are supplied from below the chamber containing sample.

b) Solvent extraction method using
    - Conventional solvents such as petroleum ether, hexane or methanol etc.
    - Supercritical fluid such as the popularly used carbon dioxide

- Subcritical water under dynamic conditions such that a constant pressure is maintained to keep the water in liquid state even under high temperature range of 100-374°C.

c) Solvent–free microwave method using microwave heating and dry distillation at controlled atmospheric pressure in the absence of any solvent.

Magnolia champaca is one of the minor fruits of Arunachal Pradesh. Its fruits are quite popular among Apatani tribes of Lower Subansiri District of the State. An attempt was made to study the antioxidant activity of essential oil extracted from this fruit.

7.2 Materials and method

(a) Sample collection and identification

Sample collection and identification was done according to 4.2 (b)

(b) Sample preparation

Samples were collected and shade dried to remove excess water components.

(c) Extraction procedure

Extraction was done by Hydrodistillation method (Tongnuanchan and Benjakul, 2014): 604 gram of dried *Magnolia champaca* fruit was manually broken into tiny pieces and put into sample holder Round Bottom (RB) Flask of Clevenger-type Apparatus. Distilled water was added to this RB as per the required volume and slowly heated by the heating mantle. The extraction was run for 48 hours to obtain the essential oil present in the sample.

(e) Tests for antioxidant activity

The essential oil obtained was subjected to following assays according to section 6.2.3 of Chapter 6

- DPPH Assay
- TEAC Assay

- FRAP Assay

(f) Statistical Analysis

All experiments were run in independent triplicate. Results are expressed as Mean ± Standard Deviation. Data were calculated usingGraphpad Prism® software version 5.03

7.3 Result

Table 7.3.1: Comparison of antioxidant activity of *Magnolia champaca* Essential oil and extract with Standard antioxidants.

Samples	DPPH	FRAP	ABTS
	(IC50)	(EC1)	(TEAC)
Magnolia champaca EO	386.433± 15.068	148.167 11.373	0.028 ±0.012
Magnolia champaca extract	347.800 ± 9.938	115.256 ± 9.695	0.209± 0.017
Ascorbic acid	4.900 0.233		
Trolox	12.733 0.940	3.308 0.291	
BHT	27.273 2.029		

7.4 Discussion

The antioxidant activity of essential oil of *Magnolia champaca* is found to be quite weak as the extract itself, as can be inferred from high IC_{50} and EC_1 values and low TEAC values (Table 7.3.1). Essential oil extraction from flowers of *Magnolia champaca* has been reported by Sinha and Varma (2016). Sisterols, mono and sesquiterpenes have been reported from the essential oil extracted from flower, fruit and leaf this plant (Sinha and Varma, 2016). The presence of sesquiterpenes may be causative of low antioxidant activity of essential oil (Thusoo *et al*., 2014).It is reported that constituents of these oils are generally obtained by phenylpropanoid routes (Thayumanavan and Sadasivam, 2003). Overall, the plant has been known for curing leprosy, fever cough and rheumatism (Perry, 1980; Hasan*et al*., 2009;Armiyanti *et al*., 2010). Fruits and flowers of this plant have been reported to be haemostatic, antihelminthic and diuretic and useful against renal diseases (Taprial, 2015); bark is

reported to be useful in treating bronchitis, chronic gastric and cardiac problems. Roots have been reported against constipation problem (Sinha and Varma, 2016). The oil produced from its flowers has been commercialized in perfumery and hair oil manufacturing companies (Barlow *et al*., 1991).

7.5 Conclusion

The essential oil of *Magnolia champaca* has low antioxidant activity. Though there have been reports of chemical constituents from this oil, there is still a need of chemical profiling to check the possible difference(s) arising out of different climatic and geographical conditions.

Chapter 8

Quantitative Estimation of Nutritional Parameters

8.1 Introduction

Nutrition is the core of growth and development of living beings from the primordial stage. WHO has defined Nutrition as “The intake of food, considered in relation to the body’s dietary needs”. Fora healthy mind and body development, balanced diet with physical exercises is a must. Deficiency of nutrition makesthe body susceptible to any kind of diseases such as protein-energy malnutrition (PEM), diabetes, osteoporosis, hypertension, chronic diseases etc. and sometimes may lead to mental retardation also. Thus, a balanced diet comprising of nutritious food is a cornerstone of a healthy population.

Fruits are an important part of a balanced diet. They are a rich source of phytoconstituents essential for biological activities. Literature reviews reveal that consumption of fruits and vegetables bring down the chances of some chronic diseases especially for the ageing population (EFSA, 2008; Schroder *et al*., 2008). It is learnt thateconomic status is a hindrance to availing nutritious food available in the global market (Darmon*et al*., 2005). In such a case, minor fruits can act as an alternative source. They have been reported to be an efficient source of nutrition (Krishnamurthy and Sarala, 2012). It is also noted that exposing minor fruits to the global population can mitigate the issues of food security around the globe (Srivastava *et al*., 2017). In this chapter, an attempt was made to estimate the quantitative nutritional parameters of minor fruits collected from Arunachal Pradesh relating to objective no.3 of the research topic.

8.2 Materials and method

8.2(a) Sample collection and identification

Sample collection and identification was done according to Section 4.2 (b)

8.2 (b) Sample preparation

Sample preparation was done according to Section 5.2(b) of chapter 5

8.3 (c) Experiments performed: Following experiments were performed:

8.2.3.1) Estimation of Protein content by Bradford assay

8.2.3.2) Estimation Free amino acid content by Ninhydrin method

8.2.3.3) Estimation of Carbohydrate by Anthrone method

8.2.3.4) Estimation of Sugar by Anthrone method

8.2.3.5) Estimation of Starch by Anthrone method

8.2.3.6) Estimation of Fat by Soxhlet method

8.2.3.7) Estimation of Fibre by Gravimetric method

8.2.3.1) Bradford method of Protein estimation

Protein estimation by Bradford method was done by modification of Maehre *et al*., 2018.

Principle

When Coomassie Blue dye comes in contact with protein, by electrostatic attraction, there is absorbance shift from 465 to 595nm. The amount of protein in sample can thus be detected by checking the absorbance at 595 nm.

Chemicals/reagents required

- Bovine Serum Albumin (BSA)
- Bradford reagent (commercially obtained)
- 1M Tris-EDTA Buffer
- Tris (hydroxymethyl) aminomethane (Tris)
- Disodium ethylenediaminetetraaceticacid (EDTA)
- Concentrated hydrochloric acid (HCl)

- Sodium hydroxide (NaOH)

Chemical preparations:

i. ***Preparation of 1M Tris solution***: 121.14 gm Tris was mixed in 800ml of distilled water and pH was adjusted to 8.0 (using HCl) with final volume as 1000ml.

ii. ***Preparation of 0.5M EDTA***: 186.1gm of EDTA was mixed with 800ml of distilled water and pH was adjusted to 8.0 (using NaOH) with final volume as 1000ml.

iii. ***Preparation of 1M Tris-EDTA buffer***: 10ml of 1M Tris solution was mixed with 2ml of 0.5 M EDTA solution followed by addition of distilled water making final volume of 100 ml. The mixture was shaken thoroughly.

The experimental procedure

Proteins from samples were extracted using 1M Tris-EDTA buffer by vortexing 10mg of powdered samples in 5mLof the buffer followed by centrifugation at 10,000 rpm for 15 mins at 0°C.BSA at various concentrations ranging from 0.2, 0.4, 0.6, 0.8, 1 mg/ml were prepared. A 96 well plate was first filed with 250µL of Bradford reagent followed by addition of 10µL of sample or standard or blank (buffer). The plate was then shaken for 30 seconds using Skan It®Ver4.0 for 30 seconds in continuous slow speed. After 30-minute absorbance at 595nm was noted using the same machine. Protein content in the samples was determined using the standard graph calibrated from BSA by linear regression. Amount of protein content was expressed as % dry weight (DW).

8.2.3.2) Total free Amino acid content by Ninhydrin method

Estimation of total free Amino acids was done according to Sadasivam and Theymoli (1987)

Principle

When Ninhydrin comes in contact with free alpha-amino acid group, decarboxylation on the amino acid takes place, resulting into formation of bluish-purple coloured product which can be detected at 570 nm.

Reagents/Chemicals required

- Ninhydrin
- Stannous chloride
- Methyl cellosolve
- n-Propanol
- 80% Ethanol
- 0.2M Citrate buffer (pH 5.0)
- Leucine

Chemical preparations:

i. ***Preparation of 0.2M citrate buffer (pH 5.0)***: 23.53gm of sodium citrate dihydrate was dissolved in few ml distilled water followed by addition of 23.05gm of citric acid and few more water about 800ml. The components were mixed thoroughly and pH was adjusted to 5.0 using HCl or NaOH as required. Then few more ml of water was added to make a final volume of 1000ml.

ii. ***Preparation of Ninhydrin solution***: 0.8gm stannous chloride was mixed in 500ml of 0.2M citrate buffer (pH 5.0). This solution was then mixed with 500ml methyl cellosolve which had 20 gm ninhydrin already dissolved in the solution.

iii. ***Preparation of diluent solvent***: Equal volume of water and n-propanol were mixed together.

iv. ***80% Ethanol***: 80% ethanol was prepared by mixing 80ml ethanol with 20ml distilled water**.**

Experimental procedure

Free amino acids were extracted by dissolving 500 mg powdered samples in 5 mL of 80% ethanol followed by filtration using Whatmann ® filter paper no.1. This filtrate was then placed on a water bath to remove the excess solvent. This concentrated filtrate was used for the experiment. The standard solution was prepared using L–leucine with concentration ranging from 1 mg/ml to 0.1mg/mL. For the reaction, 0.1mL of sample or Standard or Blank was mixed with 1mL Ninhydrin solution followed by the addition of distilled water making a total volume of 2mL. This reaction setup was then boiled for 20 minutes in a water bath. 5mL of diluents solution was then added to these tubes and mixed thoroughly followed by an incubation period of about 15 minutes. The intensity of the purple colourformed at the end of the time-period was read at 570nm against the blank.The amount of free amino acid in the sample was deduced from the standard curve calibrated from Leucine by linear regression.Results were expressed as % DW.

8.2.3.3) Total Carbohydrate estimation by Anthrone method

Estimation of total carbohydrate content was done according to Hedge and Hofreiter (1962)

Principle

Hydrochloric acid can hydrolyse carbohydrates into simple sugars. In the presence of high temperature and acidic medium, glucose is further dehydrated into hydroxymethyl furfural. The interaction between anthrone and furfural gives a green coloured product which can be detected at 630nm.

Reagents/ Chemicals required

- 2.5N hydrochloric acid (HCl)

- 95% sulphuric acid
- Anthrone
- Glucose
- Sodium carbonate

Chemical preparations:

i. ***Preparation of 2.5N HCl*:** Required % solution was prepared according to the formula $N_1V_1=N_2V_2$ as per the available stock

i. ***Preparation of 95% sulphuric acid:***Required solution was prepared using the formula $C_1V_1=C_2V_2$ as per the available stock.

ii. ***Preparation of Anthrone reagent*:**200mg of anthrone was dissolved in 100ml of ice-cold 95% sulphuric acid.

iii. ***Preparation of Standard stock solution***: 100mg of glucose was dissolved in100mL distilled water.

iv. ***Preparation of Standard working solution***: 10mL of stock solution was mixed with distilled water making a final volume of 100mL.

Experimental procedure

5mL of 2.5N HCl containing 100mg sample was boiled for 3 hours in a water bath. It was then allowed to cool down and neutralized with solid sodium carbonate until no effervescence was observed. Distilled water was added making a total volume of 100 mL, then centrifuged at 5000 rpm for 15 minutes at 37°C. The supernatant was then collected and used for the experiment. Standard glucose solution with concentration ranging from 0.01-0.1mg/mL was prepared. 1 mL of Standard, sample and blank was taken in a test tube and reacted with 4mL Anthrone reagent and boiled for another 8 minutes. The setup was cooled down rapidly by immersing in cold water and change in colouration was read at 630nm. Total carbohydrate present in samples was calculated

from the calibration curve of glucose at different concentrations (0.01-0.1mg/mL). Results were expressed as % DW.

8.2.3.4)Determination of Total Sugar

Estimation of total soluble sugar was done according to Pavan (2013)

Principle

Hot ethanol can extract sugar which on reacting with Anthrone reagent gives green colouration detectable at 620 nm.

Reagents/ Chemicals required

- 80% Ethanol
- 0.2% Anthrone reagent
- Concentrated sulphuric acid
- Glucose

Chemical preparations:

i. ***0.2% Anthrone reagent:*** 0.2gm gm anthrone was dissolved in 100ml concentrated sulphuric acid.

ii. ***80% Ethanol:*** 80ml ethanol and 20ml distilled water forming total 100ml volume.

iii. ***Preparation of Standard Glucose stock solution***: Stock solution was first prepared by dissolving 0.1gm of glucose in 100mL of distilled water.

iv. ***Preparation of Standard Glucose working solution***: 10mL of stock solution was diluted with 90mL of distilled water.

Experimental procedure

0.1 gm of samples was extracted with 10mL 80% hot ethanol placed on a hot plate for 90 minutes with intermittent vortexing. The sample solutions were then centrifuged at

4000 rpm for 30 minutes. The supernatants were collected in clean 2 mL microtubes. 0.1mL of this supernatant was then transferred to clean glass test tubes and placed in a warm water bath to evaporate off the solvent. The test tubes were then cooled down immediately till it reached room temperature. 1 mL of distilled water was then added to these tubes and vortexed briefly. In the meantime, series of glucose standard solutions were prepared with concentration ranging as 0.01, 0.02, 0.04, 0.06, 0.08, 0.1mg/mL. 1 mL of distilled water served as blank. 4mL of freshly prepared Anthrone reagent was then added to all the tubes containing samples, standard as well as blank and mixed thoroughly with the help of vortex shaker. They were then placed on a water bath at 100°C for 10 minutes and cooled rapidly by submerging on ice cold-water. Absorbance was noted at 620nm against blank. Amount of total soluble sugar content was derived from the standard curve of glucose and expressed as % DW.

8.2.3.5) Estimation of Starch by Anthrone method

Estimation of Starch content was done according to Pavan (2013)

Principle

Starch can be extracted with acid after sugars have been removed. The reaction product between starch and anthrone reagent can be read at 630nm.

Reagents/Chemicals required

- Glucose
- 80% Ethanol
- 52% Perchloric acid
- 0.2% Anthrone
- Concentrated sulphuric acid

Chemical preparations

i. ***Preparation of 0.2% Anthrone reagent:*** 0.2gm anthrone was dissolved in 100ml concentrated sulphuric acid.

ii. ***Preparation of 52% perchloric acid:*** Required solution was prepared using the formula C_1V_1-C_2V_2 as per the available stock.

iii. ***Preparation of 80% Ethanol:*** 80ml of ethanol was mixed with 20ml distilled water

iv. ***Preparation of standard glucose solution:*** Range of concentrations of glucose standard was prepared according to the previous method as given in section 8.2.3.4

Experimental procedure

100mg of the sample was homogenised with 10 mL 80% hot Ethanol to extract out sugar component. The extraction was followed by washing with hot 80% Ethanol until the sample solution gave no colouration with anthrone reagent. The residues were then warmed in a water bath to remove excess ethanol. On complete drying of samples,5mL of distilled water and 6.5mL of 52% perchloric acid (4°C) was added to these tubes. The tubes were covered with aluminium foil and kept undisturbed at 4°C for 12 hours. During this time starch is extracted out from sample by the cold acid.

On the following day, distilled water is added to these solutions, making a final volume of 100mL in a volumetric flask. The aliquots were then centrifuged at 7000 rpm for 20minutes at 4°C and supernatants were collected. 100µL of this supernatant was mixed with 900µL of distilled water making a total volume of 1mL. Thus the samples for starch analysis were ready.

4mL of anthrone reagent was added to this 1mL sample, corresponding blank and standard at different concentrations and boiled for 8 minutes in a water bath followed by rapid cooling down using ice-cold water. The absorbance was read at 630 nm.

Amount of starch content was deduced from the standard curve of glucose, multiplied by0.9 (conversion factor) and expressed as % DW.

8.2.3.6) Estimation of Crude Fat content

Crude fat content in samples was estimated according to Indrayan*et al*., 2005.

Principle

Lipids are soluble in organic solvents such as petroleum ether and hexane. The solubilised lipid can be extracted by evaporating off the solvent.

Reagents/Chemicals required

- Petroleum ether (bp40°-60°C)

Experimental procedure

5 grams of moisture-free sample was weighed in Whatman ® filter paper no 40 and packed firmly by tying with a cotton thread. The packed sample was then inserted into the glass thimble for extraction. The round bottom flask was filled with 600mL of petroleum ether solvent (bp 40-60°C). The solvent was boiled at 50°C for 6 hours for each sample. After completion of 6 hours, set up was cooled down and the sample packet was removed from the thimble. The solvent containing solubilised lipid was re-boiled at 50°C to concentrate the extracted lipid as well as to recover the solvent. 3 recovery cycles were performed but only second and third rounds were collected from thimble as the first round of recovered solvent might contain traces of sample extracts. The concentrated solubilised sample in the round bottom flask was poured out into the clean pre-weighed beaker which was then covered by porous aluminium foil. The beaker was then set on a water bath at 50°C to remove the remaining solvent. The amount of crude fat content was determined in % using the formula (Meat technology, 2006):

$$\%\text{Crude fat} = \frac{(W2-W1)}{S} \times 100$$

Where, W1= Weight of the empty beaker (gm)

W2= Weight of beaker with fat extract (gm)

S=Weight of the sample (gm)

8.2.3.7) Estimation of Crude Fibre content (Gravimetric method)

The crude fibre content in samples was estimated according to Reddy*et al*., 2017

Principle

Cellulose and lignin undergo oxidative hydrolysis and degradation when treated subsequently with acid and alkali substances. The residue so obtained is finally weighed, burned and weighed again after cooling. The loss in weight gives the percentage composition of crude fibre.

Reagents/Chemicals required

- 1.25% sulphuric acid
- 1.25% sodium hydroxide
- Alcohol
- Silica gel powder
- Petroleum jelly.

Chemical preparation

ii. ***Preparation of 1.25% sodium hydroxide solution:*** 1.25gm of sodium hydroxide was dissolved in 100ml distilled water.

iii. ***Preparation of 1.25% sulphuric acid***: Required solution was prepared using the formula $C_1V_1=C_2V_2$ as per the available stock.

Experimental Procedure

The samples were first devoid of moisture by drying in oven followed by removal of fat by Soxhlet method (8.2.3.6). 2 gm of the dried and defatted sample was boiled in 200mL 1.25% sulphuric acid for 30 minutes followed by filtering using Whatman ® filter paper no.1. The sample residue was then washed continuously with boiling water until the filtrate was no longer acidic. This residue was boiled again in 200mL of 1.25% sodium hydroxide for another 30 minutes followed by filtration and subsequently washing with 25mL of boiling 1.25% sulphuric acid, 50 mL distilled water (thrice) and 25mL alcohol. The residue so obtained was transferred to pre-weighed crucible (W1). The sample residue was then dried in the furnace at 130±2°C for 2 hours. The crucible was cooled down on completion of set timing using desiccator and re-weighed (W2). Finally, the dried sample residue was ignited at 600±15°C for 30 minutes and cooled down in desiccator to take the final weight (W3). Amount of fibre content was calculated by the formula

$$\% \text{ Crude fibre} = \frac{(W2-W1)-(W3-W1)}{\text{Weight of the sample}} * 100$$

Estimation of Nutritive Values

Nutritive value of samples was calculated according to the formula given by Indrayan*et al.*(2005) as:

Nutritive Value=(4 X % of Protein) + (9 X % of Fat) + (4 X % of Carbohydrate)

Statistical Analysis

All experiments were run in independent triplicates. Results were expressed as Mean±Standard Deviations. Data were statistically subjected to One Way ANOVA with significance level $p < 0.05$.

8.3) Result

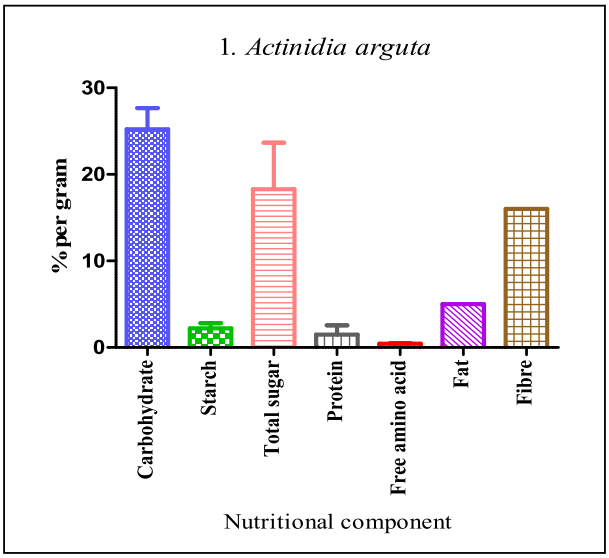
Table 8.3.1: Nutritional information of samples

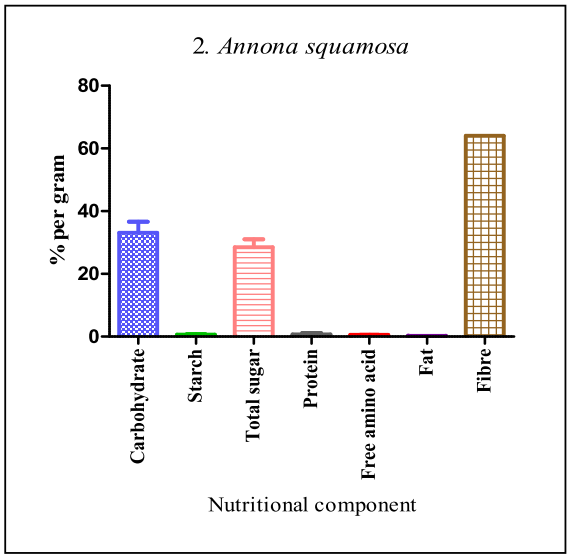
Sl no.	Samples	Carbohydrate	Starch	Total sugar	Protein	Free amino acid	Crude fat	Crude fibre
		(% DW)	(% DW)	(% DW)	(% DW)	(% DW)	(% DW)	(% DW)
1	*Actinidia arguta*	25.22±2.45	2.22 0.58	18.28± 5.37	1.49±1.07	0.45± 0.04	5	16
2	*Annona squamosa*	33.11±3.55	0.64±0.11	**28.51± 2.53**	**0.70 ±0.47**	0.54± 0.04	**0.2**	64
3	*Canarium strictum*	18.95±3.24	0.65±0.11	6.34 ±0.93	3.94 ±1.78	1.35± 0.27	8.2	25
4	*Cotoneaster microphyllus*	**74.04±4.49**	0.73±0.09	6.56±0.87	28.99 ±3.71	0.29± 0.02	2.2	25
5	*Dillenia indica*	34.24±3.41	0.56 ±0.02	15.20 ±0.60	2.44 ±0.83	0.48± 0.03	0.8	34.5
6	*Diospyros kaki*	26.46±5.51	1.23± 0.28	23.22± 1.36	**29.86 ±8.99**	**0.24± 0.03**	**0.2**	**7**
7	*Docynia indica*	30.05±6.11	**3.81± 0.21**	27.23 ±0.83	7.18 ±1.61	0.87± 0.02	0.6	17
8	*Embelia ribes*	6.67 ±1.02	**0.39± 0.01**	2.25 ±0.97	4.57 ±2.61	1.00± 0.04	1.4	53
9	*Livistona jenkinsiana*	**5.53± 1.02**	0.41 ±0.06	4.34 ±0.59	5.52 ±0.72	1.44±0.21	**37.6**	28
10	*Pyrus pashia*	28.35±5.67	0.60 ±0.05	21.23 ±1.03	5.76 ±1.22	0.54 ±0.02	0.4	19
11	*Rhus chinensis*	13.29±1.31	0.64 ±0.05	**1.86 ±0.88**	1.99 ±0.53	0.36± 0.03	2.6	**68**
12	*Spondias axillaris*	31.30±5.38	1.23±0.46	20.79 ±1.00	5.99± 1.81	0.92 ±0.05	1.6	16
13	*Spondias pinnata*	55.15±9.87	0.98±0.01	15.19 ±3.52	2.28 ±0.60	1.08 ±0.04	1.2	17
14	*Syzygiumjambos*	13.06±2.26	0.63±0.06	11.56± 0.62	2.68 ±1.91	1.18 0.18	**0.2**	19
15	*Terminallia bellerica*	19.77±7.61	0.52±0.03	7.95 ±0.60	5.13± 2.49	**3.73± 0.05**	0.8	33

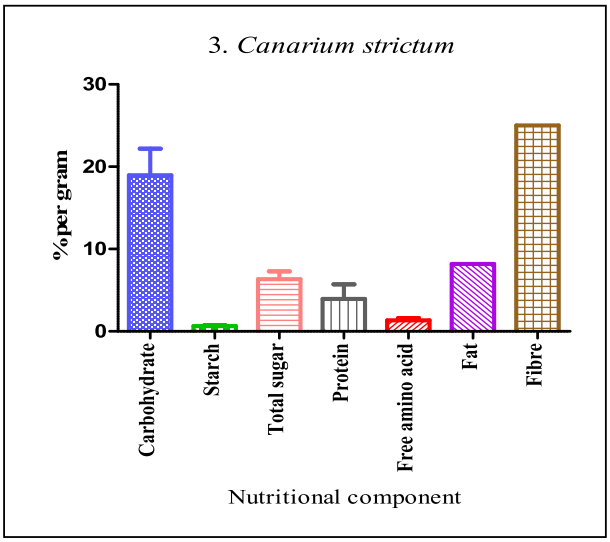
Note: DW= Dry Weight; *n*= 3 independent experiments.

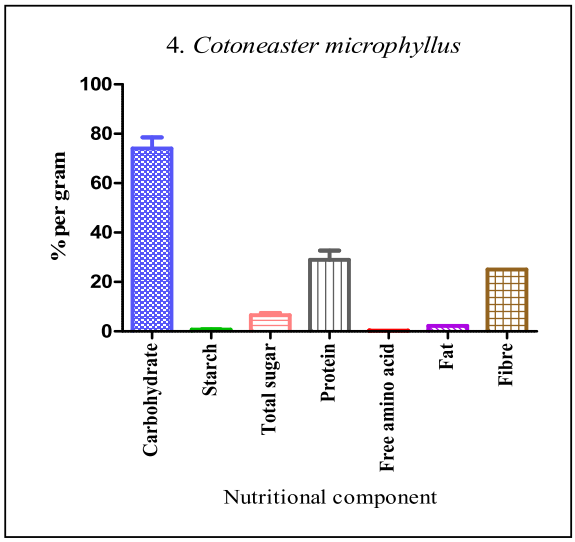
From the above table, it can be noted that carbohydrate composition is highest in*Cotoneaster microphyllus* (74.04± 4.49%)while *Livistona jenkinsiana* has the lowest value (5.53± 1.02%).*Docyniaindica*(3.81± 0.21%) has the highest starch content and *Embeliaribes*has the lowest (0.39± 0.01%). Sugar content is highest in *Annona squamosa*(28.51± 2.53%) and lowest in *Rhuschinensis* (1.86 ±0.88 %). *Diospyros kaki* has the highest protein content (29.86 ±8.99 %) and the lowest is observed in *Annona squamosa* (0.70 ±0.47 %). The free amino acid is found to be highest in *Terminalia*

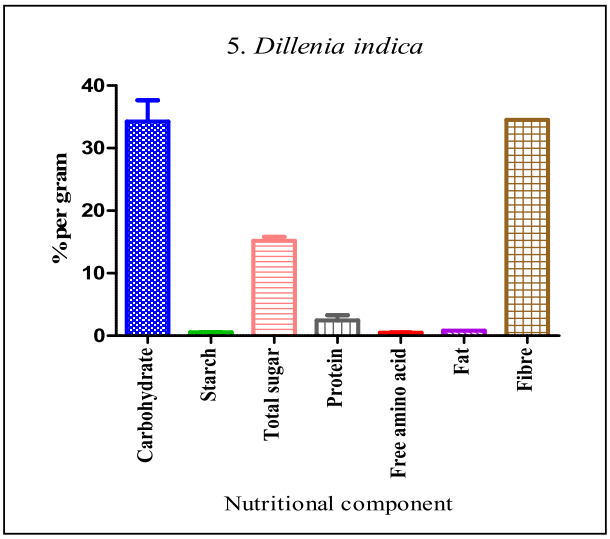
bellerica (3.73± 0.05 %)) and lowest in *Diospyros kaki* (0.24± 0.03%). Crude fat content is highest for *Livistona jenkensiana(37.6%)* and three samples are showing the same lowest value as 0.2%. *Rhus chinensis* has the highest crude fiber content (68%) while *Diospyros kaki* shows the lowest as 7% content.

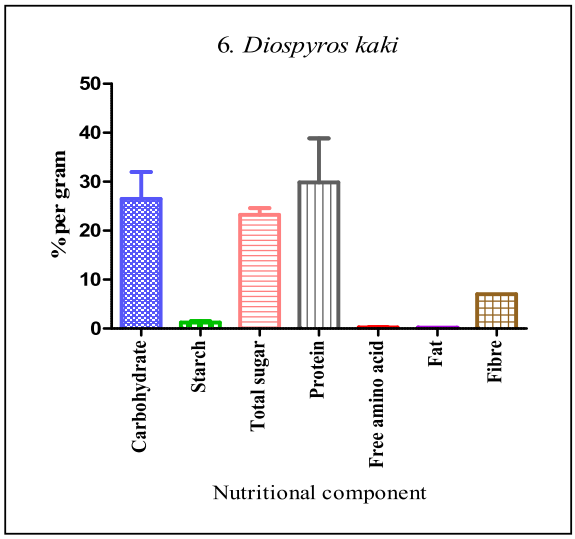

1. Actinidia arguta
% per gram
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

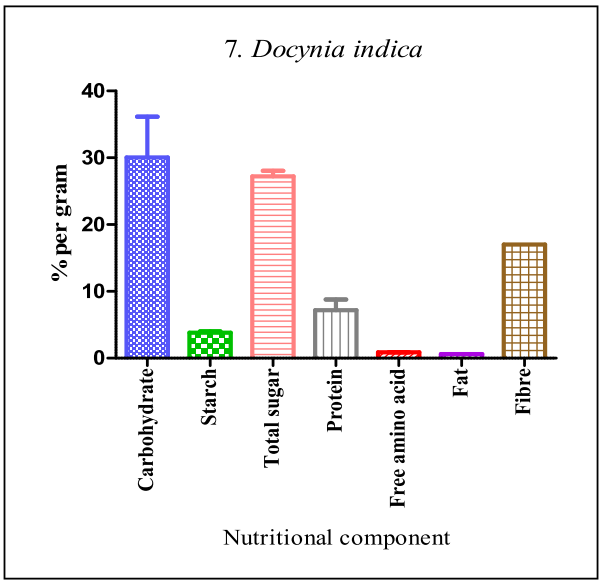

2. Annona squamosa
% per gram
80
60
40
20
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

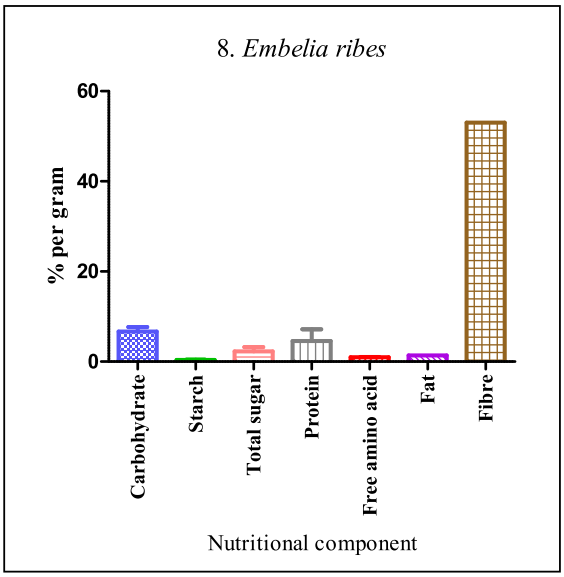

3. Canarium strictum
% per gram
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

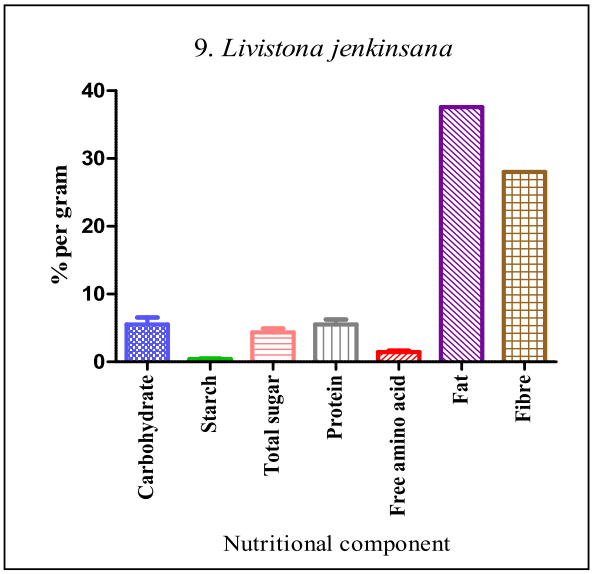

4. Cotoneaster microphyllus
% per gram
100
80
60
40
20
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

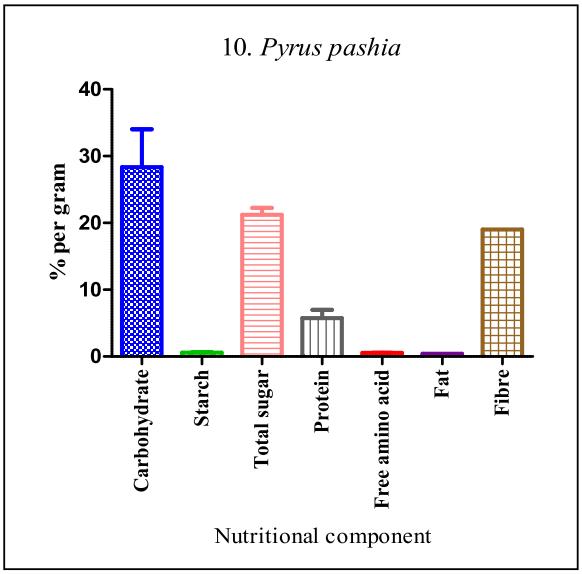

5. Dillenia indica
% per gram
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

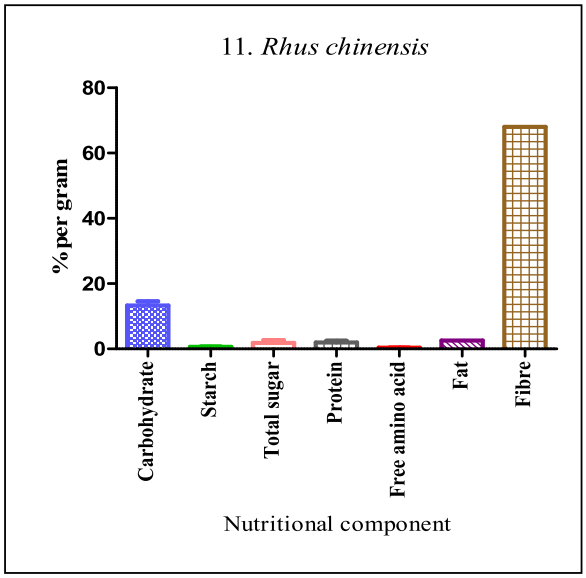

6. Diospyros kaki
% per gram
50
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

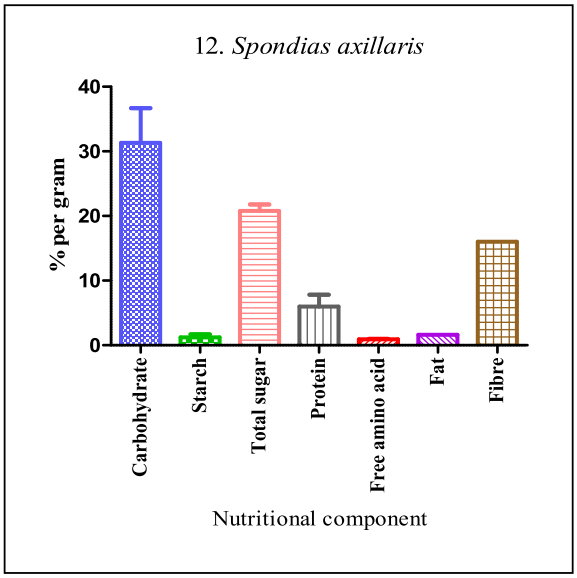

7. Docynia indica
% per gram
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

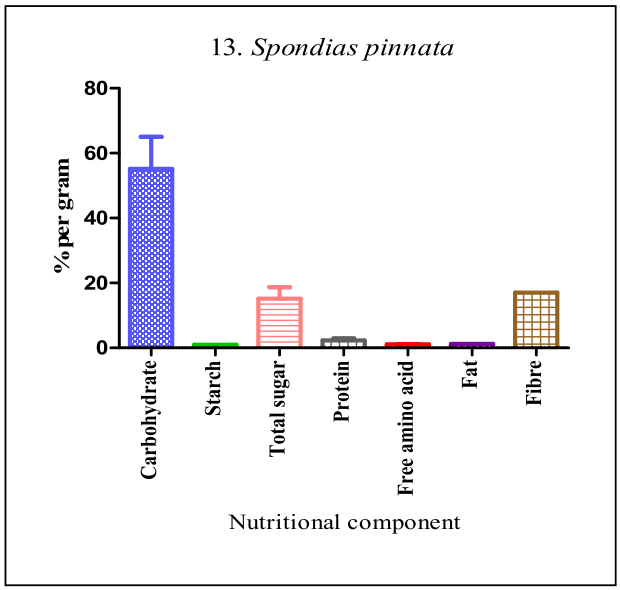

8. Embelia ribes
% per gram
60
40
20
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component

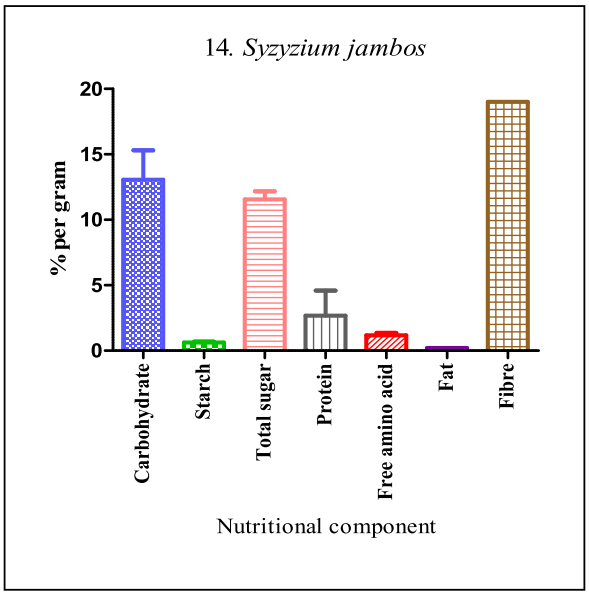

9. Livistona jenkinsana
% per gram
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component


10. Pyrus pashia
% per gram
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component


11. Rhus chinensis
% per gram
80
60
40
20
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component


12. Spondias axillaris
% per gram
40
30
20
10
0
Carbohydrate
Starch
Total sugar
Protein
Free amino acid
Fat
Fibre
Nutritional component





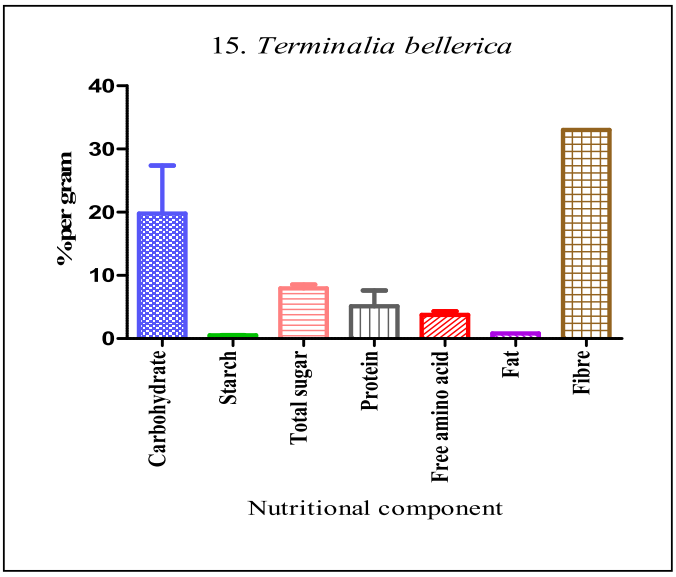


Fig8.3 (1-15): Nutritional information of each sample.

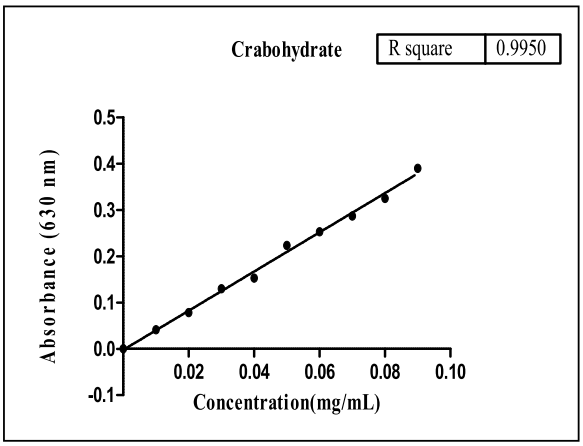


Fig 8.3(16): Glucose standard curve

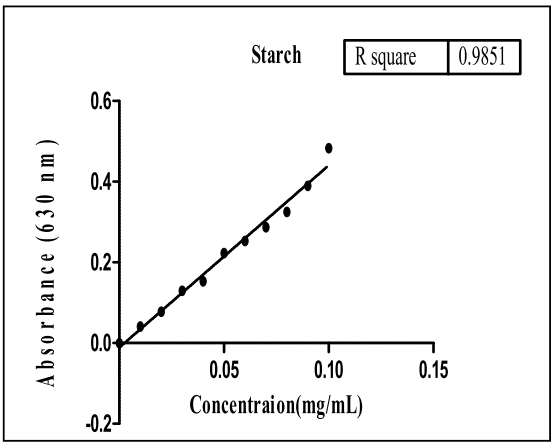


Fig 8.3(17): Glucose standard curve

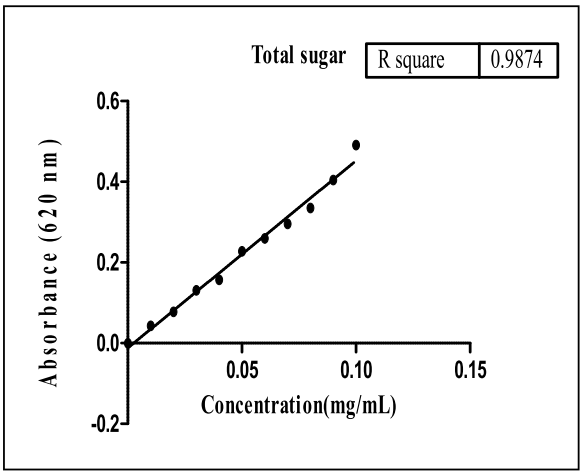


Fig 8.3(18): Glucose standard curve

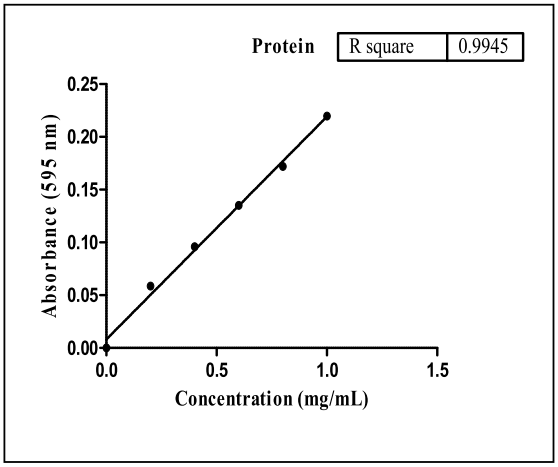


Fig 8.3(19): Bovine Serum Albuminstandard curve

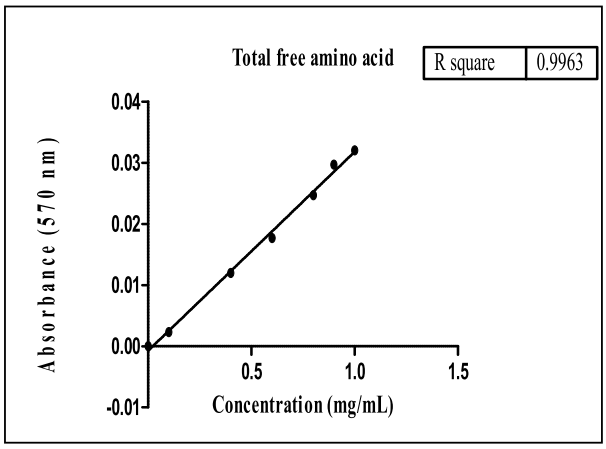


Fig 8.3(20): L-Leucine standard curve

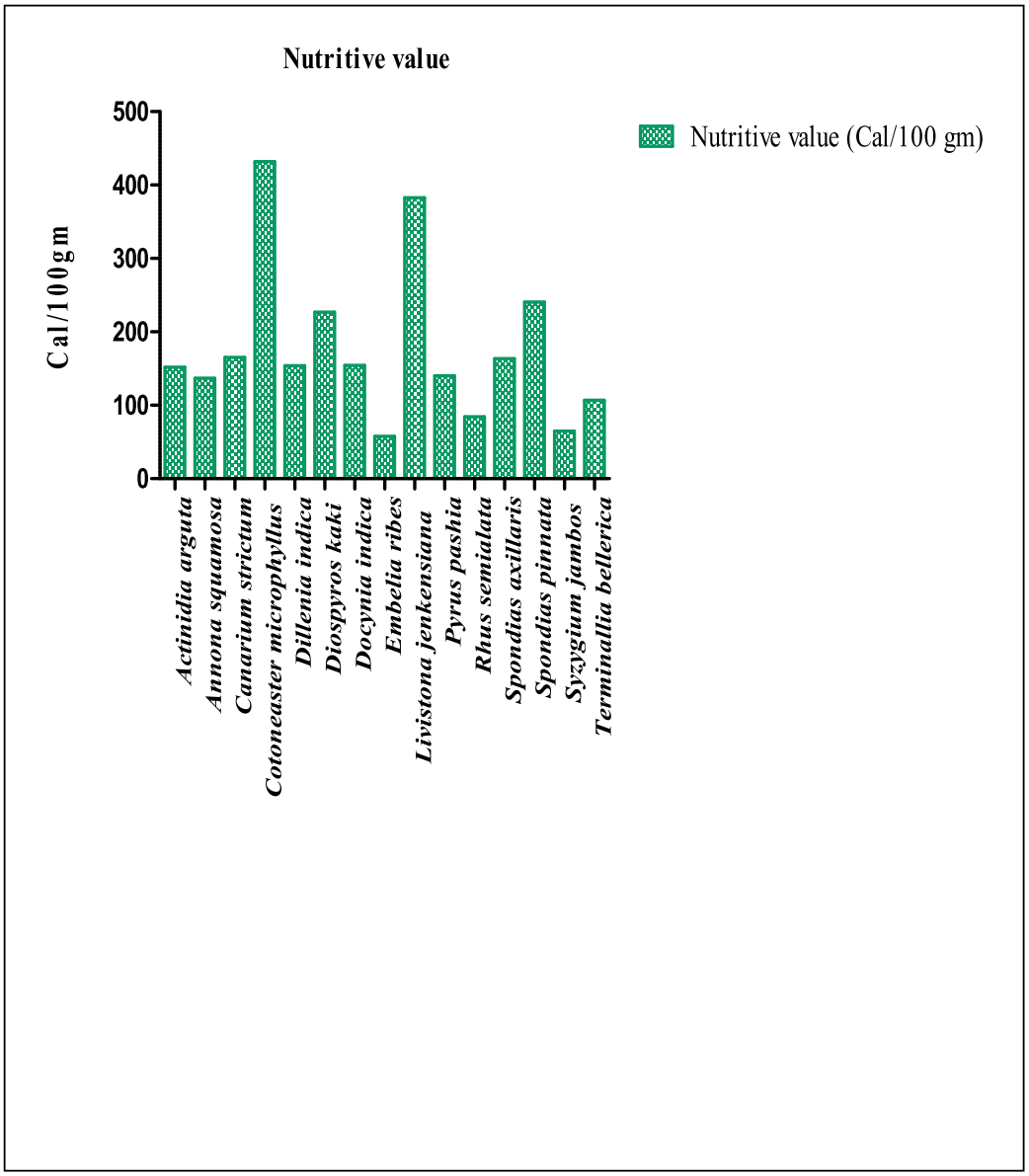


Fig 8.3(21): Graphical representation of Nutritive Value of samples

Table 8.3.2: Nutritive value of samples

Samples	Nutritive value(Cal/100gm)
Actinidiaarguta	151.84
Annonasquamosa	137.04
Canariumstrictum	165.36
Cotoneaster microphyllus	**431.92**
Dilleniaindica	153.92
Diospyros kaki	227.08
Docyniaindica	154.32
Embeliaribes	57.56
Livistonajenkinsiana	382.6
Pyruspashia	140.04
Rhussemialata	84.52
Spondiasaxillaris	163.56
Spondiaspinnata	240.52
Syzygiumjambos	64.76
Terminalliabellerica	106.8

8.4 Discussion

The result for nutritional analysis is given in Table 8.3.1. The quantity of each nutritional parameter varies in every sample as can be observed in Fig(s) 8.3(1-15). However total carbohydrate and fibre content are most prominent in all the samples. There is an appreciable occurrence of total sugar and protein content in some sample. Other parameters such as starch, free amino acid and fat content may vary highly in all of the samples or may be present in low concentrations.

Carbohydrate

Carbohydrates are the most important source of energy, which we require for full functionality of our body. Sufficient carbohydrate is required for the proper functioning of the body and brain. Consumption of carbohydrate delivers energy as well as other nutritional values because it comprises a wide range of macromolecules. As such, the

estimation of carbohydrate becomes a major part of nutritional analysis. The carbohydrate estimated includes both simple and complex groups.

Table 8.3.1 shows that almost all of the samples have an appreciable amount of carbohydrate content. The highest was found in *Cotoneaster microphyllus* (74.04±4.49%) while the least was observed for *Livistona jenkinsiana* at 5.53±1.02%. Carbohydrate content for *Spondias pinnata*(55.15±9.87%) is found to be higher than those reported from the Western Ghats of India (19.89%) by Mundaragi *et al*. (2017), Chauki and Mletha areas of Western Himalaya (23.54±0.50 % and 16.30±0.05 %) by Andola and Purohit (2010) and earlier reported as 4.5% by Goapalan *et al*. (1993). *Syzyzium jambos* also has a higher value (13.06±2.26%) than earlier reports byMundaragi *et al*. (2017) and Gopalan *et al*. (1993) as 8.71% and 8.5% respectively.The same can be observed for *Dillenia indica* which has a higher value (34.24±3.41%) than 13.4% as reported by Gopalan *et al*. (1993). However, *Docyniaindica* at 30.05±6.11% has a lower value than those reported from Sikkim (57.1%) by Sharma *et al*. (2019). *Spondias axillaris* from Sikkim at 87.07±0.01% (Pandey *et al*., 2018) also has a higher value than the present study which had 31.30±5.38%.*Rhus chinensis* from Manipur (Heirangkhongjam and Ngaseppam, 2018) also has a higher value (49.35±1.16 %) than the present study (13.29±1.31%). *Annona squamosa* also has a lower value (33.11±3.55%) than those from Madhya Pradesh (59%) as reported by Reena and Agrawal (2017). This comparison of data shows that with a change in geographical locations there are variations in % component. Overall, these minor fruits can be stated to be an amazing source of carbohydrate which can be found easily in the wilderness.

Starch content

Starchy foods have been recognized as potential "Functional food" (Vorster and Nell, 2001). Starches are digestible polysaccharides under carbohydrates. They are the chief source of energy. Starch content was found to be highest in *Docynia indica* (3.81±0.21%) followed by *Actinidia arguta*(2.22±0.58%). The least content was observed in *Embelia ribes* (0.39±0.21%).

Total Sugar

They are the simplest and smallest type of carbohydrate which can be easily absorbed by the body. They exist as a monosaccharide (glucose, fructose) and disaccharide (sucrose, lactose, maltose) and are responsible for sweet taste. They include both reducing type and non-reducing type sugars. They can bind with water molecule which becomes a major advantage for the preparation of jams and jellies (IFIC, 2012).

In the present study, *Annona squamosa* had the highest sugar content (28.51±2.53%) while the least was observed for *Rhus chinensis* (1.86±0.88%). Sugar content in *Annona squamosa* is found to be almost similar to those reported from the pulp of the same from Allahabad (21.50±10.95%) and Kerala (25.3%) by Srivastava *et al*. (2017) and Sasidharan and Jayadev, (2017) respectively.Reena and Agrawal (2017) have reported that glucose and fructose are major sugar component in *Annona squamosa* from Madhya Pradesh. Soluble sugar content in *Actinidia arguta* is found to be higher (18.28±5.37%) than those reported (3.9-9.6%) from the fresh weight of the same with sucrose at the highest level (Boyes *et al*., 1996; Nishiyama *et al*., 2008;Cui *et al*., 2013;Wojdylo *et al*., 2017)

Unlike added sugars used in food processing which are responsible for weight gain, tooth decay and type 2 diabetes (Morenga *et al*., 2012; Sheiham and James, 2014), the fruit sugars are reported to be harmless (Misra *et al*., 2016). Lack of sugar in diet had

been related to restlessness, blurred vision, and weakness (Misra *et al*., 2016). However, excess sugar consumption leads to weakening of immune system, hyperuricaemia, sucrose allergy, ageing and degeneration of eye muscles (Misra*et al*., 2016)

Protein

Proteins are building blocks of tissues and organ system as well as a defence system. They are formed by associations of amino acids linked through peptide bonds. They repair cells and tissues, carryout transportation function, and act as a catalyst too (Wu *et al*., 2014). They are an important constituent of hormones and toxin as well. Proteins are also known to be a source of other vitamins such as Vitamin B, D, E and minerals such as iron, phosphorus, zinc etc. Deficiency of protein leads to PE-M such as marasmus and kwashiorkor and stunted growth especially among children (Ghosh *et al*., 2012).

There are many methods for protein estimation. Biuret, Bradford, Lowry and Kjeldahl methods are some of the popular methods. Among them Bradford method has been reported as a better, reliable method due to its simplicity, sensitivity and minimal interferences by other components present in the sample (Berges *et al*., 1993; Maehre*et al*., 2018). Thus this method was selected based on the said criteria. During the experiment, it was taken into consideration that the buffer used for preparing the standard and extracting protein from samples were the same. This assay measures the occurrence of basic amino acid residues such as lysine, arginine and histidine, tyrosine, tryptophan and phenylalanine (Compton and Jones, 1985; He, 2011). The interaction between the dye and protein is reported to be electrostatic (Roger, 2001)

Protein content in *Diospyros kaki* was found to be highest (29.86±8.99%). However, the same from Turkey has been reported to have low protein content at 0.6% (Celik and Ercisli, 2008). The protein content of *Spondias pinnata* was found to be 2.28±0.60 %

which is almost similar to 2.13 % as reported from Western Ghats of India by Mundaragi*et al*. (2017) and 2.12±0.05 % from Chauki, Western Himalaya (Andola and Purohit, 2010). It is, however, higher than those reported from Nigeria (0.93%) by Owolarafe *et al*. (2006), Sikkim (0.70%) Khomdram *et al*. (2014), Maletha area (0.87±0.04%) in Western Himalaya (Andola and Purohit, 2010) and earlier reported as 0.7% by Gopalan *et al.* (1993). But those from Manipur (Khomdram*et al*., 2014) have been reported to have a higher value (18.92±1.9%) than the present study. Protein content for *Syzyzium jambos* is higher (2.68±1.91%) than those from Western Ghats (0.84%) of India as reported by Mundaragi*et al*. (2017) and report by Gopalan *et al*. (1993) as 0.7%. *Docynia indica* has comparatively lower protein (7.18±1.61%) content than those from Sikkim (32.2 %) as reported by Sharma *et al*. (2019). Gopalan *et al*. (1993) have reported 0.8% protein content in *Dillenia indica* which is lower than the present study (2.44±0.83%). Protein content value for *Spondias axillaris* in the present study (5.99±1.81%) is almost similar to those from Sikkim (6.31±0.58%) as reported by Pandey *et al*. (2018). *Rhus chinensis* has a lower value (1.99±0.53%) than those reported by Heirangkhongjam and Ngaseppam, (2018) from Manipur. The reported values for protein content in *Annona squamosa* has higher values, as 5.2% from Madhya Pradesh (Reena and Agrawal, 2017), 2.80±0.30% from the pulp of the same from Allahabad Srivastava *et al*.(2017) and 1.07% from Kerala Sasidharan and Jayadev (2017).

It is observed that not much accountable protein content is found in all of the collected samples except for *Diospyros kaki* and *Cotoneaster microphyllus*. National Academy of Sciences (1989) has reported that about 20 amino acids are common in the animal and plant protein which makes plant protein no lesser valueable than animal protein. However, the difference is that consuming plant-based proteins, unlike animal sources,

canreduce the chances of health problems such as constipation, obesity and cardiovascular diseases etc (Shimazu *et al*., 2007; FAO, 2010; Geber *et al*., 2013;Rosato*et al*., 2014;Shu *et al.,* 2015;Jannsch*et al*., 2017). Thus these fruits can make their tiny contributions in a noble way for a balanced diet. It is also reported that bakery products prepared from plant proteins could be consumed by diabetic persons (Agunbiade and Olanlokun, 2006)

FAO (2009) believes that shifting protein source from animal to plant would also minimise the pressure on environmental degradation. On a lighter note, plant protein could be replaced in place of animal protein for the ageing population, with weakening metabolic functionality, as they are comparatively easier to consume.

Free Amino Acids

Total free amino acids in the samples were quite low or rather undetectable as can be seen from Table 8(A). The highest was observed for *Terminalia bellerica* (3.73±0.05) while *Diospyros kaki* had the lowest value (0.24±0.03%). Jin *et al*. (2014) have identified glutamic acid and aspartic acid as major amino acids in *Actinidia arguta* along with other 20 (approx.) different amino acids.

Ninhydrin reaction has been reported to detect aminoacids such as tryptophan, which is one of the essential amino acids. Essential amino acids are those amino acids which cannot be manufactured by our body and must be obtainedfrom diets we consume while non-essential amino acids are manufactured by our body itself. The detection of the free amino acid via this method implies that these minor fruits have some low concentration of essential amino acids which are required by our body.

The functions of amino acids are numerous and lucid. Branched-chain amino acid (BCAA), valine, leucine and iso-leucine are reported to speed up muscle recovery and exercise-related muscle issues (Nosaka *et al*., 2006; Shimomura *et al*., 2006). Amino

acids play a significant role in gene expression and augment the development of skeletal muscle and small intestine (Scot *et al*., 2006; Themelis *et al*., 2017). They arealso known to be the precursor of alkaloids in plants and serotonin and melatonin in human (Takahasi *et al*., 2011; Moran-Palacio *et al*., 2014).

Fat

Fats can be stated as the storehouse of energy and medium for fat-soluble vitamins (A, D, E and K). They supply more energy than carbohydrate or protein. *Livistona jenkinsiana* had the highest fat content (37.6%) followed by *Canarium strictum*(8.2%) and *Actinidia arguta* (5%).The presence of high-fat content in *Livistona jenkinsiana*had been mentioned by Payum (2018). Fat content in *Spondias pinnata* (1.2%) is higher than earlier reported as 0.71% from Western Ghats of India (Mundaragi*et al*., 2017), but lesser than those from Chauki (12.23±0.06 %) and Maletha (12.54±0.04%) in Western Himalaya (Andola and Purohit, 2010) as well as the report (3.0%) given by Gopalan*et al*. (1993). 0.2% fat content in *Syzyzium jambos* was found to be similar with those reported from the Western Ghats (0.22%) by Mundaragi *et al*. (2017) and the value 0.2 % reported by Gopalan*et al*. (1993).*Docynia indica*had a lower value (0.6%) than those reported from Sikkim (6.7%) by Sharma *et al*.(2019). In the case of *Dillenia indica*, it was higher (0.8%) than 0.2% as given by Gopalan*et al*.(1993). *Spondias axillaris* protein content is lower (1.6%) than earlier reports (2.10±0.28%) according to Pandey *et al*. (2018). *Rhus chinensis* from Manipur (Heirangkhongjam and Ngaseppam, 2018) had so much higher fat content (12.97±0.87%) than the present study sample (2.6%). *Annona squamosa* pulp from Allahabad (Srivastava *et al*., 2017) has been reported to have 0.39±0.35% fat content while those from Kerala (Sasidharan and Jayadev, 2017) has 3.3% which is higher than the present value (0.2%). Jin *et al*., (2014) has reported 13.9-30.5% saturated fatty acids and 70.4-85.8% unsaturated fatty

acids with palmitic acid and α-linoleic acid as major saturated and unsaturated fatty acid respectively in *Actinidia arguta*.

Essential fatty acids are especially required during foetal development-throughout infancy as well as the growth period of children (Burlingame *et al*., 2009; Nandal and Bhardwaj, 2014). These minor fruits can thus provide essential fatty acids to expecting and lactating mothers as well as growing children in the region where proper infrastructure has been lacking. It is reported thatpyronoderma and weight loss are imminent repercussions of fat deficiency in the diet (Gopalan *et al*., 2004). But it should be noted that high fat (both saturated and trans-fat) intake may result in malfunctioning of coronary veins and other heart-related health issues.

Fibre

Fibres are the indigestible polysaccharide. Cellulose, hemicelluloses, lignin and pectins are some examples. They are categorized as soluble fibre and insoluble fibre.Soluble fibres are good for slow digestion and prevent excess sugar and starch absorption by the body while insoluble fibres are essential for bowel movements. They help in treating Irritable Bowel syndrome, prevent colorectal cancer, maintaining a healthy weight, keep in check cholesterol and sugar level (Dhingra *et al*., 2012), reduces obesity, gastrointestinal disorders, cardiovascular diseases, improve blood glucose, diabetes (Otles and Ozgoz, 2014)

Rhus chinensis had the highest fibre content (68%)which is higher than those reported from Manipur (22.15±0.1%) by Heirangkhongjam and Ngaseppam (2018).*Annona squamosa* has 64% fibre content which is also quite high and much higher than those reported as 11% from Madhya Pradesh by Reena and Agrawal, (2017), and 3.30±0.60% as reported by Srivastava *et al*. (2017) from Allahabad.*Dillenia indica* also showed good fibre content (17%) which is much higher than those reported (2.5%) by

Gopalan *et al.*(1993). The crude fibre content in *Spondias pinnata* was found to be 17 % which was much higher than 1.29% as reported by Mundaragi *et al*.(2017), 1% (Gopalan*et al*., 1993) and 4.03±0.05% (Chauki area) and 3.13±0.12% (Maletha area)in Western Himalaya by Andola and Purohit, (2010). The fibre content in *Syzyzium jambos* was higher in the present study (19%) when compared to reports from Western Ghats of India (1.76%) byMundaragi*et al*. (2017) and 1.2 % as reported by Gopalan *et al*. (1993). *Spondias axillaris* from Sikkim (1.55±0.30%), reported by Pandey *et al*. (2018) has lower fibre content than present study sample which had 16% for the same. In the present study, it was observed that *Diospyros kaki* had the lowest (7%) fibre content.

Nutritive value

Nutritive value gives us an idea about the potential energy content in food. As per the formula given by Indrayan*et al*. (2005) it may be defined as the cumulative sum of the product of 4 times the % of protein and 9 times the percentage of fat and 4 times the % of carbohydrate, expressed in Calorie per 100 gm.

Table 8.3(B) shows that the highest energy can be obtained from *Cotoneaster microphyllus*(431.92 Cal/100gm) followed by *Livistona jenkinsiana* (382.60 Cal/100gm).*Spondias pinnata*at 240.52 Cal/100gm had higher energy content than those reported by Mundaragi*et al*. (2017) as 94.47 Cal/100gm. The lowest was observed in *Rhus chinensis* (84.52 Cal/100gm). Mundaragi *et al*. (2017 has also reported nutritive value for *Syzyzium jambos* (40.18 Cal/100gm) which is also lower than the present study (64.76 Cal/100 gm). Nutritive value of *Spondias axillaries* as 373.18±2.28 Cal/100gm from Sikkim (Pandey *et al*. 2018) has higher nutritive value than the present test (163.56 Cal/100gm).

8.5 Conclusion

Each nutritional parameter has its vital role and overconsumption has equal side effects. Overall, all the samples have good nutritional value, based on the tested parameters. For developing countries like India where malnourishment is a continuous menace, these fruits can act as an alternative source of nutrition. Unlike the similar result pattern observed in antioxidant activity, the amount of nutritional components varied from species to species. Thus there exists no fruit as “perfect fruit” with all the required nutrition; if one parameter is high in a sample, the other parameter may below. This calls for the consumption of a variety of fruits to get maximum nutrition from nature. Throughout the discussion, it has been noted that % content for almost all parameters in every sample, varied every time a report was mentioned. It may be due to the difference in geographical conditions such as climate, altitude, temperature etc. or edaphic factors as such as pH of soil or time of harvesting or the methods how samples were gathered and processed etc. To generalize, these minor fruits from Arunachal Pradesh have decent nutritive value with high carbohydrate and fibre content. They can be a great source of energy for the rural or indigenous population as well as for people visiting fields for other activities such as research, trekking and other adventures etc. The earnest efforts required now are their introduction to the larger population and more awareness about their importance before they are erased as “useless” trees in the name of development.

Chapter 9

Correlation between Antioxidant activity and Nutritive value

9.1 Introduction

From Chapter 6 and 8 it is observed that for the antioxidant activity we had taken 32 samples out of which 15 were analysed for the nutritional parameter. These 15 samples comprised of those showing high antioxidant activity viz., *Dillenia indica, Docynia indica, Embelia ribes, Livistona jenkinsiana, Rhus chinensis, Spondias axillaries, Spondias pinnata* and *Terminalia bellerica* average antioxidant activity viz., *Actinidia arguta, Canarium strictum, Cotoneaster microphyllus, Diospyros kaki, Pyrus pashia, Syzygium jambos* and poor antioxidant activity *Annona squamosa.*

A Pearson Correlation test was conducted to check if both antioxidant activity and the nutritive value were correlated.

9.2 Materials and methods

Data collection: Data for Nutritive value was obtained from Table 8(A) and antioxidant activity from Table 6.3(A) and (B)

Statistical Analysis: The data were subjected to Pearson's correlation XY Analysis usingGraphpad Prism® software version 5.03.

9.3 Result and Discussion

From the table below, it can be noted that there is no significant correlation between antioxidant activity and nutritional value. Even the amount of phyto-constituents such as phenol and flavonoids did not make any major difference in the nutritional values of fruits.

Table 9.3.1: Summary of Linear correlation between Nutritive value and Antioxidant values

Parameter	DPPH	ABTS	FRAP	TAC	TPC	TFC
Number of XY Pairs	15	15	15	15	15	15
Pearson r	0.07059	-0.04618	0.07747	0.2530	-0.04051	0.2773
95% confidence interval	-0.4584 to 0.5626	-0.5456 to 0.4775	-0.4529 to 0.5673	-0.2980 to 0.6775	-0.5416 to 0.4819	-0.2740 to 0.6914
P value (two-tailed)	0.8026	0.8702	0.7838	0.3630	0.8860	0.3170
P value summary	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
Is the correlation significant? (alpha=0.05)	**No**	**No**	**No**	**No**	**No**	**No**
R square	0.004983	0.002133	0.006001	0.06400	0.001641	0.07689

9.4 Conclusion

Antioxidant potential and nutritional values are independent of each other.

Chapter 10

General Conclusion

A collection of 69 minor fruits from 13 districts of Arunachal Pradesh gives a positive outline about more possible numbers in the State. As the study was not possible for every nook and corner of the State, chances are that many are yet to be explored and identified. Antioxidant and nutritional potential from selected samples of the collection was quite appreciable which is in par with the hypothesis mentioned earlier. In comparison to other samples, *Livistona jenkinsiana, Spondias axillaris* and *Spondias pinnata* had higher nutritional and antioxidant value. All of these fruits are freely available in wild and consuming these fruits will be beneficial for the people.

As far as commercialisation is concerned, it was observed that few of them are/may be seasonally available only in the local market without much attraction from customers. Lack of awareness about their potency in one's wellbeing is the prime reason for such negligence because taste may not always rule out health benefit. It was also learned that fruits of *Embelia ribes*, which in under Red Listed Medicinal Plants by ENVIS, were illegally traded in some places to outsiders from indigenous people who had no or lesser knowledge about the use-value of this fruit. Such biopiracy is a major threat to biological diversity in the State besides other activities such as developmental project-related activities, anthropogenic disturbances etc.

Documenting these fruits to keep in records the genetic diversity of plants in the State and spreading awareness of about these fruits are the prime need of the hour. More field trips and a detailed study of such minor fruits seem to be an interesting and

propitious path.Further research calls for more exploration of minor fruits in the State and extrapolation of their chemical constituents. Natural calamities, lack of infrastructure, degrading ecosystem etc. have been identified as major setbacks for research in this area. However, if efforts are made then future prospects seem bright which include employment, women empowerment, entrepreneurship andcapital generation for the State government.

Summary

The present study deals with the study of minor fruits of Arunachal Pradesh with special reference to antioxidant potential and nutritional value. The thesis comprises of 10 chapters including Introduction and Literature review.

It starts with an introductory note on the general description of fruit in technical term along with details how they are differentiated in the botanical world.Their importance in maintaining a healthy life has been described.For a better understanding of the antioxidant activity, the process of oxidation has been briefed and how antioxidants prevent or break the oxidation process has been mentioned.

During research it was found that fruits consumption had increased rapidly around the world, making them costlier each passing day. Owing to situations as such, affording fruits from the market has become a challenge for the economically weaker section of societies. To overcome this problem, minor fruits had been identified as a key role player by the researchers. To better understand their role around the world, literature review was done in Chapter 2. Throughout the review, a prime focus was observed on the role of minor fruits in providing nutrition and required antioxidant to people of developing countries. The alarming issues of food security around the world were also noted to be fixed by these minor fruits. From international to national to State wise scenario, it was found that minor fruits had eminent role in upgrading the livelihood

among the rural population. It was also noted that these fruits were also a source of alternative nutrition for the indigenous population.

From Arunachal Pradesh, a total of 69 fruit samples were collected out of which ethno botanical data could be obtained for only 33 samples. By Random sampling technique, 32 samples were picked for antioxidant study. Time constrains and seasonal availability had been a major factor for low number of selection for estimating the mentioned qualities. Preliminary tests were held for 32 samples which showed that most of the samples had positive result for phytoconstituents such as phenolic compounds, flavonoids, carbohydrates and proteins etc. For deeper investigation into the antioxidant activity and nutritional value, quantitativeanalysis was done. DPPH, ABTS, FRAP, TAC, TFC and TPC were done for estimation of antioxidant activity and found that *Docyniaindica,Elaeagnuslatifolia, Embeliaribes,Ficussemicordata, Livistonajenkinsiana, Macrosolencochinchinensis, Quercussemecarpifolia, Rhuschinensis, Rosa sericea, Spondias axillaries,Spondiaspinnata* and *Terminalia bellerica* had high antioxidant potential. Hydro-distillation was done for extracting essential oil from *Magnolia champaca* which was later found to have low antioxidant activity. The experiment continued with 15 samples for estimation of major nutritional components such as protein, free amino acid, carbohydrate, total sugar, starch, fat and fiber components. These 15 samples were a combination of those samples with high, intermediate and low

antioxidant potential from the previous experiment. *Cotoneaster microphyllus*, *Livistona jenkinsiana and Spondias pinnata* were detected as highly nutritional ones based on the nutritive value calculated by the standard formula. Apart from two samples all other samples had more or less great nutritive value. A Pearson's correlation was conducted to check the relationship between antioxidant potential and nutritional values of samples. It was found that both were independent of each other. When Correlation was performed between phytoconstituents phenolic compounds and flavonoids with antioxidant activity, it was found that significant correlation existed between phenolic compounds with antioxidant activity but the case was not the same for flavonoids content in the samples.It was finally concluded thatthe samples of minor fruits collected from Arunachal Pradesh are a blend of high, intermediate and low antioxidant potential. On the nutritional scale most of them had satisfactory value. Consuming these fruits would help procure a balanced diet to the indigenous populations of the State.