CHAPTER I

INTRODUCTION

Bangladesh has the largest biodiversity of any country in the world, which abounds with a large variety of tropical and sub-tropical fruit species (Leterme et al., 2006). Fruit and vegetable consumption is considerably increasing in the daily diet, they supply high levels of biologically active compounds which imparts health benefits beyond basic nutrition. Many fruit species are unknown, and therefore, relatively few fruit species are commercially available (Mattietto et al., 2010). Many native species have unique sensory characteristics and high nutrient concentrations. Tropical fruit consumption is increasing in the domestic and international markets due to growing recognition of the nutritional and therapeutic value of fruit (Rufino et al., 2010). So fruits have a great impact both economically and nutritionally (Cardoso et al., 2011). Fruits represent an opportunity for local growers to gain access to specialized markets, by this way consumers demonstrate a preference for exotic characteristics and the presence of nutrients help to prevent degenerative diseases (Alves et al., 2008).

Antioxidants are the substances that can protect against the cell damage due to free radicals. It acts as like radical scavenger, hydrogen donor, electron donor, peroxide decomposer, enzyme inhibitor, metal chelating agents. It performs as anti-cancers, anti-aging, anti-inflammation (Cai et al., 2004). Fruits contains antioxidant compounds like phenol, vitamins, arytenoids and minerals that has chemo preventive effects (Almeida et al., 2011). Antioxidants are commonly interfering with different macromolecules such as carbohydrates, lipids, and proteins and form the food matrix (Manach et al., 2004). Fruits and vegetables also act as antioxidants are fiber, polyphones, conjugated isomers of linoleum acid, D- limolene, epigallocatechin, gallate, soy protein, isoflavones, vitamins A, B, C and E (group of tocopherols), calcium, selenium, chlorophyllin, alipharin, sulphides, catechin, tetra-drocurecumin, seasaminol, glutathione, uric acid, indoles, thiocyanates and protease inhibitors .

The antioxidant content of fresh tissues can be affected by maturity, agricultural practices, temperature and storage conditions. Different factors such as high temperature storage, ultraviolet-C irradiation, hormone treatment, among others, affect fruits and vegetable antioxidant capacity. The presence of high amounts of bioactive compounds in fresh tissue does not assure their bioavailability when they are consumed. The amount and type of antioxidant present in the fresh tissues effects the antioxidant capacity of fruits and vegetables. However, the individual contribution to the total antioxidant capacity implies rapidly. The contribution of phenols and flavonoids is higher extent than vitamin C, carotenoids, and others to the antioxidant capacity of fruits and vegetables. The high concentration of vitamin C of several fruits provides only 10–20% of the total antioxidant capacity. However, the contribution of vitamin E in fruits is significantly more to the antioxidant capacity than vitamin C does. The health benefits of fruits and vegetables are well known, information on their bioactive compounds is required to know their specific contribution to the total antioxidant capacity of the tissues.

Natural antioxidants present in foods have attracted interest because of their safety and potential nutritional and therapeutic effects (Rufino et al., 2009). Vitamin C and water soluble antioxidant are capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E is a major lipid soluble antioxidant, has the ability of chain-breaking antioxidant within the cell membrane. It also has the ability to resist lipid peroxidation in the fatty acid cell membrane. Vitamin C is responsible for regenerating vitamin E (Sies, 2013). Beta carotene and other carotenoids have also the ability to provide antioxidant protection to lipid-rich tissues. There is also prove that beta carotene may work synergistically with vitamin E (Jocab, 1995). Synthetic antioxidants are chemically synthesized and used to food as preservatives to prevent lipid oxidation (Shahidi et al., 1992). Antioxidants defense against free radical damage, and are responsible for maintaining optimum health and wellbeing (Mark Perciva et al., 1998). If we take regular consumption of antioxidative vegetables and fruits which are reducing the risk of chronic diseases (Dembinska-Kiec et al., 2008). An antioxidant rich diet has a very positive health impact in the long run (Willis et al., 2009; Sin et al., 2013). It is a well-known fact that citrus fruits (oranges, lemons, etc.) hold a high amount of natural antioxidants, such as vitamin C. Blueberries, strawberries, grapes, plums, prunes, red beans, spinach, kale, broccoli flowers, alfalfa sprouts, and more contain a high amount of antioxidants. Recent studies demonstrated that fruit-like jackfruit, araticu-domato, pindo palm, and mandacaru-de-tresquinas are held sources of vitamins C and phenolic compounds (Swami et al., 2012; Pereira et al., 2013). The factors such as genetic, chemical, or biological modification increase the antioxidant potency of fruits (Gomes et al., 2013). If the peel is concerned, extraction helps to separate this part of the fruit are found to have a good total radical antioxidative potential (TRAP) (Gorinstein et al., 2004).

Bioactive compounds have extra nutritional constituents which are in small quantities. These compounds have a great impact on physiological, behavioral, and immunological. These compounds vary on the basis of chemical structure, function. Bioactive compounds have pharmacological and toxicological effects in man and animals (Bernhoft ., 2010). Bioactive compounds prevent disease as a result of oxidative stress (Kaur and Kapoor, 2001; Bernhoft, 2010). The levels of the bioactive compounds varies in foods in composition from various fruits, vegetables and genetic factor and environmental conditions such as light, maturity and postharvest treatments. Fruit maturity stage has also a main determinant of the levels of compounds during harvesting (Deepa et al., 2007; Vallejo et al., 2003). These compounds have different beneficial effects like antioxidant activity, inhibition of enzymes, inhibition of receptor activities and inhibition of gene expression. They are the photochemical (Correia et al., 2012). The concept of bioaccessibility can be defined as the quantity or fraction release from the food matrix in the GI tract and is available during absorption (Heaney., 2001). There happens digestive transformations of food into material ready for assimilation, absorbtions of food into intestinal epithelium cells and ends at pre systemic metabolism (both intestinal and hepatic). For so nutrients, there is beneficial effects of unabsorbed nutrients (such as binding of bile salts, calcium in the tract) would be missed by absorption-based definitions. Bio-accessibility simulates gastric and small intestinal digestion.

Polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organosulfur compounds are the heterogeneous classes and they have different chemical structures such as hydrophilic or lipophilic. They have a range of concentrations both in foods and in the human body, possible site of action, effectiveness against oxidative species, and specificity and biological action (Carbonell-Capella et al., 2014). There is a correlation with the bioavailability of antioxidants (Parada and Aguilera, 2007). The modification of bioavailability of active compounds interacts with other macronutrients such as fibrin, low processed foods and beverages or proteins and polysaccharides in processed food products (Dupas et al., 2006). Ingestion of different food may take place affecting photochemical bioavailability (for example, fat enhances quercetine bioavailability in meals) (Lesser et al., 2006).

Anthocyanins bioactive compounds are found in different fruits and vegetables, and their average intake are estimated to be 180215 mg/day in the United States (Kuhnau, 1976). During digestion, anthocyanins are exposed to different environments and pH values, which have a major influence on their form (Mcghie et al., 2003). Anthocyanins are stored predominantly in the stomach (Matuschek et al., 2006). The higher stability of anthocyanins is at lower pH value (Matuschek et al., 2006). As they have low bioefficiency, they are rapidly absorbed and eliminated. The formation and metabolism of anthocyanins are instable (Manach et al., 2005). The degradation of flavonoids is greater than the anthocyanins by gut microbiota (Scheline, 1991). Citrus byproducts also have a rich source of naturally occurring flavonoids (Horowitz, 1961). The peel which contributes almost a large amount of fruit mass contains the highest concentrations of flavonoids in the Citrus fruit (Anagnostopoulou et al., 2006). Flavonoids can be classified into different groups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. These subgroups are: flavones, flavonols, flavanones, flavanolols, flavones or catechins, anthocyanins and calcones. Flavanones and anthocyanins are majorly found in citrus fruits. Flavanonol (Taxifolin) is also found in citrus fruits.

In addition, dietary intake of fruits help to displace fatty and sugar rich foods which cause chronic disease in human body. So, consumption of fruits is increasing with direct or processed form. Over the last decade, a very few number of studies have investigated on nutritional composition, microbial quality and photochemical of rare fruits of Bangladesh. But, a clear summary on available rare fruits of Bangladesh is lacking. The present study was carried out to investigate bioactive compounds and antioxidant activity of rare fruits of Bangladesh.

1.1 Objectives of the study

- To determine the concentration of bioactive compounds (total polyphenols, total flavonoids and total anthocyanins) in rare ten fruits in Bangladesh.
- To analyze the antioxidative properties of these fruits.
- To compare the significant correlation of antioxidants and bioactive compounds.

1.2 Anticipated outcomes

- Estimated the concentration of bioactive compounds in rare fruits.
- Estimated the antioxidative properties of these fruits.
- A clear relationship among antioxidants, bioactive compounds and human body were discussed.

CHAPTER II

REVIEW OF LITERATURE

Relevant literatures on antioxidant activity and bioactive compounds of rare fruits available in Bangladesh have been discussed and reviewed in this chapter.

2.1 Rare fruits of Bangladesh

Bangladesh is a tropical country and abounds with a large variety of tropical and sub-tropical fruits. There are many minor edible fruits or rare fruits that are locally available in the wild and are also cultivated, such as *Syzygium samarangense L.* (Jamrul), *Flacourtia jangomas L.* (Pani Fol), *Averrhoa bilimbi L.* (Belombo), *Garcinia cowa L.* (Cow Fol), *Manilkara zapota L.* (Safeda), *Citrus limon L.* (Katajamin), *Sabal palmetto L.* (Ashari), *Calamus tenuis L.* (Bet Fol), *Ficus carica L.* (Dumur) and *Aegel mermelos L.* (Bele) are important rare fruits which have higher nutritive value. However, there is few information available on the presence and profiling of various bioactive constituents (such as polyphenols and dietary fiber) in them.

Fruits contain vitamins and minerals in large quantities. Fruits are the oldest food of mankind**.** Nutrition scientists advise us to take at least 115 grams of fruit every day for balanced diet (Zhou, 2012) .But at present our country has the capacity to provide each of us with only 38 grams of fruits every day (Amitava and Kimberly,2014). We need fruits for economic reasons too. Most fruit trees live for years. Fruit farming is quite profitable although it may be a little expensive at the beginning. Fruits can be processed for preservation in many ways. For example, different kinds of healthy foods like jam, jelly, candy, etc. and drinks can be made from fruits. Some fruits can be dehydrated for marketing. Most fruits available in our country do not grow in the cold countries. It gives us an opportunity to export our fruits there for foreign currency. Bangladesh is a tropical country and abounds with a large variety of tropical and sub-tropical fruits. There are many minor edible fruits or rare fruits that are locally available in the wild and are also cultivated trees usually bear two kinds of flowers: female and male. The ovum of a female flower or part of it gradually grows in health, size and shape into a fruit. Many a time the whole flower evolves into a fruit. Fruits are mainly divided into two categories:

(a) Periodical and (b) Seasonal. Different fruits grow in different countries. Fruits may be classified into two other groups on the basis of the amount of time the trees take to bear them after plantation: Short term fruits and long term fruits. Short term fruits that grow on trees in two or less than two years after plantation are called short term fruits. For example: banana, pineapple, papaya are term fruits: Fruits that grow on trees in more than two years after plantation fruits are called long term fruits. For example: mangoes, jackfruits, wood apple, etc**.** Seasonal fruits may be classified on the basis of the seasons in which they grow. For example: Summer fruits, winter fruits and all season fruits (Zhou, 2016).

2.2 Antioxidants

Antioxidants are the man made or natural substances that are at low concentration in comparison with main oxidizable substrate and prevent oxidation of that substrate (Halliwell, 2007). More than 170 antioxidants have been introduced in the current literature (Zhou, 2012). There is a good impact in the body defense system against ROS (Boxin et al., 2002) and they have a good contribution to control blood pressure or blood sugar influencing substances, or can act as agents with anticarcinogenic, immunity-supporting, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic or anti-inflammatory properties (Bub et al., 2003). The fruits and vegetables act as antioxidants fiber, polyphones, conjugated isomers of linoleic acid, dlimolene, epigallocatechin, gallate, soy protein, isoflavones, vitamins A, B, C and E. Calcium, selenium, chlorophyllin, alipharin, sulphides, tetrahydrocurecumin, seasaminol, glutathione, uric acid, indoles, thiocyanates and protease inhibitors (Karakaya et al., 2001).

2.2.1 Types of antioxidants

2.2.1.1 Natural antioxidant

Many fruits and vegetables have natural antioxidants and there is a great deal of public and scientific attention (Diwani et al., 2009). There happens constant oxidative stress from free radicals which are from all parts of plants reactive oxygen species, and prooxidants generated both exogenously (heat and light) and endogenously (H_2O_2 and transition metals). Many of these tissues are effective to control free radicals, lipid oxidation catalysts, oxidation intermediates, and secondary breakdown products by the use of antioxidant system (Brown and Kelly, 2007). These antioxidant compounds act as reluctant and consist of flavonoids, phenolic acids, carotenoids, and tocopherols that can inhibit Fe^{3+} induced oxidation, scavenge free radicals and natural antioxidants are found in natural sources such as fruits vegetables and meats. There are various natural antioxidants which are available in everyday foods, the most common of which have vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin A (carotenoids), various polyphenols including flavonoids, and anthocyanins and lycopene (Ozsy et al., 2009).

2.2.1.2 Synthetic antioxidant

Synthetic antioxidants are not found naturally, they are found by chemically synthesized (Shahidi et al., 1992). These antioxidants fall into two major categories on the basis of their mode of action Primary antioxidants and secondary antioxidants. The primary antioxidants help to prevent the formation of free radicals during oxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are common antioxidants and widely used around the world for decades.

2.2.1.3 Dietary antioxidants

Ascorbates, tocopherols and carotenoids are well known antioxidants and research shows the relation of the health benefits (Boskou et al., 2005). Vitamin C, vitamin E, and beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and luteinare are well known dietary antioxidants.

2.2.1.4 Exogenous

Exogenous antioxidants bring out vitamins, flavonoids, anthocyanins, some mineral compounds. They are also able to bring out synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene and gallates (Litescu et al., 2011). Antioxidants are intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foods stuffs (Molyneux, 2004).

2.2.1.5 Endogenous antioxidants

Endogenous antioxidants are secreted from the body's metabolism may be enzymatic or non-enzymatic. One of the enzymatic antioxidants which have a good defense is superoxide dismutase. Different functions have been developed and selected defense systems against the deleterious action of free radicals because of the evolutionary conditions in living beings. Such systems are intrinsic in cells (at level intracellular and extracellular) and work together with the dietary exogenous antioxidants.

2.2.2 Mechanism of antioxidant activity in human body

When there is the balance between reactive oxygen species (ROS) formation and detoxification favors an increase in ROS levels due to Oxidative stress, and then happens to disturbed cellular function. Because of ROS leads damage to cellular macromolecules causing lipid peroxidation, nucleic acid, and protein. However, when the level of ROS exceeds this threshold, there is an increase in ROS production. This may lead to excessive signals to the cell and cause a direct damage to key components in signaling pathways. There happens an irreversibly damage of essential macromolecules by ROS. Protein-bound thiol and non-protein thiol are the major cytosolic low molecular weight sulfhydryl compound which act as a cellular reducing. They also show as a protective reagent against numerous toxic substances including most inorganic pollutants, through the –SH group. Hence, thiol is often the first line of defense that act against oxidative stress. There is evidence that oxidative stress causes irreversible damage in cellular macromolecules that leads to initiation of various diseases such as atherosclerosis, ischemic heart diseases, liver diseases, diabetes, and initiation of carcinogenesis. Antioxidants are able to inhibit reactive oxygen species production and discarding of free radicals (Shahidi and Ambigaipalan.,2015). Therefore, the review recommends that high consumption of natural foods which consists of very high antioxidants will provide more protection against toxic agents and scavenging related diseases (Adwas et al., 2019).

2.2.3 Source of antioxidants

The main sources of antioxidants are vitamin C, vitamin E, a-carotene, lycopene, selenium, polyphone, glutathione, proximate, cystine. The high amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, carotene, and lycopene are found on fruit juices, beverages and hot drinks (Ramadan-Hassanien, 2008).

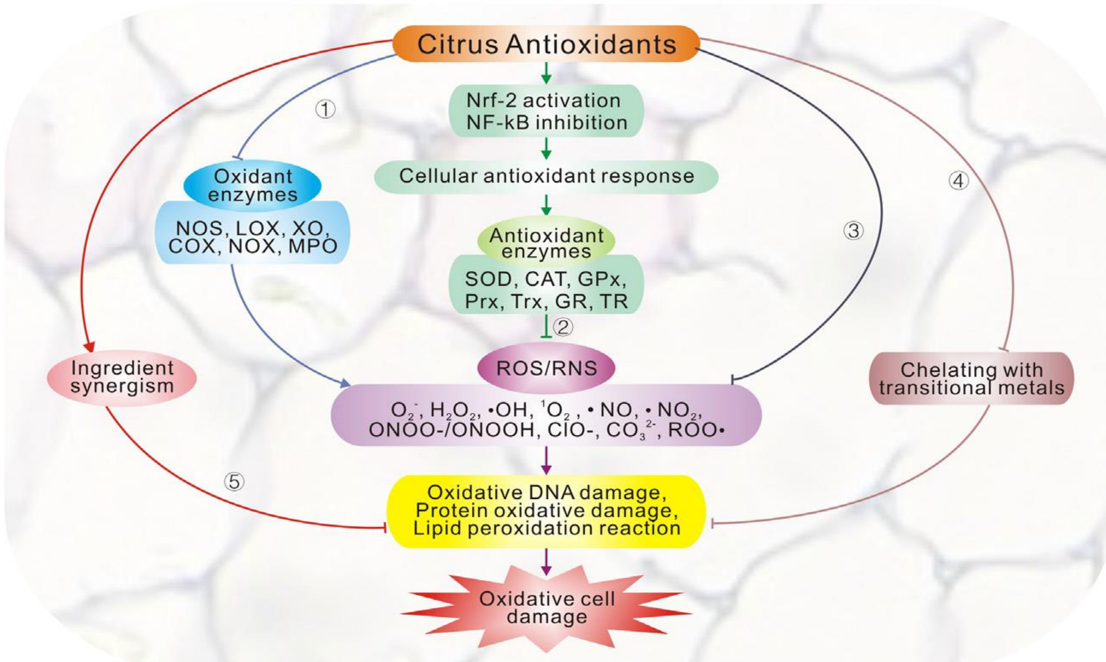

Figure 2.1: Mechanisms of antioxidant activity in human body

2.2.4 Functions of antioxidants

Antioxidants play a key role in the protective influence exerted by plant foods. The risk of chronic diseases is reduced by consuming of fruits and vegetables regularly (Dembinska et al., 2008). Studies demonstrate that an antioxidant-rich diet has a beneficial health impact in the long run of life (Sin et al., 2013). Nowadays antioxidants make a relation between radicals and oxidative stress, cancer prophylaxis and therapy, and longevity (Kalcher et al., 2009). All antioxidant work as the (antioxidant system) which are responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. However, there is a coordination system between these systems, the antioxidant (team) acts to control levels of free radical formation where deficiencies in one component impact the efficiency of others. These are hydrogen donation, electron donation by antioxidants and the addition of lipid to the antioxidants and then the formation of a complex between lipid and antioxidants. As food components work against chronic diseases, there is an attention to photo-chemicals, plant derived molecules endowed with steady antioxidant power (Peter, 2007).

Table 2.1: Different antioxidants and their functions

Compounds Name	Antioxidant Activity	References
Vitamin A	1. React with free radicals, especially $^{1}0_2$ 2. React with peroxyl radicals	Amitava and Kimberly (2014)
Vitamin C	1. Scavenge variety species of ROS 2. Give off Semi dehydroascorbic acid 3. Clear $^{1}O_2$	Amitava and Kimberly (2014)
Vitamin E	1. Restrain free radicals and quenches $^{1}O_2$ 2. Reduce ferrous iron to ferric iron to minimize catalysis 3. Synergistic effect with selenium to protect mitochondria against free radical damage and their membranes against peroxidation damage. 3. Prevent the oxidation of carotenoids, enhancing their antioxidant capacity.	Amitava and Kimberly (2014)
Minerals (Se, Zn, Cu, Mn and Fe)	1. Destroy free radicals in the cytoplasm as an essential component of antioxidant enzyme glutathione peroxidase (GSH-Px). 2. Synergistic effect with vitamin E to protect mitochondria against free radical damage and their membranes against peroxidation damage.	Amitava and Kimberly (2014)
Phenolic compounds	1. Inhibit the body's oxidant enzymes. 2. Improve the body's antioxidant enzyme activity. 3. Scavenge ROS directly. 4. Anti-lipid oxidation in vitro. 5. Decrease quality of peroxide formation in vivo.	Nakao et al., (2011)
Pectin	1. Enhance endogenous antioxidant enzymes. 2. Disposal of free radicals.	Koriem et al., (2014)
Terpenoids	1.Induce apoptosis and free radical 2. Different limonoids have variable antioxidant capacity.	Poulose et al., (2005)

2.2.4 Quantitative assessment of antioxidant activity of rare citrus fruits

Zou et al. (2016) stated that the antioxidant activity of citrus fruits and their roles in the prevention and treatment of various human chronic and degenerative diseases had increased the value. It was suggested that citrus fruits to be a good source of dietary antioxidants. The mechanism underlying the antioxidant activity of Citrus fruits, the phytochemicals in Citrus fruits introduced antioxidant activity evaluation methods, discussed the underlying factors that influence the antioxidant activity of Citrus fruits, and summarized.

Bocco et al. (1998) found that by methanol extraction (free phenolic compounds) or by alkaline hydrolysis (bound phenolic compounds) from several citrus peel and seed extracts were tested in a model system which were accelerated citronellal oxidation. Generally, the amount of antioxidants activity of seeds possessed greater than peels. The composition of all tested samples was studied by using HPLC. Methanol extracts were rich in flavones and glycosylated flavanones, whereas hydrolyzed extracts contained mainly phenolic acids and flavonols. The phenolic composition of some citrus peels and seeds was narrated for the first time. The relationship between the antioxidant activity and the phenolic composition of the extracts mechanism of action could not be cleared. Some suggestions for future study were also presented.

Zou et al. (2016) reported that citrus fruits were suggested to be a good source of dietary antioxidants. The mechanism underlying the antioxidant activity of Citrus fruits, was viewed a study on the antioxidant activity of the phytochemicals in citrus fruits. The introduced methods for antioxidant activity evaluation, discussed the factors which influence the antioxidant activity of citrus fruits, and described the underlying mechanism of action. Some suggestions for future study were also presented.

Reddy et al. (2010) observed that fruits and vegetables were rich sources of phenolic compounds and antioxidant activity (AOA). Present study was taken up to determine the AOA and phenolic content of fresh and dry fruits commonly consumed in India by two different (radical scavenging) methods and the relation to their total phenolic content (TPC) for the first time. There was a study on fourteen commonly consumed fresh fruits and ten dry fruits. The fresh and dry fruits showed marked variation between AOA and

TPC contents. The ABTS (r = 0.84) was maximum and DPPH (r = 0.77) was lesser in fresh fruits in the correlation between the TPC and AOA, DPPH activity (r = 0.97) and to a lesser extent to FRAP (r = 0.87) found in dry fruits. In general, the results indicated that majority of the fresh and dry fruits studied were rich in phenolic antioxidants with potent free radical scavenging activity imply their importance to human health.

2.3 Bioactive compounds

Bioactive compounds have the capability of modulating metabolic processes and resulting in the promotion of better health. They exhibit as a receptor activator and inhibitor of gene expression (Correia et al., 2012). The inaccessibility of each bioactive compound differs greatly and the most abundant compounds in ingested fruit are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Zhang et al.,2014). Indeed, during studying the role and impact of bioactive compounds in human health, bioavailability is not always well known (Carbonell-Capella et al., 2014).

2.3.1 Important bioactive compounds available in rare citrus fruits

Bioactive compounds are found in vegetables and whole grains . They contain various heterogeneous compounds like carotenoids, tocopherols, phytosterols, and organosulfur compounds which have different chemical structures .

2.3.1.1 Phenolic compounds

Polyphenols have more than one hydroxyl group that are attached to benzene rings. The phenolic compounds have attention for their potential as antioxidants, their great abundance in our diet and their possible role in the prevention of various diseases associated with oxidative stress (Scalbert et al., 2005). The main dietary sources of phenols are fruits and beverages (fruit juice, tea, coffee) and fewer amounts in vegetables, legumes and cereals. Fruits like apple, grape, pear, cherry and various berries contain up to 200–300 mg phenolic compounds per 100 g fresh weight; a glass of red wine or a cup of tea or coffee contains about 100 mg phenolics. The total dietary intake of phenolics is about 1 g/day (Scalbert et al., 2005).

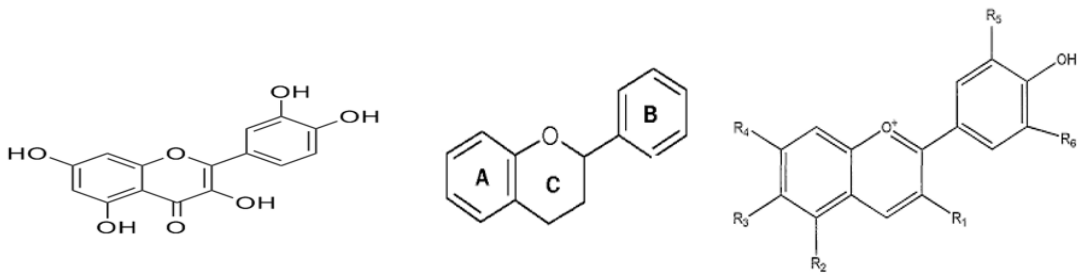

Figure 2.2: Polyphenol, flavonoid and anthocyanin

2.3.1.2 Flavonoids compounds

Flavonoids are polyphenol molecules containing 15 carbon atoms and they are water soluble. There are main three compounds such as flavored, flavanol and flavanone. Flavonoids have diverse biological properties and act as antioxidative, antimicrobial, anticarcinogenic and cardio protective. Flavonol Quercetin is a common flavonol-type flavonoid that is available in several foods such as onion, tea and apple and is consumed almost daily. In Western diet, daily intake of quercetin is estimated and the range is 0–30 mg (D'Andrea, 2015). Flavanol Catechins which are isolated into epicatechin, gallocatechin, epicatechin gallate, epigallocatechin, gallocatechin gallate and epigallocatechin gallate under flavanol group. Catechins are the major building blocks of tannins and they decrease as grapes mature (Gadkari and Balaraman, 2015). Flavanone Hesperetin, a flavanone present in citrus fruits which has low bioavailability, poor water solubility and short biological life (Parthasarathy et al.,2009). Naringenin is another natural flavanone that are rich in amount and found in citrus and grape fruits, which improves brain insulin signaling and cognitive functions (Ghofrani et al., 2015).

2.3.1.3 Anthocyanin compounds

Anthocyanins are water-soluble plant pigments, responsible for the range of colours such as red, blue and purple which are accomplished in fruits (Rufino et al., 2010). They occur primarily as glycosides or acyl glycosides of their respective aglycone anthocyanins and aglycone are found in fresh plant materials. The most common anthocyanidins which are high in plants are delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin (De Pascual-Teresa and Sanchez-Ballesta, 2008). They are available in strawberries, raspberries, cherries, berries, red grapes and cranberries. The anthocyanin composition of fruits and vegetables are diversified and vary from fruit to fruit of the same type due to the genetic and agronomic factors, intensity and type of light, temperature, postharvest treatments, processing and storage (De Pascual-Teresa and Sanchez-Ballesta, 2008).

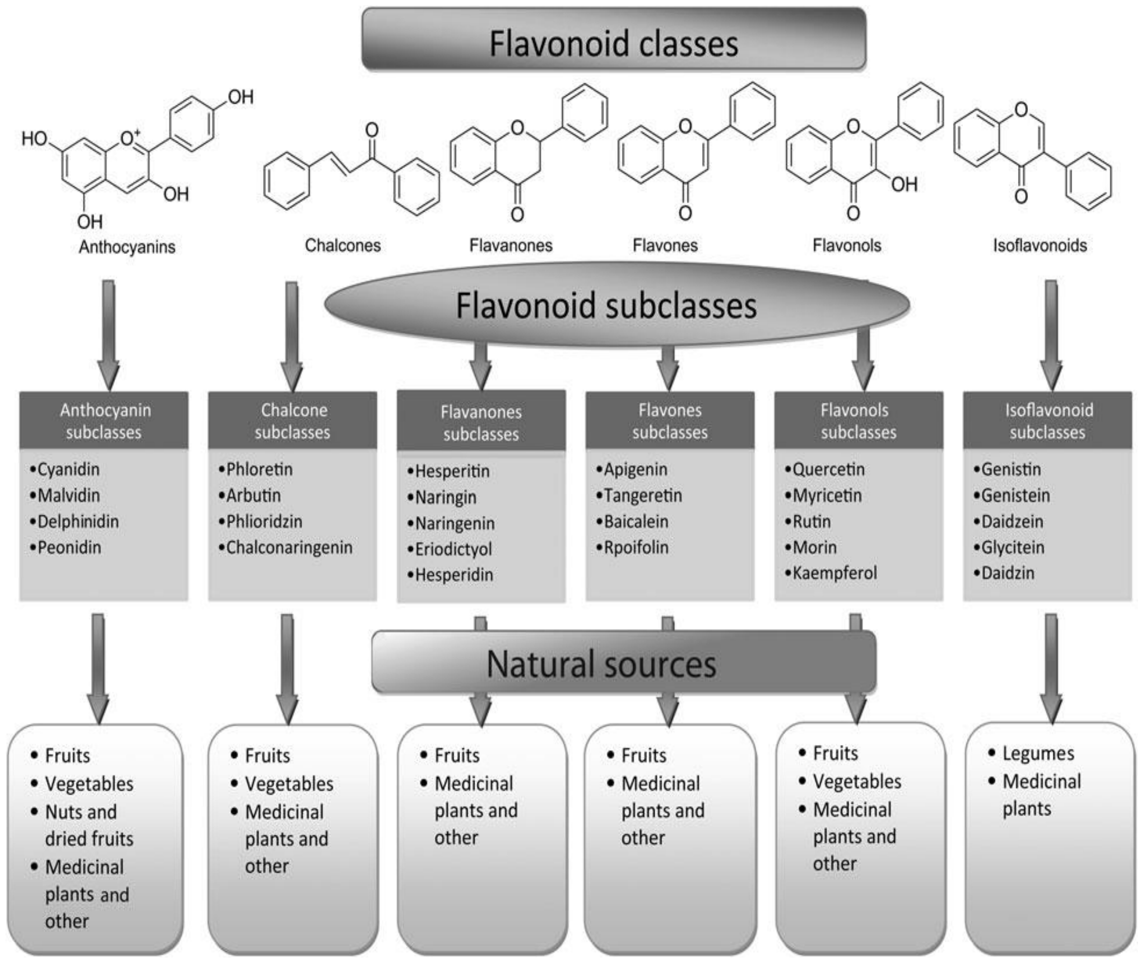

Figure 2.3: Flavonoid classes, subclasses and natural sources

2.3.2 Health benefits of bioactive compounds

Bioactive compounds have antioxidant capacity, scavenging free radicals and chelating action due to the presence of functional groups in their nuclear structure. They also assigned most of the health benefits from the consumption of flavonoids. The flavonoids act as antimutagenic and antitumoral activities. The flavonoids have the property of inhibiting many enzymes, such as oxygenases (prostaglandin synthase), required in the synthesis of eicosanoids. Thus, the flavonoids have the capacity to inhibit hyaluronidase activity and help in maintaining the proteoglycans of connective tissues. The expansion of bacterial or tumour metastases would be prevented by this process (Havsteen, 2002).

2.2: Different bioactive compounds and their biological functions

Bioactive compounds	**Health benefits**	**References**
Tannic acid	For the treatment of burns, it is used as medicinal agent	Siang (1983)
Catechin	Resistance of LDL to oxidation, brachial artery dilation increased plasma antioxidant activity, and fat oxidation	Rasool (2010)
Gallic acid	Antioxidant and potential hepatoprotective effects	Rasool (2010)
Cinnamic acid	Is a precursor to the sweetener aspartame by the means of enzyme catalysed amination to phenylalanine	Rasool (2010)
p-Coumaric acid	Antioxidant properties and potentially reduce the risk of stomach cancer	Rasool (2010)
Gallocatechin gallate	Cholesterol reduction	Rasool (2010)
Quercetin	Promotes overall cardiovascular health by encouraging blood flow	Rasool (2010)
Trans-α carotene	Antioxidant, antimicrobial, anti-inflammatory, antiallergic, anticarcinogenic, modulation of enzyme activity, antiviral and vasodilatory actions	Rasool (2010)
Trans-β carotene	Precursor to vitamin A	Rasool et al. (2010)
Violaxanthin	Reduce the risk of CVD and cancer	Li et al. (2011)
Cryptoxanthin	Used as a food colourant	Li et al. (2011)
Serotonin	Food colourant might reduce the risk of lung cancer	Li et al. (2011)
Dopamine	Reduce the plasma oxidative stress and enhance the resistance to oxidative	Li et al. (2011)
Catecholamines	Increases blood pressure, glucose levels, and heart beat rate	Choudhary and Tran (2011)
β-Sitosterol	Potential to reduce blood cholesterol levels and benign prostatic hyperplasia	Choudhary and Tran (2011)

Campesterol	Reduces the absorption of cholesterol in the human intestines	Choudhary and Tran (2011)

2.3.3 Quantitative assessment of bioactive compounds of rare citrus fruits

Wang et al. (2007) investigated that total polyphenol and flavonoid exceeded that of total carotenoid. One of the major flavanone was Hesperidin, which content was in high and the amount of liucheng and Tonkan were (5.36 ± 0.145 and 4.13 ± 0.050 mg/g db, respectively). Naringin grew rich in Peiyou and Wendun (1250 ± 0.82 and 2205 ± 11.1 µg/g db, respectively). Diosmin was the principle flavone, and Kumquat (0.699 ± 0.021 mg/g db) and lemon (0.323 ± 0.004 mg/g db) had the highest in amounts. Another one of the most abundant flavanol was kaempferol except in kendun, peiyou, and kumquat, and murcott had the highest content (1.04 ± 0.007 mg/g db). The major phenolic acid was chlorogenic and wendun and Lemon had the highest contents (103 ± 11.5 and 92.6 ± 8.90 µg/g db, respectively). Abeysinghe et al., (2007) examined the edible tissues of citrus fruits and find two flavanones (naringin and hesperidin) in citrus juice which were found by HPLC. 18.5-38.5% of Hesperidin found on the total phenolics and the species were Citrus unshiu, Citrus reticulata, and Citrus sinensis, while naringin was only found in Citrus changshanensis and 53.7% of it on the total phenolics in SM of this species.

Al-Juhaimi and Ghafoor (2013) studied that lemon (Eureka), mandarin (Kinnow) and orange (Orlando) fruits were analyzed by the extraction of juice for different physico-chemical properties, total phenolics, ascorbic acid and antiradical activities which were originated from Saudi Arabia. 8.97, 16.1 and 11.78%; 5.73, 1.37 and 1.69% titrable acidity; 79.21, 91.18 and 107.37 mg GAE/100mL total phenolics; 31.24, 53.15 and the content of ascorbic acid was 53.24 mg/100mL and 48.3, 59.19 and DPPH radical scavenging activities was 61.35%, respectively which collected from lemon, mandarin and orange . The results showed that juice from locally grown citrus fruits was of good excellence and a valuable source of health flourishing constituents.

CHAPTER-III
MATERIALS AND METHODS

3.1 Study areas and period

This experiment was conducted to determine the antioxidant activity and bioactive compounds of rare fruits of Bangladesh from January to June 2019. Samples were collected from six districts like Chattogram, Cox's Bazar, Rangamati, Kishoregonj, Faridpur and Rajshahi. Then they were transferred to the Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

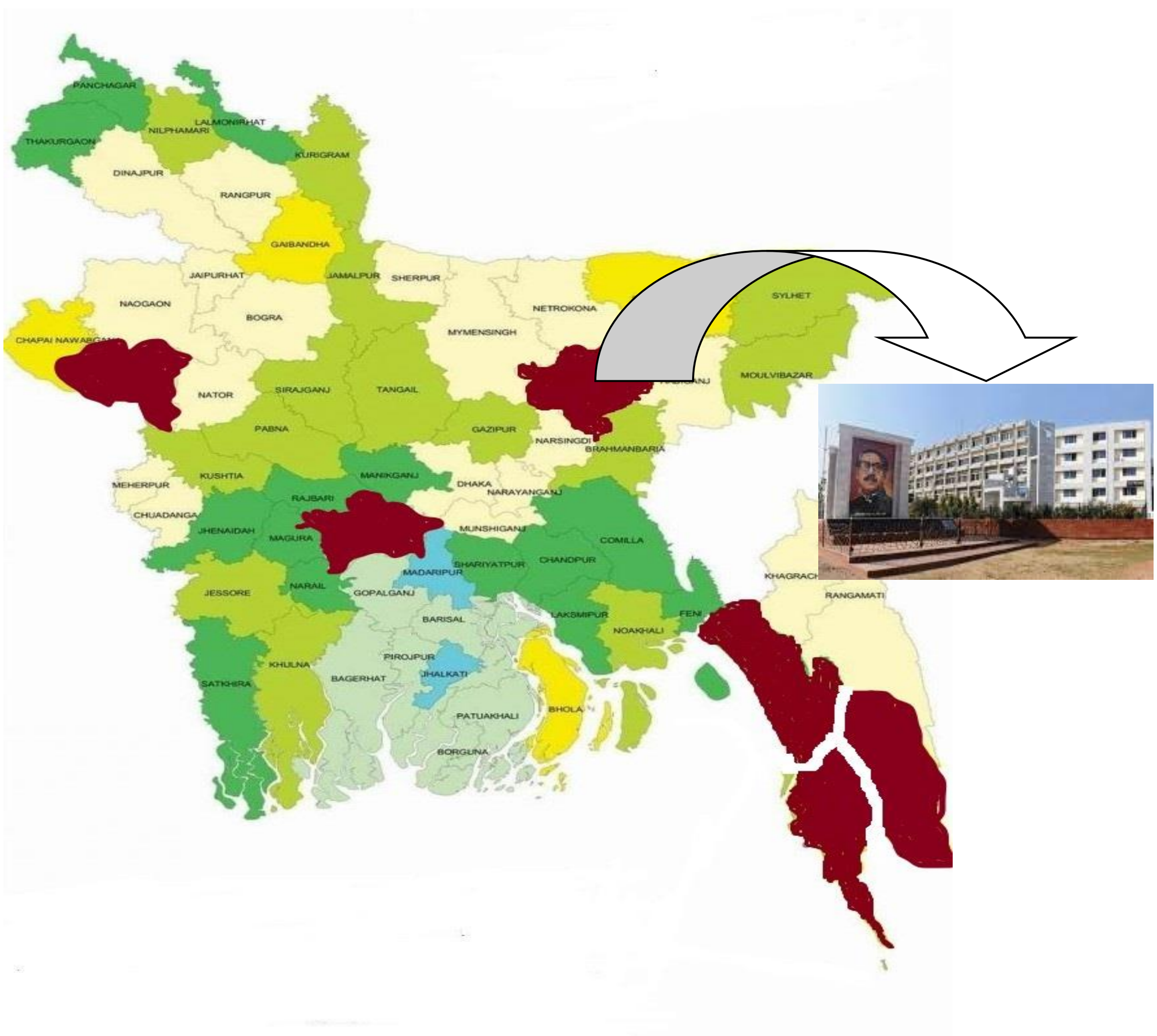

Figure 3.1: Sample collecting sides

3.2 Experimental design

Rare fruits samples were collected from different districts of Bangladesh and subjected to antioxidant activity and bioactive compounds assessment by UV-visible spectrophotometer. Antioxidant activity was measured by DPPH scavenging method and three important bioactive compounds like total phenolic contents (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) were determine by suitable standards.

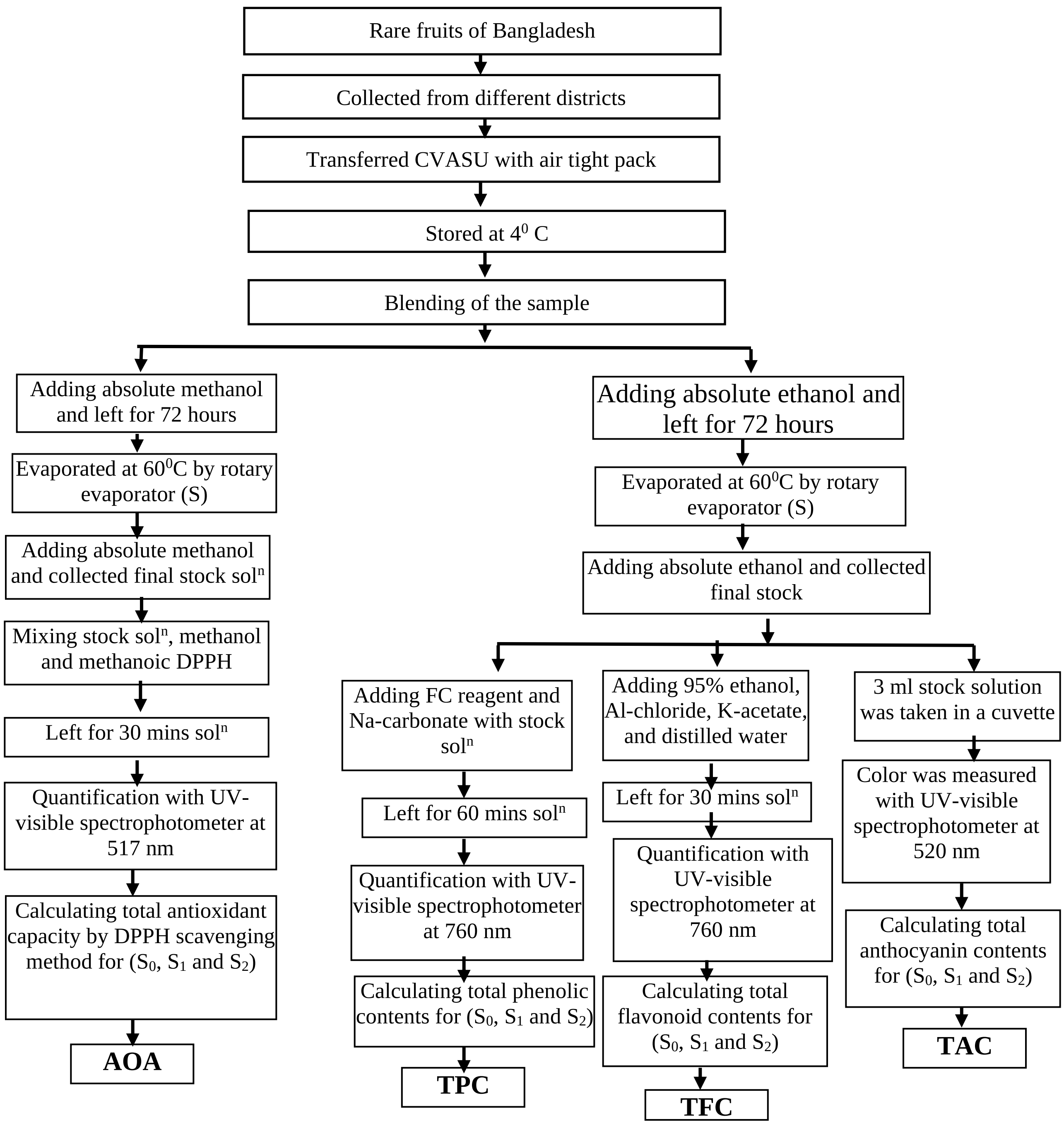

Figure 3.2: Experimental study design

3.3 Sample collection

Fruit samples like *Syzygium samarangense L*. (Jamrul), *Flacourtia jangomas L*. (Pani Fol), *Averrhoa bilimbi L*. (Belombo), *Garcinia cowa L*. (Cow Fol), *Manilkara zapota L*. (Safeda), *Citrus limon L*. (Katajamin), *Sabal palmetto L*. (Ashari), *Calamus tenuis L*. (Bet Fol), *Ficus carica L*. (Dumur) and *Aegel mermelos L*. (Bele) were collected from the local markets of six districts of Bangladesh. Fully matured and ripe fruits were selected as research sample and sealed with plastic bags. Finally they were transferred to CVASU.

3.4 Extract preparation

At first, samples were washed-up several time with water. Some fruit like Cow Fol and Katajamin juice were for squeezing for juice extraction. Others were chopped with small pieces and allowed for blending. Then for the methanioc extract, juice was mixed with absolute methanol and left for 72 hours with shaking. Again, for the ethanoic extract, juice was mixed with absolute ethanol and left for 72 hours with shaking. The filtrates were collected and stored at room temperature while the residues were re-extracted twice, each time with fresh solvent. Finally, all the filtrates were evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4 °C until further analysis (Gorinstein et al., 2004).

3.5 Chemicals and reagents

- 2, 2-Diphenyl -1-picryl hydrazyl (DPPH)
- Gallic acid
- Folin Ciocatea (FC) Reagent.
- Distilled water
- Absolute ethanol
- Absolute methanol
- Aluminum Chloride
- Quercetin
- Sodium carbonate
- Potassium acetate

3.6 Equipment

- Digital analytical balance
- Conical flasks
- Beakers
- Watch glass
- Test tubes
- Falcon tubes
- UV visible spectrophotometer
- Volumetric flask
- Electric blender.

3.7 Determination of antioxidant activity by DPPH scavenging method

Antioxidant capacity of the extracts was determined using DPPH assay as the method described by Azlim Almey et al. (2010) with slight modifications. About 6 mg of DPPH was dissolved in 100 mL absolute methanol and prepared methanoic DPPH solution. Then 1 ml methanoic extract was mixed with of 2 ml DPPH solution. Then the mixture was gently shaken and left for 30 min in dark at room temperature. The absorbance was read at wavelength 517 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA). 1 mL of methanol mixed with 2 mL of DPPH solution while methanol was used as a blank. The scavenging activity was measured as the decrease in absorbance of the samples in comparison with the DPPH standard solution. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \% \ 100$$

Trolox used as standard and TEAC compound (Trolox equivalent antioxidant capacity) was used for the calibration standard curve. The results were expressed in mg/ 100 g of Trolox equivalents per gram of powder on a dry weight (DW) basis.

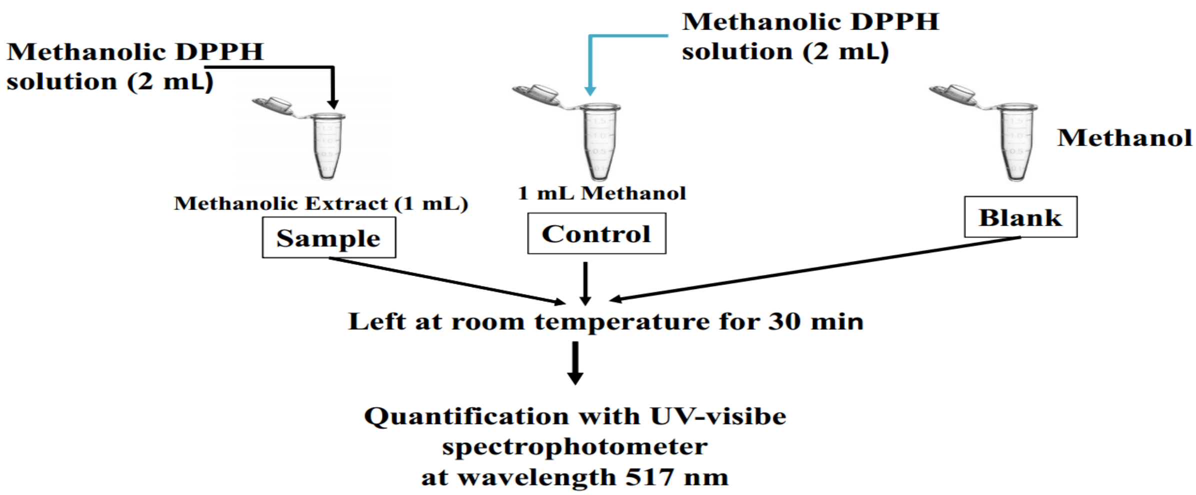

Figure 3.3 Antioxidant activity (AOA) determination procedure flow diagram

3.8 Determination of total phenolics contents (TPC).

Total polyphenol content (TPC) of the fruits were determined according to the Folin-Ciocalteu method described by Parthasarathy et al. (2009) with slight modifications. 1 ml of ethanoic extract was taken in a falcon tube and added 1.5 ml of FC reagent and left for three minutes at room temperature. Then 1.5 ml Na_2CO_3 (7.5%) was added into the mixture and left for 60 minutes. The absorbance was read at wavelength 765 nm using a UV-VIS Spectrophotometer (UV-2600, Shimadzu Corporation, USA) and ethanol was used as the blank. TPC was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

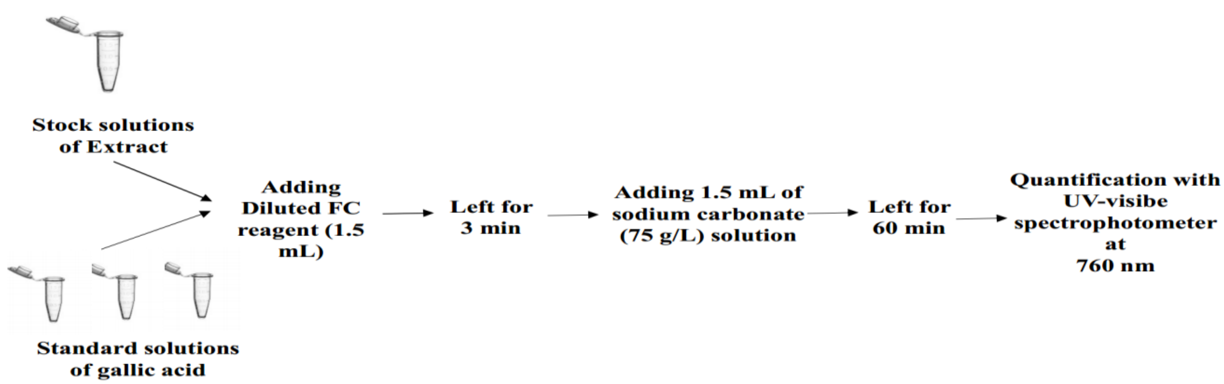

Figure 3.4 Total polyphenol contents (TPC) determination procedure flow diagram

3.9 Determination of Total Flavonoid Contents (TFC)

Total flavonoid content (TFC) of the fruit samples were determined by using the aluminum chloride colorimetric method described by Chang et al. (2002) with slight modifications. Stock solution (1 mg/mL) of extracts were prepared and aliquots of 0.5 mL of diluted extract mixed with 1.5 mL of 95% ethanol in a cuvette. Then 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water were added to the mixture in the cuvette. The mixture left at room temperature for 30 min. The absorbance was read at wavelength 415 nm in UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA) and 10% aluminum chloride substituted with distilled water of the same amount were used as the blank. Total flavonoid content in the sample was estimated by comparing absorbance of the sample extracts with a quercetin standard curve. TFC calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

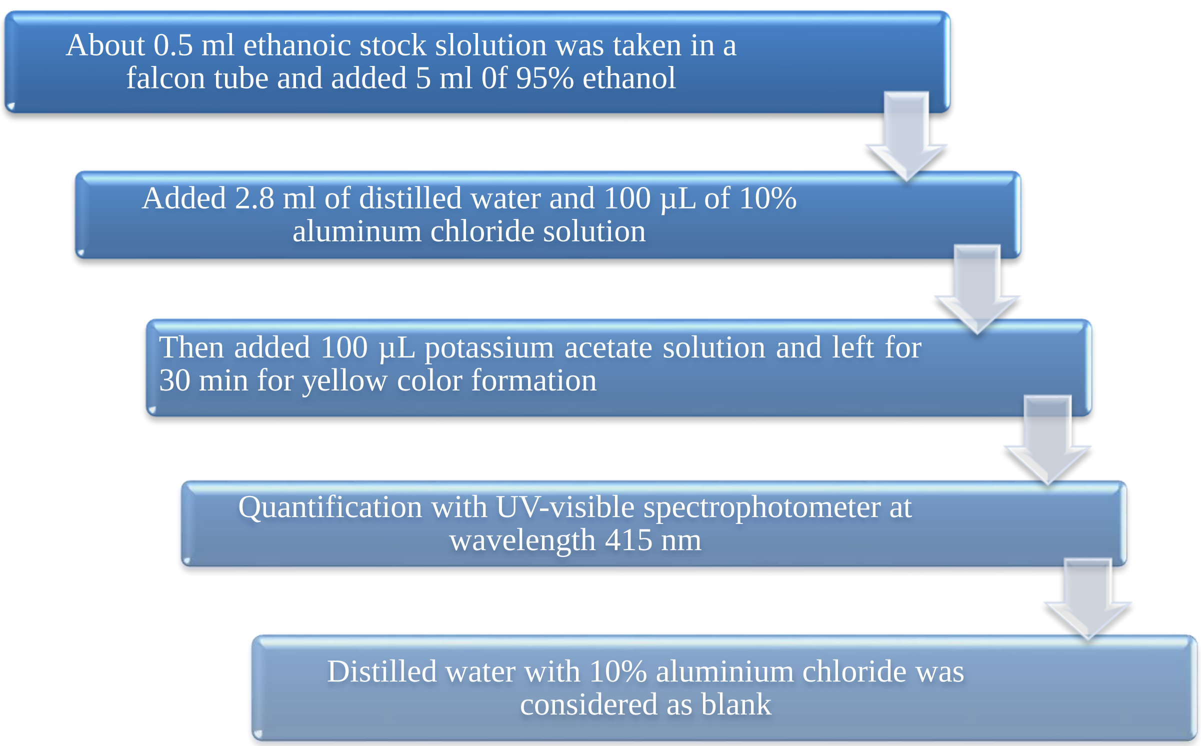

Figure 3.5 Total flavonoid contents (TFC) determination procedure flow diagram

3.10 Determination of Total Anthocyanin Contents (TAC)

Total anthocyanin content (TAC) of fruit extracts would be determined colorimetrically following the method described with slight modifications (Selim et al., 2008). About 3 mL of ethanoic extract was pipetted into a cuvette and intensity of color was measured at wavelength 520 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA). Ethanol was used as a blank. TAC was calculated and expressed as milligrams per 100 g (mg/100 g) using the following equation:

TAC= Absorbance of sample×DF×100/m×E

Where,

DF = dilution factor

m = means the weight of sampl

E = refers to extinction coefficient (55.9)

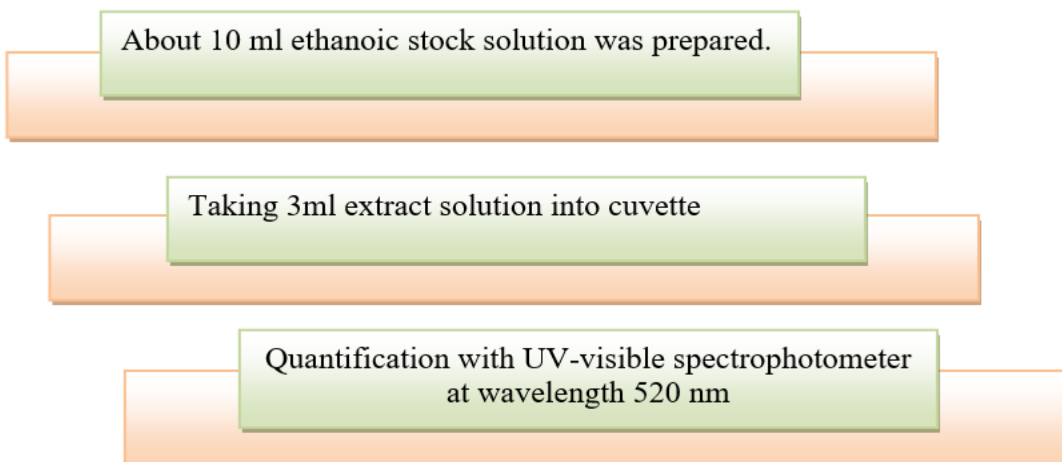

Figure 3.6 Total anthocyanin contents (TAC) determination procedure flow diagram

3.11 Statistical analysis

Data were stored in Microsoft Excel 2007 and then exported into STATATM 11.0 (Stata Corporation, College Station, TX, USA) for statistical analysis. Descriptive analysis was performed by using percentages, mean and standard deviation for different variables. Finally correlation coefficient and their significant level was measured by SPSS version 23. The level of significance was set ≤ 0.05.

CHAPTER IV
RESULTS

The results of the study are presented under the following headings. The sample of rare fruits is marked out by their local name with scientific name. Antioxidant activity and bioactive compounds of these rare fruits are presented in this chapter with necessary information’s.

4.1 Antioxidant activity of rare fruits by DPPH scavenging method

Total antioxidant activity of rare fruits was determined using DPPH assay. Antioxidant activity of the fruits is presented in table 4.1. A calibration curve was constructed by plotting percentage inhibition against concentration of trolox assay and the concentration was expressed as mg/ 100 g.

Table 4.1: Antioxidant activity (AOA) of rare fruits of Bangladesh

Serial No.	Name of the fruits	Scientific Name	Antioxidant activity DPPH (trolox equ.) mg/100 g
01	Jamrul	*Syzygium samarangense L.*	113±3.29
02	Pani Fol	*Flacourtia jangomas L.*	55±4.23
03	Belombo	*Averrhoa bilimbi L.*	115±20.76
04	Cow Fol	*Garcinia cowa L.*	94±13.20
05	Safeda	*Manilkara zapota L.*	125±1.29
06	Katajamin	*Citrus limon L.*	303±8.87
07	Ashari	*Sabal palmetto L.*	122±30.19
08	Bet Fol	*Calamus tenuis L.*	129±0.25
09	Dumur	*Ficus carica L.*	118±0.52
10	Bele	*Aegel mermelos L.*	130±3.80
-	Maximum	Katajamin	303±8.87
-	Minimum	Ashari	55±4.23
-	Average	-	130

Data are presented as mean values ± standard deviation, Number of sample =03

Among these sample, DPPH activity of fresh fruits ranged from 55 to 303 mg/100 g, with high activity being found in Katajamin and low activity observed in Pani Fol. Average DPPH activity was 130 mg/100 g. Total antioxidant activity was found in order: Katajamin>Bet Fol> Bele >Safeda>Ashari>Dumur>Belombo>Jamrul>Cow Fol>Pani Fol. DPPH is a stable free radical scavenger (maximum absorbance at 515 nm) which is scavenged from purple to yellow after it accepts an electron or a proton to become a stable diamagnetic molecule when antioxidants are encountered.

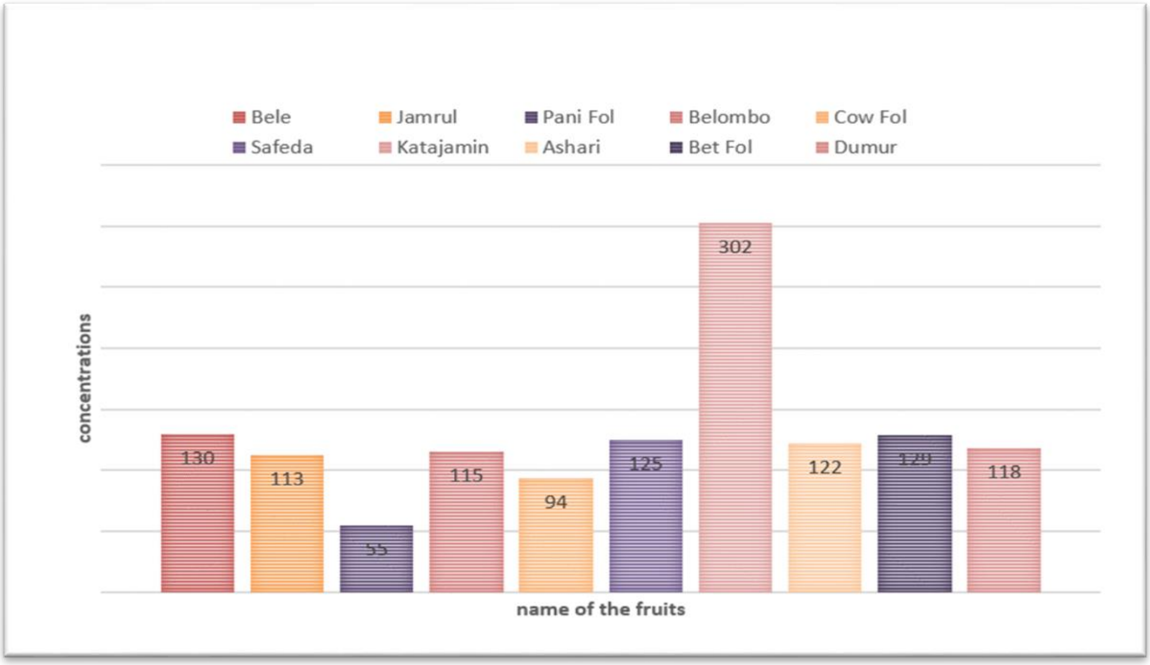

Fig 4.1: Antioxidant activity of 10 rare fruits of Bangladesh.

4.2 Determination of bioactive compounds in the rare fruits of Bangladesh

Polyphenols, flavonoids and anthocyanins are the major bioactive components which are found in fruits, vegetables coffee and animal origin and show various beneficial health-promoting activities in humans. Bioactive compounds were examined from mechanic extracts of sample with suitable standards. The results of bioactive compounds are expressed bellow.

4.2.1 Determination of Total Polyphenol contents (TPC) in rare fruits

Total polyphenol content (TPC) of fruits extracts was determined according to the Folin-Ciocalteu method. The amount of total polyphenol content in 10 rare fruits are shown in table 4.2. Here, result was expressed as mg GAE/ g.

Table 4.2: Total polyphenol content (TPC) of rare fruits of Bangladesh

Serial No.	Name of the fruits	Scientific Name	Total phenolic content (TPC) (mg GAE/100g)
01	Jamrul	*Syzygium samarangense L.*	36±0.37
02	Pani Fol	*Flacourtia jangomas L.*	48±3.72
03	Belombo	*Averrhoa bilimbi L.*	32±5.60
04	Cow Fol	*Garcinia cowa L.*	29±3.01
05	Safeda	*Manilkara zapota L.*	86±11.40
06	Katajamin	*Citrus limon L.*	141±34.98
07	Ashari	*Sabal palmetto L.*	10±2.19
08	Bet Fol	*Calamus tenuis L.*	27±12.77
09	Dumur	*Ficus carica L.*	65±9.93
10	Bele	*Aegel mermelos L.*	130±7.85
-	Maximum	Katajamin	141±34.98
-	Minimum	Ashari	10±2.19
-	Average	-	60

Data are presented as mean values ± standard deviation, Number of sample =03

Total polyphenol content of the extract ranged from 10 ±2.19 to 141±34.98 mg GAE/g (gallic acid equivalent). In this study Katajamin (141±34.98 mg GAE/g) had significantly higher total polyphenol content than others, and Ashari (10±2.19 mg GAE/g) had lower TPC and other fruits followed by Bele 130±7.85 mg GAE/g, Safeda 86±11.40 mg GAE/g, Dumur 65±9.93 mg GAE/g, Pani Fol 48±3.72 mg GAE/g, Jamrul 36±0.37 mg GAE/g, Belombo 32±5.60 mg GAE/g, Cow Fol 29±3.01 mg GAE/g, Bet Fol 27±12.77 mg GAE/g.

4.2.2 Determination of Total Flavonoid contents (TFC) in rare fruits

Total flavonoid content (TFC) of the fruits extract was determined using the aluminum chloride colorimetric method with slight modifications. The result of total flavonoid is shown in table 4.3. Here, result was expressed as mg QE/g extract.

Table 4.3: Total flavonoid content (TFC) of rare fruits of Bangladesh

Serial No.	Name of the fruits	Scientific Name	Total flavonoid content (TFC) (mg QE/g extract)
01	Jamrul	*Syzygium samarangense L.*	46±1.99
02	Pani Fol	*Flacourtia jangomas L.*	29±4.07
03	Belombo	*Averrhoa bilimbi L.*	52±5.01
04	Cow Fol	*Garcinia cowa L.*	27±5.50
05	Safeda	*Manilkara zapota L.*	31±8.55
06	Katajamin	*Citrus limon L.*	76±6.67
07	Ashari	*Sabal palmetto L.*	06±3.94
08	Bet Fol	*Calamus tenuis L.*	33±10.01
09	Dumur	*Ficus carica L.*	54±18.83
10	Bele	*Aegel mermelos L.*	68±7.86
-	Maximum	Katajamin	76±6.67
-	Minimum	Ashari	06±3.94
-	Average	-	42

Data are presented as mean values ± standard deviation, Number of sample =03

The result shows that TFC content is ranged from Ashari (06±3.94mg QE/g) to Katajamin (76±6.67mg QE/g) and the average total flavonoid concentration is 42 mg QE/g. Others fruits contain Cow Fol (27±5.50mg QE/g), Pani Fol (29±4.07mg QE/g), Safeda (31±8.55mg QE/g), Bet Fol (33±10.01mg QE/g), Jamrul (46±1.99mg QE/g), Belombo (52±5.01mg QE/g), Dumur (54±18.83mg QE/g)and Bele (68±7.86mg QE/g) with ascending order.

4.2.3 Determination of Total Anthocyanin contents (TAC) in rare fruits

Total anthocyanin content of the fruits were presented in Table 4.4. Here, result was expressed as mg TA/100 g extract. Result shows that Bet Fol (102±3.46 mg TA/100 g) maximum anthocyanin and Ashari (02±0.74 mg TA/100 g) contain minimum. Average anthocyanin content of the fruits is 30 mg TA/100 g. Total anthocyanin content was found in the following order: Bet Fol> Bele > Safeda > Katajamin > Cow Fol > Belombo > Pani Fol > Dumur > Jamrul > Ashari.

Table 4.4: Total anthocyanin content (TAC) of rare fruits of Bangladesh

Serial No.	Name of the fruits	Scientific Name	Total anthocyanin content (TAC) (mg TA/100 g)
01	Jamrul	*Syzygium samarangense L.*	03±0.11
02	Pani Fol	*Flacourtia jangomas L.*	07±1.83
03	Belombo	*Averrhoa bilimbi L.*	08±5.16
04	Cow Fol	*Garcinia cowa L.*	20±1.91
05	Safeda	*Manilkara zapota L.*	52±9.91
06	Katajamin	*Citrus limon L.*	30±0.46
07	Ashari	*Sabal palmetto L.*	02±0.74
08	Bet Fol	*Calamus tenuis L.*	102±3.46
09	Dumur	*Ficus carica L.*	04±0.45
10	Bele	*Aegel mermelos L.*	76±10.58
-	Maximum	Bet Fol	102±3.46
-	Minimum	Ashari	02±0.74
-	Average	-	30

Data are presented as mean values ± standard deviation, Number of sample =03

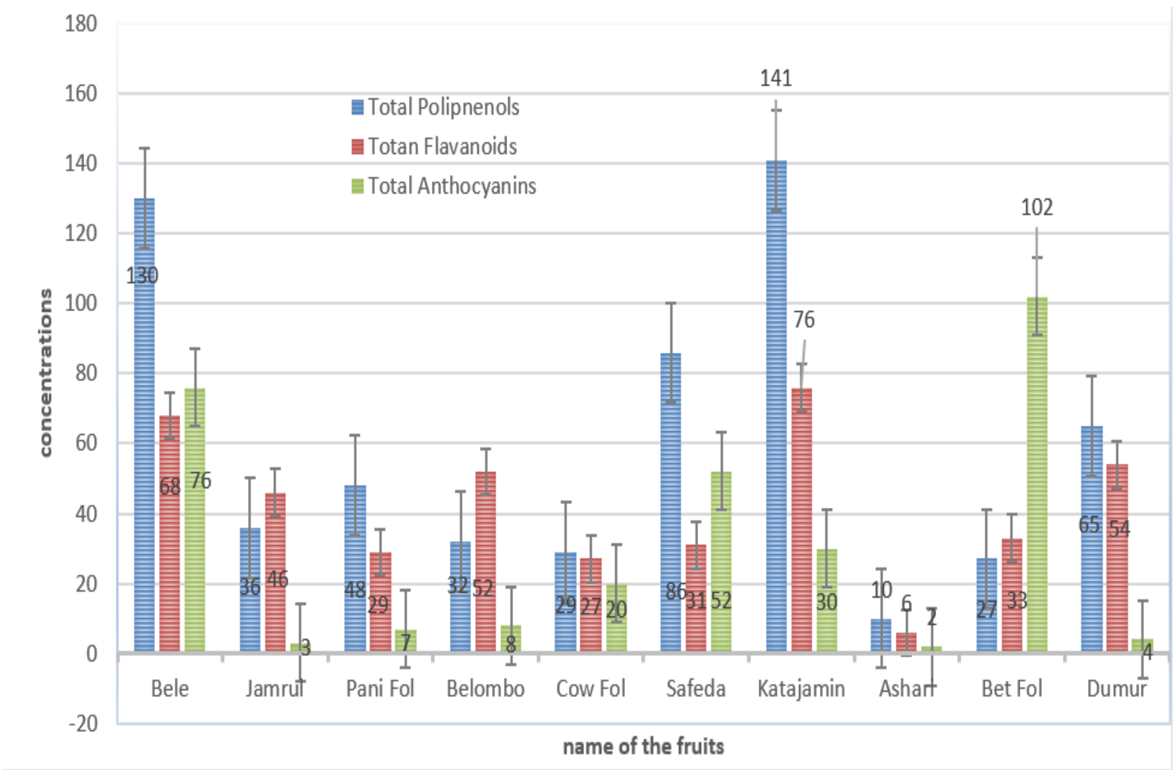

Figure 4.2: Concentration of three bioactive compounds in 10 rare fruits

4.3 Correlation between antioxidant activity and bioactive compounds in rare fruits

Correlation coefficient between antioxidant activity and bioactive compounds was expressed in table 4.4. Antioxidant activity and three types of bioactive compounds were always positively correlated and there was a strong positive correlation between AOA and TPC. But AOA and TFC were moderately correlated and AOA and TAC had very weak correlation. There were a significant (*P≤0.05*) correlation between AOC with TPC and total TFC. But the correlation between AOC and TAC was not significant.

Table 4.5: Correlations between antioxidant activity and bioactive compounds of rare fruits-

Correlations	r	r^2(%)	P
AOA vs. TPC	0.62	38	*P≤0.05*
AOA vs. TFC	0.54	29	*P≤0.05*
AOA vs. TAC	0.17	03	*P≥0.05*

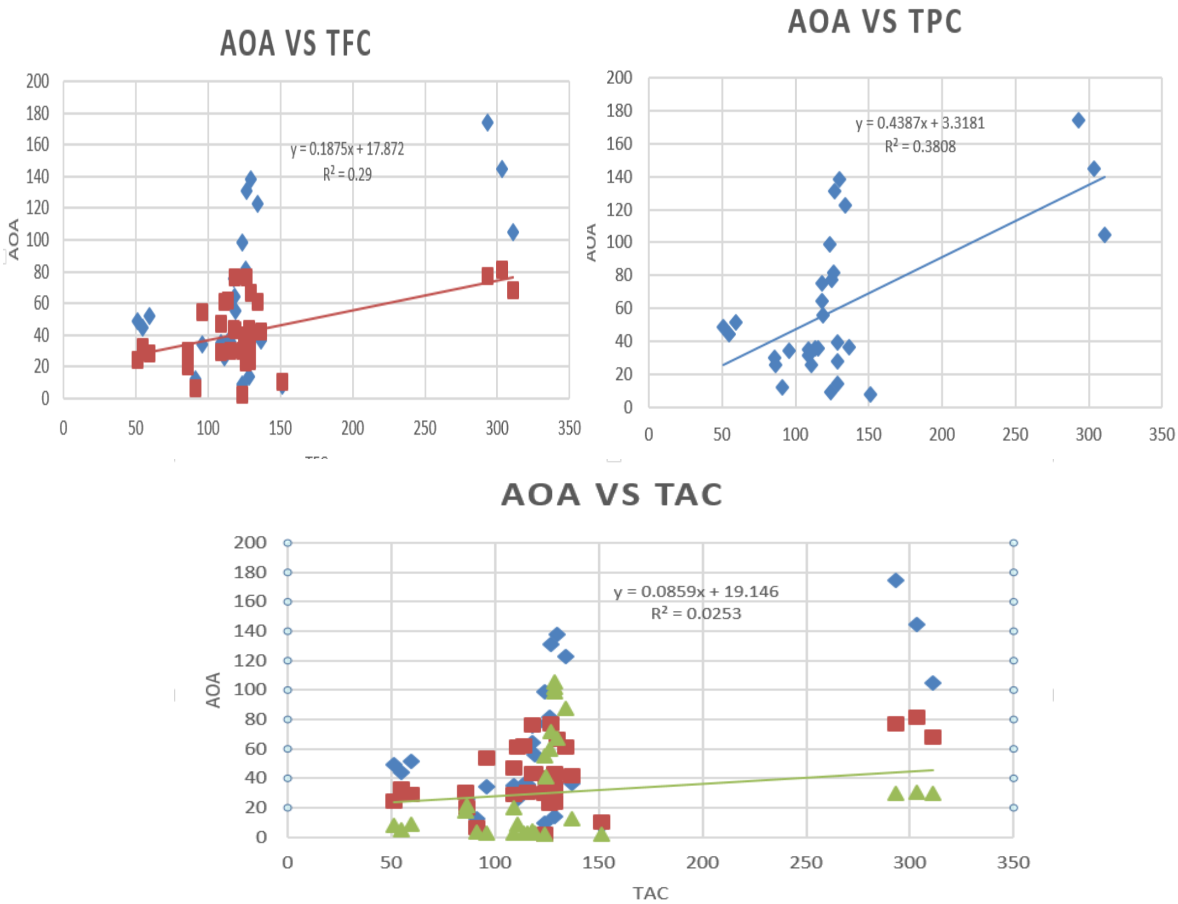

Figure 4.3: Correlation coefficient between antioxidant activity (AOA) and three bioactive compounds

Correlation coefficient among three bioactive compounds is expressed in Table 4.5. Three bioactive compounds like total polyphenol content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) were positively correlated. TPC Vs TFC were strongly correlated but TPC Vs TAC and TFC Vs TAC were weakly correlated. Correlation between TPC and TFC were significant (*P≤0.05*) but other two like TPC Vs TAC and TFC Vs TAC were not significant (*P≥0.05*).

Table 4.6: Correlations coefficient among three bioactive compounds of rare fruits-

Correlations	r (%)	r^2(%)	p
TPC vs. TFC	0.63	40	*P≤0.05*
TPC vs. TAC	0.09	0.87	*P≥0.05*
TFC vs. TAC	0.14	02	*P≥0.05*

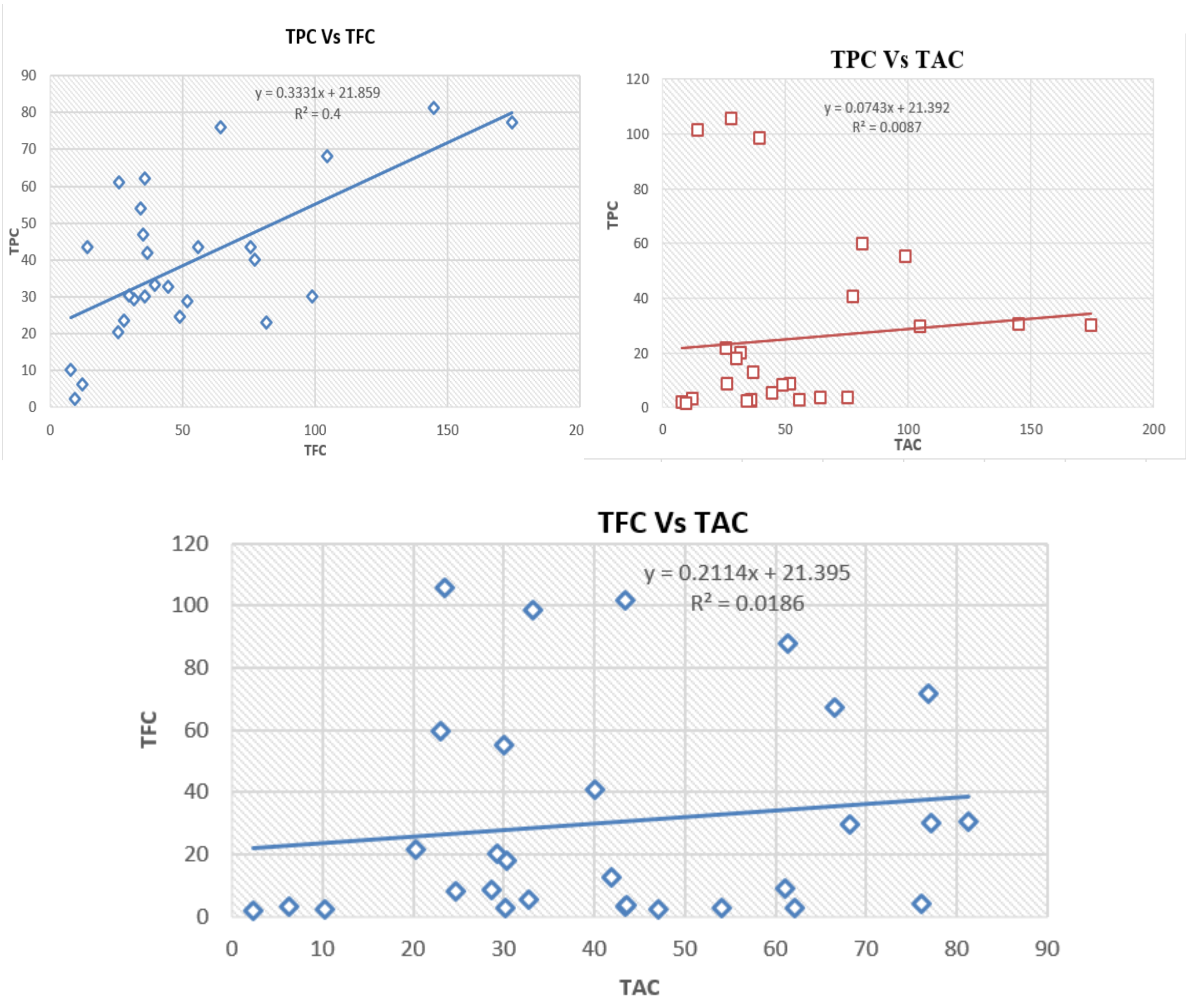

Figure 4.4: Correlation coefficient among three bioactive compounds

CHAPTER V
DISCUSSIONS

5.1 General

Antioxidant and bioactive compounds are most important photochemicals in fruits and vegetables. So it is necessary to detect the concentration of these photochemicals and to establish their relationship with human health. Again, some fruits are rare and some are endangered and limited research has been conducted on these fruits. This chapter has discussed the main findings of the present study under some broad headings.

5.2 Antioxidant activity of rare fruits

Antioxidant activity of the rare fruits was determined by DPPH assay and represented in Table-4.1. DPPH was a stable free radical which scavenged from purple to yellow after accepting an electron or proton to become a stable diamagnetic molecule when antioxidants were encountered (Singh et al., 2015). DPPH activity ranged between Katajamin (303±8.87 mg/100 g) and Ashari (55±4.23 mg/100 g) and average value was 130 mg/100 g. So, rare fruits had higher antioxidant capacity and rich sources of vitamin C, vitamin E, carotenoids, particularly b-carotene (Rice-Evans and Miller, 1996) and lycopene (Shami and Moreira, 2004) as well as flavonoids and other polyphenols (Saura-Calixto and Goñi, 2006). Antioxidant activity of the fruits was higher than some seasonal fruits available in Bangladesh (Islam et al., 2015) and similar with some Brazilian exotic fruits reported by De Souza et al. (2012). So, these fruits had higher antioxidant capacity and helped to prevent many disorder in human and animal health like arthritis, diabetes, arteriosclerosis, age-related macular degeneration, certain types of cancer, inflammation, genotoxicity, and Alzheimer disease (Ke et al., 2015; Zhang et al., 2015). Because antioxidant compounds exerted their effects through different mechanisms such as inhibiting hydrogen abstraction, binding transition metal ions and disintegrating peroxides and reactive oxygen species (ROS), and reactive nitrogen species (RON) (Diplock, 1997; Qasim et al., 2005). Antioxidant activity of the fruits from same species were also varied which might be the influence of several factors such as the time of harvest, maturity, variety, weather and soil conditions, sun exposure, location of fruit on the plant and post-harvest handling (Amira et al., 2011). DPPH scavenging activity for individual ingredients did not represent the true antioxidant activity of foods owing to the possible interactions between these components and the food matrix (Singh et al., 2016).

Positive correlations were observed for antioxidant activity and bioactive compounds. Total phenolic compounds (TPC) were strongly, total flavonoid contents (TFC) were moderately and total anthocyanin contents (TAC) had little correlation with antioxidant activity (AOA). Significant correlations ($P \leq 0.05$) were observed for TPC and TFC with AOA which was similar with some reported studies in fruits and vegetables (Kevers et al., 2007). Several other studies had reported relationships between phenolic contents and antioxidant activity; some authors found a high correlation between phenolic content and the antioxidant activity (Almeida et al., 2011) while others found no relationship (Imeh and Khokhar, 2002; Ismail et al., 2004). So, higher the concentration of bioactive compounds were higher antioxidant activity because bioactive compounds had also antioxidant and free radical scavenging activity (Bernhoft, 2010; Kaur and Kapoor, 2001). These correlations suggested that phenolic compounds, flavonoids and anthocyanins were the important factors for determining the antioxidant activity in analyzed rare fruits (Macoris et al., 2012).

5.3 Bioactive compounds of rare fruits

Fruits are the rich source of natural bioactive compounds which are essential for health promotion and disease prevention. Polyphenols, flavonoids, and anthocyanins are important group of bioactive compounds of food with plants and animal origin. So it is very important to analysis and evaluation of these compounds with respect to their sources.

5.3.1 Total polyphenol content (TPC)

In this study, Katajamin (141±34.98 mg GAE/100g) had higher TPC and Ashari (10±2.19 mg GAE/100g) had lower TPC value. Average TPC content was 60 mg GAE/100g as gallic acid equivalent. The amount of phenolic contents classification was proposed by Vasco et al. (2008) using low (<100 mg GAE/100g), medium (100-500 mg GAE/100g) and high (>500 mg GAE/100g). So, Katajamin and Bele had medium and others had low polyphenol contents. This findings were higher than previous study performed by Mamun et al. (2012) but lower than Brat et al. (2006) and Zhang and Hamauzu. (2004). Katajamin had higher polyphenol content than Lemon (36.1±0.74 mg GAE/100g) reported by Sarwar et al. (2019) but lower than Apple (179.1 mg GAE/100g) and Lychee (222.3 mg GAE/100g) reported by Brat et al. (2006).

Polyphenols contents were varied with color and water content and dark green fruits contain higher amount of polyphenols than other and recreated with the ripening process (Pavel et al., 2006). Furthermore, polyphenols were also affected by their interactions with other constituents of the food matrix and likely to interfere with the metabolism of polyphenol activity (Cheynier, 2005). Dietary requirement of polyphenols from fruits and vegetables were roughly the same which come from mango, banana, apple, orange and from many of the vegetables. Furthermore, the intake was similar for each gender, except for apple, which were consumed slightly more by women. So, our experimental rare fruits could fulfill the demand of polyphenol for Bangladeshi people and especially for rural people moreover, polyphenolic compounds had biological activity, antioxidant activity and free radical scavengers (Kahkonen et al., 1999; Sugihara et al., 1999).TPC had positive correlation with TFC with significant ($P \leq 0.05$) level which was supported by Chandra et al. (2014).

5.3.2 Total flavonoid content (TFC)

Flavonoids were the bioactive secondary metabolites present in almost plant species. Most common family members included flavonol, flavanol, flavones, flavanes, flavonols and catechins (Gadkari and Balaraman, 2015). Result showed that TFC content was ranged from Ashari (06±3.94 mg QE/g) to Katajamin (76±6.67 mg QE/g) and the average total flavonoid concentration wass 42 mg QE/g. This result was highly comparable with Lutz et al. (2015) and Harnly et al. (2006). Due to lack of studies in the literature on the flavonoids content, we decided to make the comparison with the tomato and lemon a tropical fruit that was well known for its abundance of flavonoids. TFC content of Katajamin (76±6.67 mg QE/g), Dumur (54±18.83 mg QE/g) and Belombo (52±5.01 mg QE/g) had higher than Lemon (48.99±2.61 mg QE/g) but lower than Tomato (84.84±0.28 mg QE/g) reported by Sarwar et al. (2019) and this was also supported by Singh et al. (2016).

5.3.3 Total anthocyanin content (TAC)

Anthocyanins, water-soluble antioxidant pigments, were responsible for most of the red, blue, and purple colors of fruits. In this current study, the TAC of the fruits varied from 2 to 102 mg/100g. Total anthocyanin content was higher in Bet Fol (102±3.460 mg/100g) and lower in Ashari (02±0.74 mg/100g).These results for total anthocyanin contents were compatible with others research on fruits (Carbonell-Capella et al., 2014; Barba et al., 2017).So, rare fruits were the rich sources of anthocyanin compared with other fruits there consumption helps to reduce the risk of cardiovascular disease, diabetes, arthritis and cancer (Prior et al.,2010). Anthocyanins were unstable, easily oxidized and sensitive to temperature, UV radiation, ascorbic acid and metal ions. TAC of fruits and vegetables might vary from fruit to fruit of the same type due to the genetic and agronomic factors, intensity and type of light, temperature, postharvest treatments, processing and storage (De Pascual-Teresa and Sanchez-Ballesta, 2008).Temperature had also significant impact on anthocyanis synthesis like low and high temperatures had long been considered to promote and reduce, respectively, anthocyanin synthesis in fruits (Bobinaitė and Viskelis, 2013).

5.4 Limitations of the current study

1. Here we took only some rare fruits in Bangladesh for these study but there were various types of rare fruits in various regions specially hill tracts area.
2. It was really difficult to conduct the study in limited time and limited resources.
3. In this study we selected only one season. But different season's fruits should be selected for the research.
4. We used some factor to enumerate the wavelength of TFC, TAC and TPC on the assumption basis which might not be perfect.
5. There are a various types of bioactive compounds. But we determined only three types of bioactive compounds like TPC, TFC and TAC.
6. We used UV visible for measuring the bioactive compounds but it is better to use the GC-MS for better result.

CHAPTER VI
CONCLUSION

Bangladesh is very fertile land for the growth of various fruits. 10 different rare fruits (mostly citrus) were analyzed for phenolic compounds, flavonoid contents, anthocyanin content and radical scavenging properties. These results reveal that pulp from locally grown citrus fruits are valuable sources of health benefiting bioactive components, hence they can be considered for use in food products formulation with the objectives of adding health benefits to foods such as increased antioxidant potential and bioactive compounds. This could play a great role in alleviating the malnutrition of our country. The nutrient profile of these fruits are also appealing from health point of view. The fruits hold good commercialization potential.

The tropical environment of Bangladesh is conducive for citrus production provided that appropriate agronomic practices are followed for obtaining high quality citrus fruit. This can be helpful for maximum utilization of natural foods and at the same time assist in environment protection. The analysis of the present study represent that among 10 fruits, maximum antioxidant is (303±8.87 mg/100 g) and highest amount of TPC is (76±6.67 mg QE/g) beyond the limits of WHO in several points. However, in the overall antioxidants concentration of these fruits exceeded the recommended values of WHO. This might be due to the presence of higher antioxidants sources around the respected sites of the others fruits. This exceeded level of AOA and bioactive compounds recommend a sign of presence of health beneficial which has great impact on environmental issue. If this trend of consumption continues, it may make a healthy life and in the long run can cause a good impact on human livelihood. Some other previous studies also showed that the AOA of different local fruits had high amount of antioxidants and bioactive compounds in their findings.

CHAPTER VII

RECOMMENDATIONS & FUTURE PERSPECTIVES

Rare citrus fruits constitute an important and healthy part of the human diet, mainly owing to the presence of bioactive compounds, which play an essential role in human health, also to the presence of vitamins, minerals. This chapter provides the recommendations and future perspectives of the present study on the basis of the prevalence of AOA and bioactive compounds in rare citrus fruits of Bangladesh as follows:

- The physiochemical characteristics, nutritional composition of rare citrus fruits should be evaluated. There should be created awareness about the wastage of the fruits and its possibility of being a scope for food industry.
- This present research reveals that the fruits have high concentration of AOA than other imported fruits. So therefore, further investigations should be carried out.
- The present study was carried out to determine AOA concentration and some bioactive compounds. A similar further research should to be carried out check the nutrition content and chemical profile in rare fruits along with different areas.
- The rare fruits are made available to consumers and entrepreneurs round the year if there is an application of scientific means of post harvesting handling, processing and preservation.
- The market potential of these fruits can be better exploited if fruits are made available to the consumers in a ready to eat or ready to serve form throughout the year and by this way, meet up the necessity of antioxidant in the body.
- Modern cultivation, packaging and storage condition would be developed for the betterment of rare citrus fruits.
- For the development of the socio-economic status of our country citrus fruit cultivation and consumption should be increased.

- The up gradation techniques of the fulfillment of nutritional demand and desired goal for the betterment and improvement of the nutritional approach mechanism of our country.
- The Department of Agriculture Extension (DAE) has targeted to produce additional 2,000 metric tons of citrus fruit production, by 2021.
- About 800 hectares of land will be brought under citrus fruits cultivation under the Citrus Development Project and we hope that additional 2,000 metric tons of citrus fruits would be produced by 2021.
- Citrus fruits production will go up gradually .The DAE took the five- year Citrus Development Project in 2013 to extend cultivation of orange, mandarin orange and other citrus to 67 upazilas of 17 districts in the country.
- About 60 percent of project work has already been completed and the DAE expected that the remaining work will be completed within the stipulated period.
- The Citrus Development Project is being implemented in Cox's Bazar, Bandarban, Rangamati, Khagrachari, Chittagong, Comilla, Moulavibazar, Sylhet, Norsingdi, Gazipur, Tangail, Mymensingh, Netrokona, Sherpur, Thakurgaong.
- A huge amount of foreign exchange is being spent for importing various fruits which have less antioxidant than our local fruits.

- It is possible to grow the rare fruits commercially, fulfill the national demand and save foreign exchange by eliminating the problems by developing good varieties and introducing improved management techniques. Under the Citrus Development Project, farmers are being motivated to grow citrus fruits in the favorable growing areas. Women and tribal farmers are also being involved in growing citrus through training.
- According to official data, about 135,683 metric tons of citrus fruits are being produced in 2,832 hectares area in Bangladesh. More or less citrus are grown all over the country now.

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Appendix A: Standard Curve & Sample Curve

Table: Concentration and Absorbance for Standard solution for AOA

Sample ID	Type	Ex	Conc. (ppm)	WL 517. 0	Wgt. Factor	Comments
1	Std2	Standard	1.000	0.221	1.000	
2	Std3	Standard	1.500	0.185	1.000	
3	Std4	Standard	2.000	0.133	1.000	
4	Std5	Standard	2.500	0.092	1.000	

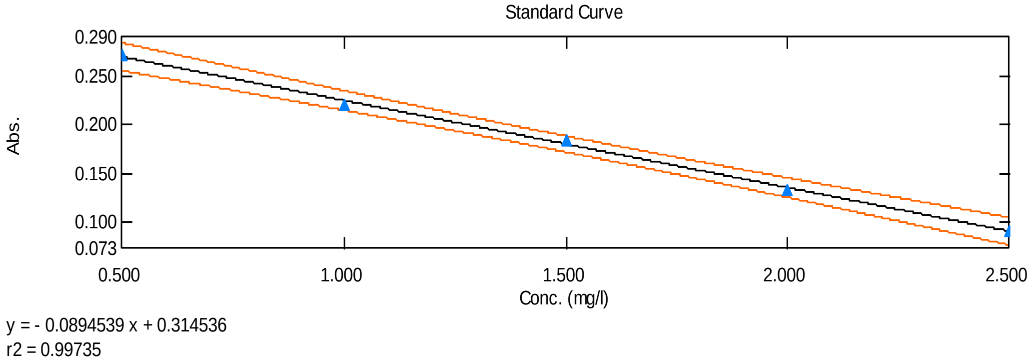

Figure: Standard curve for antioxidant capacity determination test

Table: Concentration and Absorbance for Standard solution for TFC

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt.Factor
1	Std1	Standard	1.000	0.041	1.000
2	Std2	Standard	3.000	0.088	1.000
3	Std3	Standard	5.000	0.171	1.000
4	Std4	Standard	7.000	0.234	1.000

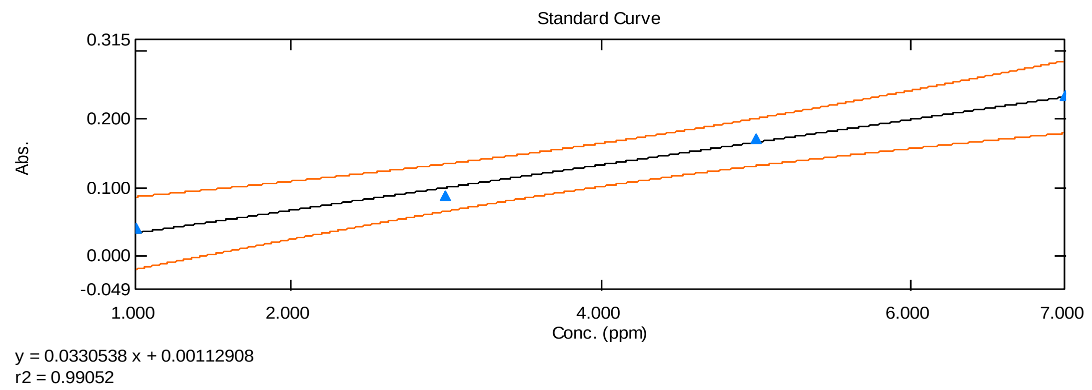

Figure: Standard curve for TFC capacity determination test

Table: Concentration and absorbance for Standard solution for TPC

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt. Factor
1	Std1	Standard	1.000	0.763	1.000
2	Std2	Standard	2.000	0.780	1.000
3	Std3	Standard	3.000	0.920	1.000
4	Std4	Standard	4.000	1.007	1.000
5	Std5	Standard	5.000	1.074	1.000
6	Std6	Standard	6.000	1.115	1.000
7	Std7	Standard	7.000	1.230	1.000
8	Std8	Standard	8.000	1.314	1.000

Standard Curve

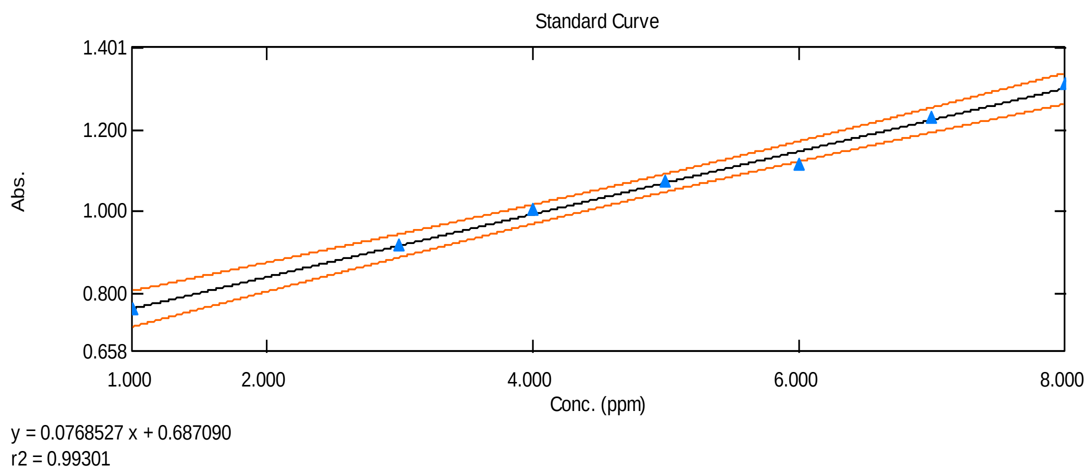

Figure: Standard curve for TPC capacity determination test

Appendix B: Picture Gallery

Figure: Jamrul

Figure: Kawfol

Figure: Panifol

Figure: Bel

Figure: Sofeda

Figure: Belombo

Figure: Dumur

Figure: Betfol

Figure: Katajamin

Figure: Asari

Figure: Stock solution preparation

Figure: Addition of Methanol

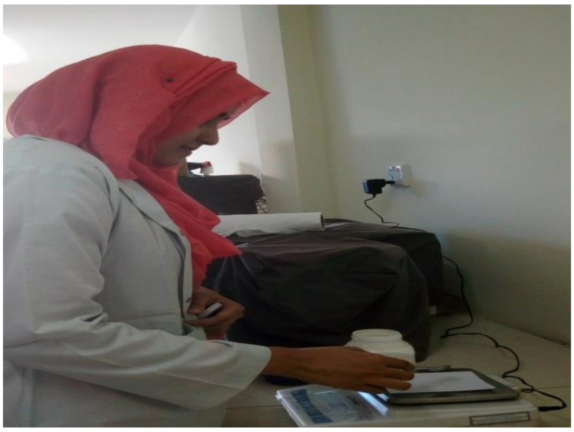

Figure: Weighting of $Alcl_3$

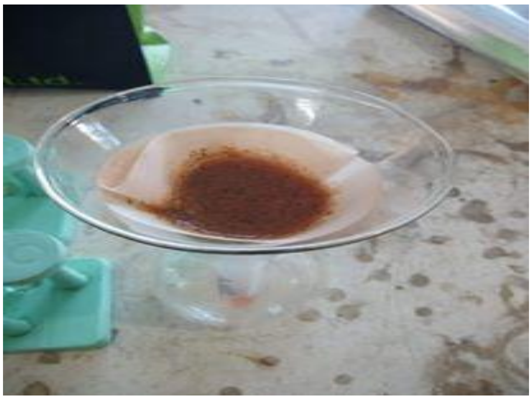

Figure: Filtration of sample

Figure: Stock solution preservation

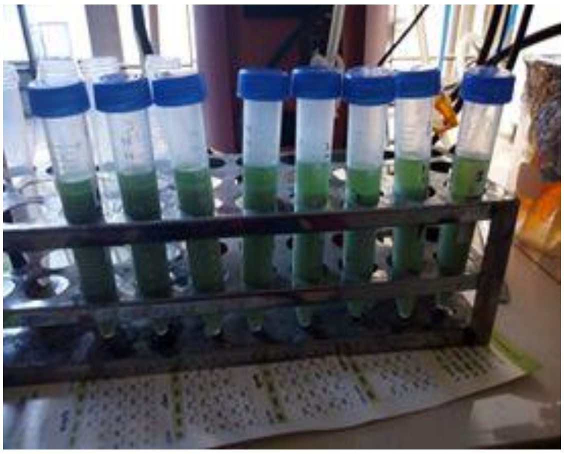

Figure: Sample with DPPH

Figure: Sample with Quercetin

Figure: Placing sample into UV

Figure: UV visible spectrophotometry

BRIEF BIOGRAPHY

Farzana Shabnam Punam passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. She obtained her B.Sc. (Hons.) in Food Science & Technology in 2017 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Department of food Science and nutrition in Applied Human Nutrition and Dietetics under the faculty of Food Science & Technology, CVASU. She has immense interest to work in different food issues including nutrition, food chemistry, quality assurance, food quality control, environmental chemistry, product development and processing, malnutrition, reduction of nutritional changes in food etc.