EVALUATION OF NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EFFECT OF *Ficus racemosa* AND *Ficus hispida* FRUITS

Fariab Hasan

Roll No.: 0118/14

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Zakir Hossain Road, Khulshi, Chattogram-4225, Bangladesh

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This is to certify that we have examined the above Master's Thesis and have found that it is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

…..……	**……**
(Md. Altaf Hossain)	**(Mohammad Mozibul Haque)**
Supervisor	**Co-supervisor**

……..
(Md. Altaf Hossain)
(Chairman of the Examination committee)

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

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PLAGIARISM VERIFICATION

Title of the Thesis: Evaluation of Nutritional Composition, Antioxidant Activity and \ Antibacterial Effect of Ficus Racemosa and Ficus Hispida

Name of the Student: Fariab Hasan

Roll number: 0118/14 **Reg. number:** 555

Department: Applied Food Science and Nutrition **Faculty:** Food Science and Technology

Supervisor: Md. Altaf Hossain

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DEDICATATION

I Dedicated My Small Piece of Work

To My Beloved Family Members and Respected Teachers.

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The Author

December 2019

Table of Contents

Table of Contents

Table of Contents

List of Tables

List of Figures

Abbreviations

%	:	Percentage
AOAC	:	Association of Official Analytical Chemists
℃	:	Degree Celcius
CFU	:	Colony-forming Unit
CVASU	:	Chattogram Veterinary and Animal Sciences University
Dl	:	Deciliter
DPPH	:	2,2-diohenyl-1-picrylhydrazyl
et al	:	Et alii/et aliae/ et alia
etc.	:	Et cetera
G	:	Gram
GAE	:	Gallic acid equivalent
Kg	:	kilogram
ml	:	milliliter
mg	:	milligram
µg	:	microgram
ppm	:	parts per million
QE	:	Quercetin equivalent
RDA	:	Recommended Daily Allowance
SD	:	Standard Deviation
SPSS	:	Statistical Package for Social Science
WHO	:	World Health Organization

Abstract

Considering the growing need to identify the alternative plant-based nutritional sources fruits of *Ficus racemosa* and *Ficus hispida* from the Moraceae family were evaluated to find out the nutritional composition including major and trace minerals, antioxidants and antibacterial activity in order to priorities their edibility and for the medicinal uses. The nutritional analysis reported that, *F. racemosa* and *F hispida* both serve as a good source of fiber, carbohydrate, minerals and bioactive compounds. Analysis showed that the fat content (9.3g) in *F. racemosa* was doubled than the *F. hispida* (4.38g) per 100 g dried weight. The average protein content of the both fruits was about 9.8g. The fiber content (26.53g) was significantly higher in the *F. hispida* than the *F. racemosa* (18.02g). The energy content *of F. racemosa* and *F. hispida* fruits were 317.2 and 242.35Kcal respectively for 100g (DW). The fruits are rich in essential minerals (Na, K, Ca, Mg, P) and sufficient trace elements (Fe, Zn, Cu). It was reported that the average potassium content of both fruits was about 1150mg. The calcium content was found higher amounts in the fruits of *F. hispida* (760mg/100g). The iron contents of both fruits were within the range of 64-68mg/100g. For the antioxidant activity, the IC_{50} value were 16.80 and 38.53 μg/ml for *F. racemosa* and *F. hispida* fruit respectively, where the value of standard ascorbic acid 9.56 μg/ml. The phytochemical screening test revealed that the alkaloids and tannins were present in the both fruits; additionally, *F. racemosa* was also positive for the saponins test. Quantitative Analysis of bioactive compounds showed that the phenolic content of *F. racemosa* and *F. hispida* were 10.42 and 5.74mg GAE /g respectively. The flavonoid content of *F. racemosa* 20.05±0.15 mg QE/g) was higher than the *F. hispida* fruits (15.97±0.75mg QE/g). The aqueous and diethyl ether extracts of both fruits did not show any zone of inhibition against *Escherichia coli* and *Staphylococcus aureus.* It can be concluded that, both fruits contain lots of essential nutrients that justify their use as food and also rich in bioactive compounds that may be responsible to cure different diseases.

Keywords: *Ficus racemosa*, *Ficus hispida*, polyphenol, flavonoid, antioxidants

Chapter 1: Introduction

In this era, people find possible ways to include more natural products in their diet and as well in the way of life. Food plants of nature have become more fascinating to the recent world for their use as value addition of food products, replacement of different synthetic chemicals and nutraceuticals. Fruits are generally eaten raw or processed, which will help to compensate for the daily requirement of calories. Different types of naturally grown wild fruits can be the alternative source of carbohydrate, protein, fat, dietary fiber and can play a significant role in human nutrition. Some of them possess medicinal value and have been used as a medicine in folk culture over a longer period. These types of plants are usually neglected; their nutritional composition and medicinal efficiency are not descriptive in literature.

Natural wild plants serve as alternative means in addition to staple food during a longer period of food shortage and considered valuable supplements for the fulfillment of nutritional needs. Fruits grown in the wild can be an alternative way of income for tribal groups and low-income groups and used for domestication (Deshmukh and Waghmode, 2011). By 2050, the global population will be expected to reach 9.8 billion. Then the food supply system of the world will be under greater stress. The demand for food will be increased by 60% higher than it is today. Environmental pollution, urbanization and soil degradation will shrink the availability of agricultural land according to the world economic forum (Grafton et al., 2015). We have to prepare for fighting this food shortage problem with systematic and preventive measures. One of them could be the utilization of existing plant resources as an alternative food source. The underutilized wild fruits found to have potential sources of nutritionally important components like mineral phytochemicals and unique health-promoting compounds in addition to their proximate composition (Hegazy et al., 2013).

Fruits are an important source of different types of phytochemicals that have been suggested as the natural source of antioxidants. Oxidative stress is the reason behind many diseases like brain dysfunction, cancer, heart disease, age-related disease, aging processes, coronary arteriosclerosis, carcinogenesis, gastric, ulcer and DNA damage (Sing et al., 2012), (Venkata et al., 2012).

Antioxidants are necessary to suppress the free radicles to eradicate the toxicity induced by free radical. Antioxidants may stop the damage occurred by the free radicals by the

process of giving electrons to the free radicals and neutralizing them. Because of the reason, they are called “free radical scavengers” (Valko et al., 2007).

The genus Ficus is having over 700 species belongs to the family Moraceae constituted one of the largest genera of medicinal plants primarily prevailing in subtropical and tropical regions throughout the world, among them *Ficus racemosa* and *Ficus hispida* are seen everywhere in Bangladesh and important for their traditional use as medicine. These plants are usually not cultivated, grown all alone with the help of nature. *Ficus racemose* is locally known as jogdummur or joggadumur. The unripe fruit is consumed as a curry and ripen one is simply as fruit in the rural parts of Bangladesh and Indian subcontinent. *Ficus hispida* is called kakdumur, khoksha, jongkidumur. This fruit is used to make curry and ripe fruits are eaten as a fruit in some tribal groups and rural parts Bangladesh. *F. hispida* is mainly cultivated for its edible fruits in places like India, Nepal, Andaman Island, Myanmar, and Sri-lanka (Kunwar and Bussmann, 2006). Different parts like bark, leave, root latex of the plant is used to cure some common diseases as part of the folk medicine culture system.

The fruits of *F. racemosa* are reported to have potential medicinal properties (Patil et al., 2006) The fruits contain flavanone, simple phenol and catechol that impart antioxidant properties. The fruit extract is used as treatment for diabetes, menorrhagia, and also used to relieve the inflammation of skin wounds (Singh et al., 2013). The fruits have the potential properties of antioxidants and hypoglycemic (Zulfiker et al., 2011). The stem bark shown was to have antioxidants (Veerpur et al., 2009) and anti-bacterial property (Mahato and Choudhary, 2005). The bark is used in inflammation, gonorrhea, scabies and mouthwash (Nair and Chanda, 2007). The bark is used as an anti-hyperglycemic agent in the treatment of diabetes. (Urooj and Ahmed, 2013). The extracts of bark leaves and fruits of *F. racemosa* is used as an anticancer anti-tumor and antimicrobial agent. (Kambli et al., 2014).

The leaves of *F. hispida* are most vital as an antidiarrheal and hepatoprotective. (Mandal, Kumar, 2002). The *F. hispida* fruit is commonly used as a tonic and coolant. The juice of the fruit is mixed with jaggery is useful as a mild purgative. A mixture of honey and fruit juice is used in the treatment of hemorrhage (Joseph and Raj 2010), the root and leaves of the plant have potential antidiarrheal activity (Mandal et al., 2000),

anti-bacterial (Kone et al., 2004) and as cardioprotective (Shanmugarajan et al., 2008), antidiabetic (Ghosh et al., 2004).

1.1 Description of *Ficus Racemosa*

Scientific Name:

Ficus Racemosa

Common Name:

Cluster Fig, Cluster tree, Country fig, Redwood fig, Gullar

Local Name:

Jaggadumur, Hulangdumur, Yajnadumur

Taxonomic position:

Domain: Eykariotes

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliophyta

Sub class: Rosidae

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: *F. racemosa*

Figure 1.1: *Ficus racemosa* Fruits

Plant Description:

F. racemosa found everywhere in Bangladesh usually in moist localities. Indian subcontinent is the original domestic of the plant. It is also found in Burma, China, Indonesia, Malaysia, and Australia. *F. racemosa* is a medium to the massive deciduous tree (15-16m) with branches. It can grow over 40 feet tall and 20 to 40 feet wide. This plant is easily noticed for its beautiful red fruits which grow on trunks or branches in clusters Figs have the hairy surface, generally 2-4 cm in size. Immature figs are light green and ripen ones are red in color. The genus call racemosa is derived from its odd inflorescence (Joseph and Raj, 2010).

1.2 Description of *Ficus Hispida*

Scientific Name:

Ficus hispida

Common Name:

Dumur, Kak dumur, Kala dumur, Devil fig, Hairy fig, Rough-leaved fig, Opposite-leaved fig

Taxonomic Position:

Domain: Eukaryote

Kingdom: Plantae

Division: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: *F. hispida*

Figure 1.2: *Ficus hispida* Fruits

Plant Description:

F. hispida plant is a decorative, flowering and additionally a fruit plant and cited as common fig, dumur or hairy fig. It belongs from its own family of “Moraceae” a medium-sized tree with branches covered with small hair-like structure. The plant grows in wilderness and fallow lands. It can be seen everywhere in Bangladesh, in addition to South and Southeast Asia and New Guinea, Australia and Andaman island. The fruit is oval-shaped and ripe fruits are yellow color. Green leaves are shaped as oval to renal and rough and hair. The name of the species 'hispida' derives from its hairy leaves (Salvi et al., 2013).

There are very few and still a lack of comprehensive investigation on the nutritional potential of wild edible fruits (Mahapatra et al., 2012). In recent years, the interest in research and development activities increased in exploiting underutilized fruit species because of their protective role against diseases and enhancing human well-being in daily life (Schreckinger et al., 2010). Descriptive information about the nutritional value of F. *racemosa* and *F. hispida* fruit are still lacking in the literature.

However, references about medicinal properties and antioxidant activities of different parts of *F. racemosa* and *F. hispida* are found in the work of literatures. Comprehensive and conclusive research about the nutritional composition of *F. racemosa* and *F. hispida* fruits indigenous to Bangladesh is not reviewed yet. Bearing this in mind this study has been undertaken to investigate the comparative value of nutritional composition, bioactive compound content and antioxidant activity of the fruits of these two species. That will probably be used in our society and green industry as a choice of healthy food and alternative medicine regarding its other functional properties.

1.3 Aims and Objectives

People of rural parts of Bangladesh and some tribal groups are consuming these fruits without knowing their nutritional needs. The study aims to let them know about the utilization of *F. racemosa* and *F. hispida* fruits as food sources with related functional properties. The objectives of the study include:

1. To compare the nutritional composition of the *F. racemosa* and *F. hispida* Fruits
2. To analyze the antioxidant activity and phytochemical compounds in both fruits
3. To examine the antibacterial effect of fruit extracts

Chapter 2: Literature Review

Relevant literature of *Ficus racemosa* and *Ficus hispida* with their health benefits related to various nutrients, bioactive and antioxidant compounds and functional properties have been reviewed. The review founding of the article is presented in this chapter.

2.1 Traditional use as food

According to Kunwar and Bussmann (2006) the fruits of *F. racemosa* and *F. hispida* is listed as edible among the indigenous people of Nepal.

F. racemosa ripe fruits have attractive flavor and eaten raw in some regions of India including Orissa, Bihar and Andhra Pradesh. Ripe fruits can be dried and ground into the powder and then used to eat with milk and sugar. This powder can also be used for preparing jelly. The dried fruit powder is used as a popular and appealing breakfast in tribal groups. Fruits are carminative, stomachic and astringent.

F. hispida fruits are greenish-yellow in color and ripe fruits are edible sometimes made into jam usually in poor households. Unripe fruits are used as a vegetable to make curry in Bangladesh (Mahapatra and Panda 2009).

2.2 Nutritive value of some wild edible fruits

Six widely grown mulberry and fig fruit were nutritionally evaluated and found to be promising sources of protein, carbohydrate, fibers and vitamins, with high energy values and essential micronutrients such as potassium, magnesium, phosphorus and iron. The Ficus groups includes *F. carica*, *F. glomerate* and *F. palmata* contains 70g/100g of carbohydrate with high energy value ranges from 316-332 Kcal/100g dry weight. The protein content ranges from 6.5 -8.6 g/100g. The fiber content is found to be highest in *F. palmata* (17.81g/100g) than *F. racemosa* (16.80g/100g) and *F. carica* (14.20g/100g). The fat content is ranges from 1.2 -2.71 g/100g. The fruits are a great source of essential and trace minerals. Potassium content is highest in F. racemosa (17.21mg/g) whereas the Calcium content is highest in *F. carica*. *F. palmata* contains higher amounts of Magnesium (6.92 mg/g) than others. The Phosphorus content ranges from 0.77-1.5mg/g (Sadia et al., 2013).

The nutrient analysis described by Nayak and Basak (2015) in 8 selected wild edible fruits indicated that wild edible fruits may be a good substitute to cultivated fruits for

their high nutrient and mineral content. The proximate analysis of *F. hispida* reported the moisture, carbohydrate, protein, and total sugar content is 82.84%, 1.75%, 1.13% and 0.63% respectively. This fruit is a rich source of minerals including Iron (6.20mg/100g), Manganese (17mg/100g), Copper (6.2 mg/100g) and Calcium (5mg/100g). The Calcium content is highest among other fruits.

A study carried out on nutritional evaluation of some edible fruit plants of Southern Odisha, India (Mishra and Mishra, 2016). It reports the nutrient value of edible fruits of 22 plants including three Ficus group *F racemosa* and *F hispida and F. benghalensis*. Most of these fruits of these plants are used by the tribal people at the time of food shortage. According to nutritional analysis the moisture percentage ranges from 63-74%. The protein content in *F. racemosa F. hispida* and *F. benghalensis* are 8.17mg/g, 26.46mg/g and 16.17mg/g respectively. The total sugar content is highest in *F. hispida* and the value is 148.5mg/g than *F. racemosa ((119.17mg/g) and F. benghalensis* (119.17mg/g). the fruits of *F. racemosa* contains 0.05 mg/g of vitamin C. This analysis indicates the scope of using wild edible fruits for the dietary supplement.

According to Jayakumar (2016), the phytochemical and nutritional evaluation of Ficus racemose Linn revealed that the leaves are rich in phytochemicals like alkaloid, carbohydrate, glycoside, saponin, phenol etc. The fruit is the source of nutrients like protein (28.125g), total carbohydrate (15.84g), total lipid (7.58g), fiber (0.544g), and minerals (2.632g), value is expressed as per 100 g of fresh weight. The fruit also contains some bioactive compounds including total phenol (1.025%), lycopene (0.0848%) and anthocyanin (0.6864 %). The fruit is reported as a rich source of minerals. The most abundant minerals are phosphorus (1312 mg/100g), Sodium (329mg/100g) and iron (315 mg/100g).

2.3 Medicinal Uses of *F. racemosa*

2.3.1 Hypoglycemic Activity

To examine the hypoglycemic activity of this plant, ethanolic extract (250mg/kg/day) of the different parts of the plant was given to alloxan-induced diabetic albino rats for two weeks. The report of the study confirmed that the blood glucose level was lowered by the application of this plant. Different compounds of the plant were isolated to check which compound possesses hypoglycemic activity. Þsistosterol isolated from the stem bark is proven to have the most potentiality among all (kar et al., 2003)

2.3.2 Hypolipidemic Activity

Fiber from the fruits was given to rats at 10% dietary level. It showed hypolipemic activity by the process of increased excretion of cholesterol through feces (Agarwal et al., 1988).

2.3.3 Wound healing

Aqueous and ethanolic extract of *F. racemosa* bark possesses wound healing ability in excised and incised wound model in albino rats. Phytochemical constituents present in bark, like flavonoid, alkaloid, saponin and tannins are known to play a considerable role in the wound healing process (Murti et al., 2012).

2.3.4 Antibacterial Activity

Various extracts of *F. racemosa* leaves were tested to see the antibacterial activity against *E. coli*, *Bacillus subtilis*, *Bacillus pumities*, *Pseudomonas aureus*. The petroleum ether extract was found to be most effective than others (Mandal et al., 2000).

2.3.5 Anthelminthic

The hot water extract of bark was evaluated on earthworm and found to have potential homicidal activity comparative to piperazine citrate. Worms become paralyzed by the use of piperazine citrate but remained alive and act normally when placed in freshwater. There is no final recovery in case of worm treated with 50mg/ml extracts of *Ficus racemosa* bark. This study suggested that it will be effective in the human body to cure parasitic infections (Chandrashekhar et al., 2008).

2.3.6 Antidiuretic

The decoction of the bark was used as an antidiuretic in the traditional medicine system of Sri-lanka. The scientific study of oral application of decoction (250, 500 and 1000mg/kg) to rats have justified the diuretic action. This decoction caused a reduction in urine Na^+ level and Na^+/k^+ ratio and in urinary osmolarity, there may have underlying multiple actions of mechanisms (Ratnasooriya et al., 2003).

2.3.7 Toxicity studies

The petroleum ether extract of *F. racemosa* did not produce toxicity at a dose of 5g/kg in mice (Deshmukh et al., 2007). Another study reported by Rao et al., 2008 that the hydro-ethanolic extract (50%) is non-toxic and safe, as no mortality or change in the behavioral patterns was observed in mice. According to a study carried out by Zulfiker et al. (2011) mortality or any considerable symptoms of toxicity was not found after oral administration of 2gm/kg body weight in mice. Even no changes in general behavior were shown up to 14 days of study.

2.4 Medicinal Use of *F. hispida*

2.4.1 Alzheimer's Disease

The study was carried out to evaluate the ameliorating effect of ethanolic extract of *F. hispida* leaf on amyloid beta-induced cognitive deficits and oxidative stress in mice. Animals were given the ethanolic extract of leaf for four weeks at a dose of 200 and 400mg/kg then received a single intracerebroventricular injection of Aβ 25-35. This study suggested that this leaf extract has a protective effect against the cognitive deficit. The antioxidant properties of this plant mat act as an underlying mechanism of this process (Sivaraman et al., 2012).

2.4.2 Anti-diarrheal Activity

The methanolic extract of *F.* hispida leaf is proven as an anti-diarrhoeal agent. A study is carried out in which methanolic extract of leaf is given to castor oil induces diarrhea and PGE2- induced enter-polling in rats model. It showed a significant inhibitory effect by the reduction of gastrointestinal motility in rats (Mandal et al., 2002).

2.4.3 Anti-hyperlipidemic

Triton-WR 1339 (Tyloxapol) induced mice model was given the methanolic extract of *F. hispida* Linn leaves to check the anti-hyperlipidemic activity against elevated triglyceride level in blood. The leaf extract was administrated a dose of 125, 250 and 500mg/kg respectively compared to Tyloxapol treated control group. This study showed a significant reduction in serum lipid parameters (Total Cholesterol, Triglycerides, VLDL and LDL level), proving that the leaf extract has potential property against hyperlipidemia disease (Shanmrugrajan et al., 2008).

2.4.4 Cardioprotective Activity

The methanolic leaf extract of *Ficus hispida* was proven to indicate cardioprotective activity. The leaf extract of the plant was indicated that it has potential activity against lipid peroxidation also has the ability to increase the level of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. This mechanism helps to reduce the glutathione activity in heart tissue induced by cyclophosphamide (Shanmugarajan et al., 2008)

2.4.5 Anti-Oxidative Activity

The methanolic extract of leaf of *Ficus hispida* has anti-oxidant activity. A study was designed to evaluate the property in Wister rats induced by Azathioprine at a dose of 50mg/kg body weight. That caused liver injury in males. The in vitro effect of the activity of this extract was determined by the enzymes like DPPH and nitric acid. Azathioprine caused liver injury which reduced the level of enzymes. The administration of leaf extract at a dose of 400mg/kg body weight given for 21 days indicated to restore anti-oxidant status within the normal range (Shanmrugrajan et al., 2008).

2.4.6 Nephroprotective Activity

The methanolic extract of F. hispida fruits showed significant nephroprotective activity than nephrocuration on cispaltin induced nephrotoxicity in a study. The male Wister albino rats were given ethanolic extract in two doses 250 and 500 mg/kg. The extract has shown partial protection against CP induced renal and functional impairment, and the protection is more significant at the higher dose (Swathi et al., 2011).

2.4.7 Anti-diabetic Activity

Traditionally *F. hispida* is herbal medicine for the treatment of diabetes. A study is reported by Islam et al., 2018 to evaluate the ethanolic extract of *F. hispida* fruits and bark in alloxan-induced diabetic rats. The report showed that intraperitoneal administration of ethanolic extracts of fruits and bark of *F. hispida* decreases blood glucose level significantly and also regulated the parameters of lipid profile.

2.4.8 Toxicity study

The methanolic extract was administrated on male Wister albino rats to evaluate the toxicity of *F. hispida* fruits on an animal model. It is reported that no animal died even at 1000mg/kg and the extract was treated as non-toxic (Swathi et al., 2011).

Chapter 3: Materials and method

3.1 Study period and Study area

The research work was conducted for a period of six months from August 2019 to December 2019. Experimental procedures were carried out in the laboratory of the Department of Applied Food Science and Nutrition, Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology, Department of Microbiology and Veterinary Public Health, Poultry Research and Training Center (PRTC) at Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2 Collection of samples and preparation

Mature and fresh fruits of *Ficus racemosa* and Ficus *hispida* were collected from the trees located in the Khulshi area in Chattogram district.

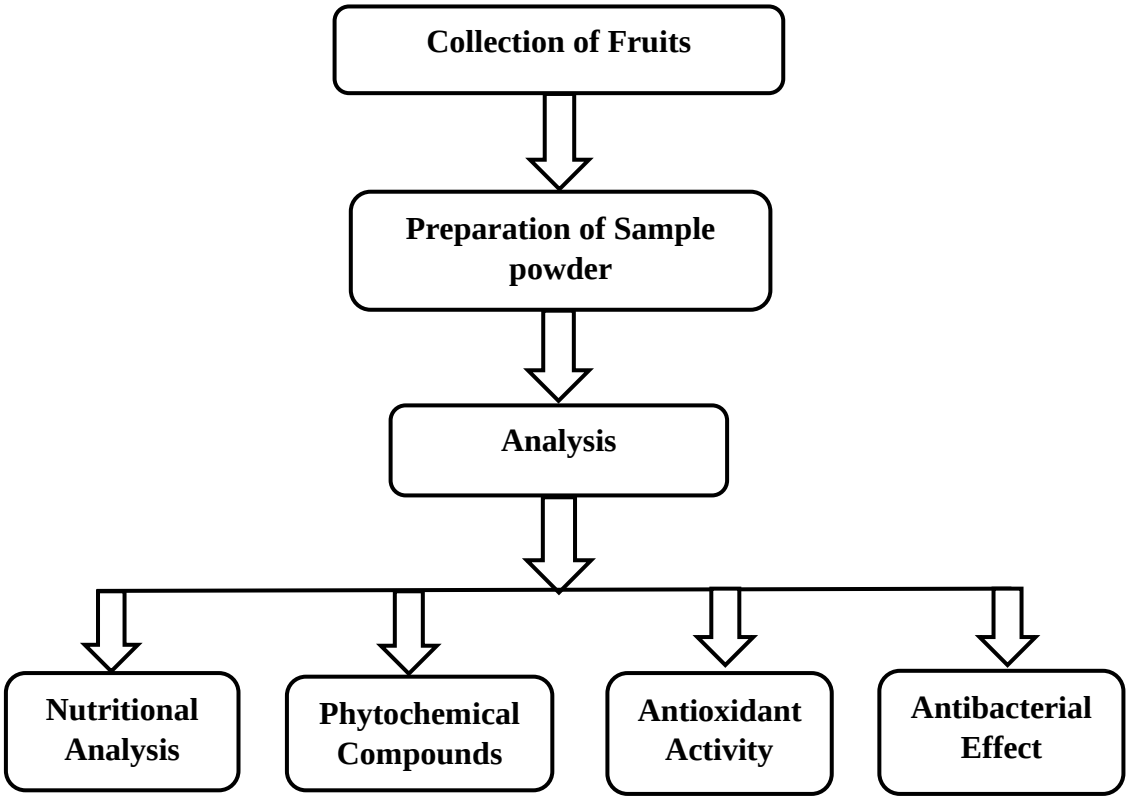


Figure 3.2: Flow chart of Experimental Procedures

Collected fruits were washed properly with water to remove any dirt particles from the fruits. Then the fruits were cut into small pieces placed the trays and dried in cabinet drier at 60℃ for two days. Dried samples were taken and ground into powder form with the help of a grinder. After that powder of samples was packed into zip-lock plastic bag and kept at 4℃ in the refrigerator for further examinations.

3.3 Proximate Analysis

The moisture content of fresh fruit was determined using the standard method of AOAC. The protein, fat, fiber and ash content of fruit samples of *F. racemosa* and *F. hispida* were analyzed on dry weight basis according to Association of Official Analytical Chemists (AOAC, 2012). Triplicate analysis of all samples was conducted.

3.3.1 Moisture Content Determination

The moisture content of fresh fruit was determined by the hot air oven drying method following the method described in AOAC. An empty crucible and 2 g of the sample were weighed in an analytical balance. The sample was taken in the crucible and dried in a thermostatically controlled oven at 105℃ for 5 hours. After that, the crucible was removed and placed in a desiccator to cool at room temperature. The sample contained crucible was weighted and the value was recorded. This step was done repeatedly until a constant weight was found out. The moisture content of the sample was calculated from the loss of weight in the sample.

$$\%\ \text{moisture content} = \frac{\text{Loss in weight}}{\text{weight of sample}} \times 100$$

3.3.2 Crude Protein Determination

The crude protein content of the sample was determined by following the Kjeldahl method. About 0.3g of the sample was weighed and taken into a digestion tube. A mixture of 72gm Potassium sulfate and 8 gm of Copper sulfate was prepared. the 4 gm of this mixture was added to the digestion tube. Then 5 ml of concentrated H2SO4 was added. Digestion was accomplished at 320℃ for 30 minutes. Then the mixture was cooled and 25 ml of distilled water and 25 ml of 40% NaOH was added.10ml of 4% boric acid was taken in receiving a conical flask and 3 drops of green bromocresol indicator was added. Cooled tube and receiving solution were placed into the distillation unit. 25ml of 40% NaOH was automatically filled into the tube. The distillation process

takes place for 3 minutes. The receiving solution turned green at the end of the process. Then the solution was titrated with 0.2N HCL until it becomes grey.

The protein content was calculated by the following formula.

Percentage of protein = percentage of Nitrogen×6.25

$$\text{The percentage of Nitrogen} = \frac{(\text{T} - \text{b}) \times \text{N} \times 14.007 \times 100}{\text{weight of sample in mg}}$$

T = Volume of the titration sample

B = Volume of titration for Blank

N = Normality of HCL (0.2)

3.3.3 Crude Fat Determination

Crude fat was determined by using a Soxhlet apparatus. The dried sample was weighted transferred into a thimble and plugged with a fat-free cotton. The thimble was placed into the fat extraction tube of the Soxhlet flask. 75 ml of anhydrous ether was taken into the flask and top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer period. At the end of the extraction, the thimble was removed and most of the ether was distilled off and collected through the Soxhlet tube. The ether from the tube was poured off as it was nearly full. When the volume of ether containing the fat particles of the sample was reached at a small volume, it was poured into a beaker using a funnel. The flask was rinsed filtered thoroughly using ether. Then the ether was evaporated on a steam bath at low heat. T was dried at 100℃ for 1 hour. After cooling, the weight was recorded carefully. The fat present in the sample is calculated by the formula given below:

$$\text{Fat} = \frac{\text{Loss of ether solube material}}{\text{Weight of sample}} \times 100$$

3.3.4 Crude Fiber

At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml 0f 1.25% sulfuric acid solution and 3-4 drops of n-octanol were added into the same beaker. N-octanol was using as an antifoaming agent. The beaker was boiled for 30 minutes at constant volume. After that, the sample was washed three times to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated and again washed the residue like before. It was washed again with 1% HCL solution in order to remove the acid. Then the residue was dried in a hot air oven at 105℃ until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660℃ for about 3-4 hours until that turned into white ash. The ash particles were weighed and calculated to determine the crude fiber content of the sample.

$$\text{Percentage of Crude Fiber} = \frac{W - W_1}{W_2} \times 100$$

Where,

W= weight of crucible containing crude fiber and ash

W1= weight of crucible containing ash

W= weight of the sample

3.3.5 Ash Content

First of all, an empty crucible was cleaned properly and dried in a hot air oven. It was placed in desiccators and cooled then the weight was recorded.2-5 gm of the sample was weighed and placed in the crucible. It was allowed to burn until all the smoke was gone from the sample. The crucible was cooled and transferred to the muffle furnace at 550-600℃ for 6-8 hours. The process ends when formation of white ash accomplished. It was cooled at 150℃ and then placed to desiccator. When it cooled to mild warm the weight was recorded.

The ash content was calculated using the following formula.

$$\text{Percentage of Ash} = \frac{W - W_1}{W_2} \times 100$$

Where W= weight of the crucible with ash

W_1= weight of the empty crucible

W_2= weight of the sample

All the analyses were done in triplicates and expressed in percentage.

3.3.6 Carbohydrate Content

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100 (per 100gm) (AOAC, 2012).

$$\text{Percentage of Carbohydrate} = 100 - (\text{Moisture \%} + \text{Ash\%} + \text{Protein\%} + \text{Fat \%} + \text{Fiber\%})$$

3.3.7 Energy Content

The energy content (Kcal/100g) was calculated by addition of multiplied values for crude protein, crude fat, and carbohydrate respectively at Atwater factors (4Kcal, 9Kcal and 4 kcal) then expressed as:

Energy value (Kcal/100g) = (Crudeprotein×4)+(crude fat×9)+(Total Carbohydrate×4)

3.3.8 Mineral Content

Ficus racemosa and *Ficus hispida* powdered samples were digested in acid solution consisted of Nitric acid and Hydrogen perchloric acid into 2:1 ratio and then evaporated in the hot plate until it became transparent. The solution was cooled and filtrated through filter paper into a 100ml standard flask and diluted to the volume with distilled water. This solution was used for mineral content determination. Mineral contents (Sodium, potassium, magnesium, calcium, phosphorus, iron and zinc) were determined by using biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox) was used for biochemical assay. The whole procedure was done in the Postgraduate Research lab under the Dept of Physiology, Biochemistry and Pharmacology at Chittagong Veterinary and Animal Sciences University. All the analyses were done in triplicates and expressed in mg/100g.

3.5 Phytochemical Compounds Analysis

3.5.1 Preparation of methanolic extract

Powdered fruit sample (50g) was weighed into a conical flask and about 50 ml of 95% aqueous methanol was added. The conical flask was placed into a water bath below 40 degrees Celsius with occasional stirring for 2 hours. It was left at room temperature for the next 24 hours. The extract was centrifuged at 3000rpm for 10 minutes and then filtrated through Whatman filter paper No.4. Supernatants were collected and stored for further experimental procedures.

3.5.2 Phytochemical Screening

Phytochemical screening of the methanolic extracts of *F. racemosa* and *F. hispida* fruits were tested for the presence of tannins, saponins and alkaloids following the standard procedures described by Bargah (2015).

3.5.2.1 Test for Tannins

The presence of tannins was determined through Braymer's test. At first 2 ml of extract was mixed with 2 ml of distilled water and then 2-3 drops of 5% ferric chloride solution was added. The formation of green precipitation indicates the presence of tannins.

3.5.2.2 Test for Alkaloids

The presence of alkaloids was detected by Hager's test. About 3ml of extract was taken in test tube and treated with a few drops of Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow color precipitate.

3.5.2.3 Test for Saponins

About 5 ml of extract was taken in a test tube. Equal volume of distilled water is also added and shaken vigorously then the test tube was warmed. The formation of emulsion or stable foam indicates the presence of saponins in the extract.

3.6 Determination of total phenol content

Total phenol content of fruit powder extract was determined by Folin-ciocalteu method as described by (Wojdylo et al., 2007).

3.6.1 Preparation of standard Gallic acid solution

About 10 mg of gallic acid was dissolved into 10ml of distilled water to make the stock solution. The concentration of the solution is 1mg/ml. Different concentrated solutions (2ppm, 4ppm, 8ppm, 16ppm, 32ppm) were prepared through serial dilution of this stock solution.

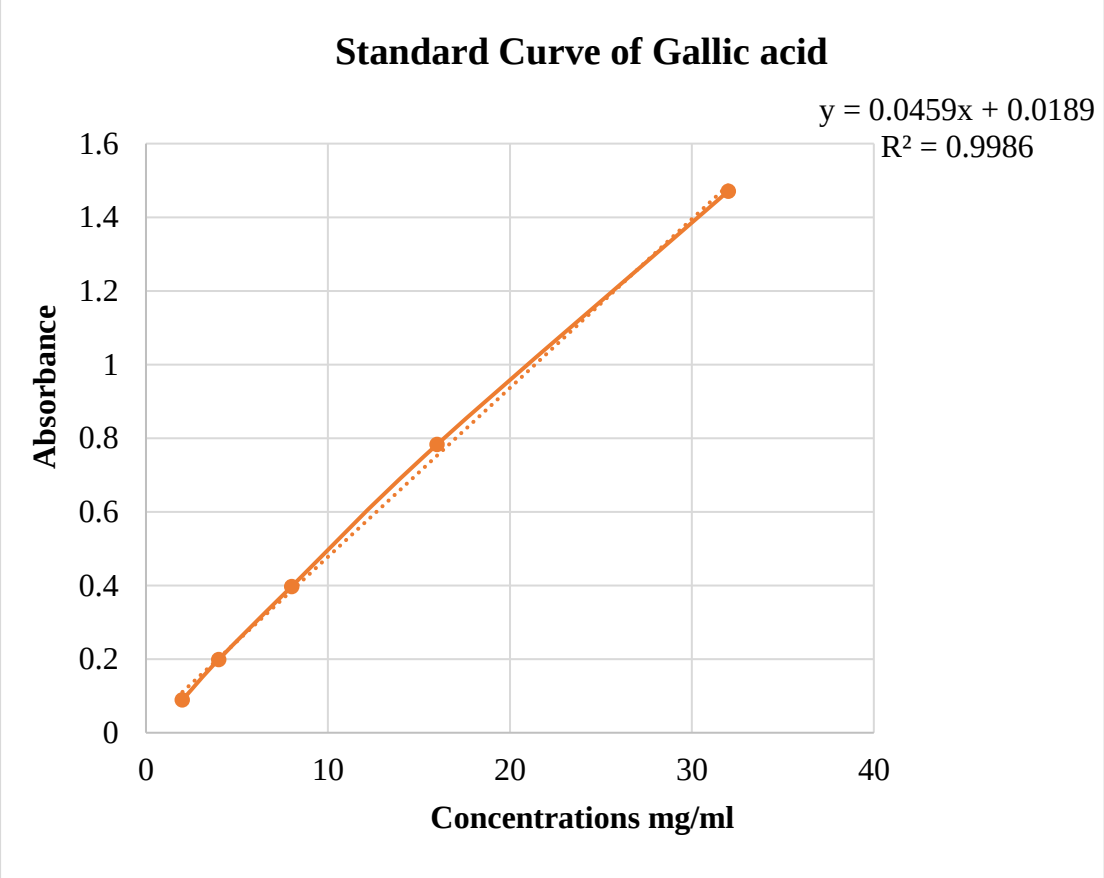


Figure 3.6: **Standard curve of Gallic Acid**

3.6.2 Procedure

1ml of sample extract or standard of different concentrations were mixed with 2 ml of 10times diluted Folin-ciocalteu regent. Then incubated at room temperature for 3 minutes and 10ml of 20% sodium carbonate was added. Then this solution was left for about an hour at room temperature for the incubation process. The absorbance was measured with a Shimadzu UV-VIS-2600 spectrophotometer against the blank

solution. The blank solution contains all the reagents without standard or sample extract. The gallic acid standard curve was used to determine the total phenol content of the sample and expressed as mg of Gallic acid equivalent (GAE) per gm of dried sample. All determinants were performed in triplicate (n=3).

3.7 Total Flavonoid Content

Flavonoid content was measured by the aluminum chloride colorimetric method as described by shah and Hossain (2014).

3.7.1 Preparation of standard Quercetin solution

About 10 mg of quercetin acid was dissolved into 10 ml of water. This is called the stock solution and the concentration of the solution in 1 mg/ml. Different concentrated solutions (6ppm, 12ppm, 24ppm, 48ppm, 96ppm) were prepared through serial dilution method.

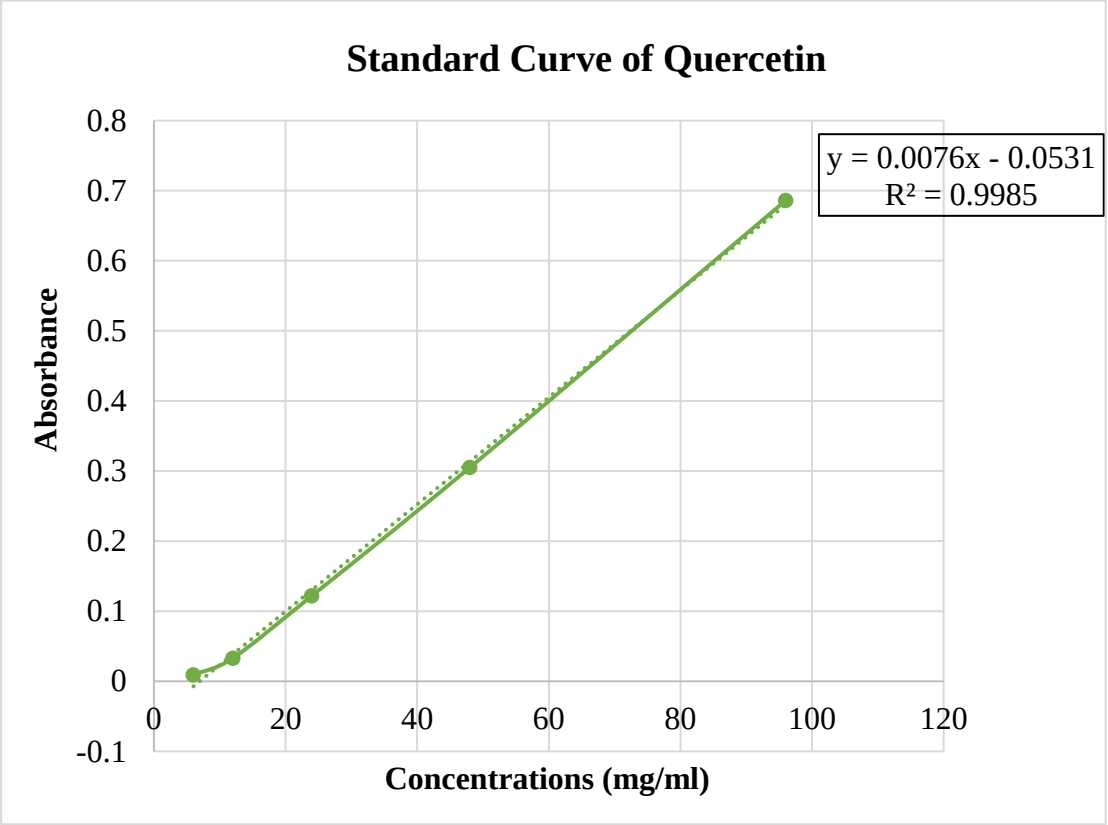


Figure 3.7: Standard curve of Quercetin

3.7.2 Procedure

At first, 0.9815 g of potassium acetate was dissolved in 10 ml water to prepare 1M potassium acetate solution.10% Aluminum chloride solution was prepared by dissolving 1g of alcl3 in 10ml of water. About 1ml of sample or standard at different concentration solution was taken in a test tube. Then 0.2ml of 10% aluminum chloride and 0.2 ml of 1M potassium acetate were added.

After that distilled water was added up to 10m ml volume of solution. The mixture of the solution was incubated at room temperature for 30 minutes to complete the reaction. The absorbance of the mixture was at 420nm against the quercetin standard curve. The result was expressed as mg of quercetin equivalent (QE) per gram of dried weight. All determinations were performed in triplicate (n=3).

3.7 Antioxidant Activity

The antioxidant activity of *F. racemosa* and *F. hispida* and ascorbic acid were determined on the basis of radical scavenging capacity on the DPPH (2, 2- Diphenyl-1- picrylhydrazyl) stable free radical. DPPH radical scavenging abilities of the crude methanolic extracts of the fruit samples were determined by the method described by (Nayira et al., 2013)

Different concentrations (2ppm, 4ppm, 8ppm, 16ppm, 32ppm) of sample extract solution and ascorbic acid were prepared with methanol.1 ml of solutions of different concentrations was made with methanol and taken in a test tube. Then 4ml of DPPH solution was added. The test tubes were kept at room temperature in dark for about an hour. Then the absorbance was read at 517nm in the UV-visible spectrophotometer. Same concentrations of ascorbic acid solutions were also prepared and used as the standard in this method.

The differences between the sample and standard solution were determined and expressed as

% of scavenging of DPPH = $(A_0-A_s)/A_0\times100$

Where A_0 is the absorbance of DPPH alone and A_s is the absorbance of sample or standard with DPPH solution of different concentrations.

3.8 Anti-bacterial Activity

3.8.1 Extraction of the Plant material

i) Aqueous extraction:

6 grams of dried powder were extracted in distilled water at 60℃ temperature in a rotary vacuum evaporator. After 2 hours, it was then filtrated with a filter paper and supernatant was collected and concentrated to make the volume one-fourth of the final volume (Parekh et al., 2005). Then it was kept at 4℃.

ii) Solvent Extraction

The dried plant sample was extracted with diethyl ether according to Parekh et al., 2005 with some modifications.9 gm of the sample was extracted with 100ml of diethyl ether in a solvent extractor. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume. It was stored at 4℃ for further studies.

3.8.2 Microorganism Used

The antibacterial activity of fruit extracts was determined against previously confirmed Pan.susceptible *E. coli* and *Staphylococcus aureus* ATCC strain. In both cases, broad-spectrum antibiotic Ciprofloxacin (5mg) was used as standard. The experiment was conducted in the Department of Microbiology and Veterinary Public Health, CVASU.

3.8.3 Inoculum

The bacteria were cultured into blood agar and incubated at 37℃ for 24 hours. Then an inoculum was prepared by transferring 3 or 4 individual colonies from the blood agar plate to a test tube containing 3ml of phosphate buffer saline. Emulsification was done in vortex machine and the bacterial suspension was adjusted to the turbidity of 0.5 McFarland Standards (equivalent to growth of 1.5×10^{8}CFU/ml)

3.8.4 Culture Media

Muller Hinton Agar was used as the culture media. A sterile cotton swab was used for the inoculation of bacterial culture homogenously on the agar plate. The plate was subjected to inoculation for about 15 minutes.

3.8.5 Determination of Antibacterial Activity

The disk diffusion method was determined by the method of Barry A.L. The extracts were applied to sterile filter paper disc in 250µg and 500µg per disk cautiously and dried to evaporate the remaining solvent. The disc containing extract was placed on agar with sterile forceps. The standard antibiotic disc was also added to each agar plate. The antibacterial activities were determined by measuring the respective zone of inhibition in millimeters.

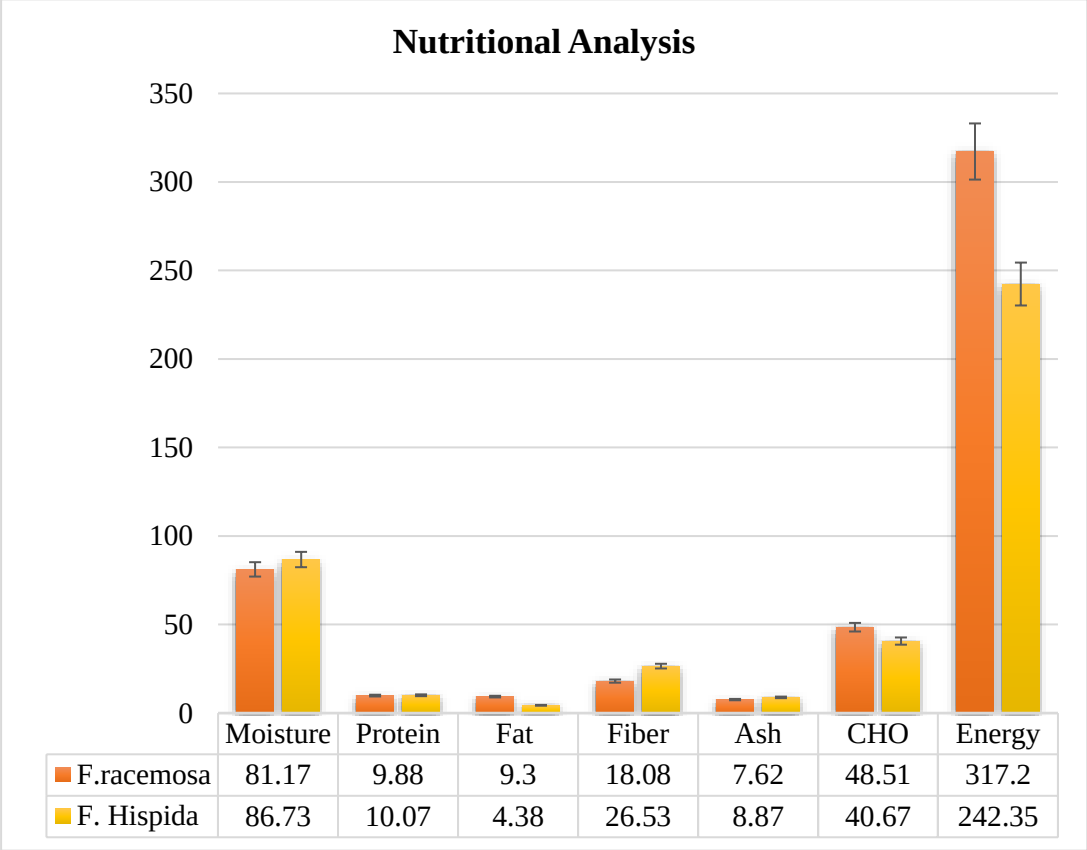
3.9 Statistical Analysis

Experimental data were stored in Microsoft Excel 2007 spreadsheet to evaluate statistical analysis. All the samples were in three replicates. Statistical analysis (mean, standard deviation and level of significance) was done in IBM SPSS 25 by using the independent sample T-test to assess the significant level of variation at 95% confidence interval. The data are presented as mean ± Standard deviations.

Chapter 4: Results

4.1 Proximate Analysis

The results of proximate analysis of *F. racemosa* and *F. hispida* fruits samples are presented in **Figure 4.1**. The moisture content is presented as fresh weight basis and the other parameters (Protein, fat, fiber, ash, carbohydrate) are on dry basis per 100g. The energy content calculated from 100g of dried weight. The results showed the fruits of *F. racemosa* and *F. hispida* are significantly (<0.05) different in terms of moisture, fat, fiber, ash, carbohydrate and energy content. The protein contents of the both fruits ranges from 9.87-10.07 g/100g and the observed values were not significantly (>0.05) different.



	Moisture	Protein	Fat	Fiber	Ash	CHO	Energy
F.racemosa	81.17	9.88	9.3	18.08	7.62	48.51	317.2
F. Hispida	86.73	10.07	4.38	26.53	8.87	40.67	242.35

Figure 4.1: Comparative Nutritional Analysis of *F. racemosa* and *F. hispida* Fruits.

4.2 Mineral Content

It is observed form the mineral content presented in **Table 4.2**, that the fruits of *F. racemosa* contained significantly (<0.001) higher amount of sodium, potassium, zinc and copper in contrast with *F. hispida* sample. On the other hand, Calcium, phosphorus and iron content are significantly (<0.001) higher in *F. hispida* fruit.

Table 4.2 Mineral Content of *F. racemosa* and *F. hispida* Fruits (dry basis)

Minerals		*F. racemosa* (mg/100g)	*F. hispida* (mg/100g)	P value
Macro-minerals	Sodium	144.80±0.20	121.6±0.40	<0.001
	Potassium	1268.33±1.26	1035±0.91	<0.001
	Calcium	280±0.20	760±1.3	<0.001
	Magnesium	150±1.20	210±0.70	<0.001
	Phosphorus	880.20±1.73	970.27±1.28	0.004
Micro-minerals	Iron	64.90±0.10	68.8±0.50	0.002
	Zinc	5.00±0.30	4.19±0.31	<0.001
	Copper	2.52±0.18	1.49±0.21	<0.001

All values are expressed as Mean± SD. The mean difference is significant (P<0.05)

4.3 Phytochemical compounds

Phytochemical screening results of methanolic extracts of *F. racemosa* and *F. hispida* fruits are given in the **Table 4.3.**

Table 4.3: Phytochemical screening test.

Phytochemical test	Extracts of *F. racemosa*	Extracts of *F. hispida*
Tannins	+	+
Alkaloids	+	+
Saponins	+	–

Here," +" indicates presence of the phytochemical

"- "indicates absences of the phytochemical.

4.4 Total Phenol Content present in sample

The ethanolic extract of *F. racemosa* and *F. hispida* fruit samples were subjected to measure total phenolic compound with a reference of gallic acid standard curve. The regression line equation of Gallic acid standard curve is used to determine the total phenolic content present in extracts of both samples. The result calculated on the basis of absorbance then calculated and presented in the **Table 4.4.**

Table 4.4 Total phenol content of F. racemosa and F. hispida Fruits

Species	Sample solution µg/ml	Dry extract gm/ml	Absorbance	GAE conc. C µg/ml	GAE conc. mg/ml	TPC as GAE A= (c×v)/m	Mean	P value
F. racemosa	1000	0.001	0.499	10.45	0.01045	10.45	10.42±0.06	<0.001
	1000	0.001	0.496	10.39	0.01039	10.39		
	1000	0.001	0.497	10.42	0.01042	10.42		
F. hispida	1000	0.001	0.280	5.69	0.00569	5.69	5.74±0.03	
	1000	0.001	0.282	5.73	0.00573	5.73		
	1000	0.001	0.285	5.80	0.00580	5.80		

All values are expressed as Mean± SD. The mean difference is significant ($p<0.05$)

Total phenolic contents available *in F. racemosa* and *F. hispida* were 10.42±0.06 mg GAE/g and 5.74±0.03 mg GAE/gm of dried sample respectively. The fruits of *F. racemosa* contains significantly (<0.05) higher amounts of phenolic compounds.

4.5 Total Flavonoid content present in the sample

The methanolic extract of F. *racemosa* and *F. hispida* fruit samples were subjected to measure total flavonoid content. Quercetin was used as reference standard. The regression line equation of Quercetin standard curve is used to determine the total flavonoid content present in extract of both samples. The result calculated on the basis of absorbance then calculated and presented in the **Table 4.5.**

Table 4.5: The Total Flavonoid content of F. racemosa and F. hispida Fruits

Species	Sample solution µg/ml	Wt. of dry extract gm/ml	Absorbance	QE Conc. µg/ml	QE conc. C mg/ml	TEC as QE A=(c×v)/m	Mean	P Value
F. racemosa	1000	0.001	0.098	19.88	0.01988	19.88	20.05± 0.15	<0.001
	1000	0.001	0.100	20.14	0.02014	20.14		
	1000	0.001	0.100	20.14	0.02014	22.14		
F. hispida	1000	0.001	0.068	15.93	0.01593	15.93	15.97± 0.75	
	1000	0.001	0.068	15.93	0.01593	15.93		
	1000	0.001	0.069	16.06	0.01606	16.06		

All values are expressed as Mean± SD. The mean difference is significant ($p<0.05$). Total flavonoid content present in F. racemosa and F. hispida fruit extracts were 20.05±0.15 mg QE/g and 15.97 mg QE/g respectively.

4.6 Antioxidant Activity

DPPH free radical scavenging assay

DPPH free radical scavenging activity of methanolic extract *F. racemosa* and *F. hispida* fruits are given in the table below. The half inhibition concentration value for ascorbic acid was 9.56µg/ml. The IC_{50} value of F. racemosa and *F. hispida* were 16.80 and 35.83µg/ml respectively. Ascorbic acid is regarded as standard antioxidant. In comparison to ascorbic acid both samples possess antioxidant activity.

Serial No	Concentration µg/ml	%inhibition of Ascorbic acid	% inhibition of *F. racemosa*	% inhibition of *F. hispida*
01	2	27.80	13.75	7.57
02	4	36.78	23.32	17.9
03	8	54.05	44.0	25.67
04	16	69.17	58.44	34.84
05	32	90.44	71.02	42.54
IC_{50}(µg/ml)		9.56	16.80	35.83

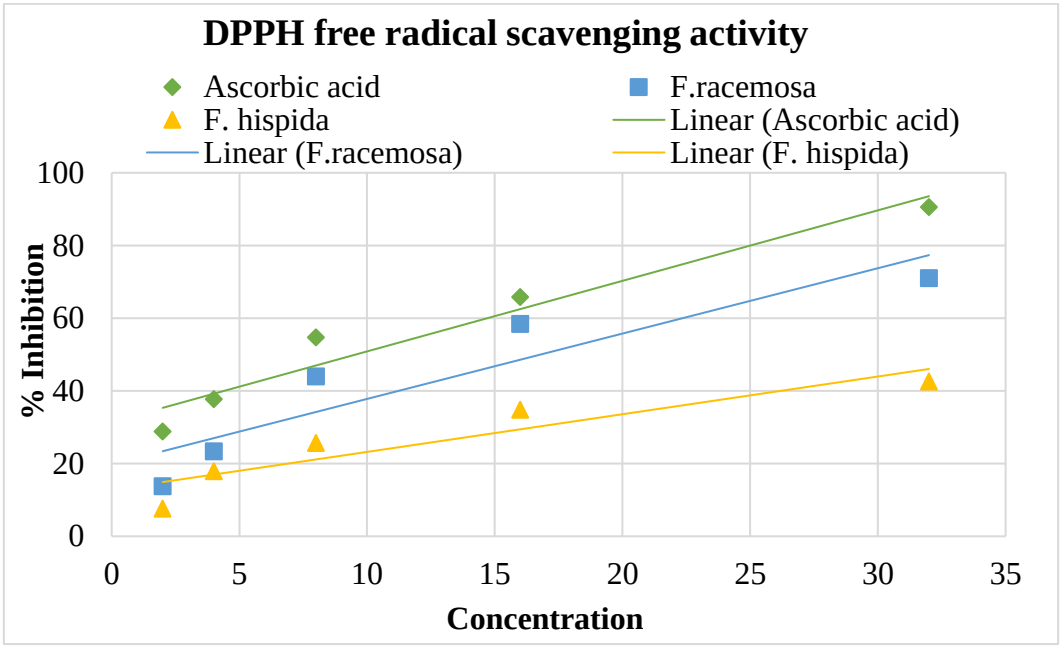


Figure 4.6: Comparative presentation of IC_{50} value of ascorbic acid, *F. racemosa* and *F. hispida* fruit extract

4.7 In vitro Antibacterial Activity

In this study, antibacterial activity of *F. racemosa* and *F. hispida* Fruit extracts was demonstrated against 2 bacterial strains and presented in the figure 4.6. In the study, standard antibiotic ciprofloxacin was used as positive control and black disk with solvent as negative control. The aqueous and diethyl ether extract of both samples were not be able to show any zone of inhibition on bacterial culture media.

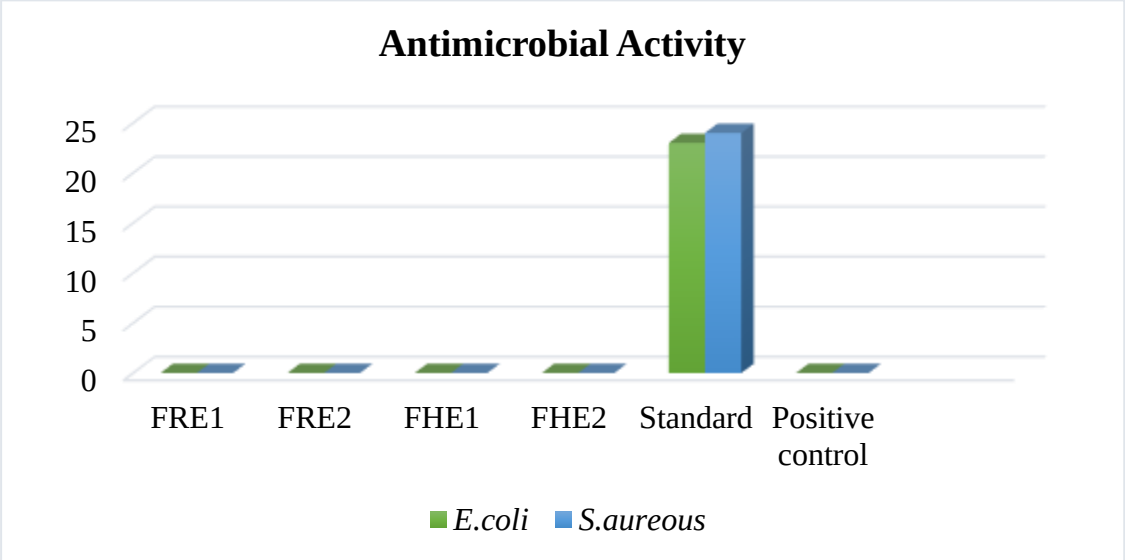
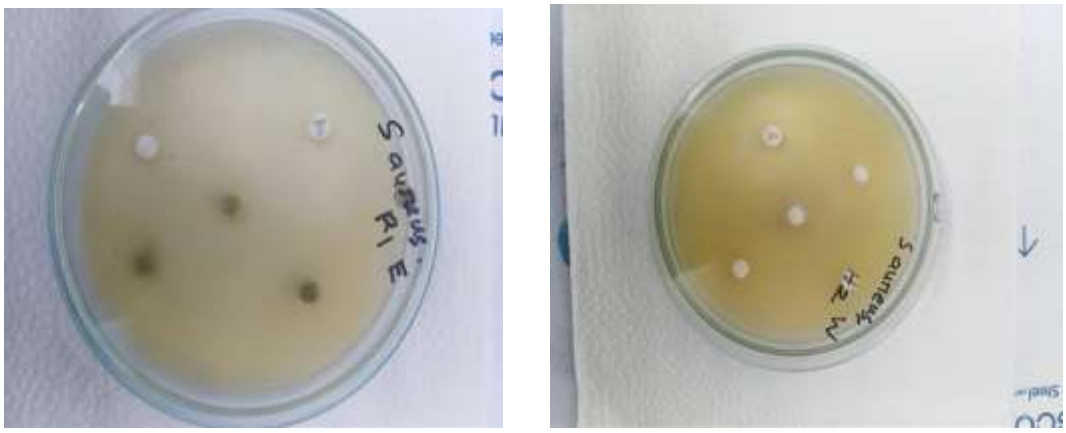


Figure 4.7.1*:* Antibacterial Activity of *F. racemosa* and *F. hispida* Fruit extracts

Here, FRE1= *F. racemosa* diethyl ether extract, FRE2 = *F. racemosa* aqueous extract, FHE1= *F. hispida* diethyl ether extract and FHE2= *F. hispida* aqueous extract.



A **A** **B**

Figure 4.7.2 A) Antibacterial activity of diethyl ether extracts of *F. racemosa* on *Staphylococcus aureus* **B)** Antibacterial activity aqueous extracts of *F. hispida* on *Staphylococcus aureus.*

Chapter 5: Discussions

In the present study, an attempt was made to evaluate the nutritional composition, mineral content, bioactive compounds, antioxidant activity and antimicrobial effects of *F. racemosa* and *F. hispida* fruits.

5.1 Nutritional Composition

5.1.1 Proximate Analysis

In the case of moisture content, *F. hispida* contains a higher moisture percentage than the *F. racemosa* fruits. The moisture content of the most common fruits and vegetables ranged within 70% to 90%. These results were higher than the moisture content of some *Ficus* species listed as wild edible fruits in India (Mishra and Mishra 2016) but close to another study according to Sadia et al. (2013). This difference can be due to the change in climate and soil. As the moisture content of fruit depends on environmental factors including rainfall, moisture percentage and type of soil etc.

Fat in food is considered as the main source of energy, essential fatty acids and vitamins. The crude fat content of *F. racemosa* (9.3g/100g) was about doubled than the fat content (4.38g/100g) *of F. hispida* fruits. The reported fat percentage is higher than wild edible figs and berries range from (1-2.7g/100g) on a dry weight basis (Sadia et al., 2013).

The protein content of *F. racemosa* and *F. hispida* are 9.87g/100g and 10.07 g/100g respectively. That value close to study the conducted for fig and berries (Sadia et al., 2013) ranges 6.5-13.5g/100g and higher than some selected dry food like dates, raisins and figs ranges 2.7-3.93g/100g (Khairuddin et al., 2017).

Fruits are rich sources of fiber which is an important component in preventing overweight, constipation, diabetes, an increase of serum cholesterol, risk of heart diseases, breast and colon cancer, hypertension, etc. (Koca et al., 2015). The crude fiber of *F. hispida* (26.63g/100g) is higher than the fiber content of *F. racemosa* (18.08g/100g) fruits. The result was slightly higher than the other wild edible figs and berries (11-17.81g/100g) reported by Sadia et al. (2013). The Recommended Dietary Allowance of dietary fiber for adult males and females is 38 and 25 g/day, respectively (Trumbo et al., 2008). Fiber is the nutrient of diet that is necessary for digestion and promoting soft stools for effective elimination so, the content of fiber in the fruit used

in our study can encourage their use in the human diet to fulfill the RDA of fiber (Vadivel et al., 2005).

The ash content of *F. racemosa* and *F. hispida* are 7.62g/100g and 8.87g/100g respectively. Ash content indicates the total mineral content of the fruit. The ash contents of both species found higher than the ash content (3.9-4.5g/100g) reported in Sadia et al. (2013). The high amounts of ash indicated that the fruits were rich in minerals. This fruit can provide considerable amounts of mineral elements in our diet.

The most abundant nutrient was found to be the carbohydrate. The carbohydrate content, *F. racemosa (*48.5/100g)> *F. hispida* (40.68g/100g) and the observed value is lower than the fruits of wild edible fruits (69-75g/100g) reported in (Sadia et al., 2013). Lower carbohydrate content and higher fiber facilitate the consumption of fruits for diabetic patients.

5.1.2 Mineral Content:

The mineral content of *F. racemosa* and F. hispida are given in the **Table 4.2**. It has been found that the fruit of *F. racemosa* was rich in potassium, phosphorus and sodium. The values for the K, P and Na increased significantly from 1268.33, 880.20 and 144.80 mg/100g respectively. The most abundant minerals in *F. hispida* fruit were potassium, phosphorus and calcium and values are 1035, 970, and 760 mg/100g respectively. The ratio of sodium and potassium in an important factor and the value of the ratio should be less than one. This ratio is also important for controlling high blood pressure and consumption of less potassium and much sodium increases the prevalence of hypertension (Saupi et al., 2009), (Tanase et al., 2013). In this study, both fruits have this ratio was less than one. So, the consumption of these fruits may be helpful to control high blood pressure. The sodium and potassium contents are higher than the value reported by the study (Sadia et al., 2013).

Calcium is an important mineral for growth, maintenance of teeth, bone, muscle and heart function (Akubugwo, 2007). The calcium content is higher in *F. hispida* (760mg/100g) than the fruits of *F. racemosa* (280mg/100g). However, the amounts of Ca are lower than fig and mulberry fruits (1094-466 mg/100g) reported by Sadia et al. 2013. The concentration of minerals depends on agricultural factors.

Magnesium plays an important role in the human body as a cofactor of many enzymes, protein, RNA and DNA synthesis in human body. The magnesium content analyzed in this study was higher in *F. hispida* (210 mg) than the *F. racemosa* (150mg) per 100g dry weight.

Iron is important for the formation of hemoglobin, normal functioning of the central nervous system (kaya and Incekara, 2000). The recommended average dietary allowance of iron is 400g/day (Gupta CP, 2014). The iron content analyzed in the study ranges from 64.8 to 68.90g/100g. According to Sadia et al. (2013) the iron content of wild fig and mulberry ranges 47-82 mg/100 g and the observed values are within the range.

It has been shown that *F. racemosa* and *F. hispida* also provides some trace minerals like Zn and Cu which are beneficial for our body. The value of Zn and Cu in *F. racemosa* and *F. hispida* are 5, 4.19 mg/100g and 2.52 and 1.49 mg/100g respectively. The ranges of Zn and Cu in fruits of Ficus groups are 5-6mg/100 gm and 3-4 mg/100mg respectively. (Sadia et al., 2013). The experimental values are close to that range.

So that these fruits are thought to be used as food materials useful in human health

5.2 Phytochemical Compounds

In the previous studies (Sen and Chowdhary, 2006; Londonkar et al., 2013) it is reported that the presence of alkaloids, tannins and saponins in *F. racemosa* fruit extracts. The presence of phytochemical in this study corelates with the above reports.

According to a study conducted by Islam et al. (2018) phytochemical screening confirmed the presence of alkaloids and tannins and absence of saponin in F. hispida extracts. The present study also agreed to this.

Total phenolic contents of *F. racemosa* and *F. hispida* fruit extracts were 10.42±0.06 and 5.74±0.03 mg GAE/gm respectively. The phenolic compounds present in the extract may contribute to antioxidant activity. They have potential antioxidant properties due to the presence of the hydroxyl group. The phenolic content of *F. racemosa* is higher than walnut and raisins. However, *F. hispida* contains lower phenolic content than *F. racemosa* but it still higher than reported in dates and dried figs (1.51-5.6 mg GAE/gm) according to khairuddin et al. (2017).

Flavonoids are the natural compounds plays important role in antioxidant activity due to their inhibition of hydrolytic and oxidative enzymes. Total flavonoid content present in *F. racemosa* and *F. hispida* fruit extracts were 20.05±0.15 and 15.97 mg QE/g respectively**.** The total phenolic content and flavonoid content of methanolic extract of *F. racemosa* fruit is 26.2 mg GAE/g and 10.63 mg QE/g according to the study reported by Sumi et al.,2016. The value of phenolic content is higher and the flavonoid content is lower than the value reported in this study. The recovery of polyphenol from plant particles depend on the solubility of phenolic compound in the solvent used for the extraction process. Furthermore, it is hard to develop a standard extraction procedure for all fruit samples (Naczk and Shahidi, 2006).

5.3 Antioxidant Activity

The model method of scavenging the stable DPPH radical is usually used to analyze the radical scavenging ability of various samples (Koleya et al., 2001) In terms of antioxidant activity, the present study suggests that the extracts of *F. racemosa* and *F. hispida* fruits have potential antioxidant activity with the IC_{50} 16.80µg/ml and 35.83µg/ml respectively *(F. racemosa>F. hispida*), where the value of standard ascorbic acid 9.56µg/ml. According to Begum et al. (2016) the antioxidant activity is slightly higher in *F. hispida* fruits with the IC_{50} value of 28.39µg/ml. The IC_{50} value of the methanolic extract of *F. racemosa* fruits was reported 8.59µg/ml with the standard ascorbic acid value of 4.15µg/ml. (Sumi. et al., 2016). The data obtained from bioactive compounds and antioxidant assay revealed that the extracts are free radical inhibitor and act as primary antioxidant.

5.4 Antibacterial Activity

From the present investigation, it is observed that, strains of *Escherichia coli* and *Staphylococcus aureus* were found to be resistant they do not have any effect against any of the extract. But both strains are inhibited by the standard antibiotic. Antimicrobial activity diethyl ether extract was previously not reported. According to a study, different solvent extracts of F. racemosa fruits showed zone of inhibition against 8 bacterial strains including *E. coli* and *S. aureus* in different concentration. In the study methanol, ethanol and petroleum ether extract showed comparatively higher antimicrobial activity (Hasan et al., 2017). In another study, petroleum ether extract was not showing any inhibitory effect against bacterial strains but benzene, chloroform

and aqueous extracts at 500 and 1000 ug/ml concentrations have shown antibacterial activity (Sandeep et al., 2013).

A similar study reported that the ethanol extracts of *F. hispida* leaves were able to show antibacterial activity against some gram-positive and gram-negative bacteria including *S. aureus* but the organisms were not sensitive to fruit extract. *Escherichia coli* were not sensitive to the extracts of leaves of *F. hispida* (Begum et al., 2016)

It is described that the antibacterial activity of plant extract depends on the degree of efficiency and different phytoconstituents of herb on the target organism. According to literature, *F. racemosa* and *F. hispida* have shown some antibacterial effect. But in this case, both fruit extracts were not able to show inhibitory activity against *E. coli* and *S. aureus*. The reason behind it can be the use of different types of solvent and method and the efficacy of the extraction procedures.

Chapter 6: Conclusion

The wild fruits represent a versatile agro biodiversity system and act as supplemental food and micronutrient source, which is inexpensive and accessible and a safety net for poor forest dwellers and a means to earn income. This study indicates that these fruits are a great source of nutrients and provide good amounts of energy. Both fruits are rich in dietary fiber and essential minerals. This can be considered as an alternative food source containing macro and micronutrients necessary for the human body. The present research also provides evidence that *F. racemosa* and *F. hispida* contains phytochemicals like alkaloids and tannins, phenolic compounds and flavonoids and saponins. These compounds contribute to the potential antioxidant activity of fruits and make them pharmacologically active. To reduce the risk of diseases caused by free radicals, the consumption of antioxidant-rich fruits and vegetables is recommended. The medicinal value of the fruits of both plants may be due to the presence of phytochemical constituents.

Chapter 7: Recommendation & future perspectives

Now a day's people are more interested in the alternative food source and plant-based medicine. These fruits grow everywhere in Bangladesh without much effort. Considering the nutritional factors this can be a low-cost source of nutrients for rural people in our country.

Future work should involve the investigation of essential vitamins and other bioactive compounds. *F. racemosa* and *F. hispida* fruit powder can be incorporated into different food products. High fiber and mineral content suggest that it has potential food value and could be recommended as a functional food ingredient. It can be regarded as a food with natural antioxidants. Further qualitative and quantitative phytochemical analysis is recommended. Fruits of both species and other parts of the plants are traditionally used to cure various diseases. Efficient methods of extraction and use of different types of solvents for extraction are recommended to evaluate the antibacterial effect. Further studies are needed to isolate and identify the active compounds and their mechanism in various disease conditions. However, studies are required in the animal model and subsequently on the human subject to prove the efficiency in curing disease conditions. This will help for the development of new medicine from plant sources and will be safe for the treatment of various diseases.

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Appendix 1: Photo Gallery

Figure: Digestion of Samples

Figure: Analysis of Mineral Content

Figure: Absorption of paper discs with fruits extracts

Brief Biography

Fariab Hasan passed Secondary School certificate examination with grade point average (GPA) 5.00 in 2009 and Higher Secondary Certificate Examination with GPA 5.00 in 2012. Fariab Hasan received The B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram (CVASU) in 2017. Now she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. She has an immense interest in exploration on clinical nutrition and dietetics to improve the health of people through proper guidelines and suggestions with a vision of improving the overall nutritional status of Bangladesh.