COMPARATIVE EVALUATION OF NUTRITIONAL COMPONENTS, BIOACTIVE PROPERTIES AND ANTIBACTERIAL ACTIVITY OF PAPAYA (*CARICA PAPAYA L.*) SEED AT TWO STAGES OF RIPENING

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Roll no: 0121/05

Registration No: 987

Session: January –June /2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

JULY 2023

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

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The Author

JULY, 2023

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Abbreviation

%	**: Percentage**
ANOVA	**: Analysis of variance**
AOAC	**: Association of Official Analytical Chemists**
°C	**: Degree Celcius**
dl	**: Deciliter**
DPPH	**: 2,2-diphenyl-1-picrylhydrazyl**
TFC	**: Total flavonoid content**
TPC	**: Total phenolic content**
et al	**: Et alii/ et aliae/ et alia**
etc	**: Et cetera**
PS	**: Papaya seed**
RPS	**: Raw papaya seed**
MPS	**: Mature papaya seed**
g	**: Gram**
ppm	**: Parts per million**
TE	**: Trolox equivalent**
QE	**: Quercetin equivalents**
GAE	**: Gallic acid equivalent**
µg	**: Microgram**
mg	**: Miligram**
QE	**: Quercetin equivalents**
SD	**: Standard deviation**
Cfu	**: Colony forming unit**

Abstract

The use of papaya including the parts usually discarded during processing, is an alternative to reduce the quantity of agro-industrial wastes. Due to their abundance of bioactive secondary metabolites, seeds have recently been the subject of further research using them as potential sources of medicines. The study reprsents comparison of quantitative estimates of nutritional component and evaluated the antioxidant and antibacterial qualities of raw and mature Carica papaya seeds.It has highlighted that mature seed contains highest levels of total polyphenol(5.14±0.009mg GAE/100 gm), total flavonoid(30.65±0.101mg QE/100gm), Vitamin A (8.06±0.03 mg/100g), protein (27.33±0.03%), fat (28.13±0.04%), fibre (20.92±0.03), potassium (23.56±0.01 mg/dl), iron (146.11± 0.02µg/dl) and phosphorus (7.22 ± 0.04). On the other hand raw seed contains highest level of carbohydrate (24.18±0.13%), moisture (8.84±0.03%), ash (10.17±0.01%), vitamin C (5.01±0.01 mg/100g), calcium (15.45± 0.07 mg/dl), zinc (9.03±0.03 mg/dl). The free radical scavenging activity was highest shown by mature seeds extract at 6.64±0.002 %; however, in vitro antimicrobial activity, mature seed showed highest zone of inhibition compared to raw seed. The results of the current investigation showed that both raw and mature seed had excellent nutritional characteristics. Consequently, papaya seed can be used in the preparation of foods for human consumption as well as for industrial purposes. The general conclusion recognized that the wasted seeds may be more cost-effectively utilized to treat oxidative stress-related illnesses and as a viable therapeutic food source.

Keywords: Papaya Seed, Bioactive compounds, Antioxidant, Antibacterial activity.

Chapter I: Introduction

The 'lungs' of our beautiful world are plants, the greatest invention of Almighty God. Plants are similar to the 'treasure box' hidden with a lot of active components that are crucial for the process of generating new drugs. In addition to being a cheap and accessible source, medicinal plants are also capable of synthesizing a variety of active chemicals that are useful in preventing and treating a wide range of illnesses. Secondary metabolite including phenols, tannins, alkaloids, flavonoids, saponins and carbohydrate are the name given to these active substances.

Plant parts such as leaf, bark, root, flower, seed, and stem can be extracted or decocted for medical purposes. One of the medicinal plants that has contributed as a treatment for a number of disorders is Carica papaya.

Fruit processing produces a lot of waste materials, including peels, seeds, stones, and oilseed meal. Legal constraints often make it difficult to dispose of these items, which is a problem in and of itself. Because these are high-value goods and their recovery may be commercially viable, new components linked to the utilisation of these wastes as by-products for further investigation on the manufacture of food additives or supplements with high nutritional content have drawn considerable interest. It is generally known that by-products are a significant source of sugars, minerals, organic acids, dietary fiber, and phenolics, which are known to offer a variety of health benefits, including anticancer, antiviral, antibacterial, cardioprotective, and antimutagenic effects.

One of the most well-known fruit trees in the genus Carica is the papaya (Carica papaya), which is commonly cultivated in tropical regions. The papaya's fresh flesh is primarily consumed, while its industrial uses primarily involve the production of sweets, jam, jelly, and pickles (Chavez-Quintal et al.,2011). The papaya fruit seeds, which make up over 20% of the fruit's weight (Chielle et al., 2016) may prove to be advantageous in the future due to their nutritive and functional component. Additionally, due to their spicy and pungent flavor, the seeds can occasionally be used in place of black pepper (Karapanagiotis et al., 2012). Due to its amazing healing properties, papaya flesh has long been used as a traditional therapeutic medicine. The seeds also have a variety of known nutritional and health advantages. Numerous phytochemicals, particularly carotenoids and polyphenols, are present in the fruit (Gayosso-Garcia et al., 2011). The antioxidant found in papaya peel and seeds

has the potential to play a significant role in the production of functional foods and nutraceuticals soon (Marchiani et al.,2011). Despite its significance, there is little information available about this largely underutilized seed. Therefore, papaya seeds which make up about 30–35% of the fruit waste are typically thrown away (Samaram et al.,2013). Papaya seeds have not yet been used commercially, despite the fact that they contain 19.1%–22.6% crude fibers, 28.2%–30.7% lipids, and 27.3%–28.3% protein.

The prevalence of papain and lipase in all organs has received a lot of attention, and some researchers believe that these two enzymes are responsible for some of the functions of papaya stated above (Varca et al., 2007) . The antioxidant activity of various secondary metabolites in the papaya organs was linked to some of the fruit's activities. (Theppakorn et al.,2004) The papaya water extract fraction was found to have the highest anti-free radical effects in early research on the DPPH, hydroxyl, and superoxide free radical-scavenging activities of certain tropical fruits (Osato et al., 1993) . Since the antioxidant activities of the other papaya seed extract fractions have not yet been investigated, it was important to do so in order to assess the prospective applications of these extracts.

Due to factors primarily related to poor hygiene, human intestinal parasitosis is a significant global health issue with significant financial implications (Awasthi and Pande ,1997) The burden is greatest in the tropics and subtropics where, particularly in children, parasites cause notable morbidity like anemia, diarrhea, and dysentery, malnutrition, apathy, and underdevelopment as well as severe acute abdominal and surgical conditions (Ene-Obong et al., 2000) The necessity for affordable and accessible local alternatives cannot be overstated because the majority of patients, particularly those from the tropics and particularly from Africa, are from low socioeconomic groups and struggle to purchase imported and expensive treatments.

According to several studies, Carica papaya seeds have proven to be an efficient anthelmintic against nematodes that are prevalent in animals (Chota A et al., 2010) . The anti-fertility, anti-implantation, and abortifacient effects of papaya seed extracts were demonstrated by Chinoy et al. in 2006 . The seeds of C. papaya have been proven to be promising anti-fertility medications in males (Lohiya et al., 2005).The Hausa word "daddawa," which means a fermented food condiment, is used to describe a native

Nigerian food condiment made from pawpaw seeds (Dakare, 2004). Rat litters are not affected by fermented seeds (Abdulazeez et al., 2009) , whereas unfermented extract had noticeable impacts (Abdulazeez et al., 2009).

When fruit is processed, almost all of the seeds are produced as lees and discarded as a horticultural by-product, posing environmental problems.In light of the aforementioned information, using papaya seeds to make new products is a viable alternative because it might raise the value of a byproduct, provide new ingredients for the food sector, and lessen the amount of agro-industrial waste that needs to be disposed of.

Significance of this study

In our country very limited study has been conducted on papaya seed. In several cultures, papaya seeds have a long history of usage in traditional medicine. They are thought to have a number of health advantages, including as antibacterial, antifungal, and anti-inflammatory qualities. These conventional claims can be supported scientifically, and analysis of the nutritional profile and phenolic content , antibacterial activity can assist identify any potential health advantages of papaya seed ingestion.It can also help to understand the differences in the health benefits of raw and mature papaya seeds.Analyzing the phenolic content and nutritional composition of papaya seeds can be beneficial for farming and plant breeding initiatives. It helps in choosing superior cultivars with appropriate phenolic and nutritional characteristics. This information can help in the creation of papaya cultivars with improved nutritional value and wellness-enhancing qualities. It can help in deciding which kind of papaya seeds to use for particular purposes. For example, depending on their nutritional composition and antibacterial activity, raw or mature papaya seeds may be the better option if we are seeking for a nutrient-rich food source or an antibacterial supplement.Overall, analyzing the nutritional profile and phenolic content of papaya *(Carica papaya L.)* seeds is important for comprehending their potential health benefits, assisting the food and nutraceutical industries, promoting agricultural practices, and further investigating their use in various applications related to human health and well-being.

Objective

- To evaluate nutritional composition, bioactive properties of both raw (green) and mature (ripe) papaya seed
- To analyze antioxidant properties of raw and mature papaya seed
- To investigate the antibacterial effect of ethanolic papaya seed (raw and mature) extracts against *E.coli* and *Staphylococcus aureus*

Chapter II: Review of Literature

2.1 Overview of papaya

A perennial fruit crop in the Caricaceae family is the papaya (*Carica papaya L.*). Since the sixteenth century, papaya has been introduced to India from its native Latin America (Mexico). Presently, Mexico comes in third, followed by Brazil and India as the top two producers. According to AtlasBig.com, Bangladesh is ranked fifteenth. Due to varying agro-climatic conditions, various crops are grown in various areas of Bangladesh. One of the many fruits that are grown around the nation is the papaya, which is also one of the most significant. The fruits range in size from medium to large (1-2 kilogram) and the plants are medium in height (180–220 cm) (BARI, 2014). Excellent quality and wonderful taste are both present. While unripe papaya fruits are frequently used as cooking vegetables, ripe fruits are consumed throughout the tropics. Papaya is a crop with several applications. Other products made from unripe papaya include salty pickles, jam, candy, nectar, puree, concentrate, slab, powder, toffee, and freeze-dried chunks, rolls, and slices (Trejo-Gonzalez and Cantwell, 2022). While completely developed but still somewhat raw fruits are used to make a perfect jelly, raw papayas can be used to make salted pickles.

2.2 Distribution and habitat

Papaya is a fruit that is indigenous to tropical America and comes from southern Mexico and Central America. Papaya was introduced to southern Florida by people who lived there before the Calusa no later than 300 CE (Morton and Julia, 1987). In the 16th century, Spaniards brought papaya to the Old World. Hawaii, Australia, India, and central Africa are currently among the countries where papaya is grown. Papaya wild populations are mostly restricted to tropical woods that have been naturally disturbed (Fay and Michael , 2007). Papaya is sporadically seen on Everglades hammocks, but it is common after large hurricanes (Brown et al., 2012) Papaya flourishes and spreads swiftly in canopy gaps in southern Mexico's rain forests, but it declines in mature closed-canopy forests.

2.3 Taxonomy of papaya

Table 2.1 The taxonomy of papaya is as follows according to ITIS (Integrated Taxonomy Information System)

Domain:	**Flowering plant**
Kingdom:	Plantae
Sub Kingdom:	Tracheobionta
Class:	Magnoliopsida
Subclass:	Dilleniidae
Super division:	Spermatophyta
Phylum:	Steptophyta
Order:	Brassicales
Family:	Caricaceae
Genus:	Carica
Speices:	*Carica papaya*

2.4 Description of papaya seed

Fruits that have had sufficient pollination may have 600 or more black seeds. The embryo is straight and spherical, and the cotyledons are flattened (Fisher, 1980). According to a research, a mucilaginous substance that coats the seeds is produced by the pluristratified epidermis of the outer integument (Roth and Ingrid , 1977). The embryo is enclosed in a gelatinous sarcotesta when it reaches physiologic maturity. A compact mesotesta as well as the exterior and inner integuments are visible underneath. The endosperm is composed of thin-walled cells with multiple oil bodies and aleurone grains that are starch-free at maturity (Fisher, 1980; Teixeira da Silva et al. 2007). Although mature photosensitive wild papaya seeds are dormant, changes in light

conditions brought on by the establishment of forest gaps may force them to sprout. (Paz and Vazquez-Yanes 1998).

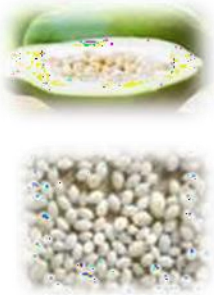

Fig 2.1: Raw papaya seed (Green)

Source:https://www.veggovilla.com/img/product img/raw_papaya

Fig 2.2 : Mature papaya seed (Ripe)

Source:https://exoticfruits.co.uk/cdn/shop/ products/papaya-seeds

2.5 Nutritional properties of papaya seed

It is essential to have complete knowledge of its chemical composition in order to get the most out of it and utilize PSs to the fullest extent possible. In a publication on the composition of PSs published by (Chan et al., 1978) defatted PSs were used for observation. The results indicated that PSs contain minerals such P, K, Ca, Mg, S, Mn, Fe, and Cu as well as 32.97% oil content, 40% crude protein, 48.9% crude fiber, 6.86% ash content, and 1.11% fatty acids. Oleic acid, which was present in high concentrations (71%), was a significant fatty acid, and several toxins were also found. The percentages of benzyl-ITC and benzyl glucosinolate in seed meal and oil, respectively, were 0.56% and 1.86% (Chan et al., 1978). Defatted and undefatted PSs were collected for observation in a different investigation by (Marfo et al., 1986). The findings approximated the composition of PSs, with undefatted PSs containing 27.8% crude protein, 28.3% lipids, 22.6% crude fiber, and 3.5% ash content and defatted PSs including 44.4% crude protein, 31.8% crude fiber, and 4.48% ash content. 0.94 percent of the mixture also contained fatty acids and minerals such P, K, Ca, Mg, S, Mn, Fe, Cu, Ni, Co, and Na. Oleic acid, one of the primary fatty acids, was present in large concentrations (79.1%), and benzyl glucosinolate (10%) was the toxin with the highest percentage of presence. This investigation found that sucrose was present in the highest proportion of all sugars, and that carotene and monosaccharide were also present but in trace amounts (Marfo et al., 1986). Investigation of the composition of papaya seed oil (PSO) found that it includes significant amounts of total phenolic and carotinoids as well as monosaturated fats and tocopherols (Malacrida et al., 2011).

Oleic, palmitic, linolenic, and stearic acids were the predominant fatty acids, and tocopherols were the key tocopherols, and cryptoxanthin was the major carotenoids (Malacrida et al.,2011) . In light of these studies, it is reasonable to conclude that PSs may be a source of crude protein, carbs, fatty acids, lipids, fiber, calcium, and phosphorus as well as some toxins.

2.6 Bioactive components of papaya seed

2.6.1 Flavonoids

Flavonoids, a group of chemical compounds with various phenolic structures, are found in a wide variety of foods, including fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine (Kumar and Pandey ,2013). Since these natural substances have many positive health effects, attempts are being conducted to isolate the so-called flavonoids from the other components. Flavonoids can be divided into numerous subgroups based on the carbon atom of the C ring that the B ring is connected to, as well as the degree of unsaturation and oxidation of the C ring. Flavonoids known as isoflavones have a B ring joined to the third position of the C ring. Neoflavonoids are compounds in which the B ring is linked in position 4; compounds in which the B ring is linked in position 2 can be further divided into several subgroups based on the structural properties of the C ring. Chalcones, anthocyanins, flavones, flavonols, flavanones, and flavanonols are some of these subclasses (Metodiewa et al., 1997).

Because they are associated with a wide range of health-promoting properties, flavonoids are crucial in a wide range of nutraceutical, pharmacological, medicinal, and cosmetic purposes. This is as a result of their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties as well as their capacity to affect crucial cellular enzyme activities. They are also known to successfully block a number of other enzymes, such as cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase (Hayashi et al., 1988).

2.6.2 Phenolic compounds

Phenolic molecules are the most widely dispersed secondary metabolites and can be found across the plant kingdom, albeit the exact variety that is present differs depending on the phylum under investigation. Around 40% of the organic carbon that circulates in the biosphere is produced biosynthetically by the malonate/acetate system, often referred to as the polyketide pathway or the shikimic acid pathway. Phenolic chemicals are produced by this system (Chapman and Ragan, 1980). Simple phenols and phenolic acids, derivatives of hydroxycinnamic acid, and flavonoids are the three primary groups of phenols found in food products (Ho et al., 1992). There is scientific proof that plant phenolics help protect against a number of chronic illnesses associated to oxidative stress, such as cancer, cardiovascular issues, and neurological issues (Dai and Mumper, 2010).

2.6.3 Antioxidants

Antioxidants are compounds that, when added to food, prevent, slow down, or stop oxidation and the degradation of food quality. Compounds known as antioxidants prevent oxidation. Antioxidants are commonly used as a component in dietary supplements with the purpose of promoting health and avoiding diseases like cancer and cardiovascular disease. Additionally, they are used as food preservatives. In actuality, this took place in the middle of the 20th century. It all started with scientists' attempts to lengthen food's shelf life. In experiments, adding antioxidants with meals that had a lot of unsaturated fat avoided rancidity, an unpleasant process in which unsaturated fats break down and produce a rancid-like smell and taste ((Baillie et al., 2009) .

Functions of antioxidants

In order for plant-based diets to have protective effects, antioxidants are essential. Consuming fruits and vegetables regularly lessens the chance of getting chronic diseases, according to studies (Dembinska-Kiec et al., 2008). An antioxidant-rich diet has been shown to offer long-term health benefits (Sin et al., 2013). Free radicals are currently connected by antioxidants to cancer prevention, preventing cell damage, and extending life (Kalcher et al., 2009). The antioxidant system, which is in charge of

defending against the negative effects of free radicals and the hazardous consequences of their metabolism, is how all antioxidants work. These include the lipidation of antioxidants, the addition of hydrogen and electrons, and the subsequent formation of an antioxidant-lipid complex.

Mechanisms

If a material stops the generation of free alkyl radicals during the initiation stage or stops the chain of free radicals from spreading, lipid oxidation can begin later or progress chemically more slowly. Free radical formation can be delayed by using peroxide stabilisers, singlet oxygen inhibitors, and metal chelating agents. Antioxidants and metal chelating substances can stop the free radical chain reaction from spreading by giving hydrogen (Brewer, 2011).

(1)R:H + O::O + Initiator → R• + HOO•

(2)R• + O::O → ROO•

(3)ROO• + R:H → ROOH + R•

(4)RO:OH → RO• + HO•

(5) R::R + •OH → R:R-O•

(6) R• + R• → R:R

(7)R• + ROO• → ROOR

(8)ROO• + ROO• → ROOR + O_2

(9)ROO• + AH → ROOH + A• (10)ROO• + A• → ROOA.

2.7 Antibacterial effect

Remarkable antimicrobial property has been demonstrated by papaya seed extracts, which makes them highly helpful in the search of new drug. Papaya seed extracts have been found to have antibacterial effects against a variety of gram positive and gram-negative bacteria (Tang et al., 1972). Such plant-based antibiotics effectively treat infectious infections while also removing most of the the unfavorable side effects connected to antibiotics generated synthetically.

In comparison to the water extract, the ethanol extract demonstrated greater antibacterial activity against the test organisms. The ethanolic extract's zone of inhibition for Staphylococcus aureus was 11.0 mm at a dosage of 100 mg/ml. With a zone of inhibition of 2.9mm and a susceptibility to the ethanol extract of 25 mg/ml, Salmonella typhi was the least susceptible (Lonkala and Reddy,2019). The test organisms were more sensitive to chloramphenicol, a common antibiotic, than to seed extract (12.2-13.2mm). The ability to get alternative antibiotic compounds from Carica papayaseeds for the creation of novel and potent antibacterial drugs is suggested by the demonstration of antibacterial activity against the test isolates.

2.8 Benefit of consuming papaya seed

According to (Pawar and Kothawade, 2022) there are several benefits of papaya seed

- **Smooth muscle action :** At concentrations ranging from 0.1 to 6.4 mg/ml, papaya seed ethanol extract effectively and irreversibly reduced jejunum contraction.As a result, the extract may lessen the isolated rubbit jejunum's capacity to contract.
- **Support for weight loss:** Papaya seeds is rich in fiber. They promote a healthy digestive system, which helps our body get rid of pollutants. They also help to control our metabolism and prevent our bodies from absorbing fat.
- **Anti-cancer properties:** Strong antioxidants called polyphenols are found in papaya seeds. they protect our body against a variety of cancers. A study that was published in the journal Cancer Epidemiology and Prevention Biomarkers reveals that eating a diet rich in beta-carotene may help prevent prostate cancer in younger men. Beta- carotene is an antioxidant that is found in papayas and may reduce the risk of acquiring cancer.
- **Lower levels of cholesterol:** Oleic acid and other monounsaturated fatty acids are abundant in papaya seeds. These fatty acids regulate blood cholesterol levels (LDL cholesterol) by reducing harmful cholesterol. Papaya seeds are rich in fiber. With the help of fiber, the body's cholesterol levels are lowered. By consuming papaya seeds, we can maintain healthy cholesterol levels in our body.

- **Diabetes:** Studies have shown that a high-fiber diet lowers blood glucose levels in people with type 1 diabetes, and it may help improve lipid, insulin, and blood sugar levels in those with type 2 diabetes. One small papaya contains roughly 3 grams of fiber or about 17 grams of carbs.
- **Heart diseases:** the high quantities of fiber, potassium, and vitamins in papaya all work to prevent heart disease. The most crucial dietary modification a person can do to lower their risk of cardiovascular disease is to increase their potassium intake while reducing their salt intake.
- **Papaya seed promotes the growth of hair:** Papaya seeds contains vitamin A, which helps to control frizzy, dry hair. To prepare a hair mask, papaya seeds can be dried, crushed, and blended with honey. Studies indicate that a shortage of protein in the body may cause hair thinning and loss. Papaya seeds promote the growth of hair since they are rich in protein and folic acid.
- **Anti-aging:** Antioxidants are essential for reducing wrinkles and fine lines on our skin. Papaya seeds contain lycopene and other antioxidants that help maintain youthful skin.
- **Acne control:** Papain, an enzyme found in papaya seeds, helps to reduce acne inflammation. Papaya seeds are also a strong source of vitamin A, which aids in the treatment of acne.
- **Treats Pigmentation:** Papaya oil, which is rich in vitamins and fatty acids, can be applied to the skin to lighten pigmentation. Papaya seeds can be used to lighten skin.
- **Reduced inflammation:** It has been demonstrated that papaya seeds are effective at reducing inflammation. Payaya seeds are rich in vitamin C and other nutrients such alkaloids, flavonoids, and polyphenols. All of these drugs have an antiinflammatory effect. As a result, they aid in the prevention and treatment of inflammation in a variety of illnesses, such as gout, arthritis, and others.

2.9 Side effects of papaya seed

Having too much when pregnant could harm the growing fetus. Consuming excessive amounts of papaya seeds might lower a man's sperm count, which can lower fertility (Ghaffarilaleh et al., 2019). If people consume too many papaya seeds, they could get

diarrhea. For breastfeeding mothers, consuming an excessive amount of papaya seeds is not recommended.The benzyl glucosinolate included in papaya seeds can result in food poisoning if consumed in excess (Okeniyi et al., 2002)When chopping the fruit, care should be taken to prevent papaya latex from coming into touch with skin.Papaya latex, an enzyme, can make the skin feel like it is burning when applied directly to it.

2.10 Dosage for of papaya seed

Papaya latex, an enzyme, can make the skin feel like it is burning when applied directly to the skin.The maximum amount of papaya seed that should be consumed each day is one teaspoon (Pawar and Kothawade, 2022).

Chapter III: Materials and Method

3.1 Area of study

The entire investigation was conducted in the labs of the Department of Applied Food Science and Nutrition, Department of Applied Chemistry and Quality Assurance, Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology, Department of Animal Science and Nutrition as well as Poultry Research and Training Center (PRTC) of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram.

3.2 Sample collection

Samples of fresh papaya were obtained from local farmer in the districts of Rangamati, khagrachori and Chattogram.

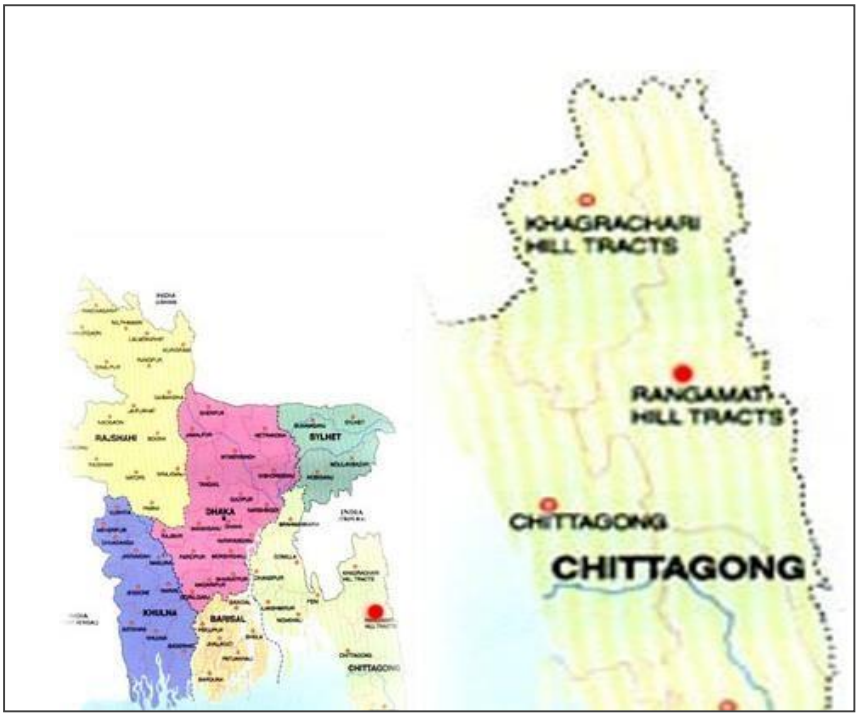

Fig: 3.1 Sampling Location

3.3 Study Duration

The experiment was conducted for a period of six months from December 2022 to May 2023.

3.4 Lay out of Experiment

First, based on availability, two types of papaya were gathered from various locations. The seeds were carefully cleaned after collection to get rid of sand, clay, and other debris.The seeds were then dried and processed into a powder. Following this, the powder sample was examined to determine its nutritional composition (moisture, ash, crude fat, protein, crude fiber, and carbohydrate), mineral contents (Potassium, Calcium, Phosphorus, Iron, Zinc), vitamin content (A & C) , bioactive compounds (total phenol, total flavonoids), antioxidant activity, and antibacterial activity (Figure 3.2).

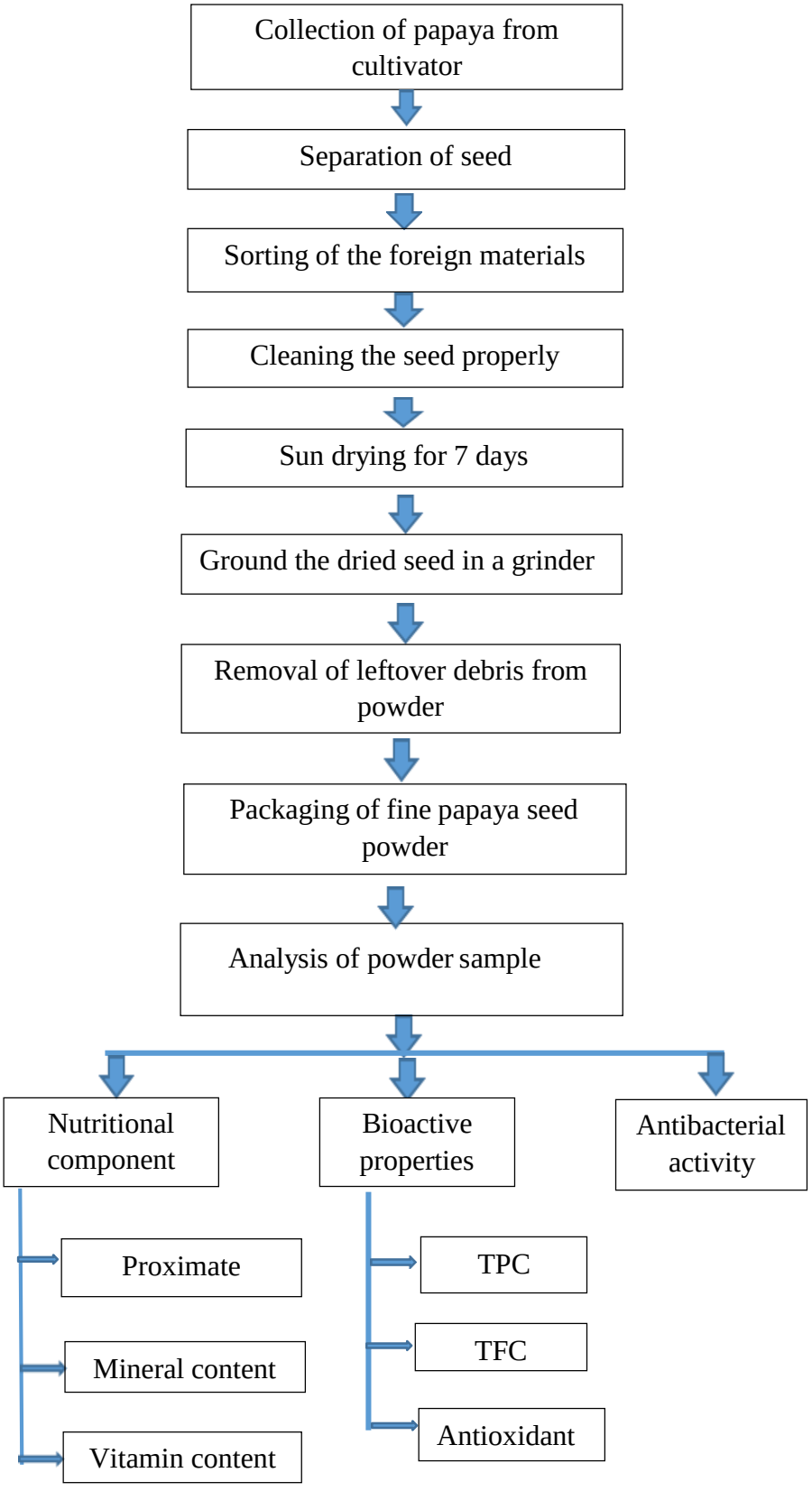

Fig 3.2: Experimental design

3.5 Preperation of sample

After collection of papaya from cultivator ,each papaya was divided into pieces and the seeds were removed. After taking out the seeds of both green and ripe papaya seed were washed with tap water to remove any residual dust and debris. Collected papaya seeds were taken in dry and clean steel tray .Then seeds were dried under the sun for 1 week at least. The dried seed were ground using a grinder. The powder samples were run through a fine (2mm mash) sieve to eliminate any leftover debris. The fine powder samples were kept in secure, labeled plastic containers at 4°C before usage.

3.6 Methods of analysis

Crude protein, total carbohydrates, crude fat, and micronutrients like vitamin C, minerals (Na, K, Mg, Ca, P, Fe, Cu, Zn), crude fiber, and bioactive compounds (total phenol, total flavonoid) antioxdant activity were estimated using standard methods of chemical analysis .

3.6.1 Proximate analysis

Using (AOAC,2016) techniques, the samples' moisture, ash, crude protein, crude fat, and crude fibre contents were determined in triplicate. The moisture content was determined by oven drying to a constant weight at 105°C. The Kjeldahl method was used to determine the crude protein concentration (crude protein for plant origin: 5.85 N). To extract crude lipid, a Soxhlet system was employed. Ash was heated to a constant weight at 550 degrees Celsius and then measured gravimetrically in a muffle furnace.

3.6.1.1 Determination of moisture content

Apparatus:

- Crucuble
- Hot air oven
- Desiccator
- Weighing balance

Procedure:

Initial sample loading was five grammes on a weight of dried, empty crucibles. After that, the crucible was placed in a thermostatically controlled air oven and dried for 24 hours at 105°C. After cooling in the desiccator and drying in the oven, the crucible was removed. A cover glass scale was then used to weigh it. The crucible was once more placed in the oven for 30 minutes of drying before being taken out, allowed to cool in a desiccator, and weighed. Up until the two subsequent weights were identical, drying, cooling, and weighing were repeated. Calculation: These weights were used to compute the percentage of moisture in food samples as follows:

Calculation: The percent of moisture was calculated as follows

% Moisture = $(W-W_1)/W_1$

W= Weight of fresh/dry sample, W1= Weight of dried sample**.**

3.6.1.2 Determination of ash content

Apparatus:

- Porcelain
- gas burner
- muffle furnace

Procedure:

The samples' ash content was assessed using the accepted AOAC methodology (AOAC, 2016). This procedure involved thoroughly cleaning and drying an empty crucible in a hot air oven. The weight was recorded after it was cooled in desiccators. The sample, which weighed 3 grams, was put into the crucible. It could burn without producing any smoke. After cooling, the crucible was moved to the muffle furnace, where it remained at 550°C for five hours. When white ash has formed, the process is complete. It was placed in a desiccator after being cooled to 150°C. The weight was taken when it reached a moderate warm temperature.

Calculation: Ash content was calculated using the following formula

% Ash = $\{(W-W_1)/W_2\}*100$

Where, W= weight of the crucible with ash; W_1= weight of the empty crucible; W_2= weight of the sample.

3.6.1.3 Determination of crude protein

Apparatus:

- Kjeldahl flask
- Condenser
- Kjeldahl digestion unit

Reagents used:

- Concentrated H2SO4 (98% pure)
- Catalyst (Potassium sulphate:Copper sulphate=9:1)
- Boric acid solution (4%)
- Sodium hydroxide (35%)
- Mixedindicator solution (Bromocresol green, methyl red)
- Standard HCl (0.2N)

For estimation of protein, the steps were followed:

Digestion:

In a Kjeldahl digestion tube, 0.3 g of sample, 4 g of catalyst, and 5 ml of H2SO4 were added. It was put in the digestion machine and heated to 320 degrees for 30 minutes of digestion. When the substance turned a light yellow, the digestion was complete.

Distillation:

After bringing the digestion tube to room temperature, 25 ml of distilled water, 25 ml of 35% NaOH, and glass blitz were added to a kjeldahl flask that already had 10 ml of 4% boric acid and a couple of drops of mixed indicator in it. The receiving solution was added to the distillation unit together with the cooled tube. The tube was automatically filled with 25ml of 35% NaOH. For three minutes, the distillation process is carried out. At the conclusion of the procedure, the receiving solution turned green.

Titration: The solution collected was titrated with 0.2N HCl solution and titer value was recorded.

Calculation: The calculation of the percent of protein in the plant sample using protein factor 5.85 is:

% $Crude\ Protein$= [{ml of titrant × Normality of acid (0.2N) × 0.014}/ Weight of sample (gm)]*100

Where equivalent of nitrogen (N_2)=0.014

% Protein = % Nitrogen × 5.85 (for plant origin

3.6.1.4 Determination of crude fat

Apparatus:

- Soxhlet apparatus
- Hot water bath
- Electric balance
- Hot air oven
- Desiccator
- Hand gloves

Procedure: The dried sample was put into a thimble after the moisture content had been established, and the top of the thimble was sealed with a wad of fat-free cotton. The thimble was put into the Soxhlet flask-connected fat extraction tube. A minimum of 75ml of anhydrous ether was added to a flask. The condenser was attached to the fat extraction tube's upper end. The sample was extracted for at least 16 hours on a water bath at 80°C. After the extraction process was complete, the thimble was taken out of the apparatus and used to collect or allow the majority of the ether to be distilled off. The ether was drained out as soon as the tube was virtually filled. Once the ether had diluted to a small amount, it was poured into a tiny, dry beaker using a tiny funnel with a cotton plug in it. The flask was properly cleaned and filtered with ether. The ether was evaporated over a steam bath at low heat for one hour, dried at 1000°C, chilled, and weighed. The weight discrepancy indicated the presence of an ether-soluble chemical in the sample.

Calculation: The presence of fat was expressed as follows:

% Crude fat= (Loss of ether soluble materials ÷Weight of sample)*100

3.6.1.5 Determination of crude fiber

Apparatus:

- Liebig condenser
- Reflux condenser
- Gooch crucible

Reagent used:

- 0.255N sulphuric acid solution
- 10.0% Potassium sulphate solution
- Asbestos- Gooch grade

Procedure:

The amount of crude fiber was calculated using the AOAC technique from 2016. The sample was first weighed at 2 g, and then it was placed in a beaker. Then, into the same beaker, were added 125 ml of 1.25% sulfuric acid solution and 3–4 drops of n-octanol. As an antifoaming agent, n- octanol was used. The beaker was heated at a steady volume for 30 minutes. The sample was then rinsed three times to get the acid off. Following a wash, 125 ml of sodium hydroxide at 1.25% and three to five drops of antifoam were added. It was cooked once again at the same volume for an additional 30 minutes. The mixture was filtered, and the residue was once again washed. To get the acid off, it was washed once again with 1% HCL solution. The residue was then dried at 105°C in a hot air oven until a constant weight was established. For cooling, it was placed in a desiccator, and the weight was noted. The remaining material was then fired in the muffle furnace at 550-660°C for roughly 3–4 hours until it transformed into white ash. The residue was then burned up to smoke.

Calculation: The ash particles were weighed and calculated to determine the crude fiber content of the sample.

% Crude Fiber = $\{(W-W_1)/W_2\}*100$

Where, W= weight of crucible containing crude fiber and ash; W1= weight of crucible containing ash; W= weight of the sample.

3.6.1.6 Estimation of total carbohydrate

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100/100gm (AOAC, 2016). Hence, it was calculated using the formula below:

% Carbohydrate = 100 − (Moisture %+Ash%+Protein%+Fat %+Fiber %)

3.6.2 Minerals determination

According to AOAC, 2010, this technology uses digestion to remove minerals from the food substance. An acid solution with a 2:1 ratio of HNO3 and HCIO4 was used to digest a sample of date seed powder. Weighing one gram of the material into a conical flask. To ensure full digestion, the flask was heated at 200W for 3 minutes after adding 7 ml of HNO3 and 3 ml of HCIO4. After cooling, the liquid was filtered via filter paper into a 100 ml standard flask, and the volume was then adjusted using distilled water. AAS (Humalyzer-3000, Origin Germany) used this solution to determine the mineral content. Measurements were made of the concentrations of several minerals, including potassium (K), iron (Fe), calcium (Ca), and zinc (Zn) and phosphorus (P).

3.6.2.1 Determination of potassium (K)

A fine turbidity of potassium tetraphenylboron is created when sodium and potassium tetraphenylboron combine. The amount of turbidity is inversely related to the sample's potassium content. To make the blank solution, 0.02 ml of deionized water and 1 ml of potassium reagent were pipetted into the cuvette. For the sample solution, a cuvette was filled with 1 ml of potassium reagent, 0.02 ml of potassium standard, and 0.02 ml of sample extract. These underwent a 5-minute retention period incubation after mixing. Within 15 minutes, the absorbance of the Standard and sample were compared to a

blank. In order to compute the ratio of sample absorbance to standard absorbance, the potassium content was expressed in mmol/L.

3.6.2.2 Determination of iron (Fe)

The transferring-iron complex is dissolved in a mildly acidic medium, releasing the iron. Ascorbic acid is used to decrease the liberated Fe to the bivalent state. An energetic molecule is produced when ferrozine and ferrous ions are combined. The intensity of the colour depends on how much iron is present in the sample. The blank solution was made by adding 1 ml of reagent to the cuvette using a pipette. For the creation of the standard, 1 ml of reagent and 200 L of standard were added. 200 L of sample extract and 1 ml of reagent were combined to create the sample solution. These were mixed, then let to sit at room temperature for 10 minutes. Standard and sample absorbance were measured in relation to a blank.In g/dL iron concentration was calculated.

3.6.2.3 Determination of calcium (Ca)

O-Cresolphthalein and calcium ions mix to form a violet complex in an alkaline medium. The reagent blank solution was prepared by mixing 1 ml of working reagent with 25 L of distilled water in a cuvette. 25 mL of the (Ca++) standard and 1 ml of the working reagent were added to the standard. 25 L of the sample extract and 1 ml of the working reagent were combined to create the sample solution. The absorbance of the sample and the standard were both determined. By dividing the sample absorbance by the reference concentration (mg/dL), the amount of calcium in the blood was calculated in mg/dL.

3.6.2.4 Determination of zinc (Zn)

Zinc, an alkaline media, and Nitro-PAPS interact to form a complex with a purple colour. The intensity of the complex that is formed is directly correlated with the amount of zinc present in the sample. The blank solution was made by pipetting 0.05 ml of distilled water and 1 ml of the working reagent into the cuvette. The standard solution was made by adding 0.05 ml of the zinc standard and 1 ml of the working reagent to a cuvette. The sample solution was made by adding 1 ml of the working

reagent and 0.05 ml of the sample extract to a cuvette. After thoroughly blending, incubate at the retention time for 5 minutes.The absorbance of the sample and standard was measured in comparison to the blank in 20 minutes.

3.6.2.5 Determination of phosphorus (P)

One millilitre of phosphorus reagent was used to prepare the blank solution, one millilitre of phosphorus reagent, ten millilitres of phosphorus standard, and one millilitre of phosphorus reagent, ten millilitres of sample extract were pipetted into the cuvette for the sample solution. The absorbance of the sample and the standard were calculated and compared to a blank. Phosphorus concentration was determined in mg/dL as sample absorbance divided by standard absorbance.

3.6.3 Vitamin analysis

3.6.3.1 Determination of vitamin A

A colorimeter was used to measure vitamin A levels. The total amount of Vitamin A in a given meal is calculated using both retinol and beta carotene contributions. Retinol and carotenoids are extracted into light petroleum and combined with alcohol to precipitate proteins. Before the colour reaction, the light petroleum is evaporated, the carotenoid-induced yellow colour intensity is measured, and the residue is subsequently dissolved in chloroform. It is taken into account how the carotenoid affected the outcome (Gibson, 1990). Trifluoroacetic acid reacts with the retinol in the sample (TFA). The presence of retinol in the sample is shown by the blue coloration of the sample and TFA response. As soon as possible after introducing the reagent—ideally, within two seconds—the blue hue must be seen. This is due to the blue hue's fleeting nature.

Each sample was prepared by mixing 100 mg of the sample in a tube with 1 ml of distilled water and 2 ml of ethanol using a vortex mixer. One cc of the supernatant was removed from the tube after it had been centrifuged for 15 minutes at 3000 rpm. First came the discovery of beta-carotene. S2 reagent (6 ml) was used to prepare the blank solution, and standard reagent (6 ml) was pipetted into the cuvette to prepare the standard. 1 ml of the sample extract, 2 ml of the S1 reagent, and 3 ml of the S2 reagent

were pipetted into a cuvette to create the sample solution. A mechanical shaker and a vortex mixer were used to thoroughly combine all of the ingredients for ten minutes.

The tubes were centrifuged at 3000 RPM for 10 minutes. The absorbance was then determined at 420 nm in comparison to the blank using 2 ml of sample supernatant, the standard, and the blank. To stop the solvent from draining and the light from harming the carotenoids, this was done right away. The retinol was then discovered. After 2 ml of the sample extract used to determine the amount of carotenes was collected, the contents of the sample cuvette were dried in a water bath that was heated to 50 °C. The sample cuvette was then filled with 100 l of S4 reagent and 1 ml of S5 reagent once the solvent had evaporated. 100 ml of the S4 reagent and 1 ml of the S5 reagent were pipetted into a cuvette to create the blank solution. 100 ml of the standard reagent and 1 ml of the S5 reagent were combined to create the standard solution. In a vortex mixer, these were properly combined. Exact 2 seconds after the reagent was added, the absorbance at

The carotene, retinol and total vitamin content were measured as follows,

Retinol (mg/L) = (0.0759 × Absorbance) + 0.1023

Carotene (mg/L) = (- 0.0167 × Absorbance) + 0.0091

Where ,0.0759 and 0.0167 are slope; 0.1023 and 0.0091 are intercept

Total vitamin A (RAE) = µg of retinol + (µg of beta-carotene / 6)

3.6.3.2 Determination of Vitamin C content

Principle: Vitamin C's market-diminishing properties influence its chemical evaluation. The amount of vitamin C in plant or animal extracts is frequently determined by how well it inhibits the dyestuff 2,6-dichloride phenol indophenols. In this instance, the colour dye triggered the oxidation of vitamin C to dehydroascorbic acid. At the same time, the dye is transformed into a colourless material. The termination point of the reaction can be easily determined. Rapid excretion and filtration are desirable since excess could be introduced into plant products by oxidised vitamin C during sampling and grinding. Oxidation occurs when metaphosphoric acid is used in the extraction process. A highly acidic solution will result in the most precise outcome. Within one

minute, the titration ought to be finished. In an aqueous solution, the dye is blue. When totally reduced, an acidic solution turns colorless (AOAC, 2016).

Reagent required:

A. Dye Solution

1. 260 mg of dye (2,6-dichlorophenol indophenols)
2. 210 mg of NaHCO3 dissolved in 100 ml of distilled water

B. Metaphosphoric acid solution (3%)

1. 7.5mg of Metaphosphoric acid.
2. 20ml of glacial acetic acid dilutes to make 250 ml with distilled water.

Standard ascorbic acid solution :25 mg of crystalline ascorbic acid dissolved in 250ml of metaphosphoric acid solution

Procedure:

A dye solution was put within the burette. Then, 5 ml of a solution of vitamin C was utilised in a conical flask. The conical flask was placed beneath the burette and dye was added drop by drop. The titration was finished when a pink tint first appeared, persisted for 20 seconds, and then disappeared. There were at least three different readings done. The same procedure was used to treat the ascorbic acid solution, but the concentration was unknown. The result was expressed as a milligram percentage, or mg%.

3.6.4 Determination of bioactive compounds

Extract preparation

1 g of the material was obtained for TPC and TFC in a Felcon tube. The combination was then given 10 ml of 100% ethanol, and it was allowed to sit for 72 hours. Continuous straining was done after a 4-hour respite. After 72 hours, the filtrate was collected, and an ethanol extract was found.

3.6.4.1 Determination of Total Polyphenol Content (TPC)

The TPC of the extracts was determined using the Folin-Ciocalteu reagent technique with a few minor adjustments (Al-Owaisi et al., 2014). According to Vergani et al. (2016), the total polyphenol content (TPC) of papaya seeds was determined using a slightly altered version of the Folin-Ciocalteu method. One millilitre of ethanoic extract was combined with 1.5 millilitres of FC reagent in a falconer tube, which was then left at room temperature for three minutes.After adding 1.5 cc of 7.5% Na_2CO_3, the mixture was permitted 60 minutes to settle.The absorbance was determined at a wavelength of 765 nm using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) with C_2H_5OH as the blank.mg of gallic acid equivalents per gram of extracts (mg GAE/g) was found to be the amount of TPC.

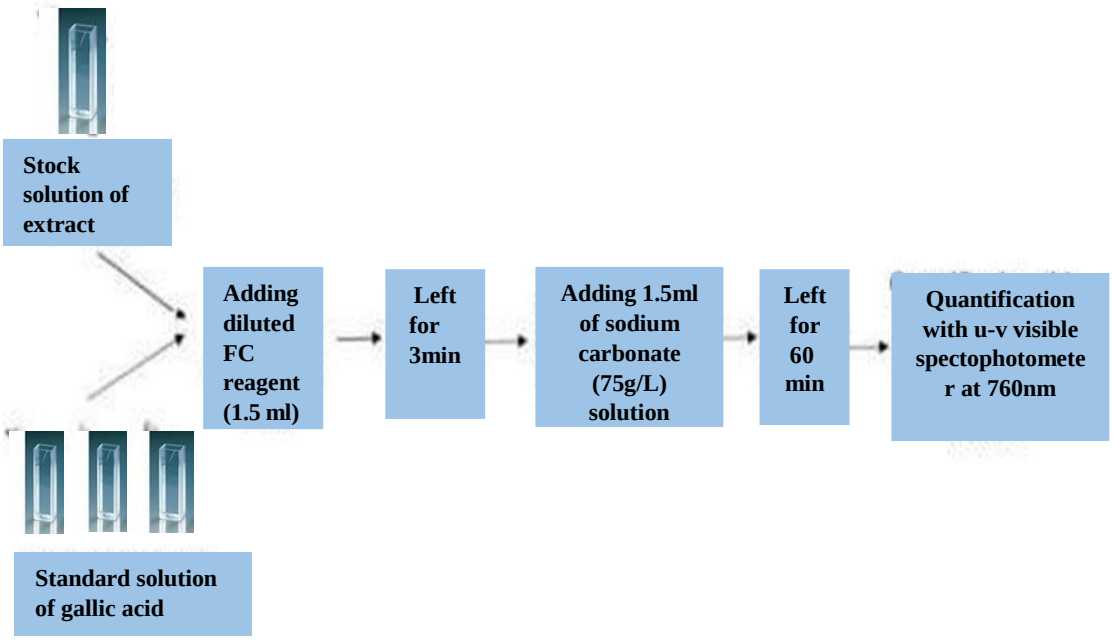

Figure 3.3 : Determination of Total polyphenol content (TPC)

3.6.4.2 Determination of Total flavonoid content (TFC)

The aluminium chloride colorimetric method reported by Chang et al. (2002) was somewhat adjusted in order to determine the samples' total flavonoid content (TFC). A stock solution of the extracts (1 mg/mL) was prepared, and aliquots of 0.5 mL of the diluted extract were diluted with 1.5 mL of 95% C_2H_5OH in a cuvette. Following that, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled

water were added to the immixture in the cuvette. The mixture was left at room temperature for 30 minutes. The absorbance was determined using a UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA) at a wavelength of 415 nm. The blank was 10% aluminum chloride substituted with the same amount of D.H_2O. The total quantity of flavonoids in the sample was calculated by comparing the absorbance of the extracts to a quercetin standard curve. Per gram of extract, TFC was determined and reported as mg of quercetin equivalents (mg QE/g).

3.6.5 Determination of Antioxidant capacity by DPPH scavenging method

Extract preparation

1 gram sample was put into the Felcon tube. The combination was then given 10 ml of 100% methanol, and it was allowed to sit for 72 hours. Continuous straining was done after a 4-hour respite. After 72 hours, filtrate was collected, and methanoic extract was found. The DPPH assay was used to determine the antioxidant mobility of the extracts with a few minor modifications from the approach described by Azlim et al. (2010). 100 mL of 100% methanol were used to dissolve around 6 mg of DPPH in order to create a methanoic DPPH solution.

Then, 1 ml of methanoic extract was combined with 2 ml of DPPH solution. The mixture was then given a gentle shake and left to stand at room temperature in the dark for 30 minutes. The absorbance was determined at 517 nm using a UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA). The control, which was created by mixing 1 mL of methanol with 2 mL of DPPH solution, employed methanol as a blank. The scavenging mobility was approximated by the decrease in absorbance of the samples compared to the DPPH reference solution. Based on extracts' ability to scavenge DPPH free radicals, the antioxidant capacity was determined using the following equation:

% of inhibition = {(Blank absorbance - Sample absorbance)/ Blank absorbance }× 100

TEAC composite (Trolox equivalent antioxidant mobility), which was also used as the standard, was used to build the calibration standard curve. The results were represented as mg/100 g of Trolox equivalents (TE) per gram of powder on a dry weight (DW) basis.

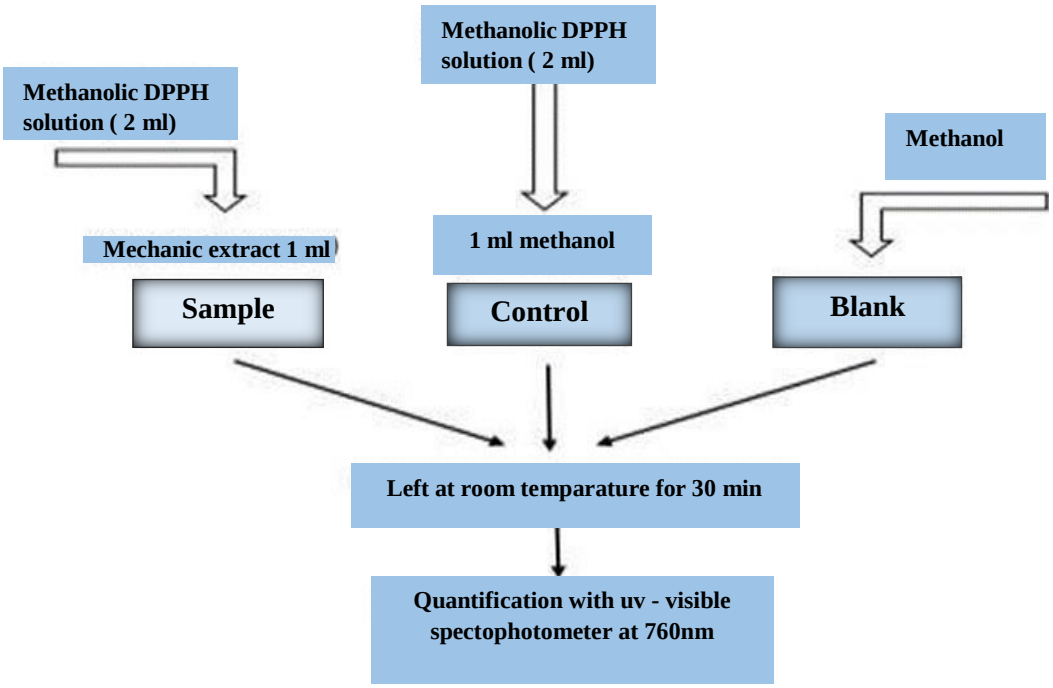

Figure 3.4: Determination of antioxidant capacity

3.6.6 Antibacterial Activity

Preparation of samples

20 gm of papaya seed powder was soaked in 60 ml of ethanol and kept at incubator at 37 º C for 48 hours. The extract was dried using a rotary evaporator and maintained in room temperature for further test. (Zaidan et al., 2005)

Test microorganisms

Antibacterial activity was tested on strains of *Staphylococcus aureus* and *E. coli*.Pure isolated cultures of Escherichia coli and Staphylococcus aureus were obtained from the PRTC (Poultry Research and Training Centre), Chattogram Veterinary and Animal Sciences University, Chattogram. In both cases broad spectrum antibiotic Ciprofloxacin (5mg) was used as standard.

Reagents and apparatus

Reagents:

- 1% Barium Chloride solution

- 1% Sulfuric acid
- Normal saline
- Distilled water

Media:

- Blood Agar
- Mueller Hinton agar

Apparatus:

- Petri dishes
- Inoculating loop
- Screw capped test tubes
- What man no 1 filter paper
- Volumetric flasks
- Pipette
- Beaker
- Spirit lamp
- Tripod stands
- Electric weight machine
- Foil paper
- Spoon
- Marker pen
- Autoclave
- Incubator

McFarland standard preparation

A solution of 1% sulfuric acid and 1% barium chloride was made. A sterile test tube with a screw-on top was used to combine 99.5 ml sulfuric acid and 0.05 ml barium chloride solution for the 0.5% McFarland standard.

Preparation of culture suspension

Each isolate's inoculum was made from a subculture. In a sterile screw cap tube containing 2 ml of sterilized saline water, 4-5 colonies of each isolate were collected. Following that, the bacterial culture was emulsified in sterile normal saline, and the turbidity was set at 1.5*108 (CFU/ml equal to 0.5% McFarland standard).

Media preparation

As instructed on the label, 38gm of Mueller Hinton agar powder was weighed and combined with 1L of distilled water. The media was then cooked to ensure thorough melting and mixing. Following mixing, the media was autoclave sterilized and placed in a water bath to cool. After cooling, the media were aseptically placed onto the petri plates and given time to consolidate. For 24 hours, the dishes were incubated at 37 °C to look for contamination.

Antimicrobial effect of samples against *Escherichia coli* and *Staphylococcus aureus*

The disc diffusion method was employed to examine the effectiveness of the extracts, and their impact was measured by taking note of the zone of inhibition surrounding the disc. Whatman No. 1 Filter paper was used to create discs with a diameter of 6 mm. .5 ml of each sample was used to impregnate the discs. The Mueller Hinton agar plates were uniformly inoculated by dipping a sterile cotton swab into the standardized bacterial suspension. They were left to dry for three to five minutes. Following that, each disc was put on the plates and lightly pressed to ensure full contact with the agar. To display overlapping of inhibitory zones, a space of at least 15 mm was kept between the plates' edges. The plates were incubated for 24 hours at 37 °C fifteen minutes after the discs were placed. Following incubation, the plates were inspected, and the diameter of the inhibitory zone for each isolate was measured.

3.7 Statistical Analysis

Descriptive statistics (mean and standard deviation) were computed for the sample in a Microsoft Excel 2019 spreadsheet where data collection and storage for statistical analysis were also carried out. The construction , coding, and recording of information were done utilizing MINITAB 19. Following that, we statistically analyzed the results of these experiments. Approximate proximate composition, mineral content, vitamins

and bioactive compound data were examined using one-way ANOVA, and the size of significant variance was assessed with a 95% confidence interval. Statistical analysis was conducted at the 5% significance level ($p<0.05$).

Chapter IV: Results

4.1 Proximate Composition

Table 4.1 displays the mean percentage with standard deviation (ME ± SD) of the proximate composition value which includes moisture, protein,fat,crude fibre,ash and carbohydrate content of both raw papaya seeed (RPS) and mature papaya seed (MPS).

Table 4.1 proximate Composition of raw and mature papaya seed.

Sample ID	% Moisture	% Ash	%Protein	%Fat	% Fibre	%CHO
RPS	8.84 ± 0.03^{a}	10.17 ± 0.01^{a}	26.16 ± 0.01^{b}	16.19 ± 0.09^{b}	14.47 ± 0.02^{b}	24.18 ± 0.13^{a}
MPS	6.06 ± 0.02^{b}	6.49 ± 0.08^{b}	27.33 ± 0.03^{a}	28.13 ± 0.04^{a}	20.92 ± 0.03^{a}	11.06 ± 0.15^{b}
P value	0.001	0.030	0.003	0.010	0.001	0.031

All values are presented as Mean ±SD. Statistically significant differences ($p< 0.05$) are indicated by different superscripted letters in each row for all the samples.

According to this analysis, MPS had a larger percentage of crude protein (27.33±0.03%), crude fat (28.13±0.04%) and crude fibre (20.92±0.03%) than raw seed (26.16±0.01% , 16.19±0.09% and 14.47±0.02% respectively).Contrarily, moisture content (8.84±0.03%),ash content ($10.17\pm0.01^{\%}$) and carbohydrate content (24.18±0.13%) of the raw seed was higher than that of mature seed (6.06±0.02%, 6.49±0.08% and 11.06±0.15% respectively).

4.2 Mineral content

Table 4.2 shows the results of the analysis of the mineral content of raw and mature papaya seed. Calcium, potassium, Iron, Zinc, phosphorus were studied as minerals.

Table 4.2 Mineral content of raw and mature papaya seed

Sample ID	Potassium (mg/dl)	Iron (µg/dl)	Calcium (mg/dl)	Zinc (mg/dl)	Phosphorus (mg/dl)
RPS	18.41 ± 0.06^{b}	130.59 ± 0.04^{b}	15.45 ± 0.07^{a}	9.03 ± 0.03^{a}	5.20 ± 0.01^{b}
MPS	23.56 ± 0.01^{a}	146.11 ± 0.02^{a}	6.20 ± 0.06^{b}	6.44 ± 0.04^{b}	7.22 ± 0.04^{a}
P value	0.041.	0.002	0.021	0.001	0.030

All values are presented as Mean ±SD. Statistically significant differences ($p< 0.05$) are indicated by different superscripted letters in each row for all the samples.

The results for two types of papaya seed (raw and mature) ranged for potassium, calcium, , iron, phosphorus and zinc from 23.56 to 18.41 mg/dl, 15.45 to 6.20 mg/dl, to 130.59 to 146.11µg/dl, 7.22 to 5.20 mg/dl, and 9.03 to 7.20 mg/dl.

4.3 Vitamin content

Table 4.3 shows the results of the analysis of the vitamin of raw and mature papaya seed. Vitamin A and Vitamin C were studied as vitamins.

Table 4.3: Vitamins content of raw and mature papaya seed

Sample ID	Vitamin A (RAE/gm)	Vitamin C (mg/100g)
RPS	2.12 ± 0.10^{b}	5.01 ± 0.01^{a}
MPS	8.06 ± 0.03^{a}	1.03 ± 0.03^{b}
P value	0.042	0.001

All values are presented as Mean±SD. Statistically significant differences ($p< 0.05$) are indicated by different superscripted letters in each row for all the samples.

Through observation of the mentioned tables derived from one way ANOVA analysis, significant differences can be discovered between the results for both Vitamin C (mg/100g) and Vitamin A (RAE/gm). Mature papaya seed (MPS) was found to have the highest levels of vitamin A (8.06 RAE/gm)than raw seed (2.12 RAE/gm). On the

other hand raw papaya seed (RPS) had more vitamin C (5.01 mg/100gm) than mature seed (1.03mg/100gm).

4.4 Bioactive compounds

Table 4.4 lists the bioactive components of raw and mature papaya seed.

Table 4.4: Bioactive compound of raw and mature papaya seed

Sample ID	TFC (mg QE / 100	TPC (mg GAE / 100	Antioxidant Capacity (mg TE / 100 g)
RPS	20.90 ± 0.030^{b}	0.47 ± 0.007^{b}	6.44 ± 0.011^{b}
MPS	30.65 ± 0.101^{a}	5.41 ± 0.009^{a}	6.64 ± 0.002^{a}
P value	0.001	0.003	0.012

All values are presented as Mean ±SD. Statistically significant differences ($p< 0.05$) are indicated by different superscripted letters in each row for all the samples**.**

According to the results, Mature papaya seed (MPS) contained the highest amount of total flavonoid concentration (30.65±0.101mg QE/100g) than Raw papaya seed (RPS) had (20.90±0.030mg QE/100g). Mature seed had the highest total phenol content measurement (5.41±0.009 mg GAE/100g). Raw seed had the lowest result (0.47±0.007 mg GAE/100g). The antioxidant capacity of papaya seed at two stages (Raw and mature) ranged from 6.44 to 6.64mg TE/100g. Mature papaya seed has the highest antioxidant capacity (6.64± 0.002mg TE/100g) than raw papaya seed.

4.5 Antimicrobial Activity

The result of antimicrobial activity of papaya seed extracts agaist *E.coli* and *Staphylococcus* aureus are given in Table 4.6

Table 4.5: Zone of inhibition of S. aureus and E. coli against Papaya seed extracts

Sample	***Staphylococcus Aureus***	***E. coli***
RPS	10 mm	11 mm
MPS	14 mm	15 mm

Table 4.6 displays the antibacterial activity of papaya seed extracts against Staphylococcus aureus and E. coli. Ethanol extract of mature papaya seed (MPS) displayed the biggest zone of inhibition against both *S. aureus* (14 mm) and *E. coli* (15 mm). On the other hand ethanolic extract of raw papaya seed (RPS)showed 10 mm inhibition zone against S. aureus and 11 mm against *E.coli.*

Chapter V: Discussion

In this research, an effort was made to assess the nutritional makeup, mineral content, bioactive substances, antioxidant activity, and antibacterial activities of papaya seed at two stages of ripening (Raw and mature).

5.1 Nutritional Composition

5.1.1 Proximate Analysis

Compared to ripe papaya seeds, which have a moisture content of 6.06%, raw papaya seeds have a moisture content of 8.84%.This disparity can be brought about by changes in the soil and climate. Because the moisture content of Seed is influenced by environmental factors such as rainfall, soil type, and moisture percentage. Raw papaya seeds had an ash content of 10.17 percent, while mature papaya seeds had an ash content of 8.84%. The results of the ash content test revealed that there were significant differences between the samples' ash contents ($p<0.05$). The results of the ash content of papaya seeds at various stages of ripening were in close accord with those reported by (Asmah R, 2014) . According to their findings raw seed and mature seed contains 10.5% and 8.2 % ash content respectively.

Mature papaya seeds had the highest protein content (27.33%), whereas raw papaya powder had the lowest (26.16%). The protein content of papayas in the mature stage was lower than what (Asmah R, 2014) had claimed (30.21%). Mature papaya seeds had a fat content value of 28.13 percent, while raw papaya seeds had a value of 16.19 percent. The fat content of raw papaya seed was higher than what (Marfo et al. 1986) reported (12.34%) and lower than what (Asmah R, 2014) claimed (18,65%).

Mature papaya seeds had the highest fiber content value (20.92%), whereas raw papaya seeds had the lowest value (14.47%). Different phases of papaya seed have lower fiber levels than (Asmah R, 2014) had claimed (RPS 17.45%, MPS 21.13%).

The mature papaya seed had the least quantity of carbohydrates, whereas the raw seed had the most. The exceptionally high carbohydrate content of raw papaya seed indicates that it is a fantastic source of energy.

5.1.2 Minerals

The mineral content of papaya seeds at different stages (raw and mature) is shown in Table 4.2. The results of the mineral contents were detected in all stages of papaya seed, and the ranges were 23.56 to 18.41 mg/dl, 15.45 to 6.20 mg/dl, 130.59 to 146.11 µg/dl, 7.22 to 5.20 mg/dl, and 9.03 to 7.20 mg/dl for potassium, calcium, iron, phosphorus, and zinc. A one-way ANOVA revealed that different stages of papaya seeds had significantly varying mineral contents ($p < 0.05$) in the case of the mineral content. Raw papaya seed contained the highest amount of potassium and iron. Mature papaya seeds had the highest levels of calcium, phosphorus, and zinc. Different phases of papaya seed powders have higher potassium and zinc levels than those reported by Marfo et al., 1986 and Malacrida et al.,2011, respectively. Raw seed contains 15.24 mg/dl and mature seed contains 20.34 mg/dl potassium (Marfo et al., 1986). In comparison to Marfo et al.,1986 and Malacrida et al., 2011, the calcium contents of mature papaya seeds were lower (16.43 mg/dl,) and greater (12.65 mg/dl), respectively. Different stages of papaya seed have higher phosphorus (RPS 4.57 mg/dl, and MPS 5.32 mg/dl,) and iron concentrations than what (Marfo et al.,1986) had previously reported (RPS 134.54 µg/dl and MPS 150.12 µg/dl).

5.1.3 Vitamins

In this comparative study of seeds two types of vitamins, which are Vitamin A and Vitamin C were estimated. Mature papaya seed was found to have the highest levels of vitamin A (8.06 RAE/gm) than raw seed (2.12 RAE/gm). Raw papaya seed has more vitamin C (5.01 mg/100gm) than mature papaya seed (1.03mg/100gm).Vitamin content is generally high in items made from plants, including papaya seed. A study revealed that ripe papaya seed contains 14.4 mg/100 g vitamin C (Asmah R, 2014). Furthermore, another study found that seeds from mature papaya contained 135 IU/mg of vitamin A where as raw seeds had 87.2 IU/mg. (Chukwuka et al.,2013). However, the nutritional content of papaya fruits varies depending on their stage of ripeness. One of the essential nutrients, vitamin C is involved in a variety of crucial bodily processes, including boosting immune system activities (Subramanian et al., 2011). L-ascorbic acid, also known as vitamin C, is an organic compound with six carbons that is hydrophilic, or water-soluble. L-ascorbic acid has the chemical formula $C_6H_8O_6$ and a molar mass of 176.13 g/mol. It is an antioxidant with a shape like glucose (Carita et al., 2019). Raw papaya seed has more vitamin C than mature papaya seed.

5.2 Bioactive compounds

An important type of dietary antioxidants are plant polyphenols (Pandey and Rizvi, 2009). These phytochemicals are now being used as next generation medicines to treat and manage a variety of disorders brought on by modern lifestyles (Tiwari, 2004). In the geriatric population, supplementing with dietary antioxidants in moderation has been demonstrated to lower oxidative stress indicators (Nelson et al., 2003).

Table 4.4 lists the bioactive substances found in papaya seed in two different stages. The results of total flavonoid content (TFC) ranged from 20.90 to 30.65 mg QE/100g. Mature Seed contained the highest amount of total flavonoid concentration (30.65±0.101mg QE/100g) where as raw papaya seed had the lowest value (20.90±0.030 mg QE/100g). The flavonoid content analysis revealed that there were significant differences between the samples in flavonoid content ($p < 0.05$).The flavonoid concentration of mature papaya seed was higher than what (Mesquita et al., 2022) claimed (25.47 mg QE/100g).

For two phases of papaya seed (raw and mature), the results of total phenolic content (TPC) ranged from 0.47 to 5.41 mg GAE/100g. Mature seed had the highest total polyphenol content measurement (5.41±0.009 mg GAE/100g). Raw seed had the lowest result (0.47±0.007 mg GAE/100g) . Total polyphenol content analysis revealed that there were significant differences between the samples' total polyphenol contents ($p<0.05$). Total polyphenol content of mature papaya seed was lower than other study. According to their findings mature seed had 34.26 mg GAE/100g TPC. (Mesquita et al., 2022).

According to the results, the antioxidant capacity of papaya seed at different stages (Raw and mature) ranged from 6.44 to 6.64mg TE/100g. Mature papaya seed has the highest antioxidant capacity (6.64± 0.002mg TE/100g).The raw papaya seed had the lowest result (6.44±0.011 mg TE/100g), however. The antioxidant capacity results revealed that samples antioxidant capacities differed considerably ($p < 0.05$). The antioxidant capacity of the mature papaya seed was lower than that of papaya as reported by other researcher (Mesquita et al., 2022) . According to their findings antioxdant capacity of mature papaya seed had 9.65 mg TE/100g.

5.3 Antibacterial Activity

The focus of this investigation is to antibacterial properties of raw and mature papaya seeds against two different harmful bacteria *S. aureus* and *E. Coli* .It is well known that the seeds of tropical fruits are full in phytochemicals, which may be crucial for regulating and controlling the number of pathogens in both people and animals' gastrointestinal tracts.

Table 4.6 displays the antibacterial activity of papaya seed extracts against Staphylococcus aureus and E. coli. From the present investigation, it was observed tha ethanol extract of mature papaya seed displayed the biggest zone of inhibition against both S. aureus (14 mm) and E. coli (15 mm). On the other hand ethanolic extract of raw papaya seed showed 10 mm inhibition zone against *S. aureus* and 11 mm against *E.coli.* With reference to papaya in particular, it has been discovered that the fruit's seeds include alkaloids, flavonoids, steroids, saponins, papain, and terpenoids with antibacterial and antiparasitic properties (Masfufatun et al., 2011). The antimicrobial effects of papaya seed extract against Salmonella enteritidis, Vibrio vulnificus, Proteus mirabilis, and Bacillus cereus have been documented by (Schinor et al., 2006) and other researchers in a number of studies.. Some studies have specifically investigated the antibacterial effects of papaya seeds.

Chapter VI: Conclusion

The current study assessed the nutritional component,phenolic content and antibacterial activity of raw and mature papaya seed . The results of this study revealed that they were high in energy, crude fat, crude protein, fiber, and minerals like phosphorus, magnesium, calcium , iron , and potassium. Nowadays value added product is a very common feature in food industry. As RPS & MPS both contain higher nutritious component so there are possibilities of use of Ca or iron fortified food products or may be used as a substitutes of Ca or Fe to meet the increasing demands of value added food products. According to the experiment's findings, papaya seed extracts (ethanolic) have antibacterial properties that are effective against both S. aureus and E. coli. Although raw and mature seeds had different nutritional contents, both were effective at preventing bacterial development. Therefore, it can be predicted that ongoing research in this area will serve as a solid foundation for the advancement of the economy, environment, and food industries. According to several studies it’s also proved that consumption of papaya seeds offers a cheap, natural, harmless, readily available immunotherapy and preventive strategy against intestinal parasitosis, especially in tropical communities. There are many prospects to convert unutilized papaya seeds into medicines, cosmetics, value-added products, and dietary supplements rather than discarding them after eating the fruit pulp. The reuse of this waste, which is rich in nutrients, will benefit both our health and the environment. So it can be said that PS, which are supposedly useless but actually have beneficial therapeutic (help fight infections, promote kidney health, protect against cancer, and enhance digestive health) and nutritional properties and are a blessing to the human body, yet dumped in the waste bin.

Chapter VII: Recommendations & future perspectives

Based on the current investigation, the following suggestions and prospects for research work are made.

- According to the findings of this study, papaya seeds are an excellent source of fat. It is therefore thought regarded as a possible source of edible oil. Oil extraction from papaya seed could be the subject of future research.
- As a good source of protein, papaya seed can be used to construct protein-based food products.
- Papaya seed has antibacterial properties against food borne disease microorganism. Seed extracts or powders can be utilized as a natural antibacterial agent. To assist prevent or treat bacterial skin infections, they can be added to topical medications like ointments, lotions, or soaps.
- Papaya seed extracts or essential oils can be added to food goods as natural preservatives, preventing the growth of bacteria and extending the shelf life of perishable foods.
- The current studies could be repeated to confirm the experimental findings.
- Similar study can be conduct on papaya seed collecting from land area.

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Appendices

Appendix A: Laboratory work

Dried papaya seed

Proximate analysis

Sample extract preparation for mineral content analysis

Antimicrobial activity analysis

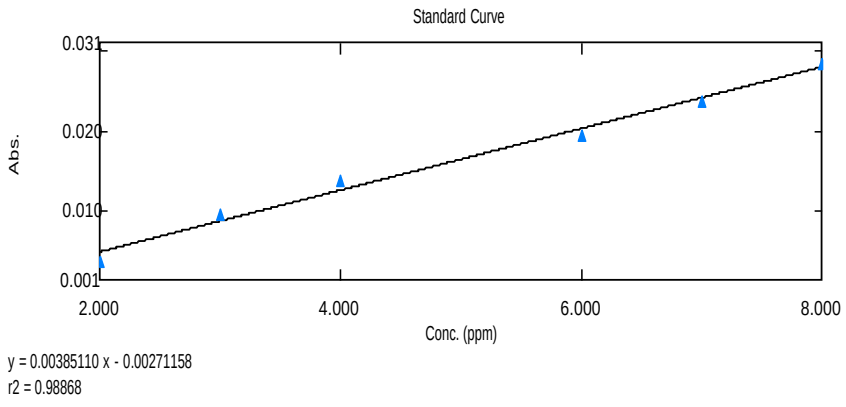
Appendix B: Bioactive compound standard curve

Total Flavonoid content (TFC)

Standard Table of Quercetin:

SL NO	Sample ID	Type	Conc	WL415.0	Wgt.Factor	Comment
1	STD1	Standard	2.000	0.004	1.000	Dilution Factor 1
2	STD2	Standard	3.000	0.010	1.000	Dilution Factor 1
3	STD3	Standard	4.000	0.014	1.000	Dilution Factor 1
4	STD4	Standard	6.000	0.020	1.000	Dilution Factor 1
5	STD5	Standard	7.000	0.024	1.000	Dilution Factor 1
6	STD6	Standard	8.000	0.029	1.000	Dilution Factor 1

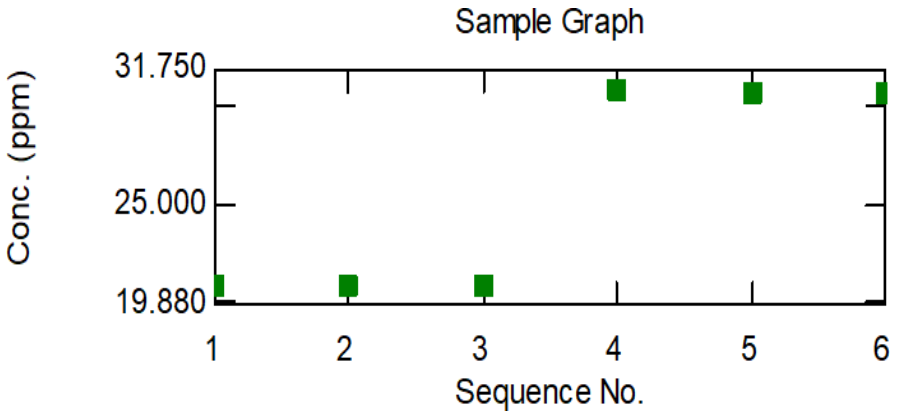
Standard Curve:



Sample Table:

SL NO	Sample ID	Type	Conc(mg/100g)	WL415.0
1	RPSP1	Unknown	20.869	0.078
2	RPSP2	Unknown	20.927	0.078
3	RPSP3	Unknown	20.913	0.078
4	GPSP1	Unknown	30.761	0.116
5	GPSP2	Unknown	30.561	0.115
6	GPSP3	Unknown	30.635	0.115

Sample graph:

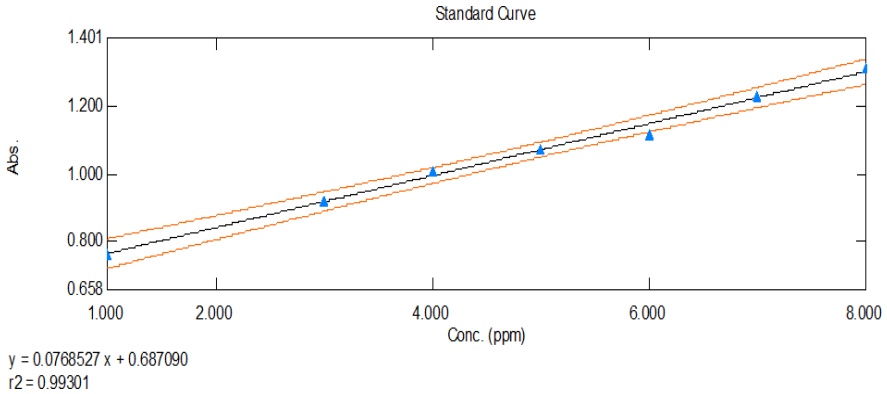


Total Phenolic content (TPC)

Standard table of Gallic Acid:

SL NO	Sample ID	Type	Conc	WL760.0	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

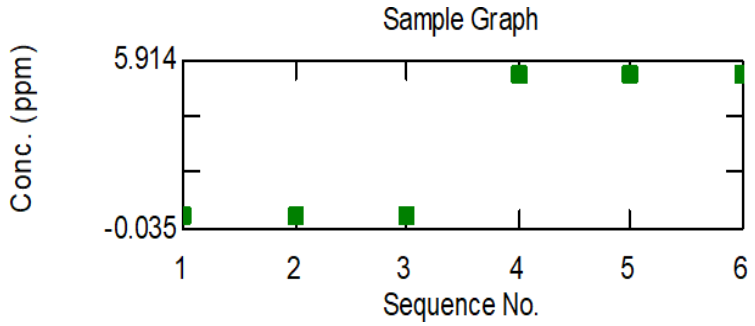
Standard Curve:



Sample Table:

SL NO	Sample ID	Type	Conc(mg/100g)	WL760.0
1	RPSP1	Unknown	0.474	0.701
2	RPSP2	Unknown	0.460	0.700
3	RPSP3	Unknown	0.471	0.700
4	GPSP1	Unknown	5.418	1.105
5	GPSP2	Unknown	5.418	1.105
6	GPSP3	Unknown	5.403	1.104

Sample Graph:

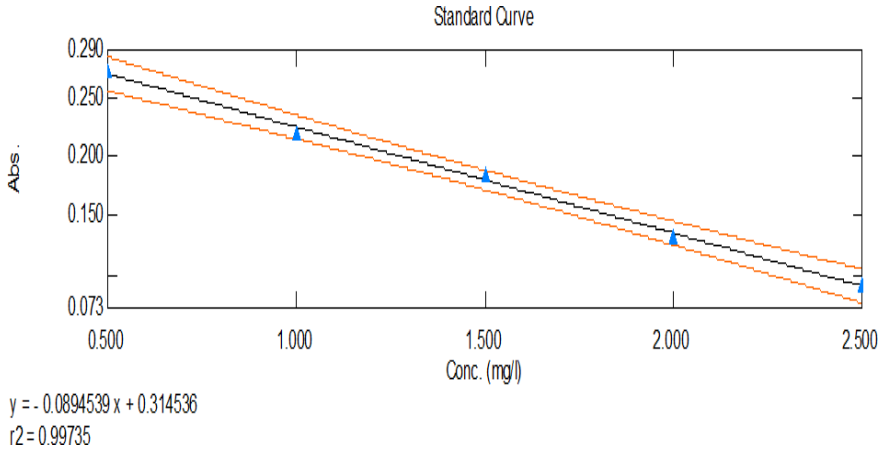


Antioxidant capacity

Standard Table of Trolox:

SL. No	Sample ID	Type	Conc	WL517.0
1	STD1	Standard	0.500	0.272
2	STD2	Standard	1.000	0.221
3	STD3	Standard	1.500	0.185
4	STD4	Standard	2.000	0.133
5	STD5	Standard	2.500	0.092

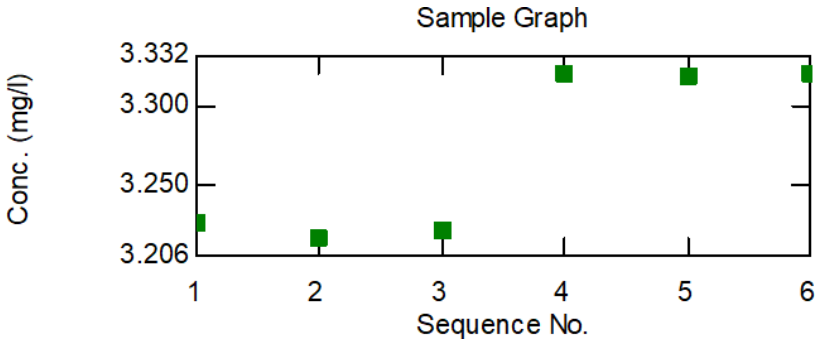
Standard Curve:



Sample Table:

SL. No	Sample ID	Type	Conc(mg/100g)	WL517.0	Comments
1	RPSP1	Unknown	3.227	0.026	Dilution Factor 2
2	RPSP2	Unknown	3.216	0.027	Dilution Factor 2
3	RPSP3	Unknown	3.221	0.026	Dilution Factor 2
4	GPSP1	Unknown	3.320	0.018	Dilution Factor 2
5	GPSP2	Unknown	3.320	0.018	Dilution Factor 2
6	GPSP3	Unknown	3.322	0.017	Dilution Factor 2

Sample Graph:



Brief Biography

Tanzila Tasmin passed the Secondary School Certificate Examination in 2012 from Dr. Khastagir Govt. Girls' High School, Chattogram. She completed Higher Secondary School Certificate Examination in 2014 from Govt. Hazi Muhammad Mohsin College, Chattogram. She earned her B.Sc. (Honors) in Food Science and Technology from Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University in Chattogram, Bangladesh. She is currently a candidate for the Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition at Chattogram Veterinary and Animal Sciences University (CVASU). She is very enthusiastic about her work to improve people's health status by providing appropriate advice and suggestions and raising public awareness of nutrition and food safety.