Chapter 1: Introduction

Guava is a well-known fruit around the world for its culinary and nutritional values (Etim *et al.,* 2020). Guava (*Psidium guajava*) is a bushes of little tree that belongs to the Myrtaceae family that is grown for its tasty nutrient rich fruits. It is one of the most common and popular fruits in our country, coming in second only to mango in terms of output. In many countries of the world like the United States, Brazil, Mexico, China, Cuba, Malaysia, India, Pakistan, Thailand, Peru, and Bangladesh are some of the world's biggest guava producers (Khatun, 2011). This fruit is called and named "Poor man's fruit" or "Tropical apple" in some countries. It is a fruit that grows both in the tropics and in the subtropics. Guava is widely grown in many countries for various consumption (Adhau and Salvi, 2014). Guavas are supposed to have originated from a region that stretched from Central America, Mexico, or northern South America all the way to the Caribbean (Morton, 1987). It was reported that in 1980 to 81 the guava production of Bangladesh was 9000 tons that rose to 506478 tons by 2018 (The daily star, 2022). Guava is eaten fresh in many places, usually by splitting into pieces or eaten like apples; it is also eaten with a touch of salt, pepper, and many other spices (masala). Guava is a popular summer snack in Taiwan, where it is offered on numerous street corners and night markets, along with packets of dried plum powder combined with sugar, salt and spices for dipping. Guava is widely eaten in East Asia with Mixes of sweet and savory dried plum powder. Guava juice is widely consumed in many countries. The fruit is also commonly used in fruit salads. Guavas are often used to produce sweets, preserves, jelly, jams, and chutneys (such as Brazilian Goiabada and Colombian and Venezuelan bocadillo), as well as a marmalade jam used over toast, due to its high pectin content (Morton, 1987). Some of the phytochemicals present in guava produce the coloring pigments of skin and flesh of the fruit, guavas which have a mixed color tone of red and orange tends to have more carotenoid and polyphenol content than which are yellow-green ones.

Psidium guajava grows in varieous countries within tropical as well as in the semitropical regions. Guava has many medicinal properties and is accepted for treating infectious disease, vertigo, skin issues, jaundice, cerebral ailments etc. It is enriched in phenolics, flavonoids, vitamins, triterpenoids, tannins, oils, essential and sesquiterpene alcohols (Altemimi *et al.,* 2017;Singh *et al.,* 2019). Guava leaves contains high level of

acid, rutin, catechin, epicatechin, isoflavonoids, and flavonoids including quercetin and guaijaverin, which have antibacterial, antioxidant and also anti-inflammatory properties (Gutiérrez-Grijalva *et al.*, 2018; Shaheena *et al.*, 2019). Though there is a large number of guava production every year, only 75-80% is directly consumed the rest is later used for making other value added products like jam, jelly, drinks, alcoholic beverage etc. which reduced the wastage (Kumari *et al.*, 2017; Jacob *et al.*,2016). Among all these products jelly is the most attractive one for its appearance and palatability.

Almost all plant components of *P. guajava* have a long history of usage in the treatment of a range of illnesses, in addition to culinary uses. Guava leaves are utilized for both medicine and food, and several research publications on their medicinal benefits, chemical composition, and pharmacological qualities have been published (Bulugahapitiya *et al.*, 2021).

By adding the extracts from the leaves of guava, a new jelly can be marked which contains more antioxidants and bioactive compound than the regular one. Focus on the needs of food producers, the current study aims to prepare rich jelly in bioactive substances. Despite the fact that it is well-researched, guava fruit is used in the making of jelly, Guava leaves, which are generally underutilized, were used in the current study. Aqueous extraction was carried out to extract bioactive components from guava leaves and their characterization has carried out with the help of Spectrophotometer. Jellies made with and without guava extract were produced in accordance with normal technique, and proximate, texture, and organoleptic analyses were all carried out to accomplish the goal.

The jelly made with guava leaf extract was proposed as a vehicle for promotion, the consumption of antioxidant-rich bioactive substances (Bulugahapitiya *et al.*, 2021). Because of the widespread evolution of microbial pathogen resistance to currently available antibiotics, the medical community is in desperate need of new antibiotic discoveries. But it has been shown that conventional plants are a superior source of antibacterial medications (Hammer *et al.*, 1999). Historical sources of new medications with anti-microbial action have ranked medicinal plants at the top. The benefits of these conventionally used therapeutic plants to human health are significant. Additionally, it is believed that plants are one of the most significant sources of secondary metabolites and essential oils (Singh *et al.*, 2010). On the other hand, the usage of medicinal herbs

has proven to be affordable, successful, and safe (Govind and Madhuri, 2006).The physiologically active, insecticidal, fungi static elements of extracted essential oils have also been demonstrated to exist. The most frequent methodology for determining antibacterial activity is the disc diffusion method. A drug that kills microorganisms like bacteria, fungus, and all protozoans or that slows their growth, is known as an antibiotic (Gadiyar *et al.,* 2017).

Different international bodies have acknowledged the growing significance of antibiotic resistance as a hazard for food safety (WHO, 2011).Given the complexity and intersectionality of the issue, cooperation and information sharing throughout the fields of agriculture, veterinary medicine, food production, and public health seem to be crucial. Because of the globalization of trade, which depends on the transportation of goods, animals, and food products, resistant bacteria have the potential to spread widely and be consumed by people all over the world. (Yang *et al.,* 2010).

Recent epidemiological studies have shown that human infections with resistant E. coli and Salmonella spp. are connected to extended illness, an increased risk of invasive disease, hospitalization, and excess mortality (Molbak, 2004). In this study, we assess the antibacterial activity of ethanolic leaf extracts of guava against pathogenic bacteria (*Escherichia coli*) to assess their potential as antibacterial agents.

Specific Objectives

i. To estimate Nutritive value and phytochemical constituent of *Psidium guajava* jelly with ethanolic guava leaf extracts.
ii. To assess shelf life of guava jelly added with ethanolic guava leaf extracts.
iii. To determine antimicrobial efficacy of ethanolic guava leaf extracts against *E.coli*.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Overview of guava

Guava (*Psidium guajava*) is a delicious fruit of the plant family Myrtaceae. Guava, a popular tree fruit of the Asian subcontinent is commonly known as 'Apple of Tropics' and local names are such as Amrud, Peru, Piyara, Koyya, Sede Pandu etc. (Zamir *et al.*, 2007). Guava fruit is commonly eaten fresh as a dessert fruit or processed as puree, juice, concentrate, jam, jelly, cheese, toffee, fruit flakes, squash, syrup, nectar, powder, wine, vinegar, ready-to-eat snacks, beverages, and dehydrated canned products (Sinha and Mishra, 2017). The root, bark, leaves, and fruit of the plant have been reported to have pharmacological properties (Seshadri *et al.*, 2020) and are used to cure a variety of ailments. malaria, gastroenteritis, spewing, looseness of the bowels, diarrhea, wounds, ulcers, toothache, sore throat, swollen gums, and a host of other symptoms have also been handled with various sections of the plants throughout herbal medicine (Biswas *et al.*, 2013).

2.1.1 Guava leaves

The leaves are opposite, oblong, three to seven inches long, and have prominent veins on the underside. Guava leaf is widely used to treat diarrhea, gastroenteritis, and other digestive issues, while the fruit of the guava has been used to raise platelets in dengue fever patients (Laily *et al.*, 2015). The guava bark is thin and has green patches on it. It is incredibly easy to dispense with it in long straps. It contains a significant volume of antimicrobial and antibacterial substances (Rahim *et al.*, 2010). Guava leaves contain variety of chemical components for example,α-pinene, β-pinene, limonene, menthol, caryophyllene (Thome *et al.*, 2019).

Figure 2.1 Guava (*psidium guajava)* with leaves

2.1.2 Origin and Taxonomy of guava

The guava plant (*Psidium guajava L.*) belongs to the Myrtaceae family. *Psidium guajava* is the most important fruit of the genus *Psidium*, which contains around 150 species. Guava is thought to have originated from an area extending from southern Mexico to or through Central America (Singh, 2011). Guava is widely known and cultivated throughout the world (Paull and Duarte, 2011).

Kingdom:	Plantae
Order	Myrtales
Family:	Myrtaceae
Subfamily:	Myrtoideae
Genus:	Psidium
Species:	Guajava
Binomial name	*Psidium guajava Linn*

2.2 Utilization and economic importance of guava and guava leaves

2.2.1 Nutritional value of guava

This delicious fruit contains nutrients. Because the fruit is high in carbohydrates, proteins, lipids, minerals, and other nutrients, it may help lower the risk of malnutrition (Youssef and Ibrahim, 2016). Among other popular fruits, guava is a good source of protein (2.3%), carbohydrates (12.16%), and dietary fiber (4.8%), according to the USDA's Food Data Central. Guava is a rich source of calcium, with 17.63 mg per 100 g. Furthermore, Guava is a good source of ascorbic acid, with 241, 86 mg/100 g, making it, along with other fruits, a prospective source of discovery and added value in food items (Bogha *et al.*, 2020).

Guava is high in lutein, zeaxanthine, and lycopene. It contains a lot of flavonoids, fructose sugar, and carotenoids (Das, 2011). Fruit is high in minerals like calcium, phosphorus, and iron, as well as vitamins like niacin, pantothenic acid, thiamin, riboflavin, vitamin A, and vitamin E. (Reddy, 2017). Guava includes polyphenolic components as well as carotenoids, which contribute antioxidant pigments to the

fruit, making it one of the most antioxidant-rich fruits (Omayio *et al.*, 2019). Guavas include essential oils, phenols, triterpenes, saponins, flavonoids, lectins, fiber, and pectin, as well as fatty acids. Among the polyphenol and glycoside esters present in guava fruit (Medina guava) are caffeic, coumaric, ferulic, cinnamic, ellagic, and rosmarinic acids. (Herrero *etal.*, 2016). Flavonoids discovered include myricetin, naringenin, epicatechin, quercetin, rutin, and apigenin (Vijaya Anand *et al.*,2020). Guava leaves contain a wide range of chemical components, including -pinene, -pinene, limonene, menthol, caryophyllene, -bisabolene, farnesene, humulene, selinene, cardinene, and curcumene, mallic acids, -copanene, -sitosterol, cineol,quercetin, tannin, guajavolide and 2-ene flavonone-2 (Thome *et al.*,2019).

2.2.2 Utilization of different part of guava

Guava is used to make a variety of processed items, including drinks, syrup, ice cream, jams, jellies, toffee, juice, and dehydrated and canned products. Additionally, different parts of guava being utilized for different pharmacological applications.

2.2.3 Food utilization

The guava has incredible potential for broad business use because of its simplicity of development, high nutrient content, and processing in a variety of consumer products (Kocher, 2011). There are a variety of products that can be made from guava processing including guava jam, juice, pulp, jellies, chocolate, wine and guava powder (generally utilized in the preparing of yogurt) and spray soluble guava extracts containing high antioxidants concentrations (Kadam *et al.*, 2012).

Guava Jams and Jellies

Jam is a semi-solid mixture obtained by cooking a fruit soft tissue with sugar. Jam is an in-between moisture food prepared by boiling fruit pulp with sugar (sucrose), pectin, acid and other ingredients (additive, coloring and flavoring materials) to a rationally thick evenness, firm enough to hold the fruit tissues in position (Rahman *et al.*, 2018).

Guava jelly is made from slightly ripe guava fruits. Jelly is a semi-solid product that is prepared by boiling clear strained fruit extracts free of pulp after adding the required amount of sugar, citric acid and pectin. It should have a total soluble solids content of at least 65 percent and a fruit portion of at least 45 percent (Jolhe *et al.*, 2020). The fruit is sliced into little pieces and cooked for around 45 minutes at low temperatures with utilizing similar measure of water and the juice extricated by filtration utilizing sifters

or muslin cloth (Kuchi *et al.,* 2014). Sugars are added to the extracted juice until it is boiled to 105 °C or a layer is formed by cooling a little segment in a spoon. The measure of sugar utilized differs relying upon the pectin content of the extracted juices, going from 0.5 kg sugar/kg juice to 0.75 kg sugar/kg juice for pectin rich juice and low-pectin juice respectively. After that, hot filling into clean and sterilized containers (Swer *et al.,* 2019).

Guava leathers

Guava leather is prepared by dehydrating the purée of fruit into a leather sheet. Leathers can be eaten as a confection or cooked to make a sauce. In the tropics, there is a scarcity of knowledge on the chemical and organoleptic properties of guava leathers. Guava leather has a higher protein and fat content. This was also noted in ash content with pawpaw leather (2.67 per cent) and guava leather (2.87 percent) (Kanwal *et al.,* 2016).

Guava Juice and Nectars

The Fresh fruits or the pulp of the guava are used to produce guava juice. Juice is separated by squeezing the guava fruit with hydraulic filter press or from the pulp after dilution with water and resulting filtration. The juice is generally not clear and requires the use of pectic enzymes to make it smoother and easier to clean. Studies by Imungi showed that the ideal conditions for removing guava juice utilizing proteolytic enzymes from Kenyan guava were 400 ppm compound at a temperature of 45-50 degree C for an hour and a half (90 minutes).

Nectars- Water is added to guava pulp or fresh juice to produce nectar. Allowed added substances or sweetners, just as sugar could conceivably be added yet the items should have at least 8.5° Brix, 25.0 percent of the guava puree or juice and acidity 0.15 percent at a pH of 3.4 – 4.8 (Omayio *et al.,* 2019).

Guava wine

The wine made from guava (*Psidium guajava* L.) is the result of anaerobic yeast fermentation, in which the sugars are converted to alcohol and carbon dioxide (Sevda and Rodrigues, 2011).

Guava leaf tea

The leaf of guava was extracted with hot water and yielded 14 percent guava leaf extract. In a summary, 100 g of dried leaves were mixed with 2 L of distilled water

(DW) and decocted at 80^0C for 30 minutes. The extracted solution was freeze dried after filtering through four layers of gauze to remove leaves (Kaneko *et al.,* 2013).

Guava leaf powder

The guava leaves were collected and manually shredded into small pieces. Subsequently, the biomaterial was washed a few times using ordinary tap water.

2.3 Medical and health benefit of guava and guava leaf jelly

2.3.1 Anti-diabetic

Blood glucose levels have been found to *Psidium guajava*. Guava fruit extract has been shown to help diabetes patients lose weight and control their blood sugar levels. Guava fruit extract was given at portions of 125 and 250 mg/kg to Streptozocin-incited diabetics. Guava Fruit Extract reduces the depletion of insulin-positive beta cells and insulin release by protecting pancreatic tissues, including islet beta cells, from oxidation. (Huang *et al.,* 2011). Quercetin, kaempferol, myricetin had inhibitory effects on sucrose, maltase, and α-amylase (Wang *et al.,* 2010).

2.3.2 Anti-inflammatory activity

Germ contamination and thymus production have been shown to be blocked by guava extract in ethyl acetate. It has the potential to serve as an antiviral agent. It has the ability to improve mRNA expression. Guava may alter the work of the heme oxygenase-1 protein. Because of this, it can be used as an anti-inflammatory agent for the skin. The lipopolysaccharide that develops nitric oxide is inhibited by guava extract in ethanol. The outflow of E2 is inhibited. In this way, it serves as an anti-inflammatory agent (Jang *et al.,* 2014).

2.3.3 Anticancer effect

A few examinations have shown that therapeutic plants of *Psidium guajava* affect human epidermal carcinoma and murine leukemia cells. (Lee and Park, 2010).

2.3.4 Antiviral Activity

Antiviral movement of guava extracts was resolved against development of A/Narita/1/2009 (amantadine-safe pandemic strain 2009) with an IC50 of 0.05 percent and development of A/Yamaguchi/20/06 (touchy strain) and A/Kitakyushu/10/06 (oseltamivir-safe strain). The development of these strains has been firmly hindered by

guava extracts. Guava tea has been shown to be effective against flu infection and has additionally been appeared to build up viral tolerance in the body (Sriwilaijaroen *et al.*, 2012).

2.3.5 Guava for Cold and Cough

Guava leaves have been shown to be beneficial in the treatment of colds and coughs. Guava is high in ascorbic acid and iron, which helps to relieve lung inflammation and mucous production while also keeping the respiratory tract free of pathogens. Fruit, especially raw fruit, or a decoction made from tender immature leaves, is extremely beneficial in the treatment of cold and cough. Because of its astringent properties, it acts by disintegrating mucus polymers, loosening cough and reducing more mucus intake, keeping the respiratory tract, mouth, and lungs free of bacteria, and inhibiting current microbial behavior. Guava has a high concentration of vitamin C, and has been shown to be very helpful in treating colds and coughs caused by bacteria or viruses. In many Indian villages, roasted ripe guava is used as a home remedy for severe cases of cough, cold, and congestion. Another study found that a hydro extract of *Psidium guajava* leaves significantly reduced coughing frequency caused by capsaicin aerosol within 15 minutes of administration when compared to a control (Kafle *et al.*, 2018).

2.3.6 Antidiarrheal activity

With the aid of antibacterial assays (microtiter plate-based assay), effect on bacterial colonization. It had been reported; Decoctionof leaves had antibacterial activity against *Vibrio cholerae* and *Shigella flexneri*. The antiglycative potential of guava leaves was investigated, and it was discovered that the extract reduced the production of advanced glycation end-products in vitro (Soman S, 2010). Gallic acid, catechin, and quercetin all displayed more than 80% inhibitory activity, whereas ferulic acid had no action. Diarrhea inhibition was dose-dependent and comparable to standard medication. The gastro intestinal motility of extract was also reduced, as determined by the distance traveled by a charcoal plug in the small intestine. The anti-diarrheal effect of extract was thus mediated by a combination of antimicrobial activity and a decrease in gastrointestinal motility (Wu, 2009).

2.3.7 Healing and cytotoxic effects

It has been reported that guava leaf extracts have potential wound healing activity. Reports are on the *in vivo* clinical and histological evaluation of traumatic lesions in the oral mucosa of rats treated with selected substances (Ravi and Divyashree, 2014).

2.3.8 Antimicrobial efficacy guava leaves

Guava (*Psidium guajava L*.) has long been used to cure a variety of ailments. Guava leaf is extensively used in Indonesia to treat diarrhea, gastroenteritis, and other digestive symptoms, while Guava fruit has been utilized to boost platelets in dengue fever patients. Many investigations have been conducted to scientifically show the efficacy of guava leaf therapy. Among them were the advantages of guava leaf as a treatment for arthritis in animals using hydro alcoholic extract (Jahagirdar *et al.,* 2010). Another study found that the flavonoids in guava leaf extract had antibacterial characteristics, while the quercetin in the extract has antidiarrheal qualities. One of the most prevalent flavonoids identified in guava leaf is quercetin. It has the ability to calm intestinal smooth muscle and prevent bowel spasms (Joseph and Priya, 2011). In vitro research involving leukemia cells, guava leaf extract demonstrated anti proliferative efficacy. Its activity was 4.37 times greater than that of vincristine. Furthermore, guava leaf water extract has been shown to be effective against a variety of microbiological strains and to have anti-rotavirus action (Mittal *et al.,* 2010). The capacity of guava leaf extract to treat many ailments has been scientifically demonstrated, however the mechanism has yet to be thoroughly described. In general, the biological features of guava have been linked to polyphenolic substances such as protocatechuic, ferulic, ascorbic, gallic, and caffeic acids, as well as quercetin (Denny *et al.,* 2010). Polyphenols are plant secondary metabolites. There has been a lot of attention in the possible health advantages of dietary plant polyphenols as antioxidants during the last decade (Pandey, and Rizvi, 2009). The polyphenol chemicals in guava fruit and leaf extract can function as an immune stimulant, leading to an increase in the immune system. Boosting the immune system can protect the body against a variety of infectious illnesses. A robust immune system is essential for staying healthy. As a result, the ability of natural compounds to enhance the immune system has long been studied (Devasagayam, and Sainis, 2002). Many synthetic and natural medicines claiming to be immunostimulants were available. They appeared to be a viable alternative to immunization and chemotherapy in the

treatment of disease. Immunostimulants derived from natural compounds may improve particular immune responses (Abasali and Mohamad, 2010).

2.4 Phytochemical component of guava and guava leaves

2.4.1 Total phenolic compounds (TPC)

Guava fruit

TPC was determined using the Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). Samples (triplicate) of 0.3 mL were introduced into test tubes followed by 1.5 mL of Folin Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexes, covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give a reading of less than 1. Total phenol contents were expressed in GAE (Gallic Acid Equivalents) (mg per 100gram fresh fruit). The gallic acid standard line has the equation $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$), where y is absorbance at 765 nm and x is concentration of gallic acid in mg/L.

The total phenolic content of guava is 247±100mg GAE/100gm (Lim *et al.*, 2006).

Leaf

Total Phenolic and Flavonoid Contents in Different Solvent Extracts of Guava Leaf-

The total phenolic and flavonoid content of a variety of solvent extract derived from guava leaf was examined. In various solvent extracts such as ethanol/water (1: 1, v/v), methanol/water (1: 1, v/v), ethanol, water, and methanol, the yield of guava leaf extracts (GLE) was determined to be 5.5 percent, 5.1 percent, 4.6 percent, 4.4 percent, and 4.1 percent, respectively (Jayachandran *et al.*,2018).

2.4.2 Antioxidant and Antioxidant activity of guava fruits and guava leaves

Antioxidant

Antioxidants, either naturally produced in situ (endogenous antioxidants) or externally provided through foods, are one of the body's methods for combating oxidative stress (exogenous antioxidants). Antioxidants help to prevent disease by neutralizing excess free radicals, protecting cells from their damaging effects, and neutralizing free radicals. Antioxidant supplements are substances that are extracted from natural sources or synthesized chemically. Naturally, they do not have the same chemical make-up as natural antioxidants found in foods. As a result, there is disagreement regarding whether

antioxidant supplements provide the same health advantages as antioxidants found in meals. Even while antioxidant supplementation is a hot topic of debate and is becoming more popular in many developed nations, the evidence is still equivocal. Although various epidemiological statistics imply that antioxidants may have a beneficial effect on many chronic diseases, systematic supplement use is hampered by a number of variables, including a lack of prospective and controlled trials, long-term effects, and dosages required for different conditions. Antioxidant supplements can also act as pro-oxidants, such as oxidative stress inducers, if eaten in quantities much exceeding the recommended dietary intakes (RDI) (Pham-Huy *et al.,* 2008).

Antioxidant activity

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2.4.3 Flavonoids

Flavonoids are a kind of polyphenol found in many different plant species. They feature more than one benzene ring in their structure and have been proved in multiple studies to be potent antioxidants and free radical scavengers. The parent chemicals are formed from Flavonoids are pigments found in higher plants that number over 4,000.

Flavonoids including quercetin, kaempferol, and quercitrin are found in about 70% of plants. Other flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones, catechins, and leucoanthocyanidins.

The total phenolic and flavonoid content of a variety of solvent extract derived from guava leaf was examined. In various solvent extracts such as ethanol/water (1: 1, v/v), methanol/water (1:1, v/v), ethanol, water, and methanol, the yield of guava leaf extracts (GLE) was determined to be 5.5 percent, 5.1 percent, 4.6 percent, 4.4 percent, and 4.1 percent, respectively (Jayachandran *et al.*,2018).The phenolic content of guava from two cultivars was shown to be dependent on the concentration of ABTS (**2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)** radical, which might be attributed to its hydrogen donating capacity. A linear rise in antioxidant activity was seen with increasing phenolic contents in two types of guava plants evaluated. The correlation coefficient (R^2) between phenolic concentrations and ABTS scavenging activity in guava cultivar peels was reported to be 0.993 (Aldabab) and 0.971 (Guava) (Anaa). For all guava cultivars tested, a linear increase of antioxidant activity was detected with increasing the phenolic concentrations (Almulaiky *et al.,* 2018).

Chapter 3: Materials and Methods

3.1 Study Area

The experiment was conducted in the laboratory of the department of Applied Food Science and Nutrition, Applied Chemistry and Chemical Technology, Department of Food Processing and Engineering, Poultry Research and Training Center (PRTC), Department of Animal Science and Nutrition and Department of Physiology, Biochemistry and Pharmacology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram.

3.2Study Duration

The experiment was conducted for a period of six months from 1st December 2021 to 20th May 2022.

3.3 Experimental Design

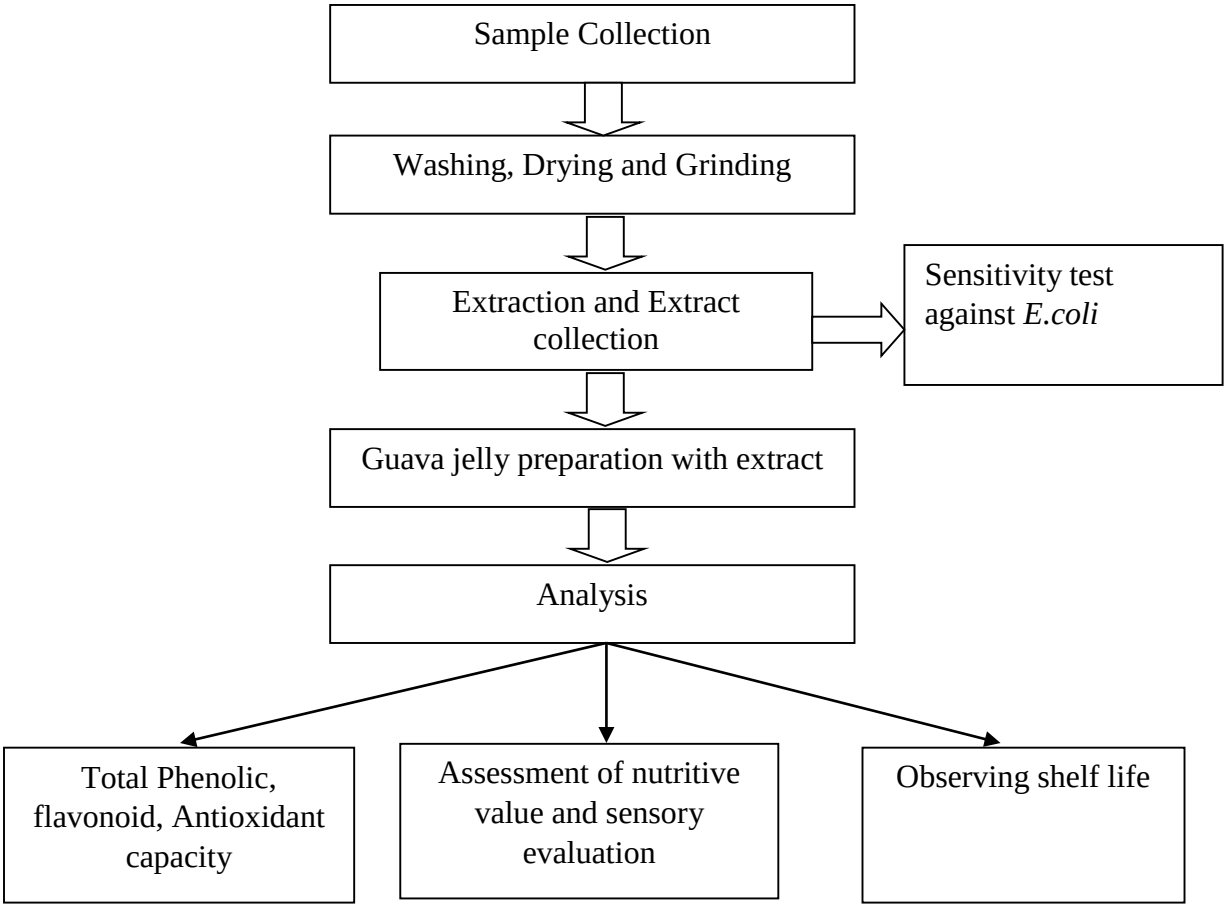


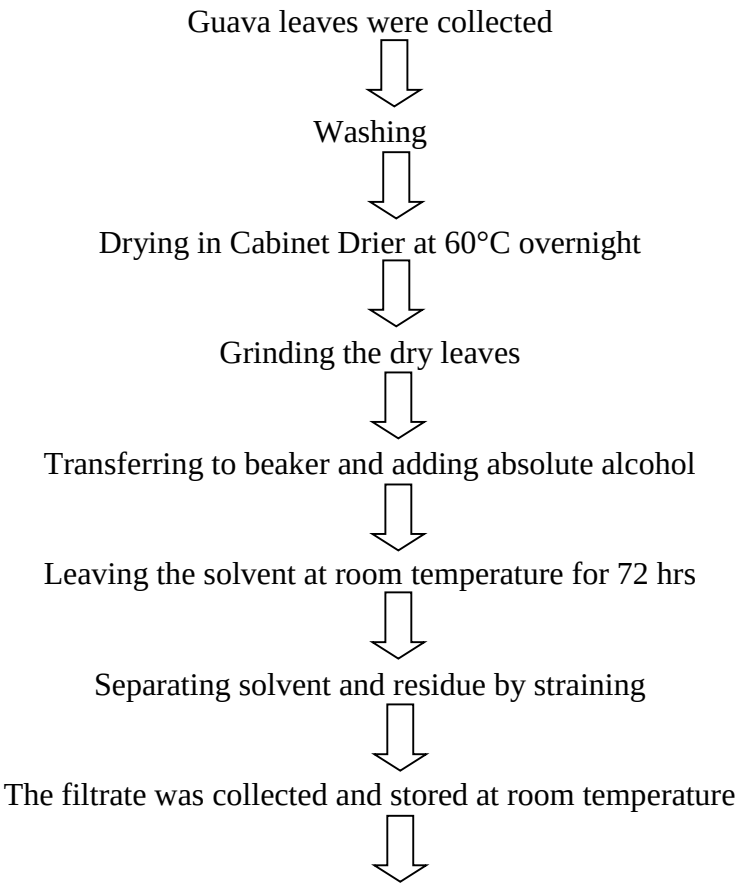
Figure 3.1 Stepwise design for the experiment

3.4 Collection of Sample Materials

Mature Guava (*Psidium guajava*) was collected from the Chattogram local market and guava leaves were collected from CVASU as experimental source material. Because the pectin concentration of guavas is dependent on maturity. The guavas were carefully chosen to ensure the desired maturity. From a scientific and surgical supply store, sugar, pectin, and citric acid were purchased. Other necessary supplies for the experiment were obtained from the laboratories.

3.5 Guava Leaves Extract Preparation

Guava leaves were collected. Collected guava leaves were washed with water, chopped into small pieces, transferred into respective beakers, added with absolute ethanol, and left to shake on a shaker for 72 hrs. at room temperature. The solvent was then separated from the plant residue by straining. The filtrate was collected and stored at room temperature while the residue was re extracted twice, each time with fresh solvent. Finally, all the filtrates were combined and evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4 °C until further analysis.



Collecting crude extract

Weighing and storing the crude extract at 4°C

Figure: 3.2 Extraction Procedures of Guava Leaves

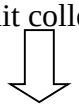
3.6 Methods of Sample Preparation

Preparation of Control Jelly (Sample A)

Guava jelly was prepared according to the preparation procedure described by Srivastava and Kumar (2005). 1 kg of mature guavas are used to preparation of jelly. The mature guavas were washed with de-ionized water to remove the external particulate or ions that could interrupt in nutrient analysis and were cut into small pieces with a stainless steel knife for easy boiling. The thin slices are boiled with water and one and half times the weight of fruit for about 30 minutes with stirring.

During boiling 3g per kg of fruit citric acid is added. The boiled pieces were crushed and strained the extract through a thick cloth to remove the suspended matter consisting of fruit tissue, seed, skin, gums and protein in colloidal form. The amount of guava juice, water, pectin, acid and sugar were calculated according to the formulation. Pectin test is done for addition of sugar. The juices of guava are boiled with the use of 1.5kg of water and sugar was added at a ratio of 1:1. Pectin was applied in product at 1%. Heating was continued with stirring. The end point was indicated by 67−68ºBrix TSS in the mixture which was measured by Refractometer. The Jelly was then filled in a glass jar. It was then covered with melted wax and cooled. After cooling the cans or jars are labeled and stored for further studies.

Guava fruit collection



Washing in cold water

Cutting into thin slices

Boiling for around 30 minutes (with water, one and a half times of fruit, stirring occasionally)

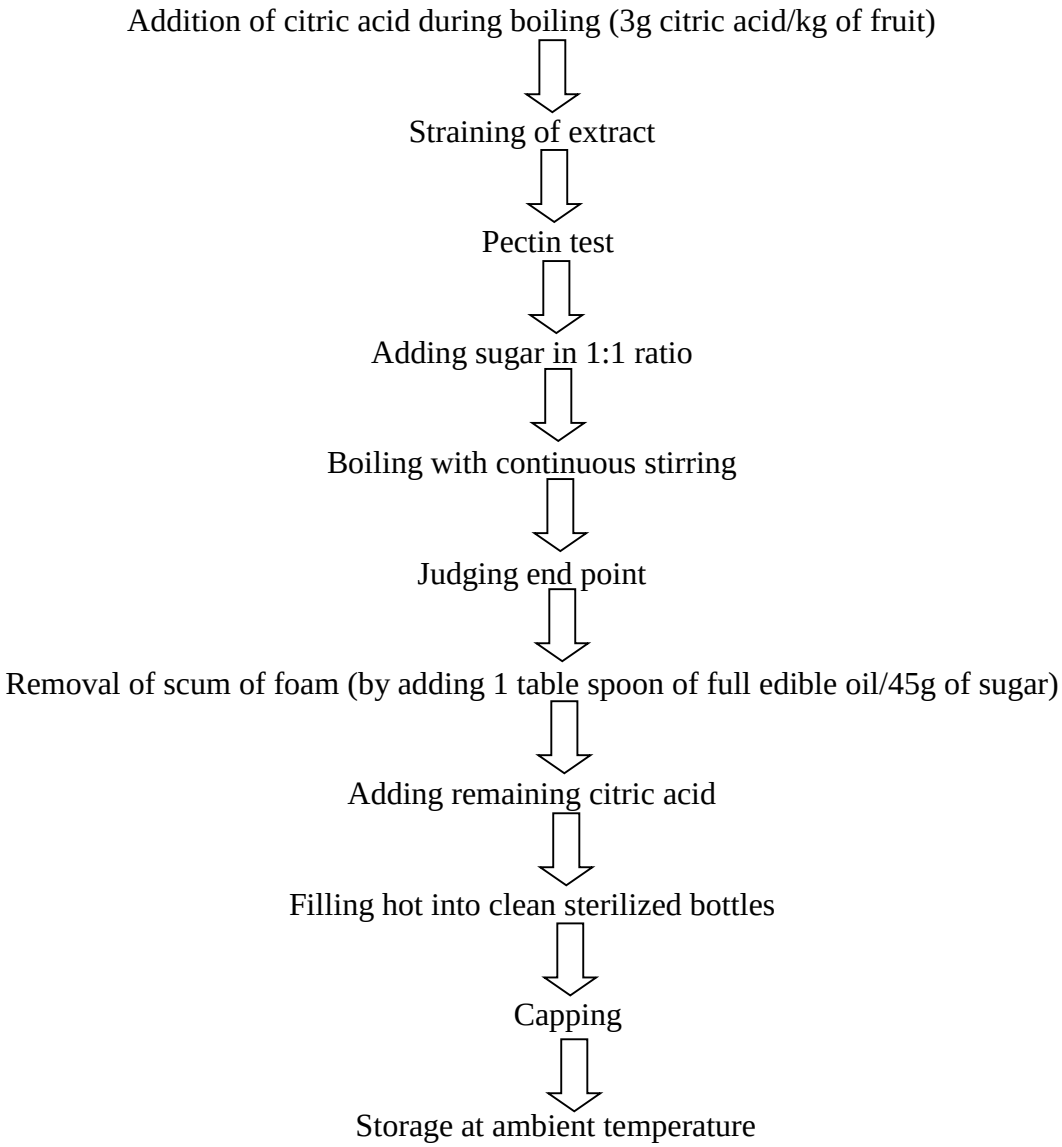


Figure: 3.3 Flow–sheet of Guava Jelly Preparation (Control) (Srivastava and Kumar, 2005)

Preparation of Treatment Jelly (Sample B, C and D)

Guava jelly was prepared according to the preparation procedure described by Srivastava and Kumar (2005). 1 kg of mature guavas are used to preparation of jelly. The mature guavas are washed with de-ionized water to remove the external particulate or ions that will interrupt in nutrient analysis and cut into small pieces with a stainless steel knife for easy boiling. The thin slices are boiled with water and one and half times the weight of fruit for about 30 minute with stirring.

During boiling 3g per kg of fruit citric acid is added. The boiled pieces were crushed and strained the extract through a thick cloth. The amount of guava juice, water, pectin, acid and sugar were calculated according to the formulation. Pectin test is done for addition of sugar. The juices of guava are boiled with water and sugar at a ratio of 1:1. Pectin was applied in product at 1%. Heating was continued with stirring. Treatment applied in use of adding guava leaves extract with three different percentages. 5%, 10% and 15% guava leaf extract were added to treatment jelly. The end point was indicated by 67−68ºBrix in the mixture which was measured by refractometer. The Jelly was then filled in a glass jar. It was then covered with melted wax and cooled. After cooling the cans or jars are labeled and stored for further studies.

Hence, the three samples could be denoted as,
Sample A - Treatment guava jelly with addition of 5% guava leaf extract
Sample B - Treatment guava jelly with addition of 10% guava leaf extract
Sample C - Treatment guava jelly with addition of 15% guava leaf extract

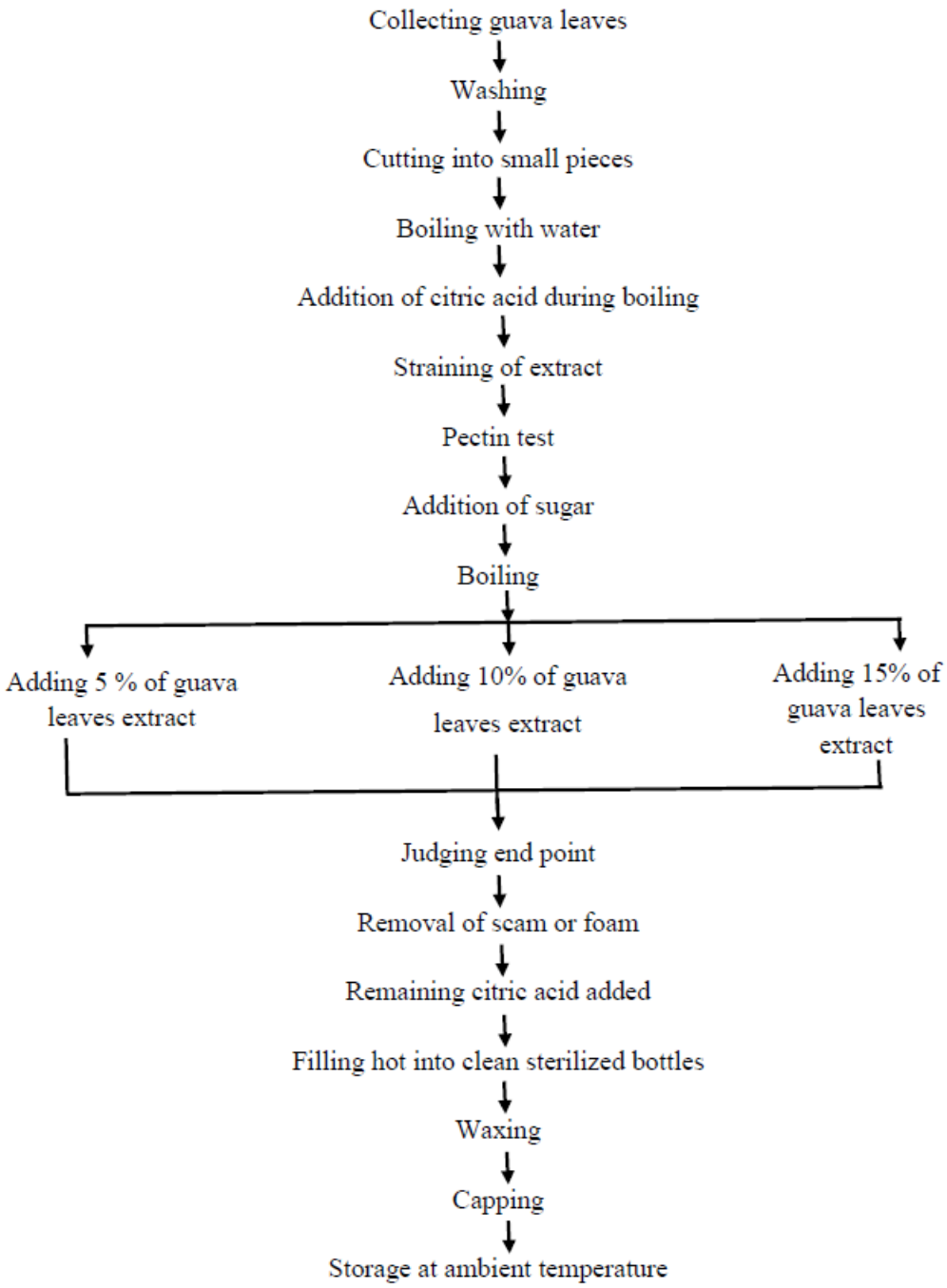


Figure 3.4 Flow–sheet of Guava Jelly Preparation (Sample B, C, D)

3.7 Physicochemical Analysis of Guava Jelly

Matured guava jellies were analyzed for moisture, total solid, ash, total soluble solid, pH, titratable acidity as per the methods of AOAC (2016). These samples were also analyzed for proximate analysis, bioactive compounds analysis and antioxidant analysis.

3.7.1 Determination of pH

Measurement of pH for aqueous solutions was done with a glass electrode and a pH meter, or using indicators. pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution (McClements and Decker, 2009).

3.7.2 Total Soluble Solids (TSS)

Total soluble solids of the fruits were found out with the help of hand refractometer. Total soluble solids (TSS) were directly recorded by digital refractometer and the results were shown as percent soluble solids (Brix) (AOAC, 2016).

3.7.3 Titratable Acidity

The percentage of acidity was determined in terms of anhydrous citric acid by titrating against N/10 NaOH using phenolphthalein indicator. Every time 10ml of juice was taken in a 100ml volumetric flask and the volume was made up to 100ml by adding distilled water then 10ml diluted juice was titrated against N/10 NaOH, using phenolphthalein as indicator. The appearance of pink color indicates the endpoint of the titration. Titration was reported thrice at the average value was recorded (AOAC, 2016). Tittratable acidity can be determined as bellow:

$$\text{Titratable acidity (\%)} = (T.V \times \text{Factor})/W$$

Where

TV = Titer value of the sample in ml

W = Quantity of the sample taken for the test in ml

Factor - Citric acid: 0.0064 (Citrus Fruit); Malic Acid: 0.0067

3.7.4 Determination of Vitamin C

Generally, Vitamin-C is determining in plant or animal extract by its reducing action on the dyes stuff 2, 6 dichloride phenol indophenols. In this matter, Vitamin C oxidized by the color dye to the dehydro-ascorbic acid. At the same time, the dye is reduced to the color less compound. S that end point of the reaction can easily determine. (AOAC, 2016).

Reagent Requirement

A) Dye Solution

- 260 mg of dye (2, 6-dichlorophenol indophenols)

- 210 mg of NaHCO3 dissolved in 100 ml of distilled water.

B) Metaphosphoric acid solution (3%)

- 15/7.5mg of Metaphosphoric acid.
- 40/20ml of glacial acetic acid dilutes to make 500/250 ml with distilled water.

C) Standard ascorbic acid solution

50/25 mg of crystalline ascorbic acid dissolved in 500 ml/250ml of metaphosphoric acid solution.

Procedure

- Dye solution was taken in the burette up to 0 marks.
- Then 5 ml Vitamin C solution was taken in a conical flask.
- The conical flask was placed under the burette and the dye was added drop wise.
- Titration was completed when pink color was appeared and stayed for 20 seconds and then disappeared.
- The reading was taken at least 3 times.
- The same procedure was performed for ascorbic acid solution of unknown concentration.
- The result was expressed as milligram percentage (mg %)

3.7.5 Moisture Content

Moisture content was determined by using the standard procedure of the Association of Official Analytical Chemists (AOAC, 2016).

Calculation: The percent of moisture was calculated as follow

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \times 100$$

3.7.6 Total Solids

Total solid was determined by methods of AOAC (2016). Percent total solid content was calculated by using the data obtained during moisture estimation using the following formula:

% Total solids = 100 – % moisture content.

3.7.7 Ash Content

Ash content was determined by methods of AOAC (2016). Ash content is the inorganic residue remaining after destruction of organic matter. 10 gram dried jam was taken in a pre-dried weighed crucible. It was then burned to charcoal. The charcoal was then taken in a muffle furnace and heat at around 600°C for 4 hours till the charcoal was completely removed. The crucible was then taken out of the furnace. Cool it in a desiccator carefully and then weighed. Calculation: The ash content was calculated by the following expression.

$$\text{Ash } \% = \frac{\text{The amount of the ash supplied sample}}{\text{Sample weight}} \times 100$$

3.7.8 Estimation of Crude Fat

Principle: Fat is estimated by dissolving food samples into organic solvents (chloroform: methanol) separating the filtrate by filtration. Placing the filtrate into separating funnels and then separated mixture is then dried to measure the extract and finally, the percentage of fat is estimated. AOAC (2016) methods using a soxhlet apparatus were used to determine the crude fat content of the samples.

Calculation: The percent of crude fat was expressed as follows expression.

$$\text{Fat } \% = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.7.9 Estimation of Crude Protein

Principle: The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also, the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples. It is an official method and it is described in different normative such as (AOAC, 2016).

Calculation

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process.

In the equations below, “N” represents normality. “ml blank” refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When boric acid is used as the receiving solution the equation is

$$\text{Nitrogen}\% = \frac{(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of the sample in gram}}$$

3.7.10 Estimation of Crude Fiber

Crude fiber is the water-insoluble fraction of carbohydrate consists mainly of cellulose, hemicelluloses, and lignin. It is estimated through digestion of fat-free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in a weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained. The crude fiber was determined according to the AOAC method (2016). Then, ignited the residue in muffle furnace up to white ash (550-600°C, 4-6 hrs).

Calculation

Calculation of the crude fiber percentage as follows:

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

Where,

W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

3.7.11 Determination of Total Carbohydrate

The carbohydrate content was determined by calculating the difference between the Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a total of the other proximate components.

Calculation: Hence it was calculated using the formula below-

% CHO = 100% - % (Protein + Fat + crude fiber + Ash + Moisture content)

3.8 Determination of Antioxidant capacity by DPPH scavenging method

Extract Preparation

- Taking 5gm of sample in falcon tube
- Adding 10ml absolute methanol and left for 72 hours
- Straining the solvent
- Collection of filtrate
- Evaporation at 60^0 c using rotary evaporator
- Collect methanolic extract

Procedure

Antioxidant mobility of the extracts was determined using DPPH assay as the process described below with slight modifications. About 6 mg of DPPH was dissolved in 100 mL absolute methanol and prepared methanoic DPPH solution. Then 1 ml methanoic extract was diluted with of 2 ml DPPH solution. Then the mixture was mildly shaken and left for 30 min in dark at room temperature. The absorbance was read at wavelength 517 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, and USA). Control prepared by mixing 1 mL of methanol with 2 mL of DPPH solution whilst methanol was used like a blank. The scavenging mobility was measured as the decrease in absorbance of the samples in comparison with the DPPH standard solution. Antioxidant capability based on the DPPH free radical scavenging mobility of extracts calculated using the following equation:

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

Trolox was used as standard and TEAC composite (Trolox equivalent antioxidant mobility) was used for the calibration standard curve. The results were revealed in mg/ 100 g of Trolox equivalent per gram of powder on a dry weight (DW) base (Azlim Almey *et al.,* 2010**).**

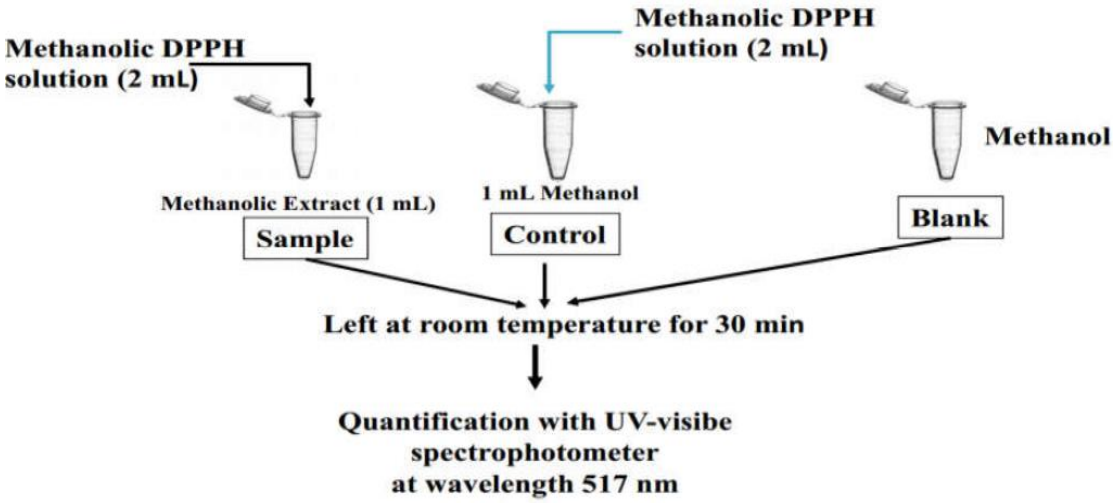


Figure: 3.5 Determination of Antioxidant Capacity

3.9 Determination of Bioactive compounds

Extract Preparation

- Taking 5gm of sample in Falcon tube
- Adding 10ml absolute ethanol and left for 72 hours
- Straining the solvent
- Collection of filtrate
- Evaporation at 60^0 c using rotary evaporator
- Collect ethanolic extracts

3.9.1 Total Phenolic Content (TPC)

TPC of the extracts were determined according to the Folin-Ciocalteu reagent method described with slight modifications (Al-Owaisi *et al.*, 2014). Total polyphenol content (TPC) of the guava jam determined according to the Folin-Ciocalteu method reported by Vergani *et al.* (2016) with slight modifications. 1 ml ethanoic extract was taken in a falconer tube and added 1.5 ml of FC reagent and left for 3 mins at room temperature. Then 1.5 ml Na_2CO_3 (7.5%) was added into the mixture and left for 60 minutes. The absorbance was read at wavelength 765 nm using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) and C_2H_5OH was used as the blank .TPC was calculated and revealed as mg of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

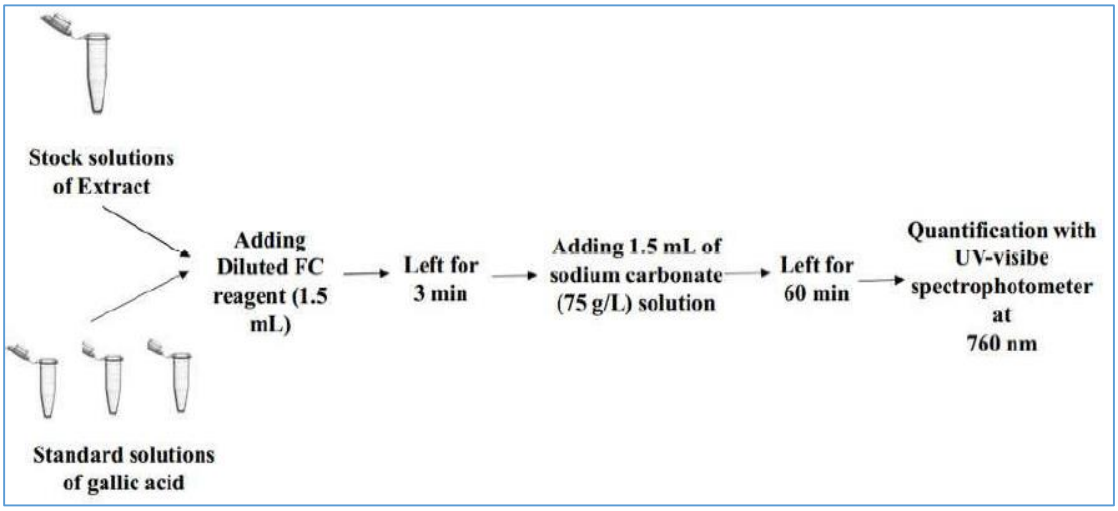
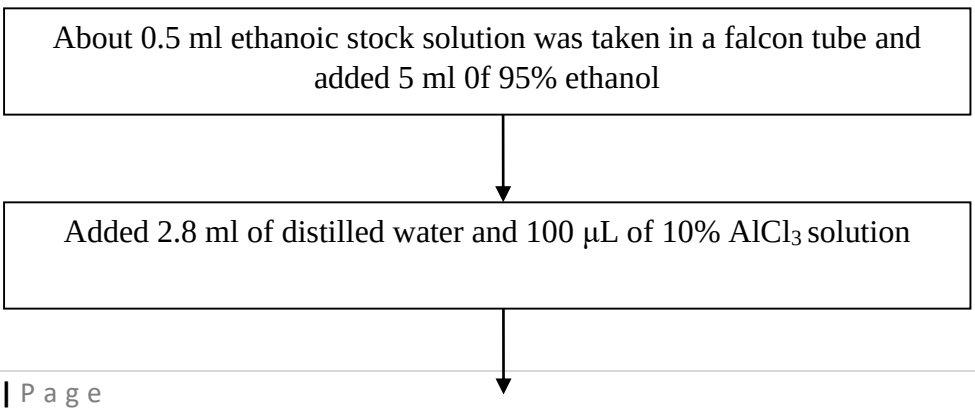


Figure 3.6 Total Phenolic contents (TPC) determination procedure flow

3.9.2 Total Flavonoid content (TFC)

Total flavonoids content (TFC) of the samples was determined by using the aluminum chloride colorimetric process reported by Chang *et al.*, (2002) with slight modifications. Stock solutions (1 mg/mL) of extracts were prepared and aliquots of 0.5 mL of diluted extract diluted with 1.5 mL of 95% C_2H_5OH in a cuvette. Then 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 mixture in the cuvette. The mixture left at room temperature for 30 min. The absorbance was read at wavelength 415 nm in UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA) and 10% aluminium chloride substituted with distilled water of the same quantity were used as the blank. Total flavonoids amount in the sample was calculated by comparing absorbance of the sample extracts with a quercetin standard curve. TFC estimated and revealed as mg quercetin equivalents (QE) per gram of extract (mg QE/g).



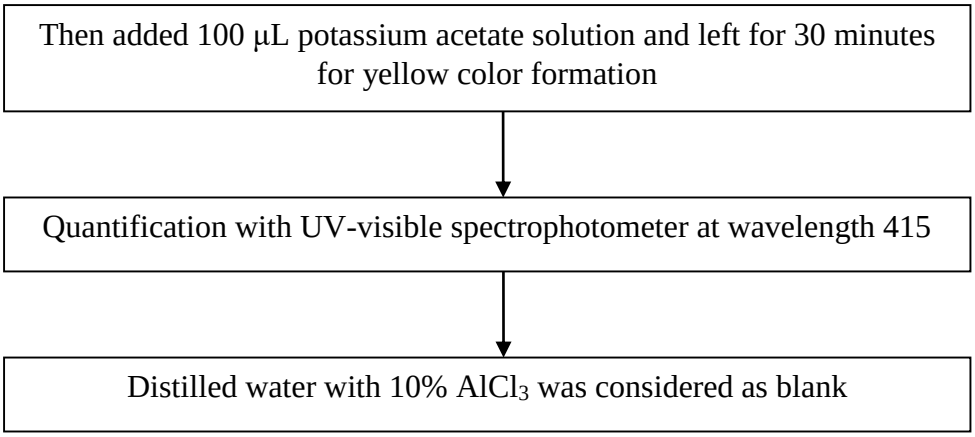


Figure 3.7 Total Flavonoid Contents (TFC) Determination Procedure Flow Diagram

3.10 Microbiological analysis

3.10.1 Aerobic plate count (Bacterial plate count)

The Aerobic Plate Count is used as an indicator of bacterial populations on a sample. Aerobic Colony Count (ACC), Standard Plate Count (SPC), Mesophilic Count and Total Plate Count (TPC) are different names of Aerobic Plate Count (APC). Total viable bacterial count (TVC) was determined through the Standard Plate Count (SPC) technique.

The test is based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. It is a generic test for organisms that grow aerobically at mesophilic temperatures (25°C to 40°C), not a measure of the entire bacterial population. APC cannot differentiate types of bacteria can use to gauge organoleptic acceptability, sanitary quality, adherence to good manufacturing practices and as an indicator of safety. Information regarding shelf-life or impending organoleptic change in a food can be provided by APC (Banwart, 2012).

Sample preparation

25 g of this well mixed guava jelly were taken in 250 ml flask. Phosphate buffer saline (0.6 M KH_2PO_4 of pH 7.2) was used for dilution of the sample. About 100 ml of the buffer saline was added to the beaker and mixed well by to-and-fro movement. The volume was made up with the same buffer water. All the apparatus, solutions and other tools used should be sterilized i.e. heated at 121^0 C for 15 minutes. The prepared sample

was then diluted to 10 times i.e. 1×10-1 time's dilution and used as stock solution (Andrews, 1992).

Dilution

A series of dilution were made as follows using 9 ml blanks. The initial 1/10 dilution (1 ml in 9 ml) was performed (b). This was mixed in a vortex mixer (c) 1 ml from (b) was taken, added to the next tube and mixed well. It was become 10-2 time's dilution. In this way, the dilution was made up to 10-6 times.

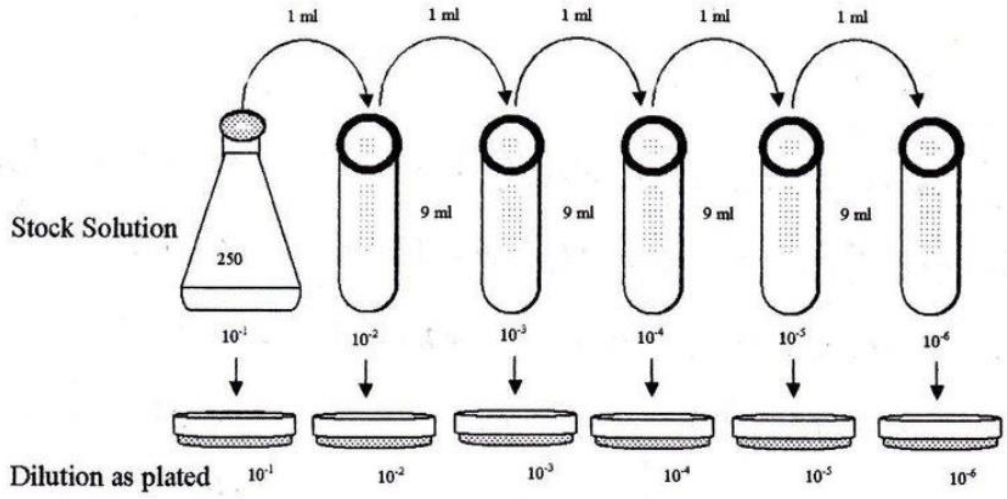


Figure: 3.8 Simple serial dilution series using with 9 ml blanks along with plating Standard plate counts

A standard plate count was used to estimate the level of microbes in the prepared and stored samples. This data could be used as the indicators of food quality or predictors for the shelf life of the product. Using a sterile pipette, 1 ml of the diluted sample was then taken into each of the sterile empty petri-dishes having nutrient agar media (Plate count agar) at a temperature of 45°C. Plates were mixed by swirling on a flat surface. After solidification of the media the plates were inverted and incubated at 37 °C for 24 hours in an incubator (AOAC, 1990; Sharf, 1966).

Counting and recording

After incubation the incubated plates were selected for counting the bacterial colony based on the number and easy of counting of the colony. The plate containing segregated, overlapping and confusing colonies was avoided. The plates containing 30 to 250 bright, cleared and countable colonies were selected.

Number of colony forming unit (cfu)/g or ml. = average cfu plate × dilution factor. The viable bacterial count was done through the steps of sample preparation, sample dilution, standard plate counts and counting and recording. The incubation was performed at 37°C for 24 hours (AOAC, 1990; Sharf, 1966).

3.10.2 Fungal analysis in jelly

Media Preparation

Sabouraud Dextrose Agar (SDA) is a selective medium primarily used for the isolation of dermatophytes, other fungi and yeasts but can also grow filamentous bacteria such as Nocardia. The acidic pH of this medium (pH about 5.0) inhibits the growth of bacteria but permits the growth of yeasts and most filamentous fungi. Antibacterial agents can also be added to augment the antibacterial effect. The SDA media is comprised of enzymatic digest of casein and animal tissues which provide a nutritious source of amino acids and nitrogenous compounds for the growth of fungi and yeasts. 10 g Mycological peptone (enzymatic digest of casein and animal tissues), 40 g Dextrose and 15 g Agar with pH 5.6 at 25^0 C are used for 1 liter SDA media.

All media used were prepared according to the manufacturer's instructions and sterilized in the autoclave at 121°C for 15 minutes. Although many selective agars exist for the cultivation and determination of mold and yeast cultures, a majority of them do not depend on strict nutritive requirements for growth. Many fungal strains will grow on Sabouraud Dextrose Agar. Methods and technique are followed here as described by Chen and Gu (2000), FSSAI (2012) and APHA (1996).

Procedure for preparation of media

At first 65 g of the medium was suspended in one liter of purified water. Then heated with frequent agitation and boiled for one minute to dissolve the medium completely. Autoclaved at 121°C for 15 minutes. Then cooled to 45°C to 50°C and poured into petri-dishes. For processing of specimen, the specimen was streaked onto the medium with a sterile inoculating loop in order to obtain isolated colonies. Then the plates were incubated at 25-30°C in an inverted position (agar side up) with increased humidity. Cultures were examined weekly for fungal growth and were held for 4-6 weeks before being reported as negative (Aryal, 2015).

Interpretation

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Examine plates for fungal colonies exhibiting typical color and morphology. Additional procedures should be performed to confirm findings. Yeasts will grow as creamy to white colonies. Molds will grow as filamentous colonies of various colors (Aryal, 2015).

3.11 Antimicrobial discs

Each of the discs was cut from Whatman's No.1 filter paper with an approximate diameter of 6 mm using a puncher. The prepared filter paper disc was sterilized by autoclaving at 121°C for15 minutes and impregnated with 30µl extract of the experimental plant. The plant extract was dissolved in 0.2% DMSO (Di-Methyl-Sulp-Oxide) to prepare four different concentrations such as 0.1 mg/µL, 0.2 mg/µL, 0.3 mg/µL and 0.4 mg/µL.

Culture and sensitivity test

E. coli was used for sensitivity test against plant extracts and. The colonies were dissolved in PBS (Phosphate Buffer Saline to obtain the optimum turbidity against the 0.5 McFarland standard concentrations. After equivocating the turbidity, the bacterial culture was ready for sensitivity test.

3.12 Preparation and components of Agar

Mueller-Hinton Agar (MHA)

To prepare 1000 mL of Mueller-Hinton agar (MHA), 10 g of tryptone, 15g of nutrient agar, 5 g of sodium chloride, and 5 g of yeast extract were weighed and added to the conical flask, 1000mL of distilled water was added and mixed. To dissolve all ingredients completely, boil for 10 minutes, sterilize by autoclaving for 15 minutes, and then sub culturing the test microorganisms.

Blood agar

To prepare 1000 mL of Blood agar,10 g of Casein, 15g of nutrient agar, 5 g of sodium chloride, and 2 g of yeast extract were weighed and added into the conical flask.1000mL of distilled water was added and mixed. To dissolve all ingredients completely, boiling for 10 minutes, and was sterilized by autoclaving for 15 minutes. Then add 50 ml of

bovine blood when the temperature decreases at 45-50°C and mix well. Then the agar is used for subculturing of the test microorganisms.

3.13 Antimicrobial Activity test of plant extracts

Agar Disc Diffusion Assay

The antibacterial activity test of crude extracts and fractionated compounds of experimental plant against *E. coli* and *Salmonella* spp.were carried out by disc diffusion method. The Sterile Muller Hinton agar was prepared for each organism as follows.

Twenty millilitres of sterile Muller Hinton agar (MHA) (maintained at 45-50°C in a molten state) was poured into sterilized Petri dishes. After solidifying Muller Hinton agar, 0.1ml of test organism was spread on MHA plate. Then the filter paper discs were placed on the surface of the agar plate at an equal distance from each other and 15 mm from the edge of the plate as described by Clutter buck *et al.*, (2007). Each disc was pressed down to ensure complete contact with the agar surface and each container was inverted and placed in an incubator set at 37°c for 24 hours. The antimicrobials susceptibility was evaluated after 24 hours by measuring zone of inhibition using plastic ruler in mm.

Chapter 4: Results

4.1 Physicochemical properties of guava jelly

pH of jelly is an important factor for optimum gel condition. In table 4.1, lowest (3.60± 0.300) pH found in sample A and C and highest (3.79± 0.010) in sample D. TSS (total soluble solids) was highest (69 degree brix) in sample D, and lowest in (66 degree brix) in sample A. The maximum value (0.30±0.399%) of acidity obtained in sample B and the least value (0.06±0.001%) found in sample A.

Table 4.1 Physicochemical properties of guava jelly

Formulation	pH (n=3)	TSS (^{0}B) (n=3)	Acidity (%) (n=3)
Sample A (control)	3.60± 0.300^a	65.96±0.036^c	0.06±0.001^a
Sample B / (5% guava leaf extract)	3.70 ±0.264^a	67.00±0.091^b	0.30±0.001^a
Sample C / (10% guava leaf extract)	3.60 ±0.300^a	66.98±0.005^b	0.09±0.001^a
Sample D / (15% guava leaf extract)	3.79± 0.010^a	69.02±0.010^a	0.09±0.002^a
p value	0.7562	0.0001	0.4514

All data are presenting Means ± Standard Deviation in Table 4.1. The presence of different superscripts along a row indicates significant difference and the same superscript shows not significant difference at ($p<0.05$). Here, n= Number of replication.

4.2 Nutritional Composition

Nutritive value of guava jelly is shown in Table 4.2, almost all samples are significantly different. Where sample C contained the highest percentage of crude fiber (1.78±0.015%) & Sample B had the largest amount of crude fat (1.29±0.010%) and sample A contains the most abundant percentage of crude protein (0.70±0.026%). The lowest percentage of crude fiber (1.47±0.010%), crude fat (1.09±0.015%) and crude protein (0.53±0.026%) found in sample A, sample D and sample C respectively.

Table 4.2: Nutritional composition of guava jelly

Formulation	Sample A (n=3)	Sample B (n=3)	Sample C (n=3)	Sample D (n=3)	p value
Moisture (%)	30.93 ± 1.850^{a}	32.70 ± 2.426^{a}	37.13 ± 5.361^{a}	36.29 ± 4.357^{a}	0.225
Crude fiber (%)	1.47 ± 0.010^{d}	1.53 ± 0.020^{c}	1.78 ± 0.015^{a}	1.60 ± 0.010^{b}	0.0001
Ash (%)	0.32 ± 0.026^{ab}	0.30 ± 0.026^{ab}	0.28 ± 0.360^{b}	0.36 ± 0.026^{a}	0.048
Crude fat (%)	1.19 ± 0.010^{b}	1.29 ± 0.010^{a}	1.17 ± 0.026^{b}	1.09 ± 0.015^{c}	0.0001
Crude protein (%)	0.70 ± 0.026^{a}	0.61 ± 0.026^{b}	0.53 ± 0.026^{c}	0.59 ± 0.026^{bc}	0.0004
CHO (%)	65.23 ± 0.750^{a}	63.13 ± 0.250^{b}	60.03 ± 0.503^{c}	60.07 ± 0.371^{c}	0.0001
Vitamin C (mg/100)	26.63 ± 0.030^{d}	27.20 ± 0.092^{c}	27.69 ± 0.015^{b}	28.40 ± 0.015^{a}	0.0001

All values are Means ± Standard Deviation. The presence of different superscripts along a column indicates significant difference and the same superscript shows not significant difference at ($p<0.05$). Here, n= Number of replication.

4.3 Phytochemical composition of guava jelly

The results of bioactive compounds (Total antioxidant content, TFC and TPC) are presented in table 4.3. There have a significantly different values found among all samples. Sample C carried the highest value of total antioxidant content (3.42±0.001 mg TA/100 mL), total flavonoid content (42.57±0.014 mg QE/100 g) & total phenolic content (8.10±0.200 mg GAE/100mL). Lowest value of total antioxidant content (1.96±0.002 mg TA/100 mL), total flavonoid content (32.87±0.001mg QE/100 g) and total phenolic content (6.40±0.100 mg GAE/100mL) found in sample A**.**

Table 4.3: Phytochemical composition of guava jelly

Formulation	**Total antioxidant content / (mg TA/100ml)**	**Total flavonoid content / (TFC) (mg QE/100gm)**	**Total phenolic content / (TPC) (mg GAE/100gm)**
Sample A (control)	1.96 ± 0.002^{d}	32.87 ± 0.001^{c}	6.40 ± 0.100^{d}
Sample B (5% leaf extract)	2.97 ± 0.001^{c}	37.33 ± 0.086^{c}	7.06 ± 0.208^{c}
Sample C (10% leaf extract)	3.42 ± 0.001^{b}	42.57 ± 0.014^{b}	8.10 ± 0.200^{b}
Sample D (15% leaf extract)	3.97 ± 0.011^{a}	46.25 ± 0.005^{a}	10.46 ± 0.152^{a}
p value	0.0001	0.0001	0.0001

All values are Means ± Standard Deviation. The presence of different superscripts along a column indicates significant difference and the same superscript shows not significant difference at ($p<0.05$).

4.4 Sensory Evaluation

There was not a significant difference ($p<0.05$) in all the sensory parameters assessed (Table 4.4). In all the parameters sample D had the highest acceptance rate. However, sample A was least accepted compared to other samples.

Table 4.4: Hedonic rating test for sensory evaluation of guava jelly

Formulation	Sample A (control)	Sample B (5% guava leaf extract)	Sample C (10% guava leaf extract	Sample D (15% guava leaf extract
Taste	7.80 ± 0.66^{b}	7.73 ± 0.46^{b}	7.07 ± 0.46^{a}	7.73 ± 0.70^{bc}
Sweetness	8.07 ± 0.47^{a}	8.20 ± 0.56^{ab}	8.33 ± 0.49^{ab}	8.60 ± 0.51^{ab}
Mouth feel	7.87 ± 0.35^{a}	7.87 ± 0.52^{a}	8.00 ± 0.48^{a}	8.60 ± 0.51^{b}
Flavor	8.00 ± 0.65^{ab}	7.93 ± 0.59^{ab}	7.87 ± 0.64^{ab}	8.20 ± 0.68^{ab}
Appearance	7.87 ± 0.35^{acd}	8.27 ± 0.70^{bcd}	7.60 ± 0.51^{ad}	8.47 ± 0.52^{bc}
Overall acceptability	7.73 ± 0.59^{b}	7.93 ± 0.80^{b}	8.00 ± 0.53^{ab}	8.60 ± 0.51^{a}

All values are Means ± Standard Deviation. The presence of different superscripts along a row indicates significant difference and the same superscript shows not significant difference at ($p<0.05$).

4.5 Microbial analysis

Microbiological characteristics are indicators of safety, quality and shelf life of prepared guava jelly. The number of bacteria that can grow and form countable colonies on nutrient agar after incubating at 37^{0}C for 24 hours is total viable bacterial count in a sample. This study was performed by standard plate count method. The total number of viable bacteria was determined by multiplying the colony-forming unit (cfu) with dilution number. The total numbers of viable bacteria in samples A (control), sample B

(5% guava jelly), sample C (10% guava jelly), and sample D (15% guava jelly) with guava leaves extract variation respectively have been shown in table 4.5 at 01, 30, 60, 90 and 120 days of storage. Initially significant amount of bacterial load was not detected but after 60 days of storage bacterial load gradually increased. Investigation on yeast and mold growth was performed at 30 days interval. No significant amount of fungal growth was detected in 7 days incubation in Sabouraud Dextrose agar. After 90 days of storage growth of yeast and mold were detected.

Table 4.5: Microbiological evaluation of guava jelly

Sample	Total viable bacteria (cfu/ml)					Yeast & Mold				
	Storage time in days					Storage time in days				
	1	30	60	90	120	1	30	60	90	120
Sample A	ND	1.3×10	1.7×10^2	2.8×10^3	2.9×10^4	A	A	A	A	P
Sample B	ND	1.2×10	1.6×10^2	2.6×10^3	2.8×10^4	A	A	A	A	P
Sample C	ND	1.1×10	1.4×10^2	2.7×10^3	2.4×10^4	A	A	A	A	P
Sample D	ND	1.1×10	1.3×10^2	2.1×10^3	2.2×10^4	A	A	A	A	P

ND= Not Detected, A= Absent, P= Present

4.6 Antibacterial activity of guava leaves extract against *Escherichia coli*

Table 4.6: Comparison of antibacterial efficacy (inhibition zone) of 10%, 20%, 30% and 40% ethanolic extract of guava leaves against *Escherichia coli*

Concentration	Mean zone of inhibition (in mm)
10%	0
20%	5
30%	10
40%	14

Chapter 5: Discussion

Everywhere across the world, guavas appreciated for its dietary benefits. The fact that guavas are rich in folic acid, dietary fiber, potassium, and dietary minerals led to its inclusion on the list of super fruits. Traditional medicine also makes extensive use of guava plants fruits, leaves and other parts. The guava tree is cultivated on a wide scale primarily on commercial purposes since each component of the tree has monetary advantages.

Physicochemical properties of guava jelly

Fresh guavas possess chemical properties that are remarkably like those described in a 1972 assessment by the US Department of Health, Education, and Welfare. According to their findings, fresh guava includes 0.4% pectin, 4.5% reducing sugar, 3.5% non-reducing sugar, 8.9% total sugar, 80.61% moisture, 0.70% total ash, 1.280% acidity, and 19% TSS (Vibhakara *et al.,* 2006).

From Table 4.1 and Table 4.2 I discovered that, sample A yielded pH 3.60, TSS 65.96%, 0.32% ash, 0.06% acidity and 30.93% moisture. Sample B comprised of pH 3.70, 67.00% TSS, 0.30% ash, 0.30% acidity, and 32.70% moisture. Sample C incorporated in pH 3.60, TSS 66.98%, 0.28% ash, 0.09% acidity, and 37.13% moisture. Sample D showed pH 3.79, 69.02% TSS, 0.36% ash, 0.09% acidity, and 36.29% moisture. Similar trend was reported in case of red guava jams (NS kumar *et al.,* 2021). The moisture and ash in the jelly developed from varieties of guava were in the range of 33-38.5% and 0.08–0.19% respectively (Joshi *et al.,* 2017).

Effects of treatment combinations and storage times on TSS, titrable acidity and ascorbic acid have been determined. The TSS of samples increased with an increase in storage time at room temperature. The impact of treatment combinations and the length of storage has been shown to be significant. The study also showed that TSS increased with an increase in storage time, regardless of storage conditions. The rise in total soluble solids during storage may be due to the conversion of insoluble to soluble fraction. Similar findings have been documented while working on guava jelly (Jollhe *et al.,* 2020)**.**

A slight discrepancy could be the result of measuring inaccuracy or instrument error. This study's variety distinctions have indeed been impacted by the soil's nutrients, the

growing area's composition, and inefficient quantification or instrumental error. It should be mentioned that the type of guava plant employed in this experiment is not disclosed. Due to the addition of extra sugar throughout formulation, manufactured jelly possesses greater sugar content.

Moisture content

It was clear that the moisture content (%) of the samples increased with increasing storage times at room temperature (25-30 ° C). About 7% humidity increased in 120 days of storage. The combined effect of composition and storage time was significant. The study also found that the moisture content (%) increases with increasing storage time when stored at room temperature. A similar trend was reported by (Singh *et al.*, 2012) for grape jelly during storage. The increase in moisture content is due to the hydrolysis of sugars into alcohol, carbon dioxide and water during storage (Kuchi *et al.*, 2014). The moisture content also increased due to addition of guava leaves extract. Sample C, the guava jelly which contains 10% of guava leave extract contain higher amount of moisture (37.13%).

Total soluble solid

It is evident that the TSS of the tests increases with increasing periods of capacity at room temperature (25-30°C). The influence of the different compositions and capacity periods has been remarkable. From Table 4.1, the highest amount of TSS increase (65.96% to 69.02%) was found in the sample D (addition of 15% guava leaves extract). It is also worth noting the combined influence of composition and capacity period. The reasoning that TSS expands at room temperature (25-30°C) with a period of increasing capacitance is overstated.

Nutritional composition of guava jelly

Nutritive esteem of guava jelly is appeared in Table 4.2, nearly all tests are altogether diverse. Where Sample C contained the most elevated rate of crude fiber which was 1.78±0.015% and sample B had the biggest percentage of crude fat (1.29±0.010%) and sample A contains the largest rate of crude protein (0.70±0.026%). The lowest rate of crude fiber (1.47±0.010%), crude fat (1.09±0.015%) and crude protein (0.53±0.026%) found in sample A, D and C individually.

Consumer acceptability of guava jelly

Sensory quality of guava jelly attributes viz. taste, mouth feel, flavor, appearance and overall acceptability were evaluated by serving the jellies to panelists who have assigned scores following the hedonic scale from 1 to 9 (A globally used scale for sensory evaluation). Sensory analysis data from Table 4.4 illustrates that the jelly made from guava with the addition of 15% guava leaves extract (sample D) had the highest overall acceptance score of 8.60±0.51. It could be because of the way the food tastes, how sweet it is, or how it looks. Similar trend was reported in case of red guava jams (kumar *et al.,* 2021).

In all the sensory characteristics tested, there was no significant difference ($p>0.05$) (Table 4.4). Sample D achieved the highest acceptance rate across all metrics. Sample A, on the other hand, received the least amount of acceptance (7.73±0.59) when compared to the other samples.

Microbial study of guava jelly

Safety, quality, and shelf life of prepared guava jelly are all determined by microbiological features. The total viable bacterial count in a sample is the number of bacteria that can grow and form countable colonies on nutrient agar after being incubated at 37^0C for 24 hours. The bacterial count was not consistent during this time period, as shown in Table 4.5, and there was significant variability over time. In one day, no total live bacteria were found in either treatment. In 30 days and 60 days, the bacterial count was (1.3×10) cfu/ml and (2.9×10^4) cfu/ml in sample A, and in sample D it was (1.1×10) cfu/ml and (2.2×10^4) respectively. For a production of high-quality fruit juice and jelly, free from any microbial contamination, use of good quality raw material is essential. Jellies obtained from fruits which are damaged and bruised contain more bacterial count than the juice obtained from sound fruits (Wolford and Berry, 1948).

During the 90 days of storage, no yeast or mold were identified in the guava jelly, as shown in Table 4.5. Mold, according to (Koburger, 1971), is an aerobic creature that cannot thrive in low-oxygen environments. On the other hand, yeast can grow in both aerobic and anaerobic environments. The acid/alkaline needs for yeast and mold growth in a variety of food products are highly diverse, ranging from pH 2 to pH 9. Yeast and mold growth were avoided by keeping jellies in an airtight bottle.

When it comes to microbial pathogen development rates, timing is crucial. When determining a product's shelf life, food makers take into account the concept of time as it relates to microbial development. The term "shelf life" refers to the time between when a product is manufactured and when it is meant to be consumed or used. A variety of parameters, ranging from organoleptic quality to microbiological safety, are utilized to determine a product's shelf life. The microbiological safety of the product is the most important factor to consider in this report. The Uniform Open Dating Regulation stipulates that a perishable food product's shelf life must be specified in terms of a sell-by date (Mahajan, 2018).

Antibacterial activity of guava leaves extract against *Escherichia coli*

The efficacy of 30% and 40% ethanolic extract of guava leaves were found to be much effective against *Escherichia coli* than 10% and 20% of that. Ethanolic extract contains tannins as well as flavonoids which had been reported to demonstrate good antibacterial activity. This difference in composition can be attributed to the difference in concentrations of various components of guava leaves in the extract.

In a study it was found that agar well-diffusion method was administered for microbiological assay that had been reported to have more sensitivity than other methods like disc diffusion method. Results of the present study portrayed almost similar efficacy of 20% ethanolic of guava leaves extract and 0.2% chlorhexidine. Other than leaves, extracts from other parts of guava had also been found to own activity against bacteria. He also showed the efficacy of 20% leaf extract being much more than the extract of 5%. It was also researched the ethanolic and methanolic extracts from guava roots and found these to have activity against L. *acidophilus*. ((Jain, *et al.,* 2014*).*

Another research showed gradual increase in the inhibition zone of *Escherichia coli* with the respective increase in the concentration of the guava leave extract which is 1,3,5,10 and 20 (µg/ml). (Dhiman, *et al.,* 2011).

Chapter 6: Conclusion

The objectives of the present study were to observe effect of guava leaf extract on preparation and changes in qualities of guava jelly with no preservatives for storage. Microbiological status and sensory evaluation of guava jellies were also evaluated. Matured guava was collected from the local market and prepared jelly with three formulations with Guava leaf extract variation and stored at room temperature (25−30°C).

Increased global supply has intensified competition in all food commodities. Product quality and consistency is the key for long-term profitability. To make a good quality jelly, the fruit must contain the proper amount of pectin and acid. Changes of chemical parameter for all different composition were similar during 90 days storage period. Color and texture were slightly different for 5%, 10% and 15% leaf extract treated jellies. Phytochemical components flavonoid, total phenolic compounds and antioxidant differed significantly after preparation but no significant difference in overall nutritive value was observed.

The usage of *psidium guajava* leaf extract for treating various diseases by the population is justified by the presence of numerous bioactive components in it. The results of the phytochemical studies confirmed the traditional use of the *psidium guajava* plant for medicinal purposes. They also showed that some of the plant extracts contain compounds with antimicrobial properties that can be used as antimicrobial agents in new medications for the treatment of infectious diseases brought on by various pathogens. In comparison to commercial drugs made chemically, using guava leaf as a herbal treatment is more beneficial. In this investigation, guava leaf extract's susceptibility to *E. coli* varied. However, guava leaf extract showed a nearly identical zone of inhibition across a range of concentrations and dosages as several commercial antimicrobials. That might be conceivable since *psidium guajava* has chemicals that are almost identical to the active components of widely available antimicrobials.

Chapter 7: Recommendations and Future Perspectives

Nowadays, more than half of the people suffer malnutrition in our country, in these situation guava jelly with its leaf extract could be a good source of the nutrients and energy as these are available in rural areas of Bangladesh. We have been concluded with good findings in the area of developing this jelly. It is also resulted with its commercial value and better marketability. Modern food industries can adopt the procedure form medium and large scale of production. On the basis of present investigation, the following suggestions and prospects are made for the further research work.

a) The present studies may be repeated for confirmation of the experimental findings.
b) The composition may be modified further and may try for making mixed jam with various recipes with different ratio of fruit.
c) Such types of research should be done for other fruits like papaya, mango etc. available in markets especially for off season.
d) Modern packaging and storage condition would be developed for the betterment of guava jelly with its leaf extract.
e) The findings will be helpful from therapeutic point of view as it has medicinal value.
f) Although the sample size was sufficient to perform statistical comparisons between analytical data. Our conclusion should be considered with caution because of the small number of analyzed samples and results would need to be confirmed with another larger study.

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Appendices

Appendix A: Questionnaire for Hedonic test of guava jelly

Name of the Taster: ……

Date: …….

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as color, flavor, texture and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describe your sense and feeling about the sample please give a reason for this attribute. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us. For Taste/Flavor/Mouth feel/Appearance/Overall Acceptability

The scale is arranged such that; Like extremely =9, Like very much =8, Like moderately =7, Like slightly=6, Neither like nor dislike =5, Dislike slightly =4, Dislike moderately =3, Dislike very much =2, and Dislike extremely =1.

Here,

A- Control guava jelly without addition of guava leaf extract

B- Treatment guava jelly with addition of 5% guava leaf extract

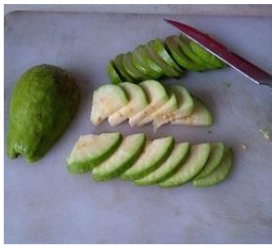
C- Treatment guava jelly with addition of 10% guava leaf extract

D- Treatment guava jelly with addition of 15% guava leaf extract

Hedonic	Taste						Flavour						Mouth feel						Sweetness						Appearance						Overall Acceptability					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Like Extremely																																				
Like very much																																				
Like moderately																																				
Like slightly																																				
Neither like or dislike																																				
Dislike slightly																																				
Dislike moderately																																				
Dislike very much																																				
Comments																																				

Appendix B: Photo Gallery

Fresh guava



Slicing guava

Boiling

Jelly preparation

Color variation due to extract

Final product

Sensory evaluation

Plate: Product development

Determining brix in refractometer

pH determination

Acidity determination

Weighing sample

Crude fiber determination

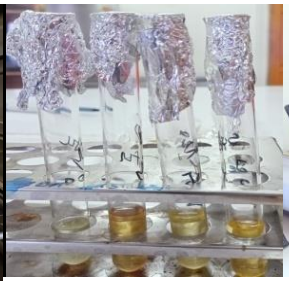
Protein digestion

Fat determination

Rotary evaporator

Filtration

Drying guava leaves



Ethanolic extract preparation

Working in UV spectrophotometer

Checking absorbence

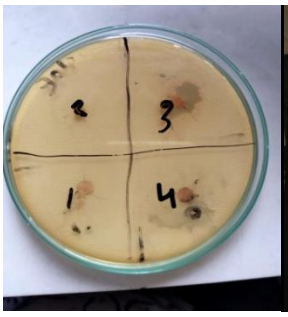
Applying sample in bio-safety cabinet

Counting bacterial colony

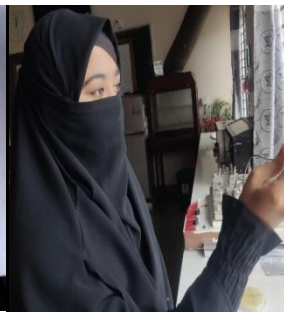
Yeast found at day 120

Autoclave

Heating agar



Zone of inhabition



Measure zone of inhabition of sensitive bacteria

Plate: Laboratory work

Brief Biography

This is Shymoon Nahar Khanam, daughter of Anwarul Islam Khan and Fardaoush Ara Begum from Anowara upazila under Chattogram district of Bangladesh. Shymoon Nahar Khanam passed the Secondary School Certificate Examination in 2010 from Aparnacharan city corporation girls' high school, Chattogram, and then Higher Secondary Certificate Examination in 2012 from Chittagong Govt. Women College, Chattogram. She obtained her B.Sc. (Hons.) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. 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, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition. With her best knowledge and expertise, she hopes to deliver competent veterinary medical treatment and sustains the norms of professionalism in the future