Evaluation of Anti-Microbial Activity and Minimum Inhibitory Concentration of *Flacourtia jangomas* Fruits

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Evaluation of Anti-Microbial Activity and Minimum Inhibitory Concentration of *Flacourtia jangomas* Fruits" submitted by MD. Atikur Rahman of summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor on February 2023.

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Ethics Statement

There were no human or animal experiments conducted for this study.

Abstract

The objective of this study was to determine the particular antibacterial components of *Flacourtia jangomas* fruit. The antimicrobial screening used the Kirby-Baurer disk diffusion technique and the Broth dilution method, both of which are considered "standard procedures." Kirby-Baurer disk diffusion technique shows that at a concentration of 500g/disc, methanol extract demonstrated exceptional efficacy against the vast majority of test microorganisms. *Bacillus cereus* QL 29 performed the best among the test organisms, whereas *Sacharomyces cerevaceae** fungus exhibited the least susceptibility to the extract. In Minimum Inhibitory Concentration test highest MIC value is 256 µg/ mL which shows 7 bacteria and lowest MIC result is 128 µg/ mL which is showed by 5 bacteria where the test was done on 12 bacteria. The *Flacourtia jangomas* fruit has been shown to exhibit significant antibacterial activity in both method.

Keywords: *Flacourtia Jangomas*, antimicrobial effect, Kirby-Baurer disk diffusion, Minimum Inhibitory Concentration.

Dedication

Dedicated to my family, Teachers and Friends

Acknowledgement

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List of Acronyms

MIC	Minimum Inhibitory Concentration
OD	Optical Density
DMSO	Dimethyl Sulfoxide
INFS	Institute of Nutrition and Food Science
CLSI	Clinical and Laboratory Standards Institute

Chapter 1

Introduction

1.1 General Introduction

Plants are likely one of the most major suppliers of medicines. The medicinal plants are extensively utilized around the globe and conduct for a large source of staple product for the old medication and plant medication industry, as well as a substantial percentage income and health security of the worldwide population. Bioactive compounds, such as volatile oils, alkaloids, flavonoids, phenolics, tannins, and glycosides, have a great lot of pharmacological activity potential and may be used therapeutically. These bioactive compounds are available in a vast range, and more are extracted daily from medicinal plants. Therefore, medicinal plants are an important source of raw materials for drug development. (Talukder.C, Saha.S, Islam.M, 2012)

Herb *F. jangomas* is sometimes serves as old medication. *F. jangomas*, which belongs to the Flacourtiaceae family, may reach heights between 5 and 10 meters. For the purpose of obtaining cytotoxic and antibacterial compounds, scientists are now researching a vast array of plants. Due to the fast growth of drug-resistant microorganisms, scientists have investigated novel antibacterial lead agents, particularly those derived from plant extracts. Plant extracts containing antimicrobial chemicals may have therapeutic use in the treatment of resistant microbial strains because they may limit bacterial growth or kill the pathogens in a manner distinct from that of conventional antimicrobials. The cytotoxic test may also facilitate more research towards the creation of anticancer medicines. Consequently, the goal of this study is to undertake a thorough investigation of the existing data about its pharmacological characteristics (Parvin.S, Kader.A, Sarkar.G, Hosain.S (2011))

Bacteria are the underlying cause of several diseases, including those affecting the skin, respiratory tract, urinary tract, gastrointestinal tract, central nervous system, and brain. These infections, particularly UTIs, may be deadly if not appropriately or at all treated. Antibiotics are used worldwide to treat bacterial illnesses, but bacteria are developing resistance to them. In recent years, antibiotics have been overused, which has accelerated research into alternative medicines. In the manufacturing of the great majority of antimicrobial medications, natural plant-based components are used. Since the earliest days of human civilization, plants have played a vital role in maintaining and enhancing human health. The ability of plants to produce antimicrobial chemicals has paved the way for the synthesis of innovative natural products with the potential to replace conventional antibiotics. Considering the great potential of plant extracts to cure infectious diseases caused by drug-resistant superbugs, it is astonishing that less than 5 percent of plant species have been examined for their ability to produce antimicrobial chemicals (B. Mirzaei, 2017)

Antimicrobial, MIC, and medicinal characteristics of *F. jangomas* discussed in this article.

1.2 Classification of medicinal plant

Plant or natural medications are mostly obtained from spermatophytes, also known as seed plants, of which the Angiospermae phylum is regarded as the superior group. To make it easier to discover a certain kind of natural medicine, they have been categorized according to a variety of distinctive qualities. (Shah & Seth, 2010)-

Table 1 Classification and characteristics of medicinal plant

Classification	Characteristics
1. Alphabetical classification	The generic and brand names of medications are listed in English and Latin alphabetical order. This classification system is used by the Pharmacopeias.
2.Taxonomical classification	It is based on their classification within the plant kingdom. (superkingdom, kingdom, category, division, family, phylum, class, family, and order)
3.Morphological classification	Organ or piece of a plant that is used for medicinal purposes, such as leaves, roots, bark, etc.
4.Pharmacological classification	Describes the drug's pharmacological action and therapeutic properties, including whether it kills bacteria, reduces pain, produces vomiting, etc.
5.Chemical classification	By categorizing drugs based on their basic chemical constituents, such as alkaloids, glycosides, tannins, etc.
6.Chemotaxonomical classification	This categorization is based on a taxon's chemical similarity.

1.3 Importance of Antibiotics

Antibiotics were formerly believed to be "magic bullets" that could heal any bacterial illness, but bacteria have developed resistance to them. In the United States alone, 99,000 individuals each year are killed by hospital-acquired infections (HAIs) caused by antibiotic-resistant bacteria. Antibiotics have been freely accessible and have spread around the globe as a result of widespread misinformation. The global use of antibiotics was projected to reach 63,151 tons in 2010, and this quantity is estimated to climb by around 67% by 2030. The misuse of antibiotics enables microorganisms to develop defensive mechanisms that render them resistant to treatment.

In current use, germs resistant to antibiotics are sometimes referred to as "Superbugs." It is anticipated that 444 million people would be affected by 2050 if the number of superbugs continues to climb, and as a consequence, the birthrate will plummet.

Despite the fact that the 1930s through the 1960s were known as the "golden age" of antibiotics, scientists were unable to keep up with the rate of invention due to the increasing resistance of bacteria (Aslam et al., 2018). Due to the frightening pace at which bacteria and viruses are gaining resistance, the world might soon enter a "post-antibiotic era" if effective new antibiotics and antimicrobials are not discovered. Since the present crop of antibiotics has a small therapeutic window and a conventional mode of action, it is vital that new antibiotics be identified (Mahlapuu, Hkansson, Ringstad, and Bjorn, 2016). In order to combat the war against antibiotic-resistant microbes, a significant amount of money is being invested in the search for new antibiotics, and a number of suggestions have been made to accelerate the search. (Aslam et al., 2018)

1.4 Brine Shrimp Lethality Bioassay

Cytotoxicity *F. jangomas* extracts was evaluated using a brine shrimp lethality Process. It is simple to learn, inexpensive, and only needs a small sample size. Once the active component has been discovered, more specialized and expensive bioassays may be employed as a backup to this method's initial screening. Bioassays of brine shrimp mortality are highly informative of cytotoxicity and pesticide effectiveness. (Krishnaraju et al., 2005)

1.5 Taxonomic classification of *F. jangomas*

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Violales

Family - Flacourtiaceae

Genus - Flacourtia

Species - *Flacourtia jangomas (Lour.)* Rausch -Indian plum

1.6 Morphology of F. jangomas

F. jangomas Lour (Family: Flacourtiaceae), which is extensively spread in Bangladesh's Chittagong Hill Tracts, Cox's Bazar, and Sylhet region as well as throughout south-east Asia, is also known locally as Painnagola, Lukluki, Paniamra, Flacourtia species may be shrubs or trees; their branches contain simple, branching spines and sympodial growth. The leaves are simple, alternate, lanceolate-ovate to elliptic-oblong in shape, with an entire margin, serrate-dentate to pinnately veined, occasionally 3-5 pliveined at the base, an acute to acuminate apex, lateral veins upcurved, thicker near the midrib and diminishing toward the margins, a petiole,

and minute early caducous or absent stipules. Pedunculate axillary and terminal raceme fascicles with many flowers. Flowers are unisexual (dioecious) or bisexual (sometimes) and hypogynous, with articulate pedicels, oval, chartaceous, scalelike bracts, four to seven imbricate, connate at the base but free above, a ciliate edge, and a subpersistent petal ring. To use a common phrase, male flowers: There are fifteen to thirty stamens with filiform filaments that are continuously connected to the disk cup; the anthers are two-loculed and globose; the pollen has three cells; and there is no pistil. Female flowers contain a superior ovary that is globose and surrounded by a disc; three to ten incompletely loculate and connate carpels; two ovules in each locule; a free or almost nonexistent style; and a stigma that is either short bilobed or retuse. The fruit is an indehiscent berry or drupe that is ellipsoid-globose when ripe but obtusely constricted and ribbed when dried. Each locule contains two pyrenes superposed. Pairs of ovoid or rectangular seeds with a thin coriaceous covering and no arillate radicles are counted. Each branch may support up to twenty fruits, and fruit clusters normally vary in size from two to seven. The fruits, which are 2–2.5 cm in diameter and originally greenish but become reddish purple as they mature, resemble cherries. Each fruit contains four to fourteen tiny, jagged seeds with very sharp edges. The styles, which consist of four or five tiny projections with black tips, are gathered in a circle at the top of the fruit. Malaysian trees produce fruit periodically, with peak output in June and July. Each year, each tree produces 40-100 kg of fruit on average. In three to five months, the fruit will ripen. (Rahman.M, Habib.M, Hasan.M, Islam.A, Khan.I(2011)

Figure 1 F. jangomas fruit (Mishra et al)

1.7 Traditional use

The fruits of the *F. jangomas* tree are appreciated for their delectable sweetness and pleasant acidity. When ripe, the fruits become a rich red or purple hue and are edible fresh or used to produce jams and collect. Fruit is firm, bluish-green in color rather soft. The fruit may be used to make juice, syrup, jam, marmalade, pickles, and sauces, among many other things. To soften the texture of very astringent fruits, they are rolled between the palms of the hands. It is used to make jellies when it is harvested when it is still slightly immature. In Indian cuisine and Keralan medicine, fruits are highly valued. In Indonesia, the young, acidic shoots are edible. The wood is red or scarlet in color, has a tight grain, is robust and brittle, is durable and polishes well. Tool handles and construction blocks are two of its numerous agricultural uses. Tamil Nadu, Kerala, and Karnataka are three Indian states that gather wood for timber on occasion. It is a popular alternative to more expensive timbers like as teak. This plant provides a food source for Bactrocera tryoni, a fruit fly native to Queensland. (Sasi.s, Anjum.N 2018)

1.8 Phytochemistry

Several plant species, like *F. jangomas*, need scientific inquiry. There is a dearth of study on the phytochemical features of the plant. Recently, a series of cytotoxic diterpenes were identified from Casearia sylvestris, a member of the Flacourtiace family. Unfortunately, only a limited number of species' phytochemicals have been recorded, and the chemistry of this family is presently poorly understood. Terpenoids, alkaloids, flavonoids, tannins, lignans, flavanolignans, glucosides, coumarins, and isocoumarins are only a few of the several chemical classes discovered to be produced by the Flacourtiaceae. Tannins are mostly derived from the plant's bark, but the leaves and young shoots are also rich in tannins. Also reported are xanthones, quinones, limonoids, and phenazines. It has been discovered that the stem and bark of *F. jangomas* contain two limonoids: limolin and jangomolide. It has been shown that *F.*

jangomas contains bioactive compounds such as corymbulosine, tremulacin, hydnocarpic acid, and chaulmoogric acid. The stem bark and fruit were removed for the coumarin ostruthin. Heartwood was discovered to contain the butyrolactone lignan disaccharide ramontoside and the steroids beta-sitosterol and its beta-D-glucopyranoside. Fruits are rich in protein, fat, sugars (fructose, glucose, and sucrose), amino acids, vitamin C, and minerals like calcium, potassium, phosphorus, iron, magnesium, salt, manganese, copper, and zinc. Fatty acid analysis revealed the presence of palmitic, hexadecadienoic, stearic, oleic, linoleic, alpha-linolenic, and a few more minor unidentified acids in lipids. Additionally, proline, hydroxyproline, methionine, alanine, glycine, and valine were identified in the fruit extract. Potassium is highly bioavailable in mature *F. jangomas* fruits, indicating that they might be utilized as a healthy source of potassium in the human diet. Fruits include several antioxidants and reducing agents, such as anthocyanin, alkaloids, beta-carotene, flavonoids, tannins, saponins, amino acids, and phenolic compounds. Unripe fruits contain several phytochemicals, including flavonoids, alkaloids, tannins, and total phenols. The physicochemical properties and mineral content of F. gangomas fruits from Bangladesh were investigated. In a methanolic extract of fruits from Assam, India, flavonoids, phenols, tannins, terpenoids, and saponins were detected, but not alkaloids. The following table details the chemical compounds identified and reported from different *F. jangomas* tissues. (Sasi.s, Anjum.N 2018)

1.9 Pharmacological Activity

F. jangomas is renowned in the pharmaceutical industry for its astringent, acrid, refrigerant, stomachic, diaphoretic, analgesic, stomachic, anti-inflammatory, and antibacterial characteristics. It seen to treat asthma, tumors, jaundice, toothaches, and diarrhea, among other diseases. Fruits' anti-diabetic benefits have long been acknowledged. Ripe fruits are high in beneficial nutrients, such as monounsaturated fats, which are more prevalent than their polyunsaturated counterparts, and fiber, which helps you feel full for longer. Zeaxanthin, Beta-

carotene, lutein, retinol all present in high amounts, and they all contribute to the maintenance of normal blood and fibrinogen levels in body. High amounts of vitamin C (ascorbic acid), vitamin B3 (niacin) are incude. Potassium, which is abundant in ripe fruits, is known to have a function in blood pressure regulation, while phosphorus and magnesium are crucial for preventing osteoporosis. Numerous pharmacological effects, including as analgesic, anti-inflammatory, antibacterial, anti-diarrheal, antiviral, antioxidant, and anti-amylase activity, have been researched in connection to this tree. A summary of pharmacological study on the elements of the plant. (Sasi.s, Anjum.N 2018)

1.10 Literature Review

It is a unique fruit tree that provides several health advantages to people. Time of harvest; fruit at its optimum ripeness Low in fat and rich in monounsaturated fatty acids rather than polyunsaturated fatty acids; high fiber and protein content. Evaluation of a leaf and stem extract of *F. jangomas* in alloxan give diabetic research animal administered with the gold standard anti-diabetic, glibenclamide. When the ethanolic extract of *F. jangomas* leaves was examined in an in vivo antidiarrheal model using castor oil to induce diarrhea.(Mishra et al)

1.11 Rational of the Study

F. jangomas thrive in Sundarbans and other saline, humid regions of Bangladesh. Despite the numerous active phytochemical components discovered in plants by both old and new research, trees are most commonly utilized for their wood. By examining these molecules and their ability to adapt to abiotic and biotic stresses, we can increase the potential for the development of new medicines and make significant therapeutic advances in Bangladesh's healthcare system. This study investigated and analyzed the Antimicrobial Activity of *F. jangomas* against bacteria and fungus, as well as the presence of these phytochemical substances.

1.12 Study Aim

The study's goal is to assess the antibacterial efficacy of *F. jangomas* and identify its phytochemical components.

1.13 Objectives of the Study

Objectives are-

- In order to isolate the phytochemicals of *F. jangomas*
- This research goal to determine if *F. jangomas* have antibacterial characteristics.

Chapter 2

Methodology

2.1 Collection and Identification of the Plant

The Bangladesh National Herbarium in Mirpur, Dhaka, properly identified the *F. jangomas* leaves collected in November 2011 in Savar, Bangladesh (Accession no. DCAB- 87043). In addition, a voucher specimen was deposited for future investigation.

Figure 2 F. jangomas Plant with flower (*Mishra et al).*

2.2 Bacterial Strains

In this study 14 bacterial species are used and those are *Bacillus cereus QL 29, Bacillus megaterium QL 38, Sarcina lutea QL 166, Bacillus subtilis QL 40, Staphylococcus aureus ATCC25923, Sarcina lutea QL 166, Salmonella paratyphi A AM16590, Escherichia coli ATCC 25922, Salmonella typhi AM 16406, Shigella dysenteriae ATCC 26131, Shigella sonnei ATCC 25931, Shigella boydii ATCC13147, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneomoniae ATCC 10031,* These were collected as pure culture from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

2.3 Fungi

In this study 3 fungi species are used and those are *Candida albicans*, Aspergillus niger*, Sacharomyces cerevaceae*.* The Institute of Nutrition and Food Science at the University of Dhaka in Bangladesh supplied these pure cultures.

2.4 Kirby-Bauer Disk diffusion method

This method, which is widely recognized as the gold standard, is used to test for antibacterial activity. This method involves seeding Mueller Hinton with a bacterial suspension and allowing it to sit for twenty-four hours. 25 sterile tweezers are used to remove a 6mm-diameter sterile filter paper disk from an oil sample-containing vial. After 24 hours of incubation, a clear halo should form around the disks if the extract sample used in this study is effective at inhibiting the growth of microorganisms. As the zone surrounding the disk grows larger, the sensitivity of the microorganism increases. (Boukhraz et al., 2016)

2.4.1 Forming agar medium

Initially, 7.6g of Mueller-Hinton agar was dissolved in 200 mL of distilled water. After adding the agar powder to the water, the mixture was rapidly agitated to dissolve the powder. After 60 minutes of autoclaving at 121°C, the mixture was cooled to between 45°C and 50°C. Then, eight clean Petri dishes were inoculated with a sterile solution of Mueller-Hinton agar. In each 100-millimeter Petri dish, 25 milliliters of Mueller-Hinton agar solution were put. The solution was allowed to cool to ambient temperature and solidify.

2.4.2 Pre-culturing the bacterial strains

Strains of bacteria were isolated from medium that had been frozen at very low temperatures and stored for a long period. To aid in the recovery of the bacteria, a nutrient agar medium was prepared by combining 5.6 grams of nutrient agar with 200 milliliters of distillation water and

then autoclaving the combination. 13 clean Petri dishes were then infected with the nutritious agar solution. The bacterial strains were then transferred to the new nutrient agar medium using a sterile loop to remove them from the old culture media. The bacteria-containing nutrient agar medium was cultured for 24 hours in order to rejuvenate them.

2.4.3 Forming bacterial suspension

By dissolving 0.25 grams of nutritious broth in 10 milliliters of distillation water, a bacterial suspension was created. In the same manner, eight distinct broth mediums were used to establish sixteen distinct bacterial and fungus suspensions in the conical flasks. The broth medium was then autoclaved at 121 degrees Celsius for 60 minutes. As the soup cools, it is served at room condition. Then, each strain was taken separately from the nutrient agar plates, dipped into the nutrient broth medium, and forcefully mixed to generate the bacterial suspension. After creating the bacterial suspension, it was put in an incubator with shaking for 24 hours. Because bacteria may grow on the suspension. After 24 hours, the absorbance of the bacterial suspensions was assessed. Since an absorbance value between 0.1 and 0.2 was desired, the suspension was diluted with freshly made nutritious broth until the desired concentration was achieved.

2.4.4 Preparation of disc

The diameter of the Whitman paper disc was a meager 6 millimeters. All of the disks were autoclaved in one test tube. The discs were then treated with a methanol mixture of different proportions that had been previously prepared. Then, it was allowed to absorb the remaining liquids for a further 10–15 minutes.

2.4.5 Procedure

The antibacterial activities of *F. jangomas* on fifteen distinct microorganisms were investigated using freshly prepared Mueller-Hinton agar. Then, a cotton swab was dipped into a bacterial and fungus suspension made by suspending bacteria and fungus in nutritional broth medium. To drain extra fluid, cotton swabs were gently pressed against the tube. Using the swab, the bacterial fungus solution was initially spread in one direction on the nutrient agar plate. After then, it accelerated in a straight line before deviating to the diagonal. The swab was used to streak the strains along the outermost diameter of the nutrient plate. The sixteen Petri dishes were handled in precisely the same manner. After that, we let the agar plates to dry for 5 minutes. After waiting 5 minutes, each disc holding the extract and methanol combination was gently placed on the plate using forceps. One antibiotic disk was used per plate. The bacterial growth in each Petri dish was observed after 24 hours of incubation at 37°C. The procedure was performed in a biosafety cabinet with laminar airflow.

2.5 Broth dilution method

Broth dilution is often used to determine the MIC of antimicrobic substance including antibiotics and other compounds that kill (bactericidal activity) or limit the growth of bacteria (bacteriostatic activity). A preset number of bacterial cells are injected into a liquid growth medium containing geometrically increasing amounts (typically a twofold dilution series) of the antimicrobial medication to perform a broth dilution. Macro- dilution is testing with a total volume of 2 ml, while microdilution is testing on microtiter plates with r500 ml each well. After incubation, the presence of turbidity or sediment indicates that the organism has grown. (Wiegand et al., 2008) The minimum inhibitory concentration (MIC) of a substance is the lowest concentration at which fungal growth is inhibited for a certain length of time. The lowest

inhibitory concentration is expressed in micrograms per milliliter. (Rodriguez-Tudela et al., 2008).

2.5.1 Forming bacterial suspension

The medium for the bacterial suspension was created by combining 0.25g of nutritious broth with 10ml of distillation water. In the eight conical flasks containing bacterial suspensions, the same approach was utilized to prepare broth medium. The whole medium broth was autoclaved for 60 minutes at 121 degrees Celsius. The broth medium is then allowed to cool to room temperature. Lastly, the bacterial and fungus suspension was made by transferring each strain from the nutrient meadi to the nutrient broth medium and forcefully combining the two. After preparing a fresh bacterial suspension, the combination was allowed to rest for a full day in an incubator that was agitated. In order to cultivate bacteria on this solution. The absorbance of these bacterial cultures was measured after 24 hours. Since 0.1% to 0.2% absorbance was selected. With the addition of freshly prepared nutritious broth, the concentration of the suspension was adjusted to the proper level.

2.5.2 Concentration

Antimicrobial agent concentration is the total amount of the agent per unit of liquid. The unit of concentration is micrograms per milliliter. Although theoretically feasible, the conversion between ug/mL and mg/L is discouraged.

2.5.3 Procedure

To test the antibacterial activity of *F. jangomas* against 12 unique microorganisms, a freshly generated. The bacteria were then diluted in nutrient broth medium and dispersed by dipping a glass spreader into the bacterial suspension. The in vitro antimicrobial activity of fruit extracts from *F. jangomas* species was evaluated using the MIC (minimum inhibitory concentration)

method, in which the extracts were diluted until 80% of the tested microorganisms' growth was visibly inhibited compared to the control medium (for bacteria) or completely inhibited (for fungi) (for fungi). The minimum inhibitory concentration (MIC) was determined in vitro utilizing broth dilution techniques with Mueller-Hinton medium (Biocorp) buffered at pH 7.1 (for bacteria) or pH 5.6 supplemented with 2% glucose (for fungi), containing twofold dilutions of the tested extracts (at final concentrations of 512, 256, 128, 64, and 32 µg/ mL). The experiments were done primarily in accordance with the CLSI (Clinical and Laboratory Standards Institute) criteria, with a few modifications. To achieve reliable findings, a control consisting of the medium without the investigated compounds was conducted. The materials were incubated overnight at 37 degrees Celsius after being dissolved in DMSO (Dimethyl sulfoxide), a typical commercial solvent derived from trees as a byproduct of paper production. By adding 32, 64, 128, 256, and 512 µg, the concentration of DMSO was raised. Visual inspection and spectrophotometer measurements of optical density (OD) at 630 nm were used to verify the existence of growth. The OD was obtained immediately after the ocular examination. The formula used to determine growth inhibition for test wells at each dilution of plant fruit extract:

Percentage of inhibition = (OD of control - OD of test)/ (OD of control) × 100%

Chapter 3

Result

Both Disk Kirby-Bauer Disk diffusion method and broth dilution method result are given below

Table 2 Result of Antimicrobial effect of F. jangomas flesh extract

Antimicrobial effect of F. jangomas flesh extract		
Microorganisms	Zone of inhibition (Diameter in mm)	
	MFJ 500 µm/disc	Kanamycin (30 µg/disc)
Bacillus cereus QL 29	25	30
Bacillus megaterium QL 38	16	32
Bacillus subtilis QL 40	18	33
Staphylococcus aureus ATCC25923	17	31
Sarcina lutea QL 166	18	34
Escherichia coli ATCC 25922	17	31
Salmonella paratyphi A AM16590	19	32
Salmonella typhi AM 16406	22	33
Shigella dysenteriae ATCC 26131	21	31
Shigella sonnei ATCC 25931	23	34
Shigella boydii ATCC13147	21	32
Pseudomonas aeruginosa ATCC 27853	19	30
Klebsiella pneomoniae ATCC 10031	19	31
Candida albicans*	10	33
Aspergillus niger*	11	35
Sacharomyces cerevaceae*	10	34

These were collected as pure culture from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh

Table 3 Minimum Inhibitory Concentration Result

Minimum inhibitory concentration (MIC)	
Microorganisms	MIC µg/ mL
Bacillus cereus QL 29	256
Bacillus megaterium QL 38	256
Bacillus subtilis QL 40	128
Staphylococcus aureus ATCC25923	128
Sarcina lutea QL 166	256
Escherichia coli ATCC 25922	256
Salmonella paratyphi A AM16590	128
Salmonella typhi AM 16406	128
Shigella dysenteriae ATCC 26131	256
Shigella sonnei ATCC 25931	128
Shigella boydii ATCC13147	256
Pseudomonas aeruginosa ATCC 27853	256

3.1 Result of Disk method

The antibacterial activity of plant extracts from *F. jangomas* was first evaluated using the disc diffusion method against a variety of microbes. Common diseases were caused by these microbes. The study's plant extracts exhibited variable antibacterial and anti-fungal efficacy against all tested diseases, according to the findings. Different bacteria and fungus show different zone of inhibition zone. We employed normal kanamycin discs for purposes of comparison (30 mg each). In this study, the antimicrobial effectiveness of methanol extract of *F. jangomas* was evaluated. We discovered that given on table 2.

The zone of inhibition created by the crude methanol extract of the plant ranges from 0 to 30 millimeters. At a concentration of 500g/disc, methanol extract demonstrated outstanding efficacy against the vast majority of test microorganisms. Bacillus cereus QL 29 fared the best against the test substances and Sacharomyces cerevaceae* fungi, which shown low susceptibility to the extract.

3.2 Result of MIC

The development of test organisms was inhibited in test tubes with concentrations below and above the minimum inhibitory concentration (MIC). Concentration of plant extract needed to prevent growth of 12 different bacteria. The minimum inhibitory concentration (MIC) for Bacillus cereus QL29, Bacillus megaterium QL38, Sarcina lutea QL166, Escherichia coli ATCC 25922, Shigella dysenteriae ATCC 26131, Shigella boydii ATCC13147, Pseudomonas aeruginosa ATCC 27853 was 256 g/mL; for Staphylococcus aureus ATCC, Salmonella paratyphi A AM16590, Salmonella typhi AM 16406, Shigella sonnei ATCC 25931 Minimum inhibitory concentration = 128 g/mL. The result given into table 3.

Chapter 4

Discussion

The global growth of antibiotic resistance is a big concern. In recent years, an increasing number of human pathogenic bacteria have acquired multiple resistances, because people aren't using market antimicrobial medications for treating infections the right way. It is hypothesized that the extensive use of broad-spectrum antibiotics, the use of immunesuppressing

medications, and the persistence of HIV infections have all contributed to this worrying increase. Every year, millions of people die as a result of the growth of antibiotic-resistant microorganisms. This has forced researchers to seek out alternate sources of antibacterial chemicals, such as medicinal plants28. The selection of plant material for this study was guided by ethnobotanical knowledge on the plants' historical use in treating bacterial infections. Upon fractionation and extraction with an organic solvent (methanol), it was observed that certain fruit sections of *F. jangomas* inhibited the target bacterium in a dose-dependent manner. Therefore, the experiment findings imply that *F. jangomas* has antibacterial characteristics. The zone of inhibition test for antimicrobial activity reveals a linear connection between fraction concentration and their capacity to inhibit microbial growth. By fractionating *F. jangomas* fruit antibacterial activity is significantly boosted. Table 1 details the capacity of therapeutic fruits to suppress the development of microorganisms and fungi.

Chapter 5

Conclusion

In South Asia, raw and fried *Flacourtia jangomas* fruits are particularly popular. These fruits are used as a diarrhea remedy. The roots are said to relieve toothache, but the dried leaves may aid bronchitis.

The results of this study indicate that the fruit of *F. jangomas* has high antibacterial activity against a variety of bacteria and fungi. Plants belonging to the *F. jangomas* species may be proposed for use in the manufacture of antibacterial drugs based on the findings of this study.

Future prospect

In South and Southeast Asia and India, it is possible to gather fruit from the tree *F. jangomas*, also known as the Indian Coffee Plum. The antibacterial properties of *F. jangomas* have been investigated by scientists, who discovered that it is efficient against bacteria, fungus. Fruits of *F. jangomas* exhibit antibacterial activity with a minimum inhibitory concentration (MIC) indicative of future improvement. Antibiotic-resistant bacteria are a growing concern in medicine, but more research into this plant antimicrobial properties might lead to the development of new antimicrobial drugs that are effective against these pathogens. Also, if the bioactive components responsible for *F.jangomas* antibacterial activity are identified and extracted, new natural antimicrobial drugs may be developed. Possible applications for these compounds include pharmaceutical use and food preservation. Perhaps, *F. jangomas* might be used to produce natural antibacterial agents for food preservation, hence reducing the need for synthetic preservatives. It may also be used to generate new all-natural skin care products that efficiently combat bacterial and fungal skin diseases.

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