Evaluation of Anti-diarrheal Activity of Methanolic Flesh extract of Flacourtia jangomas on mice

By

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This project is submitted to the School of Pharmacy in substantially satisfies the Requirements for the Bachelor of Pharmacy (Hons.) degree

School of Pharmacy

BRAC University

November, 2022

Declaration

It is hereby declared that

1. The project submitted is my own original work while completing degree at Brac University.
2. The project 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 project 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

"Evaluation of Anti-diarrheal Activity of Flacourtia jangomas Methanolic Flesh extract on Swiss albino mice" submitted by Nusrat Jahan Susmitaof summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on November, 2022.

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Ethics Statement

State University of Bangladesh, Department of Pharmacy has granted ethical permission.

Abstract

Flacourtia jangomas is rare fruit, rarely found in hill tracks of Bangladesh. This is a less researched fruits but have many phytochemical constituent. It contain many pharmacological activity as well. The fact that *F. jangomas* is now thought to be an excellent reservoir of chemicals such phenol, tannin, alkaloids, flavanoids, and terpenoids that may be a better source to cure diarrhea is a major driving force behind this research. This study tested the *F. jangomas* flesh in vivo antidiarrheal effect on Swiss albino mice. In order to compare the test results, Loperamide was utilized as the reference medication. Three concentrations of *F. jangomas* (15mg/ml, 30mg/ml and 45mg/ml) of each extract showed antidiarrheal activity. The results have been elucidated as number of feces in every 1hr. The number feces counted till 4hrs. The antidiarrheal activity of methanolic flesh per hour until 4hrs extract of *F.jangomas* at 100,200and 300mg/kg concentration showed number of feces time respectively is 10.83, 9.67 and 8.65 in 4hrs. *F. jangoma* showed the effectiveness of antidiarrheal activity 14.52%, 23.68%and 32.91% respectively. All the result were significant ($P<0.001$) for the antidiarrheal activity. This study highlights the significance of *F. jangomasin'*s use as an antidiarrheal agent and the need for additional research to identify the active ingredient that causes this effect.

Key words*: Flacourtia. jangomas,* anti-diarrheal activity, castor oil, methanol, Swiss albino mice

Dedication

I dedicate the research work to my loved family, the helpful seniors from my university and my honorable supervisor, Dr. Farhana Alam Ripa (Assistant Professor, School of Pharmacy, BRAC University)

Acknowledgement

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List of Acronyms

THPs	Traditional Heath Practitioners
T and CM	Traditional healing and Complementary medicine
GABA	Gamma-aminobutyric acid
SEM	Standard Error of the Mean
ANOVA	Analysis of Variance
SE	Seed Extract
FE	Flesh extract
WHO	World Health Organization

Chapter-01

1 Introduction

1.1 General information

Humans have employed trees and their products over thousands of years to treat a wide variety of ailments. Before the advent of modern medicine, numerous societies created knowledge known as traditional medicine, sometimes known as indigenous or herbal medicine. From the very beginning of life, human beings were mostly depends on the nature for their existence. From there, plants have been playing a vital role for mankind. Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants (Sofowora, 1982). According to the World Health Organization (WHO), medicinal plants are those that have qualities or substances that can be utilized therapeutically or those that synthesize metabolites to create valuable medications (WHO 2008). Plant-based medicines are well known for their efficacy, accessibility, and affordability (Iwu et al., 1999). Herbal remedies can be made from complete plant parts or mostly from the leaves, roots, bark, seeds, and flowers of various plants. They are applied topically, orally, or through inhalation (Westh et al., 2004).Medicinal herbs are more significant to the health of individual and community the development of pharmacopoeial, non-pharmacopoeial, or synthetic medications has long relied on the use of medicinal plants as a rich supply of components. Aside from that, these plants are essential to the growth of human cultures all across the world. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Due to the availability of natural chemicals, medicinal plants are a key source of molecules with therapeutic qualities. The presence of phytochemical components in medicinal plants makes them useful for treating human illnesses and an important component of healing.

Finding plants with healing properties and looking for agents to treat various illnesses have always been important factors in the discovery of modern pharmaceuticals, going all the way back to a very early stage. From ancient times to the present, scientists have been looking for therapeutic plants and herbs that can improve healthcare systems and be effective in the elimination of illnesses and human suffering. Therefore, during this journey, scientists learned about more than 80% of modern medications that have been derived either directly or indirectly from medicinal plants. According to a source, more than 5000 medicinal plants are distributed in 200 families in the flora of Bangladesh and over 33 medicinal plants are being use in the many rural areas as herbal medicine nowadays they are very well familiar because of their healing properties.

1.2 Importance of medicinal plants and some herbs with their medicinal values

1. Cinnamon and sandalwood work wonders as astringents. In particular, sandalwood is used to stop the release of blood and mucous.
2. Certain antipyretic herbs, including *Chirayta*, black pepper, sandalwood, and safflower, are suggested by traditional Indian medicine practitioners to suppress fever and the generation of heat brought on by the condition.
3. Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sag e are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.
4. By removing the metabolic poisons, several herbs are utilized as blood purifiers to improve or change a chronic illness. These are likewise referred to as "blood cleaners." A person's immunity is increased by some herbs, which lessens illnesses like fever.

5. Many different herbs are used as tonics, including giloe, golden seal, aloe, and barberry. They can also be nourishing and revive both healthy and sick people.
6. An open wound or new cut can be effectively treated with honey, turmeric, marshmallow, and liquorice. These plants are known as vulnerary herbs.

1.3 Types of medicinal plants across the world

According to the research on plants, globally, there are about 320,000 different plant species, from enormous sequoias to seagrass meadows. 320,000 plant species are thought to exist worldwide. These are vascular plants by far the majority. Although, so many different plants exist in the world, but not every plants have the medicinal property in it. Worldwide 72000-77000(17-18%) plants are currently utilized for medicinal purpose.

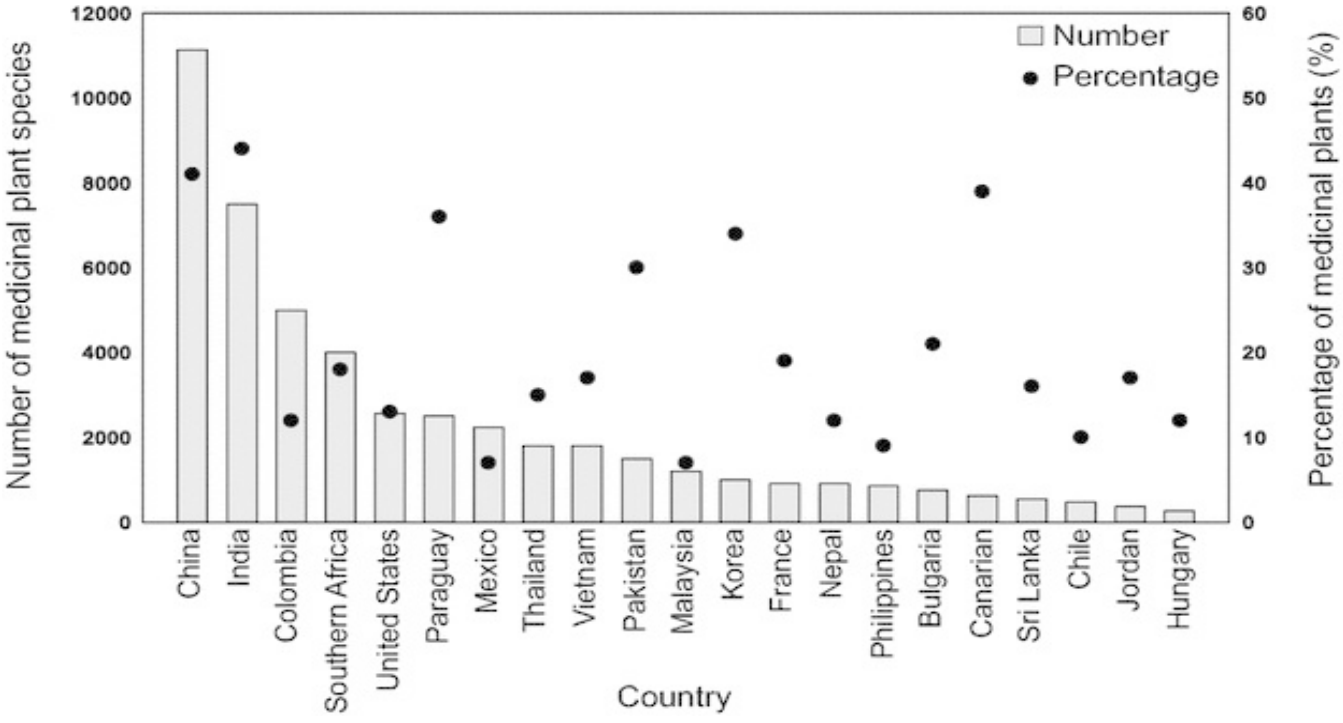


Figure 1.1: Number of medicinal plant exists in different country and percentage (Chen., 2019)

1.4 Classification of Important medicinal plants

The automatic classification and identification of medicinal plants will provide farmers and everyday people with access to medical information, hence increasing the production of these

essential plants. Since there are so many various kinds of medicinal plants, it is possible to categorize them accordingly to the function, longevity, active components, aromatic plants, and natural products. Below is a list of them:

✓ According to habit

1. **Herb:** *Centella asiatica*
2. **Shrub:** *Hiptage benghalensis* – malpighiaceae
3. **Tree:** *Aegle Marmelos* – Rutaceae
4. **Climber:** *Tinospora cordifolia* – Menispermaceae
5. **Epiphyte/Parasite:** Cuscuta reflexa – Cuscutaceae

✓ Medicinal plants based on diseases treated

1. **Cancer :***Plumbago zeylanica*- Plumbaginaceae
2. **Diabetes:** *Coccinia grandis*– Cucurbitaceae
3. **Cardiac:** *Calotropis procera*– Asclepiadaceae
4. **Dysentery:** *Aegle marmelos*– Rutaceae
5. **Asthama :***Acalypha indica*- Euphorbiaceae

Figure 1.2: Morphological description of the Plumbago zeylanica L (Shukla.et al., 2021)

- ✓ **Based on the uses of plant**

1. Whole plants: *Centella asiatica* – Apiaceae

2. Root: *Aristolochia indica* – Aristolochiaceae

3. Stem/Stem bark: *Acacia catechu*- Mimosaceae

4. Leaf : *Justicia adhatoda* – Acanthaceae

5.Fruits: *Aegle marmelos* **–** Rutaceae

1.5 Medicinal plants available in Bangladesh

Bangladesh is a subtropical country with a large natural supply of medicinal plants. Even in the early 1980s, the Ayurvedic and Unani herbal medicine producers in the nation, collect their 80% of supplies from domestic natural forests and imported the other 20% of their needs. According to Bangladesh Agricultural Research Institute (BARI), there are 722 species of medicinal plants in Bangladesh. As opposed to 4,000 in India, 700 plants are used in Bangladesh for medicinal purposes. Of them, 255 plants are utilized by the manufacturers of Ayurvedic and Unani medicines. According to a study, there are more than 86,000 Kavirajs, or doctors, in Bangladesh's more than 1600 villages. This means that there are approximately 2/3 Kavirajs for every 500–800 people, and the most intriguing fact is that these doctors use a variety of medicinal plants and their parts to treat patients. Those plant pieces must have undergone a crushing or maceration process. The juices from the plant parts are then gathered and prepared to create the medicine, which is then applied topically, orally, or both, to the affected area to treat it (Md. Shahadat Hossan, 2010). In addition, a single medicinal plant can heal a variety of ailments due to its varied actions.

- ***Phyllanthus niruri* L**

Phyllanthus niruri L. belongs to the Phyllanthaceae family.It is referred to as Bhui amla or Bhui amloki in Bengali. It also goes by the name Tamalaki. Along with fenugreek, cumin seeds, or milk, extract of the roots and leaves or crushed powder of dried leaves and roots are utilized. It has anti-malarial and anti-hepatitis B virus viral properties. It has strong antibacterial, antifungal, and antifilarial properties.

Figure 1.3: Whole plant of Phyllanthus niruri L (Wahyuni S., 2010)

- ***Ocimum sanctum* L**

Ocimum sanctum L. belongs to Labiatae family. In Bengali it is known as Tulsi It is well-known for being holy basil and basil. The substance utilized is an aqueous infusion of leaves. The main ingredient in tulsi, eugenol, is responsible for reducing a variety of illnesses or symptoms. Tulsi is most frequently used to treat the common cold. Viral hepatitis and viral encephalitis patients experience less pain because to tulsi preparations. Tulsi and black pepper are both used as a malaria preventative. It has potent anti-helminthes, Aspergillus niger, Plasmodium vivax, and Plasmodium falciparum action.

Figure 1.4: a) whole plant b) leaves of Ocimum sanctum L *(Tran A., 2020)*

- ***Hiptage benghalensis* L**

Hiptage benghalensis L. belongs to Malphighiaceae family. Among Bengalis, madhobi lota is actually referred to as a flower. It is also frequently called Myrtle. Leprosy and other disorders can be treated with flower and root extracts. Whole plants, leaves, branches, roots, barks, fruits, seeds, tubers, and gum are all included in kavirajes.

Figure 1.5: Hiptage benghalensis L *(Uddin. , 2013)*

- ***Swertia chirata***

Swertia chirata is the scientific name for Chirata. A well-known plant called chirata is used to treat conditions like fever, diabetes, liver disease, and stomach pain. Here, the entire plant provides therapeutic and medicinal benefits.

Figure 1.6: Swertia chirata (Rijal D., 2013)

- ***Azadirachta indica***

The scientific name of Neem is *Azadirachta indica Neem* leave is used specially for therapeutic purpose. Moreover, Neem leaf and its constituents have been shown to have anticarcinogenic, anti-inflammatory, antidiabetic, antispasmodic, antimalarial, antifungal, bactericidal, antiviral, antioxidant, antitumoral, and chemopreventive properties.

Figure 1.7: Neem Leaves (Mollik, 2020)

1.6 Chemical components of medicinal plants

The following are some of the frequently occurring chemicals that give plants their therapeutic (as well as harmful) characteristics (Motaleb et al., 2011).

1. Alkaloid and amines

a. Pyridine group

b. Tropane group

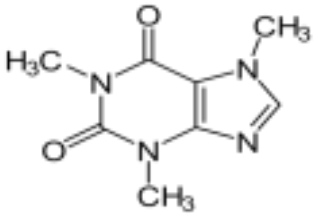
c. Quinoline group

d. Quinolizidile group

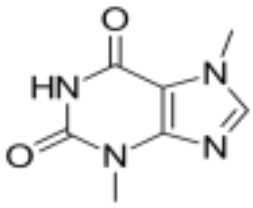
e. Indole group

f. Steroidal group

g. Alkaloid amines



Theobromide



Caffeine

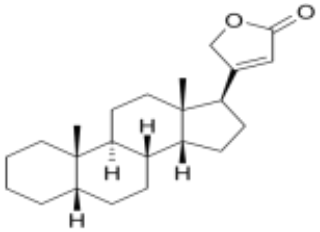
Figure 1.8: Structure of some alkaloids and amines (Mulherjee et al.,2015)

2. Glycosides:

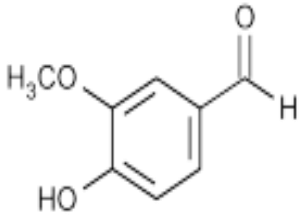
a. Anthraquinone glycoside

b. Cardiac glycoside

c. Saponin glycoside



Cardenolide



Vanillin

3. Volatile or essential oils

4. Fixed oils.

Figure 1.9: Some common glycosides and volatile oils (Sekhar et al.,2018)

1.7 Antidiarrheal activity of plants

A clinical condition diarrhea is characterized by the fast pass of semisolid or liquid stools via the digestive tract. When there is an imbalance between secretion and absorption in the small intestine, secretory diarrhea develops. This disorder can be caused by parasites and bacteria. The main cause of secretory diarrhea is the active secretion of chloride and bicarbonate ions. The presence of phytochemicals such terpenoids, tannins, and flavonoids in the plant extract may be responsible for this antidiarrheal activity, which are shown to inhibit the prostaglandin

release and intestinal absorption of electrolyte. Osmosis-driven water flow into the intestines is caused by the secretion of these electrolytes. The main course of treatment is supportive care with replenishment of intestinal fluid losses using oral rehydration solutions or isotonic intravenous fluids. Overall, reducing chloride release into the digestive tract is likely to lead to decreased stool volume and frequency, alleviating the symptoms of diarrhea. (J. Daniel Dubreuil, 2013). One of the top causes of death that can be prevented in underdeveloped nations is diarrheal illness, which primarily affects children and newborns. In accordance with data from the WHO and UNICEF, there are over 2.5 billion cases of diarrheal disease worldwide each year, and 1.9 million children under the age of 5 pass away from the disease each year, with the majority of these deaths occurring in underdeveloped nations. 78% of all diarrhea-related infant deaths take place in Africa and Southeast Asia. A prospective source of novel antidiarrheal medications is medicinal plants. Due to this, the WHO has supported research on the use of conventional medicine in the treatment and prevention of diarrheal illnesses. *(M. Belay, A. Assefa,2018)*

1.8 List of some plants that have antidiarrheal activity

Numerous studies and evaluations have been done and are still being done on diverse plant species in an effort to identify novel potential antidiarrheal molecules and determine their potential mode(s) of action through the use of various screening techniques. This results in a list of plants and the chemicals in them that have potent antidiarrheal properties. From those, the following plants are listed:

- ***Justicia schimperiana*** **(***Family*-Acanthaceae)

It has been claimed that the Libo Kemekem area in northwest Ethiopia uses the leaf of Justicia schimperiana as an antidiarrheal medication. It is a typical shrub found in wet montane forests,

frequently next to streams and rivers, in evergreen sage on hill slopes, in forest clearings, and on coffee plantations.

Figure 1.10: Justicia schimperiana(Darbyshire et al.,2015)

- **Leea indica**

Roots and leaves of Leea indica have been traditionally used as treatment for diarrhea and dysenteryin India and other countries**.**

Figure 1.11: Leea indica (Akhilesha S. 2013)

- ***Indigofera spicata* Forssk**

The traditional use of the stems *of Indigofera spicata* Forssk, has been supported by significant antidiarrheal efficacy. Further research is required, and it's possible that the plant

could provide a novel medicinal agent for diarrhea. (Birru, E.M., Asrie, A.B., Adinew, G.M., 2016)

Figure 1.12: Indigofera spicata Forssk *(Birru EM., 2019)*

1.9 Plant Extracts as a Source of Compounds Used in Anti-diarrheal activity

Natural products are a wonderful starting point for treating human ailments. According to the survey of World Health Organization (WHO), plants serve as the primary treatment for maximum people of the world's population's health problems. Dilrukshi Jayawardene and colleagues, 2021) the secondary metabolites that plants produce, play the significant role in defensive systems of plants. Secondary metabolites include terpenoids, tannins, and flavonoids, which are bioactive phytochemicals with strong anthelmintic effects and a significant role in helminth management in ruminants. (2018) (Lanusse et al.) These organic substances exhibit promising antidiarrheal properties.

- **Terpenoids:** Terpenes are the most abundant group of plant volatiles that inhibit biochemical targets such as acetylcholinesterase, GABA, and tyramine receptors. It also interacts with glutamate-gated chloride channels and P-gp. Terpenes are polymers made of the 5-carbon component isoprene.

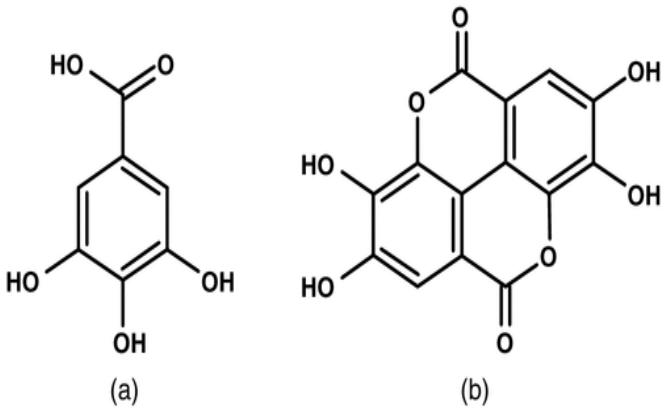


Figure1.13: Structure of Terpenoids(Boot,2020)

- **Tannins:** Water-soluble polyphenols called tannins—also called tannic acid which found in a variety of plant diets. In experimental animals, they have reportedly been linked to reductions in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility

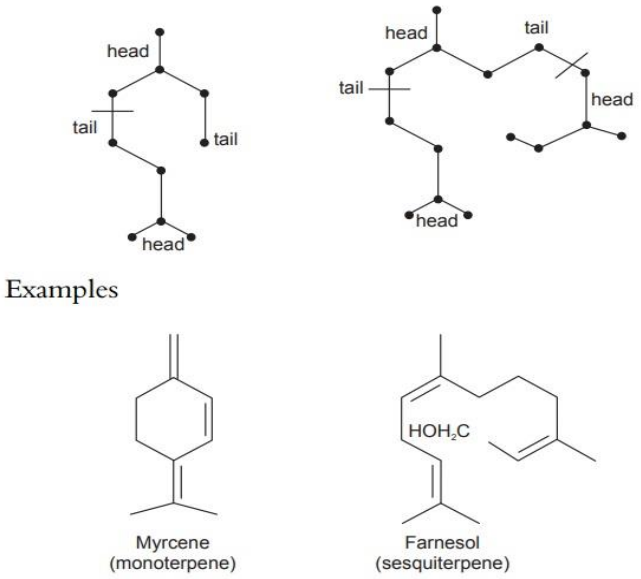


Figure 1.14: (a) Hydrolysable tannins (b) condensed tannins (Drabble & Nierenstein, 1907)

- **Flavonoid**: It is a group of chemical product that have various structures of phenol are found in fruits, vegetables, cereals, bark, stems, branches, flowers, tea, and wine. Some efforts being undertaken to withdrawn the extract of the components known as "flavonoids" because these natural compounds well known for positive impacts on body. *F. jangomas* contains this which gives anti-diarrheal effect.

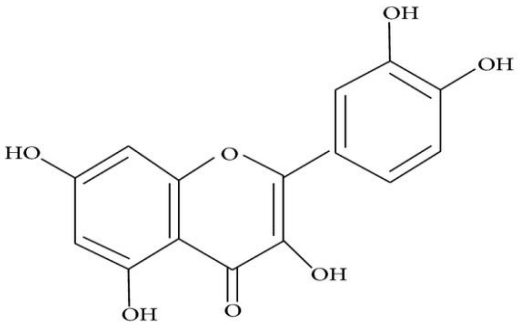


Figure 1.15: General Structure of flavonoids (Mulherjee et al.,2015)

1.10 Information about *Flacourtia jangomas*

Plants have been used as a source of medicine from the very early stage of life to the present. The plants were initially the mainstay of herbal or ethnomedicine, which was practiced in China, Middle East,and South America in addition to India. A major part of the traditional information was later documented, and subsequently store into other organized medical systems, including Ayurveda, Chinese, Yunani, Sidha, Tibetan, and other systems. Villagers still largely rely on folk medicines to treat frequents illnesses like cough, cold, and fever, headache, body ache, constipation, dysentery, burns, cuts, and scalds, boils, ulcers, skin diseases, and respiratory issues, despite the significant development of rural health services. A paradigm shift from chemical medication molecules to the herbal and supportive systems of medicine has occurred during the past ten years. A lack of modern curative therapies for the treatment of chronic diseases and public awareness of how to prevent various diseases may be to blame for the resurgence of these drugs. Improper use of chemical drugs that results in bacterial resistance. Additionally, technological developments in the fields of phytomedicine and alternative medicines have better positioned the scientific community for future discoveries of potent bioactive chemicals for treating a variety of ailments. It is expected that 5–15% of the plants utilized to make crude pharmaceuticals in recent years have had their potential for bioactive chemicals examined. According to the WHO, over 50% of the people rely on their

herbal and supplementary system of medicine in nations like China, Africa, Indonesia, India, Japan, Singapore, The Republic of Korea, etc. Therefore, guidelines and coordinated efforts are required to ensure their quality control, stability, and standardization parameters. A well-known plant in the genus *Flacourtia* called *F. jangomas* has a variety of therapeutic benefits. The species was once classified in the extinct family Flacourtiaceae and given the honorary name Étienne de Flacourt (1607-1660), a governor of Madagascar. It is indigenous to the subtropical. A number of the genus' species are grown for their fruits and for ornamentation.

Figure 1.16: Different parts of F.jangoma (leave, fruit, tree, seeds)(Missra D. 2020)

1.11 Taxonomical classification

Kingdom : Plantae

Subkingdom : Viridiplantae

Infrakingdom : Streptophyta (Land plants)

Superdivision : Embryophyta

Division : Tracheophyta (Vascular plants)

Subdivision : Spermatophytina (Spermatophyes)

Class : Magnoliopsida

Superorder : Rosanae

Order : Malpighiales

Family : Salicaceae (Willows)

Genus : Flacourtia

Species : jangomas

- ✓ **Synonyms:** Flacourtia cataphracta Roxb. Ex Willd., Stigmarota jangomas Lour.

1.12 Different names

In Bangladesh it is known in different names in different areas. Among them,

i)Lukluki,

ii)Tepafol,

iii)Paila are common in many areas**.**

- ✓ **In other countries**

Arabic : Talisfir, Zarnab

Assamese : Paniyal

Bangladesh : Painnagola

Bengali : Tipafol, Luluki

Brazilian : Cereja-De-Cameta

Chinese : Yun Nan Ci Li Mu

English : Indian coffee plum, Indian sour cherry

Thai : Takhob

1.13 Traditional uses of *F. jangomas*

The tasty, luscious fruits of *F. jangomas* have a deliciously tangy flavor. When the fruits are ripe, they are dark crimson or purple and can eat raw or used to make jam and preserves. The meat of the fruit is fairly juicy, f greenish-brown in color. It is stewed as a treat and transformed into sauces, pickles, jam, syrup, juice, and jam marmalade. Fruits can occasionally be astringent; to lessen astringency, they are rolled between the hands. Jellies are produced from it when it's just little underripe. Fruits are incredibly important to Indian cuisine and medicine, particularly in Kerala. In Indonesia, people consume the young, acidic shoots. Closed grained, hard, brittle, long-lasting, and polishable, the wood is red or scarlet in color. For blocks or farming equipment, it is utilized. In the Indian states of Tamil Nadu, Kerala, and Karnataka, the wood is occasionally harvested for timber. It is frequently utilized as a less costly option to Teak and other high-priced wood. Bactrocera tryoni, the Queensland fruit fly, is thought to have this plant as one of its principal hosts**.**

1.14 Botanical description

F. jangomas, also known as Paniala, Indian plum, or coffee plum, is a member of the Flacourtiaceae family. It is a little grown tree that can occasionally reach a height of 14 m and grows to a height of 6 to 10 meters. Older trees' trunks and branches have less thorns than young trees, which have woody thorns. When young, leaves are alternate, deciduous, pale pink, spirally organized; they are rarely ovate-lanceolate, long point toothed, very thin, glossy on both surfaces, and have an elliptic, serrated blade. Axillary racemes with a sub-corymbose, glabrous inflorescence are present. Dioecious flowers with four or five ovate triangular petals

and a white to greenish color have a honey-like scent before or with the young foliage. Female flowers are solitary, while male flowers are filamentous, glabrous, and either alone or in clusters. There are different trees for the male and female blooms. From December through April, flowers and new leaves with a stunning fresh green color both appear. The ellipsoid berries' subglobose, dull brownish red or purpleThen blackish, pulp's greenish- yellow color, and 4-5(-10) flat seeds ripen from March to July. Seeds are used to spread the tree. However, since seeds take a while to germinate, propagation is typically accomplished by in-arching or budding onto self-seedlings. Birds consume ripe fruits and disseminate them widely, enabling a very broad distribution of the species.

Figure 1.17: Tree of F.jangomas (Mallik.2016)

1.15 Origin and geographic scope

Fruit tree *F. jangomas* is a lowland semi-cultivated species with a questionable wild restriction. Although the origin of its wild organ is unknown, it is believed to have come from India and spread over the tropical regions of East Africa and tropical Asia. However, it has recently eluded cultivation in a number of locations. It is native to Uttar Pradesh's North-Eastern Terai region, particularly in the division of Gorakhpur. It is a rare fruit tree in Gorakhpur. The region from which the data were gathered is located in the northeastern corner of the state of Uttar

Pradesh between the latitudes of 26°5' and 27°29' north and 83°4' and 84°26' east. Visits were made to the following tribal communities: Lachhmipur, Madhaulia, Nichlol, Rajhain, Ramgarh,. A study of ethnopharmacology of the Gorakhpur forests revealed that the wild Flacourtia plant species is currently quite scarce there urgent conservation measures are required. Though, in Bangladesh it is not grow naturally, it's being cultivated here for trading purpose. Most of the tine it is being imported from other country.

1.16 Ethno-pharmacology

F. jangomas is a significant fruit tree with significant medicinal and dietary value. Fruits are revered in the Indian medical system as a remedy for poisonous diseases and doshas. The fruits are useful for diarrhea and bilious disorders. Additionally, it is used to treat toothaches, nausea,

Figure 1.18: Seeds and flesh of F. jangomas (Veeresham C.,2012)

digestive problems, acid reflux, and bleeding gums. Fruits have also been prescribed for enlarged spleen and jaundice. After being decocted, the leaves are used to cure piles and bleeding dysentery. Pharmaceutical companies use various plant parts to treat a variety of illnesses, including pre- and postnatal blood purification, asthma, and other conditions. The treatment of intermittent fever involves the use of barks. The roots are diuretic, alexipharmic, pleasant, and refrigerant. The astringent and stomachic leaves and young shoots have a rhubarb-like flavor.. In traditional south Indian medicine, the plant is also used to treat diabetes.

F. jangomas is one of those plants whose phytochemistry hasn't been well researched from a scientific standpoint. The Flacourtiaceae elaborates huge area of substances, such as terpenoids, alkaloids, flavonoids and tannins, lignans and flavanolignans, glucosides based studies. This tree contain both tannin and a fixed oil, the tannins being mostly found in the bark, leaves, and early shoots. Xanthones, quinones, and phenazines have been found. The root and skin *of F. Jangomas* were found to contain the limonoids limolin and jangomolide. There have been reports of bioactive substances in *F. jangomas*, including corymbulosine, tremulacin, hydnocarpic acid, and chaulmoogric acid. A coumarin called ostruthin was produced from the fruit and stem bark. In contrast to the heartwood, where ramontoside, a butyrolactone lignan disaccharide, sitosterol, and its -Dglucopyranoside were reported, the bark contained the phenolic glucoside ester flacourtin. Nutrients, protein, fat, sugars, amino acids, vitamin C, and minerals with calcium, potassium, phosphorus, iron, magnesium, salt, and zinc are abundant in fruits. The existence of palmitic, stearic, oleic acid, alpha-linolenic, and a few minor unidentified acids are found through fatty acid analysis of fats. Furthermore, proline, methionine, alanine, glycine, and valine are exists in the amino acid extract of dried, ripe fruits. Studies on simple reducing sugars and related alditol acetates using paper chromatography revealed the existence of arabinose, glucose, fructose, and galactose. The ripe fruits of *F. jangomas* contain a large amount of potassium, which is highly bioavailable and may therefore be an excellent source for an adequate intake of potassium.

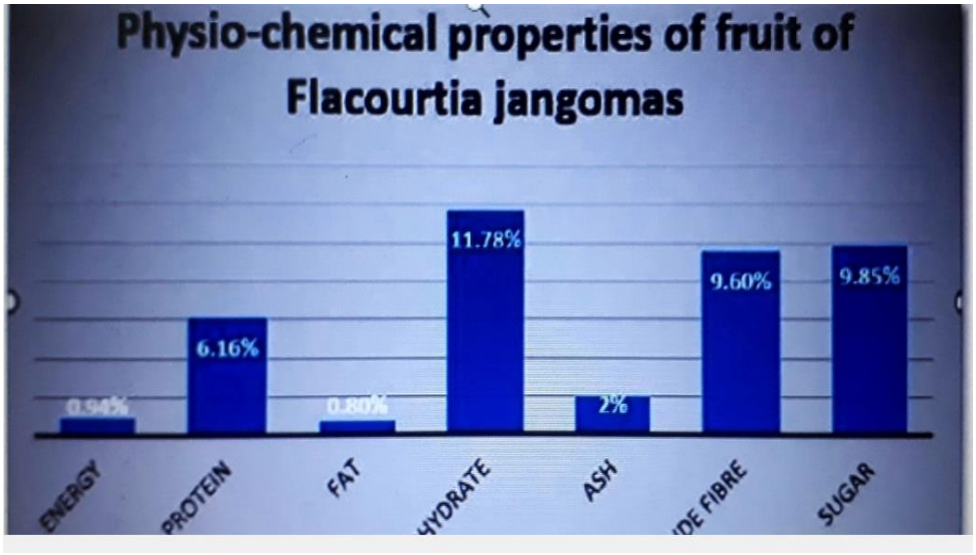


Figure 1.19: Flacourtia jangomas-physio-chemical properties of fruit (Tiwari, P., Kumar, B., 2017)

1.17Therapeutic benefits

This tree produces a distinctive fruit that is both incredibly nutritious and beneficial to our general health. The ripe fruits have a larger concentration of monounsaturated than polyunsaturated fatty acids, as well as a high fiber content, good protein content, and low fat level. Beta-carotene, which is present in substantial amounts, is followed by lutein and zeaxanthin, retinol, and phylloquinone (vitamin K), all of which are crucial for the control of hemoglobin and fibrinogen in the human body.

1.18 Parts utilized in the study

F. jangomas fruit flesh and seed extract was employed in this work to conduct pharmacological research.

1.19 Literature Review

This plant's roots, stems,fruit and leaves have all been the subject of several research in the past. Below is a list of the reported studies summary**:**

- **Mishra.T et all,2020 -** Alkaloids, tannins, glycosides, proteins, flavonoids, and other chemicals have been found to be extracted from different parts of the plant, and these

chemicals have a variety of pharmacological properties, including anticancer, antifungal antidiuretic, antioxidant, anti-fatigue , antimicrobial and anti-diarrheal.

- **Sarker et al., 2018**-The results of the inquiry show that the plant's leaves (LE) and aerial roots (AR) contain methanol extracts with antihyperlipidemic, antiatherosclerotic, and cardio protective properties. Sprague Dawley rats that had developed diabetes as a result of the alloxan experiment were used.
- **Jagdishprasad .T.V, 2017**-According to the fruit of *F. jangomas* is used to treat liver conditions, and the skin of the tree using to treat malaria. Synthetic and biological activity have supported ethnomedical usage.
- **Ali. M,2011**-The plant's bark's methanol extract has been shown to have antihyperglycemic action in oral glucose tolerance tests in Swiss albino mice loaded with glucose. It has also been shown to have antinociceptive activity in acetic acid-induced gastric pain writhing in the same animal model. However, the results of the experiment demonstrated strong antihyperglycemic (more effective than glibenclamide to treat diabetes when it was administered at the fixed doses that are mentioned in the article) and antinociceptive activities, validating the plant part in the treatment of diabetes and pain.

1.20 Rationale of the Project:

F. jangomas is very important medicinal plant for Bangladesh, which has been used for many years to treat a variety of illnesses including antianalgesic, diabetes, acne, scarring, rashes, piles, diarrhea, dysentery, colic, and acidity. By using a qualitative phytochemical test, many chemical components, including glycosides, alkaloids, steroids, tannin, saponin, resin, terpenoids, and carbohydrates, have so far been identified. All components are significant since they are essential to the production and development of medicines**.**

1.21 Aim of the Project:

This study looked on the antidiarrheal abilities of *F. jangomas* in vivo**.**

1.22 Objective of the project:

Due to their accessibility for drug discovery, natural products, especially medicinal plants, have attracted significant attention in the pharmaceutical research area in recent years. Because they are less expensive to treat with, less toxic, and have less adverse effects than synthetic medications, many pharmaceutical lead compounds today are derived from plants. Other than this, herbal treatments are inexpensive, secure, and socially acceptable. And as a result, the development of the herbal drug sector received all of the attention in the globe. This analysis demonstrated that our experimental plant might have more bioactive chemicals than previously thought. We look into the fruit meat and seeds of the plant since they have a lot of medicinal benefits for the use in herbal medicine to treat a variety of disorders.

The primary goal of this project is to identify *F. jangomas*'s antidiarrheal properties. Additionally, the goal of the current experiment was to identify antidiarrheal action**.**

1.23 Present Study Protocol:

The most important motto of the current investigation is to evaluate the therapeutic value of several *F. jangomas* extracts. The study protocol must outline how the experiment will be conducted. Below are the study protocols:

1. Preparation of fruit and seed extract from *F. jangoma.*
2. Using mice to test the anti-diarrheal properties of crude extracts.

Chapter 2

Methodology

2.1 Extract preparation of *F. jangomas*

2.1.1 Collection and identification

Fresh fruits of *F.jangomas* were collected for pharmacological investigation. Those were collected from the tree Mathbaria, Barishal, Bangladesh in January, 2022. *F.jangomas* was recognized and verified by Dr. Farhana Alam Ripa, Assistant professor,BRAC Univercity,School Of Pharmacy and Bangladesh Herbarium taxonomist, Mirpur, Dhaka (DACB Accession No: 87043) and conserved in their laboratory for future investigation.

2.1.2 Plant sample preparation:

The experimental plant parts (fruits) underwent simultaneous tap water cleaning to remove any dirt, followed by a second wash with distilled water to sterilize them. The fruit was then allowed to dry in fresh air. Flesh and seeds were divided into two separate bowls after drying. Once more, seeds were washed in fresh water to eliminate any remaining flesh. It was properly dried after being bathed once more to prevent contamination. For a very little length of time, flesh and were kept apart. After drying seeds were crushed into powder.

Figure 2.1: Separation of seeds from flesh

Figure2.2: Separated dried seeds of F. jangomas

2.1.3 Method of extraction

Three glass jars were taken, cleaned well, rinsed with methanol, and dried as well. First of all, 5kg of fresh and dried *F. jangoms* was taken. Separated flesh and seeds were poured in different jars. As fleshes were more in amount than seeds it took 4 jars and another one jar was allocated for seeds. The whole powder of the seed was taken in that separate jar. Then, methanol solvent was poured into the jars according to the need. It was poured in the way that flesh and seed remained covered up to half inch from the flesh and seeds in the jar. The five jars were sealed with its content air tightly so that any kind of foreign particles could not enter into the jar as well as air. Those jars were kept for 2 weeks (14 days) at room temperature (22-25) ℃ and a secure place as it was congaing methanol (because it is flameable liquid). At the 1st day, the lid of the jars were being opened to remove the gas. It was continued for 2-3 days with stirring as seeds and flesh will release the gas frequently. To improve extraction, it was occasionally shaken and stirred. However, a multiple layer phase was seen, with the upper phase containing the methanol solution and the lower phase exhibiting sediment. The double-phased sample solution with methanol is shown here.

Figure2.3: Mixture of flesh and grinned seeds with methanole

2.1.4 Fractionation of the Extracts:

Using a clean cotton cloth, the extracts from *F. jangomas* were coarsely filtered, and the sediments were carefully removed for fractionation**.** Then it was again stored in the cleaned jars accordingly. After that, it was concentrated by running a rotary evaporator (Heidolph) at 100 revolutions per minute. The filtrate was once again evaporated at ambient temperature before being deposited separately.

The experimental plant components were extracted using a fractional method, concentrated by letting the solvent evaporate, and then collected in three different Petri plates. The extracts were then divided into various bottles and given the names "methanolic extract of *F. jangomas* seeds and flesh as FJMS, FJMF.

2.1.5 Steps of Methodology and Diagram

1. Collection of plant material (fruit)
2. Separation of Seeds from flesh
3. Drying of the material
4. Extraction

Figure 2.4: Diagram of extraction method

2.2 Experimental Procedure

2.2.1 Drug:

Loperamide was used for this study which was obtained from Square Pharmaceuticals Ltd., Bangladesh

2.2.2 Chemicals:

1. Methanol
2. Tween 80

2.2.3 Mice collection:

Adult Swiss Albino mice were used in this study (male/female). Average Weight of the mice was 30g. The mice were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept under standard environmental conditions for 14 days for acclimatization. They were given food according to the ICDDRB formulated food. Twenty- four mice were used for this study.

Figure 2.5: Swiss albino mice (Tripati H. 2018)

2.2.3 Test and standard drug preparation

For the flesh extract of *F. jangomas*, three different extracts were prepared at the concentration 15mg/ml, of 30mg/ml and 45mg/ml. Tween-80 was used as a control and loperamide was used as standard drug.

2.2.4 Anti-diarrheal test/ Castor oil induced method

The antidiarrheal impact was assessed, using castor oil induced method.

- Firstly mice were distributed into five section, control, standard and three extraction sector.
- Mice were fasted for 3 hrs.

- After that, tween 80, distilled water, loperamide and flesh extracts were given orally to respective group.
- 1hr later, 0.2ml of castor oil is given orally. Diarrhea was assessed each hour for 4hrs. Stool count was also determined per hour.

2.2.5 Antidiarrheal activity

The beginning of diarrhoea was significantly ($P< 0.001$) increased and the frequency of stools was significantly ($P <0.001$) decreased in the castor oil-induced diarrhoea model in vivo antidiarrheal test of F. jangomas leaves compared to control. The methanolic flesh extract demonstrated 14.52%, 23.68%, and 32.91% inhibition of defecation at doses of 100, 200, and 300 mg/kg, respectively, while standard loperamide demonstrated 72.93% inhibition of defection at dose of 100 mg/kg. This result indicates there was not very promising but slightly antidiarrheal activity by the prolongation of latent period as compared to control and standard.

2.2.6 Statistical Analysis

The experiment's overall values (experimental result) are expressed as mean standard error of the mean (SEM). Here, ANOVA (Analysis of Variance) was used to compare all the data that had been collected, and the Dunnet's Test came next using SPSS 16.00 (USA). However, at $P <0.01$, the values may be regarded as statistically significant.

Chapter 3

Result

3. Result

3.1 Results of Antidiarrheal Activeness of *F. jangomas* methanolic extracts on Swiss albino mice

Antidiarrheal activity with various concentrations of methanolic extracts of F. jangomas and reference standard loperamide is given in the provided result table.

Table 1: *Effects of F. Jangomas extracts on castor oil-induced diarrhea in mice*

Group	**Treatment**	**Number of feces in 4h**	**% inhibition of defecation**
Control (I)	Tween 80 solution	12.67±0.82	---
Standard (II)	Loperamide 100 mg/kg	2.67±4.58**	72.93
III	*F. jangomas* 100 mg/kg	10.83±0.41*	14.52
IV	*F. jangomas* 200 mg/kg	9.67±1.21*	23.68
V	*F. jangomas* 300 mg/kg	8.50±0.84*	32.91

*Every value represents mean ± SEM for n = 6, ** All the data were analyzed by ANOVA Followed by Dunnet's test.significant at p < 0.001, and * p < 0.05 vs. control.*

From the value of the table I found the antidiarrheal activity of methanolic flesh per hour until 4hrs extract of *F.jangomas* at 100,200and 300mg/kg concentration showed number of feces time respectively is 10.83, 9.67 and 8.65.

Chapter- 04

Discussion

In the present work, I examined the antidiarrheal properties of methanolic solvent extracts of *F. jangomas* flesh in Swiss albino mice. For studying how medications work, in vivo techniques using whole animal models are regarded as effective. Although the anti-diarrheal action of the meat extract was evident, it did not quite match the standard value of Loperamide. Ricinoleic acid a component of castor oil, increases prostaglandin production and causes diarrhea. Prostaglandin is involved in the patho-physiological processes that take place in the gastrointestinal tract. The withdrawal liquid presumably stopped diarrhea by stopping formation of prostaglandins. Finally I can say from the experimental value, the methanolic seed extract of *F.jangomas* have the antidiarrheal activity but it is not as significant as standard drug as Loperamide. Further study could be done to utilize the plants extract.

Chapter-05

Conclusion

People have depended solely on plants for the treatment of illnesses from the beginning of human society, but as modern cultures spread, scientists began to invest in various synthetic pharmaceuticals in addition to herbal medicines to treat many dangerous diseases. In order to examine the antidiarrheal activity of *F. jangomas*, we have selected various sections for this excursion, including the seed and flesh. It contains a variety of organic substances, including proteins, polyphenols, tannins, flavonoids, alkaloids, saponins, steroids, and terpenoids, which have been shown to have antidiarrheal effect in swiss albino mice. However,the existence of those major second line biotransformation**,**the various extracts of trial plants exhibit notable antidiarrheal action against diarrhea. My research on albino mice has produced encouraging findings, leading to the development of a method for synthesizing novel antidiarrheal medications from natural sources.

To sum up, it can be said that the results of the investigation make it abundantly clear that our experimental plant, *F. jangomas*, is a valuable resource in the search for new antidiarrheal medications. However, my work was a preliminary effort and will need more participation, thorough research, and a human-based model to establish our findings for potential therapeutic effects. In addition, pre-formulation studies and a depiction of active ingredients are required for the development of a viable dosage form.

Future prediction

The extracts of *F. jangomas* are the sole parts of the plant that are studied in this experiment. Other extraction techniques and more thorough phytochemical screening can be used in the future to learn more about the bioactivities of the plant. Because they will aid in the discovery of various structurally bioactive molecules. Therefore, proper screening of the various *F.*

jangomas sections may reveal more diverse bioactive chemicals with antidiarrheal activities at various concentrations. Additionally, the antidiarrheal activity against various microbes can be researched and contrasted. This will make it easier to comprehend how the extracts interact with different bacteria. Substantial antidiarrheal properties are present in all of the *F. jangoma* fruit seed extracts that have been studied. But more investigation is required to identify the precise mechanisms at play as well as the chemical components in charge of the pharmacological effects.

Chapter-06

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