

Study of Thrombolytic and Anti-arthritic Activities of
Flacourtia jangomas Fruit

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 (Hons.)

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

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Abstract:

One of the most important medicinal plants is *Flacourtia jangomas*, often known as Indian plum. It is extensively distributed in South-East Asia and Bangladesh's Chittagong Hill Tracts, Cox's Bazar, and Sylhet area and is used by medical experts to cure a variety of human disorders. Although no research has been done to confirm it, it was thought that *F. jangomas* fruit also had thrombolytic and anti-arthritic activity. In this in vitro study, methanolic extract of *F. jangomas* fruit flesh were prepared separately. Later, it was discovered that all extracts had a successful thrombolytic action, with the percentage of clot lysis reaching up to $56.77 \pm 7.52\%$ for the Streptokinase(SK) extract and $38.95 \pm 4.81\%$ for the MFJ extract of fruit flesh. Additionally, it had an anti-arthritic impact and had a percentage of protein denaturation up to $50.02 \pm 3.34\%$, which was more effective than half of the typical effectiveness of $88.45 \pm 4.05\%$. This made this research successful in terms of both efficacy in *F. jangomas*.

Keywords: *F. jangomas*, Thrombolytic, Anti-arthritic, Streptokinase(SK).

Dedication

Dedicated to my parents

Acknowledgement

I'd like to start by offering my gratitude to the All-Powerful Allah, our Creator, the source of our life and strength, as well as our knowledge, patience and wisdom, for his bounties and kindness. All thanks to the All-Powerful Allah, and I would want to convey my appreciation for giving me the perseverance, fortitude, thankfulness, and support I needed to finish this job. Without the assistance of the persons who are warmly acknowledged below, this study would not have been finished.

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List of Acronyms

BSA	Bovine Serum Albumin
SK	Streptokinase
MFJ	Methanolic Flesh extract Values of <i>F. jangomas</i>
ME	Methanol Extract
tPA	Tissue Plasminogen Activator
DMSO	Dimethyl sulfoxide
MIC	Minimum Inhibitory Concentration
FE	Fruit Ethanol Extract
BE	Bark Ethanol Extract
Conc.	Concentration

Chapter 1

Introduction

From the dawn of time till the present, people have employed plants as a origin of medicine. They once made up the majority of folk or ethnomedicine, which was used not just in India but also in nations like China, the Middle East, Africa, and South America. A large percentage of this traditional knowledge was gradually codified, arranged, and finally transferred into formal medical systems like Ayurveda, Chinese, Yunani, Sidha, Tibetan, or other systems (Sasi et al., 2018). The most accessible source of conventional treatments is herbal medicine. Since the beginning of time, people all over the world have used medicinal plants as traditional treatments to treat a wide range of maladies.

Many Bangladeshi plants have undergone bioactivity research in the past based on their historical usage, and many bioactive compounds have been found in different natural product laboratories (Talukder et al., 2012). Bioactive compounds with pharmacological activity, such as volatile oils, alkaloids, flavonoids, phenolics, tannins, and glycosides, may be used therapeutically. These bioactive chemicals come in a wide variety, and more are being extracted from therapeutic plants every day. So, a key source of starting materials for the development of new drugs is medicinal plants (Talukder et al., 2012).

Villagers still largely rely on herbal folk medicines for the treatment of common illnesses like cough, cold, and fever, headache, body ache, constipation, dysentery, burns, cuts, and scalds, boils, ulcers, skin diseases, and respiratory issues, among others, despite the significant development of rural health services (Dutta et al., 2017). However, recent years have seen a rise in interest around the globe in the use of phytopharmaceuticals as supplementary or alternative medicine, either for the prevention or amelioration of various ailments. Approximately 75–80% of the world's

population is now being treated by traditional medicine, making this mission very significant. As a result, WHO's goal is to increase the quality and efficacy of this medication.

In traditional medicine, plants serve as the main therapeutic medium (Dutta et al., 2017). In the classical and eastern worlds, plants have been utilized for healing for twenty-five millennia. Today, modern medicine has replaced many plant-based medications with synthetic alter in many nations, but it is important to note that about 30% of pharmaceutical preparations are derived directly or indirectly from plants.

Furthermore, we must acknowledge that many industrialized nations have a parallel industry for plant goods (herbalism) that is not necessarily scientifically accurate, particularly in recent years on the wave of naturism and ecology (Sebastian, 2017). Now, it is crucial that the plants used for healing be effective, which means that they include biologically active principles, are maintained in a way that the active principles are retained for a fair amount of time, and are in sufficient concentrations and do not contain dangerous substances (Marini-bettglo, 1980).

In traditional medicine, it is customary to utilize herbs to treat burns, dermatophytes, and infectious disorders. Based on taxonomic and ethnopharmacological data, the antibacterial efficacy of various medicinal plants' aqueous and methanol extracts was assessed in vitro using the agar diffusion technique. Researchers looked into the antibacterial activity of five different plants' leaves from various families that have been used traditionally for therapeutic purposes (Dubey et al., 2013). All of the chosen plants' powdered leaf components underwent water and methanol extraction (Marini-bettglo, 1980).

Following research supports the use of traditional medicines (herbal extracts) to treat a variety of illnesses, including diarrhea, gastrointestinal tract disorders, throat infections, ear infections, fever,

and skin disorders (Satish & Girish, 2008). *Flacourtia jangomas* (Lour.), one plant that is often used in conventional medicine. *Flacourtia*-related plants are recognized to provide a range of medicinal benefits. 15 species of flowering shrubs and small trees belonging to the Salicaceae (Willow) family make up the genus *Flacourtia*. The genus was once classified in the extinct family *Flacourtiaceae* and given the honorary name of Étienne de *Flacourt* (1607-1660), a governor of Madagascar. It is indigenous to the tropics and subtropics of Africa and Asia. A number of the genus' species are grown for their fruits and for ornamentation (Nath et al., 2021).

In the Caribbean, where residents use its fruits in cocktails and cuisines, they are highly adored. *Flacourtia* species may also be found in India. It expanded to several regions of the globe, including China, Florida, northern South America, Puerto Rico, Sri Lanka, and Puerto Rico. The phytochemical, ethnomedical and pharmacological features of *F. jangomas* are discussed in this paper (Sasi et al., 2018). Uncultivated, wild tropical fruit called *F. jangomas* has a wide range of uses in the culinary and medical sectors. In order to manufacture different pulp-based items while simultaneously increasing consumer acceptability, equipment and processing techniques will need to take into account the fruit's rheological qualities (stationary and dynamic shear properties). Understanding rheological properties is also necessary for forecasting other features, such as the mass and heat transfer coefficients for product creation, as well as packaging and storage techniques (Marini-bettglo, 1980).

The results of this study will be highly beneficial to companies who process food in order to produce goods using *F. jangomas* pulp (Nath et al., 2021). Ethnic groups investigate the healing potential of various plant species. Infusions, decoctions, and combinations made from various plant parts, including fruit, leaves, roots, stems, and bark, have traditionally been used as preliminary health remedies by ethnic societies (Benny et al., 2010).

Recent years have seen an increase in interest in plant research globally due to the production of secondary metabolites. In the search for new medications or microbial endophytes are a possible source for the production of strong and unique metabolites with potential pharmacological importance (Aly et al., 2010). A plant's internal endophytes may be the source of a variety of important traits and medicinal powers. Additionally, several studies have shown that endophytes create bioactive compounds that support plant growth and increase plants' resistance to diseases (Shukla et al., 2015).

1.1 Habitat and Morphology

F. jangomas (Lour.) A member of the *Flacourtiaceae* family, Raeusch is also known as Paniala, Indian plum, or coffee plum (now placed in *Salicaceae*). It is a modest deciduous plant with a height range of 6 to 10 meters, with the potential to infrequently reach 14 meters. While woody thorns are found in young trees, the branches of older plants are thornless (Tripathi et al., 2018). Ranging from pale brown to copper-red to buff-colored, with smooth, lenticelled bark that flakes into thin lamels. White Yung branches that are mainly glabrous or puberulous with many suborbicular lenticels. The oval, serrated, pappery, 7.0–11.0 cm × 3.5–4.0 cm leaf blades include 3-6 bundles of secondary roots. The leaves are alternate, perennial, soft pink when immature, swirly arranged, and seldom lobed, elongated tip jagged, and very slender. The stalk is 6.0-8.0 mm in length (Sasi et al., 2018). The inflorescence is composed of axillary racemes that are 1.0–2.0 cm long, subcorymbose, pubescent, and few bloomed with the male being 1.5–3.0 cm and the female being 1.0–1.5 cm long.

Dioecious flowers with four or five ovate triangular petals that range from white to greenish in color and have a honey-like scent are produced before or together with the young leaves (Sarma & Mahanta, 2020). Pedicels are only 0.5–1 millimeters thick (-1.5 cm). Oval, obtuse, 4-5, pubescent on both sides, and 2 mm in length, the sepals are ovate. White or yellow (orange) in color, the disk is fleshy and whole or slightly lobed. Female flowers are solitary whereas male flowers are filaments that are depilated, secluded or in bunches (Tripathi et al., 2018).

On different trees, you may find the male and female flowers. Anthers, which are numerous and oval to suborbicular in shape, make up the androecium. Two ovules are present in each of the ovary's six locules, which are initially flask-shaped before becoming subglobular. Each of the 4-6 styles has a large, bilobed, wedge shaped stigma. These styles connate form a separate, 1 mm high column, not or somewhat open near each apices. Between December and April, flowers begin to bloom together with new leaves that are a very attractive shade of fresh green (Sasi et al., 2018). The fruit may be consumed raw or used to make jam.

Due to the bark's advantageous phytochemicals with therapeutic benefits, it is used for a variety of uses. Ayurveda, Yunani, and other terms have been researched and recorded by ancient civilizations in India, China, and other nations (Nath et al., 2021). Villagers in India and other nations still use these herbal cures and easily accessible fruits as treatments for several common illnesses like cough, fever, stomach issues, etc., despite the fact that modern medicine has made significant progress in this area.

Around the globe, the plant has a long history of usage in conventional remedy to medicate a variation of illnesses. Tannins, minerals, ascorbic acid, tartaric acids, amino acids, and phenolic compounds are among the bioactive components found in *F. jangomas*. Both gram-positive and gram-negative bacteria were effectively inhibited by its strong antibacterial activity. In addition to

its therapeutic benefits, fruits are a wonderful source of vitamins, minerals, protein, carbohydrates, and dietary fiber (Sarma & Mahanta, 2020).

F. jangomas fruits are oval, prolate(1.5–2.5 cm in diameter), pale brown or purple, becoming blackish having green-yellow mush that ripens from March to July and contains 4-5(-10) plain seeds. Solitary, brief style columns of 4-6-min apical points are present at the tips of the seeds. Using seeds, the plant is multiplied. But since seeds take a long time to germinate, plants are often propagated via inarching or budding upon self-seedlings (Anjum et al., 2018)

Plant Profile (Sasi et al., 2018):

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Malpighiales

Family : Salicaceae

Genus : *Flacourtia*

Species : *jangomas*

1.2 Medicinal Uses of *F. jangomas*

Biliousness, fever, and digestive ailments are historically treated using *F. jangomas* fruit. Diarrhea, toothaches, bleeding gums, piles, and limb weakness are all treated with leaves and bark. A prior

research used streptozotocin and alloxan-induced diabetic mice to assess the anti-diabetic properties of *F. jangomas'* leaves and stem. Investigations and reports were made about the antimicrobial and cytotoxic qualities of roots (Anjum et al., 2018). Limolin and jangomolide, two limonoids, were discovered by phytochemical analysis of the stem and bark. To support the notion that the plant may be used in folk medicine, pharmacological studies on the leaves of *F. jangomas* were conducted after a literature review and based on its traditional usage. These studies looked for antioxidant, analgesic, and antidiarrheal properties (Talukder et al., 2012).

The tribes Pangiae and Oncobaeae's specific species of *Flacourtiace* are arguably most recognized for the seed oils they generate. Leprosy and other ailments have traditionally been treated with "Chalmoogra oil," which is made from the seeds of the spices *Taraktogenos kurzii* and *Hydnocarpus*. Sulfur medicines have now replaced this oil. Asthma is treated in India using dried *Flacourtia jangomas* leaves (Parvin et al., 2011). *F. jangomas* leaves and bark are used to cure a number of conditions, including gout and liver diseases as well as rheumatoid arthritis. However, the roots of its related species, *F. indica*, are pleasant, cooling, depurative, alexipharmic, and diuretic. In vitiated situations of pitta and vata apathies, toxic bites, skin problems, pruritus, erysipelas, stranguria, nephropathy, and psychopathy, they are helpful. Scabies and pruritus can be treated using the leaves.

The fruits are useful for treating straggler's jaundice, gastropathy, and splenomegaly since they are sweet, aperitif-style, digestive, and diuretic (Kumar Patro et al., 2013). Asthma, bronchitis, and hepatic problems are treated using dried leaves. Fruits are used for edible purposes by indigenous Indians as food. The leaves, bark, and roots are useful portions. Fruit is bitter. Dried leaves have astringent, tonic, and carminative properties. External and internal application modes (Hossain et al., 2011). To treat skin conditions, jaundice, and tumors, *F. jangomas* plant components are used

as astringents, acids, refrigerants, stomachics, diaphoretics, and anti-inflammatory agents (S.K.Gousia et al., 2013).

Dried *Flacourtia jangomas* leaves are used to treat asthma in India (Shirona T.K., 2014). As one of the primary active ingredients in remedies for dermatitis, scorpion venom, colds, stomachaches, gastritis, and a variety of other ailments, *couroupita guianensis* is regarded as a plant having significant therapeutic value (C.Alagesaboopathi et al., 2013). Various elements of the cannonball tree have antibacterial, ulcer-healing, anti-inflammatory, antioxidant, antinociceptive, antihelminthic, and anticancer activities (George, 2016). The fruit has a delicious acidic flavor and is quite cooling (Kumar Patro et al., n.d.).

The young stems and leaves, which have a rhubarb-like flavor and are said to have astringent and stomachic characteristics, are used for diarrhea and weakness. It is said that the leaves contain diaphoretic qualities. Mature fruits of *F. jangomas* accommodates a healthy quantity of potassium, which is highly bioavailable and may thus be an excellent source for ensuring an adequate consumption (Kumar Singh et al., 2010).

Table 1: Several F. jangomas plant components and their use as medicines for a variety of human ailments (S.K.Gousia et al., 2013).

Parts (Used)	Medicinal Uses in Common Disease and characteristic	Disease Category
Leaves and bark	Diarrhea, toothaches, bleeding gums, piles, and limb weakness	GIT problems.
Woods and root ashes	Remedy for snake bites.	Tropical disease and zoonotic disease.
Bark	Gout and rheumatism.	Arthritis.

Root ashes	Kidney disease.	Chronic kidney disease.
Fruit	Nausea and vomiting as well as appetizing, carminative, and bilious illnesses.	GIT problems.
Leaves	May help with scabies and itch.	Skin conditions.
Twig	Used as toothbrush for toothache and oral infection.	N/A

1.3 Ethnopharmacology and Phytochemistry

A branch of medical anthropology that deals with the treatment of human and animal ailments is ethnomedicine, commonly referred to as traditional medicine in this nation. This depends not only on the handwritten records of those who practiced in the past, but also on those who practice, gain experience, and then pass on their expertise to the next generation in various locations (Gupta et al., 2015). These ethnomedicinal reports are sometimes used in anthropological study as well as novel medication development. The mangrove forest Sundarban is a repository for several species that are commercially extremely useful for the provision of different medications while offering strong timber for building materials and food such as bee honey, sea crabs, and river fish. *F. jangomas* is a well-known and widely used remedy in the southern region of Bangladesh to cure a variety of illnesses that affect the local population. Locals cure skin conditions, stomach discomforts, hepatic ailments, along with many other common health issues, using the leaves, roots, and bark of *F. jangomas* (Gupta et al., 2015).

Moreover, having great nutritional and therapeutic value, *F. jangomas* is a significant fruit tree. Fruits are thought of in the Indian medical system as a remedy for poisonous diseases and vitiated doshas. Biliary diseases and diarrhea are treated with the fruits. The leaves after being decocted are used to cure diarrhea, dysentery, and piles in addition to treating diabetes, toothaches, and bleeding gums (Tripathi et al., 2018). Asthma, pre- and postnatal blood purification, and a number of other illnesses are treated pharmaceutically using various plant components. Fever with sporadic onset is treated with barks. In addition to being diuretic and alexipharmic, the roots are pleasant and cooling. They may be helpful for conditions including anemia and asthma. Astringent and stomachic, the immature shoots and leaves have a rhubarb-like flavor (Tripathi et al., 2018)

The fruits are used to treat nausea, diarrhoea, fevers, biliousness, thirst, and other ailments as well as to overcome digestive issues. To stop diarrhoea, the leaf decoction is consumed. For skin eruptions and ulcers, powdered roots are used as a poultice. They are also kept in the mouth for toothaches (Ahmed and Ramaswamy, 2006).

Biliary disorders, bleeding gums, toothaches, piles, and limb weakness may all be treated with a decoction of the bark. The leaves and bark are given to sore teeth and bleeding gums to cure toothaches, piles, and weakness in the limbs. The bark infusion is gargled to treat hoarseness. In order to treat bronchitis and cough, dried leaves are powdered (Ahmed and Ramaswamy, 2006). Fruits have a special place in the management of digestive and stomachic disorders; they quench thirst, are beneficial for biliousness and fevers, and they also ease nausea. For better digestion, the fruits are consumed in Burma (Yusuf et al., 2007). Asthma is managed in India with dried leaves. The pulp extracted from the roots is utilized to cure herpes infections, while a decoction of the leaves is utilized in Malaysia to medicate diarrhea and aid with digestion (Sasi et al., 2018). To induce labor, the fruits or a decoction of the leaves are consumed in Cambodia, Laos, and Vietnam.

To treat wounds, ulcers, and to calm an irritated throat, a paste of the roots is administered. In cases of jaundice and splenomegaly, fruits are administered (Ahmed and Ramaswamy, 2006).

In the tribal villages of the Western Ghat, ground bark mush is also utilized for the treatment of a number of common maladies. In liver-related illnesses, fruits are also employed. In south Indian traditional medicine, the astringent, acrid, sour, cooling, and stomachic plant is utilized to medicate a wide range of conditions, including diabetes, inflammation, skin illness, jaundice, tumors, nausea, and dyspepsia (Yusuf et al., 2007). The astringent and stomachic leaves and immature shoots that have a rhubarb flavor. It is beneficial for toothaches and bleeding gums since the leaves and bark are somewhat acidic and bitter. For diarrhea, piles, limb weakness, bleeding gums, and stomatitis, the leaves and bark are said to be helpful. As gargle, bark infusion is used (Anjum et al., 2018).



Figure 1: F. jangomas tree with leaves and fruits (Barbhuiya et al., 2020).

F. jangomas is one of those plants that hasn't been well studied scientifically. Studies on the plant's phytochemical components have been determined to be limited. Of fact, it wasn't until recently that plants in the *Flacourtiace* family came to light, thanks to the identification of a number of cytotoxic diterpenes from *Casearia sylvestris*. However, data on phytochemicals are recently obstructed to a small number of species (Sebastian, 2017). According to research so far, the *Flacourtiaceae* family produces a wide range of chemical classes, such as glucosides, alkaloids, terpenoids, flavonoids, tannins, lignans, and flavanolignans. While tannins are mostly found in the bark of the plant, they are also abundant in the leaves and young shoots. The plant also contains a fixed oil. Xanthonenes, quinones, limonoids, and phenazines have also been mentioned in studies (Sebastian, 2017).

According to reports, the stem and bark of *F. jangomas* contain the limonoids limolin and jangomolide. A number of bioactive substances have been identified in *F. jangomas*, including corymbulosine, tremulacin, hydnocarpic acid, and chaulmoogric acid. Ostruthin is a coumarin that was produced from the stem bark and fruit. A butyrolactone lignan disaccharide known as ramontoside, a steroid called -sitosterol, and its -Dglucopyranoside were discovered in the heartwood while the phenolic glucoside ester flacourtin was found in the bark (Chukwuma et al., 2018). Fruits were said to be a great origin of vitamins, minerals, protein, fat, sugar (including fructose, glucose, and sucrose), amino acids, and vitamin C. These minerals included zinc, potassium, manganese, calcium, copper etc. When fatty acids in fats were analyzed, it was discovered that oleic, hexadecadienoic, α -linolenic, stearic, palmitic including little more small anonymous acids were present (Srivastava et al., 2009).

Additionally, glycine, hydroxyproline, alanine, proline including valine were identified in amino acid extracts from dried, ripe fruits. Studies utilizing paper chromatography upon simple reducing

sugars and associated alditol acetates revealed the presence of arabinose, glucose, fructose, and galactose (Tripathi Y et al., 2018). The mature fruits of *F. jangomas* accommodates a large quantity of potassium, which is highly bioavailable and may thus be an excellent source for ensuring an adequate consumption. According to studies, the plant's leaves and stem accommodates secondary metabolites for example phenolic acid, tannins, sugar, saponins, steroids, and flavonoids like rutin, luteolin and quercetin. It also contains isocoumarins, coumarins, quinones, flavonolignans, phenazines, xanthones etc.

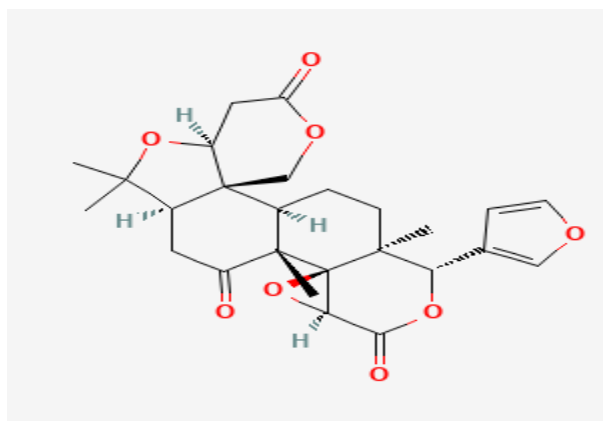
Fruits have beta-carotene, phenolic chemicals, anthocyanin, flavonoids, saponins, amino acids, which show that they are excellent antioxidants and have strong reducing power. Fruits that are still green also include phytochemicals such flavonoids, alkaloids, tannins, and total phenols (Sasi et al., 2018). The physicochemical composition and mineral content of Bangladesh were studied. Fruits from Assam, India, were extracted in methanol, and a phytochemical analysis of those extracts indicated the existence of saponins, flavonoids, alkaloids, phenols and saponins however, were not identified. The bulk of the functional ingredients were found to be present in the fruit's methanol extract, which also had 20 mg/g and 2 mg/g of total phenol and flavonoid contents, respectively (Anjum N et al., 2018).

Table 2: Phytochemical constituents obtained from F. jangomas (Sarma & Mahanta, 2020).

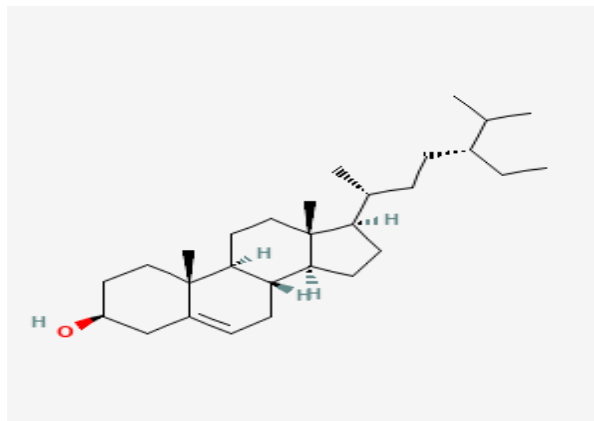
<i>F. jangomas</i> plant parts observation	Phytochemical Constituents
Crude protein	4.21%
Total carbohydrate	14.1%
Crude fiber	1.01%
Total lipid	0.16%
Ash content	0.735%

Total phenolics compounds	390 mg GAE/100g dm
Total flavonoids	6.66 mg QE/100g dm
Ascorbic acid	105.63 mg/100g
Antioxidant activity IC50 (DPPH method)	11µg/ml

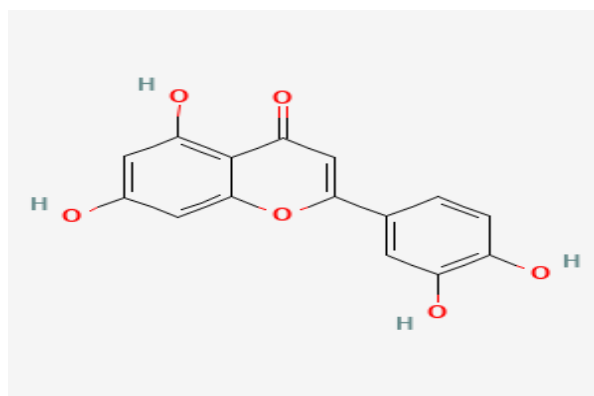
The fruit's rheological characteristics (dynamic and stationary shear properties) will be very helpful over establishing the machinery and screening methods needed for its extracting of various pulp-flourished goods whilst too enhancing customer acceptance. Understanding rheological characteristics is also crucial for forecasting other metrics, including temperature and mass shift coefficient, considering production creation as well as packaging and storage methods. The research's conclusions will be highly beneficial for the food processing businesses in creating *F. jangomas* pulp-based products (Rifna et al., 2019). Studies have been shown that various phytochemical constituents present in *F. jangomas*; such as mucilages, carbohydrates, gums, glycosides, phytosterols, phenolic compounds, alkaloids, saponins, terpinoids and protiens etc. (R. et al., 2018). Using standardized techniques, prepared plant extracts were examined for the presence of several phytochemical elements in *F. jangomas* (Wagner et al., 1984). An established methodology was utilized to check for the existence of substances for example: glycosides, steroids, alkaloids, tannins, flavonoids etc.



(a) Limonoid (limonin)



(b) Steroid (β -sitosterol)



(c) Flavonoid (Luteolin)

*Figure 2: Chemical structures of some phytochemical chemical constituents present in *F. jangomas* (Tripathi et al., 2018).*

In the current research, the ethanol extract of *Flacourtia jangomas* leaves was pharmacologically examined to pursue and assess the antioxidant, antidiarrheal and analgesic actions in order to give a acceptable guide that can be used in the future to continue a modern route of exploration. With an IC₅₀ value of 11 g/mL compared to the conventional ascorbic acid's IC₅₀ value of 5 g/mL, the extract significantly inhibited the DPPH radical scavenging activity in a mass-reliant approach. The Folin Ciocalteu's reagent was used to determine the extract's total phenol concentration, which was 601.03 mg GAE/100 g of dried plant elements (Saha S et al., 2012). In a dose-reliant approach,

the extract demonstrated substantial ferric reducing power when compared to normal ascorbic acid in the reducing power experiment. This was supported by high absorbance that rose with concentration. The extract (250 and 500 mg/kg) showed considerable ($p < 0.001$) dose-dependent reduction of the writhing reflex in the acetic acid-induced writhing test, which was highly equivalent to the diclofenac sodium reference medication (Talukder et al., 2012).

The hot-plate test results showed a dose-dependent approach, statistically remarkable improvement in the extract's ability to lower pain threshold levels ($p < 0.001$). Castor oil-induced diarrhea sample was utilized in an in vivo antidiarrheal test, and the extract (250 and 500 mg/kg) remarkably ($p < 0.001$) and dose-dependently accelerated the start of diarrhoea and reduced the overall amount of faeces differentiated to the control. The findings of the current research showed such the extract may have certain bioactivities, that call for more research, such as LC-MS to separate bioactive chemicals and to pinpoint underlying processes (Mondal et al., 2012).

Table 3: Discovered pharmacological activity of F. jangomas by using a variety of chemical test techniques and several solvent extraction techniques (George et al., 2017).

Solvent extraction and plant part(s) tested	Observed activity	Test method
Ethanollic extracts of leaves.	Anti-amylase activity	2, 2-Diphenyl picrylhydrazyl (DPPH) radical scavenging activity.
	Analgesic activity	TLC plates, DPPH radical scavenging activity, acetic acid induced writhing and hot-plate test.

	Antidiarrheal activity	Model for diarrheal induction by castor oil(<i>Swiss-Albino</i> mice). DPPH radical scavenging activity.
	Anti-microbial activity	In vitro microbiological screening, Disk Diffusion Method.
	Antioxidant activity	DPPH test for measuring free radical scavenging activity.
Methanolic extract of <i>Flacourtia jangomas</i> (MEFJ) leaves and stem	Anti-diabetic activity	Phytochemical screening, Acute toxicity test.
Ethanollic extracts of pneumatophores	Antibacterial activity	Minimum Inhibitory Concentration (MIC) Method.
Ethylacetate extract of <i>F. jangomas</i> leaves	Cytotoxic activity	Brine shrimp lethality bioassay method.
Utilizing in vitro techniques, ethanol and aqueous extract of leaf extracts	Anti-inflammatory activity	Inhibition of protein denaturation and inhibitory assay <i>proteinase</i> inhibitory assay. HRBC membrane stability assay, Cyclooxygenase inhibitory assay, Nitric oxide inhibitory assay.
Methanolic extract of both leaf and stem powder	Characterization of chromatography	Bioautography Screening, ¹ H NMR, HPLC, FTIR spectral analysis, and TLC (qualitative and quantitative DPPH assay),

The starch-iodine technique was used to measure the activity of α -Amylase. Because of the existence of proanthocyanidins and tannins in medicinal plants, a variety of biological actions, including anthelmintic, antibacterial, antineoplastic and cytotoxic, are shown to be effective in humans. Due to this, they provide a defensive mechanism against both invasive harmful parasites and herbivores.

Additionally, *proanthocyanidins* are a kind of flavonoid chemical polymers that have been shown to be effective against diarrhea. *Catechins*, *proanthocyanidins*, and other beneficial flavonoids have shown effective in several trials as agents. The microbe *Entamoeba histolytica lectin* and the *Shigella dysenteriae* toxin, which are the major causes of dysentery difficulties, have also been employed as wonderful antioxidants and may also block dysentery problems (Dubey et al., 2013).

This discussion has shown the specific plant's ethnomedical use in treating a range of gastrointestinal conditions. So, it is possible that this plant has a vast array of herbal remedies that may be used to cure diarrhea. Free chemical radicals may produce reactions within the body that have the opposite of the desired impact owing to their side chain features, which makes them very harmful. With the aid of phytochemicals, the body may reduce its level of free radicals, and subsequently that road can lead to a much better body system, that may be obtained from *F. jangomas* (Dutta et al., 2017)

In the test known as the brine shrimp assay of *F. jangomas* sections extracts, no toxic effects nor harmful interactions were discovered. There may have been in vitro experiments done, and from all of them it was discovered that *F. jangomas* had very effective anti-diabetic, anti-microbial, antioxidant, anti-amylase, analgesic, antidiarrheal, and anti-cancer capabilities within human body systems (Table 3).

1.4 Literature Review

Flacourtia jangomas Lour (Family: *Flacourtiaceae*), which is extensively spread in Bangladesh's Chittagong Hill Tracts, Cox's Bazar, including Sylhet region as well as across south-east Asia, is also known locally as Painnagola, Lukluki, Paniamra, Indian plum, and coffee pulm. It has already been discovered that following plant is utilized by both the local healthcare providers and the rustic residents of Bangladesh's southern region. That knowledge made it obvious that this tree has several ethnomedicinal qualities (Newaz Khan et al., 2011). It has been shown via several prior investigations that this specific tree has medicinal effectiveness for various ailments. According to reports, *F. jangomas* bark and stem contain the limonoids limolin and jangomolide. A number of bioactive substances have been identified in *F. jangomas*, including chaulmoogric acid, tremulacin, hydnocarpic acid, and corymbulosine (Chukwuma et al., 2018). From earlier research, it has been observed that *F. jangomas* has various effects including Antibacterial activity, Antidiabetic and Anti-fungal activity, Anti-amylase activity, Analgesic activity, Antidiarrheal activity, Antioxidant activity, Cytotoxic activity, Hypoglycemic and Anti-microbial activity etc. (Barbhuiya et al., 2020).

As stated by locals and local medical professionals from the southern region of Bangladesh, it is now thought through several reviews studies that *F. jangomas* tree also demonstrates effectiveness for arthritis and thrombolytic issue. However, no in vivo investigation was been out to support that claim. In current investigation, the anti-arthritis and thrombolytic effectiveness of *F. jangomas'* fruit flesh extracts will be examined in vivo. It is clear that, of all the things the *F. jangomas* is capable of, treating the rural residents in the southern portion of Bangladesh is one of its best uses.

This research was done to determine whether or not *F. jangomas* had any thrombolytic or anti-arthritic properties. If this is done, local healthcare providers may treat additional ailments with the same plant rather than only treating infections at this time. Due to the fact that the southern region of the country lags faraway beyond in the use of modern medications, this may be able to assist healthcare professionals in treating a variety of illnesses as well as greatly contribute to the ethnomedicine report, which will aid in the creation of novel pharmaceuticals from the ongoing research.

The study's objective was to separate the various components of the *F. jangomas* and test their effectiveness for thrombolytic and anti-arthritic effects in vitro. Although it was discovered in some earlier studies that *F. jangomas* has thrombolytic and anti-arthritic activity, the entire study was based on phytochemical research, and the leaves, roots, and bark were extracted into various solutions to determine its new efficacy. However, this research will represent that different techniques of ingredient extraction have varying degrees of effectiveness in addressing the problem.

1.5 Study Protocol

The objective of the following study was to look into any possible medical advantages of the fruit flesh extract from *F. jangomas*. It is essential to establish a study protocol outlining the trial's procedures. The following is an overview of the study's procedures:

- i. The fruit was collected from the desired plant.
- ii. The fruits were sliced into tiny pieces and then fruit pulp was prepared.
- iii. Then *F. jangomas* fruit flesh extraction was prepared with methanolic extract.
- iv. Thrombolytic and Anti-arthritic activity of the fruit was observed.

Chapter 2

Methodology

2.1 Plant Materials Collection

The fruit was taken in September 2022 after it had reached maturity from the Jahangirnagar University campus in Savar Dhaka-1342, Bangladesh. It was recognized by specialists at the Bangladesh National Herbarium in Mirpur, Dhaka (Accession number: DCAB87043). Also stored there for future use and further research was a voucher specimen. After being sliced into tiny pieces, the fruit pulp was prepared (Razibul Habib et al., 2012).

2.2 Preparation of Extraction

Ripe fruits were carefully cut into thin slices, and turned into pulp to create the methanol extract of *Flacourtia jangomas* fruits (MEFJ). Using 250 ml of methanol over 48 hours, 50ml of the fruit pulp extracted. The extract ultimately weighed 10.9g. The extract was stored in tiny aliquots at -20°C until use, and care was taken to avoid repeatedly freezing and thawing the extract vials (Shova et al., 2018). Cold extraction was used to extract a part of the fruit pulp using equal amounts of methanol. Each component of the sample was stored for about 72 hours while being continually stirred. All of the extracts were filtered off and evaporated using a rotary evaporator to dryness (45°C) at low pressure. 2.5 g (0.25%) was the yields of the methanol (ME) extracts. Finally, the extracts underwent a 4° Celsius thermal defatting process (Razibul Habib et al., 2012). With frequent shaking and stirring, 100 g of ground powder was steeped in 500 mL of ethanol in a glass vessel for seven days as part of the cold extraction process. Filtering the extract with a clean, white cotton plug twice produced a clear solution by removing any remaining plant material. The ethanol in the extract was removed using a rotary evaporator. Drying resulted in the production of a dark

purple, gooey crude extract. Dried plant material was yielded at 7.35%. Before conducting the experiment, the extract was kept in a refrigerator at 4 °C (Talukder et al., 2012).

Each fruit portion was extracted with ethanol. To achieve this, fruits were steeped in 650 cc of ethanol along with dry powder for a week. The components were placed in the jars, which were then shaken gently to prevent any substance spillage. The gaseous substances within the jars were released after careful shaking of the containers (Shova et al., 2018).. After that, additional solvent was added, and it was shaken as well as swirled while the powdered plant parts of *F. jangomas* absorbed it.

For an additional week, this approach was maintained. Following that, Whatman filter paper was used to filter the combination of the plant fruits and solvents. The filtrates, which had been previously filtered from fruit flesh to 50 ml, were then evaporated in a water bath to produce the ethanolic extracts. Then, they were left to fan dry so that a thick extract for fruit flesh in a dark purple hue could be produced. The undeveloped stage of fruit flesh is what is meant by this (Shova et al., 2018).

To make it stable in a separating funnel, methanolic extract and ethanol were first combined. This process was let to sit for a few minutes. Because methanol has a lower specific gravity than ethanol, it was obvious from the data in Table 4. that the methanolic extract component of the solution was at the top and the ethanol portion was at the bottom. Ethanol was added to one beaker from the bottom after being poured into the separation tube. When it was finished, the top half was transferred into a different beaker containing methanolic extract, and the second portion of the solution was used to perform the next experiments. A rotating vacuum evaporator was finally used to completely remove all solvents from the extracts while under decreased pressure (Tubon et al., 2019).

Table 4: Specific Gravity for the following Elements (Tubon et al., 2019).

Constituents	Specific Gravity
Ethyl Acetate	0.907
Methanolic extract	0.647
Ethanol	0.787
Methanol	0.8

2.3 In vitro Thrombolytic Activity

Thrombolysis is the pharmacological term for the breakdown (lysis) of clots in the circulation. It is sometimes referred to as clot busting. It functions by stimulating tissue plasminogen activator (tPA)-mediated fibrinolysis by the intravenous infusion of analogs. The protein called tissue plasminogen activator (tPA) ordinarily activates plasmin in the human body (Tabassum et al., 2017).

2.3.1 Instruments and Reagents

Digital shaking incubator, Eppendorf tube, Microliter Pipettes/ Micropipette, Centrifuge machines and Weight machine.

2.3.2 Extract Solution Preparation for Thrombolytic Test

The extract was diluted in 10 ml of distilled water at a concentration of 100 mg. To ensure a thorough mixing, it was afterwards agitated ferociously in a vortex mixer. It was left overnight after the combination. The soluble supernatant was then removed by slowly pouring the solution, and it was afterwards filtered via filter sheets (Whatman No. 1). In the end, a solution was

discovered, and it was prepared for the in vitro thrombolysis investigation (Amin Sikder et al., 2011).

2.3.3 Clopidogrel Solution Preparation

This mixture served as both a reference solution for the thrombolytic test and a stock from which 100µl of the in vitro thrombolysis procedure's total volume was drawn (Amin Sikder et al., 2011).

2.3.4 Test Procedure for Thrombolytic Test

Healthy human volunteers' vena cava was used to collect blood, which was then transported into 18 pre-weighed, sterile Eppendorf tubes with a 500µl capacity each. After that, these tubes were left incubated for 45 minutes at 37°C. As a consequence, once clot augmentation took place, the serum from all those tubes was entirely cleared, but the clot was unaffected at all for the subsequent trial. The Eppendorf tubes containing a clot underwent a second weighing to ascertain the clot weight (Jakaria et al., 2017).

$$\textit{Clot weight} = \textit{Bodyweight of clot containing tube} - \textit{Weight of tube alone}$$

Then, clots formed within each of the tubes. Each plant's extract—fruits, leaves, roots, and barks—amounted to 100 l, which was put into the test tubes. Following a 90-minute incubation period at 37 °C, all of the tubes containing each plant extract were combined. Following that, each tube had evidence of clot lysis.

After the incubation, the tube had some fluid in it, which was completely eliminated. After the blood clotting had been broken up, the tube was once again weighed to make a note about the weight change. In percentage form, the changes that were seen in the tube immediately before and immediately after the clot lysis were reported (Amin Sikder et al., 2011).

$$\% \text{ clot lysis} = (\text{Weight of the clot lysis} / \text{Weight of clot before lysis}) \times 100$$

It was discovered that the fruit flesh of *F. jangomas* inhibited the thrombolytic action. As a positive and negative (non-thrombolytic) demand, clopidogrel and water were utilized. The test was done many times using blood samples from several people (Tabassum et al., 2017).

2.4 In-vitro Anti-arthritic Activity

The technique of denaturing bovine serum proteins was used to investigate the in-vitro anti-arthritic efficacy.

2.4.1 Instruments and Reagents:

BSA(Bovine Serum Albumin), UV Spectroscopy, Beaker, Test tube, Micropipette, Pipette, Weight machine.

2.4.2 Preparation of Reagents

0.5% Bovine Serum Albumin (BSA):

Bovine serum albumin (BSA) 500 mg has been dissolved in 100 ml of purified water (Bs et al., 2014).

2.4.3 pH 6.3 Phosphate Buffer Saline:

0.2gm of KCl, 0.24gm of KH₂PO₄, 8gm of NaCl and 1.44gm of Na₂HPO₄ were dissolved in 800ml distilled water. After then, 1N HCl was used to elevate the pH to 6.3. Following pH correction, distilled water was used to create a complete solution with a volume of 1000ml (P et al., 2019).

2.4.4 Arthritic Test Methodology:

Test solution: The 0.5ml of solution included 0.05ml of test solution with varied concentrations together with 0.45ml of bovine serum albumin(BSA) (0.5% W/V aqueous solution).

Test control solution: The 0.5ml solution was composed of 0.05ml of distilled water and 0.45ml of BSA (0.5% W/V aqueous solution).

Product control: The solution was prepared in an amount of 0.5 ml, consisting of 0.05 ml of the test solution and 0.5 ml of distilled water.

Standard solution: A 0.5 ml solution containing 0.45 ml of BSA(0.5% w/v aqueous solution) and 0.05 ml of diclofenac sodium at different concentrations was added (Subramoniam, 2013).

Procedure: 0.05 ml of test medicines at conc. of 100, 250, and 500 g/ml and diclofenac sodium at concentrations of 50, 100, and 250 g/ml were used as the reference drugs, respectively. To each of them, 0.45 ml BSA(0.5% w/v) was combined. After that, the samples underwent a 20-minute incubation at 37 °C. Later, the temperature was increased to 57°C and held there for 3 minutes. All of the samples were preserved for subsequent cooling. After the respective solutions had cooled, 2.5 ml of phosphate buffer was added to each of them. The absorbance was measured at 255 nm using a UV-Visible spectrophotometer. Protein denaturation at 100% is represented by the control. The outcomes were contrasted to those of diclofenac sodium (Jakaria et al., 2017).

To calculate the % inhibition of protein denaturation, the given formula was used:

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{optical density of test solution} - \text{optical Density of control}}{\text{optical density of test}} \times 100 \right]$$

Chapter 3

Results

3.1. Result for Thrombolytic Test (In vitro clot lysis effects of *F. jangomas* fruit flesh extract)

Table 5: percentage(%) of lysis for thrombolytic activity of *F. jangomas* flesh with standard solution and water.

Sample	% of lysis
Water	14.22±1.16
Streptokinase (SK)	56.77±7.52*
MFJ	38.95±4.81*

Note: MFJ = Methanolic Flesh extract Values of *F. jangomas* are indicated as Mean ± S.E.M

(n=3). Data was analyzed using ANOVA preceded by Dunnett test, *p<0.05 compared with negative control.

3.2. BSA Method for Anti-arthritic Efficacy Results

Table 6: *F. jangomas* flesh extract's in vitro Anti-arthritic efficacy (% inhibition of denaturation for flesh extracts compared to diclofenac sodium standard)

Sample	% of Inhibition				
	Concentration(µg/ml)				
	100	200	300	400	500
Diclofenac sodium	66.46±1.01	75.41±1.54	81.13±1.62	83.42±6.69	88.45±4.05
MFJ	50.02±3.34*	56.89±3.75*	61.32±3.79*	69.69±7.00	70.26±6.39*

Note: MFJ = Methanolic Flesh extract Values of *F. jangomas* are indicated as Mean ± S.E.M

(n=3). Data was analyzed using ANOVA preceded by Dunnett test, *p<0.05 compared with negative control.

Chapter 4

Discussion

Thrombolytic and Anti-arthritic Activity of *F. jangomas* Fruit Flesh

In all of the studies conducted using the flesh extract of the *F. jangomas*, it was discovered that practically every portion of the tree that was removed had thrombolytic activity in an in vitro test. More thrombolytic action may be obtained from a component if its percentage of lysis is higher (Choudhary et al., 2015). In case of fruit flesh extract the maximum result was obtained from the extract of Streptokinase(SK) ($56.77 \pm 7.52\%$) and the lowest result was got from the Methanolic Flesh extract Values of *F. jangomas* ($38.95 \pm 4.81\%$).

Once the standard data from Clopidogrel, one of the most popular thrombolytic drugs utilized by all medical experts, was obtained, all the highest percentages of lysis were compared (Table 5). Following the comparison, it became clear that all of the highest lysis percentages were quite near to the reference percentage of 24.054%. Thrombolytic activity must exist in all of the extracts mentioned before, and they were undoubtedly capable of dissolving blood clots, it can be concluded with ease (Jakaria et al., 2017). From a different angle, it became apparent that one of the most crucial elements was that, in every instance involving fruits, leaves, roots, and barks, chloroform extracts invariably had the lowest percentage of lysis (P et al., 2019).

However, ethanol and ethyl acetate, the other two extracts, had lysis percentages that were almost as high as the maximum ones. It may be claimed that it might be desirable to forgo the extraction with methanol when using these plant parts to manufacture a pharmaceutical for commercial use since it was unable to properly separate the contents from the plant organs. Finally, it can be concluded from this study on phytochemicals that *F. jangomas* has thrombolytic action that can

be used to humans after all the testing. The process used to extract the plant's elements is crucial to take into account since various extraction techniques have varied levels of effectiveness. The local healthcare professionals or other researchers may benefit from this information by receiving it, since it will improve their understanding of *F. jangomas* from an ethnomedical perspective.

Earlier research and literature suggest that protein denaturation is one of the main causes of the known medically as rheumatoid arthritis (Alamgeer et al., 2015). The development of auto-antigens in the majority of rheumatic illnesses may occur owing to *in vivo* denaturation of the proteins, as was first hypothesized and subsequently supported by evidence (Sree et al., n.d.). Additionally, the modification of hydrogen, hydrophobic electrostatic, and disulfide bonding was thought to be a component of the process of denaturation. Anti-arthritic medications may include substances with the capacity to stop protein denaturation. Accordingly, in the context of our study, the more denaturation will be inhibited, the more effective the plant will be at combating arthritis (Bs et al., 2014).

Following the *in vitro* research, the outcomes of this investigation were now taken. The current study's findings indicate that all of the extracts used in this study, including the fruit flesh from *F. jangomas* from table 6, were capable of inhibiting protein denaturation, which is unquestionably an anti-arthritic potential in a dose-dependent way in comparison to that of diclofenac sodium, that was collected as a standard. All of the extracts employed in this investigation shown a possible percentage of inhibition of protein denaturation, despite some variations in the findings at various doses. In contrast to the thrombolytic study, various extraction techniques this time demonstrated varying potential effectiveness in terms of the anti-arthritic activity. However, it has been shown that *F. jangomas* ability to prevent protein denaturation makes it certain that it has anti-arthritic properties on its own.

Chapter 5

Conclusion

It may be concluded that *F. jangomas* has certain elements that will have both thrombolytic and anti-arthritic effects on humans after the whole investigation was carried out in vitro using the various portions of the plant. According to the research's findings, various extraction techniques had varying degrees of success in curing ailments. The same extraction technique should not be used to extract items from several branches of the same plant.

Additionally, During the components were differentiated with the outcomes of the standard data along with drugs, it was surprising that for each instance, the data were imminent to the standard data, that strengthened the following experiment's ethnomedicinal statement for designing modern adequacy out of an ancient origin of natural medications that would aid the rural mankind among the southern part of Bangladesh to meet a good medications for numerous disorders.

Additionally, it will enable the researchers to concentrate more heavily on the ethnomedical reports. It is crucial and required to identify the bioactive substances that are present in these natural sources and are accountable for these actions. Bioassay-guided fractionation is one method that may be used to accomplish this. The present study should serve as a starting point, a crucial instrument for upcoming chemical screening and biological testing, and it must provide new paths for the creation of innovative medications to treat thrombolysis and arthritis.

According to the results of the in vitro clot lysis investigation that was conducted, it could be shown that the fruit flesh extracts from *F. jangomas* predominantly exhibited moderate thrombolytic activity. This could be as a result of the many phytochemical kinds that are found on it. It is reasonable to presume that these extracts are potential sources of organic thrombolytic

drugs. This is simply a preliminary research; in order to fully use the extract's medical and pharmaceutical potential, phytochemical and pharmacological aspects need be completely studied (Tabassum et al., 2017).

Future Aspects of *F. jangomas*

After doing the whole experiment in vitro utilizing the different plant parts, it can be said that *F. jangomas* has certain components that will have both thrombolytic and anti-arthritic effects on people. The fruits of *F. jangomas* have showed promise for more research to find new bioactive components. The fruit extract has the ability to treat oxidative and non-oxidative damage brought on by reactive oxygen species. Investigations into ethnomedicine may provide crucial hints for identifying and creating new medicines from historically utilized medicinal plants (Sarma & Mahanta, 2020).

The present research demonstrated the in vitro way of determining the plant *F. jangomas*' effectiveness to have anti-arthritic impact and thrombolytic efficacy, however the utmost crucial work to complete is in vivo investigation. To determine if it can have a negative impact on either human or animal bodies, an animal study should be conducted soon or in the near future.

Furthermore, concerning for treating thrombolytic and arthritic problems, it is also possible to create novel extraction techniques that allow for the absorption of more ingredients than the ones used now and increase the proportion of protein lysis and denaturation (Dutta et al., 2017). Additionally, investigations on the adverse effects, medication interactions, effects on children, effects on pregnant women, and other factors should be carried out to ensure that no patients of any age or condition will be harmed. To uncover greater effectiveness against new diseases of this specific plant, the computational analysis may also be carried out in the future.

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