Phytochemical & Antidiarrheal Activity of

Nypa Fruticans wurmb fruit shell

By

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A project 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/our own original work while completing a 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/We have acknowledged all main sources of help.

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Approval

The project titled "Phytochemical & Antidiarrheal Activity of "*Nypa Fruticans wurmb*" fruit shell submitted by Samia Sultana Tinni (19146032), of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

Ethical permission has been achieved from the Department of Pharmacy, Jahangirnagar University.

Abstract

The objectives of this study were to determine the phytochemical composition and evaluate the potential antidiarrheal activity of the methanol extract obtained from *N. Fruticans wurmb* by its fruit shell. The phytochemical screening was conducted using qualitative analysis, whereas the antidiarrheal test was assessed using the castor oil induced method. Based on the findings of the phytochemical investigation, the methanol extract of the *N. fruticans wurmb* fruit shell included carbohydrates, saponin, resin, flavonoids, phenolic compounds, alkaloids, tannin, steroids, glycosides, and fixed oil. In the analysis of antidiarrheal activities, loperamide HCl served as the reference standard. In compared to Loperamide HCl (78.1%), diarrheic faeces were reduced at 200mg/kg, 400mg/kg and 600mg/kg with an inhibition of 23.15%, 37.49% and 59.42%. These results demonstrated the significant antidiarrheal action of the *N. Fruticans wurmb* methanolic shell extract.

Keywords: Phytochemical, Anti-diarrheal, *N. fruticans,* Loperamide

Dedication

This work is dedicated to my parents, siblings and friends for their love and constant support.

Acknowledgement

I am grateful to almighty Allah for providing me the opportunity to work with such wonderful people from the school of pharmacy who have always been idealistic and encouraging throughout my journey.

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List of Acronyms

STD	Standard sample
TAC	Total Antioxidant Capacity
TPC	Total Phenolic Content
WHO	World Health Organization
NO	Nitric oxide
OGTT	Oral Glucose Tolerance Test

Chapter 1

Introduction

1.1 Background

The phrase "medicinal plant" is used to describe a wide variety of plant species that are part of the practise of herbalism ("herbology" or "herbal medicine"). It includes both the scientific study of plants and their practical application in medicine. (Fitzgerald et al., 2020)

The term "herb" comes from the Latin "herba" and the obsolete French "herbe." The term "herb" is now commonly used to refer to any non-woody plant part, whether it be the fruit, seed, stem, bark, flower, leaf, stigma, or root. In the past, the term "herb" only referred to non-woody plants, such as grasses and flowers. In addition to being used in food, flavonoids, medicine, and perfume, these therapeutic plants are also used in various spiritual practices. (Shaheen et al., 2019),

Plants have been utilized as medicine by humans as far back as the Stone Age. Unani texts, Chinese texts, and Egyptian papyri all included discussions on plants. Around 4000 years ago, herbal remedies were used by Unani Hakims, Hindu Vaids, and the cultures of the Mediterranean and Europe. In addition to the widespread use of herbs in traditional medical systems like Unani, Ayurveda, and Chinese Medicine, they also played an integral role in the healing rites of ancient Roman, Egyptian, Iranian, African, and American cultures. (Shaheen et al., 2019)

Traditional medical treatments continue to be frequently employed in many circumstances. In recent times, there has been a surge in the attention given to the utilisation of botanical substances as a potential reservoir of therapeutic agents for diverse human afflictions. This heightened interest can be attributed to several factors, including the insufficiency of available pharmaceuticals, the exorbitant expenses associated with treatments, the adverse effects associated with numerous synthetic drugs, and the emergence of resistance to existing medications used for infectious diseases.

Among the world's oldest cultures, India is also known for its wealth of medicinal plants. Many medicinal and aromatic plants are taken from India's woods and processed for use in the pharmaceutical and perfume industries. As stated by various researchers (Ahmad et al., 2021).

About eight thousand different herbal remedies are recognized by the AYUSH systems of India. Ayurveda, Unani, Siddha, and Folk (tribal) Remedies make up the four major indigenous medical systems. In India, Ayurveda and Unani are the most well-known and widely practiced medical traditions. (Ahmad et al., 2021)

Recent WHO estimates suggest that over 80% of the global population uses herbal treatments to meet at least some of their primary healthcare requirements. (Ekor, 2014) More than 75% of the world's population uses plants or plant extracts for their primary source of medicine, according to recent data. Historically, humans have used more than 30 percent of plant species for therapeutic purposes. Plant-based pharmaceuticals are responsible for up to 80% of the drugs taken in quickly developing nations like India and China, while only accounting for up to 25% of the drugs consumed in industrialized nations like the United States. Consequently, economies in places like India place a much higher value on medicinal plants than elsewhere. Indigenous medical practices constitute the backbone of rural health care, and their producing countries supply almost two-thirds of the world's medicinal plants. (Rahman et al., 2022)

Because of this lack of adverse reactions, many people consider using medicinal herbs to be a safe option. The primary advantage is that they don't conflict with nature in any way. Herbal medicines are effective for both young and old, male and female. (Shaheen et al., 2019)

The ancients just assumed that plants could cure a wide variety of ailments. They did extensive investigation and experimentation to determine the efficacy of several herbs with therapeutic potential. Most drugs developed in this fashion have no unwanted reactions or side effects. This is contributing to the worldwide rise in popularity of herbal remedies. These herbs have been used medicinally for thousands of years, and they have shown effective in treating a wide range of internal ailments that had previously been considered intractable. Several common maladies are treated by medicinal plants like aloe, tulsi, neem, turmeric, and ginger. In many sections of the nation, these are regarded as natural cures. It is a well-known fact that many customers use basil (also known as tulsi) for pooja, brewing black tea, manufacturing medicines, and other daily activities. (Niyati Kumari & Mahalakshmi, 2019).

1.1.1 A Historical Overview of Medicinal Plant

Plants and herbs have a rich history of being used as medicine. They have a lengthy history of use in medical settings. Religion and the use of medicinal plants go back centuries. Many countries' cultural practices were influenced by them. They are currently thought of as medical practice in equal parts art and science. No one knows for sure when it happened, and the search for new plants is where doctors find all of their medical herbs. Nearly 800 therapeutic plants are cataloged in the ancient Egyptian Ebers Papyrus. Herbalism's "golden age" was between the 1400s and 1600s. (Petrovska, 2012).

1.1.2 Phytochemical Screening of Medicinal Plants

Extracting, identifying, and testing for the presence of medicinally active chemicals in plants is what is meant by "phytochemical screening of medicinal plants." Alkaloids, flavonoids, carotenoids, glycosides, tannins, antioxidants, and other phenolic compounds are only a few examples of bioactive chemicals found in plants (N. Gandhiraja, 2009). Phytochemicals are substances that occur naturally in plants and play a vital part in the plant's defense against a wide variety of microbes that can be hazardous. These components are extracted from medicinal plants and then identified through the use of a variety of separation techniques. (Lone, 2013).

1.1.3 Bangladesh’s stand in Medicinal plants

The World Health Organisation (WHO) provides a definition of traditional medicine as a comprehensive collection of information, expertise, and methodologies derived from ideas, beliefs, and accumulated experiences that are shared throughout many cultures. These practises are employed within the realm of healthcare to both treat and prevent illnesses. (Haque et al., 2018) Pharmaceutical plants are regarded as one of the most common areas of conventional medicine. Since Bangladesh is situated in the greatest deltaic plain—the Ganges–Brahmaputra delta—and is mostly made up of flood plains with a subtropical monsoon climate, it has an extensive variety of medicinal plants. The nation possesses a diverse array of plant species owing

to its distinctive geographical location, consistent access to untainted freshwater, and favourable climatic conditions. (Mukul, Biswas and Rashid, 2017). More than 6000 plant species, including bryophytes, pteridophytes, gymnosperms, angiosperms, algae, and ferns, are projected to exist there; of these, 455-747 are said to contain therapeutic agents (Flora, 2021).

In Bangladesh, the forests, coastal regions, shrubs, and vacant land near the canal are all naturally home to medicinal plants utilized in traditional medicine. There are four different traditional medicinal systems that may be found in the nation: homoeopathic, ayurveda, unani, and traditional. Kavirajes (Ghani, 1998) are those who use the folk or traditional medical system and primarily treat patients using local medicinal plants. Rural residents rely on them for their medical needs because the medications are easily accessible, efficient, and affordable. There are more than 87000 villages in Bangladesh, and most of them have one or two Kaviraje practitioners. Their understanding of medicinal plants provides them with enough knowledge to conduct scientific studies and create safe and effective medications. Also, earlier studies on ethnomedicine point to the effectiveness of the therapeutic garments worn by various Kavirajes in the management of a particular illness (Rahman et al., 2001).

1.2 Sample Plant (*N. Fruticans wurmb*)

The Nipa palm, scientifically referred to as *Nypa fruticans wurmb*, is a palm species indigenous to the coastal regions and estuaries of the Indian and Pacific Oceans. It is also known as the nipa palm (or simply nipa, from the Malay word nipah). (Farid Hossain, 2015a) It is the only palm tree that has been shown to survive in the mangrove environment. The root of the tree is really underground, with just the leaves and flower stalks visible above ground. The maximum diameter of the trunk is 45 cm, while the maximum length of the leaves is 6 m. This palm has a globose inflorescence with female flowers at the very tip and male blooms lower down on the branches. (Farid Hossain, 2015b) It may be eaten and produces a sweet sap that is mostly utilized in the production of alcoholic beverages, syrup, sugar, and vinegar. The seed is picked when the fruit is still young and consumed uncooked. Traditional medicine relies on plant components to alleviate symptoms including pain and inflammation (such as those caused by toothaches, headaches,

ulcers, and centipede bites). The leaves are prized for their use in thatching structures and weaving high-quality baskets and mats.

The Nypa palm can be grown from either seeds or sucker cuttings to produce new plants. Catkins-like inflorescences of red or yellow male flowers are found on the lower branches of the plant, and globular clusters of female flowers can be found at the very top of the plant. The flower produces a globular cluster of woody nuts that can measure up to 25 centimetres (10 inches) across, and this cluster is produced on a single stalk. The mature nuts fall off the ball and float away with the tide. Sometimes, while they are still submerged in water, they begin to germinate. The Sundarbans, which are located in Bangladesh, include the biggest continuous mangrove forest in the world. This is the only site in Bangladesh where the Nipa palm grows naturally. The leaves of the Nypa plant are the primary commodity produced by the plant. These leaves are widely utilized as thatching materials and provide a source of income for people of Bangladesh's Sundarbans Impact Zone (SIZ) who are economically dependent on the forest.

Figure 1: Different parts of *N. Fruticans* (Bobrov et al., 2012)

1.2.1 Ecology

It is particularly resilient to situations that are both freshwater and brackish, which demonstrates the diverse ecological ranges that it may thrive in. It can also be found inland, but only to the extent that the tide can carry the floating seeds inland. It is the only species of palm that does particularly well in mangrove environments.(Widodo et al., 2020) It is known that long-tailed macaques (Macaca fascicularis) eat the nipa palm's fruits. In the Padas Damit Forest Reserve, proboscis monkeys have been seen consuming the inflorescences. Orangutans in Borneo eat the hearts and shoots of nipa palms. (Numbere, 2019).

1.2.2 Taxonomic Tree

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Arecales
Family:	Arecaceae
Genus	*Nypa*
Species	*Fruticans*

Table 1: Taxonomical classification of *N. fruticans* wurmb("Nypa Fruticans (Nipa Palm)," 2022)

1.2.3 Distribution and Habitat

Nipa palms have a preference for inhabiting regions characterised by abundant nutrient-rich sediment and the presence of sluggish river or tidal currents. The floating nuts have the potential to be found in locations extending inland to the furthest extent influenced by tidal movements. These organisms are frequently found in the coastal areas and river systems that drain into the Indian and Pacific Oceans, spanning from India to the Pacific Islands. When the palm tree's surrounding environment experiences desiccation, it may exhibit a certain degree of resilience and endurance. Despite being commonly referred to as a "mangrove palm" and exhibiting growth in coastal regions, this particular plant possesses only a limited level of salt tolerance and is susceptible to mortality when subjected to immersion in saline environments. The estuary water with high salinity is more suitable to the organism. (Dewi Astuti et al., 2020)

It is believed to be native to the regions of Queensland and the Northern Territory in Australia, as well as the Ryukyu Islands, Bangladesh, India, Sri Lanka, the Andaman and Nicobar Islands, Myanmar, Cambodia, Thailand, Vietnam, Borneo, Java, Maluku, Malaysia, Singapore, the Philippines, Sulawesi, Sumatra, the Bismarck Archipelago, New Guinea, Solomon Islands, Caroline Islands, and Australia. It appears to have been naturalised in Nigeria, the Society Islands of French Polynesia, the Mariana Islands, Panama, and Trinidad. Iriomote Island, off the coast of Japan, and neighbouring Uchibanari Island comprise the distribution's northernmost limit. (Dewi Astuti et al., 2020)

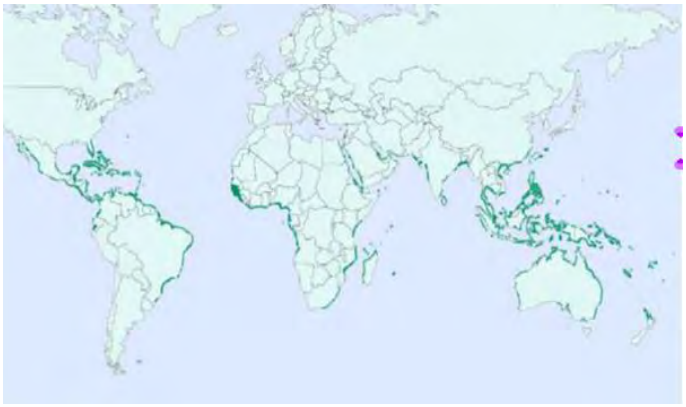

Figure 2: Distribution of Nypa(Dewi Astuti et al., 2020)

1.2.4 Plant Type

Perennial

Aquatic

Seed propagated

Vegetatively propagated Woody (Dewi Astuti et al., 2020)

1.2.5 Botanical Features

N. fructicans is a gigantic, invasive, pleonanthic, armless, creeping palm that bears fruit despite the fact that it is monoecious. Up to 45 cm in diameter, prostrate or underground (a rhizome) stem with regular and dichotomous branching, bending leaf scars above, and roots below. The petiole is fairly thick, and there can be as many as 163 leaflets on each leaf. The leaves are grouped in whorls of three to five on each plant. The form of the leaf is linear, and its dimensions range from 1.2 to 1.5 metres in length and from 6.5-8.6 centimetres in width. The texture is coriaceous, and the midrib has appressed brown scales on the lower surface. The leaf length ranges from 4.5 to 14.2 metres.(Dewi Astuti, together with others, 2020) The protogynous inflorescence is a solitary, erect, multibracteate inflorescence that can reach a maximum length of 2.1 m and has a stout, terete peduncle that can reach a maximum length of 2.4 m; the rachis is typically shorter than the peduncle, terete, and ends in a globose head of female flowers that is surrounded by numerous short, It is dark to black in colour, has a smooth exocarp, a fibrous mesocarp, and a thick endocarp made of interwoven fibrous threads, and measures 10-15 x 6-8 cm. The fruit is a drupe, developing on a carpet; it is compressed and unevenly angled; it takes the form of a pyramid; and it is between 15 and 20 by 6 to 8 centimetres in size. The seed is an elongated oval and is distinguished by its adaxial grooves, its basal hilum, and its homogeneous endosperm. Germination occurs on the infructescence (viviparous); the plumule swells and forces the fruit to the surface of the plant. (“Nypa Fruticans (Nipa Palm),” 2022)

1.2.6 *N. fruticans wurmb* in Bangladesh

Golpata (*N. fruticans wurmb*) is a well-known palm that can be found in both wild and cultivated settings along the coast of Bangladesh. The plant species under consideration is not only found naturally in specific regions of mangrove forests that are under government control, but it is also extensively grown via private endeavours in plantations established by rural farmers along the coastal areas, namely in the southern region of Bangladesh. The Sundarbans is recognised as the largest contiguous expanse of mangrove forest globally.(Chaudhury & Naithani 1985, Akhtaruzzaman 2000). Demand for Golpata goods is currently exceeding supply across the country. According to Shiva (1994), Golpata might be widely used in Bangladesh's commercial construction and healthcare sectors. Golpata plays a crucial part in the livelihood economies of both rural and urban Bangladesh. Nearly eighty percent of the homes in the Sundarbans region are constructed from Golpata (Faizuddin et al., 2000a), making it an essential resource for the region's estimated 50,000 inhabitants. The growing need for housing and other reasons necessitates the expansion of output through the creation of plantations and the sustainable management of the natural resource. It is worth noting that despite this, several local inhabitants residing near the Sundarbans opt to independently cultivate Golpata on their own property. (Islam et al., 2020)

1.2.7 Traditional use of *N. fruticans wurmb*

Humans have found many uses for the Nipa palm, including thatching roofs, dividing rooms, weaving mats and hats, harvesting sugar from the arteries sap, making aromatic tea from the leaf blade, and treating illnesses with the young seed. The purpose of the newly developed shoots is as a vermicide. Nipa palm ash has been used for centuries as a pain reliever, particularly for toothache and migraine. Leaves, petioles, stem wood, fruit scraps, and other dried plant matter are all utilised as fuel. Nipa palm rhizomes are often utilised in fishing because they help the net stay afloat. Nipa palm, according to farmers, is a great bait for luring large fish from the depths. Molasses and alcoholic beverages are produced from the juice. Tapping the palm for drinks like wine or toddy is a practise that has deep roots in the cultures of the South Pacific and Southeast Asia.(“Nypa Fruticans (Nipa Palm),” 2022b) Nypa palm has considerable commercial potential in Bangladesh's construction and healthcare industries. It serves as a place to live as well as for other essential

functions. The long, pinnate leaves (fronds) can be used to make thatch for roofs. You may make brooms, baskets, mats, and sun hats out of the leaflets and midribs. Sweet and jelly-like, the white endosperm of immature seeds is a popular snack. In several parts of the world, cigarettes are wrapped in the cuticle of newly unfurled leaves. Traditional medicines derived from the nipa palm include treatments for a variety of ailments, including herpes with juice from young shoots and toothaches and headaches with ash from burned nipa palm material. (Dewi Astuti et al., 2020) The Nipa palm is one of the palms that is utilised in the production of salt. In Nigeria, the hard shell, also known as the mesocarp, is effectively utilised in the production of a variety of fashion accessories, including buttons, necklaces, and other items. Nipa fronds are a common sail material for the regional fisherman. In the past, thatching roofs was accomplished using a wide variety of materials, including leaves, umbrellas, raincoats, hats, mats, brooms, baskets, cigarette wrappers, and ropes, in addition to the leaves themselves. Many people in Malaysia, India, and Bangladesh eat both the vinegar and the alcoholic "toddy" that are produced from the sweet fluid that is extracted from the flower stem. This alcoholic beverage is made using the fruit of a variety of palm plants, including the coconut palm. The gelatinous endosperm of immature seeds is edible and may be eaten fresh or stored in 'heavy syrup.' The seeds that have hardened from matured fruits are used as vegetable ivory and buttons. In addition, the treatment of conditions such as headaches, toothaches, and herpes is recommended to involve the use of palm leaves, roots, and young shoots in traditional medicine. Just before it opens its petals, the inflorescence is cut apart so that the delicious sap may be extracted. When they are still young, Nipa Palm shoots can be eaten. The petals of the flower, when steeped in hot water, produce a pleasant tea. The unripe immature fruits are described as being white, translucent, and hard like jelly. They have not yet reached their maturity. Attap chee is the usual term for these ingredients when they are used in regional desserts. The flowering stalks (peduncles) of palm trees may be tapped to extract palm sap, which has a long history of being processed across South and Southeast Asia into treacle (also known as molasses), amorphous sugar (known as "gula malacca"), alcohol, and vinegar. "Toddy" is a locally manufactured beer that is created from the sap of the sugar cane palm tree. In Indonesia and Malaysia, it is called "nera," and in the Philippines, it is called "tuba."

1.3 Rational of the Study

N. Fruticans wurmb found widely in the Sundarbans and other wet, salty regions of Bangladesh. The trees are widely utilized for their timber despite the fact that numerous research, both old and new, have found that the plants contain many active phytochemical elements.

The medical industry of Bangladesh may be able to generate new pharmaceuticals and make substantial advances in the treatment of current ailments if it conducts study into these chemical compounds and their natural potential to develop in an abiotic and biotically inhospitable environment. This research was conducted to analyse the phytochemical components of Nypa and its antidiarrheal potential.

1.4 Aim of the Study

The purpose of this research is to identify the phytochemical components of *N. fruticans wurmb* and investigate whether or not the plant have antidiarrheal properties.

1.5 Objectives of the Study

The following are the goals of this study:

1) To identify the phytochemical ingredients of *N. Fruticans wurmb* fruit shell.

2) To investigate the antidiarrheal efficacy of *N. Fruticans wurmb* fruit shell**.**

Chapter 2

Materials and Methods

2.1 Experimental Design

A member of the *N. Fruticans wurmb* family was the subject of investigation in this particular piece of research. The findings of the research on the fruit shell will be broken down into two categories:

● Phytochemical Analysis

● Biological Analysis

2.2 Analysis of the Phytochemical Profile of *N. Fruticans wurmb* fruit shell

2.2.1 Plant Material Collection and Preparation

In order to study the phytochemical properties of the *N. Fruticans* plant, fresh fruits shells were harvested. These are from the Sundarbans, or mangrove forest, in Bangladesh's Bagerhat district. This specimen was collected in Mirpur and Dhaka and later authenticated by a taxonomist at the Bangladesh National Herbarium (DACB Accession number: 87898). The BRAC University of Bangladesh Phytochemical Laboratory maintained the material. The shells were air-dried for a few days and then oven-dried for 24 hours at 400C or less so that they would ground more efficiently. The dried roots were ground into a coarse powder using high-capacity grinders.

Figure 3: Morphology of *Nypa* fruit shell (Bobrov et al., 2012)

2.2.2 The Process of Plant Material Extraction

In a conical flask, 2L of methanol was added to 500g of fruit shell powder. The flask was covered with aluminum foil and left for 14 days, during that period it was shaken and stirred occasionally. After transferring the combined substances to a rotary evaporator set to evaporate the solvent at 50°C Celsius, the mixture was filtered twice: first through a new cotton plug and then through filter paper.

2.3 Methods of the Experiment

In order to assess the plant's qualitative chemical structure, a crude extract of *N. Fruticans wurmb* was placed through a phytochemical screen. This screen looked for the presence of carbohydrates, glycosides, tannins, alkaloids, saponins, resin, phenols, flavonoids, steroids, and fixed oil. Additionally, it looked for the existence of these components.

2.3.1. Test for carbohydrates (Usman et al., 2010a)

Molisch Test: In order to conduct the Molisch test, first 2 millilitres of the shell aqueous extract were placed into a test tube. Next, 2 drops of a freshly prepared alcoholic solution of alpha naphthol at a concentration of 10% were added to the test tube. After that, the test tube was turned upside down, and 2 millilitres of highly concentrated sulfuric acid was allowed to flow down the side. This produced an acid layer beneath the water. When carbohydrates are present, a dark purple solution is produced when a crimson or reddish violet ring forms at the interface between the two layers and the mixture is left to stand or shake. This phenomenon is known as the "ring of crimson" or "ring of reddish violet." After that, it remained up for close to two minutes before being placed back down once more. After that, 5 millilitres (mL) of water were added to the solution in order to wet it down. A hazy, bluish-violet precipitate will appear very immediately upon analysing plant material for its carbohydrate content.

Fehling's Test: For the Fehling's test, 2 milliliters of the shell material's methanolic extract was combined with 1 milliliter of a solution made by mixing Fehling's solutions A and B (in equal volumes). Then, for a few minutes, the concoction was brought to a boil. When reducing sugar is present in plant extract, a crimson or brick-red precipitate will form.

2.3.2 Test for Glycosides (Shaikh & Patil, 2020)

Keller Killiani test: After dissolving 1 ml of the extracts in an equivalent amount of glacial acetic acid and allowing the liquid to cool, 3 drops of ferric chloride were added in order to perform the Keller Killiani test. This was done so that the results of the test would be accurate. The interior of the tube was then treated with two millilitres of a strong sulfuric acid solution. The presence of glycosides was demonstrated by the formation of a ring with a crimson-brown colour at the interface between the two layers.

2.3.3 Test for Tannin:

Ferric chloride test: The ferric chloride test identified tannins by reacting the extracts with a few drops of ferric chloride solution, producing a black precipitate.

Lead acetate test: The presence of tannins was determined by adding a few drops of a 1% lead acetate solution to 1 milliliter of extracts, which resulted in the production of a large, crimson precipitate.

Alkaline Reagent test: Tannins were detected by the formation of a yellow to crimson precipitate after treating 2 mL of extracts with a solution of sodium hydroxide.

2.3.4 Test for Alkaloids (Shaikh & Patil, 2020)

Mayer's test: Observing a cream-colored precipitate following the addition of a few drops of Mayer's reagent (Potassium Mercuric Iodide Solution) to 1 mL of each extract, Evans (1997) concluded that the presence of alkaloids was present.

Wagner's test: Upon the addition of 1 mL of Wagner's reagent, which consists of iodine in potassium iodide, to 1 mL of each extract, a precipitate with a reddish brown coloration was seen. As a result of this discovery, it has been established that alkaloids indeed have a presence.

Dragendorff's reagent test: A volume of one millilitre of each extract was combined with a volume of two millilitres of Dragendorff's reagent. The existence of the alkaloids was confirmed by the observation of an orange precipitate development with the addition of 2 mL of diluted hydrochloric acid to the mixture.

Hager's test: The confirmation of the presence of alkaloids was achieved by the observation of a vivid yellow precipitate that formed upon the combination of 2 mL of each extract with a little quantity of Hager's reagent, which is a saturated solution of picric acid.

Tannic acid test: When 10% tannic acid was added to the extracts, a pale yellow-brown precipitate occurred, confirming the presence of the alkaloids.

$FeCl_3$ test: Each extract was diluted to 2 mL with a ferric chloride solution of a few drops. A yellowish precipitate indicated the presence of the alkaloids.

2.3.5 Test for Saponin (Usman et al., 2010b)

The powdered shell material weighed around 0.1 g, and it was allowed to dissolve in about 10 mL of water. The ingredients were then let to boil for a few minutes. The liquid was filtered thereafter. Once the filtrate had cooled, around 5 mL was diluted with water and shaken quickly. Stable foam formations even after heating are characteristic of saponins.

Alternatively, you may do this test by mixing together 0.5 g of alcoholic extract from the plant and water. It is possible to get the same effects.

2.3.6 Test for Resin (Shaikh & Patil, 2020)

Over low heat, around 5-10 mL of acetic anhydride was used to dissolve a tiny amount of chloroformic or ethanolic extract. After that, around 0.05 mL of sulphuric acid was added while the solution was still warm. If resin is present in the plant extract, it may turn the solution a vivid purple-red that quickly fades to violet.

2.3.7 Test for Phenol (Usman et al., 2010b)

Ellagic acid test: Evans (1997) claims that phenols can be detected by treating the extracts with a few drops of 5% (w/v) glacial acetic acid and then 5% (w/v) sodium nitrite solution, yielding a murky brown color.

Phenol test: The interaction of 2 mL of the extracts with 1 mL of ferric chloride solution produced a vivid colour, proving the presence of phenols.

2.3.8 Test for Flavonoids (Shaikh & Patil, 2020)

Zinc-HCl reduction test: Each extract had some zinc dust and strong HCl added to it. The creation of a deep red colour indicated the presence of flavonoids.

Lead- acetate test: The addition of a few drops of lead acetate solution to 1 millilitre of any extract precipitated a reddish brown colour, proving the presence of flavonoids.

2.3.9 Test for Steroids (Shaikh & Patil, 2020)

Liebermann Burchard's test: Each extract was diluted with the acetic anhydride solution to a volume of 1 millilitre. A few drops of strong sulfuric acid were used to treat the test tube walls, resulting in a reddish brown ring at the junction, which proved the presence of steroids.

Salkowski test: Chloroform and sulfuric acid were used to get the volume of the extract down to 1 millilitre. When steroids were present, a red hue became noticeable.

2.4 Biological Analysis

2.4.1 Anti-diarrheal activity

Principle

The antidiarrheal effect of *N. Fruticans wurmb* fruit shell extract was tested in mice using a castor oil-induced diarrhea model. According to the protocol, 1 milliliter of analytical-grade castor oil was used to cause diarrhea in each mouse. Separate mouse poop counts were recorded. Antidiarrheal activity was determined by comparing the treatment groups' observations to those of a positive control group.

Experimental animal

This study required young (Swiss-albino) mice weighing between 25 and 35 grammes. We got our mice from the ICDDR, B (International Centre for Diarrheal Diseases Research) at their Animal Resource Department. The mice were housed in ideal conditions, with a constant temperature of 21°C and a light/dark cycle of 12 hours every day. Dietary supplements were given to the animals. Due to their sensitivity to environmental changes, they were quarantined for three to four days in an ideal setting. No compromises were made in terms of ethics when using animals in experiments.

Layout of an Experiment

The twelve mice were randomly split into four groups, each with three mice: Group I, Group II, Group III, and Group IV. The fruit shells of *N. Fruticans wurmb* were extracted using methanol at two different concentrations (200 and 400 mg/kg body weight) and given to separate groups. All of the mice were precisely weighed, and space was prepared for both the control and test groups, before the experiment began.

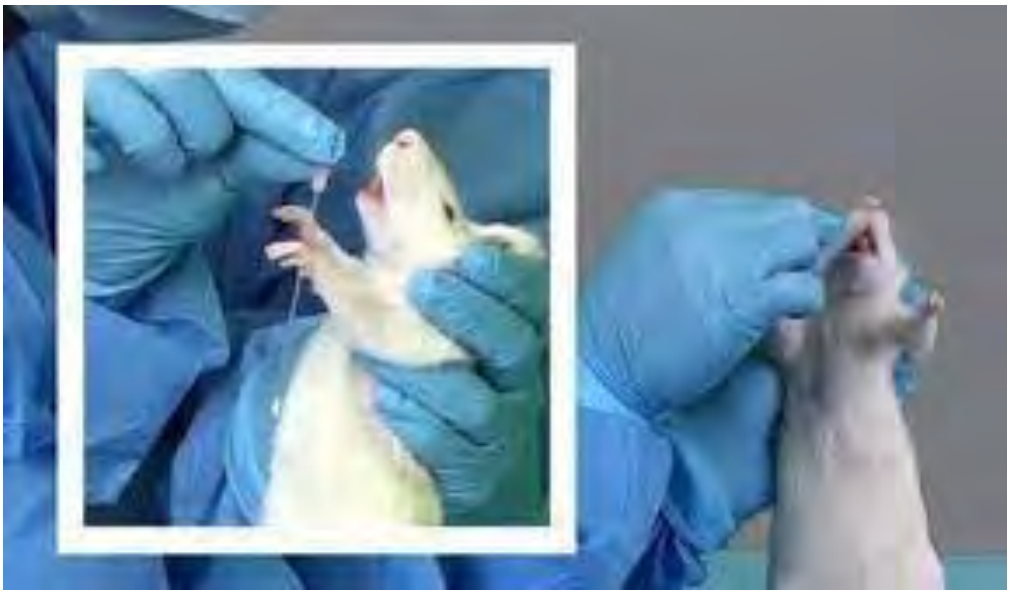

Figure 4: Oral feeding of test sample to mice(Yang et al., 2023)

Preparation of Test Materials

When preparing extracts at dosages of 400 mg/kg body weight and 200 mg/kg body weight of mice, the extracts were carefully weighed before being diluted in 0.8 mL distilled water and given orally. The reference standard dose of loperamide HCl was 2 mg per kg of patient weight. The mice were given 100 mL of a solution containing the standard, which had been dissolved in distilled water.

Procedure

There were a total of nine mice used, with three in the control, three in the positive control, and three in the test groups. In the study, participants in group-I (the control group) were given an oral dosage of 0.2 mL/kg of distilled water. The second group, known as the "positive control," was given the antimotility drug loperamide HCl at a dosage of 2 milligrammes per kilogramme of body weight.

N. Fruticans wurmb methanolic fruit shell extracts were given orally to Groups III and IV at doses of 200 and 400 mg/kg, respectively. Each group's mice were kept in separate plastic cases with absorbent paper below. Absorbent paper was replaced on the hour. Shell extracts, Loperamide HCl, and water were administered an hour later, and then 1mL of castor oil was given to each mouse. The effects of castor oil on diarrhea in mice were monitored by checking on them every hour for four hours. Diarrhea can be triggered in mice by observing any change in their feces or fluid output. Every hour, we recorded how many of each mouse's diarrheic feces had stained the adsorptive paper.

Chapter 3

Result

3.1 Initial Phytochemical Testing

Modified Kupchan Partition Method (Van Wagenen et al. 1993) was used to separate the methanolic fruit shell extracts of *N. Fruticans wurmb* into the individual components of petroleum ether, dichloromethane, and carbon tetrachloride. Carbohydrates, glycosides, saponin, steroid, resin, flavonoids, phenols, tannins, and alkaloids are all present in the aforesaid fractionates, as shown by preliminary phytochemical investigation and summarized in Table 2.

Table 2: Screening of bioactive phytoconstituents of shell extract of Nypa fruticans wurmb fruit

Phytoconstituents	NFS
Carbohydrate	+++
Glycoside	+++
Tannin	+++
Alkaloid	+++
Saponin	++
Resin	++
Phenol	+++
Flavonoid	+++
Steroid	++
Fixed oil	-

Note: NFS= methanolic fruit shell extract, "+" specifies the existence and "-" shows the absence of any phytochemical group. Bioavailability key: (+++) ve = strong intensity, (++) ve = Moderate intensity, (+)ve weak intensity,(−) ve = Absence.

3.2 Antidiarrheal activity

N. Fruticans wurmb fruit shell methanol extract was tested for antidiarrheal action at doses of 400 mg/kg and 200 mg/kg. Table 4 displays the total amount of diarrheal faeces produced by each mouse. Samples of *N. Fruticans wurmb* methanolic extract at 200 and 400 mg/kg body weight reduced the total amount of diarrheal faeces by 23.15% and 37.49%, respectively, compared to the control group (78.1%) (Table 3). The results of the experiments showed that the antidiarrheal activity of the methanolic extract of *N. Fruticans wurmb* fruit shell was much higher than that of the reference drug Loperamide.

Group	Dose	Total no of feces in 4 hrs	Percentage % inhibition of defecation
G-I-Control	Tween 80 solution	10.67±0.58	---
G-Il-Standard	2 mg/kg	2.0± 0.33* *	81.25
G-III-NFS	200mg/kg	8.2±0.91	23.15
G-IV-NFS	400mg/kg	6.67±1.21*	37.49
G-V-NFS	600mg/kg	4.33±1.21*	59.42

The values are demonstrated as mean±STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. **P<0.01; *P<0.05 significant compared to the control.

Chapter 4

Discussion

Plants may be utilized in a wide range of different ways as potential medicinal resources. The pharmaceutical industry makes extensive use of several therapeutic plants, either in the form of crude extracts or as raw materials. The component of the plant that contains the pharmacologically active ingredient can be isolated and utilized in the form of tinctures, powders, tablets, capsules, or fluid extracts. And the most popular method of making economical use of the plant resources is to follow the appropriate extraction and purification technique, which may isolate the selected compounds and allow them to be consumed as active medications. (Jain, 2016) Because of this, phytochemical screening of the plant is extremely important in the initial stage of plant-based investigations. This is due to the fact that it identifies the elements that are present in the plant and provides a focus for the research. In the current investigation, several phytochemical screening techniques were carried out on the methanolic fruit shell extract of *N. fruticans wurmb*. These processes indicated the existence of elements within this particular plant. These constituents included carbohydrates, glycosides, saponin, steroid, resin, flavonoids, phenols, tannins, and alkaloids. Given the existence of all of these components, it is reasonable to hypothesize that the plant may have significant potential as a therapeutic substance.

The experimental methodology involved the utilisation of the castor oil-induced diarrhoea approach to evaluate the potential antidiarrheal effects of fruit shell extracts in a model of mice. The quantification of the reduction in the volume of diarrheal stools was conducted by the use of extracts derived from fruit shells. At dosages of 200 and 400 mg/kg, the methanolic extract of the roots of *N. fruticans wurmb* demonstrated a reduction in the amount of diarrheal feces produced by mice of 23.15% and 37.49%, respectively. In this particular investigation, the positive control or standard was determined to be loperamide HCl. The current investigation shown that a methanolic shell extract of *N. fruticans wurmb* at a concentration of 200 and 400 mg/kg promotes degradation of diarrheal stools in comparison to Loperamide HCl's level of effectiveness.

Chapter 05

Conclusion:

Research on *N. fruticans wurmb* fruit shell phytochemical composition and anti-diarrheal activities has shown it to be a promising natural resource. Bioactive substances with antioxidant, anti-inflammatory, and antibacterial characteristics were found through phytochemical research. These chemicals include carbohydrates, glycosides, saponin, steroid, resin, flavonoids, phenols, tannins, and alkaloids. (Hafizi Sukairi et al., 2019) Evidence suggests that the fruit shell of *N. fruticans wurmb* has strong anti-diarrheal activity, suggesting it may be useful as a therapy for diarrhea. *N. fruticans wurmb'* bioactive components help with this by lessening the frequency and intensity of diarrhea attacks. (nugroho et al., 2022) These chemicals have antibacterial and anti-inflammatory effects, meaning they can stop the growth of bacteria responsible for diarrhea. These results show that fruit shell of *N. fruticans wurmb* has the potential to be an effective natural treatment for diarrhea. To further investigate its potential applications in the development of new anti-diarrheal medicines, and to identify and isolate the individual chemicals responsible for its anti-diarrheal activity, more research is needed.

As a whole, *N. fruticans wurmb* fruit shell shows promise as a source of bioactive chemicals with anti-diarrheal characteristics, which could help those who suffer from diarrhea and lead to the discovery of new, all-natural remedies for the ailment. (Prasad et al., 2013)

Future

This finding stimulates future inquiry of Nypa fruticans and helps to our understanding of the medicinal potential of natural products. The wurmb fruit has the potential to be a source of various active pharmaceutical ingredients. In addition to its use in medicine, the extract may also have benefits in other fields, such as agriculture or biotechnology. For instance, research may investigate its viability as a component in cosmetic products or as a biopesticide.

It is possible that there will be an increase in attention paid to the sustainable harvesting and conservation of N. fruticans as a valuable resource in response to the growing interest in natural products and traditional medicine. Researchers from a variety of disciplines, including pharmacology, biochemistry, botany, and traditional medicine, might work together to get a thorough understanding of the qualities of the extract as well as the possible applications of the extract.

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