

In Vivo Antidiarrheal Activity of
Nypa fruticans wurmb Fruit Pulp

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

BRAC University

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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 have acknowledged all main sources of help.

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Approval

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Ethics Statement

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

Abstract

Nypa fruticans wurmb has long been utilized in traditional medicine to treat a variety of illnesses including diarrhea. The study was conducted to evaluate the antidiarrheal property of methanolic fruit pulp extract of *N. fruticans*(NFS). The antidiarrheal activity of NFS extract tested in an experimental model (Swiss albino mice) of castor oil induced diarrhea in 3 different doses - 200, 400 and 600 mg/kg. A significant ($p<0.05$) reduction in the frequency of wet stools and delaying the onset of diarrhea as compared with the control group were observed in rodents treated with methanolic crude extract at doses 400 and 600 mg/kg. However, the effect increased dose dependently and the 600 mg/kg NFS extract produced a comparable effect(66.68%) with the reference drug (Loperamide) in mice. Therefore, our result proved that *N. fruticans*(NFS) pulp has potent antidiarrheal activity. Moreover, the investigation's promising findings may provide a framework for further studies in this area.

Keywords: *Nypa fruticans wurmb*, Antidiarrheal, Castor Oil, Loperamide, Methanolic, Traditional

Dedication

This work is dedicated to my parents and faculties who have showed unconditional support throughout the journey.

Acknowledgement

First and foremost, I want to show my gratitude to Almighty Allah.

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List of Acronyms

MNSP	Methanolic extract of the <i>N.fruticans wurmb</i> pulp
ANOVA	One-way analysis of variance
ICDDR, B	International Centre for Diarrhoeal Disease Research , Bangladesh
Mg	Milligram
ml	Milliliter
SEM	Standard error of mean
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Any plant that contains compounds that have therapeutic value or serve as building blocks for the creation of effective medications in one or more of its organs is considered medicinal. This description enables one to distinguish between plants that are considered medicinal but have not yet undergone a comprehensive scientific investigation, and medicinal plants whose constituents and therapeutic qualities have been scientifically proven. Traditional medicine has long employed a variety of plants. Certain treatments appear to be effective, but there may not be enough scientific evidence to support them (double-blind trials, for instance). These kinds of plants ought to be considered medicinal. (Sofowora et al., 2013) Medicinal plants have been a key source of important bioactive compounds as well as preparations for human medical therapies that are both curative and preventive. An estimated 80% of people on the planet regularly rely on traditional medicine and related products to meet their healthcare needs, particularly in developing nations. A large number of ill individuals in developing nations combine traditional and conventional medicine. In remote rural communities of developing countries, traditional medicines are likely the only accessible natural remedies and are typically less expensive than modern ones. Because traditional healers are close by and have a thorough understanding of both their patients' conditions and culture, rural residents favor traditional medicine. (Mbuni Y.M et al., 2020)

A vast range of plant species that are used in herbalism—also known as "herbology" or "herbal medicine"—are referred to as "medicinal plants." It covers both the actual use of plants in medicine and their scientific study. "Herb" is derived from the Latin "herba" and the archaic

French word "herbe." These days, any non-woody plant part, be it a fruit, seed, stem, bark, flower, leaf, stigma, or root, is referred to as a "herb". The word "herb" used to exclusively apply to non-woody plants, like flowers and grasses. These healing plants are used not only in food, flavonoids, medicine, and perfumery, but also in a variety of spiritual practices. (Fitzgerald et al., 2020)

Humans have used plants for medicinal purposes since the Stone Age. Plants were discussed in Chinese texts, Egyptian papyri, and Unani texts. Herbal remedies were used about 4,000 years ago by Hindu Vaidis, Unani Hakims, and Mediterranean and European cultures. Apart from their extensive application in conventional medical systems such as Unani, Ayurveda, and Chinese Medicine, herbs were also essential to the healing practices of ancient Egyptian, Roman, Persian, African, and American cultures. (Numbere, 2018)

According to recent estimates from the WHO, more than 80% of people worldwide get at least some of their primary healthcare needs from herbal remedies. Recent data shows that over 75% of the world's population gets their primary medical care from plants or plant extracts. (Fitzgerald et al., 2020) Over thirty percent of plant species have been used medicinally by humans in the past. In rapidly developing countries like China and India, up to 80% of prescription medications are made from plants, compared to up to 25% in industrialized countries like the United States. (Numbere, 2018) As a result, medicinal plants are valued far more highly in economies like India's than they are elsewhere. The foundation of rural health care consists of indigenous medical practices. As per the World Health Organization (WHO, 2013), approximately 80% of the sick population in developing nations, such as Bangladesh, receives their primary healthcare (PHC) from traditional healing. The West gave rise to what is now referred to as "conventional medicine." (Lestari & Noor'an, 2019)

1.1.1 Plant Parts Utilized in Medicines

Different parts of plants such as bark, root, pulp, tuber, fruit, flower etc are utilized to treat various diseases. For example: In traditional medicine, plant flowers have long been used. Flowers such as chamomile and cloves are examples. Pollen, maize stigmas, and saffron stamens are examples of flower parts that are also utilized. Fruits have long been utilized in medicine.

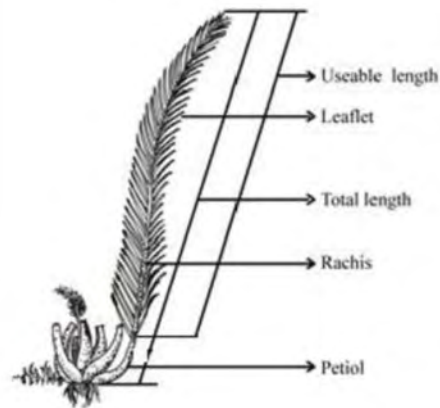


Figure 1 : Green leaves of *N. fruticans wurbm* (Numbere, 2018)

1.1.2 Background of Medicinal Plants

A lot of the products that are marketed as "traditional herbal medicines" have undoubtedly been used historically, which is implied by the term "traditional" use of herbal medicines. For their medical needs, a significant section of the populace in many developing nations turns to traditional healers and their arsenal of therapeutic plants. For historical and cultural reasons, herbal medicines have frequently retained their popularity, despite the possibility of modern medicine coexisting with such traditional practices. Particularly in developed nations, these products are now more widely available in the market. Certain ingredients that originated from traditional healing systems are now being marketed for purposes never considered in the traditional settings. Ephedra use for weight loss or improving sports performance is one example. While strict manufacturing standards apply to herbal medicines in some nations, this is not

always the case. Herbal products are sold under the same safety, efficacy, and quality standards as other drug products in Germany, for instance, where they are referred to as "phytomedicines." In contrast, the majority of herbal products available in the USA are sold and controlled as dietary supplements, a product category that does not need product pre-approval based on any of these factors.

1.1.3 Importance of Medicinal Plants

Medicinal plant is used for treating many diseases and help to fight against bacteria, fungi and so on,

- Alkaloids help to treat pain
- Volatile oil works as an antiseptic.
- Gum-resins have analgesic properties.
- The use of fixed oil reduces acidity.

1.1.4 Traditional Medicinal Plants of Bangladesh

The definition of traditional medicine given by the World Health Organization (WHO) is an extensive compilation of knowledge, skills, and techniques based on concepts, convictions, and life experiences that are common to many different cultures. These methods are used in the healthcare industry to treat and prevent diseases. In traditional medicine, one of the most common specialties is pharmaceutical plants. Bangladesh boasts a wide range of medicinal plants because it is primarily composed of flood plains with a subtropical monsoon climate, and it is located in the world's largest deltaic plain, the Ganges–Brahmaputra delta. Traditional medicine is defined by the World Health Organization (WHO) as an extensive body of knowledge, skills, and practices derived from concepts, convictions, and accumulated

experiences that because of its unique geographic location, steady access to uncontaminated freshwater, and favorable climate, the country is home to a wide variety of plant species. There are expected to be more than 6000 plant species there, including bryophytes, gymnosperms, angiosperms, algae, and ferns; of these, 455-747 are thought to contain medicinal agents. Medicinal plants used in traditional medicine are naturally found in Bangladesh's forests, coastal areas, shrubs, and undeveloped land near the canal. The country may have four distinct traditional medical systems in place: homeopathic, ayurvedic, unani, and traditional. Kaviraja are practitioners of folk medicine, or traditional medicine, who treat patients primarily with medicinal plants found in the area. Because the drugs are readily available, effective, and reasonably priced, rural residents rely on them for their medical needs. In most of Bangladesh's more than 87,000 villages, there are one or two Kaviraje practitioners. Their knowledge of medicinal plants gives them the necessary skills to carry out research and develop secure and efficient pharmaceuticals.

1.2 Introduction to the Sample Plant: *N. fruticans wurmb*

An essential part of the mangrove vegetation found in East Asia is the *N. fruticans wurmb*. It's among the oldest palms still in existence. The "Sundarbans," the world's largest continuous mangrove forest tract, is the only area in Bangladesh where Nipa palms naturally occur. It grows in the Indian and Pacific Oceans' estuaries and coastlines. It is a tall, erect palm without a stem that has an extensive root system and an underground rhizomatous stem. It is well-suited to withstand swift flowing water. (Md Mizanur Rahman, 2023) Nipa palm plays a crucial role in Bangladesh's rural and urban livelihood economies. Around 80% of the homes in the "Sundarbans" neighborhood are made of Nipa palm, which is essential to the locals' way of life. Its sap could potentially be used to make vinegar, alcohol, and sugar. When it comes to mitigating

the effects of hurricanes, cyclones, and tsunamis, nipa palms are the first line of defense, minimizing damage in coastal areas. This paper provides an overview of the nipa palm, taking into account its origin, distribution, habitat, uses, and significance. (Hossain & Islam, 2015) A figure showing the plant *N. fruticans wurmb* is given below:



Figure 2 : N. fruticans wurmb

To generate new plants, the Nypa palm can be cultivated from seeds or sucker cuttings. Catkins: such as the red or yellow male flower inflorescences that grow on the plant's lower branches, and Female flowers are found at the very top of the plant in globular clusters. A single stalk of woody nuts, up to 25 centimeters (10 inches) in diameter, is produced by the flower in the form of a globular cluster. (Benmouloud, 2021) When the tide comes in, the mature nuts slide off the ball and drift away. On occasion, they will start to germinate while they are still submerged in water. It can be found in the largest continuous mangrove forest in the world which is the Sundarban's. They are widespread from India to the Pacific Islands on the coasts and rivers that flow into the Indian and Pacific Oceans. (Das, 2015)

1.2.1 Ecology of *N. fruticans wurmb*

Because of the distinctive horizontal rhizomes that grow underground of Nipah palms, coastal areas and river banks can be stabilized, preventing waves and approaching water from eroding the land-water margins. (Md Mizanur Rahman, 2023) Nipah palms, which grow along coastlines like mangroves, serve as buffer zones by absorbing the force of approaching waves, storms, and strong winds. Since they hold on to the silt and sediments that the nearby waters have deposited, they are in fact crucial elements of land progression (coastline extension). (Rozainah et al., 2000) It can withstand both freshwater and brackish conditions especially well, indicating the wide range of ecological conditions it can tolerate. To the extent that the tide can carry the floating seeds inland, it can also be found inland. It is the only kind of palm that thrives especially in mangrove habitats. The fruits of the nipa palm are known to be consumed by long-tailed macaques (*Macaca fascicularis*). Proboscis monkeys have been observed eating inflorescences in the Padas Damit Forest Reserve. In Borneo, orangutans consume the shoots and hearts of nipa palms. (Numbere, 2019).

1.2.2 Geographical Location or Habitat of *N. fruticans*

Nipa, along with other real mangroves, makes up the wetlands affected by tides that are primarily found in Southeast Asia. It is especially prevalent in estuaries that retain suspended sediments, acting as a belt of transition between freshwater swamps and mangroves. (Lestari & Noor'an, 2019) The tropical Indo-West Pacific Region, which stretches from Sri Lanka through Southeast Asia to northern Australia and the western Pacific islands, is the only area where nipa is currently found. The northern boundary of nipa distribution is Iriomote Island in Ryukyu, Japan, where nipa also sporadically occurs. However, because of the lower average temperatures, the individual plants were only occasionally found on the island and were unable to establish a

population. (Mantiquilla J. A et al., 2022) A lot of nutrient-rich sediment and slow-moving river or tidal currents are characteristics that nipa palms prefer to live in. The locations where tidal movements have the greatest influence and extend inland are those where floating nuts may be found. From India to the Pacific Islands, these organisms are commonly found in the coastal regions and river systems that empty into the Indian and Pacific Oceans. The palm tree may show signs of resilience and endurance when the environment around it becomes desiccated. Although this plant is often called a "mangrove palm" and grows in coastal areas, it has a low tolerance to salt and can die if submerged in saltwater. (Lestari & Noor'an, 2019)

1.2.3 Taxonomic Classification

The taxonomic classification of *N.fruticans wurmb* is given below:

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

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

The fruit of the plant *N. fruticans wurmb* has been shown in following Figure 3.



Figure 3: *N. fruticans wurmb* fruit

1.2.4 Plant Type

- Perennial
- Aquatic
- Seed propagated
- Vegetatively propagated Woody (Lestari & Noor'an, 2019)

1.2.5 Biological and Ethnobotanical Characteristics of Nipa Palm

N. fruticans wurmb is a massive, invasive, armless, pleonanthic, creeping palm that produces fruit in spite of its monoecious nature. prostrate or underground (a rhizome) stem up to 45 cm in diameter, bent leaf scars above, roots below, and regular, dichotomous branching. Petiole consists of up to 163 leaflets per leaf, which is rather thick. Each leaf on a plant is arranged in whorls of three to five. (Uhl, 1972) The leaf has a linear shape, and its length and width range from 1.2 to 1.5 meters and 6.5-8.6 centimeters, respectively. There are appressed brown scales on the lower surface of the midrib, and the texture is coriaceous. The length of the leaf varies from 4.5 to 14.2 meters. With a maximum length of 2.1 m and a robust, terete stem that can reach 2.4 m, the protogynous inflorescence is a solitary, erect, multi bracteate inflorescence. The

rachis, which is usually shorter than the peduncle and terete, terminates in a globose head of female flowers that is encircled by numerous short, Its smooth exocarp, fibrous mesocarp, and thick endocarp, which is composed of interwoven fibrous threads, give it a dark to black color. Its measurements are 10-15 x 6-8 cm. The fruit, a drupe, grows on a carpet and is compressed, unevenly angled, and shaped like a pyramid. It measures between 15 and 20 by 6 and 8 centimeters. (Tsuji et al., 2011)

A figure showing the morphology of *N. fruticans wurmb* fruit has been shown in figure 4.

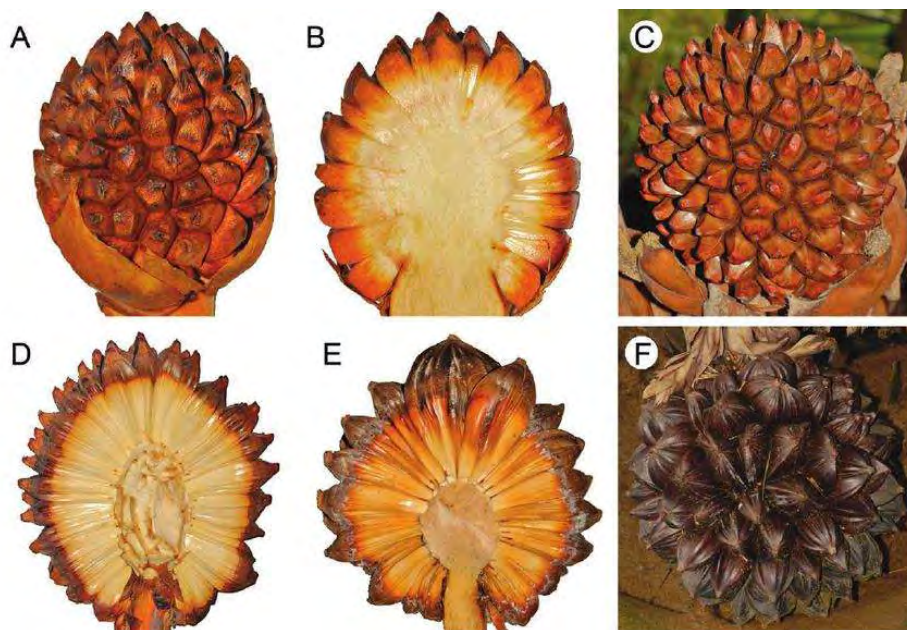


Figure 4: Morphology of *N. fruticans wurmb* fruits (Бобров et al., 2012)

1.2.6 Traditional Use of *N. fruticans wurmb* in Bangladesh

The utilization of native knowledge is the only method used to manage *N. fruticans wurmb* on nearby plantations. As per reports, Golpata is utilized primarily for fishing, molasses, wine, housing, food, fuel, building fences, medicines, and wrapping cigarettes. Fruit kernels that are still immature are consumed. The juice is used to make alcohol and molasses known as Gur in the area. It has been reported that recently developed shoots are used as a vermicide. Golpata ash

is used as an analgesic for headache and tooth pain. Fuel is made from dry leaves, petioles, stem wood, leftover fruit, etc. Golpata rhizomes are widely used in fishing, allowing the net to float above the water's surface. Farmers also mention that deep-water fish are drawn to Golpata when it is in a river or the sea. (Hamilton & Murphy, 1988) Along Bangladesh's coast, the well-known palm Golpata (*N. fruticans wurmb*) grows in both wild and cultivated environments. In addition to existing naturally in certain areas of government-managed mangrove forests, the plant species under consideration is also widely produced through private initiatives on plantations founded by rural farmers along coastal regions, specifically in Bangladesh's south. The Sundarbans are acknowledged as the world's largest continuous mangrove forest. (Iftekhar & Islam, 2004) In Bangladesh's commercial construction and healthcare industries, golpata may be widely used. In Bangladesh, golpata is essential to the livelihood economies of both the country's rural and urban areas. Golpata is used to build nearly 80% of the homes in the Sundarbans region, making it a vital resource for the area's 50,000+ estimated population. In order to meet the increasing demand for housing and other needs, plantations must be established in order to increase output and manage natural resources sustainably. It is noteworthy that in spite of this, a number of the locals who live close to the Sundarbans choose to grow Golpata on their own land on their own initiative. (Arora et al., 2013)

1.3 Rationale of this Study

N. fruticans wurmb is widely distributed in Bangladesh's wet, saline regions such as the Sundarbans. The plants contain a large number of active phytochemical elements, despite the fact that numerous studies—both recent and historical—have found that the trees are widely used for their timber. Studying these chemical compounds and their inherent ability to develop in an abiotic and biotically inhospitable environment could help Bangladesh's medical industry

produce new pharmaceuticals and make significant strides in treating diarrhea. The purpose of this study was to examine *Nypa*'s potential antidiarrheal properties.

1.4 Aim of the Study

The purpose of the study is to identify the antidiarrheal property of the pulp of *N. fruticans wurmb.*

1.5 Objective of the Study

The objective of the study is given below:

- The different parts of *N.fruticans wurmb* have different medical aspects that can be utilized to treat various diseases.
- The prominent purpose of this experiment is to determine the antidiarrheal activity.
- To determine dangerous sides of the extract.

Chapter 2

Materials & Methods

2.1 Preparation of Plant Extracts (*N. fruticans wurmb*)

The pulp extraction of *N. fruticans wurmb* consists of three major steps

1. Identification and collection of *N. fruticans wurmb* sections
2. Drying of the parts of *N. fruticans wurmb*
3. Extraction separately of all parts of *N. fruticans wurmb*

The plant extraction process of *N. fruticans wurmb* has been shown in the following Figure 5.

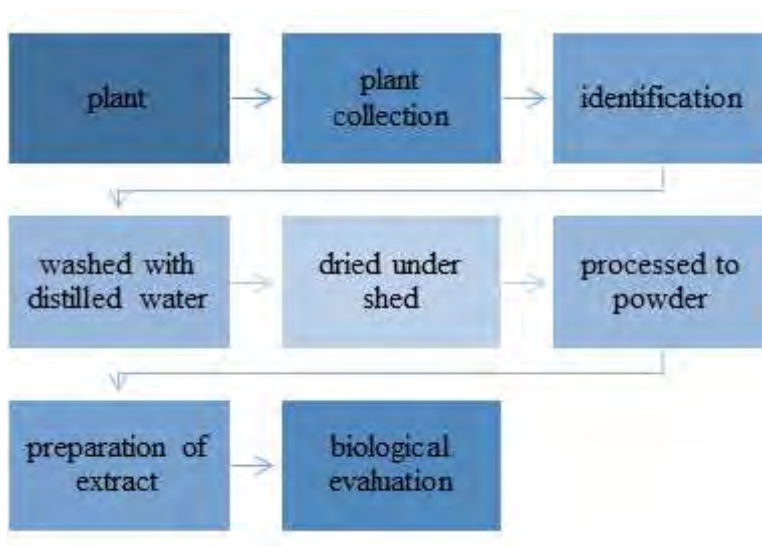


Figure 5: Steps involving plant extraction

2.1.1 The Process of Plant Material Extraction

In a conical flask, 2L of methanol was added to 500 g of fruit shell powder. The flask was covered with aluminum foil and left for 14 days, during that period it was shaken vigorously and stirred occasionally. After transferring the combined substances to a rotary evaporator set to evaporate

the solvent at 50 Celcius, the mixture was filtered twice: first through a new cotton plug and then through filter paper.

2.1.2 Reagents and Drugs

Due to its strong anti-diarrheal property, loperamide is used as a standard in animal models. It is used to evaluate the efficacy of other antidiarrheal medications by serving as a benchmark or reference material. In this case, loperamide is used to compare the antidiarrheal effect of novel drugs or treatments with one another and to set a baseline reaction to diarrhea.

2.1.3 Statistical Method

One way ANOVA followed by Dunnett's test, $p < 0.05$ was considered to be significant (Lalan et al.,2015)

2.1.4 Experimental Animal

The Animal Resources Branch of the International Center for Diarrheal Diseases and Research (ICDDR) in Bangladesh provided the Swiss Albino mice used in this investigation. Swiss albino mice weighing between 25 and 35 grams were required for this investigation. The mice were kept in an ideal condition. Additionally, a 12-hour daily cycle of light and dark was maintained. Supplements were given to mice for their diet. They were placed in a perfect environment and quarantined for three to four days because they were sensitive to changes in their surroundings. While using animals in experiments, no ethical concessions were made.

2.1.5 Ethical Consideration

The Department of Pharmacy at Jahangirnagar University has approved the test guidelines. Additionally, the institutional animal ethical committee's guidelines for animal testing were strictly adhered.(Zimmermann,1983) In animal research, mice are chosen according to

the 3Rs (Replacement, Reduction, and Refinement) principles. By improving and streamlining experimental protocols, these principles aim to reduce the number of animals used and mitigate any potential decrease.

A figure of the experimental animal is shown in figure 6.



Figure 6: Swiss Albino Mice

2.2 Plant Extract Pharmacological Investigation of *N. fruticans wurmb*

The determination of pharmacological activity (antidiarrheal effect) is obtained from the crude extraction of *N. fruticans wurmb*.

2.2.1 Antidiarrheal Study that effect of the MNSP Extract

Antidiarrheals function by slowing down the bowel's movement to reduce the frequency of bowel movements and reducing the amount of fluids and electrolytes that enter the colon. This allows the body to absorb more fluids, which reduces diarrhea and promotes the formation of larger, more solid stools. The antidiarrheal effect of *N. fruticans wurmb* extract was tested in

Swiss albino mice and compared to a standard dose of Loperamide. The antidiarrheal action is triggered by the castor oil induced diarrhea model.

2.2.2 Castor Oil Induced Diarrhea Model

Through the use of a castor oil-induced diarrhea model, the antidiarrheal property of *N. fruticans wurmb* pulp were evaluated in mice. The procedure required administering each mouse one milliliter of analytical grade castor oil to induce diarrhea. Number of feces after 4 hours of administration were recorded. Comparing the observations of the treatment group with those of a positive control group allowed for the determination of antidiarrheal activity.

2.2.3 Layout of the Experiment

Five groups of a total thirty mice were formed where Six mice were assigned to each group. Group I, Group II, Group III, Group IV, Group V. Following two different concentrations of methanol (200 and 400 mg/kg body weight), the pulp of *Nypa fruticans wurmb* was extracted and distributed among distinct groups. Before the trial started, every mouse was carefully weighed, and areas were set aside for the test and control groups.

2.2.4 Preparation of Test Materials

The extracts were carefully weighed before being diluted in 0.8 ml of distilled water and administered orally at dosages of 400 mg/kg body weight and 200 mg/kg body weight of mice. Loperamide HCl was administered at a reference standard dose of 2 mg per kg of patient weight. A solution containing the standard, dissolved in 100 milliliters of distilled water, was administered to the mice.

The groups related to treatment therapy are shown in Table 2.

Table 2: Groups linked to the treatment therapy

Group number	Treatment
Group 1	1% Tween 80 in water
Group 2	Loperamide which is used as a standard
Group 3	NFS extract 200 mg/kg
Group 4	NFS extract 400 mg/kg
Group 5	NFS extract 600 mg/kg

2.2.5 Procedure

There were a total of thirty mice used, with six in control, six in the positive control, and six in the test group. In the study, participants in group-I(the control group) were given an oral dosage of 0.2 ml/kg of distilled water. The positive control group or the second group was given the antimotility drug loperamide HCl at a dosage of 2 mg per kg of body weight.

Oral feeding of sample mice has been shown in the following figure:

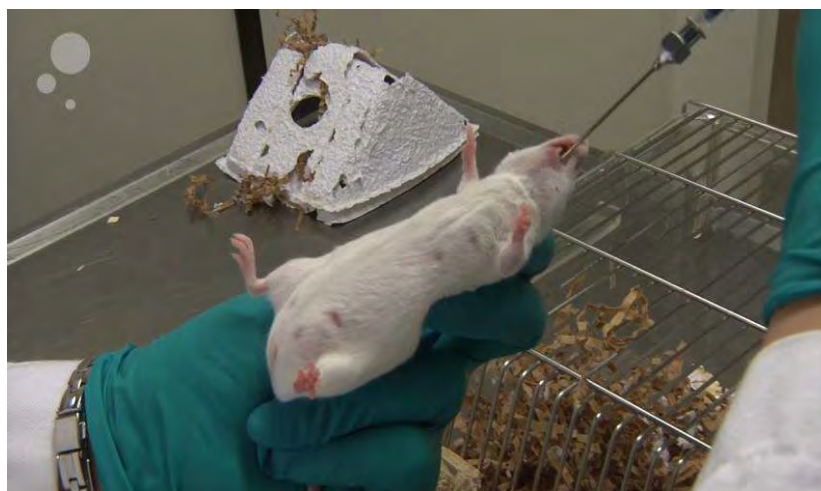


Figure 7: Oral feeding of test sample to mice

N. fruticans wurmb methanolic pulp extract was given orally to Group III, IV, V at doses of 200, 400 and 600 mg/kg respectively. Each group's mice were kept in separate plastic cases with absorbent paper below. Absorbent paper was replaced in an hour. Pulp extract, Loperamide HCl

and water were administered an hour later and then 1 ml 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 4 hours. Diarrhea can be triggered in mice by observing any change in the feces or fluid output. Every hour, we recorded how many of each mouse's diarrhea feces had stained the adsorptive paper.

A flowchart showing the procedure of the experiment using castor oil to induce diarrhea is given below:

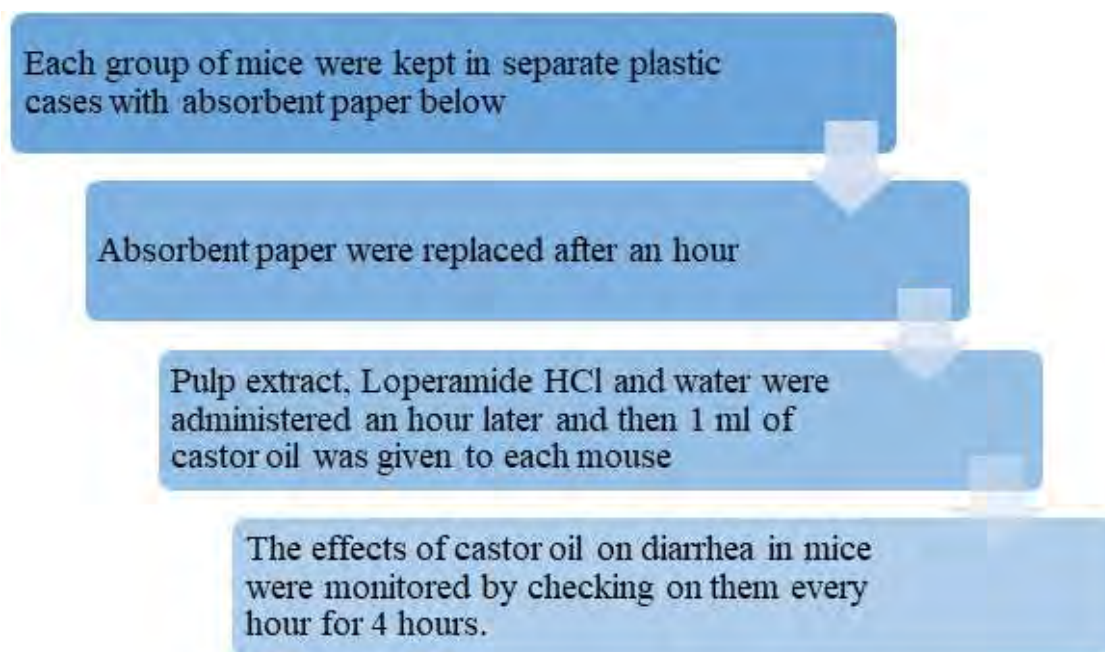


Figure 8: Flow chart of the procedure

Chapter 3

Result

3.1 Antidiarrheal Activity

At doses of 600 mg/kg , 400 mg/kg and 200 mg/kg, the antidiarrheal properties of *N. fruticans wurmb* pulp methanolic extract were evaluated. Each mouse produces a total of x number of diarrheal feces in 4 hours, as shown in Table 3. Comparing samples of the pulp of *N. fruticans wurmb* methanolic extract at 200, 400 and 600 mg/kg body weight to the control group (78.1%), the total amount of diarrheal feces was reduced by 36.17%, 57.14% and 66.68% respectively .The methanolic extract of *N. fruticans wurmb* pulp exhibited significantly greater antidiarrheal activity than the reference medication Loperamide, according to the experiment results. Effects of *N. fruticans wurmb* methanolic pulp extracts on castor oil-induced diarrhea in mice are given below:

Table 3: Effects of *N. fruticans wurmb* methanolic pulp extracts on castor oil-induced diarrhea in mice

Group	Dose	Total no of feces in 4 hours	Percentage % inhibition of defecation
G-I-Control	Tween 80 solutions	17.50±2.43	-----
G-II Standard	2 mg/kg	2.67± 1.03* *	84.74
G-III-NFS	200 mg/kg	11.17±1.17	36.17
G-IV-NFS	400 mg/kg	7.50±0.55*	57.14
G-V-NFS	600 mg/kg	5.83±2.14*	66.68

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

Diarrhea is characterized by fecal urgency and incontinence linked to an imbalance in the intestinal absorption and secretion mechanism, frequently with hypermotility, which causes an excessive loss of water and electrolytes in the feces. Diarrheal illnesses are still a major public health concern in Bangladesh. Indeed, in 2020 alone, these diseases claimed the lives of over 35,000 people. One of the main causes of morbidity and death for Bangladeshi children under the age of five is diarrheal disease. (Kamal et al., 2022) Without any scientific proof, people frequently use various parts of medicinal plants as cures for a variety of illnesses, including diarrheal disease. As a result, it's critical to assess the safety and effectiveness profiles of medicinal plants used in conventional medicine. By assessing these plants' effects on gastrointestinal motility, water and electrolyte secretion, and animal models, several studies have scientifically validated the use of traditionally used antidiarrheal plants. To have safe and affordable therapeutic alternatives, there is an urgent need for newer, safer, more affordable, and more effective antidiarrheal medications. Therefore, using three antidiarrheal experimental models, the study was designed and carried out to verify the purported antidiarrheal effect of *N. fructicans wurmb* pulp. (Oratai Neamsuvan et al., 2012)

A substantial decrease in the frequency of diarrheal stools was observed in the study of the impact of a methanolic extract of *N. fructicans wurmb* pulp on experimental diarrhea in rats induced by castor oil. (Oratai Neamsuvan et al., 2012) Additionally, the peristaltic index was inhibited, the volume of intestinal contents, and the intestinal secretions of electrolytes were all decreased. (Hafizi Sukairi et al., 2019) Ricinoleic acid, which is present in castor oil, irritates the

intestinal mucosa, promotes gastrointestinal secretion, and increases intestinal peristalsis. In order to assess the possible antidiarrheal effects of fruit pulp extracts in a mouse model, the castor oil-induced diarrhea approach was used in the experiment. (Rose et al., 2015) By using extracts from the pulp, the amount of diarrheal stools that were reduced could be measured. Mice's production of diarrheal feces was reduced by 36.17%, 57.14% and 66.68 respectively, when the methanolic extract of *N. fruticans wurmb* pulp was administered at 200, 400 and 600 mg/kg. It was found that loperamide HCl was the positive control or standard in this specific investigation. The results of the current study indicate that, in contrast to the effectiveness of loperamide HCl, a methanolic pulp extract of *N. fruticans wurmb* at concentrations of 200, 400 and 600 mg/kg promotes the degradation of diarrheal stools.

Chapter 5

5.1 Conclusion

Studies on the antidiarrheal property of *N. fruticans wurmb* pulp have demonstrated its potential as a natural resource. Through biological research, there is evidence that *N. fruticans wurmb* pulp has potent anti-diarrheal property, indicating that it could be a valuable treatment for diarrhea. The bioactive ingredients of *N. fruticans wurmb* pulp aid in this by reducing the frequency and severity of diarrheal episodes.(Desta et al., 2021) These substances can prevent the growth of the bacteria that cause diarrhea because they have antibacterial and anti-inflammatory properties. These findings suggest that the pulp of *N. fruticans wurmb* may be a useful natural remedy for diarrhea. More research is required to determine and isolate the specific chemicals causing its anti-diarrheal activity, as well as to explore its possible uses in the creation of novel anti-diarrheal medications.(Sadiq et al., 2020) The *N. fruticans wurmb* pulp as a whole exhibits promise as a source of bioactive compounds with anti-diarrheal properties, which may benefit those who experience diarrhea and aid in the development of novel, all-natural treatments for the condition.

5.2 Future

This discovery advances our knowledge of the potential medical uses for natural products and encourages further research on *N. fruticans wurmb*. It is possible to extract different active pharmaceutical ingredients from the pulp. Apart from its application in medicine, the extract might also be advantageous in other domains like biotechnology or agriculture. For example, studies could look into its potential as a biopesticide or as a component of cosmetics. Given the growing interest in natural products and traditional medicine, it's possible that the sustainable

harvesting and conservation of *N. fruticans* will be a valuable resource. Scholars from diverse fields such as pharmacology, biochemistry, botany, and traditional medicine may collaborate to gain a comprehensive comprehension of the characteristics of the extract and its potential uses.

Chapter 6

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