In vitro Anti-arthritic Effect of *Nypa fruticans* Wurmb Fruit Pulp Extract

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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

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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.

Student's Full Name &Signature:

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Approval

The thesis titled "*In vitro* Anti-arthritic effect of *Nypa fruticans* wurmb Fruit Pulp Extract "submitted by Afsana Rahman Roma (19346029), of summer,2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Despite the discovery of many medications, such as medicinal herbs, there is still a need to offer better, safer, and more effective drug sources for rheumatoid arthritis, an autoimmune inflammatory disease. The current study aimed to evaluate Nypa fruticans Wurmb fruit pulp extract's antiarthritic potential in patients with rheumatoid arthritis. To evaluate the anti-arthritic effect of this methanolic fruit pulp extract, *in vitro* suppression of protein denaturation (bovine serum albumin) was performed. In this investigation, five distinct test concentrations (100, 200, 300, 400, and 500 µg/ml) were used. The observed action was shown to be dose-dependent.

Using the protein denaturation method, the chosen extract at 500 µg/ml demonstrated the highest level of protection (76.28±1.05%), while the standard medication offered 96.77±0.78% protection. Our conclusion was that, in comparison to the usual medication, diclofenac sodium, NFP extract demonstrates strong anti-arthritic activity at varied concentrations. However, to verify its effectiveness and mode of action, more pharmacological research utilizing separate active components must be done.

Keywords: Nypa fruticans Wurmb, Anti-arthritic, Bovine serum albumin, protein denaturation, Diclofenac sodium.

Dedication

I dedicate this thesis to my parents.

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:

BSA	Bovine Serum Albumin
MNF	Methanolic fruit pulp Extract of *N. fruticans*
ANOVA	Analysis of Variance
RA	Rheumatoid Arthritis
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
TNF-α	Tumor Necrosis Factor-Alpha

Chapter 1

Introduction

1.1 Background

Herbal medicine has grown significantly in recent decades, being widely recognized in both developed and developing countries due to its natural origins and low frequency of adverse effects. Herbal treatments provide a variety of therapeutic agents for the treatment of interior disorders that are frequently regarded as resistant or incurable by traditional medical systems. Arthritis is an autoimmune disorder that causes joint discomfort, swelling, and restricted movement. With a global incidence rate of 2.03%-year, arthritis is a significant autoimmune disorder that is now treated with a variety of commercially available drugs that either improve the clinical state or reduce symptoms. But a variety of adverse effects are frequently associated with these medicines. Because of this, it is imperative to investigate therapies that provide better efficacy and safety profiles; in this regard, natural or nature-derived medications have been shown to be incredibly beneficial. About 1% of people worldwide suffer with rheumatoid arthritis, however its precise etiology is uncertain. Advances in understanding its pathophysiology have resulted in the creation of novel therapy techniques, which have helped patients. Rheumatoid arthritis, if left untreated, can progress to a systemic inflammatory illness, causing irreparable joint damage and increasing the chance of death. Alfred Garrod was the first to distinguish between rheumatoid arthritis and gout, calling it "Primary Gout." Inflammatory illnesses, especially rheumatoid disorders, are a prominent source of morbidity globally, particularly among the working population, and are sometimes referred to be the "king of human miseries." Inflammation is a physiological defense process that responds to tissue damage caused by trauma, toxic chemicals, or microbial pathogens.

People have been utilizing plants as a source of medicine from ancient times and continue to modern healthcare. Mangroves are made up of ferns, trees, bushes, and palms that grow in the intertidal area, which is above sea level. Mangrove forests are different and unique types of ecosystems, habitat to specialist plant species that have grown to live in extreme conditions where few other plants can. By excreting extra sodium through specialized glands in their leaves or by compartmentalizing it inside their tissues, mangroves plants are able to withstand excessive salinity levels. The mangrove plant *N. Fruticans*, also referred to as Nipa palm, is found in the subtropical and tropical regions of the Southeast Asian continent and Oceania. Considering its possible therapeutic benefits, which include anti-inflammatory and anti-arthritic effects, it has been utilized in a variety of traditional remedies. *N. fruticans* has significant benefits over other biofuel-alcohol crops, study on the crop has recently concentrated on its possibilities for use as a biofuel crop.

Table 1: Classification of *N.fruticans* (*Nipa fruticans* (nipa palm). (2022). In *CABI Compendium*.)

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

1.2 Habitat and distribution

In ecosystems, the wide range of trees and shrubs that comprise the predominant plant community in tidal morasses along the coast along defended tropical and tropical coastlands are applied to as mangroves. Nipa is a type of mangrove plant that thrives in an environment with year-round temperatures ranging from 20 to 350 degrees Celsius and 1,000 millimeters of rainfall every month. Only this particular palm (Arecaceae) can survive and adapt to the mangrove environment. They can be found in rivers that clear into the Indian and Pacific Oceans as well as on strands from India to the Pacific Islands. Native to China (Hainan), Southeast Asia such as Indonesia, Malaysia, Thailand etc., Australia (Queensland and Northern Territory), and the Asia-Pacific Islands like Solomon, Mariana etc. this plant is a native species. This plant prefers brackish waters surrounding river mouths and will perish if exposed to pure saltwater. Still, this species may live in streams, tidal plains, if freshwater overflows and high tides come off. As long as the tide can hold this plant's seeds, they can be found inland. Nipa can endure temporary drying in its natural habitat. The horizontally creeping rhizome along the riverside has the ability to stabilize the soil and stop soil erosion. A distinctive feature of *N. fruticans* is its rapid recovery following disruption. After, damage new leaves can swiftly reappear, shielding the tree from storm winds and providing important products for the community (Duke, 2011).

1.3 Phytochemicals in fruits

Nipa palm's main chemical components are flavonoids, alkaloids, polyphenols, phenolics, and saponins. It also includes, among other things, steroids, diterpenes, phenol hydroquinone, hexane, triterpenoids, and chloroform. *N. fruticans* contains fruit, commonly referred to as nipa palm sap (NPS), which may be collected to obtain substantial quantities of sap. NPS is recognized as a source of traditional medicine utilized in the treatment of several ailments. Utilizing the 2,2-

diphenyl-1-picrylhydrazyl (DPPH) test shown significant antiradical action in NPS, with an IC_{50} value of 33.36 g/ml (Yahaya *et al.,* 2021). The FRAP, CUPRAC, and DPPH radical-scanning experiments have been used to evaluate the anti-free radical activity of nipa. Terpenoids are a broad group of organic compounds that come from plants. Terpenoids in *N.fruticans* may have an anti-arthritic effect by lowering inflammation and regulating immune responses. *N.fruticans* phenolic acids have anti-free radical and anti-oxidative damage effects for joint tissues (Ms & Mv *et al.,* 2018).

1.4 **Uses of *N.fruticans***

As public awareness of health issues grows, natural components in medicinal products are favored since they are thought to be more affordable, easier to obtain, and unlikely to have long-term negative effects on the human system. Some naturally occurring medications or herbal remedies have been handed down from generation to generation and have emerged as the community's preferred options. One such medication is made from Nipa. People have been using nipa for a long time to create a range of traditional medicines. *N. fruticans*, also known as nipa or nipah, is a palm species. The mangroves, particularly nipa, are plants with diverse advantages that may be used to meet the everyday needs of coastal populations, substantiates this further (Gazali, Nufus *et al.,* 2019). The nipa plant can be processed to use almost all of its parts in traditional medicines. Nipa leaves, wood, roots, and young shoots are occasionally used medicinally (Tsuji *et al.,* 2011). The parts of nipa (leaves, roots etc.) were commonly used as analgesics, sedatives, and agents for alleviating excess wind in the body to address ailments such as asthma, leprosy, tuberculosis, throat inflammation, liver disorders, and snake bites (Rahmatullah *et al.,* 2010). Nipa palm vinegar (NPV) in the local community, is a traditional preparation made through sap fermentation. In East Asia, people frequently eat it. NPV is given before bedtime and before meals, mixed into drinking water. Consumption of nipa vinegar has been shown to reduce postprandial hyperglycemia among people with type 2 diabetes after consuming a meal with a moderate glycaemic index (Liatis *et al.,* 2010). The local population in Malaysia has the belief that nipa vinegar can cure diabetes. Scientific research demonstrates that vinegar has an anti-diabetic effect by selectively inhibiting intestinal glucose transporters, which delays the absorption of carbohydrates from the small intestine and lowers postprandial hyperglycemia (Yusoff *et al.,* 2015). In Southeast Asia, there is a common belief that NPS has medicinal properties for treating fever, gout, kidney stones, strengthening energy, helping digestion, and curing specific chronic diseases and metabolic syndromes such as diabetes and hypertension.

Immature seeds are snacked on for their white endosperm, which tastes sweet and jelly-like. In several parts of the world, people wrap cigarettes with the cuticle of immature leaves. The nipa palm has been used to produce many traditional remedies; the juice from fresh leaves treats herpes, while ash from burned nipa material alleviates toothaches and headaches as well as materials for salt extraction. Early attempts to make buttons from the "plant ivory," or endocarp of mature fruits, were unsuccessful due to fungal attack, and plastic materials have mainly taken their place. In Nigeria, the hard shell (mesocarp) is used to make buttons, necklaces, and other fashion items. The fronds of nipa are commonly used as sails by local fishermen. Nipa is also used in non-medical settings, such as: roofing materials, handicrafts, and as a renewable energy source (Nugroho *et al.,* 2022).

1.5 Different pharmacological activities of *N. fruticans*

Mangroves are among the natural resources that have been used for many years as medical remedies. (Mahmud *et al.,* 2014) stated that mangrove plants have been demonstrated to possess phytochemicals with noteworthy anticancer, antibacterial, antinociceptive, and antioxidant properties. Additional investigation has also been conducted on the phytochemical composition of nipa, one of the plants in the mangrove environment. Study by (Reza *et al.,* 2011), for example, showed that methanol extract of nipa plant twigs and leaves have analgesic and antidiabetic properties. The results of other studies have also been conducted. Nipa leaf extract has the potential to boost peripheral glucose utilization, induce an extra pancreatic mechanism that produces an antihyperglycemic impact, and stimulate pancreatic cells' residual function, according to (Reza *et al.,* 2011). Similar to this, (Yusoff *et al.,* 2015) study investigated postprandial hyperglycemia in response to aqueous extract (AE) in order to determine the mechanism of antidiabetic action (Kumar & Pola, 2023).

1.5.1 Anti-inflammatory activity

Mangrove plant extract have proven to be quite effective in traditional medicine applications. Research reveals that one of these functions includes strong anti-inflammatory qualities. Mangrove extracts' capability to function as strong anti-inflammatory drugs depends on their flavonoid composition. Flavonoids are a class of secondary metabolites that are commonly present in plants and are usually made up of 15 carbon atoms. Most flavonoids have a direct impact on the enzymes that are responsible for the inflammatory process. Furthermore, many flavonoids have the ability to prevent the activation of adhesion molecules, such as blood neutrophils, that are necessary for an inflammatory response (Middleton *et al.,* 2000). *N. fruticans*, a plant renowned for its numerous medicinal properties, has generated considerable interest. *N. fruticans* ability to reduce inflammation is recognized for its bioactive components, which include of tannins, saponins, flavonoids, and phenolics. Together, these substances affect important inflammatory pathways, which may have therapeutic effects for a range of inflammatory diseases. Because phenolic compounds neutralize free radicals, they lower oxidative stress, which is a major contributing factor to inflammation. They're well- known for their antioxidant rates. Pro-inflammatory mediators are subsequently downregulated as a result. Flavonoids limit the activation of nuclear factor kappa B, a critical regulator of seditious processes, and inhibit the product of pro-inflammatory cytokines similar as interleukin- 1β (IL- 1β), interleukin- 6 (IL- 6), and tumor necrosis factor- alpha. *N. fruticans*, tannins have been demonstrated to inhibit cyclooxygenase- 2, an enzyme that's involved in the synthesis of prostaglandins that promote inflammation, hence lowering inflammation.

1.5.2 Antibacterial Activity

An antibacterial is a therapeutic medicine that eliminates germs, specifically pathogenic bacteria that are capable of causing harm to humans. To efficiently eradicate germs, antibiotics must have a high degree of selectivity, signifying that the medication should be very detrimental to bacteria. Antibacterial agents can also be used to prevent the spread of various pathogenic bacteria. The majority of natural antioxidants reported in the literature also exhibit antibacterial activities (Lourenço *et al.,* 2019). Subsequently, research performed phytochemical analysis of nipa plant leaves to investigate their antibacterial properties. They evaluated many extracts against E. coli, Klebsiella pneumoniae, Staphylococcus aureus etc. Nipa includes a wide array of biological constituents, including alkaloids, cardiac glycosides, polyphenols, phlorotannin's, saponins (Ebana *et al.,* 2015). The results indicated that a concentration of 5% and higher completely inhibited the growth of E. coli when using the leaf ethanol extract. Next, a separate investigation demonstrated that the most potent concentration of crude nipa leaf extract for inhibiting Aeromonas hydrophila and Streptococcus Agalactiae was 60%. The minimum bactericidal concentration (MBC) for A. hydrophila bacteria showed the lowest growth at a value of 1.33a±0.52, while the highest growth was observed at a value of 4.33b±0.82. The minimum bactericidal concentration (MBC) for S. Agalactiae bacteria ranged from 2.17a ± 0.75 to 4.67b ± 0.52.(Sari 2017).

1.5.3 Antioxidant Activity

An antioxidant is a substance that inhibits or prevents the oxidation of other molecules, thereby protecting cells from damage caused by free radicals. Researchers have shown significant interest in natural antioxidants derived from plants because of their organic source and reduced negative side effects (Lourenço *et al.,* 2019). Furthermore, consuming exogenous antioxidants including ascorbic acid (also known as vitamin C), network tocopherols (also known as vitamin E), carotenoids, and polyphenols is becoming more and more popular among the general population. These antioxidants can be found in various natural food products like fruits, vegetables, cereals, and drinks. This is because it is believed that these items can enhance the antioxidant defense system. Furthermore, mangrove plants feature a greater abundance of chemicals that exhibit antioxidant properties in comparison to other substances. According to (Choi *et al.,* 2022), nipa has chemical compositions such as phenolic acids and flavonoids.

In addition, nipa contains phenolic compounds, alkaloids, and flavonoids that have been proven to possess antioxidant effects. According to (Margaretta and Handayani *et al.,* 2011), these substances have been scientifically demonstrated to possess antioxidant properties. Antioxidants are capable of neutralizing free radicals and blocking autoxidation processes such as lipid oxidation. Phenolic chemicals and flavonoids in biological systems function as agents that scavenge free radicals and possess antioxidant activity. Hence, the advantageous impact of the bioactive chemicals found in NPS may exhibit antioxidant properties, thereby offering a safeguard against chronic illnesses. (Yahaya *et al.,* 2021) found that nipa contains ascorbic acid, which exhibited the most potent antioxidant activity. Its IC_{50} value was measured at 21.29±0.74 g/ml. Based on the research conducted by (Sowndhararajan and Kang., 2013), it has been found that there is a direct relationship between the IC_{50} value and antioxidant activity. Specifically, a lower IC_{50} value indicates a higher level of antioxidant activity. The antioxidant capabilities of the sample were assessed using the DPPH radical scavenging activity test, which yielded positive findings. Specifically, the EC_{50} value was determined to be 112.90 mg/ml (Sukairi *et al.,* 2018). The antioxidant chemicals found in nipa have the ability to counteract free radicals in human body. These compounds show promising potential for being developed into nutritious meals and beverages, supported by scientific proof and validation (Thyagarajan & Sahu, 2017). In order to determine the antioxidant activity of NPV, three *in vitro* antioxidant assays were employed: 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azinobis-3-ethyl benzothiazoline-6- sulphonic acid-free radical scavengers, and a reducing power assay (Yusoff *et al.,* 2015). The chemical analysis of the NPV aqueous extract identified the presence of acetic acid, that made up 35.25% of the extract's composition. Subsequently, the ethyl acetate extract derived from nipa induces a notable antioxidant activity due to its elevated phenolic content. Therefore, it can be inferred that the ethyl acetate extract contains the antioxidant component (Lobo *et al.,* 2010).

1.5.4 Immunomodulatory Effects of *N. fruticans*

N. fruticans has anti-inflammatory, antibacterial and antioxidant properties, but beyond this it also has immunomodulatory effects. The plant able to modify the immune response in arthritis by affecting the generation of cytokines and immune cell activity, which would lessen excessive inflammation and tissue damage. According to research, *N. fruticans* may affect the activity of different immune cells involved in these processes:

Macrophages: Macrophages can be polarized into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes, and they are essential to the inflammatory response. Research has demonstrated that extracts from *N. fruticans* can affect the polarization of macrophages. For example, studies by (Rahman *et al.,* 2022) showed that *N. fruticans* extract could promote the M2 phenotype of macrophages over the M1 phenotype, hence decreasing the generation of pro-inflammatory cytokines like TNF-α and IL-1β.

T lymphocytes: T cells are important for adaptive immunity and are implicated in the rheumatoid arthritis (RA) pathogenesis. By influencing their differentiation and cytokine production, *N. fruticans* may have an effect on T cell activity. According to a study by (Zhang *et al.,* 2023), extracts from *N. fruticans* may have a role in controlling autoimmune reactions in arthritis by preventing T cell multiplication and lowering their release of pro-inflammatory cytokines. Dendritic Cells: DCs are immune response-initiating, immune-regulatory cells that deliver antigens. Studies have suggested that DC function may be impacted by *N. fruticans*. According to a study by (Kumar *et al.,* 2021), extracts from *N. fruticans* may change how DCs mature and lessen their capacity to activate T cells, which may lessen arthritis's overall inflammatory response.

1.5.4.1 Modulation of Cytokine Expression

Signaling proteins called cytokines mediate and control immunological responses. Malfunctioning cytokine synthesis contributes to joint damage and chronic inflammation in arthritis. Dysregulated cytokine production is a contributing factor to joint injury and chronic inflammation in arthritis. *N. fruticans* seems to affect the synthesis of a number of important cytokines related to arthritis: TNF-α, also known as tumor necrosis factor-alpha, is a key modulator of inflammation in arthritic conditions. TNF-α overproduction is linked to tissue damage and joint inflammation. *N. fruticans* extracts have the ability to considerably lower TNF-α levels in both *in vitro* and *in vivo* models of arthritis, as shown by a study by (Lee *et al.,* 2022). The plant's capacity to suppress TNF-α synthesis at the transcriptional level or to encourage TNF-α breakdown may be the cause of this effect. Interleukin-1 Beta (IL-1β) is an additional pro-inflammatory cytokine that is essential to the etiology of arthritis. In animal models of arthritis, *N. fruticans* extract successfully reduced IL-1β levels, according to research by (Singh *et al.,* 2024). This decrease is probably related to how the plant affects immune cell function and how it modifies inflammatory pathways. Interleukin-6 (IL-6): IL-6 plays a role in arthritis-related systemic inflammation as well as the acute phase response. *N. fruticans* has been demonstrated in studies to lower IL-6 production, which may lessen the inflammatory response linked to arthritis in the system. For instance, NF- κB, a transcription factor that controls IL-6 expression, was found to be inhibited by *N. fruticans* extract in a study by (Chen *et al.,* 2023), which suppressed IL-6 production (Kang & Hyun, 2020).

1.6 Objective of the study

This study objective to examine the possible antiarthritic characteristics of *N. fruticans* fruit pulp. The study will evaluate the safety and efficacy of *N. fruticans* fruit pulp in alleviating arthritic symptoms, specifically pain and inflammation. Understanding how the pulp interacts with different inflammatory pathways and receptors may open the door for the development of novel therapeutic treatments, therefore understanding the underlying mechanisms of action responsible for its antiarthritic properties is a major focus. It will further evaluate any possible adverse effects related to its use. This thorough analysis wants to establish a scientific foundation for the use of *N. fruticans*. The application of fruit pulp in arthritis treatment, complementing current therapeutic alternatives in rheumatology and inflammatory disorders. This study tries to fill this gap by investigating the antiarthritic activities of *N. fruticans* fruit pulp, therefore laying the groundwork for its potential use as a natural therapeutic agent. This research evaluates the safety profile of *N. fruticans* fruit pulp, addressing concerns regarding herbal treatments and ensuring that therapeutic uses are supported by scientific data. This study is justified by the necessity for effective, safe, and alternative therapies for arthritis, as well as the potential of *N. fruticans* fruit pulp as a feasible alternative, emphasizing the necessity of comprehending its processes and safety to improve treatment approaches in rheumatology and the management of inflammatory diseases.

1.7 Aim of the test

Evaluating *N. fruticans*' (fruit pulp) anti-arthritic qualities *in vitro* aims to assess the plant's possible therapeutic benefits in arthritis models. This involves analyzing its anti-inflammatory properties by measuring decreases in inflammation indicators, such as swollen joints or high cytokine levels. The study seeks to ascertain if extract of *N. fruticans* may alleviate arthritis-related discomfort by behavioral evaluations, including paw pressure measurements. Another purpose is to examine alterations in joint structure and damage by histological analyses or imaging modalities, providing information toward an improvement of joint health. The study wants to evaluate the efficacy of *N. fruticans* in comparison to standard anti-arthritic medications, aiming to determine its potential as an alternative or adjunctive treatment, thereby facilitating the development of new natural therapeutic options for arthritis management with reduced side effects with respect to conventional drugs.

1.8 Justification for the Research

Arthritis and associated inflammatory disorders provide considerable health issues, impacting millions worldwide and resulting in chronic pain, decreased mobility, and decreased quality of life. Present therapeutic alternatives, such as nonsteroidal anti-inflammatory medications (NSAIDs) and corticosteroids, sometimes entail adverse effects and may not yield sufficient alleviation for many individuals. This emphasizes the need for alternative therapies that are both effective and safe. *N. fruticans*, an exotic fruit pulp, has demonstrated potential in conventional medicine for its anti-inflammatory properties. However, scientific investigations about its usefulness and safety in regard to arthritis are still constrained. This study tries to fill this gap by investigating the antiarthritic capabilities of *N. fruticans* fruit pulp, laying the foundation for its potential use as a natural therapeutic agent. This research evaluates the safety profile of *N. fruticans* fruit pulp, addressing concerns regarding herbal treatments and ensuring that therapeutic uses are supported by scientific data. This study is justified by the necessity for effective, safe, and alternative therapies for arthritis, as well as potential of *N. fruticans* fruit pulp as a possible alternative, emphasizing the necessity of comprehending its processes and safety to improve treatment approaches in rheumatology and the management of inflammatory diseases.

1.9 Literature Review

- **(Middleton *et al.,* 2000; Prabhu *et al.,* 2019; Cohen *et al.,* 2020):** Mangrove plant extracts, particularly from *N. fruticans*, exhibit strong anti-inflammatory activity due to their rich flavonoid, tannin, saponin, and phenolic content, which modulate key inflammatory pathways and reduce oxidative stress.
- **(Lourenço *et al.,* 2019; Ebana *et al.,* 2015; Sari *et al.,* 2017):** The antibacterial activity of *N. fruticans* leaves, characterized by their diverse bioactive compounds such as alkaloids, glycosides, and saponins, has been demonstrated to effectively inhibit the growth of pathogenic bacteria, including

  E. coli, Klebsiella pneumoniae and Aeromonas hydrophila with significant inhibitory effects observed at concentrations as low as 5% and minimum bactericidal concentrations (MBC) reported between 1.33 and 4.67.
- **(Lourenço *et al.,* 2019; Blanch *et al.,* 2020; Yahaya *et al.,* 2021):** The antioxidant activity of *N. fruticans* is attributed to its rich composition of bioactive compounds, including phenolic acids, flavonoids, and ascorbic acid, which effectively scavenge free radicals and demonstrate significant potential for health benefits and commercial applications.

1.10 Study Protocol

The next study's goal was to discover any possible health advantages of *N. fruticans* fruit pulp extract. Establishing a study protocol that describes the steps involved in the experiment is crucial. An outline of the study's methods is given below:

i. The target plant's fruit was harvested.

ii. After the fruits were cut into small pieces, fruit pulp was prepared.

iii. Methanolic extract was then used to prepare the fruit pulp extraction of *N. fruticans.*

iv. The fruit's anti-arthritic properties were observed.

Chapter 2

Methodology

2.1 Plant Materials Collection

The world's biggest contagious mangrove forest, the Sundarban forest is situated in the southwest of Bangladesh, between the rivers Baleswar and Harinbanga. It borders the Bay of Bengal. This is where the leaves, roots, fruits and bark of *N. fruticans* have been collected during April – May. A taxonomist from the Bangladesh National Herbarium in Mirpur, Dhaka [DACB Accession number: 42,861] authenticated it.

2.2 Preparation of Methanolic Extract of *N. fruticans*

The plant materials were cleaned with water before the analysis in order to get rid of any dirt and mud. After that, further air drying was done on the parts to get rid of extra moisture and water. The *N. fruticans* pulp was cleaned in a dry environment in preparation for coarse grinding. The specific portion of the plant examined in the study was the fruit pulp of *N. fruticans*. The Nipa palm fruit's pulp was precisely removed while maintaining the fruit's integrity. After the pulp was separated, it was carefully diced into small pieces. This is typically done by carefully removing the outer fibrous layers and extracting the soft, edible pulp inside. The quality of the pulp is essential, as it directly impacts the yield and effectiveness of the extract. Once the pulp is obtained, it is crucial to handle it with care to prevent any degradation or contamination. The pulp is then ready for the next stage in the extraction process. This step is crucial because it significantly enhances the material's surface area, promoting improved solution penetration during extraction. The finer the pieces, the more efficient the extraction process will be, as smaller particles allow the solvent to interact more thoroughly with the plant material. Grinding can be done manually or using a grinder, depending on the volume of pulp being processed. Following the process, a homogeneous slurry is prepared from the pulp. This involves mixing the finely chopped pulp with a small amount of solvent, typically distilled water or another suitable liquid, to create a consistent mixture. The purpose of creating a slurry is to enhance the extraction of soluble compounds and ensure that the plant material is well-dispersed within the solvent. The slurry should be blended until a smooth consistency is achieved, free from lumps. The next step, 2 liters of 95% methanol are introduced to the jar containing the pulp slurry. Once the methanol is added, the jar is sealed and maintained at a controlled temperature of 25°C for three days. This incubation period is essential as it allows sufficient time for the methanol to permeate the plant material and extract the desired compounds. During this time, the mixture is occasionally stirred and shaken. This agitation helps to mix the components thoroughly, enhancing solvent interaction with the plant material. Stirring also prevents sedimentation of the pulp, ensuring that the extraction process is uniform throughout the solution. After the incubation period is complete, the next critical step is filtration. The solution is filtered three times using Whatman No. 1 filter paper, which is known for its fine porosity and ability to retain even small particulates. Following the initial filtration, the solution is further passed through a sterilized cotton bed. This additional filtration step is crucial for ensuring that the final extract is free from any residual impurities, providing a cleaner product for subsequent analysis. Once the filtration is complete, the resulting crude extract is carefully collected. This extract is now labeled as the methanolic extract of *N. fruticans* (MNF). At this stage, the extract is ready for further analysis and experimentation.

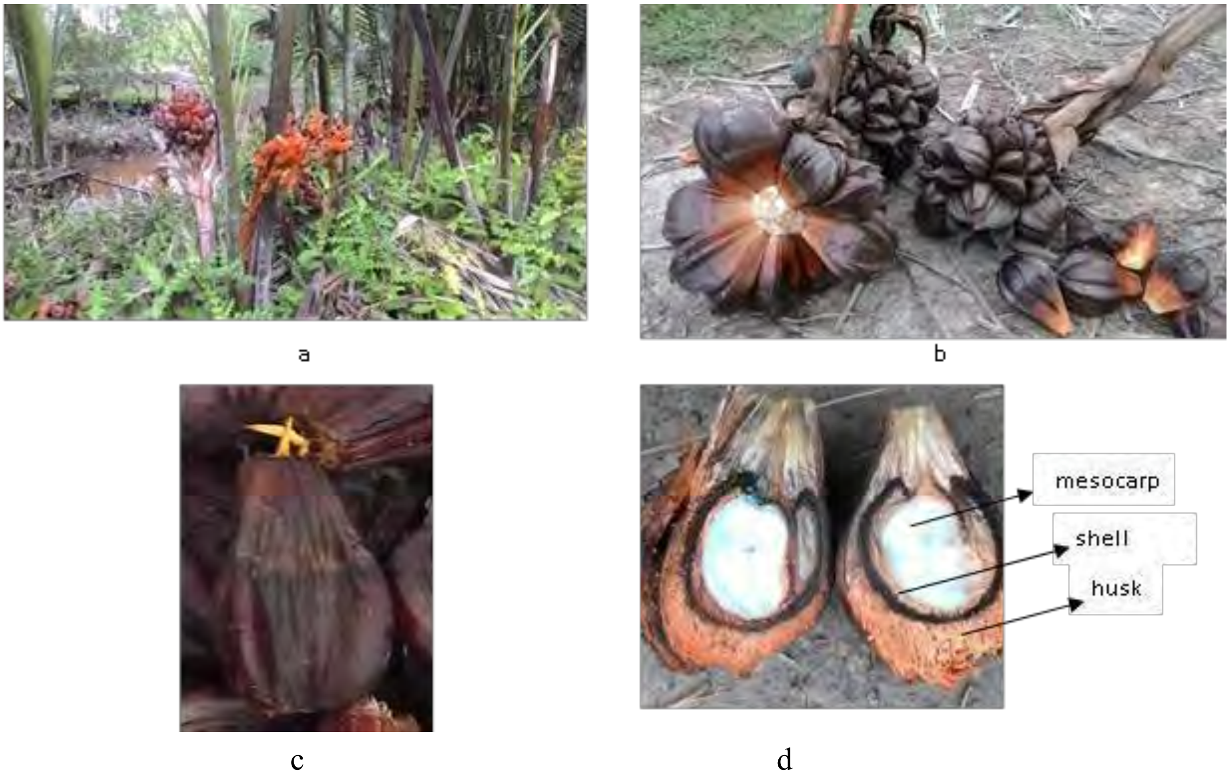

FIGURE 1: Nipa trees and the fruits (a), bunches of mature Nipa fruit (b), sprouted fruit (c), cross section of Nipa fruit (d). (Sari *et al.,* 2017)

2.3 Statistical Analysis

An analysis of variance (ANOVA) was conducted to estimate the effects of newness and bleaching duration on the functional properties of the flour. Dunnet's multiple range test was employed to identify significant differences among the means. The data analysis was performed using SPSS software version 28. Variations in the means of different groups were considered significant at $p < 0.05$.

2.4 Reagents and Equipment

The substances used to conduct the experiment were 0.5% Bovine Serum Albumin, Diclofenac Sodium test tube, beaker, weight machine, pipette and magnetic stirrer. All chemicals utilized in this experiment were classified as analytical laboratory grade.

2.5 Assay for Bovine Serum Protein Denaturation

2.5.1 Formulation of Bovine Serum Albumin (BSA) 0.5%

Materials: 500 mg of Bovine Serum Albumin (BSA), 100 ml of distilled water, Phosphate Buffer Saline (PBS) at pH 6.3 (if necessary) in a clean beaker.

The process:

1. 500 mg of BSA weighed using a precise balance
2. Poured the BSA powder in a dry, clean beaker
3. Poured 100 ml of distilled water into the beaker with the BSA in it.
4. Gently mix the mixture with a magnetic stirrer until all of the BSA has totally dissolved.
5. Assured that the solution was homogenous and checked for any undissolved particles.

Result: The finished product is a homogeneous, clear solution of bovine serum albumin.

2.5.2 Preparation of pH 6.3 Phosphate Buffer Saline (PBS)

In order to make the solution, 800 milliliters of distilled water were used to dissolve 8 grammes of sodium chloride (NaCl), 0.2 grammes of potassium chloride (KCl), 1.44 grammes of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 grammes of potassium dihydrogen phosphate (KH_2PO_4). The pH was subsequently adjusted to 6.3 using 1N HCl, and the solution volume was increased to 1000 ml using distilled water.

2.6 Investigation of Antiarthritic Activity using the Protein Denaturation Method

A previously developed technique with a few minor adjustments was used to evaluate the plant extract's antiarthritic activity (Ripa *et al.*, 2024). The only ingredients in the negative control solution were 0.05 ml of distilled water and 0.45 ml of aqueous bovine serum albumin (BSA). The solutions for the NFP extract and positive control comprised 0.45 ml of BSA (5% aqueous solution) and 0.05 ml of various doses (100, 200, 300, 400, and 500 µg/ml) of the reference drug, diclofenac sodium, together with the corresponding sample. 1N hydrochloric acid (HCl) was used to bring the pH of each solution down to 6.3. Using a UV-visible spectrophotometer, the samples were heated to 57°C for three minutes after being incubated for thirty minutes at 37°C. The absorbance was measured at 660 nm. Total denaturation of the protein is simulated by the negative control. The outcomes were contrasted with sodium diclofenac's. The method of (Ripa *et al.*, 2024) was used to compute the percentage inhibition of protein denaturation: Percentage of inhibition = [100 − (Optical density of test solution − Optical density of product control)] ×100.

Chapter 3

Result

3.1 Result for Anti-Arthritic

Using the denaturation of bovine serum protein technique, the *in vitro* anti-arthritic activity of *N. fructicans* fruit pulp extract was assessed, with diclofenac sodium acting as the standard reference. The antiarthritic activity of the assessed extract was investigated at five distinct doses ranging from 100 to 500 µg/ml. Table 2 displays all of the findings. In a concentration-dependent way, the tested extract was found to inhibit BSA denaturation. Notably ($p < 0.01$), NFP showed a maximum percentage inhibition of 76.28±1.05% at the highest dose of 500 µg/ml, compared to Diclofenac sodium (96.77±0.78%).

Table 2: *In-vitro* anti-antiarthritic activity evaluated by bovine serum protein denaturation method:

Sample	**% of Inhibition**				
	Concentration(µg/ml)				
	100	**200**	**300**	**400**	**500**
Diclofenac sodium	75.48±0.98	79.75± 0.52	86.53±0.88	94.12±0.86	96.77±0.78
NFP	58.48±0.82	64.32±0.53*	69.34±0.78*	74.26±0.75*	76.28±1.05**

The values are demonstrated as mean±STD (n=3); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the control.

Chapter 4

Discussion

The current investigation assessed the anti-arthritic effect of the methanolic extract of *N. fruticans* using *in vitro* models, specifically the prevention of protein denaturation. This specie of mangrove possesses significant potential as a natural anti-arthritic agent. The results indicate that active components of *N. fruticans* may be responsible for the anti-arthritic property shown by *N. fruticans* extract. Arthritis is a medical disorder characterized by the presence of swelling and inflammation in one or multiple joints of the body. With concerns about the side effects of synthetic anti-inflammatory medications, there is growing interest in natural remedies free from artificial substances. Tissue protein denaturation is a well-established factor contributing to inflammatory and arthritic conditions. Previous study indicates that protein denaturation is a leading cause of rheumatoid arthritis. In rheumatic disorders, autoantigens may arise due to protein denaturation *in vivo*, as hypothesized and supported by evidence (Sree *et al.,* n.d). It was also believed that changes in hydrogen, hydrophobic electrostatic, and disulfide bonding were involved in the denaturation process. Materials with the capacity to stop protein denaturation may find use as anti-arthritic medications. According to (Sinica *et al.,* 2013) stated that, in the overall context of our study, the plant will have more anti-arthritic efficacy the higher the percentage of denaturation inhibition that occurs. Anti-arthritic drugs may contain chemicals that prevent protein denaturation. Our work suggests that inhibiting denaturation increases the plant's effectiveness in treating arthritis (Bs *et al.,* 2014). Following the *in vitro* research, the results of this investigation were obtained. According to the result (Table 2) indicate that fruit pulp from *N. fruticans* inhibited protein denaturation, anti-arthritic potential in a dose-dependent manner compared to diclofenac sodium, the standard. The methanolic extraction and Bovine Serum Albumin test showed varied levels of anti-arthritic efficacy *N. fruticans* fruit pulp capacity to prevent protein denaturation.

Chapter 5

5.1 Conclusion

In conclusion, from the explanation above, it is conceivable that *N. fruticans* has been shown in experimental tests to have an effective anti-arthritic effect. Nipa produces active compounds such as phenolics, saponins, flavonoids, and tannins, which are valuable as raw materials for products, particularly in anti-arthritic activity. One of the primary causes of RA is protein denaturation, which results in the synthesis of autoantigens. Based on the results, it may be inferred that the plants fruit pulp extract inhibits protein denaturation, which could regulate the synthesis of autoantigens. In several tropical nations, according to traditional beliefs, nipa is regarded as an herbal treatment for a variety of conditions, including fever, gout, kidney stones, digestive disorders, chronic diseases, and metabolic syndromes such as diabetes and hypertension. Nipa has demonstrated antioxidant, anti-inflammatory and antibacterial. Nipa is employed not just for medicinal purposes but also for roof houses, cow pens, and garden huts, as well as for crafting broomsticks, handicrafts, and fishing implements, in addition to providing food, beverages, and renewable energy fuel. The outcomes of the current investigation indicate that *N. fruticans* may serve as a possible anti-arthritic agent. Additional study is crucial to completely understand its therapeutic potential and to inform the public about the significance of nipa as a sustainable resource for the future. We need to persist with the inquiry utilizing *in vivo* models. Additional research utilizing isolated active compounds and pharmacological analyses at the molecular level may be conducted to validate its efficacy and explore its mechanism of action.

5.2 Planning of Future Study

The present study demonstrated the development of the anti-arthritic properties of the plant *N. Fruticans* using an *in vitro* methodology; nevertheless, the most significant goal at this point is to conduct an *in vivo* investigation. To determine whether the experiment will have any adverse effects on human or animal bodies, it should be conducted on animals as soon as possible. Furthermore, in order to address thrombolytic and arthritic problems, a new extraction technique with higher constituent absorption rates and higher protein denaturation and lysis percentages could be developed. Studies on the drug's adverse effects, interactions with other drugs, effects on children, effects on women who are pregnant, and other issues should be carried out as well to ensure that no patients of any kind, regardless of age or condition, will be harmed. In the future, the computer analysis can be carried out to discover more efficacy against novel diseases affecting this specific plant. To improve overall efficacy, *N. fruticans* may be used in conjunction with other treatments. Its chemicals may interact with other alternative therapies or currently available anti-arthritic medications, according to research.

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