Analgesic and CNS Depressant Activities of Methanolic Shell Extract of *Nypa fruticans* Wurmb Fruit in Swiss Albino Mice

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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August, 2023

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 thesis titled "Analgesic and CNS depressant activities of methanolic shells extract of *Nypa fruticans* Wurmb fruit in Swiss albino mice" submitted by Rafiya Rafa Lubaba (Student ID: 19346050) of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.)

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Ethics Statement

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

Abstract

The study explored the analgesic and CNS depressant activities of a methanolic shell extract from *Nypa fruticans* Wurmb fruit. Methanolic shell extract of *N. fruticans Wurmb* fruit was acetic acid induced technique was used to test the analgesic efficacy in mice. An evaluation of CNS depressant activity used the hole cross test. Different doses (200, 400, and 600 mg/kg) were administered to mice, and results indicated significant dose-dependent analgesic and CNS depressant activities ($p<0.05$). The extract exhibited enhanced analgesic effects, achieving 51.16% inhibition at 600 mg/kg, whereas the standard drug Indomethacin achieved 79.54% inhibition. At 600 mg/kg, the extract reduced locomotor activity and exploratory behavior, similar to standard drug diazepam. These findings highlighted the notable analgesic and CNS depressant activities of the methanolic shell extract from *N. fruticans* Wurmb fruit.

Keywords: *Nypa fruticans* Wurmb fruit, CNS Depressant, Analgesic, Acetic Acid Induced Writhing method, Hole Cross method.

Dedication

Dedicated to my Family, Teachers and Friends

Acknowledgement

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List of Acronyms

CNS	Central Nervous System
NFS	*Nypa fruticans* Shell
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
ICDDR, B	International Centre for Diarrhoeal Disease Research, Bangladesh

Chapter 1

Introduction

1.1 General introduction

Plants have been utilized for their medicinal properties for millennia. Around 10% of vascular plants serve as medicinal plants (Joppa et al., 2011) , and their total estimated species count lies between 350,000 to nearly half a million (Pimm et al., n.d.) . Throughout history, plants have been utilized for medicinal purposes, and their applications continue to be relevant in modern times.

Numerous societies across different eras have employed plants to address various ailments and maladies. Presently, plant-derived medications still hold significant importance in the field of healthcare. A multitude of plants are employed in medicine, either in their natural state or as extracts and compounds. The medicinal attributes of plants led to the development of pharmaceutical drugs derived from specific plants possessing these advantageous properties (Jones et al., 2006) . Herbal medicine represents a form of treatment that utilizes plant-based remedies to address a broad spectrum of health conditions. Many herbs find application in traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and Native American medicine. The contribution of plants to the advancement of modern medicine has been substantial, and they continue to offer vital treatments for numerous health conditions. Additionally, contemporary medicine largely depends on chemicals produced from plants for various therapeutic purposes.

The significance of medicinal herbs was crucial as therapeutic agents and valuable resources for producing both traditional and modern medicines. They provided alternative remedies that offered significant opportunities for generating income, employment, and foreign currency for developing countries. Various traditional healing herbs and their components were proven to possess medicinal properties, capable of preventing, alleviating, or curing several human diseases. It was estimated that a significant percentage, around 70-80%, of the global population primarily relied on traditional, predominantly herbal medicine to fulfill their primary healthcare requirements (Rahman et al., 2013) .

Medicinal plants are plants that have been used for their therapeutic properties for centuries. They contain various compounds that can have positive effects on human health. Some well-known examples include:

- Aloe Vera: Used for its soothing properties on burns and skin irritations.
- Peppermint: Known for its ability to ease digestion and relieve headaches.
- Ginseng: Used to boost energy, improve mental clarity, and enhance immune function.
- Turmeric: Contains curcumin, which has anti-inflammatory and antioxidant properties.
- Lavender: Often used in aromatherapy for relaxation and stress relief.
- Echinacea: Thought to support the immune system and help prevent colds.
- Chamomile: Used as a mild sedative and for its anti-inflammatory effects.
- Garlic: Known for its potential cardiovascular benefits and immune system support.
- Ginkgo Biloba: Often used to improve memory and cognitive function.

Comprehensive research was carried out globally to verify their efficacy, leading to the creation of plant-derived medications. The annual global market worth of medicinal plant products exceeded $100 billion. A medicinal plant was defined as any plant containing substances in one or more of its parts that could be used for therapeutic purposes or as building blocks for producing valuable pharmaceuticals (Kunle, 2012) .

Modern science, particularly during the Renaissance era, performed a significant part in the creation of chemical analysis and advanced instrumentation like the microscope. These advancements made it possible to isolate the beneficial components present in medicinal plants (Reeds, 1976) . Over time, these active constituents were synthesized in laboratories to create pharmaceutical medicines (Atanasov et al., 2015) . Over time, as the use of medications increased, so did the frequency of directly using medicinal plants in contemporary medicine (Gertsch, 2009) . The frequency of employing plants for medicinal purposes in contemporary medical practice rose over time as the usage of pharmaceuticals did (Arceusz et al., 2010) .

Medicinal plants offer a wide range of potential benefits due to the bioactive compounds they contain. Some of the benefits include:

- Natural Healing: Since ancient times, a variety of traditional medical systems have used medicinal plants to treat a wide range of medical issues.

- Holistic Approach: Many medicinal plants provide a holistic approach to health, addressing not only the symptoms but also the underlying causes of various ailments.

- Minimal Side Effects: Compared to some pharmaceutical drugs, medicinal plants often have fewer and milder side effects.

- Rich Source of Bioactive Compounds: Medicinal plants contain compounds like alkaloids, flavonoids, terpenoids, and polyphenols that can have positive effects on health. These compounds often have antioxidant, anti-inflammatory, and antimicrobial properties.

- Support for Chronic Conditions: Some plants, like turmeric and garlic, may assist in managing long-term diseases like diabetes, heart disease, and arthritis.
- Immune System Support: Certain plants, such as echinacea and elderberry, are believed to enhance the immune system and help the body fight off infections.

- Mental Health Benefits: Plants like lavender and chamomile are known for their calming and anxiety-reducing effects, promoting better mental well-being.

- Digestive Health: Many plants, including ginger and peppermint, can aid digestion, soothe digestive discomfort, and alleviate issues like indigestion and bloating.

- Skin Care: Aloe vera, tea tree oil, and neem are used in skincare products for their soothing, antimicrobial, and healing properties.

- Traditional Wisdom: Medicinal plants are often integral to cultural and traditional healing practices, passing down knowledge through generations.
- Cost-Effectiveness: Medicinal plants can offer cost-effective alternatives to expensive pharmaceutical medications.

Consequently, numerous regions around the world persist in depending on traditional medicine, which entails the direct utilization of medicinal plants, owing to its economical nature (Salmerón-manzano & Manzano-agugliaro, 2020) .

1.2 Analgesic Activity

Pain is a debilitating aspect of numerous medical conditions, and effectively managing pain is a crucial therapeutic priority. According to official definitions, pain is an unpleasant sensory and emotional sensation associated with existing or potential damage to tissues (Moretti et al., 2018) . It functions as an alert indication and primarily acts as a safeguarding reaction, although frequently resulting in substantial unease and having the potential to generate a range of unfavorable consequences (Harsoor, 2011) . Analgesics are medications utilized to treat or alleviate pain. Conventional pain-relieving medications, particularly opioids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), stem from natural sources (Shojaii & Abdollahi Fard, 2012) . However, many synthetic compounds acting through the same mechanisms have been developed. Unfortunately, certain synthetic compounds are linked to significant undesirable outcomes, such as ulceration, gastrointestinal bleeding, addiction susceptibility, breathing difficulties, drowsiness, and nausea, among other effects (Ene et al., 2008) . Despite the advancements in pain therapies, the medical field continues to seek secure, efficient, and

powerful analgesic medications to address various painful conditions, particularly chronic pain. Herbal therapy presents an intriguing possibility for treating opioid dependence and withdrawal (Hijazi et al., 2017) .

1.3 CNS Depressant

Anxiety and depression are prevalent psychiatric disorders that affect a significant number of individuals globally. By 2020, it was already anticipated to be the second most significant contributor to the global burden of disease, following closely behind heart disease (Smith et al., 2008) . The general population's estimated prevalence of depression ranges from 5% to 8% (Currie & Wang, 2004). Currently, approximately 121 million individuals are estimated to be impacted by depression, and suicide stands as one of the most prevalent consequences (Richelson, 2001) . However, the medications employed to address diverse neuropsychiatric and neurological conditions such as anxiety, depression, schizophrenia, epilepsy, and parkinsonism frequently carry significant adverse impact or unwanted interactions with other drugs or food (Kwan & Brodie, 2001) .

These conditions can be experienced by people at various stages of their lives. While there are several medications available for the treatment of anxiety and depression, their effectiveness is often limited, and they can be costly. As a result, ongoing research aims to develop more potent, well-tolerated, and cost-effective drugs. Plant derived medicines have emerged as promising sources of psychiatric therapies, spanning a wide range of possibilities. This is evidenced by the extensive investigation and screening of numerous traditional medicines for their potential

in psychotherapeutic applications, particularly in rodent models. The aim is to identify novel plant-based compounds that could provide effective treatments for anxiety and depression.

1.4 Background information of *Nypa fruticans* Wurmb

Nypa fruticans Wurmb (NF), a member of the Araceae family, is classified as an "underutilized" plant (Chunxiao et al., 2011) . It is also known by other names such as *Cocos Nypa* Lour., *Nypa fruticans* unb., and *Nypa palm*. This monoecious palm thrives in brackish water and has an upright stem, lacking a trunk, with common fruits sprouting from the ground.

Nypa fruticans, also referred to as the mangrove palm or *N. palm*, is a kind of palm tree that is indigenous to the coastal regions of Southeast Asia and parts of the Pacific Islands. It is well-adapted to brackish or saline water and is often found in mangrove ecosystems.

- Habitat and Distribution: *N. fruticans* typically grows in coastal areas where freshwater and saltwater mix, such as estuaries and tidal flats. It can form dense stands in mangrove swamps and along riverbanks.

- Physical Characteristics: The *N. palm* has a unique appearance with feathery fronds that can grow up to 9 meters (30 feet) in length. Its leaves are used to thatch roofs and make various traditional products.

- Uses: Various parts of the *N. palm* are used by local communities for multiple purposes. Some common uses include:

- Thatching Material: House roofs are frequently thatched with leave and shelters due to their water-resistant properties.
- Construction Material: The trunks are used as construction material for buildings and huts.
- Edible Shoots and Fruits: The young shoots of the *N. palm* are edible and used in local cuisines. The fruits, which are small and spherical, can also be consumed.
- Medical Uses: In several traditional medical systems, various illnesses are treated using various plant parts.

- Economic Importance: *N. fruticans* contributes to the livelihoods of coastal communities through its various uses. Its thatching material, in particular, is valued for its durability and resistance to water.

- Ecological Importance: *N. palm* play a crucial role in mangrove ecosystems by providing habitat for various organisms and helping to stabilize coastal areas.

- Environmental Impact: In some regions, the expansion of *N. palm* populations can contribute to changes in local ecosystems and impact biodiversity.

- Conservation: Like many mangrove species, *N. fruticans* face threats from habitat destruction, coastal development, and pollution. Conservation efforts aim to protect and restore mangrove habitats to ensure the survival of species like the *N. palm*.

Figure 1: *Nypa fruticans* Wurmb fruit bunch.(Prasad et al., 2013)

It is commonly located in India, Malaysia, Indonesia, the Philippines, and certain regions of Queensland, Australia (Tamunaidu & Saka, 2011) . The sap obtained from the flower cluster stem of this plant is ingested as a drink by native communities, and the youthful fruits are consumed (Tang et al., 2010) . The sap functions as a precious sugar source and is employed in creating confections, vinegar, drinks, and alcoholic beverages. The *N. fruticans* Wurmb fruit is abundant in carbohydrates, dietary fibers, minerals, and vitamin A. Initially, various parts of *N. fruticans* Wurmb including the leaves, stem, and roots, fruits have been employed to treat conditions such as asthma, leprosy, tuberculosis, sore throat, liver disease, snake bites, pain relief, sedation, and as a carminative (Padmaja et al., 2002) . Moreover, recent studies have shown that methanol extracts from the stem and leaves of *N. fruticans* Wurmb possess antidiabetic and analgesic properties (Hamilton & Murphy, n.d.) . The fronds, husk, shell, and leaves of the nipa palm, which have a -cellulose content ranging from 28.9 to 48.2%, are prospective raw materials for fuels and chemical production, according to earlier studies (Tamunaidu et al., 2013).

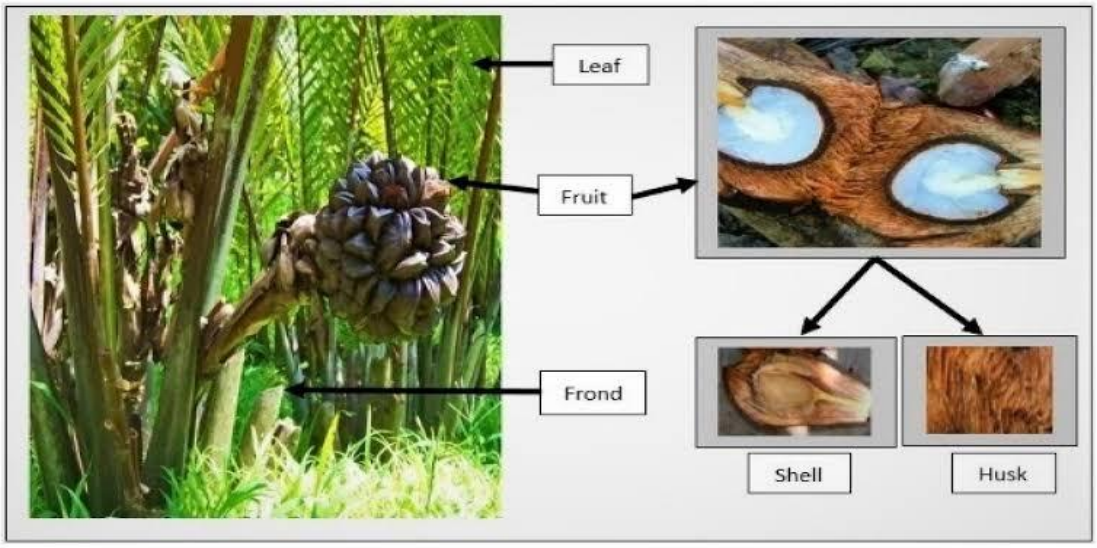

Figure 2: *Nipa palm* byproducts that serve as raw materials for chemicals and energy (Tamunaidu et al., 2013)

1.5 Objective of the study

Objectives of your study, "Analgesic and CNS depressant activities of methanolic shells extract of *Nypa fruticans* Wurmb fruit in Swiss albino mice," summarized in two points:

1. Analgesic Evaluation: Determine the potential analgesic effects of the methanolic shell extract derived from *N. fruticans* Wurmb fruit by conducting pain response tests in Swiss albino mice, aiming to measure its ability to reduce or inhibit pain.
2. CNS Depressant Assessment: Investigate the CNS depressant activities of the extract by conducting behavioral studies or tests in mice, aiming to evaluate its sedative or depressive effects on the central nervous system.

By accomplishing these objectives, the purpose of the study is to understanding of the potential analgesic and CNS depressant properties of methanolic shell extract of the *N. fruticans* Wurmb fruit, providing valuable insights into its therapeutic potential and pharmacological profile.

1.6 Significance of the Study

The significance of my thesis topic, "Analgesic and CNS depressant activities of methanolic shell extract of *N. fruticans* Wurmb fruit in Swiss albino mice," can be summarized as follows:

- Exploration of Natural Analgesics: Investigating the analgesic properties of the methanolic shell extract of *N. fruticans* Wurmb fruits provides an opportunity to explore potential natural alternatives for pain management, potentially reducing dependence on synthetic drugs.

- Central Nervous System (CNS) Effects: Understanding the CNS depressant activities of the extract can contribute to the knowledge of its pharmacological profile, aiding in the development of therapeutic interventions targeting neurological conditions or disorders.

- Animal Model Relevance: Utilizing Swiss albino mice as the animal model helps establish the extract's effects in a widely accepted and accessible model, enhancing the translational potential of the findings to human applications.

- Novel Research Approach: Investigating the specific extract from the shell of *N. fruticans* Wurmb fruit adds to the existing body of knowledge, potentially revealing new and unique bioactive compounds with analgesic and CNS depressant properties.

- Promoting Sustainable Resources: Focusing on a fruit extract from *N. fruticans* Wurmb fruits highlights the potential of utilizing underutilized or overlooked natural resources, fostering sustainability and conservation efforts while exploring their therapeutic value.

Chapter 2

Methodology

2.1 Plant material collection and preparation

A sample of the fruit shell from the *Nypa fruticans* Wurmb fruits plant was obtained from Mangrove Forest Sundarban, Bangladesh (accession number DACB 87898). A skilled taxonomist accurately identified the plant specimens. The shell was allowed to dry in the sun over a period of time. Later, plant materials were thoroughly dried in an oven at a relatively low temperature for 24 hours to aid in better grinding. Subsequently, the dried shell was coarsely powdered using an electric blender for a lab. With that, samples that are in powder intended for the experiment were prepared and stored in an airtight container to avoid contaminating. After that, they were stored in a cool, dark and dry atmosphere.

2.1.1 Plant Material Extraction

Fractional extraction involves using a customized Kupchan partitioning method (Ashikur Rahman et al., 2016) . Around 200 grams of the powdered material were placed into a tidy, circular-bottomed flask filled with two liters of methanol. A cotton plug and aluminum foil were used to seal the flask, which was then left undisturbed for 14 days while being periodically shaken and stirred. After that, the entire combination was filtered twice: once through cotton and once again using Whatman No. 1 filter paper. A Heidolph rotary evaporation device was used to concentrate the filtrate that resulted from that process. After being exposed to air to dry, the concentrated extract produced a solid residue. Only 2 grams of the plant's entire dried,

powdered body made up the raw methanol extract. This extract was kept at -22 °C in a refrigerator until its utilization was required (Emran et al., 2015) .

2.2 Experimental animals

Swiss-albino mice were employed for the purpose of therapeutic research, sourced from the animal lab at Jahangirnagar University and ICDDR, B. Swiss-albino mice, regardless of sex, aged between 4 and 5 weeks and weighing approximately 20-25grams.The mice were housed in clean and dry polypropylene cages, maintaining a 12-hour light-dark cycle with a relative humidity of 45–55% and a temperature of 25.2°C. To ensure their hydration and nutrition, enough food and water were given.

2.2.1 Experimental design

A total of twenty-five experimental animals used in experiment selected at random and separated into five groups: Group I, Group II, Group III, Group IV, and Group V, with each group comprising five mice. Each group was subjected to a distinct treatment, which included a control group, a standard group, and varying doses of the extract derived from different fractions of the plant's shell. Before administering any treatment, the mice were weighed accurately, and the doses of the reference, control, and test samples were changed correspondingly. It was required to distinguish between each mouse across a group during the treatment since it was difficult to detect the physiological responses for every one of the mice receiving the same treatment. The mice were labeled M-1 (Mice 1), M-2 (Mice 2), M-3 (Mice 3), M-4 (Mice 4), and M-5 (Mice 5). This allowed for the identification and tracking of each mouse throughout the study.

2.2.2 Ethical approval

Ethical permission was obtained from the Department of Pharmacy at Jahangirnagar University and it was made sure that all procedures followed the institutional animal ethics committee's rules.

2.3 Pharmacological investigation of plant extracts

The medicinal effect of the experimented extracts was assessed through pharmacological investigations, which included evaluating their:

- CNS depressant activity
- Analgesic activity.

2.3.1 Analgesic activity of methanolic shell extract of *Nypa fruticans* Wurmb fruit

An analgesic drug, also known as a painkiller, is used to relieve pain. The analgesic test in the experiment was conducted implementing the acetic acid-induced writhing method. This technique is commonly employed to assess the effectiveness of substances in reducing pain sensations.

2.3.1.1 Preparation of drug and chemical solution

To prepare the standard the recommended dosage of indomethacin, which is 10 mg/kg body weight, a specific amount of Indomethacin was collected and diluted in a solution adding 0.9% saline. Each mouse was subsequently given 0.5 ml of the standard orally.

A crude extract was prepared at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg according to the mice's body weight. To achieve this, the doses were weighted and combined with the

suspending agent tween 80. Saline water was then mixed in with the other ingredients, and the last amount was adjusted. This was followed by giving each of the mice 0.5 ml of the solution.

2.3.1.2 Preparation of acetic acid solution

To prepare the Acetic Acid solution, 0.7 ml of Acetic Acid was diluted by adding it to 100 ml of distilled water.

2.3.1.3 Acetic acid-induced writhing method

The Acetic acid-induced writhing (Sukul et al., 2017) test is a technique used to observe analgesic behavior. In this method, mice were administered intra-peritoneal acetic acid to induce pain sensation. Indomethacin was considered as the standard drug for comparison.

The experiment began with orally administering normal saline, along with extracts at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, as well as the standard drug. After a span of 30 minutes, an intraperitoneal injection of a solution of 0.7% v/v acetic acid was given to induce pain. For the ensuing 5 minutes, no writhing occurrences were recorded, followed by a 15-minute period of observing writhing episodes.

During this observation period, each one of the mice was put on a surface for observing, and the quantity of writhing episodes was documented. It is noteworthy that the mice might display both complete and partial writhing, and two incomplete writhing episodes were considered equivalent to one complete writhing episode. This method facilitated the assessment of the analgesic impact of the tested extracts and their comparison with the standard drug.

The flow chart is the procedure of analgesic activity of methanolic shells extract of *Nypa fruticans* Wurmb fruit by acetic acid induced writhing technique.

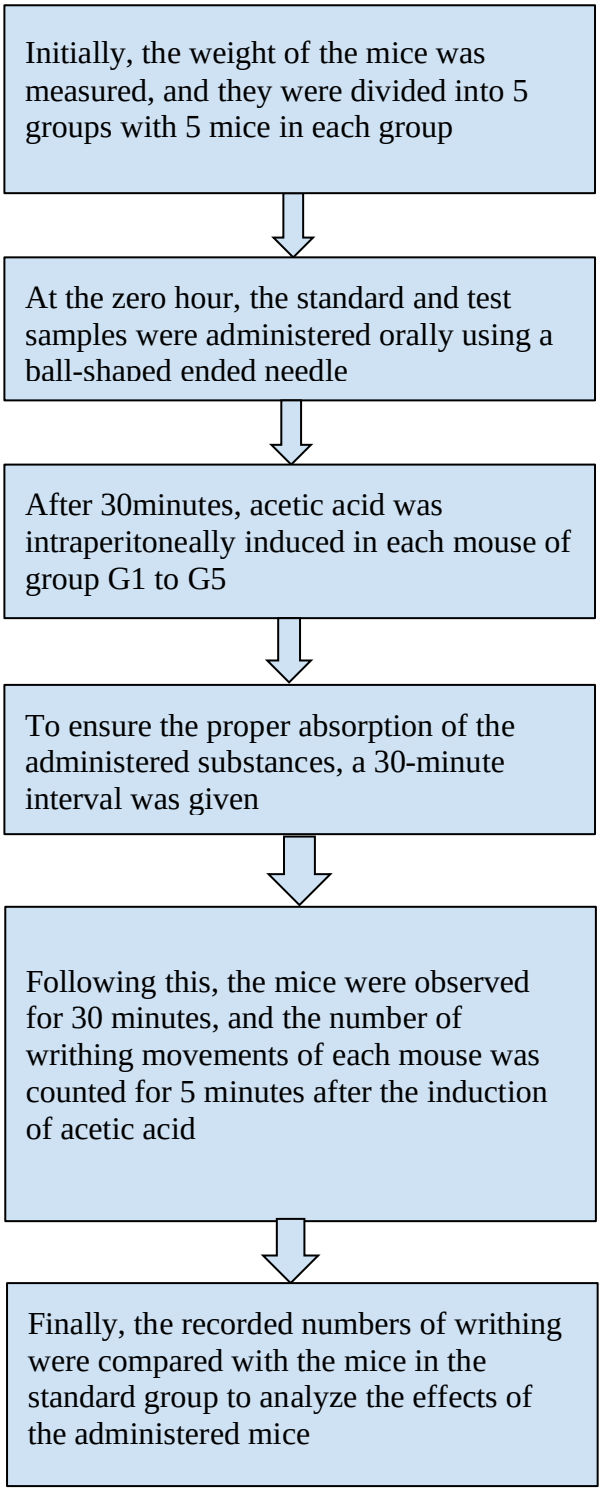

Figure 3: Process of acetic acid induced writhing method for analgesic activity on mice (Sukul et al., 2017)

2.3.2 CNS Depressant effect of methanolic shells extract of *Nypa fruticans* Wurmb fruit:

CNS Depressant drugs are substances that reduce brain activity. They are often prescribed by doctors to treat conditions such as panic attacks, anxiety, and insomnia. These drugs mainly activate the GABA neurotransmitter, which results in decreased brain activity.

In the experiment, the CNS depressant action of *Nypa fruticans* Wurmb fruit methanolic shell extracts was observed in rodents. The comparison was made using the common diazepam. Utilizing the results of the CNS depressant action by the hole cross technique.

2.3.2.1 Preparation of drug and chemical solution

To prepare the standard the 10 mg/kg diazepam dosage that is advised body weight, a specific amount of diazepam was gathered and dissolved with 0.9% saline in a solution.

After then, each mouse received 0.5 ml of the standard orally.

For the formulation of the crude extract, doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg were prepared based on the mice's body weight. This was achieved by measuring the doses and blending them with the suspending agent Tween 80. The final amount was subsequently modified after adding saline water to the remaining ingredients. The mice that were treated were subsequently given 0.5 ml of the solution after that.

2.3.2.2 The Hole Cross Technique

The primary objective of the hole cross test (Gawade, 2012) is to assess the impact of extract on locomotion and exploration effects of the extract on mice using a hole-board apparatus.

The hole-board used for the test was sized at 30 x 20 x 14 cm. Within the box, a steel divider with a hole of 3-centimeter diameter was positioned at the center. This arrangement enabled the observation and measurement of the mice's behavior as they explored and crossed through the holes in the board. This facilitated an understanding of their locomotor and exploratory behaviors in response to the tested extract.

The procedure for evaluating the CNS depressant effect of the methanolic shell extract of *Nypa fruticans* Wurmb fruit through the hole cross test is outlined below:

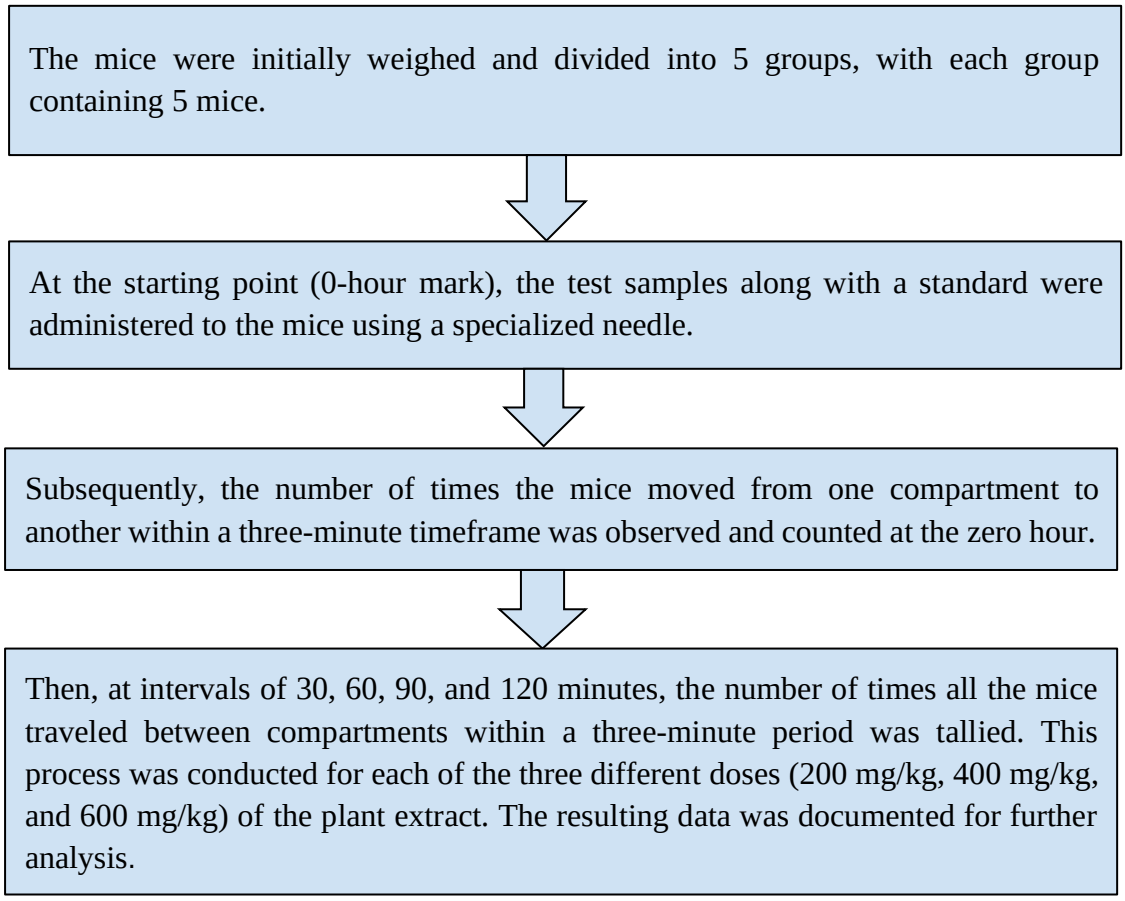

Figure 4: Process of hole cross method for CNS depressant activity on mice (Gawade, 2012)

2.4 Statistical Analysis

The results acquired from the experiment are expressed as mean ± standard deviation (STD), with a sample size of 6 (n=6). Statistical analysis was conducted using One-Way Analysis of Variance (ANOVA), followed by Dunnett's test. Within the framework of Dunnett's test, significance was assessed with a threshold of *P<0.05, indicating statistical significance when compared to the control group.

Chapter 3

Result

3.1 Analgesic activity of methanolic shell extract of *Nypa fruticans* Wurmb fruit in mice

In the acetic acid-induced writhing test, the methanol extract obtained from the shells of *Nypa fruticans* Wurmb fruit significantly and progressively reduced the occurrence of acetic acid-induced writhing in mice following oral administration. At a dosage of 200 mg/kg body weight, the extract exhibited a writhing inhibition of 21.85%. Similarly, at 400 mg/kg body weight, the extract displayed a writhing inhibition of 32.54%, and at the highest dosage of 600 mg/kg body weight, the extract demonstrated a notable writhing inhibition of 51.16%. This indicates that the plant extract exhibited analgesic activity, with the dose of 600 mg/kg body weight coming particularly close to the standard drug indomethacin, which achieved a writhing inhibition of 79.54% at a dose of 10 mg/kg body weight.

Table 1: Analgesic activity of test samples by Acetic acid induced writhing method in mice

Groups	Treatment	Dose, route	No. of writhing	Percent inhibition
Group-I (Control)	1% Tween 80 in water	0.1 ml/10 gm body weight	35.83 ± 1.09	
Group-II (Standard)	Indomethacin	10 mg/kg	7.33 ± 0.81*	79.54
Group-III (Extract)	NFS	200 mg/kg	28.00± 0.89*	21.85
Group-IV (Extract)	NFS	400 mg/kg	24.17 ± 1.33	32.54
Group-IV (Extract)	NFS	600 mg/kg	17.5. ± 1.05*	51.16

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

3.2 CNS depressant activity of methanolic shells extract of *Nypa fruticans* Wurmb fruit in mice

In the hole cross test, animals were administered various doses of methanolic shell extract obtained from *Nypa fruticans* Wurmb fruit (at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg). The decline in locomotor activity was observed during the second observation period (30 minutes) and persisted consistently up to the fifth observation period (120 minutes) for the extracts. Particularly at the higher dose (600 mg/kg), the reduction in activity was comparable to that induced by the standard drug diazepam, which was employed as a reference to evaluate the impact of the plant extract on the central nervous system (CNS) by inducing depression.

The extract led to a decrease in spontaneous motor activity, indicating that this effect could be attributed to CNS depression, as decreased locomotor activity aligns with a common

characteristic of many neuroleptic substances. Throughout the observation period, the CNS maintained a state of depression, and the results achieved were statistically significant.

Table 2: CNS Depressant activity of test samples by Hole cross method

Group	Treatment	Dose	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-G1(Control)	1% tween 80 in water	10ml/kg	129±0.89	126.4±1.74	123.4±1.62	120.0±1.09	117.2±1.16
Group-G2(Standard)	Diazepam	1mg/kg	118.4±1.2	73.6±0.33*	45.8±0.58*	28.6±0.88	13.3±0.78*
Group-G3 (Extract)	NFS	200mg/kg	127.2±1.23	98.0±0.56*	66.2±1.88	39.4±0.92*	30.2±1.28
Group-G4(Extract)	NFS	400mg/kg	121.5±1.02	76.2±0.89	48.2±1.33*	26.1±0.87*	17.8±0.87
Group-G5(Extract)	NFS	600mg/kg	117.6±1.15	88.3±1.43*	60.2±1.55	33.6±0.92*	24.1±0.78*

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

Chapter 4

Discussion

Our current research findings demonstrate that the methanolic shells extract obtained from the *Nypa fruticans* Wurmb fruit exhibits dose-dependent analgesic and central nervous system (CNS) depressant activities.

In mice, the acetic acid-induced writhing test, commonly employed to assess analgesic drugs, is associated with visceral pain. The crude residue derived from the plant, administered at varying doses (200 mg/kg, 400 mg/kg, and 600 mg/kg body weight), exhibited noteworthy analgesic activity when compared to the standard drug Indomethacin. Notably, the higher dose (600 mg/kg) displayed greater analgesic efficacy against acetic acid-induced pain in mice. A localized inflammatory response that results in the release of unbound arachidonic acids from tissue phospholipids via cyclooxygenase (COX) and prostaglandin production is what causes the writhing that is caused by acetic acid. This causes the writhing reaction being connected to increased PGE2 and PGF2 levels in peritoneal fluids, as well as lipoxygenase-related goods. Prostaglandin levels in the peritoneal cavity are increased, which through raising capillary permeability in response to inflammatory discomfort. The acetic acid-induced writhing test proves effective in evaluating analgesics that act peripherally. A substance that diminishes the number of writhing responses is deemed to possess analgesic properties, possibly achieved through the inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The marked reduction in pain observed with the plant extract might stem from the presence of analgesic components that influence prostaglandin pathways. Furthermore, acetic acid-induced abdominal writhing is reported to indirectly stimulate neurons sensitive to other drugs, including narcotics and centrally acting agents, by releasing endogenous mediators (Wang et al., 2006) .

The potential analgesic effects of the methanolic extract of *N. fruticans* Wurmb fruit shell, as well as its distinct solvent-soluble fractions, were investigated using the acetic acid-induced writhing inhibition method. In this evaluation, the experimental animals were given doses of the crude methanolic extract and the various partitioned fractions, namely at 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. This test was designed to gauge the capacity of these extracts and fractions to alleviate pain, considering different levels of dosage.

The outcomes of the acetic acid-induced writhing test highlight the substantial suppression of acetic acid-triggered writhing in mice due to the three fractions of the methanol extract derived from *N. fruticans* Wurmb fruit shell. Various percentages depict the observed writhing inhibition at distinct dosage levels. Specifically, at a dose of 200 mg/kg body weight, NFS exhibited a writhing inhibition of 21.85%. For a dose of 400 mg/kg body weight, NFS displayed a writhing inhibition of 32.54%. Furthermore, at a dose of 600 mg/kg body weight, NFS showcased a writhing inhibition of 51.16%.

The methanolic extract of *N. fruticans* Wurmb fruit displayed a decline in both movement frequency and amplitude in the hole cross test conducted on mice, indicating its central nervous system (CNS) depressant effects. Reduction in locomotor activity serves as an indicator of lowered alertness, implying CNS depressant activity (Dey et al., 2011) . This reduction in spontaneous motor activity could be attributed to the impact of the plant extract on the Central Nervous System (Rakotonirina et al., 2001) .

The hole cross test resulted in a notable decrease in locomotion among the mice. Gamma Amino Butyric Acid (GABA) plays a critical role as the primary inhibitory neurotransmitter within the central nervous system (Hepsomali et al., 2020) . It is intricately involved in a

multitude of physiological functions linked to a diverse spectrum of psychological and neurological disorders, including epilepsy, depression, Parkinson's syndrome, and Alzheimer's disease (Kumar et al., 2013) . Various drugs have the potential to impact the GABA system through different mechanisms. This can include enhancing GABA-mediated postsynaptic inhibition by modifying GABA receptors through allosteric interactions, directly increasing the conductance of chloride ions, or indirectly reinforcing GABA-induced chloride conductance while simultaneously reducing the activity of voltage-activated Ca2+ channels – a mechanism reminiscent of the effects observed with barbiturates. Considering these intricate mechanisms, it is plausible to propose that the extract might operate by bolstering GABAergic inhibition within the central nervous system. This could potentially result in membrane hyperpolarization and a reduction in the firing rate of essential neurons in the brain. As an alternative explanation, the extract could directly activate GABA receptors, heighten the affinity of GABA for its receptors, or prolong the duration of GABA-gated channel opening. These possibilities underscore the extract's potential to modulate GABAergic signaling within the CNS, leading to alterations in neuronal activity and ultimately influencing behaviors such as locomotion (Amabeoku & Kabatende, 2012) .

The administration of NFS (at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg) to mice elicited a reduction in locomotor activity that followed a dose-dependent pattern. This reduction was comparable to the effect observed with the standard drug diazepam. In the control group, there was minimal fluctuation in the number of times the mice traversed from one chamber to another over the 0 to 120-minute timeframe. However, the groups that were treated with the plant extract at the specified doses exhibited a significant decline in movement when compared to their initial values during the 0 to 120-minute period. These findings from the result suggest that the NFS extract, similar to diazepam, influenced the locomotor activity of the mice, leading

to a reduction in movement. The dose-dependent reduction further indicates that higher doses of the extract were associated with a more pronounced effect on locomotor activity.

The result table illustrates the effect of NFS extracts at different doses (200 mg/kg, 400 mg/kg, and 600 mg/kg) on locomotor activity. The locomotor activity lowering effect of the extracts became evident during the 2nd observation period (30 minutes) and persisted until the 5th observation period (120 minutes). The extracts significantly reduced locomotion activity, which was comparable to the standard drug diazepam. At a dose of 200 mg/kg, the NFS extracts exhibited a depressant activity of 30.2±1.28. At 400 mg/kg, the NFS extracts showed a depressant activity of 17.8±0.87, and at 600 mg/kg, the NFS extracts displayed a depressant activity of 24.1±0.78. In comparison, the standard drug diazepam exhibited a depressant activity of 13.3±0.78 at a dose of 1mg/kg.

These findings suggest that the NFS extracts, much like diazepam, effectively lowered locomotor activity in a manner that was dependent on the dosage administered. The sustained impact witnessed across the observation period indicates the potential of NFS extracts to influence and regulate locomotion activity.

Chapter 5

Conclusion

In conclusion, the research conducted on the analgesic and CNS depressant activity of methanolic shell extract of *Nypa fruticans* Wurmb fruit demonstrates its potential as a valuable natural resource in the field of medicine. The findings of this study provide compelling evidence that *N. fruticans* Wurmb fruit exhibits significant analgesic properties and possesses CNS depressant effects. The experimental results indicate that the extracts derived from *N. fruticans* Wurmb have a notable analgesic activity, as evidenced by their ability to reduce pain response in various animal models. This suggests that *N. fruticans* Wurmb fruit may contain bioactive compounds that interact with pain pathways in the body, offering potential applications in the development of new analgesic drugs. Furthermore, the CNS depressant activity observed in this study indicates that *N. fruticans* Wurmb fruit extracts have a calming effect on the central nervous system. These findings imply the presence of compounds that can modulate neurotransmitter systems, potentially leading to the development of therapeutic agents for conditions involving overexcitation of the CNS, such as anxiety and insomnia. The results also highlight the importance of further exploration and identification of the active constituents responsible for the observed analgesic and CNS depressant effects. Isolating and characterizing these compounds could pave the way for more targeted research, including pharmacological studies, toxicity assessments, and potential clinical applications. Overall, the analgesic and CNS depressant activities demonstrated by *N. fruticans* Wurmb fruit provide a promising foundation for future investigations in the field of natural medicine. The potential development of novel analgesic agents and CNS depressants from this fruit holds great significance, offering alternatives to synthetic drugs that often come with adverse side effects.

Future

This research contributes to our understanding of the therapeutic potential of natural products and encourages further exploration of *Nypa fruticans* Wurmb fruit as a potential source of medicinal compounds.

Beyond medicinal applications, the extract might have other uses in biotechnology or agriculture. Research could explore its potential as an ingredient in cosmetic products or as a biopesticide, for example.

As interest grows in natural products and traditional medicine, there may be increased attention to the sustainable harvesting and conservation of *N. fruticans* as a valuable resource.

Researchers from various fields, such as pharmacology, biochemistry, botany, and traditional medicine, might collaborate to gain a comprehensive understanding of the extract's properties and potential applications.

References

Amabeoku, G. J., & Kabatende, J. (2012). Antinociceptive and anti-inflammatory activities of leaf methanol extract of cotyledon orbiculata L. (Crassulaceae). *Advances in Pharmacological Sciences, 2012*. https://doi.org/10.1155/2012/862625

Arceusz, A., Radecka, I., & Wesolowski, M. (2010). Identification of diversity in elements content in medicinal plants belonging to different plant families. *Food Chemistry*, *120*(1), 52–58. https://doi.org/10.1016/j.foodchem.2009.09.068

Ashikur Rahman, S. M., Mahfuzur Rahman, M., Aslam Hossain, M., & Rashid, M. A. (2016). Chemical and Biological Investigations of Leaves of Abroma augusta Linn. In *Bangladesh Pharmaceutical Journal* (Vol. 19, Issue 2).

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. In *Biotechnology Advances* (Vol. 33, Issue 8, pp. 1582–1614). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2015.08.001

Chunxiao, W., Jingjing, L., Yire, X., Jingning, L., Kai, K., Liang, S., Yi, L., & Rasco, B. (2011). Biosynthesis of a novel recombinant peptide derived from hPTH(1-34). *Protein Expression and Purification*, *79*(1), 156–163. https://doi.org/10.1016/j.pep.2011.04.017

Currie, S. R., & Wang, J. L. (2004). Chronic back pain and major depression in the general Canadian population. *Pain*, *107*(1–2), 54–60. https://doi.org/10.1016/j.pain.2003.09.015

Dey, P., Chandra, S., Chatterjee, P., & Bhattacharya, S. (2011). Neuropharmacological properties of Mikania scandens (L.) Willd. (Asteraceae). *Journal of Advanced*

Pharmaceutical Technology and Research, *2*(4), 255–259. https://doi.org/10.4103/2231-4040.90883

Emran, T. Bin, Rahman, M. A., Uddin, M. M. N., Rahman, M. M., Uddin, M. Z., Dash, R., & Layzu, C. (2015). Effects of organic extracts and their different fractions of five Bangladeshi plants on in vitro thrombolysis. *BMC Complementary and Alternative Medicine*, *15*(1). https://doi.org/10.1186/s12906-015-0643-2

Ene, K. W., Nordberg, G., Bergh, I., Johansson, F. G., & Sjöström, B. (2008). Postoperative pain management - The influence of surgical ward nurses. *Journal of Clinical Nursing*, *17*(15), 2042–2050. https://doi.org/10.1111/j.1365-2702.2008.02278.x

Gawade, S. P. (2012). Acetic acid induced painful endogenous infliction in writhing test on mice. In *Journal of Pharmacology and Pharmacotherapeutics* (Vol. 3, Issue 4, p. 348). https://doi.org/10.4103/0976-500X.103699

Gertsch, J. (2009). How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. In *Journal of Ethnopharmacology* (Vol. 122, Issue 2, pp. 177–183). https://doi.org/10.1016/j.jep.2009.01.010

Ghannadi, A., Hajhashemi, V., & Jafarabadi, H. (2005). An Investigation of the Analgesic and Anti-Inflammatory Effects of Nigella sativa Seed Polyphenols. In *JOURNAL OF MEDICINAL FOOD J Med Food* (Vol. 8, Issue 4).

Hamilton, L. S., & Murphy, D. H. (n.d.). *Use and Management of Nipa Palm (Nypa fruticans, Arecaceae): a Review I*.

Harsoor, S. S. (2011). Emerging concepts in post-operative pain management. In *Indian Journal of Anaesthesia* (Vol. 55, Issue 2, pp. 101–103). Indian Society of Anaesthetists. https://doi.org/10.4103/0019-5049.79872

Hepsomali, P., Groeger, J. A., Nishihira, J., & Scholey, A. (2020). Effects of Oral Gamma-Aminobutyric Acid (GABA) Administration on Stress and Sleep in Humans: A Systematic Review. In *Frontiers in Neuroscience* (Vol. 14). Frontiers Media S.A. https://doi.org/10.3389/fnins.2020.00923

Hijazi, M. A., El-Mallah, A., Aboul-Ela, M., & Ellakany, A. (2017). Evaluation of Analgesic Activity of Papaver libanoticum Extract in Mice: Involvement of Opioids Receptors. *Evidence-Based Complementary and Alternative Medicine*, *2017*. https://doi.org/10.1155/2017/8935085

Jones, W. P., Chin, Y.-W., & Douglas Kinghorn, A. (2006). The Role of Pharmacognosy in Modern Medicine and Pharmacy. In *Current Drug Targets* (Vol. 7).

Joppa, L. N., Roberts, D. L., Myers, N., & Pimm, S. L. (2011). Biodiversity hotspots House most undiscovered plant species. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(32), 13171–13176. https://doi.org/10.1073/pnas.1109389108

Kumar, K., Sharma, S., Kumar, P., & Deshmukh, R. (2013). Therapeutic potential of GABAB receptor ligands in drug addiction, anxiety, depression and other CNS disorders. In *Pharmacology Biochemistry and Behavior* (Vol. 110, pp. 174–184). Elsevier Inc. https://doi.org/10.1016/j.pbb.2013.07.003

Kunle. (2012). Standardization of herbal medicines - A review. *International Journal of Biodiversity and Conservation*, *4*(3). https://doi.org/10.5897/ijbc11.163

Kwan, P., & Brodie, M. J. (2001). Neuropsychological effects of epilepsy and antiepileptic drugs. In *Lancet* (Vol. 357, Issue 9251, pp. 216–222). Elsevier B.V. https://doi.org/10.1016/S0140-6736(00)03600-X

Moretti, L. S., Candini, V., Cárdenas, F., Conn, H., Fabbro, F., Muñoz-Navarro, R., & Adrian Medrano, L. (2018). An appraisal of the fit of a cognitive behavioural model of headache in University students. *Journal of Behavior, Health & Social Issues*, *9*(2), 54–61. https://doi.org/10.1016/j.jbhsi.2018.01.003

Padmaja, R., Arun, P. C., Prashanth, D., Deepak, M., Amit, A., & Anjana, M. (2002). Brine shrimp lethality bioassay of selected Indian medicinal plants. In *Fitoterapia* (Vol. 73).

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, † T M, Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., & Sexton, J. O. (n.d.). *The biodiversity of species and their rates of extinction, distribution, and protection Background Rates of Species Extinction*.

Prasad, N., Yang, B., Kong, K. W., Khoo, H. E., Sun, J., Azlan, A., Ismail, A., & Romli, Z. Bin. (2013). Phytochemicals and antioxidant capacity from Nypa fruticans Wurmb. Fruit. *Evidence-Based Complementary and Alternative Medicine*, *2013*. https://doi.org/10.1155/2013/154606

Rahman, M. M., Masum, G. Z. H., Sharkar, P., & Sima, S. N. (2013). Medicinal plant usage by traditional medical practitioners of rural villages in Chuadanga district, Bangladesh. *International Journal of Biodiversity Science, Ecosystem Services and Management*, *9*(4), 330–338. https://doi.org/10.1080/21513732.2013.841757

Rakotonirina, V. S., Ngo Bum, E., Rakotonirina, A., & Bopelet, M. (2001). Sedative properties of the decoction of the rhizome of Cyperus articulatus. In *Fitoterapia* (Vol. 72).

Reeds, K. M. (1976). Renaissance humanism and botany. *Annals of Science*, *33*(6), 519–542. https://doi.org/10.1080/00033797600200481

Richelson, E. (2001). Pharmacology of antidepressants. In *Mayo Clinic Proceedings* (Vol. 76, Issue 5, pp. 511–527). Elsevier Ltd. https://doi.org/10.4065/76.5.511

Salmerón-manzano, E., & Manzano-agugliaro, F. (2020). Worldwide research on low cost technologies through bibliometric analysis. In *Inventions* (Vol. 5, Issue 1). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/inventions5010009

Shojaii, A., & Abdollahi Fard, M. (2012). Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum . *ISRN Pharmaceutics*, *2012*, 1–8. https://doi.org/10.5402/2012/510795

Smith, A. J., Sketris, I., Cooke, C., Gardner, D., Kisely, S., & Tett, S. E. (2008). A comparison of antidepressant use in Nova Scotia, Canada and Australia. *Pharmacoepidemiology and Drug Safety*, *17*(7), 697–706. https://doi.org/10.1002/pds.1541

Sukul, A., Haque, S., Poddar, S. K., Hossain, Md. S., Niloy, K. K., & Saha, S. K. (2017). Comparative physicochemical, anti-inflammatory, and analgesic activity assay of synthesized chromium and nickel complexes of indomethacin. *Cogent Chemistry*, *3*(1), 1302312. https://doi.org/10.1080/23312009.2017.1302312

Tamunaidu, P., Matsui, N., Okimori, Y., & Saka, S. (2013). Nipa (Nypa fruticans) sap as a potential feedstock for ethanol production. *Biomass and Bioenergy*, *52*, 96–102. https://doi.org/10.1016/j.biombioe.2013.03.005

Tamunaidu, P., & Saka, S. (2011). Chemical characterization of various parts of nipa palm (Nypa fruticans). *Industrial Crops and Products*, *34*(3), 1423–1428. https://doi.org/10.1016/j.indcrop.2011.04.020

Tang, S. Y., Hara, S., Melling, L., Goh, K. J., & Hashidoko, Y. (2010). Burkholderia vietnamiensis isolated from root tissues of nipa palm (Nypa fruticans) in Sarawak, Malaysia, proved to be its major endophytic nitrogen-fixing bacterium. *Bioscience, Biotechnology and Biochemistry*, *74*(9), 1972–1975. https://doi.org/10.1271/bbb.100397

Wang, X., Traub, R. J., & Murphy, A. Z. (2006). Persistent pain model reveals sex difference in morphine potency. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *291*(2). https://doi.org/10.1152/ajpregu.00022.2006