Assessment of CNS Depressant Activity of *Nypa fruticans* Wurmb Fruit Pulp

By

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

School of Pharmacy

BRAC University

February 2024

Declaration:

It is hereby declared that

1. The thesis submitted is my own original work while completing my 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 reference.
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 "Assessment of CNS Depressant Activity of *Nypa fruticans* Wurmb Fruit Pulp" submitted by Tasmim Farhana Anika (20146019) of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy in February 2024.

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Ethics Statement

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

Abstract

Nypa fruticans Wurmb (Family: Arecaceae) or Nipa palm is locally known as "Golfol" in Bangladesh which is of great interest now for its multiple uses. The present investigation was carried out to evaluate the CNS depressant activity of methanolic fruit pulp extract of *N. fruticans* (MNF) on *Swiss albino* mice model by following the standard protocol of the hole cross test at three different dosages (200, 400 and 600 mg/kg body weight) against the standard diazepam. Here, MNF extract showed maximum suppression of motor activity (only 3.40 ± 0.49 number of movements) at 600 mg / kg after 120 minutes ($p < 0.05$). Therefore, the current study's findings indicate potent and dose-dependent CNS depressing action of MNF extract which can be used as a new source for the development of novel sedative-hypnotic drug. However, further investigation is required to find out the responsible phytoconstituents for the CNS depressant activity of MNF.

Keywords: *Nypa fruticans* Wurmb; CNS depressant; Diazepam; Hole cross test; Sedative-hypnotic; Phytoconstituents.

Dedication

This work is dedicated to my respected parents and my beloved elder brother for their continuous support and unwavering belief in me.

Acknowledgement:

First and foremost, I want to show my gratitude to Almighty Allah for giving me the strength and ability to overcome all the difficulties and fulfill this project. Without Allah's mercy, it could never be possible to accomplish the purpose of the work.

Firstly, I would like to thank my supervisor Dr. Farhana Alam Ripa (Associate Professor, School of Pharmacy, BRAC University) for her constant guidance, continuous support, words of encouragement and invaluable advice. From the beginning to the end of my project, she instructed me, inspired me, and showed immense patience to complete my work.

I would also want to express my sincere gratitude to the honorable Professor, Dr. Eva Rahman Kabir, (Dean, School of Pharmacy, BRAC University). I would also like to thank Professor, Dr. Hasina Yasmin (Program Director and Assistant Dean, School of Pharmacy, BRAC University) for providing me with huge knowledge to make my journey easy and convenient. I am also grateful to all of the faculty members of the School of Pharmacy for their help and guidance whenever I needed it.

Finally, I want to express my gratitude to all the lab officers and staff members who greatly assisted me throughout the project. They provided constant direction, support, and most importantly, a cooperative attitude.

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List of Acronyms:

MNF	Methanolic fruit pulp Extract of *N. fruticans*
SEM	Standard Error of Mean
WHO	World Health Organization
mg	Milligram
mL	Milliliter
GABA	Gamma-aminobutyric acid
ANOVA	Analysis of Variance
ICDDR,B	International Center for Diarrheal Disease Research, Bangladesh

Chapter 1

Introduction

1.1 General Introduction

From the very beginning of human civilization, people utilized various resources from the environment including food and medicine. Plants play a major role amongst these resources by supplying essential services and maintaining the balance in the ecosystems. The knowledge regarding medicinal plants has been carried out through generations after generations with increasing demand of medicinal plants. This knowledge became more complete and precise with the evolving civilization. Medicinal plants hold vital importance across various cultures in the modern era (Jamshidi-Kia et al., 2017).

The term "medicinal plant" indicates a variety of plants with significant medicinal properties. Before recorded history, early humans discovered the use of plants in every sector of everyday life including in clothing, shelter, food, fuel etc. Even medicinal plants are part of oldest science topics in countries like China, Greece, Egypt, and India. Medicinal plants were commonly used as drugs, disinfectants, and aromatic agents in ancient Persia which indicates the use of these plants for treating diseases since the early stage of human life (Hamilton AC., 2004). Plants have been the first treatment of choice of humans from the beginning of human civilization. In recent times, over 50,000 plant species, which is almost a tenth of all plants, are widely being used in the field of pharmaceutical and cosmetic industry (Halberstein RA., 2005). Medicinal plants for its unavoidable therapeutic benefits can aid the development process of novel drugs. Plant parts like seeds, roots, leaves, skin, flower, fruits, or the entire plant may possess physiological effects on the living organisms. Interaction of these substances may result in both beneficial and harmful consequences.

However, the rising awareness about the toxicity and adverse effects of conventional and allopathic medicines have increased the demand for safe use of herbal medicines which have subsequently decreased the use of chemical drugs (Jack DB., 1997).

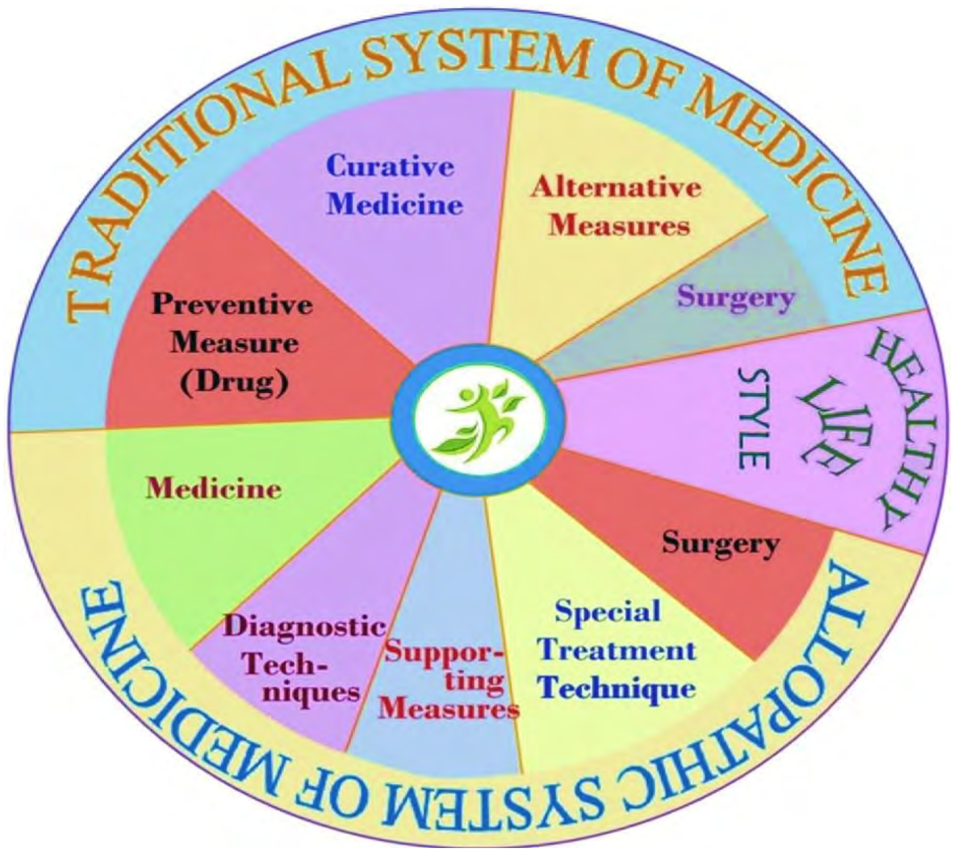

Figure 1: Benefits of traditional medicines over allopathic medicines (Source: Sen and Chakraborti,2017)

Despite the significance of medicinal plants, these are not evenly distributed and used throughout the world. These are being primarily collected from wild sources. In fact, the demand for these wild sources has grown by 8%-15% annually in Europe, North America, and Asia in recent years (Verma et al., 2008).

On the contrary, countries like Malaysia, Korea, France, Chile, Nepal lack in number and percentage of medicinal plant species.

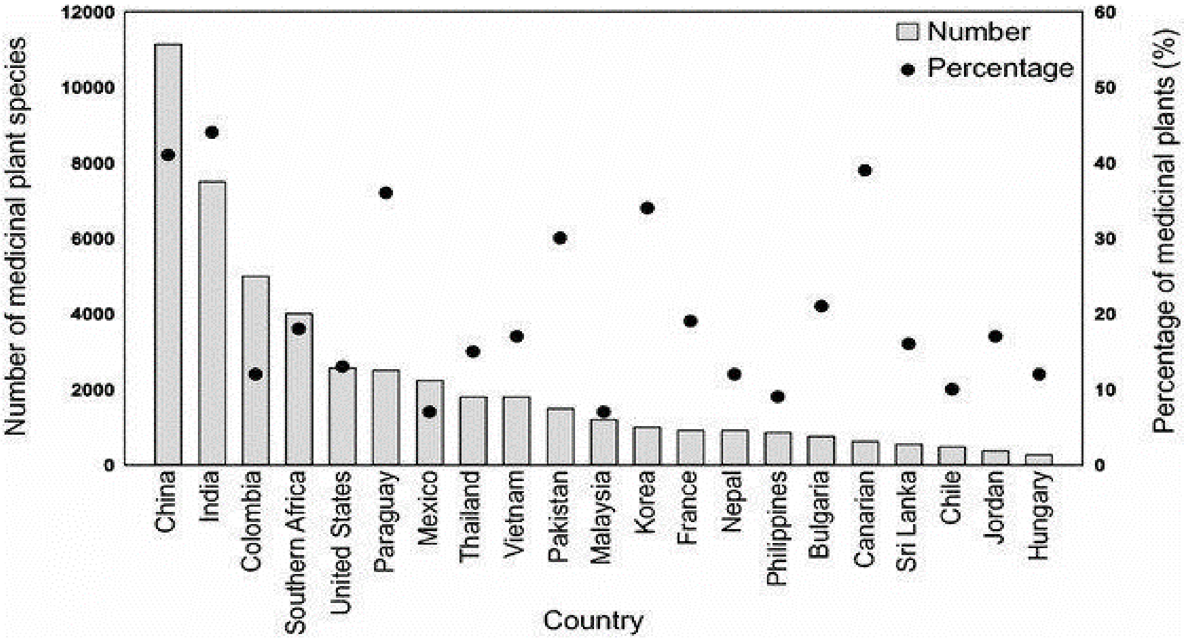

Figure 2: Number and percentage of medicinal plant species in different countries (Chen et al., 2016)

Furthermore, the people of underdeveloped continents like Asia, Africa and Latin America are backward and hence lack inventiveness to produce or use modern drugs by utilizing new technology. So, for these 3rd world countries herbal medicine is the only option to cure illness as well as earn money by importing these (Adodo, 2013). Therefore, this medicine is a call to fight against all obstacles.

In South Asia, Bangladesh is situated on the fertile Bengal delta. Bangladesh has not only rich and fertile soil but also a favorable climate that allows optimal environment for the growth of multiple types of medicinal plants for which these plants possess major significance in the traditional healthcare practices of Bangladesh. These plants have been used in several forms of traditional medicine, including Ayurveda, Unani, and Herbal medicine, as cures for different diseases and ailments. Among thousands of medicinal plants, some common ones include Neem, Turmeric, Aloe Vera, and Tulsi. These medicinal plants are vital for minor health issues as well as for severe diseases such as diabetes, hypertension, and even cancer, which highlight their vast importance in healthcare (Rahman et al., 2022).

Figure 3: Common medicinal plants in South Asia (A. Aloe barbadensis Miller, B. Cordyceps sinensis, C. Vaccinium macrocarpon, D. Trigonella foenum-graecum, E. Glycyrrhiza glabra, F. Syzygium aromaticum, G. Curcuma longa, H. Azadirachta indica, I. Zingiber officinale, J. Illicium verum)

For the future, the field of medicinal herbs is of great importance as around half a million plant species across the globe have not been discovered yet. These unexplored species may possess valuable components which play a key role in novel treatments in the distant future.

As we keep discovering this particular are, we might find that these plants can be helpful in treating many diseases and change the perspective of people regarding healthcare and medicine.

1.1.1 What are Medicinal Plants

A medicinal plant can be defined as any form of plant life which contains substances of therapeutic properties in its various parts or organs which can be used for the development of drugs. These substances can either be directly used for therapeutic purposes or can be used as a base for the synthesis of drugs.

Medicinal plants are different than normal plants in the sense that medicinal plants encompass healing properties from various diseases whereas non-medicinal plants do not possess any medicinal use.

1.1.2 A Historical Overview of the Use of Medicinal Plants

Medicinal plants are the oldest and most widespread form of medication which is proved from various previous evidence. Most medicines were directly derived from plant or animal sources until the last century. Traditional medicines along with synthetic drugs are still the treatment of choice for several diseases.

Other primates could also relate with the curing properties of various plants. Several species of monkeys and apes consume specific botanical species containing various chemical components which act as analgesics, anti-microbials, anti-inflammatories, antiseptics, anti-psychotics, immunostimulants, immunosuppressants, anti-diarrheals, digestive aids, and fertility regulators.

Emperor Sheng Nun (2730 BC – 3000 BC) compiled the first pharmacopoea	The Egyptian Papyrus Elber was written at about 1500 BC described some very important medicinal plants which are still used today

Traditional medicine practitioners worthy of mention

Hippocrates (460 BC); who is known to be the father of modern medicine wrote a book named *Materia Medica* containing over 400 simple remedies	Theophrastus of Athens (370 BC); a biologist and botanist had written the book *Historia Plantarium*

Figure 4: Brief History of Traditional Medicine

A recent review article has revealed that many animals as well as humans choose some of the same plants to treat the same types of diseases, injuries, and health issues (Huffman MA, 1997). Archaeological evidence has also proved that medicinal plants were regularly used by people in ancient times.

1.1.2.1 Written Evidence of Medicinal Plants

Around 5000 years back, the earliest written evidence of medicinal plant for drug preparation was found on a Sumerian clay slab from Nagpur(Qiu J., 2007). Over 27 centuries BC, Egyptians and Chinese first used plants as medicine which was further adopted by Ancient Greeks like Hippocrates and Aristotle. The School of Medicinal Plants was established by Greek scientist, Theophrastus. Mesopotamians and Egyptians also documented plant usage on clay tablets and Eber Papyrus respectively. Later, 600 medicinal plants were described in *De Materia Medica* encyclopedia by Pedanius Dioscorides. Another reference, Pen Ts'ao Ching Classic of Materia Medica, appeared around 200 CE. Thus, ancient herbalists globally recognized and extensively used the medicinal properties of plants, with their knowledge which serve as a basis for conventional medicine (Khan, 2014).

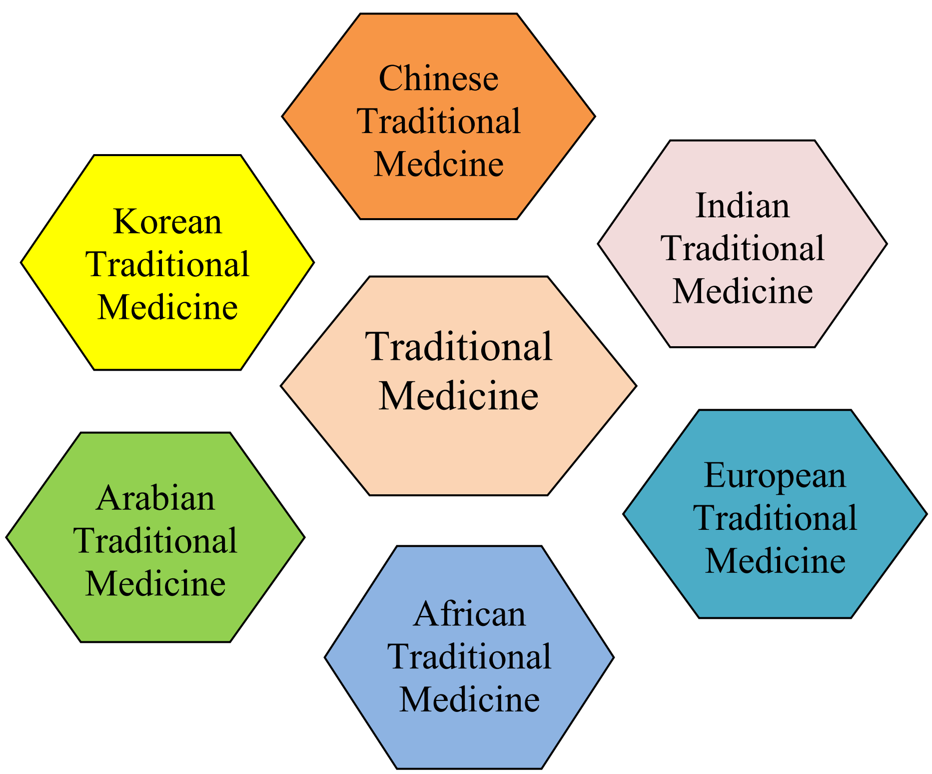

Figure 5: Written evidence of traditional medicine

1.1.2.2 Ancient Egyptian History of Medicinal Plants

The ancient Egyptians were renowned for their skills related to health and medicine. For gastrointestinal disorders they prescribed sedatives, analgesics, and various remedies. For urinary tract and common cold they prescribed medicines (Nunn JF, 1996). They used plants not only internally but also topically and even inhaled some as vapor. The credit for early medicinal use of wine, marijuana, castor oil, opium, mints, and beer go to the Egyptians. They were the first to use such drugs which were later on known as medically significant. Ancient Egyptian wrote the Ebers Papyrus where about 700 medicinalplants were enlisted including garlic, juniper, cannabis, castor bean, aloe vera etc. (Oakes e al., 2003). Furthermore, they invented wine infusion process of herbs for the use of medicinal purpose. These innovations of the Egyptians further influenced the Greek and Roman for discovering traditional medicine.

1.1.2.3 Ancient Greek History of Medicinal Plants

The Greeks significantly contributed to pharmaceutical sciences, especially phytopharmaceuticals. Aristotle and Theophrastus both described 500 crude drugs in their works. Hippocrates, the father of allopathic medicine, believed that many diseases were due to imbalances in normal human physiology, hence, treatment was focused on restoring this balance. He identified almost 400 plant-originated medicinal substances. Claudius Galen Pergamum introduced the concept of pharmaceutical formulation and prepared vegetable drugs using extraction techniques, known as Galenical. He wrote about 300 books on plants (Khan, 2014).

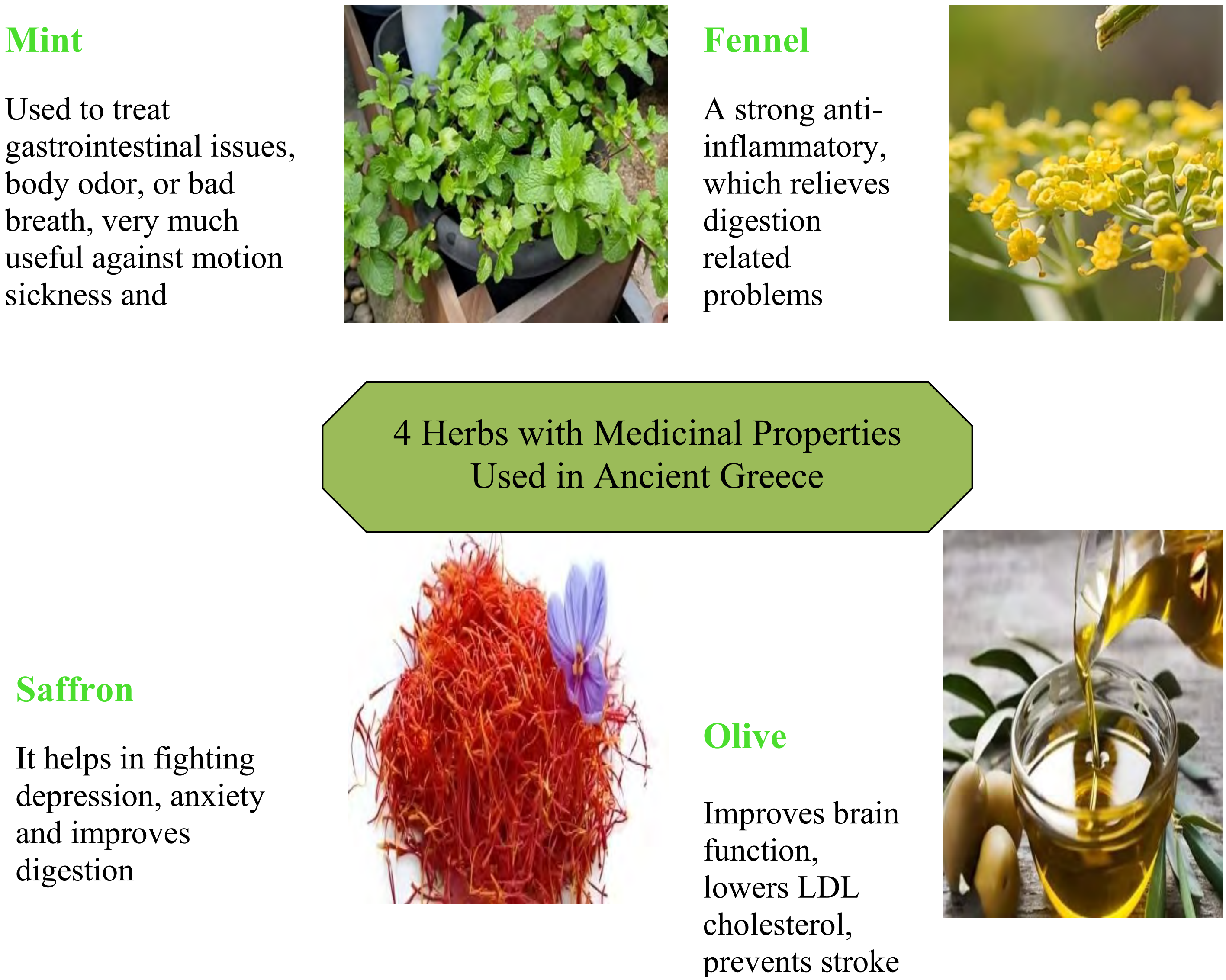

Figure 6: Medicinal Plants used in Ancient Greece

1.1.2.4 Traditional Chinese History of Medicinal Plants

Chinese medicine system is free from any types of external influence because of its unique theories and treatment approaches. Fu His (2953 BC) pioneered traditional Chinese medicine, addressing exogenous factors in pathology. Emperors Shen Nong and Hong Ti developed it further. Chinese pharmacopoeia Pen Tsao offered numerous remedies. Shen Nong Ben Cao Jin (22-250 AD) is a crucial text in this field. Cao Yuan Fang (550-630) wrote Zhu Bing Yuan Ji Lun, a standard medical reference. Wang Tao (702-772) contributed significantly, using tongue diagnosis.

Li Shizen's Ben Ca Gang Mu (1596) is a comprehensive pharmacopoeia with 1894 prescriptions.

Traditional Chinese medicine was generational knowledge, which was formalized into academic training in the 1950s (Khan, 2014).

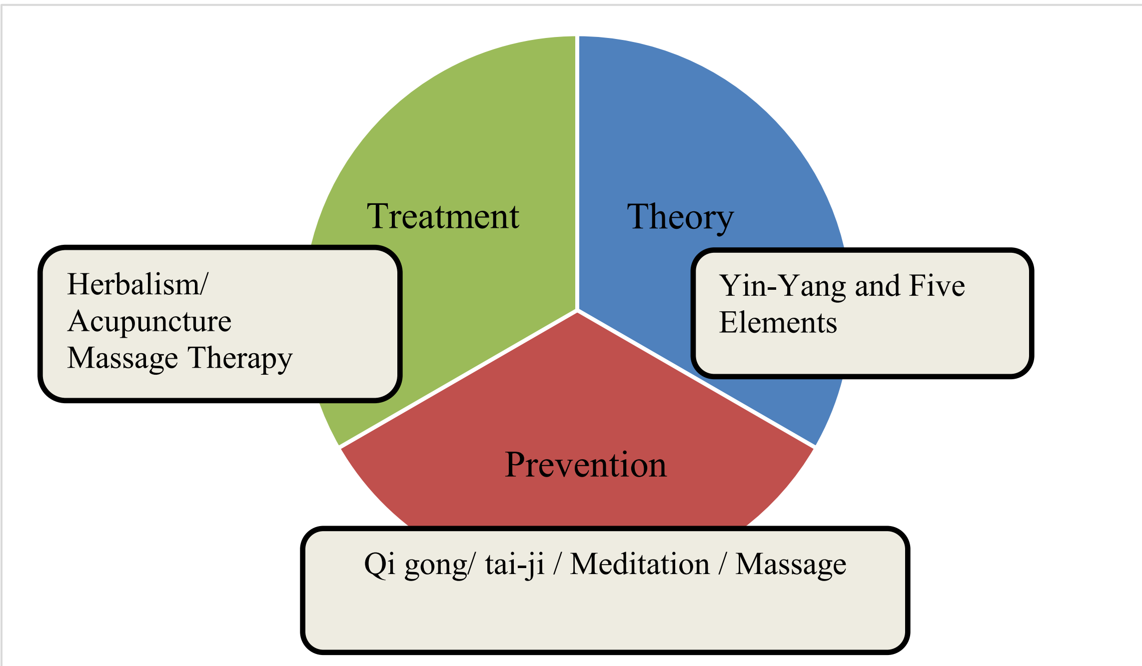

Figure 7: Traditional Chinese Medicine (TCM) System

1.1.2.5 Traditional Indian History of Medicinal Plants

Ayurveda, the oldest health care system, is described in ancient texts like Rig-Veda and Atharva-Veda, circa 5000 BC. The Sanskrit term means 'knowledge of life' and represents a holistic healing system which perceives health as a balance of seven basic tissues, with disease resulting from an imbalance (Khan, 2014).

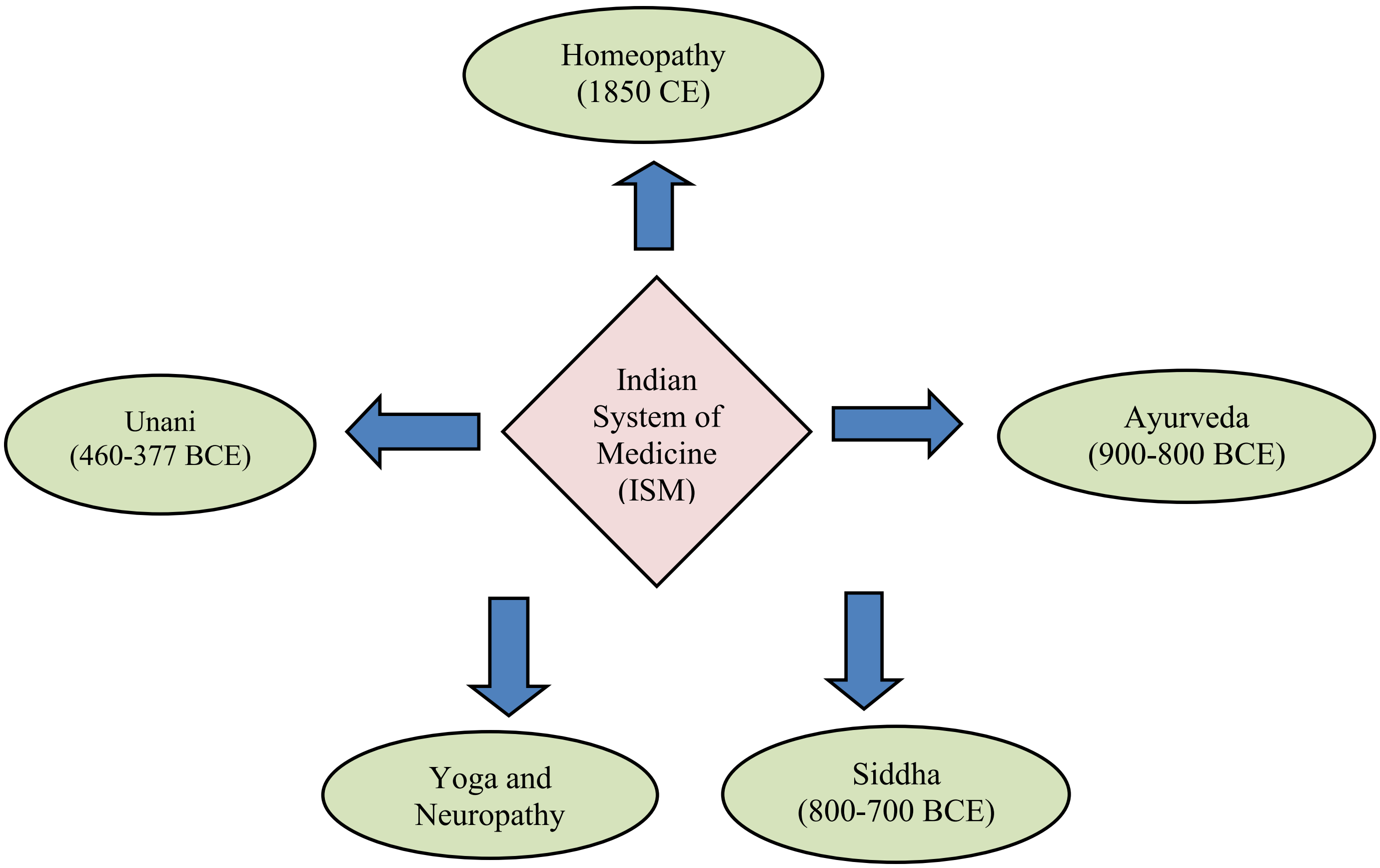

Figure 8: Indian System of Traditional Medicine

1.1.2.6 Golden Age of Medicinal Plants (The Arab Period)

Arabs made significant advancements in science and medicine right after the fall of the Roman Empire. They had introduced diet control, basic pharmacy practices, exercise and translated Greek and Roman Books. They also defined and distinguished the roles of physicians and pharmacists which later on led to the development of each individual field. The contribution of the Muslim chemist Jaber Bin Hayan in isolating vital chemicals was noteworthy. As mentioned in Al Quran and Al Hadis, hygienic lifestyle principles are highly recommended and maintained in Arab system of traditional medicine. Renowned scientists like Ali Ibn Rabban Al Tabri and Abu Ali Al Hussan ibn Sina (Avicenna) authored important works on drugs, poisons, and antidotes, contributing to both healing and understanding of toxic aspects of various plants.

1.1.2.7 Ancient Traditional Medicine Practices in South and East Asia

Southeast Asian region is rich in Traditional Medicine. Since centuries after centuries the countries of the region have been practicing it as traditional medicine was the principal medicine dealing with health and disease. In the following table, popular traditional medicines used in the South and EastAsian regional countries are mentioned:

Country	Name of Traditional Medicine System
Bangladesh	Ayurvedic and Unani
Bhutan	So-wa-rig-pa
India	Ayurvedic, Unani, Siddha, Yoga
Indonesia	JAMU and Acupuncture
Maldives	Dhivelhibeys
Myanmar	Ayurvedic, Chinese

Nepal	Ayurvedic
Sri Lanka	Ayurvedic, Unani, Siddha
Thailand	Thai medicines (Combination of Ayurvedic and Chinese medicine)

Table 1: Traditional Medicine Practices in South and East Asia (Source: cited at Alam, 2007)

1.1.2.8 Historical Background of Traditional Medicine in Bangladesh

The medicines of Bengal, were enriched over generations, was taught in homes and physicians' houses in the 18th century, with Ayurvedic and Unani literature being written. Modern healthcare came with the 1835 establishment of Calcutta Medical College (CMC), as prior schools mainly produced Ayurvedic and Unani practitioners. In 1874, Dacca Medical School was established. Healthcare was primarily curative until the mid-19th century. Despite shunning indigenous medicine, the British recognized its importance, especially for rural healthcare, from 1867. Post 1835, allopathic medicine professionalization and drug standardization began. In the 19th and 20th centuries, some in Bengal furthered indigenous medicine. After independence, the Bangladeshi constitution identified health as a basic need and used traditional medicines hugely for treatment purposes (Rahman and Hossen, 2017). Over the last two decades, medicinal plants and ethnomedicinal studies have been carried out in different parts of Bangladesh, receiving significant attention from the government and pharmaceuticals sector.

1.1.2.9 Plant Based Products in the Modern Era

Over the years, significant advancements have been made in the field of traditional medicine. With different structural parameters, medicinal plants continue to be a valuable source for the development of new drug compounds. With the advancement of modern technology, the development process of plant-derived drugs has become more efficient and easier. Effective use of such technologies has led to the innovation of clinically useful molecules. From 2000 to 2003 timespan, around 15 naturally derived compounds were discovered which are now undergoing either phase III clinical trials or the registration stage of drug development process. Recently it has been established that, 100 times higher hit rate can be obtained from natural products than the synthetic drugs. However, not all the molecules discovered are approved for clinical application because only 1 molecule out of 5000 can successfully progress all stages of drug discovery and development.

1.1.3 Medicinal Plants and Conventional Medicine

Phytotherapy is the study of the use of extracts from natural origin as medicines or health-promoting agents. Currently, clinical, pharmaceutical, and chemical studies of traditional drugs, primarily derived from plants, form the basis of many early drugs. These include Aspirin (from willow bark), Digoxin (from Foxglove), Morphine (from the Opium poppy), Quinine (from Cinchona bark), and Pilocarpine (from Maranham Jaborandi). It is estimated that over 50% of available drugs are somehow derived from medicinal plants (Harvey AI., 2008).

1.1.4 Drug Discovery of Natural Compounds

Traditional Processes of Discovering Natural Drugs

Traditional methods test plant materials for pharmaceutical purposes. If any activity is detected, the extract is fractioned, and the active compound is then isolated and identified later on. This process is known as bioassay-guided fractionation which is guided by different biological tests at each stage. Alternatively, a direct isolation method can be used for the same purpose. This method involves isolation of various natural components to measure their biological activity. However, this process can be slow, inefficient, and does not guarantee the successful isolation of lead compounds from screenings (Sarker SD., 2013).

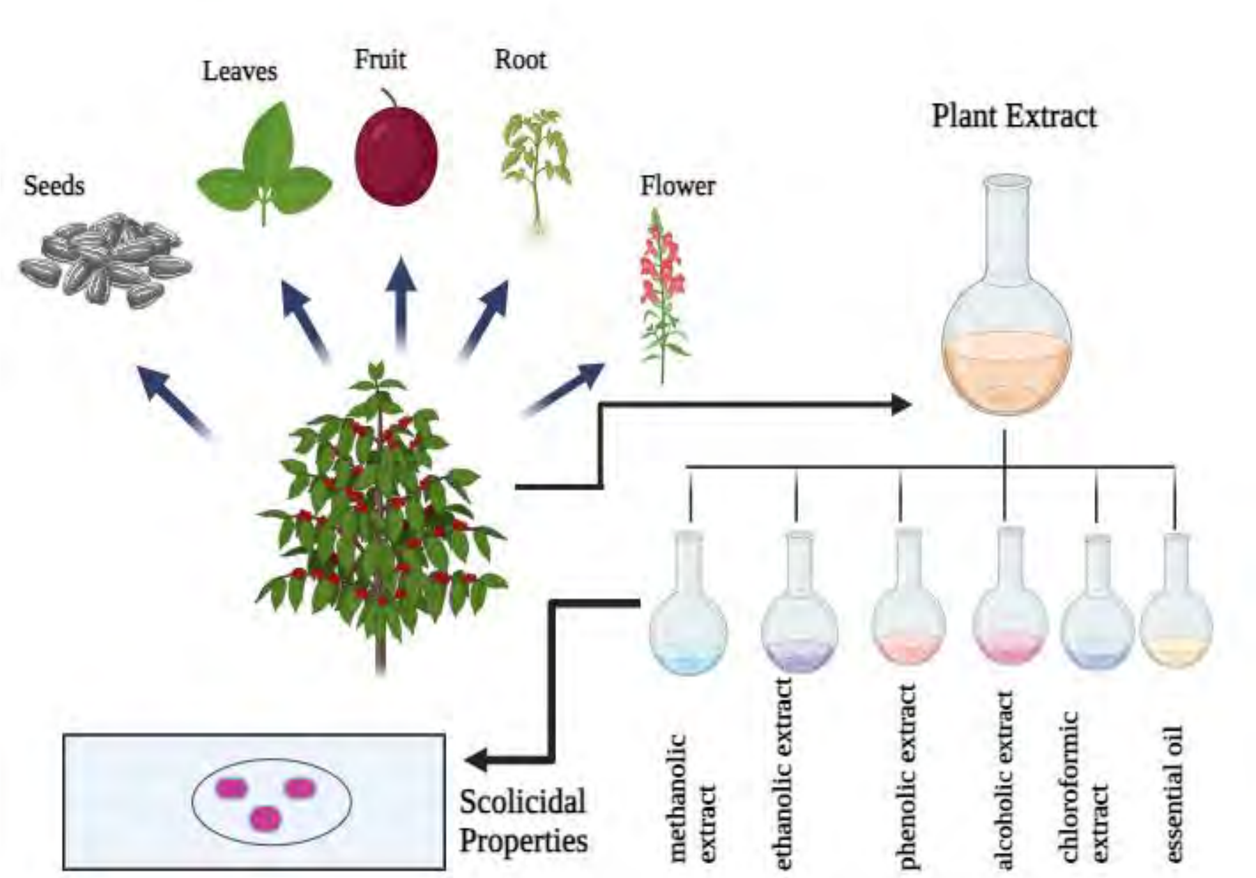

Figure 9: Traditional Process of Discovering Natural Drugs

Modern Ways of Discovering Natural Drugs

Modern methods for discovering natural drugs include high-throughput screening (HTS). To incorporate natural products into HTS programs, a library of natural compounds must be established. Previously, creating such a library was complex, time-consuming, and challenging. However, new, and advanced technologies for the isolation and identification of natural products have significantly improved this process. The most effective results can be achieved with a library of fully identified natural products. This allows scientists to rapidly isolate lead compounds, expediting the progression of novel drug formulation. These advancements can streamline processes such as full or partial synthesis, in vivo experiments, and clinical trials (Sarker SD., 2013).

Figure 10: Modern Process of Discovering Natural Drug

1.1.5 Medicinal Plants widely used in Bangladesh

Bangladesh, a South Asian country where approximately 75% of the population resides in rural areas, has around 80% of its inhabitants depending on plant-based ethnomedicine for primary healthcare needs. These include treatment for fever, coughs, colds, diarrhea, and dysentery. The country is estimated to have about 6500 plant species, with over 500 possessing medicinal value. About 250 of these are regularly used for healthcare purposes. According to Bangladesh's Directorate General of Drug Administration, there are 528 plant-based medicine manufacturers (Unani 272, Ayurvedic 201, and herbal 55), with a total of 11,290 registered medicines (Unani 6630, Ayurvedic 4110, and herbal 550).

There are no restrictions on the sale of medicinal plant products. However, the quality of these medicines often falls short due to multiple obstacles and alack of stakeholder goodwill (Rahman et al., 2022).

Bangladesh, with its diverse flora, is home to many medicinal plants that are widely used in traditional medicine. Some of the most common ones include:

- **Neem *(Azadirachta indica)***: It is known for its anti-bacterial and anti-fungal properties, hence used for the treatment of various skin conditions.
- **Turmeric *(Curcuma longa)***: It is a primary active component, curcumin, has anti-inflammatory and antioxidant benefits.
- **Garlic *(Allium sativum)***: It is used for heart and blood system-related conditions.
- **Aloe Vera**: It is used for skin conditions and also has benefits when ingested.
- **Ginger *(Zingiber officinale)***: It is often used in the treatment of various types of stomach problems.
- **Tulsi *(Ocimum tenuiflorum)***: Known as holy basil, which is used to treat many conditions, including the common cold, headache, stomach disorder, inflammation, heart disease, and malaria.

1.1.6 Important Chemical Constituents of Medicinal Plants

Due to the presence of various chemicals, plants exhibit medicinal effects. Primary metabolites play indispensable roles in plant nutrition and reproduction. On the other hand, secondary metabolites protect them against bacteria, fungi, and animals. These metabolites play a significant role in treating diseases. The key chemicals that contribute to the value of a plant include tannins, glycosides, alkaloids, volatile oil, gum resins, vitamins, and minerals.

Chemical Constituents	Therapeutic Effect
Alkaloids	Anesthetics, Cardioprotective and Anti-inflammatory agent
Glycosides	Analgesic, Antibacterial, Antifungal, Antiviral, Cardiotonic, Anticancer effects
Tannins	To treat gastro-intestinal problems as well as tonsilitis, pharyngitis, hemorrhoids
Volatile Oil	Antiseptic, emollient, antiemetic, antiparasitic
Gum-Resins	Anti-inflammatory

Table 2: Valuable plant constituents with their therapeutic effects

Drug name	Source	Indication
Aspirin	*Salix spp*	Analgesic, anti-pyretic, cardiovascular drug
Atropine	*Atropa belladonna*	Anti cholinergic, pupil dilation
Morphine	*Opium poppy*	Analgesic

Digoxin	*Digitalis lanata*	Cardio tonic

Table 3: Some very common medicaments derived from plants (Source: Rahman et al., 2022)

1.1.7 CNS Depressant Activity of plants

Central Nervous System (CNS) depressants are substances that can slow down brain activity, making them useful for treating anxiety and sleep disorders. One interesting area of research is the CNS depressant activities of various plant extracts. Drugs that lead to CNS depression mostly work on GABA, serotonin, dopamine, adrenergic receptors. Most commonly used CNS depressive drugs are Barbiturates, Benzodiazepine, Opioids, etc.

Plants, often used in traditional medicine, are now being studied scientifically for their potential benefits. They can contain a wide variety of compounds, including alkaloids, flavonoids, and terpenes, that may interact with the nervous system to induce relaxation, sedation, or hypnosis. Plant extracts with potential CNS depressant activity can be found all over the world. For example, valerian root and lavender are used in Europe for their calming effects, while kava and passionflower are used in the Pacific Islands and Americas, respectively (Anucha, 2023). In addition to their potential therapeutic uses, these plant extracts are also of interest for their safety profile. Many of them have been used safely for centuries in traditional medicine, suggesting that they might be a natural, safe alternative to synthetic drugs. However, more research is needed to carefully and significantly understand the mechanisms behind their action, efficacy, and safety.

Plant species	Study Evidence	Used for	Parts Used
Achyranthes aspera L.	Attenuate epilepsy, anticonvulsant, Increase cognitive function	Epilepsy	Whole plant
Amaranthus viridis L.	No recorded experiment	Epilepsy	Leaves
Enhydra fluctuans Lour	CNS depressant	Nervous system disorder	Whole plant
Phoenix slvestris (L.)	CNS depressant	Nervous debility	Roots

Table 4: Medicinal Plants Exerting CNS Depression (Source: Rahman et al, 2022)

1.1.8 Herbal Medicines Against CNS Disorders from Bangladesh

In Bangladesh, the Fabaceae family contributes the highest number of species used to treat various Central Nervous System (CNS) disorders, followed by the Rubiaceae, Lamiaceae, Cucurbitaceae, Vitaceae, Euphorbiaceae, Malvaceae, and Zingiberaceae families. The most frequently used species, in decreasing order, are *Asparagus racemosus*, *Centella asiatica*, *Stephania japonica*, *Aegle marmelos*, *Coccinia grandis*, *Tabernaemontana divaricata*, *Bacopa monnieri*, *Abroma augusta*, and *Scoparia dulcis* (Uddin and Zidorn, 2020).

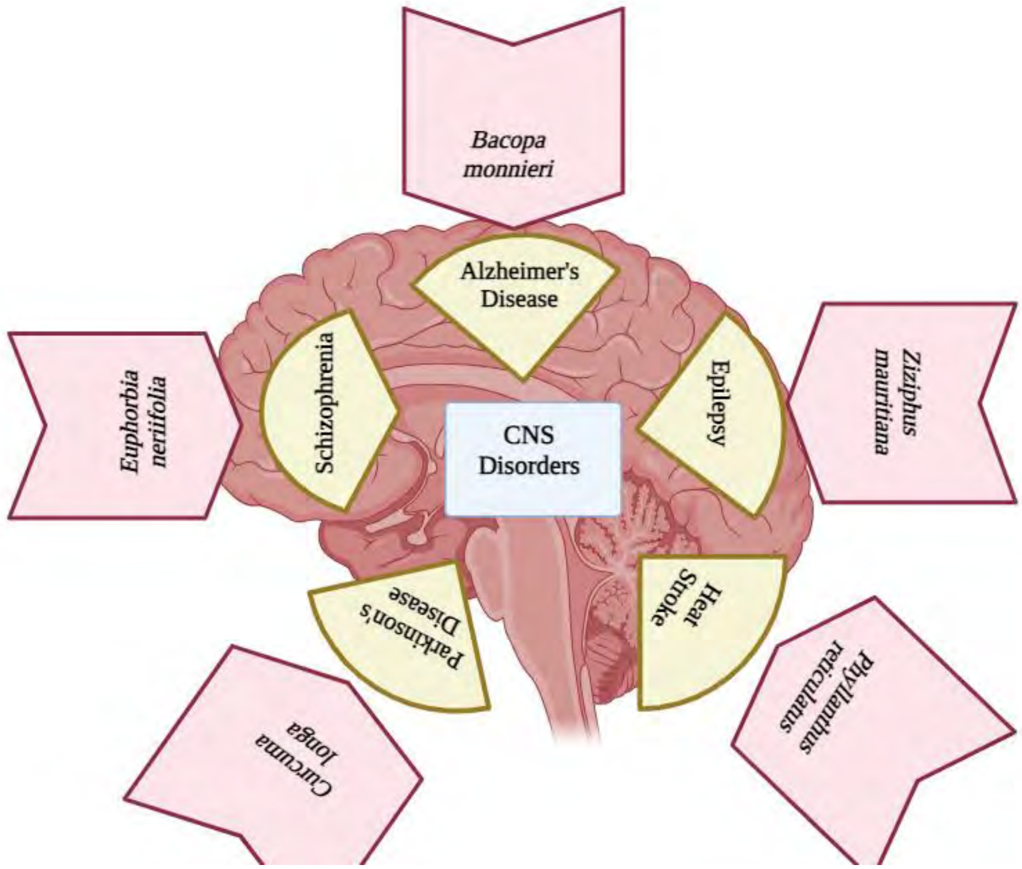

Figure 11: Plants in Bangladesh to treat several CNS Disorders

1.1.9 Mechanism of Action of CNS Depressants

Most of the CNS depressant drugs work on the gamma-aminobutyric acid (GABA), dopamine, serotonin, opioid, cannabinoid, and adrenergic receptors. For example, drugs working on GABA receptor are barbiturates, benzodiazepines, propofol. All of these drugs are allosteric agonists for GABA receptor thus initiating the inhibitory action. Drugs that target cannabinoid receptor are dronabinol, tetrahydrocannabinol. These are also agonists for cannabinoid receptor. The drug that has been working as an agonist on adrenergic receptor is dexmedetomidine.

Aripiprazole and clozapine are serotonin antagonists that inhibit the 5-HT2A receptor to provide the CNS depressant effect. Drugs working on the opioid receptor are morphine, fentanyl etc. Haloperidol, chlorpromazine, antagonizes D2 receptor to reduce the excitation of the brain (L. Eaddy, 2013).

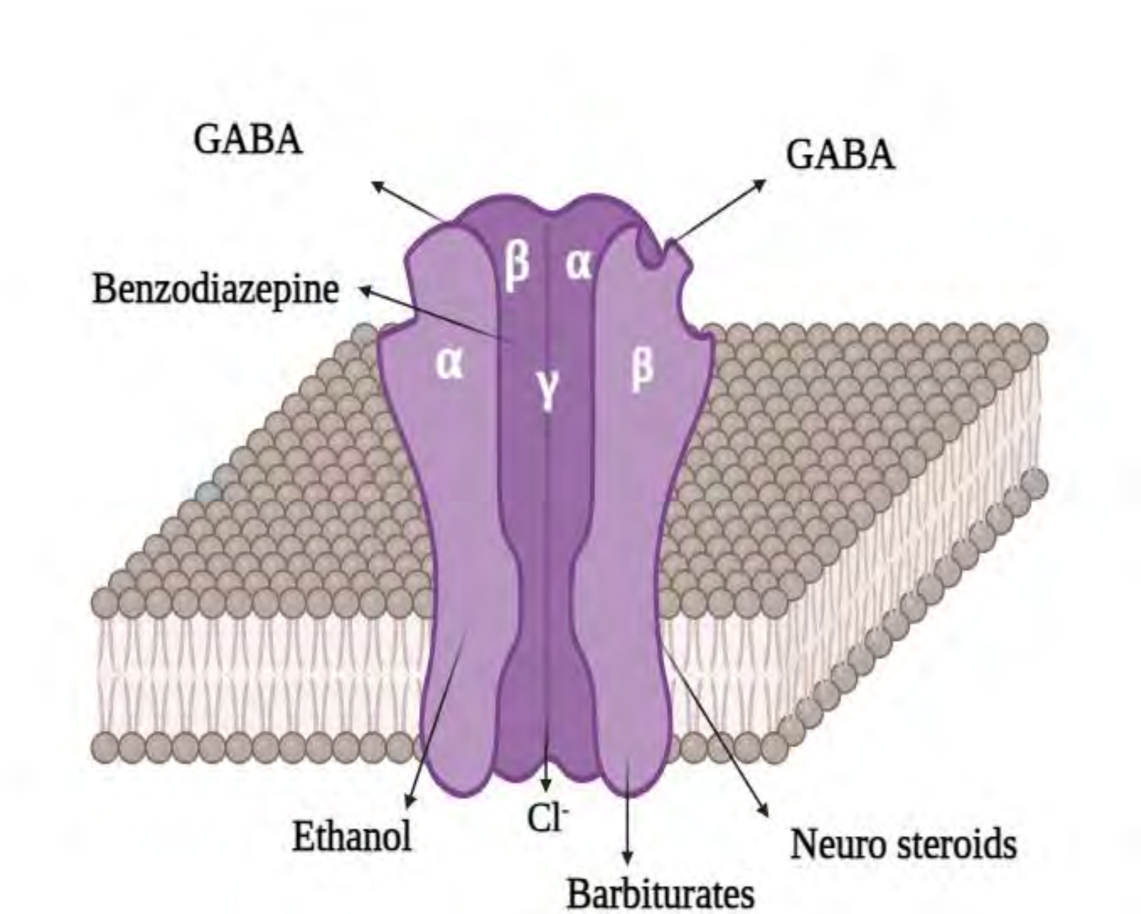

Figure 12: Mechanism of Action of CNS Depressant Drugs

1.2 Review of *N. fruticans* Wurmb

N. fruticans Wurmb, belongs to the Arecaceae (or Palmae) family. This mangrove species is distributed throughout Asia, Oceania, and the east coast of Africa. It is a trunkless palm with tall, erect leaves ranging from 3-9m long, and has a short, horizontal underground stem called a rhizome, accompanied by a dense root system. Nipa naturally grows along the banks of rivers and streams within mangroves and requires regular inundation. It can regenerate naturally or be artificially propagated by planting seedlings along the muddy banks of mangrove forests or on exposed shorelines. Seedlings are typically raised on nursery beds regularly inundated by tidal water. Once they reach about 25cm in height, usually after two months, they are suitable for planting. Harvesting of Nipa leaves generally begins when the plant is five years old and is done annually. The harvesting period is typically during the dry months, from October to February, with all leaves harvested except the unopened leaf and the leaf next to it (M. Khairul Alam, 2021).

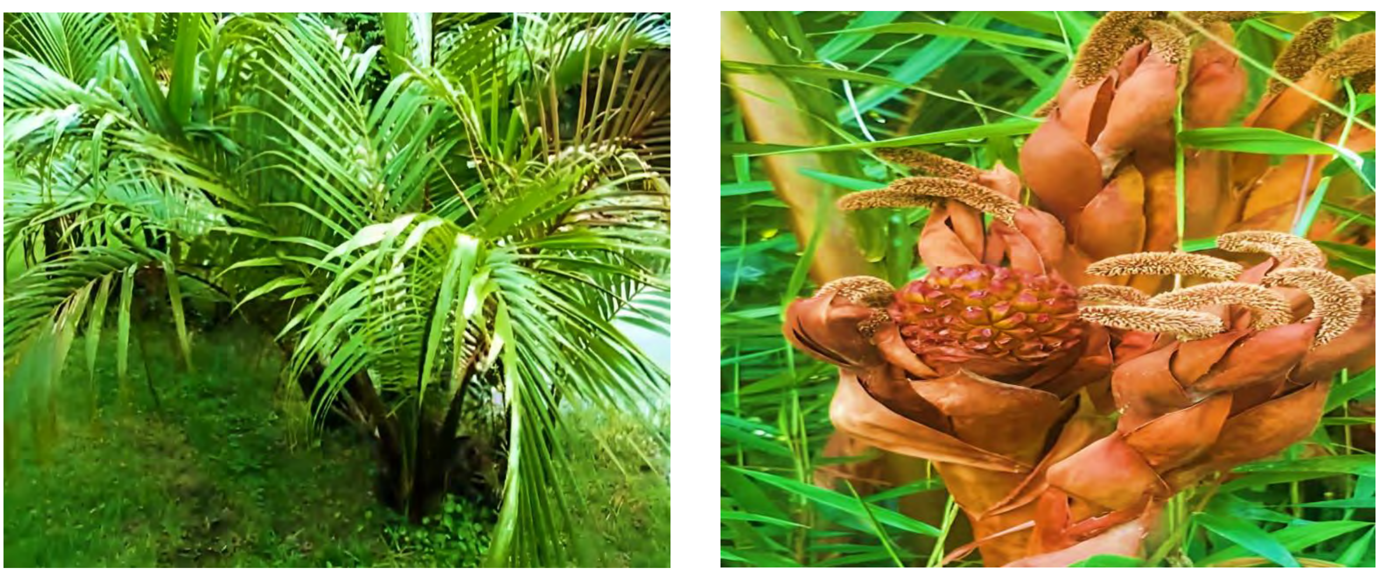

Figure 13: N. fruticans Wurmb plant

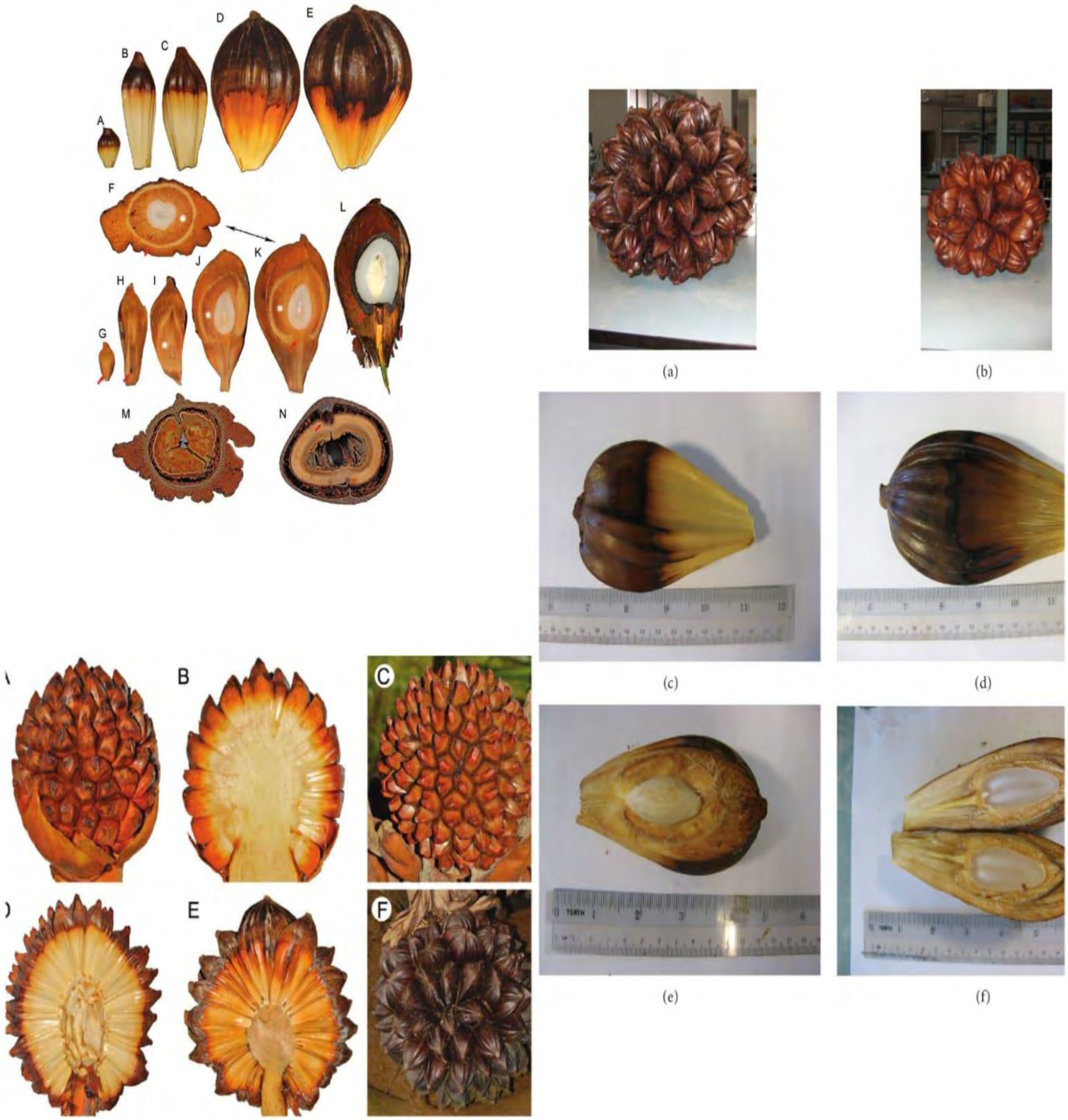

Figure 14 : Morphology of N. fruticans Wurmb fruit (Source: Bobrov et al., 2012)

1.2.1 Taxonomical Classification of *N. fruticans* Wurmb

Kingdom: Plantae

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: Lilianae

Order: Arecales

Family: Arecaceae

Genus: *Nypa*

Species: *N. fruticans* Wurmb (Vinit K et al., 2018)

1.2.2 Synonyms and Different names of *N. fruticans* Wurmb

Synonyms for *N. fruticans* Wurmb are *Cocos nypa* Lour, *Nipa arborescens* Wurmb, *Nipa fruticans* Thunb, *Nipa litoralis* Blanco. *N. fruticans* Wurmb are commonly known in different names in different countriesor regions such as:

Bengali names: Gol pata, gol pati, om gach

English names: Nipa, Nipa palm, Mangrove Palm, Nipah Palm

Indian names: Gulga, Neerthengu, Nipamu

Chinese names: Shui ye

Indonesian names: Buah atap, buyuk

Myanmar name: dane (Pucccio, n.d.)

1.2.3 Description of *N. fruticans* Wurmb

Nipa palms have roots, stems, leaves, flowers, fruits, and seeds, each possessing different characteristics and therapeutic significance. The stems of the plant usually grow underwater, with only leaves and flower stalks above the surface which gives the plant a trunkless appearance. Different parts of the plants are described below:

Roots: Since Nipa roots are only found in unstable soil, sand, or mud, the clumps of the plant can be easily washed out to sea. The fibrous roots of the Nipa plant can reach approximately 13 meters in length.

Trunks: The trunk of Nipa palm is a 60 cm thick rhizome which spreads along the ground, submerged in mud and water, reaching half a meter in length like the *Metroxylon spp.* tree.

Leaves: The compound pinnate leaves emerge from the rhizome, standing height of 7-10 meter. As the Nipa plant ages, the sturdy petiole turns brown. Like coconut leaves, the ribbon-shaped leaflets taper at the end and can reach 60-130 cm long and 5-8 cm wide. The young leaves are yellow in color whereas the old ones are green, each stalk containing 25-100 strands.

Inflorescences: Each strand contains 4-5 male flowers and 2-3 female flowers, each of 5cm length. Female flowers of a 25-30 cm diameter ball at the end, while male flowers are panicles, strand-like, are yellowish red with pollen protruding from the sheath. These flowers can be tapped for juice, with optimal sap production for four to five months after their emergence.

Fruits: The fruit is more similar to a coconut with a smooth exterior, fibrous layer, and a hard shell. This fruit is like a oval stone in shape and reddish-brown in color. It grows in tight clusters of 30-50. Upon ripening, it falls into water, floats until it finds a new place for growing (Nugroho et al., 2020).

Figure 15 : Some parts of N. fruticans Wurmb (A. flower a) female, b) male, B. Fruit, C. Tree with roots, rhizomes andleaves)(source: Nugroho et al., 2020)

Pulp: *N. fruticans* Wurmb has the potential to be made into pulp which is located inside the center of the fruit which is surrounded by a hard shell. The pulp contains cellulose which is of great significance. Although the extraction of the pulp is somewhat difficult, it is used for multiple purposes including culinary purposes and for nutrition as well.

Figure 16: Pulp of the N. fruticans Wurmb fruit

1.2.4 Habitat and Distribution of *N. fruticans* Wurmb

Nipa is a mangrove plant that thrives in temperatures of 20-35°C and with an evenly distributed rainfall of 1,000 mm/month. It is the only palm from the Arecaceae family that can adapt and survive in the mangrove ecosystem. Originally native to China (Hainan), South Asia (Sri Lanka and Bangladesh), Southeast Asia (Indonesia, Malaysia, Thailand, Myanmar, Cambodia, Singapore, Vietnam, and the Philippines), Australia (Queensland and Northern Territory), and Pacific Islands (Solomon, Mariana, Bismarck Islands, New Guinea, and the Caroline Islands), it has now been introduced to other regions such as Cameroon, Guyana, Marianas, Nigeria, Panamá, Society Islands, and Trinidad-Tobago (Rajaprabu NA, 2023).

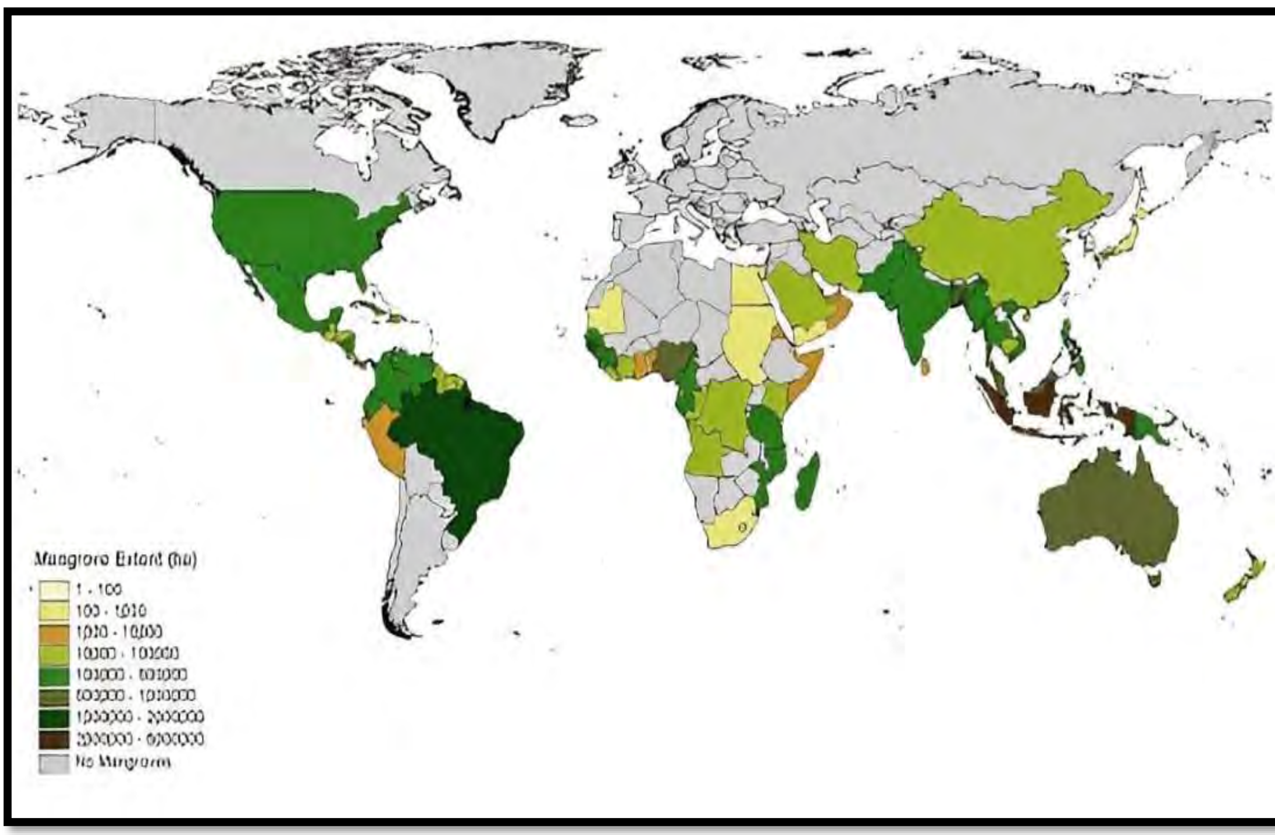

Figure 17: Distribution of N. fruticans Wurmb in the world (Source: Rajaprabu NA, 2023)

1.2.5 Phytochemical Composition of *N. fruticans* Wurmb fruit

Phytochemical analysis is a method used for identifying both primary and secondary metabolites in plants. Compounds that support the plant's growth, development, and survival under changing climate environment are referred as the primary metabolites. While on the contrary, compounds which protect plants from local disturbances like pathogens are called secondary metabolites which are often referred to as bioactive substances (Yahaya et al. 2021).

Name of the compounds	Percentage	Importance
Water	13.57 %	Heat Conductivity
Ash	1.04 %	Affects the nutritional value of food and aids in whitening teeth
Protein	7.04 %	For muscle development and growth of human beings
Carbohydrates	61.53 %	Produces energy
fat	0.06 %	Low level of cholesterol
Energy level	106 Kcal	Source of energy
Maleic acid, cinnamic acid, chlorogenic acid, and kaempferol	1.8-2.1 %	Antioxidant properties
Phenolic contents	7.02%	Antioxidant activity
Flavonoid contents	6.89 %	Antioxidant activity
Polyphenols	0.2 %	Natural Antioxidants

Table 5: Phytochemicals found in N. fruticans Wurmb fruit

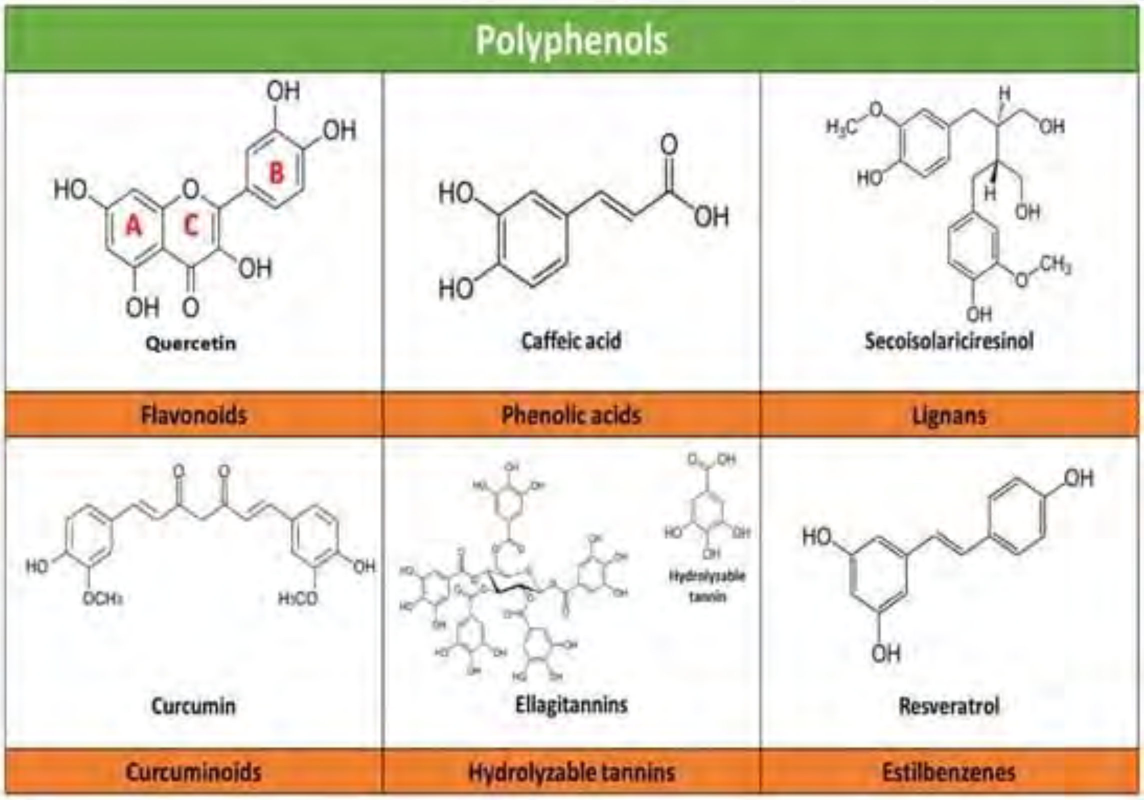

Figure 18 : Structures of phytochemicals found in N. fruticans Wurmb fruit *(Source: Arias-Sanchez et al., 2023)*

1.2.6 Medicinal Uses of *N. fruticans* Wurmb

Nipa exhibits a range of therapeutic applications including antioxidant, anti-inflammatory, antibiotics, antidiabetic, and analgesic effects (Nugroho et al., 2020). Traditional medicines that are prepared from this plant contain no side effects, toxicity and provide enhanced efficacy and safety.

Different Parts	Medicinal Use
Nipa Palm Vinegar (NPV)	Antidiabetic
Nipa Palm Sap (NPS)	Treat gout, fever, kidney stones, and metabolic disorders
Ashes from burnt nipa	Treat headache and toothache
Fresh Leaves	Treat ulcer, thrush

Table 6: Medicinal uses of N. fruticans Wurmb plant

1.2.7 Plant part used for the study

N. fruticans Wurmb fruit pulp has been used for evaluating the CNS depressant effect of the plant.

1.3 Objective of the study

The objective of the study is to understand the potent CNS depressing effects of the *N. fruticans* Wurmb fruit pulp. This study is performed in order to determine whether the pulp of this particular fruit can be used as an alternative source for the treatment of CNS associated conditions such as anxiety and insomnia. Furthermore, the research also focuses on the establishment of the safety and efficacy of this treatment approach.

Moreover, this study also leads to further investigation for figuring out the possible mechanisms of action responsible for CNS depression by *N. fruticans* Wurmb fruit pulp because by understanding how the pulp interacts with various receptors, it can be used as a source for the development of novel sedative and hypnotics. Other objectives include identifying the phytochemicals in the fruit pulp that may be responsible for its CNS depressant effect and also identifying any possible side effects related to the use of the plant. This comprehensive study is a scientific basis for the use of *N. fruticans* Wurmb fruit pulp in the field of mental health treatment and neurology.

1.4 Literature review

A number of studies were conducted on different pharmacological effect of different parts of the mangrove species named *N. fruticans* Wurmb which are mentioned below:

- **Bunyapraphatsara et al., 2003:** Described about the strong antilipid peroxidation effect of the inflorescences of *N. fruticans* Wurmb .
- **Zumbroich 2009 :** Ashes of the shell of *N. fruticans* Wurmb fruit were used for treatingblackening of teeth.
- **Kang et al., 2020 :** Described about the neuroprotective effect of *N. fruticans* Wurmb by

inactivating TRPV1 using nerve crush injury in rat.

- **Kang and Yae- Hyun 2020:** Described about the antinociceptive and anti-inflammatory effects of *N. fruticans* Wurmb by the inactivation of TRPV1.
- **Park et al., 2022 :** Evaluated whether the ethyl acetate fraction from *N. fruticans* Wurmb has a modulatory role in the MAPK signaling pathway and inhibition of the IκB/NF-κB signalingpathways which might exhibit anti-inflammatory effect.
- **Fitri et al., 2023:** Described about the phenolic and flavonoid contents from *N. fruticans* Wurmb fruit extracts exert antihyperglycemic effect.
- **Chatatikun et al., 2023 :** Talked about the antioxidant and ant tyrosinase activity exerted by nipa palm vinegar.
- **Mitra et al., 2024:** Analyzes whether the phytochemicals found in nipa palm can be used in cancer research.

1.5 Research scope for the study

Since there has been no study on the CNS depressant effect exerted by the *N. fruticans* Wurmb fruit pulp, the research scope for this study requires a detailed analysis. This experiment will provide a comprehensive examination of the phytochemical composition, and the mechanism of actions of the fruit pulp that are responsible behind its CNS depressive action. This research will also involve conducting a comparative analysis with other potent known CNS depressants. The main moto is to provide a thorough knowledge about the potential therapeutic use of the *N. fruticans* Wurmb fruit pulp in order to treat conditions related to abnormalities of the central nervous system.

Chapter 2

Materials and Methods

2.1 Identification and Collection of plant

The plant sample under study is *N. fruticans* Wurmb, collected from the mangrove forest of Sundarbans inthe Bagerhat district, Bangladesh. It was authenticated by a taxonomist from the Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession number: 42,861).

2.2 Preparation of plant extract

The initial step in the process involved thoroughly washing the entire plant with water. This was done in order to remove any impurities. The specific part of the plant that was used for the study was the *N. fruticans* Wurmb fruit pulp. This pulp was carefully isolated from the Nipa palm fruit, ensuring its integrity.

Once the pulp was isolated, it was finely chopped into small pieces. Then homogenous slurry of the pulp extract was prepared, and it was then filled into a jar. Following that, 2 liters of 95% methanol was added. This added methanol served as the mother solvent for assuring that the solution was stable and ready for further study. The solution was left in this condition at 25ºC for three days. During this time, it was occasionally stirred and shaken to ensure that all the components of the solution were properly mixed. The solution was then filtered for three times through a Whatman No. 1 filter paper and a sterilized cotton bed. This rigorous filtration process ensured that only the purest form of the solution remains. Following the filtration, the crude extract was obtained, ready for further analysis and study and labeled asmethanolic extract of *N. fruticans* Wurmb (MNF).

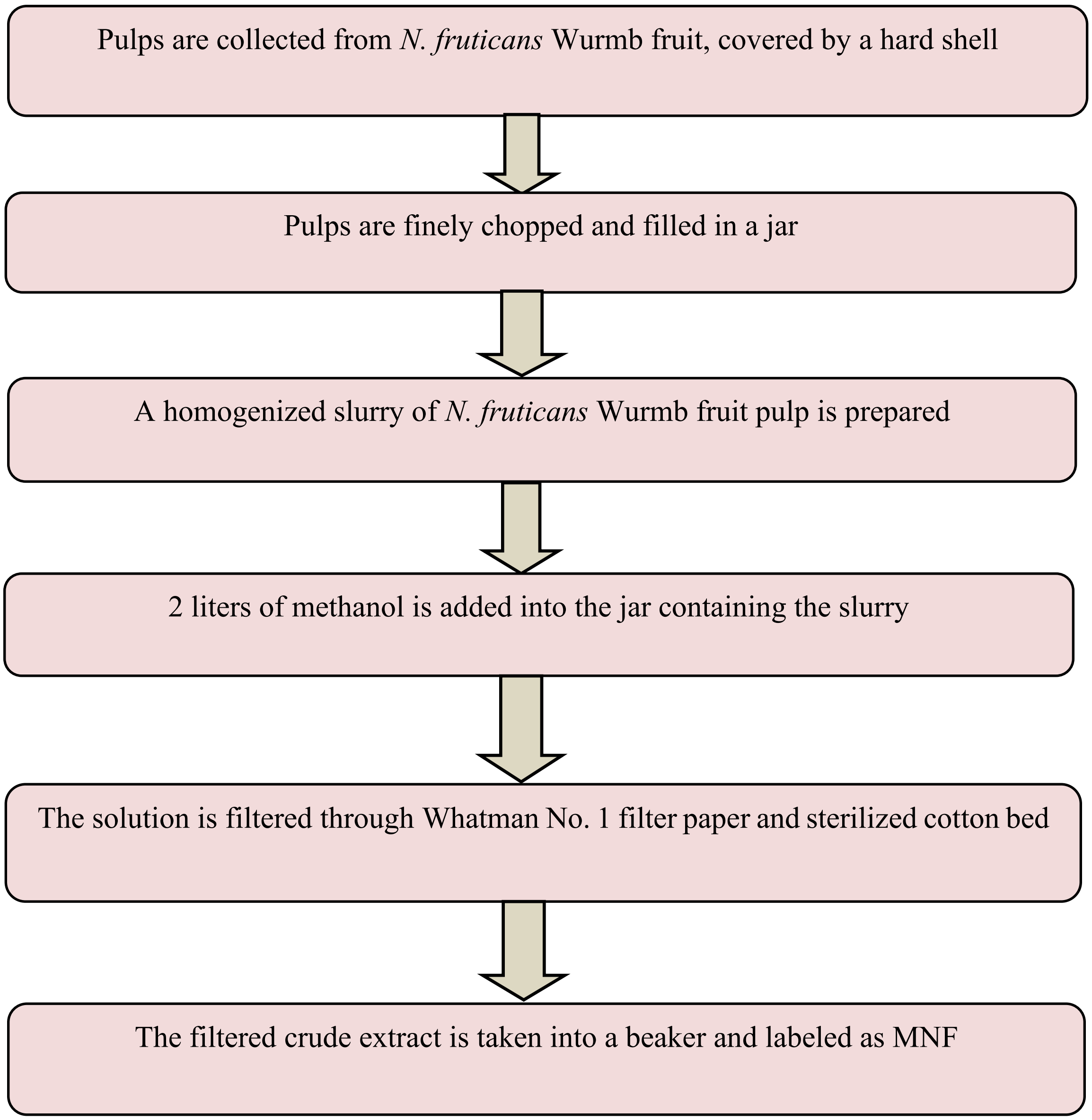

Figure 19: Flowchart of preparation of methanolic extract of N. fruticans Wurmb

2.3 Drugs, Reagents and Equipment Used in the Experiment

Drugs, Reagents & Equipment	Company Name
1% Tween 80 in water which is used as vehicle	BDH Chemicals Ltd.
Diazepam which is used as standard drug	Square Pharmaceutical Ltd, Bangladesh
Normal saline solution (0.9% NaCl) Beximco Infusion Ltd.	Beximco Infusion Ltd.
Electronic weight machine	Denver Instruments M-220/USA

Table 7: List of Drugs, Reagents and Equipment

2.4 Preparation of Chemicals and Drugs

Initially, a dosage of 1mg/kg diazepam was prepared. This diazepam was subsequently dissolved in 0.9 percent NaCl saline water. Each *Swiss albino* mouse was then administered 0.5 mL of this solution intraperitoneally. Dependent on the body weight of each mouse, MNF extract was prepared at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg. Following this, doses were calculated, and a small amount of Tween 80 was added to each preparation. The saline solution was then incrementally added. After calculating the final volume, each mouse was given 0.5 ml of preparation.

2.5 The experimental design for evaluation of CNS depressant effect

For the evaluation of CNS depressant effect, the hole cross method was followed. Here, 25 *Swiss albino* mice were used. They were divided into five groups, each containing five mice. The mice were all healthy and weighed accurately before starting the experiments. Each group underwent specific tests to measure CNS depressant activity. The mice were marked, and doses were adjusted according to their body weight to ensure the accuracy of the test results. The five groups consisted of as the following:-

Group	**Treatment**	**Dose**	**Route of Administration**
Group-G1 (Control)	1% Tween 80 in water	10mL /kg	Orally
Group-G2 (Standard)	Diazepam	1mg/kg	Intraperitoneally
Group-G3 (Extract)	MNF	200mg/kg	Orally
Group-G4 (Extract)	MNF	400mg/kg	Orally
Group-G5 (Extract)	MNF	600mg/kg	Orally

Table 8 : Experimental design for evaluation of CNS depressant activity of MNF Extract

2.6 Animals used in the study

Swiss albino mice which were about 3–4 weeks old and 20 to 25 g in weight, were used in this study. The mice were collected from the laboratory of Pharmacology at Jahangirnagar University, Savar, Dhaka, Bangladesh. These mice model was kept in cages made of polyvinyl with soft wood bedding under standard environmental conditions as per the guideline i.e. a temperature close to the room temperature which is 25 ± 2 °C, and a relative humidity of 55–65%, and 12-hour exposure of

Light and dark cycle for 14 days prior to the experiments. As instructed in the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) which was generated by the Swiss Academy of Medical Sciences and the Swiss Academy of Science, all the experimental steps had been performed. All experimental protocols were approved by The Institutional Animal Ethical Committee (IAEC;15.03) of ICDDRB, Bangladesh. During the time of habituation, many experimental procedures had been performed upon the mice in order to scrutinize the CNS depressant effect, while ensuring that animals faced minimal discomfort and distress. All of these experimental procedures were performed by following the standard protocol under the careful supervision of highly experienced researchers. For assuring overall good health of the mice models, health checks were performed regularly including both physical and behavioral changes examination and monitoring. Whenever any symptoms of abnormalities were seen it was addressed straightaway and proper actions were taken to cure those (Sultana et al., 2018).

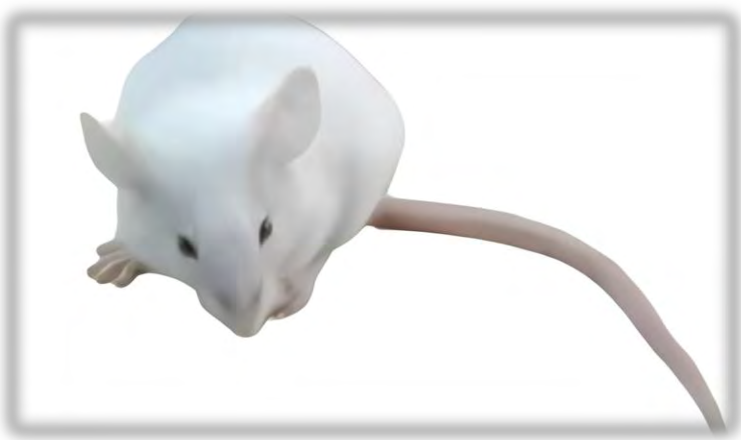

Figure 20: Swiss albino mice of 3 to 4 weeks old

2.7 Ethical Approval

The guidelines for the particular experiment and animal testing were approved by the Department of Pharmacy, Jahangirnagar University.

2.8 Hole Cross Test

The most reliable behavioral change can be measured with an intense emotional response towards a new stimulus of the environment. The method which had been used here was followed according to the directions mentioned by Takagi et al. The agenda was to figure out the changes in the emotional behavior of rodents by conducting the hole cross test. A partition made up of steel was placed in the center of a 30x20x14 cm cage. At the height of 7.5 cm there was a 3 cm diameter hole whichallowed only one mouse at a time to cross the hole. A light was placed just at the opposite side of the hole of similar height and same setup was maintained on the other side of the plate in order to interrupt the mice whenever it crossed the hole.

This action was counted and documented straightaway. If the plant extract has depressing activity, then it might lower the movements of the rodents gradually. The number of movements of the rodents through the hole of one chamber to another chamber were counted at an interval of 30 minutes at respectively 0 minute, 30 minutes, 60 minutes, 90 minutes, and 120 minutes after the intraperitoneal administration of reference drug Diazepam (1mg/kg). Diazepam was administered with the help of a needle. Similar procedures were being performed for the methanolic fruit pulp extract of *N. fruticans* (MNF) extract at doses of 200 , 400 and 600 mg/kg body weight (Ripa et al., 2014). The MNF extract would give different number of movements at different doses which can be further analyzed after performing the hole cross test. The procedure for conducting a hole cross test accurately is mentioned in the flowchart below:

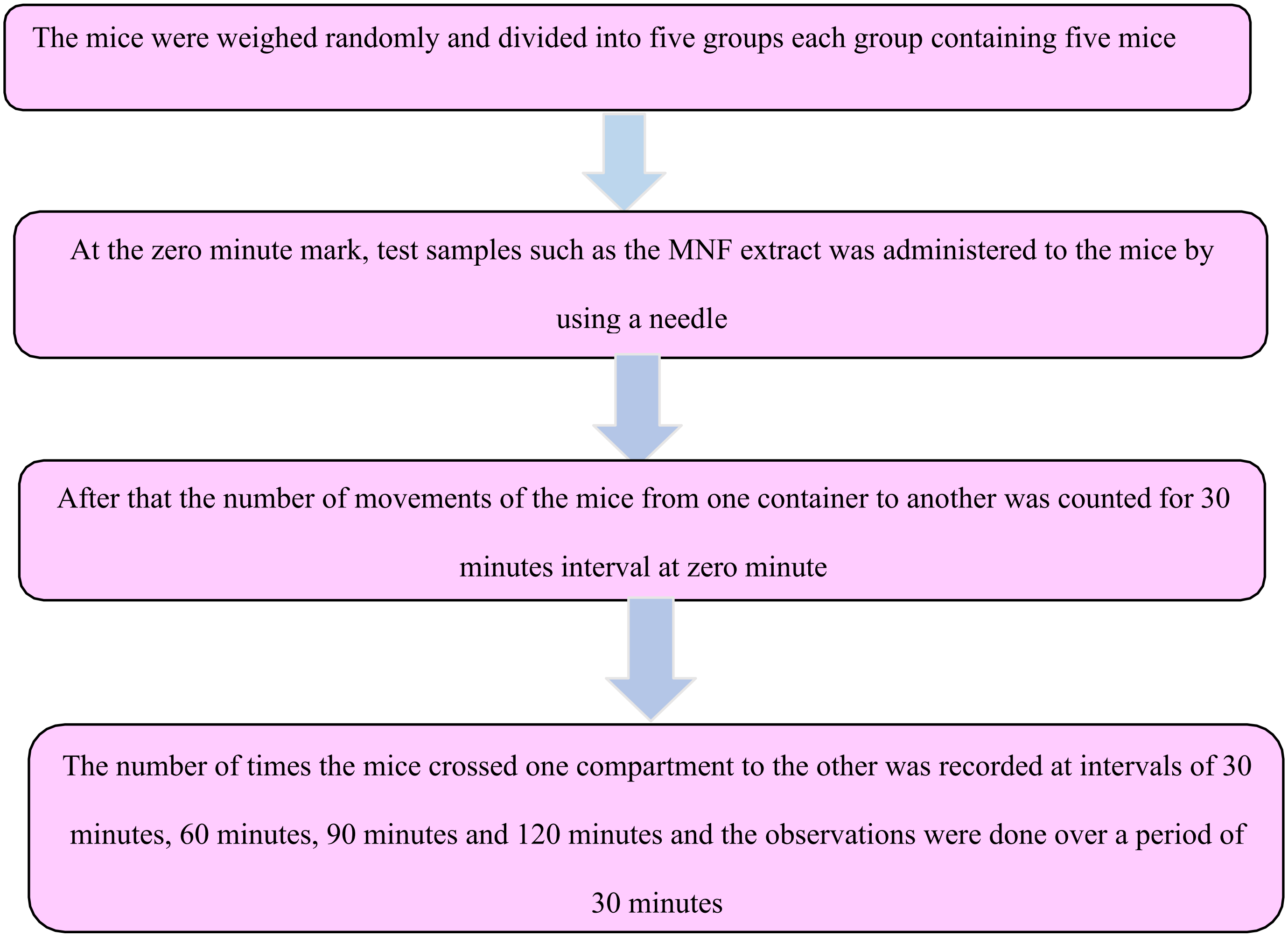

Figure 21 : Flowchart of Hole Cross Test for evaluation of CNS depressant effect from N. fruticans Wurmb fruit pulp

Figure 22: Hole cross test on Swiss albino mice

2.9 Statistical Analysis

All values in the test are put forward as mean ± standard error of the mean (SEM). These data were analyzed statistically by using Analysis of Variance (ANOVA) and Dunnet's multiple comparisons test through SPSS version 26 (USA). At $p < 0.05$, differences between the means of various groups were contemplated significant.

Chapter 3

Results

3.1 Results of Hole Cross Test for *N. fruticans* Wurmb extract

The plant *N. fruticans* Wurmb is considered to contain potential medicinal benefits due to the presence of essential phytochemicals for which it possesses many pharmacological effects as well. In order to investigate the CNS depressant activity of *N. fruticans* Wurmb fruit pulp a pharmacological test such as hole cross test was performed on *Swiss albino* mice. In the test, the MNF extract exerted a gradual dose-dependent reduction in the locomotion activity in the test animals (at all the dose levels: 200, 400 and 600 mg/kg body weight) in comparison to the reference drug diazepam. For the control group, the rodents showed negligible variation in the number of holes crossed from one chamber to another from 0 minute to 120 minutes. However, groups treated with the MNF plant extract at the mentioned dosages demonstrated a significant decrease in movement from their initial value over the same time frame. All the data obtained were dose dependent and statistically significant ($p<0.05$). Following are the outcomes of the test:

Group	Treatment	Dose	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-G_1(Control)	1% tween 80 in water	10ml/kg	25.20±1.72	22.80±0.76	21.00±0.63	21.00±0.75	19.20±1.47
Group-G_2(Standard)	Diazepam	1mg/kg	21.20±1.47	11.20±1.17*	5.40±0.80*	2.00±0.89*	1.40±0.48*
Group-G_3(Extract)	MNF	200mg/kg	20.40±1.02	18.40±1.09	13.20±1.16*	10.20±0.75*	7.20±0.74*

Group-G_4 (Extract)	MNF	400mg/kg	23.40±0.49	18.00±1.09*	11.80±0.75*	9.80±0.74	5.20±0.75*
Group-G_5(Extract)	MNF	600mg/kg	23.00±0.63	13.60±1.02*	8.00±1.67	9.20±0.75*	3.40±0.49*

Table 9: Data from a Hole cross method for MNF extracts

All values were put forward as mean ± SEM (n=5). The data was statistically analyzed by using a One-Way Analysis of Variance (ANOVA), followed by Dunnett's test, $p<0.05$, which stipulates statistical significance compared to the control.

Chapter 4

Discussion

From previously published journals, the phytochemical screening of the plant extract has ensured the presence of alkaloids, tannins, flavonoids, phenols, terpenoids, proteins, carbohydrates etc. for which the plant is thought to possess several medicinal benefits. Hence, the current study focused on the evaluation of the CNS depressant effect of methanolic extract of pulp of *N. fruticans* Wurmb (MNF) in mice. A significant step in observing the pharmacological effect is to monitor the reduced locomotor activity of the mice model. Hole cross test was conducted to obtain accurate results regarding the effect of the MNF extract on the CNS in mice. The extracts demonstrated significant dose-dependent CNS depressant effects in the hole cross test viz, 7.20±0.74 number of movements for 200mg/kg dose, 5.20±0.75 for 400mg/kg dose and 3.40±0.49 number of movements for 600mg/kg dose after 120 minutes in comparison to the standard drug which is diazepam ($p<0.05$).

This effect might be related to the consequence of the plant extract on the GABA neurotransmitter. There are various muscle relaxant, anxiolytic, and sedative-hypnotic drugs which exert their therapeutic effects through $GABA_A$. An assumption is made that flavonoids and neuro-active steroids which are one of the vital phytochemicals of *N. fruticans* Wurmb can function as benzodiazepine-like molecules as many of these may act as ligands for the $GABA_A$ receptors in the CNS (Verma A et al., 2010). The sedative effect may be co-related to the interaction with these benzodiazepine-like compounds. The MNF extract may enhance the GABAergic inhibition in the CNS through membrane hyperpolarization, which basically reduces the firing rate of critical neurons in the brain, or it may be due to the direct activation of the GABA receptor by the extracts (Kolawole OT et al., 2007). Hence, the phytoconstituents might be the possible cause for the CNS depressant activity of the MNF extract (Ripa et al., 2015).

Chapter 5

Conclusion

5.1 Conclusion

For thousands of years, nature has bestowed medicinal gifts upon us that serve as natural sources for modern drugs. One such gift is *N. fruticans* Wurmb, a plant with various pharmacological activity. The hole cross test was conducted to evaluate the CNS depressant activity of the fruit pulp of the plant. Recent study results indicate that MNF extracts of the plant's pulp at doses of 200 mg/kg, 400 mg/kg, and 600mg/kg exhibited significant dose-dependent CNS depressant activity compared to the standard diazepam ($p<0.05$). This activity is evident through demonstration of numerous mechanisms, including its potential for reducing anxiety as well as its sedative effects. These unique properties of this fruit pulp make it a promising candidate for developing new therapeutic agents in order to manage various disorders which are related to the central nervous system. However, more research is required to understand the exact mechanisms involved and identify the chemical constituents responsible for the pharmacological activities. With further detailed research, it may be possible that novel sedative-hypnotic medications could be developed using this plant.

5.2 Future work

The pulp of *N. fruticans* Wurmb fruit, along with its potential CNS depressant activity might be a key resource in developing new treatment approaches for conditions such as anxiety and insomnia. In addition to its medicinal applications, the extract might also be beneficial for other fields including agriculture and biotechnology. Therefore, a multidisciplinary team of researchers in fields such as pharmacology, biochemistry, and traditional medicine could collaborate to fully understand the advantageous properties and potential applications of the extract.

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