EVALUATION OF ANTIOXIDANT 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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Ethics Statement

No animals were used or harmed during this experiment

Abstract:

Nypa fruticans wurmb (Family: Arecacea), popular as tropical palm is now drawing attention in the medicinal field beacuse of its Multipurpose benefits. Antioxidants are necessary medicinal elements which helps in preventing oxidative stress in human body and scavenging the harmful effect of free radicals. In this study, the antioxidant activity of the methanolic extract of *N. fruticans* fruit pulp (NFM) was determined using DPPH(2,2-diphenyl-1-picrylhydrazyl), against the standard antioxidant ascorbic acid. Here, the IC_{50} value of Ascorbic acid and NFM extract was 33.16 µg/ml and 62.99 µg/ml accordingly. Recent research finding emphasizes NFM extract as a moderate potent antioxidant containing component which can be a new source of antioxidant which can be use for discovering new drug compounds. Although further research is required to explore the antioxidant potentials of NFM extract to discover its pharmaceutical importance as a natural antioxidant agent.

Key words*:* *Nypa fruticans* pulp, Antioxidant, DPPH method, Ascorbic Acid, scavenging

Dedication

This work is dedicated to my beloved parents who has inspired me and supported me in every step of my life.

Acknowledgement

All the praising belongs to Lord Krishna, who has given me the physical and mental strength to complete this project paper with great patience. Also, I would like to express my heartfelt gratitude to work with a very kind hearted, supporting faculty of our department Dr. Farhana Alam Ripa (Associate professor, school of pharmacy, Brac University). without her valuable guidance and immense support from my supervisor I could not have been finish my proper so easily.

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List of Acronyms

TCM	Traditional Chinese Medicine
WHO	World Health Organization
BHT	butylated hydroxytoluene
BHA	butylated hydroxyanisole
NMF	Methanolic extract of *N.fruticans* wurmb Pulp
ml	Milliliter
ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber

Chapter:1

Introduction:

1.1 Background:

Going back to the prehistoric time frame, we can observe that our earliest ancestors embraced medicinal herbs to alleviate infections, eradicate diseases, ranging and calm nervousness. A number of compounds derived from different kinds of plants, especially sub-parts of woodlands, have been administered traditionally since ancient times to treat a wide range of diseases. Every one of those substances comes within the category of "Herbal Medicine(Dias et al., 2012)." The holistic approach of natural medicine is considered as the most efficient method to treat a variety of diseases and prolong human life. The oldest known written record of people using medicinal herbs to make different therapies has been found on a Sumerian clay slab which might have been used 5000 years ago. Furthermore, this experienced approximately 12 recipes and identified roughly 250 surnames of medicinal plant.

Figure 1: Herbal Medicine and their widespread use (Awortwe et al., 2018)

These medicinal plants frequently include different plant components which are comprised of of different rich elements that can be used in an assortment of ways for developing medications preparations. Since they have the unique synergistic effect, these kinds of drugs are currently in high demand for managing some uncommon medical conditions. Several

uncommon conditions, including cancer, can potentially be prevented via growing by several plant components. Due to the toxicity and adverse reactions of allopathic and conventional treatments, the need for medicinal products has grown as humanity has progressed. This also lessens the need for pesticides in pharmaceuticals, and plant-based medicine maintains the forefront in healing a variety of conditions, reducing human anxiousness, and ensuring human safety and health(Petrovska, 2012).

Due to a lack of technological assist at the time, people relied heavily on plants for therapeutic purposes and to alleviate illnesses. Individuals have traditionally found it challenging to figure out the root causes of their symptoms due to a lack of understanding regarding plant substances that both safe and effective for use as treatment(Hosseinzadeh et al., 2015). Although it's unclear precisely when, when, and under what circumstances medicinal plants were originally employed and it's possible they were initially employed to treat an extensive list of disorders. It is believed that herbal medicine was originally used our our ancestors(Sofowora et al., 2013).

1.2 History of accessible medicinal plants:

The history of plants for medicinal purposes is thoroughly integrated into the culture of humanity, with their application going backwards thousands of years. These plants have served as essential to traditional therapeutic approaches across various cultures and remain in affecting contemporary medicine.

Ancient Records:

Sumerian Civilization- The earliest documented makes up of medicinal plants come from the Sumerians, who documented various herbs, including opium, on clay tablets from 2600 B.C. This emphasizes the long-standing relationship that exists between humans and herbal medicine. The another one is -The Papyrus of Ebers, beginning around 2900 B.C., is an

ancient Egyptian document that describes over 700 plant treatments, including different forms of treatment like pills and ointments. It serves like a single of the earliest comprehensive therapeutic instruction manuals.

Chinese Materia Medica: The culture of Chinese herbal medicine celebrates an ancient history, highlighted by handwritten ones that include the Shennong Herbal (~100 B.C.), which describes 365 medicinal plants. Traditional Chinese medicine continue to be extensively utilized, with an important percentage of the population relying on these traditional techniques for health care

Mediaeval to Early Modern Age:

In the Middle Ages, European the monks preserved herbal knowledge, while Arabic scholars enriched Greco-Roman documents. Furthermore, Avicenna's Canon Medicine established a significant impact on health care practices in both Eastern and modern Western cultures. The Renaissance triggered a renewed phase of interest in herbalism, which brought about the commercialization of hundreds of herbals that recorded regional plant purposes around Europe.(Aligabi, 2020)

1.3 Contribution of medicinal plants:

1.3.1. As traditional Medicine:

Over the time of history, various countries have created their own structures for herbal medicine-

- **Traditional Chinese Medicine:** This medicine history incorporates more than 12,000 crop chemicals, focusing on customizing therapy with the hep of some difficult medicinal preparation(Dias et al., 2012).

- **Ayurveda in India:** This ancient beneficial technique combines nutritional habits and botanical remedies and has been around used approximately 5,000 year.
- **European Herbalism**: In the Dark Ages, monasteries maintained Greco-Roman knowledge while introducing regional herbs into their methods of operation. The Arabs contributed significant achievements by establishing pharmacies and furthering herbal knowledge(Dias et al., 2012).

Evidence of the application of medicinal plants in conventional medical procedures dates to the days of ancient civilizations that include the Sumerian, Egyptian, and Greek ones .Medicinal plants possess played an integral part in the healing process of a variety of conditions throughout traditional Chinese medicine and Ayurvedic treatments . The main source of health care in many developing nations, particularly in rural regions, is

Traditional medicine, which includes herbalism for the purpose to treat significant ailments in humans. the Santal people living in Bangladesh use an extensive range of medicinal plants . With the goal alleviate 179 different symptoms or ailments, traditional healers in the geographic area of Chimborazo, Ecuador, employ 153 distinctive medicinal plants . Ayurveda and Traditional Chinese Medicine (TCM)constitute two leading conventional medical systems that have historically employed plant remedies for the ages for treating a broad variety of conditions. Traditional treatment with herbs persists as many people's primary option for healthcare, especially in remote rural regions in developing countries. Traditional medical wisdom contributed as well much to the development of modern procedures in medicine(He et al., 2019).

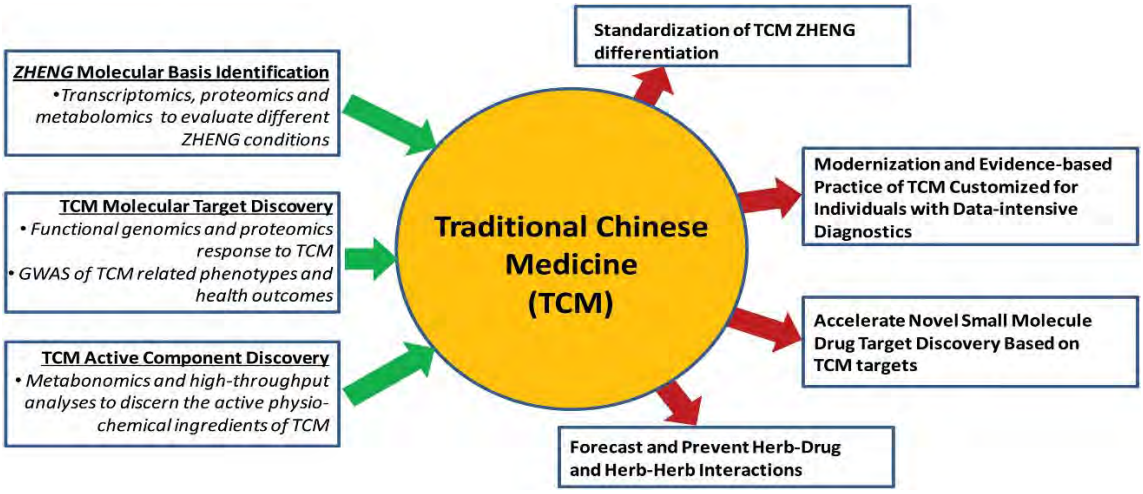

Figure 2: Traditional Chinese Medicine (Wang & Chen, 2013)

1.3.2. As alternative of synthetic drugs:

Pharmaceutical preferences for treatment are shifting toward synthetic medication largely a result of the Industrial Development and the subsequent development of organic chemistry. This was because to the simplicity of which natural substances could be obtained, the flexibility with modifications to structure to create potentially safer and more active medications were possible to carry out, and the steadily increasing financial position of the chemical companies. In addition, the use for raw materials has been connected to enchantments and faith throughout the evolution of humanity, and numerous different cultural perspectives on illness and wellness are currently present. Such an approach was obviously in contradiction to the contemporary way of existence established in developed western nations, where medications produced from renewable resources were considered as either a low-income or unskilled person's option or as simply a religious superstition with no application in real life(Ilaria, 2023).

An estimated 60% of tumour fighting and anti-infectious drugs currently in research trials or readily accessible on the market contain natural sources Organic substances may be used as

lead compounds, permitting the advancement of biomimetic synthesis, the design and careful development of new medications, and the discovery of novel medicinal properties not yet linked to commonly used substance. he curiosity in drugs manufactured from plants originates to a variety of reasons, such as the fact that conventional medicine can be ineffective (e.g., have adverse effects and unsuccessful therapy), that incorrect or abusive use of synthetic drugs may cause side effects and other problems that an important proportion of the world's population doesn't have access to traditional pharmaceutical treatment, and that "natural" products are harmless due to ecological awareness and folk medicine(Campos et al., 2019).

Some available synthetic drugs are provided in the bellow figure.

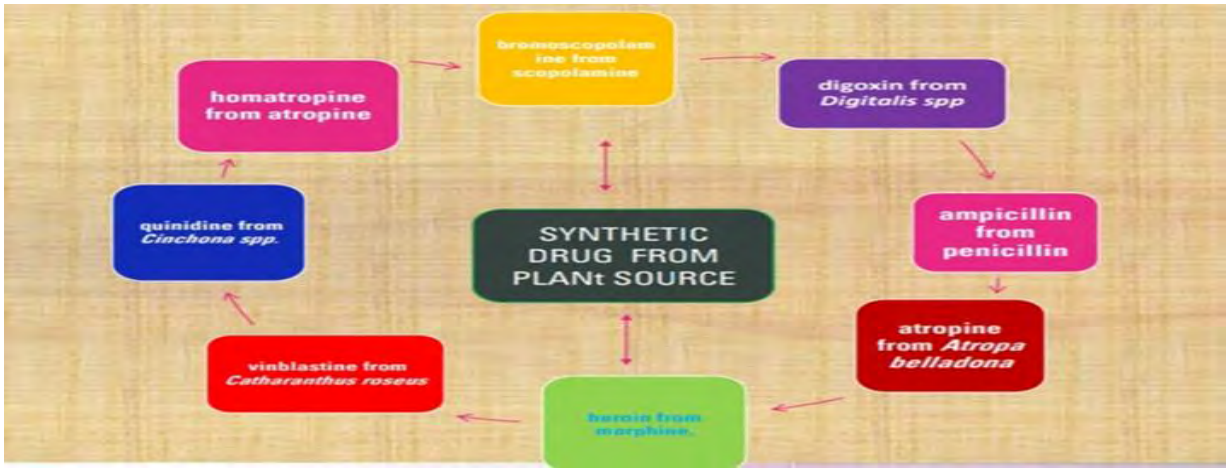

Figure 3: Available synthetic drugs from the plant source.

1.4 Available Medicinal Plants in Bangladesh Having Antioxidant Potentials:

The antioxidant competencies of many different kinds of Bangladeshi medicinal plants have been investigated. Here are some of the prominent instances:

- ***Scoparia dulcis***: . *B.Sapida* (Latkan) is a different kind of delicious fruit which is normally grown in the region of Bangladesh, Nepal, India, Myanmar, and some parts

of China this Plant is unique in nature because of having an IC_{50} concentration of 173.36 µg/ml, which made the leaves and root extracts of this plants serving a remarkable antioxidant activity for the help of mankind. This plant possesses some pharmacologically active biomolecules which includes flavonoids, alkaloids, proteins, as well as carbohydrates, and glycosides(Rashid et al., 2018).

- ***Curculigo orchioides :*** *Curculigo orchioides* this species is available generally around the Indian sub-continent including some region around Bangladesh. The root extract of this plant demonstrates a potential antioxidant action. It contains numerous kinds of phytochemicals, which includes flavonoids, alkaloids, proteins, carbohydrates, and glycosides which made this plant popular among this region as a great source of antioxidant(Rashid et al., 2018).
- ***Pandanus fascicularis:*** Among the plants under examination, the roots, leaves, fruits parts of this plant used in unani and other folk medicine over the decades. The root preparation of *Pandanus fascicularis* displayed scientifically highest level of antioxidant activity, having an inhibitory concentration (IC_{50}) of 21.87 µg/ml. This plant did nonetheless demonstrate dose-dependent toxicity, with an LD_{50} of 25.64 µg/ml(Rashid et al., 2018).

1.5.1: Antioxidants:

Antioxidants are generally those substances that support the body in eliminating harmful free radicals that are not useful or generally harm causing. Antioxidants are prevalent in diets which are generally originated from plants, collected from plant source. Antioxidants can play a vital role in maintaining the healthy relationship between antioxidants and free radicals in the human body, helps human body to fights against oxidative stress.(Pham-Huy et al., 2008).lycopene, and vitamins A, C, and E (alpha-tocopherol) are some examples of dietary

antioxidants .Selenium which is a mineral substance and it is often considered as a dietary antioxidant.(Davis et al., 2012)

1.5.2: Antioxidant potential of Medicinal plants:

Usually, in medicinal plants there are the non-enzymatic antioxidants which are presents and they are huge in number. They may be helpful in defending over oxidative stress, which plays an important part in the appearance and treatment of many diseases. Mostly these antioxidant compounds can be produced by plants automatically and they are easily available for use(Rudenko et al., 2023).

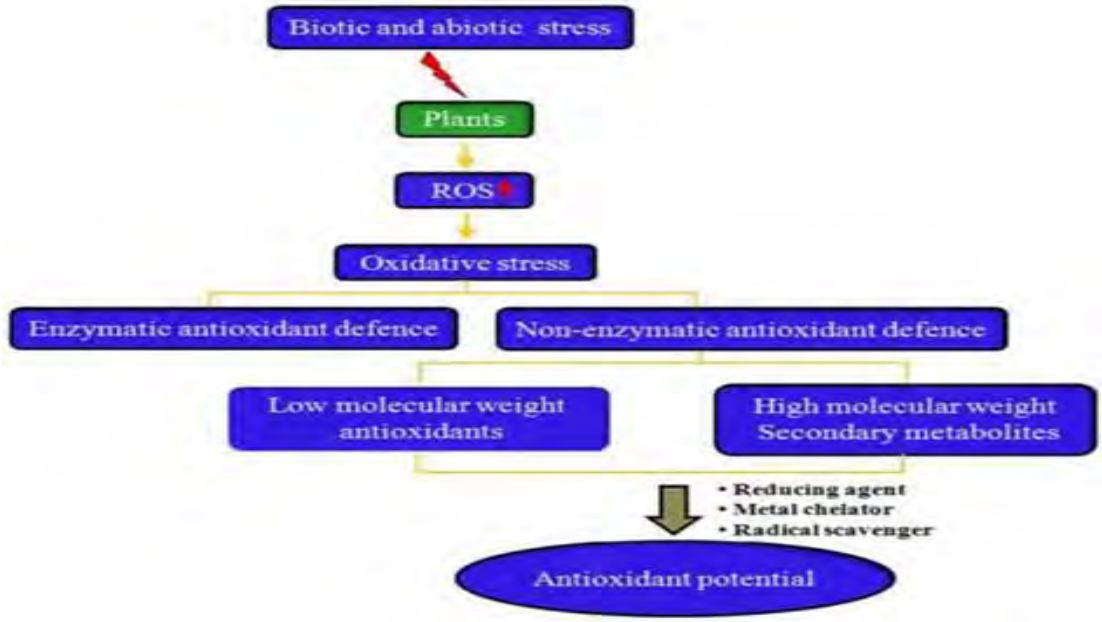

Figure 4: Antioxident potential of Plants.*(Kasote et al., 2015)*

Several studies have been executed from the ancient times to present days to study the potential of antioxidant compounds. Generally, in vitro studies have shown, extracts from various kinds of medicinal plant which is collected from the different plant parts, that include

stems, and their roots, tree bark, leaves, and fruits and vegetables and seeds that demonstrate significant antioxidant activity. secondary metabolic pathway products named phenolic compounds could possibly be responsible for their antioxidant activity capabilities(Yu et al., 2021).

1.6: Types of Antioxidants:

Table 1: Classification of antioxidants based on mode of actions stated in (Flieger et al., 2021).

Mode of action	**Examples of antioxidants**
Antioxidant that reacts with weak O-H or N-H bonds in peroxyl radicals to destroy chains.	Phenol, Naphthol
Antioxidants that break chains by reacting with alkyl radicals	Quinones, Nitrones, Iminoquinones
Antioxidants that hydroperoxide-degrade	Sulphide, Phosphide, Thiophosphate
Deactivating antioxidants with metals	Diamine, Hydroxyl acids, Bifunctional compounds
Antioxidants that break the cycle of oxidative degradation	Aromatic amines, Nitroxyl radical, Variable valence metal compounds
antioxidants functioning in combination with other antioxidants	Phenol sulphide in which the phenolic group reacts with peroxyl radical and sulphide with hydroperoxide.

1.7.1: Free Radicals:

A free radical is defined as any molecular structure that has the potential to exist independently without any interference and contains a minimum of a single unpaired electron in its atomic orbital. The unpaired electron basically keeps radicals that are unpaired making them highly reactive, as they aim to stabilize themselves by donating or retaining electrons from other molecules. The scientific understanding of radicals that are free has improved significantly revealing their complicated roles in both wellness and ailments of human being. While these substances are necessary for some particular biological functions, at the same time excessive quantities may result in negative impacts on cells and tissues (Lobo et al., 2010).

1.7.3. Common Disease caused By Free Radicals:

Free radicals, also called oxygen radicals, are destabilizing compounds that can exert substantial damage upon cells, which leads to many different illnesses. They are naturally generated in the human body during metabolic processes, but they may also be produced by external factors such as contamination, cigarette smoking, and radiation. The build-up of free radicals may end up in oxidative stress, linked to several medical conditions. Free radicals might cause damage on DNA, which leads to mutations that could bring about the beginning of cancer(Lobo et al., 2010). They are connected with multiple cancer types, particularly pulmonary, breast, and colorectal cancers, attributable to their potential to produce abnormalities in chromosomes and trigger cancer-causing genes. Oxidative stress has been suggested to be linked to the metabolic disorders linked to obesity, probably influencing inflammation and sensitivity to insulin. Conditions include Parkinson's and Alzheimer's correspond to oxidative damage in nerve cells. Free radicals may trigger death of neurons, which results in decline in cognition and impairment of movement(Lobo et al., 2010).

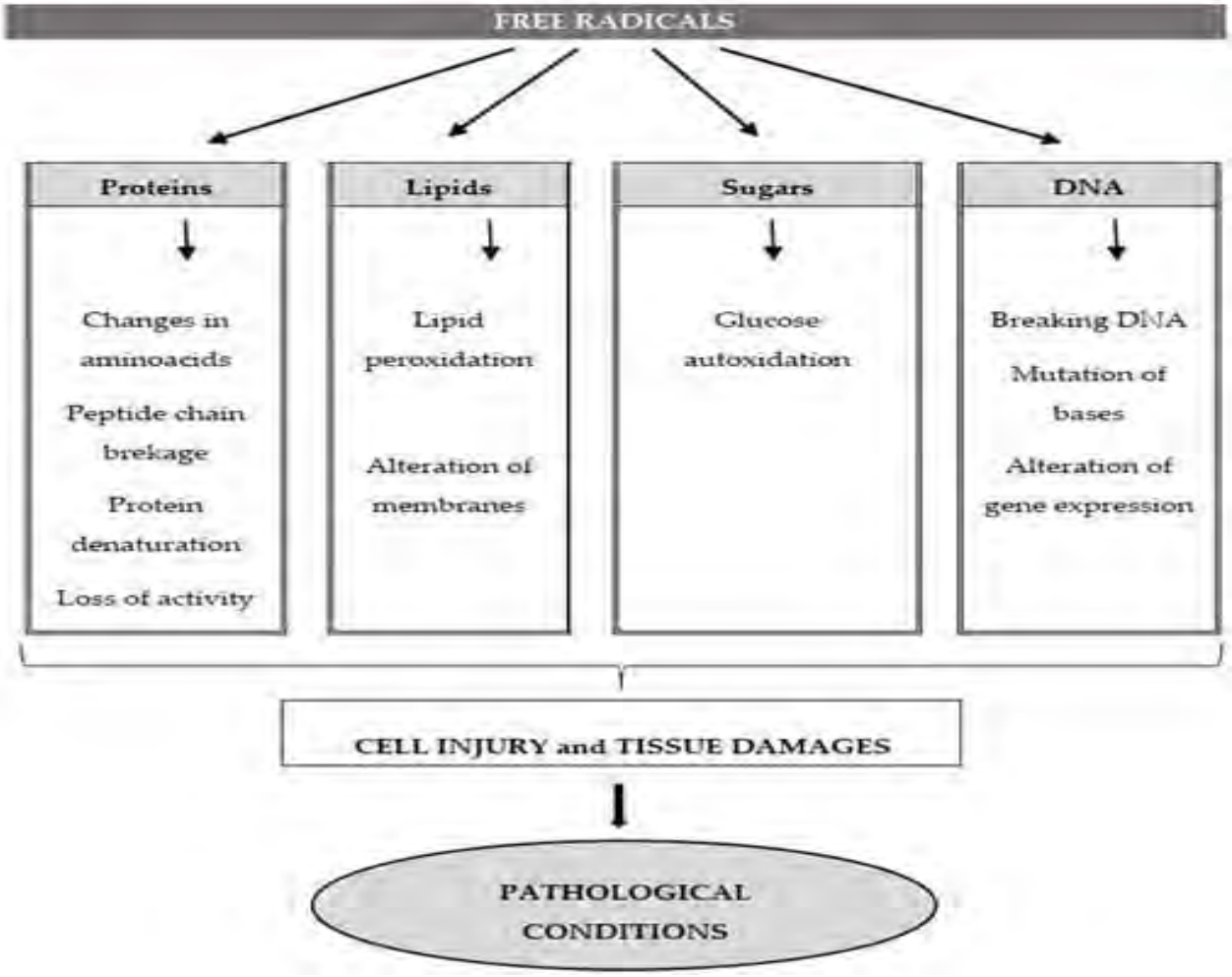

Figure 5: The molecular target of free radicals and occurrence of oxidative damages (Martemucci et al., 2022)

1.8. DPPH Method And its significance:

The DPPH assay is a widely used method for evaluating the antioxidant properties of compounds or plant extracts(Baliga et al., 2013). It focusses on measuring the magnitude of the antioxidants' property to scavenge the extremely stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl).When DPPH comes into contact with an ingredient that may give away a single atom of hydrogen, that is subject to a decrease in concentration, which results in the diminishing of its extreme colour that's violet (Kedare & Singh, 2011). The previously spectrophotometric evaluation of this change in colour appears at nearly 520 nm. The

antioxidant's capability to neutralise free radicals can be measured by the intensity of a colour change.

The assay for DPPH is simple, explosive and does not require any of the advanced equipment(Gulcin & Alwasel, 2023). It has been further refined and automated by employing techniques that include sequential injecting evaluation which allows high-throughput screening for multiple samples. Two The IC50 value is an established approach for measuring the antioxidant activity, since it indicates the quantity of the antioxidant essential to neutralise fifty percent of the DPPH radicals. The DPPH assay is a method which is frequently used for determining the antioxidant activity of chemicals or plant extracts; nevertheless, it has certain drawbacks when compared to other antioxidant experiments.

Investigation has shown that the ABTS analysis may be more efficient than the DPPH assay for determining the antioxidant capacity of a wide variety of foods(Gulcin & Alwasel, 2023). The ABTS assay is based on the ability to inhibit of the pre-formed ABTS, which radical cation, while the DPPH assay utilises the stable DPPH radical(Gülçin & Alwasel, 2023).

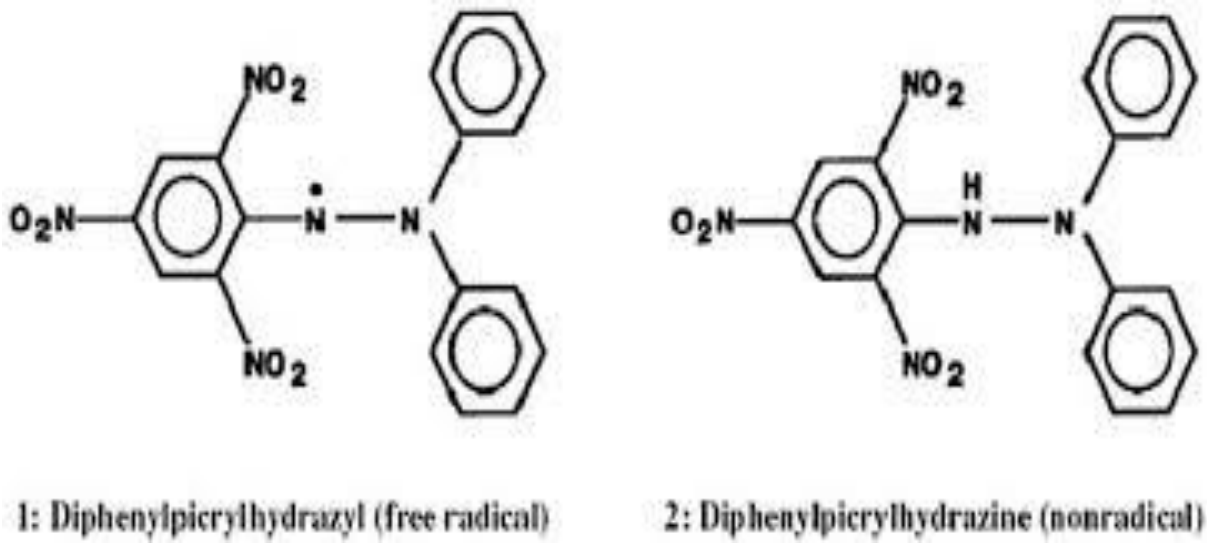

Figure 6 : Free radical reduced form of DPPH *source*(Lewoyehu & Amare, 2019)

Z•+AH→ZH+A•$Z•+AH→ZH+A•$ -------- (Reduction Reaction)

In this reaction, Z• indicates the DPPH radical, AH is here the hydrogen donor (antioxidant) part, *ZH* is basically the reductase part which is reduced form of DPPH,

A• is the free radical which accumulates basically as an result of the antioxidant. A quantitative evaluation of DPPH can be performed by the elimination of the hue of violet indicates an appearance of the reduction of DPPH in different time region. (Baliyan et al., 2022)

1.9. Background of *N. fruticans* wurmb Plant:

There are many types of synthetic antioxidant elements are being used in in different Industries. Synthetic antioxidants like, butylated hydroxy toluene (BHT), and BHA (butylated hydroxy anisole) has prohibited their applications in foods due to their high cancer-causing ability. As a result, now a day's consumers have become more concerned regarding renewable antioxidants. Natural antioxidants are generally drawing attention in scientific research because they are considered safer and healthier compared to synthetic antioxidants as they are obtained from natural sources rather then chemical reactions (Pokorný, 2007).Scientists have discovered that consuming lots of elements filled with antioxidants containing fruits and vegetables decreases the probable possibility of diabetes, certain types of cancer, and coronary heart disease. These antioxidants advantages are generally depending on the compounds named carotenoids, anthocyanins, phenolic acids and flavonoids.(Lewoyehu & Amare, 2019).

The 2011–2020 UN "Decade on Biodiversity" strives to promote consciousness around the world about unnoticed foods while promoting their consumption around general people. Along with that ,scientific research and development is at present concentrating towards understudied fruit antioxidants evaluation.(Aitken, 2011)However, due to inadequate marketing and advertising is responsible for many of these fruits being unknown to general

people. Among many natural antioxidant sources *N. fruticans* wrumb is popular, which is packed with antioxidants benefits. It can be a great alternative of synthetic antioxidant element.

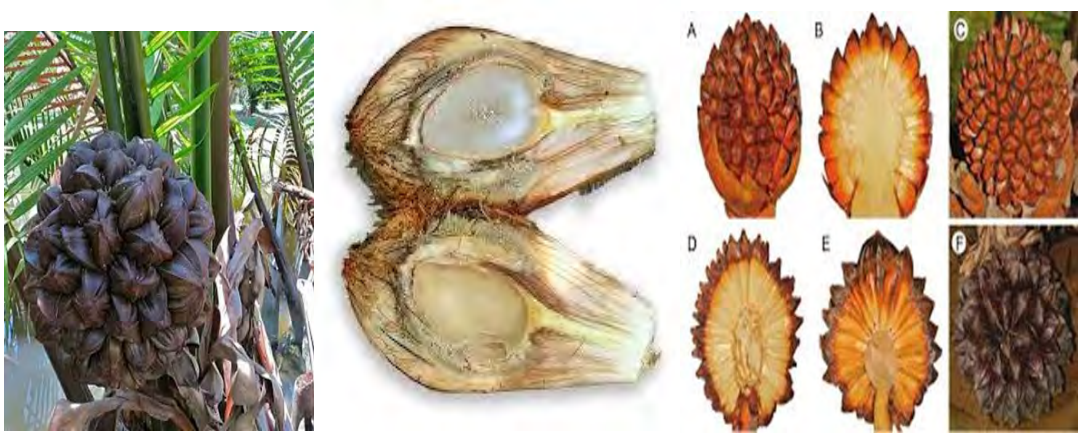

Figure7:Description of different parts of *N.friticans* plant (Bobrov et al., 2012)

1.10. Distribution of *N.fruticans:*

Palm species *N. fruticans,* frequently recognised as ‘‘Nipa Palm or Mangroves Palm”.Which possess some specific characteristics compared to other palms. Nipa palm's stem generally grows under the ground surface but its leaves and flower stem expand up to 9 feet above the. This palm tree grows mostly in tropical locations including the Philippines, as well as India, Malaysia, Queensland, even in Australia.

N. fruticans' inflorescence is basically hemispheric containing female flowers at the very top and catkin-like red or yellow males on the bottom place. In this palm plant there is lignified nuts present which create a long cylindrical cluster on every stem of the plant from the blossom. These nuts after that split and move along with the electricity to expand itself inside the water region.(Lim, 2012)

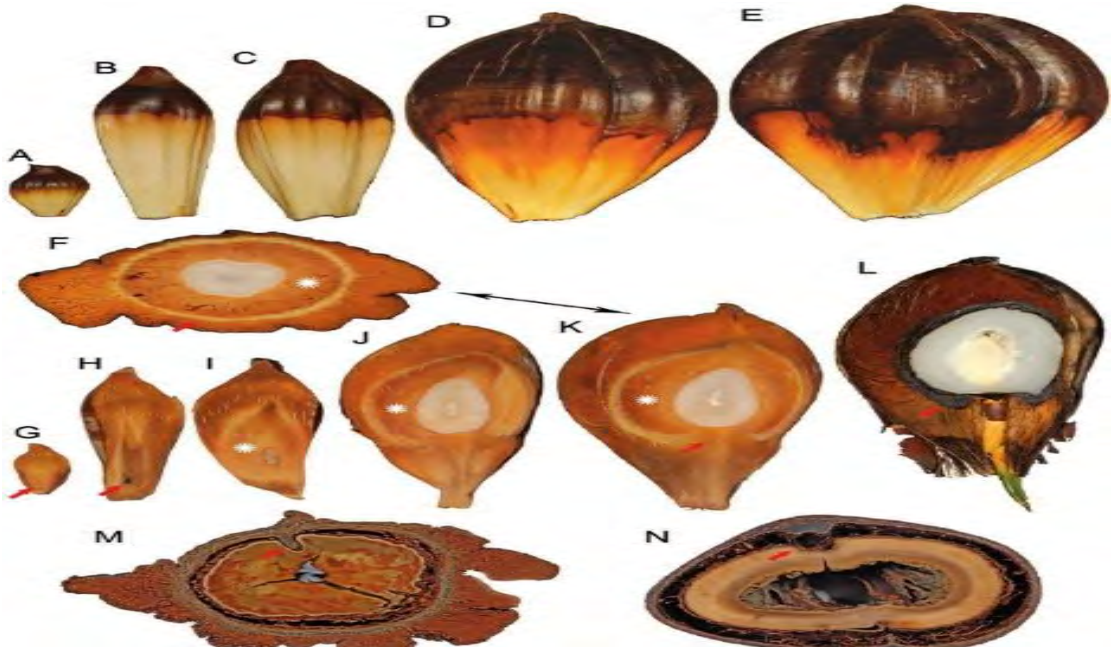

Figure 8 :The internal parts of the plant and their classification (Tamunaidu & Saka, 2011)

This palm species develops rapidly due to its ability to embraces soft mud and slow speed tidal and river waters. It is common in coastal regions and aquatic environments that remain across the Indian and Pacific Oceans from India to the Pacific Islands. Despite being known as "mangrove palm," *N. fruticans* is able to tolerate modest amount of salt and it generally exists above seawater. The plant originates overall in China, as well as in India, the Southeast Asian region, many Pacific Island (Bobrov et al., 2012) . (Bobrov et al., 2012)(Bobrov et al., 2012)(Bobrov et al., 2012)

1.11. Local Name of *N. fruticans* wurmb:

Nipa (*N.fruticans*) is a palm species that has been popular coastal region of the different parts of the world due to its different benefits and the contributions in the managements of different diseases in mankind .It is well in different areas in different names. As like –

Table 2. The diversity of Naming of *N. fruticans* wurmb in different areas:

Country	Local Name
Austalia	***rola, ki-bano, tacannapoon***
Bangladesh	***golpata, nipa palm***
Myanmar	***dani***
China	***shui ye***
India	***gabna gulag, nipumu***
Singapore	***attappalm***
Sri Lanka	***gim-pol***
Thai	***lukchaak, atta***
Vietnam	***dừanước, dùalá***

1.12. Classification:

Table 4: The taxonomical classification of *N. Fruticans*:

Subclass	Taxonomical Classification
King	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Lilianae
Order	Arecales
Family	Arecaceae
Genus	*Nypa*
Species	*N.fruticans* Wurmb

(Tsuji et al., 2011)

1.13. Morphological Classification:

1. Trunk: The trunk part of *N. fruticans* plants generally grows under the ground, raising a recumbent or subterranean root that can grows easily up to 45 cm in diameter. This adjustment allows it persist in coastal and estuary that occurs geographical regions where high tides submerge it.

2. Leaves: Nipa palm leaves are generally long and soft, reaching a length of 9 meters (30 ft) from the ground. They are palm -shaped plant, increases from the underground stem. Ecstatic nipa leaves then stays straight however being partially immersed in water.

3. Flowers: The inflorescence of *N. fruticans* wurmb contains an accumulation of female flowers at the top of the plant where, catkin-like male flowers in the downstairs part of the plant. Male blossoms of this plant are red or yellow in colour. This plant pattern draws all together all the extremely small insects and honeybees which occurs the pollination of plant.(Tamunaidu & Saka, 2011)

4. Fruits: *N. fruticans* wurmb contains globular clusters of woody nuts like fruit structure that can measure up to 25 cm (about ten inches) maximum. Each fruit comprises multiple seed segments which is generally wrapped around by an outer fibrous husk. Fully developed seeds might start to migrate away from the group of seeds along with the tide precisely because of their airborne characteristics.

5. Roots, Rhizomes: The plant stays grounded in the mangrove muck because of the presence of its robust roots. The horizontal rhizomes enable the plant to formulate dense stands and produce offspring in a natural process .(Bobrov et al., 2012)

1.14. Chemical Composition of *N. fruticans* wurmb:

The nipa palm, *Nypa fruticans*, contains a complex chemical constitution in nearly each of its parts, including the fronds, fruits, and kernel. Below includes a comprehensive description which is derived from the currently available information about this plant-

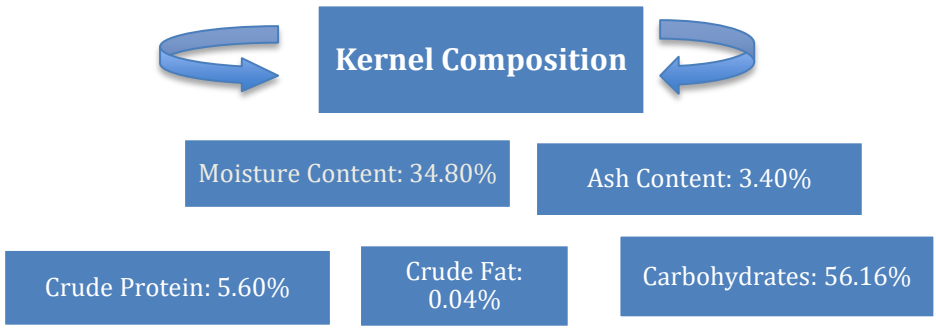

Figure 9: the chemical compounds in the kernel part (Tamunaidu & Saka, 2011)

Table 4. chemicals compounds present in different parts of the plant -

Frond Composition	• Cellulose: 24.6% • Hemicellulose: 22.4% • Lignin: 35.0% • Organic Extractives: 5.4% • Ash Content: 9.5% (Evelyn et al., 2022)
Fruit Composition	• Chlorogenic Acid • Protocatechuic Acid • Kaempferol Additionally, the fiber analysis of palm fruit flour shows: • Crude Fiber: 46.18% • Acid Detergent Fiber (ADF): 63.03% • Neutral Detergent Fiber (NDF): 83.01% • Hemicellulose: 19.98% • Cellulose: 62.35% (Dewi Astuti et al., 2020)
Secondary metabolites	• Alkaloids, Steroids • Triterpenoid (Dewi Astuti et al., 2020)

1.15. Disease Management Profile:

The nipa palm, called *N. fruticans,* is a plant with versatility and this is beneficial for an extensive spectrum of healthcare applications considering to its numerous components. That which follows is an in-depth analysis of the literature highlighting its therapeutic applications.

Leaves: *N. fruticans* leaves and their many different uses for healing – **Antimicrobial Action:** Cataplasm or lotion constructed from fresh leaves may be used to treat ulcers. The antibacterial characteristics of the extract of the leaves have been proven to be exceedingly successful against a number of infectious agents which might involve bacteria such as E.coli and staphylococcus aureus(Prasad et al., 2013a).

Diabetes Management: This plant has shown a potential evidence in the management of diabetes. Animal studies have demonstrated that methanolic extracts of young as well as old leaves exhibit significant antihyperglycemic effect (Dwi Nugroho et al., 2022).

Stem:

Pain Relief and Antinociceptive Properties: Among those the numerous advantages of the stem includes its analgesic effects, which have rendered it an increasingly common option for the alleviation of pain resulting from an array of medical conditions. **Antidiabetic Potential**: The stem's extracts, similarly those who were of the leaves, have antidiabetic characteristics, which assist with controlling glucose levels in rats with diabetes(Prasad et al., 2013b).

Roots:

Traditional Treatments: The condition of asthma, leprosy, and tuberculosis are just some of the traditional diseases being treated through their roots. Furthermore, they have been

employed for the treatment of snake bites and problems with the liver.

Fruits:

Nutritional and Medicinal Value: Neither the ripened and unripe fruits of *N. fruticans* possess an abundance of useful phenolic compounds and antioxidants, which at first contributes to both their nutritional and medicinal value. Whenever it concerns preventing diseases developed through oxidative stress, the outermost layer of fruit that is unripe has been found to be the most effective antioxidant.

Hepatoprotective Effects: Studies indicates that fruit extracts could assist with regulate liver-related disorders by demonstrating a shielding effect on the liver.

The Sap framework: The health benefits of the inflorescence sap is improved while it is consumed as a sugary beverage. Vinegar and alcohol produce are two of its well-established traditional application.:

Phytochemical ingredient- Phytochemicals derived from *N. fruticans* wurmb comprise phenolic acids including kaempferol and chlorogenic acid as well as flavonoids. Its antioxidant properties and general wellness positive aspects are connected in a significant way to these substances, thereby rendering it a crucial instrument in traditional medicine. At the end of the *N. fruticans* is a potent medicinal herbal which possesses multiple applications contingent upon the component of the plant, that indicates highly for its potential application in preventing and curing illnesses. Further research into the effects upon its phytochemical constituents may enhance its use as modern medicine(Tinni, 2023).

1.16. Rational of this study:

N. fruticans has great potential as a provider of natural antioxidants, with multiple health advantages. This plant is generally distributed near the Sundarbans region of Bangladesh.

The investigation emphasizes the need of sustainable harvesting and commercial use of *N. fruticans*, which can boost local economies while additionally promoting preservation of the environment. Its numerous uses in medical products, food, and nutraceuticals highlight its significance as an underutilized resource(Sudirman & Wardana, 2024). subsequent studies could focus on optimizing the extraction process to increase the generation of bioactive compounds from *N. fruticans*.

1.17. Aim of this Project:

The present investigation of the *N. fruticans* antioxidant activity mainly employed to study the antioxidant capacity of the fruit pulp with the use of the technique called, DPPH radical scavenging assay. Another important parameter is to compare the presence antioxidant properties in the *N. fruticans* (NF) pulp with the standard Ascorbic Acid.

1.18. Objective of this project:

The objective of this study is to-

1.Understand the DPPH method's principles for evaluating a plant extract's antioxidant activity.

2.Study the implementation of the specific parameters known as "IC_{50}" (Inhibitory concentration to give 50% impact), and how this is utilised in the interpretation of experimental data collected from DPPH method.

- The primary interest of this study is to analyze known phytocompounds present in medicinal plant extracts. Also, to find antioxidant source compare to the traditional Ascorbic Acid in contrast.

1.18. Literature Review:

- **(Bae & Park, 2016**): Described the potentials of *N.fruticans* in the generation of pro-inflammatory cytokines inhibition .
- **(Kang & Hyun, 2020):** Showed evidence of nipa plant in managing antinociceptive effects in rat models involving sciatic injury from crushing.
- **(Imra et al., 2016**):Demonstrated strong antioxidant and antibacterial activities of this palm species in different research field.
- **(. et al., 2023)** : This study described about how herbal extracts of N. fruticans used to treat diarrhea and also discussed about various different mechanisms.
- **(Garba & Abdullahi, 2019):** This study shows evidence of Nipa palm carrying potential as a biofuel crop.

Chapter 2:

Methodology

2.1. Collection of plant:

The study focuses on *N. fruticans* Wurmb, a plant found in the Sundarbans mangrove forest in Bangladesh's Bagerhat district. A taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession number: 42,861).

2.2 Preparation of the plant Extract:

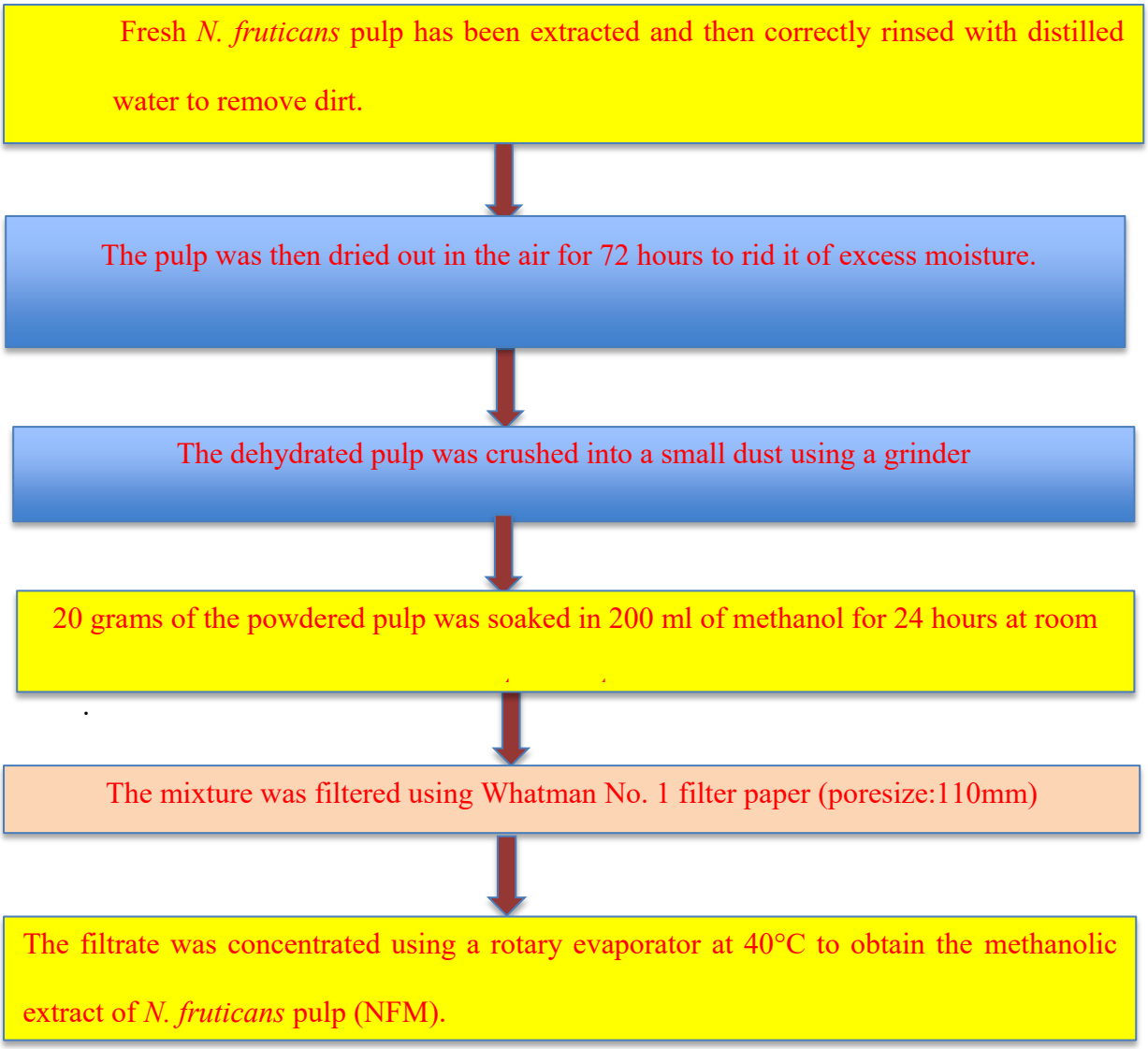

Figure 10. Flowchart of preparation methanolic extract of N.fruticans Wurmb (NFM)

2.3. Reagents and chemicals Used in the experiment:

Reagents and elements necessary for estimating DPPH scavenging activity are describing bellow-

2.3.1. Name of reagent-

1. DPPH (1, 1-diphenyl-2-picryl hydrazyl)

2. Methanol

3. L-ascorbic acid

2.3.2. Reagent preparation:

To prepare a 0.004% (w/v) DPPH solution, at first 2 mg of DPPH was dissolved within 50 mL of distilled water. After that the dissolved solution was refrigerated at 4°C cooling the solution till before use.

2.4. Preparation of sample and standard:

In the beginning, the stock solution of the sample was prepared by diluting 120 mg of pulp extract in approximately 10 mL of methanol. This took place in order to get a concentration of 12 mg/mL. here basically, serial dilution of the sample stock solution was performed to create six concentrations of the sample and all of them were serially diluted. The resulting concentrations were as follows: 100, 80, 60, 40, 100, and 20 μg/mL. With the help of same technique, the preparation of standard L-ascorbic acid was made by the six serial dilution ranging from 100 to 0 μg/mL.

2.5. Preparation of blank solution:

To make the blank solution, 3 milliliters of methanol had been used.

2.6. Procedure:

1. Begin by diluting 1ml of the sample and standard (L-ascorbic acid) fractions by volume in independent clean test tubes.

2) Subsequently, 2 milliliters of DPPH solution (0.004% w/v) was added to every test tube.

3) Following that, the tubes were allowed to incubate at ambient temperature for half an hour. Afterwards, the solutions and control (DPPH and methanol) were measured for absorbance.

taken using a spectrophotometer (U-2910 UV-Vis Spectrophotometer) at 517 nm in comparison to a blank solution of methanol.

4) Lastly, the coefficient of variation was used for calculating the free radical scavenging activity percentage (% FRS) in the manner described below:

% Inhibition of free radical scavengers = $(A_0 – A_1) / A_0 \times 100$

Whereas, A_0 = The absorbance of the control

A_1 = The absorbance of the sample/standard

Chapter :3

Result:

In this study the Antioxidant activity of *N.fruticans* wurmb pulp was evaluated using the DPPH method ,which is a widely used technique for determine the free radical scavenging potential of various substances. Here the IC50 value of N.fruticans wurmb pulp explains its capacity as a moderate potential antioxidant with the standard Ascorbic acid. These finding explain this plant as a great source of natural antioxidant

Here, DPPH radical scavenging activity of sample *N.fruticans* and the standard-ascorbic Acid is given bellow with their respective IC_{50} values-

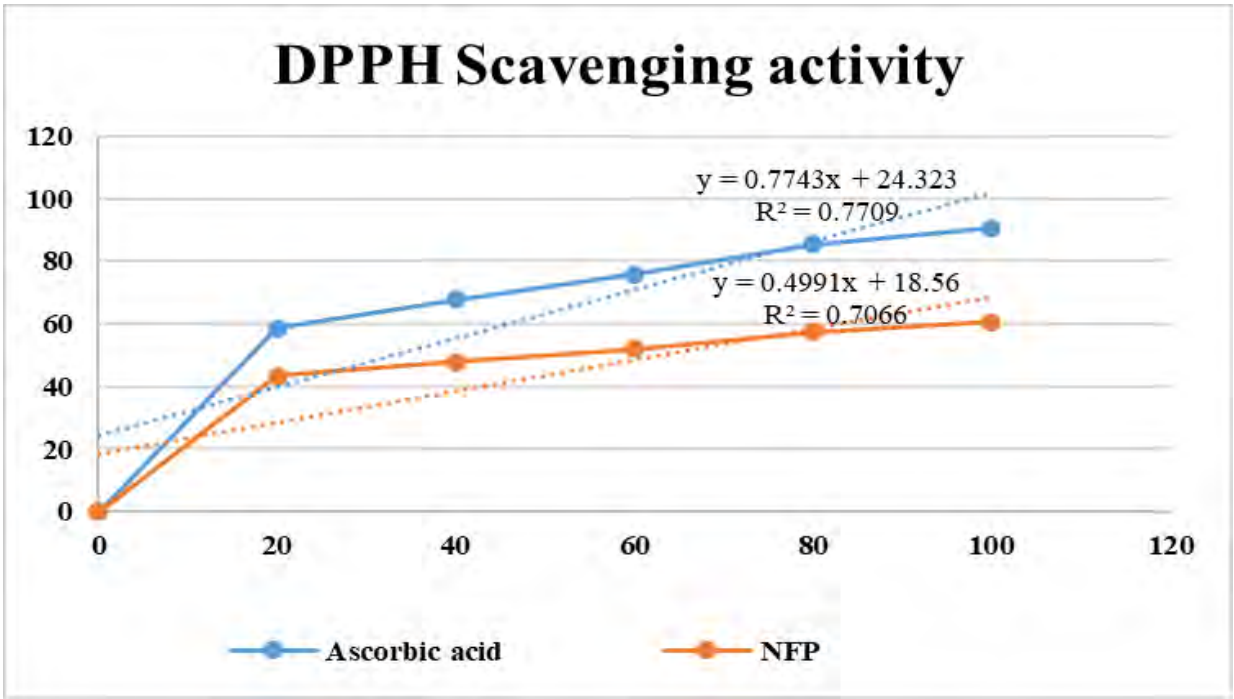

Figure11: analysis of the DPPH radical scavenging activity of ascorbic acid and N. fruticans wurmb pulp:

The evaluated IC_{50} value of ascorbic acid 33.16 µg/ml and the NFM extract IC_{50} value was 62.99 µg/ml.

Table 5: IC_{50} vlues of *N. fruticans* wrombs methanolic extract and standard ascorbic acid:

Evaluated product	**IC_{50} Value**
NFM extract	62.99 µg/ml.
Ascorbic Acid	33.16 µg/ml

Chapter :4

Discussion:

Natural plants are the great source of antioxidants from the ancient time which are drawing attention for lots of research purpose. These natural can be a great alternative to serve the mankind to reduce the oxidative stress in human being and can contribute in the discovery of new drug molecules. In this study antioxidant activity of *N. fruticans* wurmb (NFM) pulp, determined by using the DPPH method, where displays notable evidence on how effective it is in comparison with ascorbic acid. The IC_{50} value of ascorbic acid is 33.16 µg/mL on the other hand, the sample from *N. fruticans* wurmb (NFM) includes an IC_{50} value of 62.99 µg/mL, indicating a reduced antioxidant capacity. The IC_{50} value of 62.99 µg/mL for NFM demonstrates moderate antioxidant activity, as the value is higher than That of ascorbic acid.

Previous research has shown that developed nipa fruit pulp emphasizes improved antioxidant capacity, which indicates that ripeness increases antioxidant capacities in the fruit pulp (Khairi et al., 2022). *N. fruticans* wurmb contains numerous components for example-flavonoids, including other substance called kaempferol and quercetin, which basically helps to improve its antioxidant features. The greater amount of presence of these compounds reveals the potential of *N. fruticans* wurmb as an alternative antioxidant source, although its effectiveness is less to that of ascorbic acid (Raharjo et al., 2024). *N. fruticans* wurmb (NFM) pulp displays a moderate antioxidant potential, even though its IC_{50} value indicate it may be less powerful than ascorbic acid.

Chapter 5:

Conclusion

5.1 Conclusion

In conclusion, the DPPH antioxidant examination of *N. fruticans* wurmb pulp indicates significant promise as a natural antioxidant source as compared to the standard ascorbic acid. The investigation showed that the antioxidant potential of *N.fruticans* wurmb pulp is dependant onto the fruit's ripeness, with the maturation of fruit there is a significant range of an increased antioxidant capacity. The antioxidant effects of Nypa fruticans sap displayed great findings, including multiple extracts revealing beneficial DPPH radical scavenging activity, however less potent than ascorbic acid, that had an IC_{50} of 62.99 µg/ml.

Therefore, the antioxidant potential of *N. fruticans* wurmb pulp positions itself as a great competitive candidate for the development of functional foods, dietary supplements, or even pharmaceuticals aimed at enhancing human health by reducing chronic diseases.

5.2 Future work

This discovery improves our knowledge of the possible therapeutic uses of natural products and encourages additional studies on *N. fruticans* wurmb to discover its potential. Several active therapeutic compounds may be obtained from the pulp of this plant. In alongside it can be use in the field of medical treatment. The extract has other uses which could potentially be advantageous in other sectors including biotechnology or agriculture

Further studies might focus on the enhancement of its antioxidant activity through processing or by incorporating it with various other natural antioxidants. Even, in future appropriate extraction parameters might involve that can enhance the activity and the potential of antioxidants.

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