Antioxidant and Hypoglycemic Effect of *Nypa Fruticans wurmb* fruit shell

By

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A thesis submitted to School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. BRAC University

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Declaration

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Approval

The project titled "Antioxidant and Hypoglycemic Effect of *Nypa Fruticans* fruit shell" submitted by Saosan Salmin Alam (19146060) of Spring-2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on December 2023.

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Ethics Statement

The ethical permission for animal experiments has been provided by the pharmacy department of Jahangirnagar University.

Abstract

Plant extract-based natural medicines are becoming more and more popular as a substitute for synthetic drugs, which raises safety concerns. This study examined *Nypa fruticans's* hypoglycemic and antioxidant impact. We have chosen to assess the antioxidant and hypoglycemic qualities of the methanolic extract of *N. frutican's* fruit shell (MNF) to demonstrate this. So, DPPH (1, 1 diphenyl-2-picryl hydrazyl) test was done for the percentage scavenging of methanolic extract of *N.fruticans* in contrast to Ascorbic acid for antioxidant activities This study shows that the *N.fruticans* fruit shell sample's methanolic extract has an IC_{50} value of 50.59 g/ml, which is indicative of its antioxidant properties, compared to the potent antioxidant ascorbic acid's IC_{50} value of 44.11 g/ml. Swiss albino mice were given 10% glucose solution as a reference medication and allowed to undergo the Oral glucose tolerance test (OGTT) for one to three hours. Following an hour, the NFS600 glucose level declined to 12.17±2.36, which is almost identical to the usual 8.58±1.84. The remaining outcomes followed one another. Because of this, the methanolic shell of *N. fruticans* has antioxidant and hypoglycemic properties that may help advance medical research in the area of diabetes diagnosis.

Keywords: *Nypa fruticans* Hypoglycemic, Antioxidant, DPPH,

Dedication

Dedicated to my parents, who have guided me through every step of my life and have brightened my world since I was a child.

Acknowledgment

Alhamdulillah, all the praise is due to Allah (SWT), who has blessed me with the physical and mental health necessary to complete this project paper patiently.

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Above ground, I owe my family gratitude for inspiring me to work harder and complete this last paper. May Allah SWT continue to favor us

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List of Acronyms

DPPH – 1,1 diphenayl-2-picryl hdryzyl

N.fruticans -Nypa fruticans

WHO-World Health Organization

ME-Methanolic extract

ICDDR, B- International Centre for Diarrheal Disease Research ,Bangladesh

MNF-Methanolic extract of the fruit shell of *Nypa fruticans* OGTT-Oral

Glucose Tolerance Test

Chapter 1

1.1 Introduction

The condition known as diabetes mellitus is a complex metabolic disease of the endocrine system characterized by hyperglycemia caused by abnormalities in insulin production, action, or both. The kidneys, heart, skin, and brain are just a few of the organs that are impacted by this metabolic disorder's numerous macro- and micro-vascular problems. One theory for the pathogenesis and progression of diabetes and related vascular problems is that oxidative stress is a major element in these processes (Yusoff et al., 2015). In every given year, the use of herbal medicine is pervasive and expanding. Healthcare practitioners need to be conversant with this therapeutic approach because many herbal medications have high pharmacological activity and hence have the potential to produce negative side effects and drug interactions (Sweiss et al., 2023).The goal of the study is to evaluate the methanolic extract of *N. Fruticans* shell hypoglycemic and antioxidant capabilities to apply the results to the human body for a more effective anti-diabetic and anti-oxidant medicine. The inaugural experiment on Swiss albino rats was conducted for this purpose, and the results are explained in more detail below. The hypoglycemic characteristic and antioxidant features aid in the management of diabetes and Alzheimer's disease, respectively.

1.1.1 Herbal Medicine

Since the dawn of human civilization, herbal medicine has been used by mankind for healing. Herbal products have fallen out of favor with the rise of "modern medicine," especially in western nations. However, the usage of herbal medicine for healthcare has been progressively rising in recent years. likewise, are growing worries about the quality, efficacy claims, and safety of herbal items used in nutraceuticals, health foods, cosmetics, and herbal medicine. Despite the fact that herbal items have historically been used safely, several herbal products, including herbal medicines, have been linked to serious side effects. The two main causes of the negative effects are thought to be unintentional and intentional adulteration. Because herbal therapy takes a comprehensive approach, evaluating its claimed efficacy can be

challenging. The procedures utilized for modern pharmaceuticals are modified in order to provide useful methods for evaluating the efficacy of herbal therapy. The importance of upholding herbal medicine standards has been emphasized, and practical methods of ensuring their quality by analyzing their chemical fingerprints using contemporary instruments are highlighted. The necessity for quick documentation arises from the fact that traditional herbal medicine knowledge is widely dispersed throughout the world at the family and community levels, and especially so among indigenous people (Mosihuzzaman, 2012).

1.1.2 History of Herbal Medicine

Long before recorded history, people employed plants for medical purposes. Plant medicines have been described in Chinese and Egyptian papyrus writings dating back as 3,000 BC. Herbal medicines are used in traditional medicinal systems such as Ayurveda and Traditional Chinese Medicine. Native American and African indigenous societies also used botanicals in their healing traditions. Researchers found that the same or related plants were commonly used for the same purposes by humans all throughout the world. When chemical analysis first became practical at the beginning of the 19th century, scientists began to extract and modify the active ingredients from plants. Over time, the usage of herbal treatments decreased in favor of the use of medications as chemists started creating their versions of plant substances. Approximately one-fourth of pharmaceutical drugs are derived from plants (Mount Sinai, 2000).

1.1.3 Medicinal Plants

A plant is said to be medicinal if one or more of its organs contain elements that can be used therapeutically or as building blocks to make beneficial drugs. It is possible to distinguish

between plants whose therapeutic properties and constituents have been scientifically proven and plants that are thought to be medicinal but have not yet undergone thorough scientific research by using this description (Sofowora et al., 2013).

According to WHO data, almost 80% of the world's population still uses herbal medicines,

and many contemporary drugs have their origins in medicinal plants. Classic examples of natural chemicals that have been utilized for a long time as sources of therapeutic remedies are salicylates (from willow bark), quinine (from cinchona), ergotamine (from diseased rye), and digitalis (from foxglove). Biological, molecular, botanical, and phytochemical methods are all employed in the multifaceted process of finding novel medications from natural sources. Different research methodologies and plant selections are employed, according to studies on the antibacterial in vitro activity of recognized medicinal plants used to treat infections in Mexico. To verify the effectiveness of chemicals and plant extracts, defined experimental techniques and in vivo pharmacokinetic investigations are needed. It offers a multitude of knowledge on the antibacterial medicinal herbs that are often used in Mexico and have been the focus of scientific study. We anticipate that this evaluation will be helpful for future study since it provides a good information tool for selecting the most significant plants and their potential antibacterial properties (Sharma et al., 2017)

Considering this, the creation of pharmaceuticals derived from medicinal plants remains an essential, unexplored area in which a thorough investigation might surely result in important findings against a range of pharmacological targets. Paradoxically, the potential advantages of plant-based medicines are leading to a situation where unscientific resource exploitation occurs all over the world (Sen & Samanta, 2015).

1.1.4 The position of Bangladesh in medicinal plants

Bangladesh, a subtropical nation, offers an abundance of readily accessible medicinal plants. In the past, herbal medicine businesses in the nation sourced 20% of their supplies from imports and 80% of their needs from natural forests. There are 700 medicinal plants in the nation, 255 of which are used to make Ayurvedic and Unani medications. However, a lot of value chain participants and farmers are ignorant about the viability and sustainability of

these plants. In the 1990s, commercial production started, primarily in the Natore region (Helal Uddin, 2022)

Bangladesh has a long history of using medicinal plants, and it remains a crucial aspect of the nation's healthcare system. Diabetes mellitus and its treatment have recently come under the spotlight of concerns regarding public health in Bangladesh due to the disease's sharply rising prevalence (Ocvirk et al., 2013).

1.2 Literature Review

1.2.1 *N. fruticans* and its morphology

N.Fruticans, also known as Nipah Palm, Mangrove Palm, Nipah, Attap, Water Coconut falls under the family Arecaceae(Palmae) (National park, 2023) One of the oldest living palms, the nipa palm, is an essential part of the East Asian mangrove environment. It grows in the Sundarbans, the world's biggest mangrove forest, and is employed for a variety of things, including construction, food, fuel, medicine, cigarette packaging, wine, and fishing. The sap of the palm tree can be used to make sugar, vinegar, and alcohol. It also acts as a first line of defense against hurricanes, cyclones, and tsunamis, minimizing coastal damage (Farid Hossain, 2015).

Nipa is a monoicous, pleonanthic palm that also displays viviparous germination, much like many other mangrove species. The nipa palm's dichotomously branching underground rhizome grows to a maximum length of 50 cm, from which it sprouts leaves that can grow up to 10 meters in height. Rather than appearing to grow from an erect trunk, the leaves of this species sprout from the ground. As the younger leaves emerge from the center of the crown, the older ones push them aside before they wither and fall off, leaving behind scars or bulbous leaf bases. A single leaf can grow up to eight meters in height, and the cluster's diameter may reach up to 75 centimeters. The full crown may feature six to eight active leaves and twelve to

fifteen bulbous leaf bases at any one moment. In a plantation, leaf harvesting usually starts with plants that are 6–7 years old. A slanting cut that keeps the angle at 45 degrees is used. The cutting height above the ground is influenced by the planting density. When cutting, one should do so at a height of 7 or 8 cm above the ground in cases of high density and 5 or 6 cm in cases of low density. January and February were used for harvesting since fresh shoot development begins

in March(Farid Hossain, 2015).

Figure 1: *N. fruticans* [5] **(Farid, 2015)**

1.2.2 Taxonomical Classification and its origin

Table 1:Taxonomical classification of *N.fruticans*

kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Division	Tracheophyta
Subdivision	Spermatophytina

Class	Magnoliopsida
Superorder	Lilianae
Order	Arecales
Family	Arecaceae
Genus	*Nypa*
Species	*fruticans*

Asia was once supposed to have been the initial range of this species, which then spread to Europe, Africa, and America. Its current range is restricted to the tropical Indo-West Pacific region, which includes the Western Pacific islands, Northern Australia, and Sri Lanka. This suggests that the climate is changing and/or that adaptable genotypes that can withstand a wider range of environmental conditions are disappearing (Farid Hossain, 2015).

1.2.3 Traditional use of *N. fruticans*

The Araceae family member *N. fruticans Wurmb.* (*NF*) is regarded as a "underutilized" plant. NF Thunb., Cocos nypa Lour., and Nypa palm are further names for this species. This monoecious palm grows in brackish water, has an erect stem, is trunkless, and often sprouts fruits from the ground. It is commonly found in the Philippines, Indonesia, Malaysia, India, and some areas of Queensland, Australia. The native peoples eat the young fruits while drinking the sap (obtained from the flower stalk), which is useful for making vinegar, beverages, confectionery, and alcohol. The fruit is also a good source of fiber, minerals, vitamins, and carbohydrates. The leaves, stem, and roots of NF are traditionally used for treating asthma, leprosis, TB, sore throats, liver disease, snake bites, pain relief, sedative, and carminative properties. Recent studies have demonstrated that *N.fruticans* stem and leaf

methanol extracts have anti-diabetic and analgesic effects (Prasad et al., 2013).

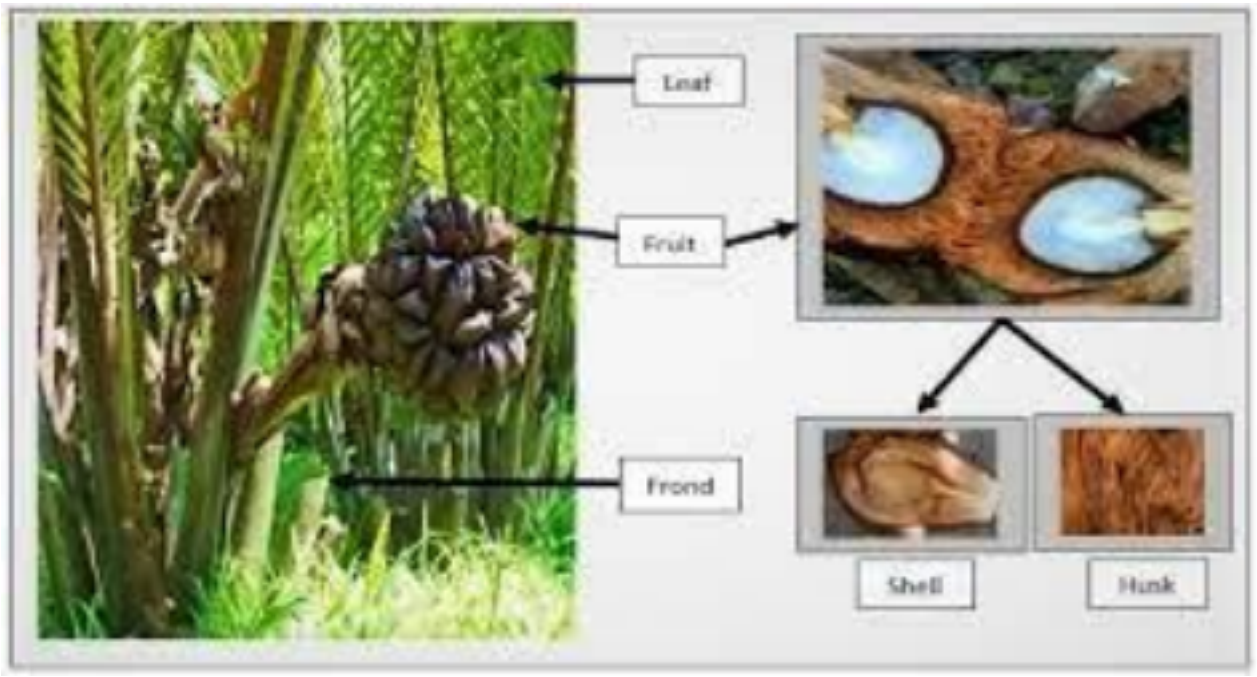

Figure 2:Potential parts of *N.fruticans*(Evelyn et al., 2022)

Chapter 2

2.1 Methodology

2.1.1 Collection of plant parts and identification

In 5th March 2023, the leaf was collected from the Sundarbans, or mangrove forest, in Bangladesh's Bagerhat district. The Bangladesh National Herbarium in Mirpur, Dhaka, where the DACB accession number 87898 has been deposited, acknowledged it.

2.1.2 Extraction of plants

Generally, the first plants on the plantation to have their leaves gathered were those that were between seven and eight years old. A 45° angle is maintained by the slanting cut's use. The cutting height above the ground is dependent on the density of the plants. When cutting, it is done at a height of 7 or 8 cm above the ground in high density situations and 5 or 6 cm in low density situations. Since the formation of new shoots begins in March, harvesting is conducted

in January and February(Farid Hossain, 2015).

2.2 Experimental Animal

To examine the pharmacological effects of the substance under investigation, Swiss-albino mice were used as the test animals. The Jahangirnagar University in Savar's animal research institute provided the study's animals. Each mouse was 30-35 grammes in weight and was 4-5 weeks old. The "International Centre for Diarrheal Disease Research, Bangladesh" (ICDDR, B) reviewed and approved the work. The mice were provided with ICDDR, which is B's favored diet for rodents, as well as frequent access to water in a setting that was conducive to their well-being. Multiple plastic cages purchased from BIK firms in India were used to house

the animals, while softwood shavings purchased from nearby lumber stores were used as bedding.

Every mouse was housed in a cage with a 12-hour cycle of light and darkness, and the environment was regulated at 23.2°F and 60.10% relative humidity. Before the actual experiments started, the mice spent a week becoming accustomed to the lab environment.

The above processes were carried out in compliance with the ethical guidelines approved by the institute's committee for the ethical research of animals**.**

2.3 Design of the experiment:

2.3.1 The DPPH method's free radical scavenging activity

In organic solvents such as methanol or ethanol, the absorbance drop at 517 nm is commonly measured to assess DPPH radical scavenging capabilities. Methanol consumption is not advised due to its dangerous nature. Analyses in 1 mL or 3 mL cuvettes were performed using a UV-vis spectrophotometer. A freshly created stock solution of 10−3 M DPPH radicals in ethanol or methanol was prepared for this purpose before analysis. To make the DPPH

solution, 3 mL of the stock solution was diluted to 50 mL with methanol and covered with aluminum foil to keep out light. The absorbance values were established at 1.00 ± 0.200. Subsequently, 0.5 mL of extract was combined with 3 mL of DPPH working solution, mixed, and allowed to sit in the dark for 30 minutes. Antioxidant agents cause the purple tint in the reaction media to disappear. A reference sample was prepared in a similar way using 0.5 mL of solvent. A freshly prepared DPPH radical solution exhibits its greatest absorbance at 517 nm. Each analysis was run in three duplicates, and the absorbance at 517 nm was recorded. The blank is the reaction mixture that has no test chemicals in it. Using a range of antioxidant doses, the antioxidant concentration that scavenges 50% of the initial DPPH radicals in a specified but arbitrary time

interval is identified. The antioxidant's capacity to remove DPPH radicals increases with decreasing IC_{50} values. In this regard, biochemistry frequently uses the IC_{50} value to assess the capabilities of various antioxidants to scavenge radicals. The radical scavenging affinity is quantitatively described by the IC_{50} values. The IC_{50} value is among the most useful methods for assessing DPPH radical scavenging affinities because of all these factors. The following formula was used to determine the maca extracts' radical scavenging activity (RSA):

$$\text{RSA}\ (\%) = \left(\frac{\text{Ac}-\text{As}}{\text{Ac}}\right) \times 100$$

or

$$\text{RSA}\ (\%) = \left(1 - \frac{\text{As}}{\text{Ac}} \times 100\right)$$

where Ac is the absorbance of the control sample (which may include plant extracts or pure chemicals) at 517 nm and as is the absorbance of the test sample at that same wavelength. The graph that plotted the scavenging % versus the test sample concentration (µg/mL) was used to compute the IC_{50}. The amount of DPPH radicals drops dramatically when exposed to radical remover(Gulcin & Alwasel, 2023).

2.3.2 Hypoglycemic activity

Using a Oral glucose tolerance test (OGTT) , *N. fruticans* hypoglycemic action was demonstrated. Following a fortnight of administering the plant extracts, the animals were

allowed to fast for 12–14 hours while still having unrestricted access to water, and their blood glucose levels were assessed four times during this period. A 1 mL/kg amount of glucose solution (10%) was given orally. After the glucose solution was administered, blood samples were taken 30, 60, and 120 minutes apart (Tafesse et al., 2017)

The percentage of blood glucose reduction can be obtained using the following equation:

$$\%\text{blood glucose reduction} = \left(\frac{\text{BG control} - \text{BG test} \times 100}{\text{BG Control}}\right)$$

The average blood glucose level for each group is indicated here by BG.

Chapter 3 Result

3.1 Result of Antioxidant activity

It was shown that concentration affected the proportion of DPPH radical scavenging. The graphs and table below demonstrate the results of the DPPH scavenging activity using the IC_{50} value of the methanolic extract of *N. fruticans* and standard ascorbic acid. Result of Antioxidant activities**:**

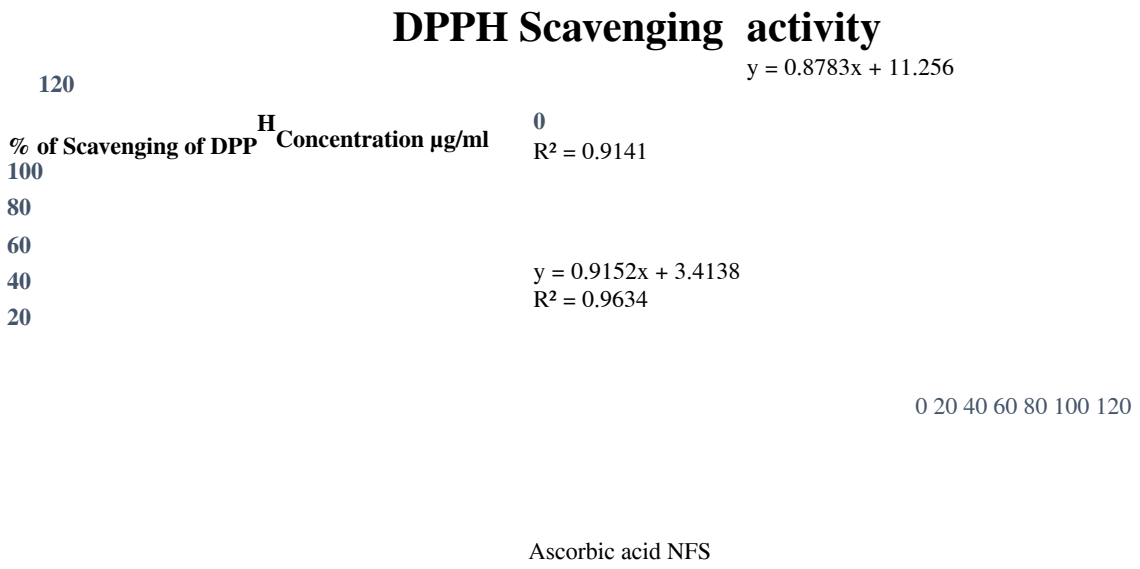

Figure 3: Percentage scavenging assay of DPPH

The IC_{50} value of standard ascorbic acid is 44.11 µg/ml and methanolic extract of *N. fruticans* 50.59 µg/ml which emphasis on the greater antioxidant properties.

Table 2: IC_{50} values of *N.fruticans* methanolic extract and standard ascorbic acid

IC_{50} value of NFS	IC_{50} value of STD ascorbic acid
50.59	44.11

3.2 Result of hypoglycemic activity:

This table displays the glucose levels over time for the experimental mice that were given 10% glucose injections, the control group, the standard group, and all other groups. And as time goes on, these findings about the methanolic extract of *N. fruticans'* hypoglycemic impact are seen**.**

Table 3: Hypoglycemic activity of different mice

Group	1 Hr	2 Hr	3 Hr
Control	20.27±1.49	16.07±2.23	13.2±1.64*
Std (Metformin)	8.58±1.84*	7.33±0.94*	3.9±0.44
NFS 200	18.75±4.4*	16.38±3.79*	12.23±3.8
NFS 400	15.28±2.29*	12.7±2.87	9.73±2.82*
NFS 600	12.17±2.36	9.58±1.8*	7.9±1.49*

Chapter4

Discussion:

4.1 Scavenging DPPS technique

A stable diamagnetic molecule, DPPH is a nitrogen-centered free radical that readily receives an electron. During the course of the experiment, we observed that this activity had risen in comparison to the growth of the sample *N. fruticans* methanolic extract. The DPPH absorbs at

517 nm and has a rich purple color because it has one odd electron.

Therefore, when the DPPH takes an electron, the absorbance changes, leading to its decolorization. The DPPH-scavenging activity of *N.fruticans* methanolic extract (IC_{50} 50.59 g/ml as opposed to ascorbic acid IC_{50} 44.11 g/ml) was then plotted about concentration in the graph. Therefore, *N. fruticans* methanolic extract shows greater antioxidant activity than ascorbic acid.

4.1.2 Hypoglycemic activity:

Herbal medicines are becoming more popular among diabetics who want to reduce their health risks. In the experiment, we saw that rats with hypoglycemia had a considerable drop in serum glucose. In the 200 mg/kg, 400 mg/kg, and 600 mg/kg groups, 10% glucose was given, and the results for 1, 2, and 3 hours revealed a significant decrease in their glucose level. These results were reported ($p<0.05$ & $p<0.01$). The diabetic 600mg/kg body weight caused the highest reduction to 7.91±49mmol/L on furthering the experiment with the methanolic extract of *N. fruticans,* which is close to the standard value. We can therefore conclude from this study that the methanolic extract of *N. fruticans* increases the absorption of insulin by encouraging the ingestion of glucose.

Chapter 5

Conclusion

Scientists are interested in natural antioxidants from plants because of their natural source and lack of harmful side effects. Natural food products are rapidly using exogenous antioxidants such ascorbic acid, network tocopherols, carotenoids, and polyphenols. In comparison to other substances, mangrove trees have higher antioxidants. Nipa, a plant having salicylic acid, can lessen salt stress and boost antioxidant activity. Nipa's great antioxidant potential can be put to use in the food and pharmaceutical industries for profit. Nipa has antioxidant-capable phenolic compounds, alkaloids, and flavonoids to fight free radicals and autoxidation events in lipid

oxidation. These compounds provide a defense against chronic disorders by acting as free radical scavengers. With an IC_{50} value of 44.11g/mL, nipa has the greatest antioxidant activity of any plant. With a great deal of promise for commercialization as healthful foods and beverages, NPV's antioxidant capabilities can help the body fight off free radicals (Nugroho et al.,2022) Blood sugar (glucose) levels in people with diabetes mellitus are unusually high because not enough insulin is being produced by the body to meet those needs. According to the current early experimental findings, *N.fruticans Wurmb*. significantly reduced blood sugar levels in glucose-induced (Reza et al.,2011).

5.1 Future perspective:

N. fruticans fruit shell extract has strong hypoglycemic and antioxidant properties, making it useful as a diabetic medication. By selectively inhibiting intestinal glucose transporters, it decreases postprandial hyperglycemia by delaying the small intestine's absorption of carbs (Yusoff, Ahmad, et al., 2015)

Ultra-high-performance liquid chromatography was used to identify and quantify eight phenolic components from *N. fruticans* endosperm extracts. The three main phenolic constituents were kaempferol, protocatechuic acid, and chlorogenic acid. As a result, this fruit may be utilized as a natural antioxidant source (Prasad et al.,2013b).

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