Studies on the phytochemical and Pharmacological potential of the root and bark of Moringa oleifera.

PROJECT WORK

THIS IS A PROJECT REPORT THAT FULFILLS SOME OF THE REQUIREMENTS FOR A BACHELOR OF PHARMACY DEGREE. IT HAS BEEN SUBMITTED TO THE DEPARTMENT OF PHARMACY AT DAFFODIL INTERNATIONAL UNIVERSITY.

SUBMITTED TO

DEPARTMENT OF PHARMACY, FACULTY OF HEALTH& LIFE SCIENCES

DAFFODIL INTERNATIONAL UNIVERSITY

SUBMITTED BY

Student ID: 201-29-401

Batch: 23rd

Department of Pharmacy

Daffodil International University

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Date of submission:

APPROVAL

A project paper investigating the medicinal properties of the root and bark of Moringa oleifera has been submitted and accepted by the Department of Pharmacy at Daffodil International University for partial fulfillment of a Bachelor of Pharmacy degree.

BOARD OF EXAMINERS

Professor Dr. Muniruddin Ahmed

Chairman

Head of the Department,

Department of Pharmacy

Daffodil International University.

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- **Internal examiner----------**
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DECLARATION

I hereby declare that this project report is done by me under the supervision of **Dr. Ekramul Haque Professor**, Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for a Bachelor's or any degree award.

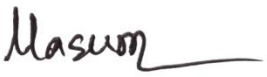
SUPERVISED BY:

DR. Md. Ekramul Haque

Professor

Department of Pharmacy,
Daffodil International University

SUBMITTED BY:



Md. Masum Parvez

ID: 201-29-401

Batch: 23rd
Department of Pharmacy,
Daffodil International University

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Md. Masum Parvez

DEDICATED TO MY BELOVED PARENTS, RESPECTED TEACHERS, AND WELL-WISHERS.

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ABSTRACT

Moringa oleifera Lin is the most commonly found to grow wild in Bangladesh and its leaves and fruits are considered to be a superfood. the present study reports the phytochemical screening and some biological works from the bark and root bark extracts. The phytochemical test showed the presence of alkaloids, glycosides, steroids, flavonoids, tannins, gum, and three biological works as Hepatoprotective activity was performed by SGPT test against silymarin (as a standard) and anti-glycemic activity was performed as well as antilipidemic activity were performed as against atorvastatin (as a standard) using Wister albino mice. Both bark and root extracts exhibited strong hepatoprotective activity, with bark showing 72.33±4.58 and root 74.33±11.85. The antilipidemic activity was notable in both extracts, with bark at 60.44±4.61 and root at 68.66±11.56. The anti-glycemic activity was observed in both extracts, with bark at 4.93±0.42 and root at 3.23±0.18. These findings highlight the potential of Moringa oleifera bark and root bark as sources of bioactive compounds that possess significant hepatoprotective, antilipidemic, and anti-glycemic properties. Therefore, further exploration and research are warranted to determine their potential therapeutic applications.

CHAPTER ONE: INTRODUCTION

1.1 GENERAL INTRODUCTION

Medicine stands as the world's most profound miracle, safeguarding the well-being of humanity. Across epochs, humans have harnessed diverse botanical components for healing wounds. The realms of pharmacy, medical science, and practice, alongside their primary reservoirs, wield pivotal roles in uncovering novel drug compounds, whether synthesized or sourced from nature. The saga of natural medicine, steeped in significance and renown, underscores the journey of primitive societies. Through relentless experimentation, our ancestors discerned the nutritional value of flora and fauna, distinguishing them from their toxic counterparts. The saga continues, as medicinal plants persist as indispensable allies in alleviating human afflictions. The pursuit of enduring health and vitality impelled ancient civilizations to explore their immediate environs, experimenting with various botanicals, animal derivatives, and minerals to formulate diverse therapeutic remedies. Across millennia, the refinement of these remedies ensued through a process of trial, and error, empirical observation, and deliberate experimentation, culminating in the discovery of what we now recognize as "medicine." In cultures such as those of India, China, and the Arab/Persian world, this knowledge was meticulously documented, shaping the foundations of structured medical systems and becoming integral to their respective material medical. Yet, among the ancient systems of medicine, Ayurveda stands as a paragon of theoretical sophistication and practical insight. With roots deeply embedded in history, the Babylonians, as early as 3,000 B.C., exhibited an awareness of numerous medicinal plants and their properties, some of which persist in contemporary usage. The earliest traces of plant-based medicinal practices in the Indian subcontinent can be traced back to the Rig Veda (4,500–1,600 B.C.) [1] [2]

1.2 STATUS OF MEDICINAL PLANTS IN BANGLADESH:

Being a sub-tropical nation, Bangladesh boasts abundant natural reserves of medicinal plants. In the early 1980s, local herbal medicine companies, primarily Ayurvedic and Unani, relied heavily on domestic forests, satisfying 80% of their requirements locally and importing the remaining 20%. However, this trend has since reversed. Presently, a staggering 80% of the demand is fulfilled through imports, with only 20% sourced domestically. Despite Bangladesh's rich biodiversity, the Bangladesh Agricultural Research Institute (BARI) reports a modest count of 722 medicinal plant species, significantly lower than India's 4,000. Out of these, a mere 700 plants find application in traditional medicine within Bangladesh, with 255 species specifically utilized by Ayurvedic and Unani medicine manufacturers.[3]

1.3 PLANTS IN TRADITIONAL MEDICINE:

The reliance on traditional herbal medicine is widespread, with an estimated 70-80% of the global population depending primarily on these remedies for their healthcare needs. Moreover, the demand for herbal medicine is not only substantial but also steadily increasing. In India, for instance, the market for Ayurvedic medicines is expanding at a remarkable rate of 20% annually, while in China's Yunnan province alone, the quantity of medicinal plants harvested has surged by tenfold over the past decade.[4][5]

This escalating demand is compounded by various factors, including population growth and the insufficient availability of Western allopathic medicine in many developing nations. [6] As a consequence, pressure on gathering sites, such as the Gori Valley in the Indian Himalayas, has intensified, with the annual harvesting period for medicinal and aromatic plants (MAP) extending from 2 to 5 months.[7][8]

1.4 AN OVERVIEW OF M. OLEIFERA

***Moringa oleifera*,** recognized as the miracle tree, is commonly found in Tropical and subtropical regions worldwide, with its origin in Asian countries including Bangladesh, India, Afghanistan, and Pakistan. The moringa family surrounded 13 species, and *M oleifera*, in particular, has obtained property for its multifaceted uses in nutrition, and fertilizer. Noticeably, Moringa exhibits a unique resilience to sunshine. [9] The leaves of *M oleifera* are rich in minerals, calcium, and potassium, dried leaves, boast an oleic acid content of approximately 70%which having application in the formulation of moisturizers. The tree's bark is honored for its efficacy in accosting divers' ailments such as ulcers, toothaches, and hypertension. Roots play a role in treating toothache, helminthiasis, and paralysis. The tree is renowned for its remarkable properties in accosting malnutrition in infants and lactating mothers.[10] The current review aims to present a comprehensive summary of the current understanding of *M oleifera* pharmacological activity, worldwide research analysis, toxicological characteristics, and phytochemical composition. In the Phytochemistry overview, the genus Moringaboasts the identification of over 90 compounds with significant therapeutic potential These isolated synthetic compounds possess a diver's array of flavonoids, sterols, terpenes, tannins, saponins, fatty acids, glycoside, and polysaccharide.[11] [12]

1.5 THE BENEFITS OF NATURAL MEDICINE:

People have practiced natural medicine for centuries, before the advent of modern technology, doctors and their patients had to rely on natural techniques and herbal remedies to treat illness or heal injuries, everything is to like from the common cold to life-threatening conditions such as cancer, diabetes heart disease have been intended to be successfully treated without the use of modern-day medical equipment.[13]

CHAPTER TWO: PURPOSE OF STUDY

2.1 EXPLORING THE THERAPEUTIC POTENTIAL OF MORINGA OLEIFERA: A PATHWAY TO NEW DRUG DEVELOPMENT IN BANGLADESH"

Medicinal plants, containing therapeutic substances in their organs, have been integral to healthcare for years. Plants serve as sources for both medicines and key ingredients in drug formulations. However, the process from plants to pharmacologically active compounds is complex and requires a multidisciplinary approach. Selective collection based on chemical taxonomy and ethnobotanical knowledge increases the likelihood of discovering active compounds. In Bangladesh, where approximately 75% of the population relies on traditional and herbal medicine for primary healthcare, the diversity of local plants is crucial. With around 500 medicinal plant species in Bangladesh, over 300 are commonly used in traditional medicine preparations.[14] Extensive research has been conducted worldwide on various species within the Amaranth and *Moringa oleifera* families, given their potential medicinal properties. In Bangladesh specifically, efforts have been made to explore the chemical composition and biological activity of Moringa oleifera plants. These studies aim to identify and validate the presence of multiple medicinal compounds within *M. oleifera* grown in Bangladesh, highlighting the importance of understanding the therapeutic potential of local plant species for healthcare applications.[15] Therefore, the main goal is to explore the possibility of developing a new drug from *M. oleifera.* which is used to treat various diseases.

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CHAPTER THREE: PLANT REVIEW

PLANT PREVIEW

3.1 Scientific name

Scientific name:*Moringa oleifera*

Family:*Moringaceae;*

3.2Taxonomic Classification:

- Kingdom: Plantae;
- Sub-kingdom: Tracheobionta;
- Super division: Spermatophyta;
- Division: Magnoliophyta;
- Class: Magnoliopsida;
- Sub class: Dilleniidae;
- Order: Capparales;
- Family: Moringaceae;
- Genus: Moringa;
- Species:oleifera

Moringa root

Moringa bark

Fig 1: Different parts of *MoringaOleifera* (From the Internet)

[16]https://www.researchgate.net/figure/Moringa-oleifera-left-bark-pieces-right-bark-powder_fig2_266870872

3.3SYNONYMS [17]

- Drumstick tree
- Ben oil tree
- Horseradish tree
- Miracle tree
- Malunggay (in some regions)
- Sajina (in certain cultures)
- Behenbaum (in German)
- Sahijan (in various languages)
- Kamunggay (in certain regions)
- Shevaga (in Marathi)

3.4COMMON VERNACULAR NAMES [18]

- Drumstick tree
- Horseradish tree
- Ben oil tree
- Miracle tree
- Malunggay (**in the Philippines**)
- Sajina (**in Bengali**)
- Shevaga**(in Marathi**)
- Munaga (**in Telugu**)
- Murungai (**in Tamil**)
- Sehjan (**in Hindi)**
- Mulaga**(in Malayalam)**
- Zogale (**in Hausa**)
- Kamunggay (**in some regions**)
- Saijan (**in Punjabi)**
- Sigru (**in Sanskrit)**

3.5 PLANT DESCRIPTION/MORPHOLOGY

The ***Moringa oleifera*** tree thrives in rapidly growing loamy and well-drained sandy soils, ideally at elevations around 500 meters above sea level. Typically, it is small to medium-sized with naturally trifoliate leaves and flowers arranged on an inflorescence 10–25 cm long. The fruits, often referred to as "pods," are usually trifoliate. The trunk tends to grow straight, although sometimes poorly formed, with disorganized branches forming an umbrella-shaped canopy. The seeds, brown in color, possess a semi-permeable hull, with each tree capable of producing about 15,000–25,000 seeds Annually. [19][20]

3.6 THE PLANT PART UTILIZED IN THE STUDY [21]

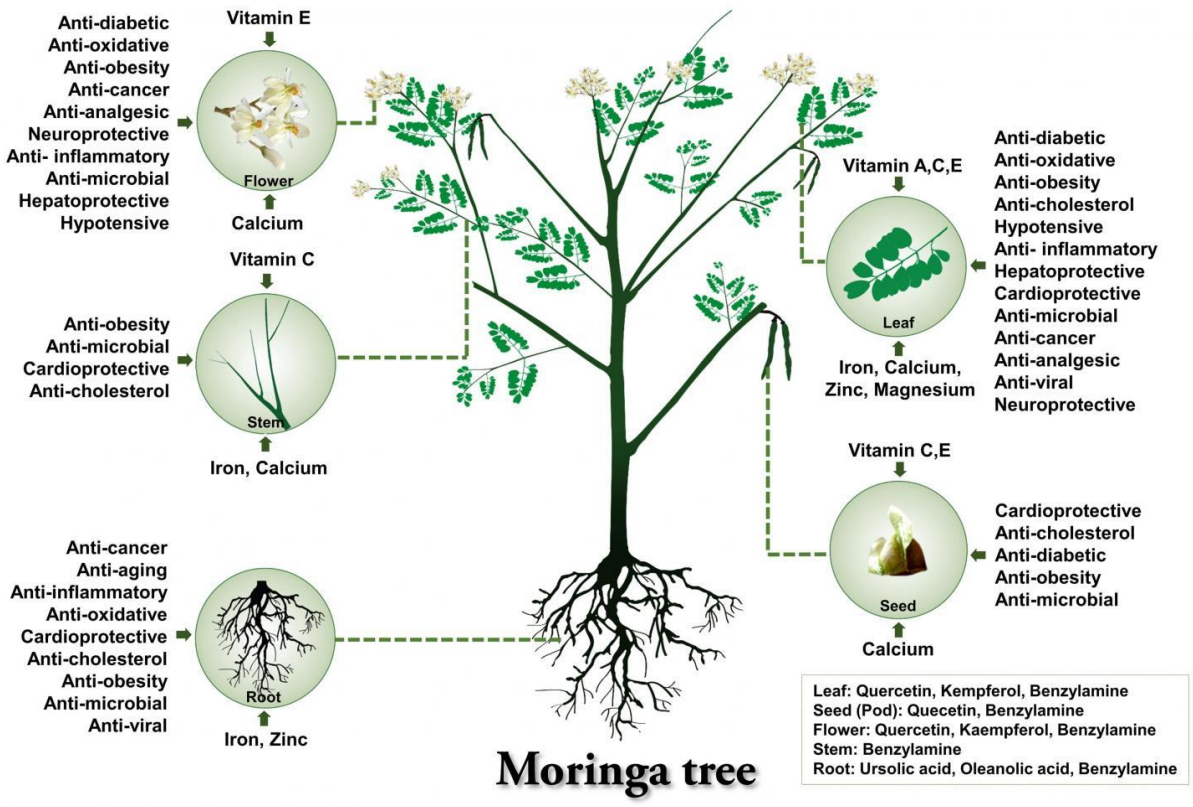


Fig 2:Image of moringa plant parts

https://twitter.com/NCBS_Bangalore

Fig 3: Image of Moringa bark powdered

3.7 TRADITIONAL USES:

For a long time, people in countries like Asia have been eating a plant called M. oleifera because it is good for them. They can eat almost all parts of the plant and it has been used by pregnant and breastfeeding women in Africa, India, and Nicaragua for a long time.[22] [23]***M. oleifera*** is good for you because it has many important nutrients like minerals, amino acids, and trace elements. It's just as good for you as spirulina. In India, Pakistan, the Philippines, Hawaii, and some African countries like Nigeria, people eat different parts of a special tree as food. In Malaysia, people eat the seeds of the tree, while in other places, they use the young leaves in salads or vegetable dishes, or make them into seasonings for everyday cooking.[24]The Moringa tree seeds have special powers that make them stick together and fight off harmful germs. People in Asia, Africa, and other countries use these seeds to clean dirty water. They can also be turned into a type of fuel called biodiesel and used to feed animals because they are very nutritious.[25] [26]***Moringa oleifera*** is a plant that has been used for a long time to help people feel better. In places like India and Thailand, it is used to treat things like paralysis, infections, and fevers. It is also known for making people feel healthier and younger. This review explains how the traditional uses of *M. oleifera* plants could be connected to their modern medical benefits. It talks about how the plant's chemical components can help with farming and nutrition but also mentions some potential risks. Overall, this information can help scientists create new medicines, farming products, or healthy foods.[27] [28] [29]

CHAPTER FOUR: LITERATURE REVIEW

4.1 EVALUATION OF THE POLYPHENOL CONTENT ANTIOXIDANT PROPERTIES OF METHANOL EXTRACTS OF THE LEAVES STEM, AND ROOT BARKS OF MORINGA OLEIFERA LAM.

Medicinal plants can help prevent and treat cancer and other diseases caused by damage to our bodies. One plant called Moringa oleifera is used as both food and medicine in Nigeria and other places. Scientists studied different parts of the plant and found that they contain special substances called polyphenols, which can help fight off harmful molecules in our bodies. The extracts from the leaves, roots, and stem bark of the plant showed strong antioxidant effects, which means they can protect our bodies from damage. This is why people have been using this plant as medicine for a long time. It is also possible that these antioxidant effects can help prevent cancer, but more research is needed to be sure.[30]

4.2ANTIDIABETIC PROPERTIES OF MORINGA OLEIFERA: A REVIEW OF THE LITERATURE

More and more people are getting diabetes, which can be very serious and expensive. Medicinal plants could be a good way to treat diabetes because they are cheaper and have fewer side effects than medicine. A review looked at 36 studies, including ones about how people use plants for medicine and ones that tested the plants in labs. The studies found that different parts of the plants, like leaves and roots, can be used to treat diabetes. This is important because it shows that using plants could be a helpful way to fight diabetes in places where people don't have a lot of money.[31]

4.3 COMPARATIVE EFFECTS OF MORINGA OLEIFERA ROOT BARK AND MONENSIN SUPPLEMENTATIONS ON RUMINAL FERMENTATION, NUTRIENT DIGESTIBILITY, AND GROWTH PERFORMANCE OF GROWING LAMBS

We did two experiments to see how adding Moringa oleifera root bark (MRB) and monensin (an antibiotic) to the diets of ruminants (like lambs) affects their digestion. In the first experiment, we used a machine to simulate digestion and found that MRB reduced

methane production without any negative effects. In the second experiment with real lambs, we saw that MRB improved digestion and overall health, similar to monensin. This shows that MRB could be a good supplement for ruminant diets.[32]

4.4 ANTIBACTERIAL ACTIVITY OF BARK EXTRACTS OF MORINGA OLEIFERA LAM. AGAINST SOME SELECTED BACTERIA

We tested different extracts from the bark of the Moringa oleifera tree to see if they could kill bacteria. We found that all the extracts were able to stop the bacteria from growing, with the ethyl acetate extract being the most effective. The bacteria Staphylococcus aureus was the most easily killed by the extracts. This shows that Moringa oleifera could be a good natural way to treat infections caused by bacteria that are hard to kill with antibiotics.[33]

4.5 BIOACTIVE COMPONENTS IN MORINGA OLEIFERA LEAVES PROTECT AGAINST CHRONIC DISEASE

Moringa Oleifera is a plant that grows in Asia and Africa and is very important for people there. It has been studied because it has lots of good things in it, like vitamins and special substances that can help our bodies stay healthy. The leaves of the Moringa Oleifera plant have been studied the most and they are helpful for many different health problems, like high cholesterol, high blood pressure, diabetes, liver disease, and even cancer. Some studies have been done on animals and cells, but there is not as much information yet about how it helps humans. But overall, it is known that the leaves of Moringa Oleifera are really good for our hearts, diabetes, cancer, and fatty liver.[34]

4.6 MORINGA OLEIFERA: MIRACLE PLANT WITH A PLETHORA OF MEDICINAL, THERAPEUTIC, AND ECONOMIC IMPORTANCE

Moringa oleifera is a very important plant that grows in forests but can also be grown in gardens. It is known by different names in different regions. It is very healthy to eat and has many good effects on our bodies. The different parts of the plant, like the leaves and seeds, are full of good things like protein and vitamins. It can help with many health problems like inflammation and diabetes. [35]

4.7 NOVEL INSIGHTS ON THE ANTI-OBESITY POTENTIAL OF THE MIRACLE TREE, MORINGA OLEIFERA: A SYSTEMATIC REVIEW

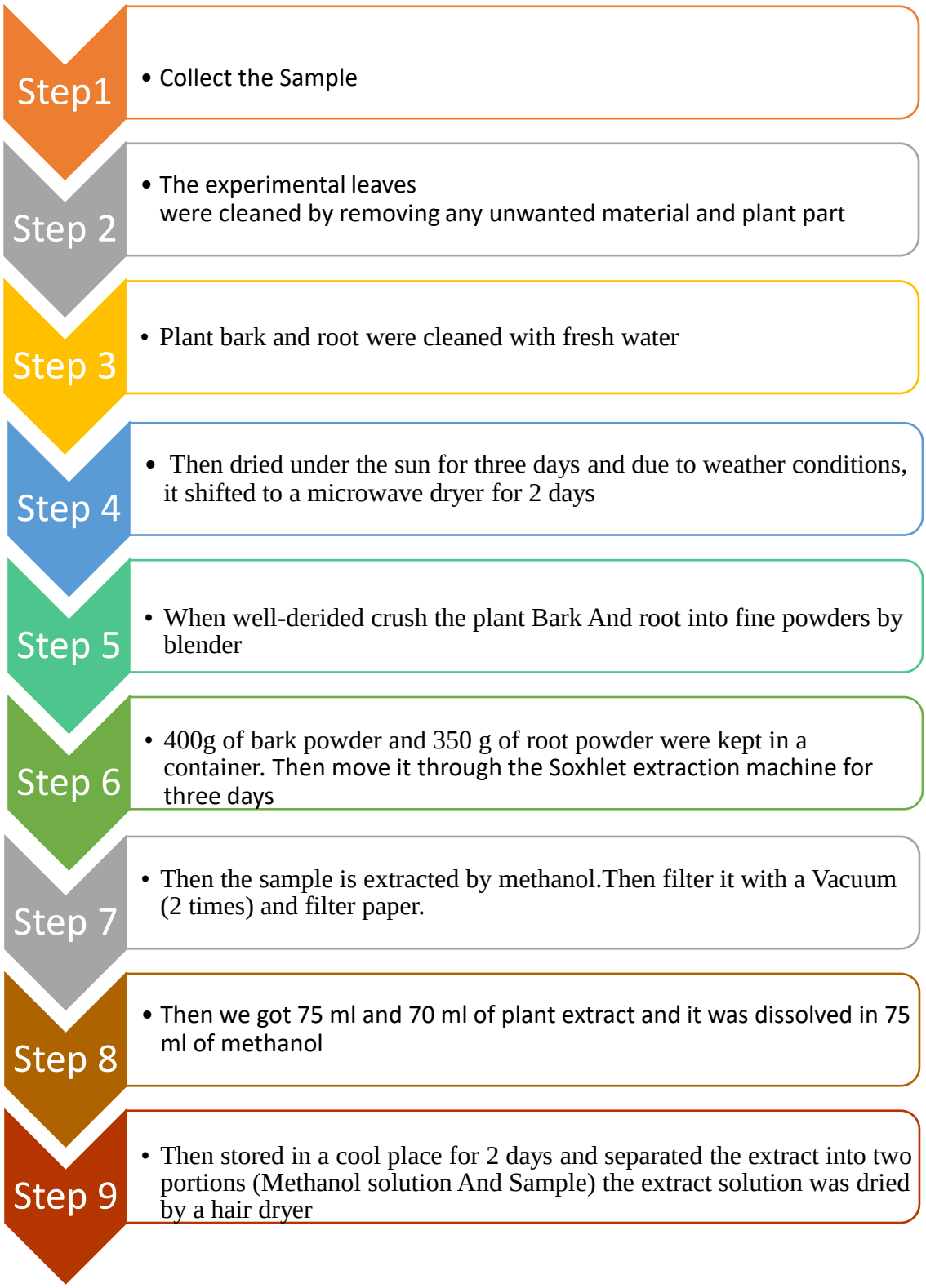
Moringa oleifera (MO) is a plant that has caught the interest of many scientists because it is packed with nutrients and helpful compounds that could be used in medicine. In this review, researchers looked at 36 studies that tested whether MO could help with obesity. They found that MO extracts made from its leaves, and sometimes a particular compound in MO called isothiocyanates could help with weight and cholesterol levels in mice, rats, and a few human studies. MO seemed to work by improving cholesterol levels, and weight, and regulating genes that are important for fat storage, blood sugar, and hormones. However, there is still limited research on how MO affects obesity in humans.[36] [37] [38]

4.8 MORINGA OLEIFERA LAM. AND DERIVED PHYTOCHEMICALS AS PROMISING ANTIVIRAL AGENTS: A REVIEW

People have been using plants for medicine for a long time. One plant called Moringa oleifera is especially helpful for many health problems. It can be eaten in different forms like leaves, fruits, and seeds. The World Health Organization thinks it's important to study natural products like plants for medicine. Scientists are interested in finding new ways to treat viruses because current medicines have some problems and can be expensive. Moringa oleifera has been used in traditional medicine for lots of different conditions like malaria, diabetes, and headaches. It has also shown promise in fighting viruses like HIV and herpes. Some studies have found specific chemicals in the plant that help fight viruses, but we still need more research to understand how it works. It's important to keep studying this plant and make sure it is protected.[39] [40][41]

CHAPTER FIVE: METHOD AND MATERIALS

5.1 PREPARATION OF PLANT FOR THE TEST:



5.2 PLANT COLLECTION:

The experimental plant Moringa oleifera root was collected from the Daffodil Smart City area and the bark was collected from my hometown Bogura. The bark and root of the plant were fresh.

5.3 PREPARATION OF THE MORINGA BARK AND ROOT (DRYING AND GRINDING)

The plant was sourced in its natural form, with any undesirable materials or components carefully removed after collection. To preserve the plant's integrity and prevent any chemical degradation, it was refrained from washing or cleansing the collected plant parts to avoid the risk of hydrolysis, oxidation, or other forms of deterioration. The bark was meticulously separated and left to dry under the gentle morning sunlight, between 10 am to 12 pm, to maintain optimal temperature levels. Instead of direct sunlight, it was opted for indirect sunlight due to the cooler temperatures. Subsequently, a grinder was employed to transform the bark and root into a fine powdered form. To ensure its long-lasting quality, the powder was then securely stored in an airtight container, maintaining a dry, cool, and dark environment for future use.

5.4 SOXHLET EXTRACTION

Soxhlet extraction is when you keep taking out something from a mixture over and over again in a loop. A warm, natural liquid that can dissolve things. Soxhlet extraction is a method used when we want to get a certain substance out of another substance, but it's not very good at dissolving in a liquid. This method helps us separate the substance we want from other things that can't dissolve in the liquid. It can work on its own without someone watching it, and it can reuse a little bit of liquid to dissolve a lot of stuff.[42] [43]

Procedure

Step1
- Throughout the whole process, a solid material is placed in a thimble, which is connected to a flask containing methanol.

Step 2
- Soxhlet extractor has a condenser attached to it

Step 3
- The solvent is heated until it reaches its boiling point and starts to evaporate

Step 4
- The vapor travels up to a distillation arm and enters the chamber where the thimble is located.

Step 5
- The solid material in the chamber gradually fills with warm solvent, allowing the desired compound to dissolve.

Step 6
- When the chamber is almost full, the solvent is emptied back into the distillation flask through a U-shaped siphon.

Fig3: **Soxhlet Extraction on our research lab DIU**

5.5 FILTRATION

Filtration is the process where the extract is filtered by a vacuum filter two times and sterilized cotton and filter paper to filter the extract. The suitable solvents are soaked and go through the funnel. During a glass container tool, the filter was collected, and the filtration process continued. [4

Figure 4: Filtration machine (Source of Internet)

https://www.mrclab.com/

5.6 ROTARY EVAPORATION

We used a special machine called a rotary evaporator to remove the liquid from the extract. We heated it to a temperature between 64 and 65 degrees Celsius and spun it around at a speed of 34-38 times per minute. The liquid that turned into gas was then collected in a container called a beaker that can hold 100 ml. after collecting the original 75 ml of methanolic extract. The pure extract was collected and covered with aluminum foil before being placed under a fan to evaporate any remaining solvent. A hot hairdryer was then used to further remove moisture from the extract. Then we will collect the dried extract.[45] [46]

Fig 5: Rotary evaporation machine Source of our lab

5.7 PHYTOCHEMICAL ANALYSIS

Test Material:

Methanolic extract of *moringa oleifera* bark and root

5.7.1 INSTRUMENT / APPARATUS

- Electronic balance
- Funnel
- Filter paper
- Glass rod
- Stand with a clamp
- Cotton
- Measuring Cylinder
- Test-tube
- Spatula

5.7.2 REAGENTS

- Mayer's reagent
- Dragendorff's reagent
- Benedict's reagent
- DPPH solution
- 5.5% of Ferric chloride solution
- 10% potassium dichromate solution
- Dilute sulfuric acid
- Distilled waters.
- Aqueous sodium hydroxide
- Conc. HCl
- Conc. H2SO4.

Sample of moringa bark and root: The tests listed below were conducted to identify various chemical groups.[47][48][49]

5.7.3 TEST FOR ALKALOIDS:

Mayer's reagent: The addition of 2 ml of the extract, 0.2 ml of dilute hydrochloric acid, and 0.1 ml of Mayer's Reagent results in the formation of a yellowish-buff-colored precipitate, indicating the presence of an alkaloid.

Dragendroff's reagent: A solution containing 2 ml of the extract and 0.2 ml of diluted hydrochloric acid was prepared. To test for the presence of alkaloids, 0.1 ml of Dragendroff's reagent was added. The formation of an orange-brown precipitate indicates the presence of alkaloids

Figure 6: Mayer's Test for Alkaloid Alkaloid

Figure 7: Dragendroff's Test for

5.7.4 TEST FOR GLYCOSIDE:

A small amount of dried plant extract was mixed with water and sodium hydroxide. The lack of a yellow color indicated that there were no glycosides present in the plant

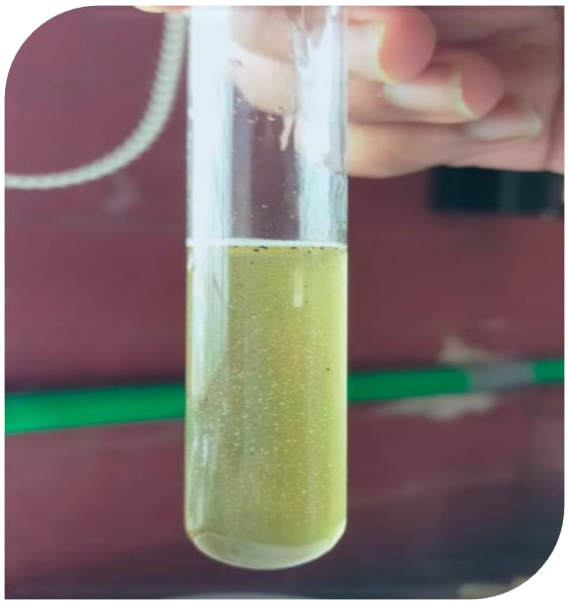


Fig 8: Glycoside test

5.7.5 TEST FOR STEROIDS:

10 mg of extract dissolved in 1 ml of chloroform. The chloroform layer is a reddish-brown color indicating the presence of steroids

Fig 9: Steroid test

5.7.6 TEST FOR GUMS:

5 ml solution of extract. Molish reagent and sulfuric acid. If Red –a red-violet ring is produced at the junction of two liquids, it indicates the presence of gum.

Fig 10: Gum test

5.7.7 TESTS FOR FLAVONOIDS:

1 ml solution of ethanolic extract Few drops of conc. HCl was added to the extract. If an Immediate red color is formed, it indicates the presence of flavonoid

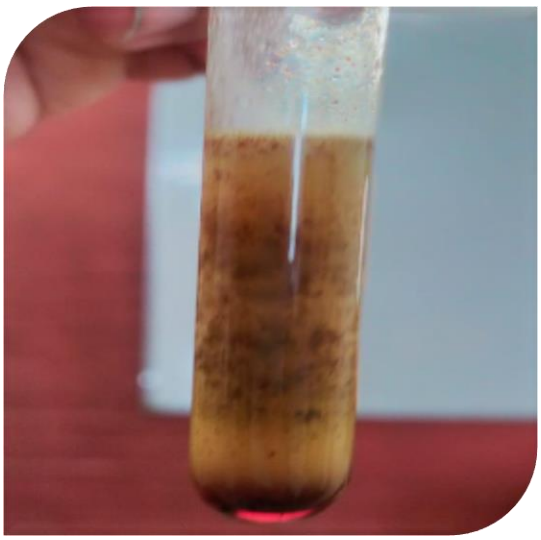


Fig 11: Flavonoid test

5.7.8 TESTS FOR SAPONINS: 1 ml solution of the distilled water was diluted to 20 ml Shaken in a graduated cylinder for 15 minutes. If a centimeter layer of foam is formed, it indicates the presence of saponin.

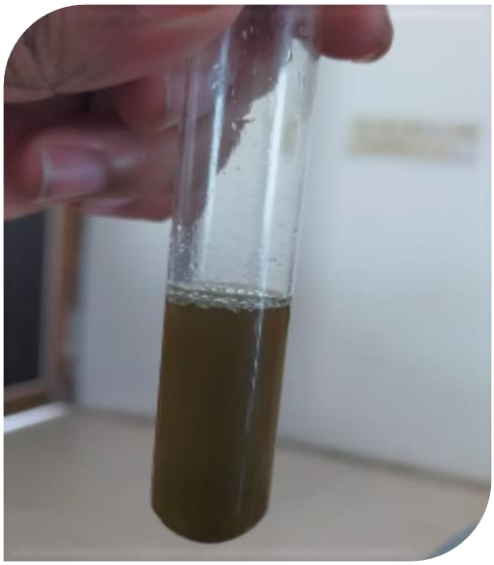


Fig 12: Saponin test

5.7.9 TESTS FOR TANNINS:

5 ml solution of extract 1 ml of 5% of Ferric chloride solution. Greenish black precipitate indicates the presence of tannin.

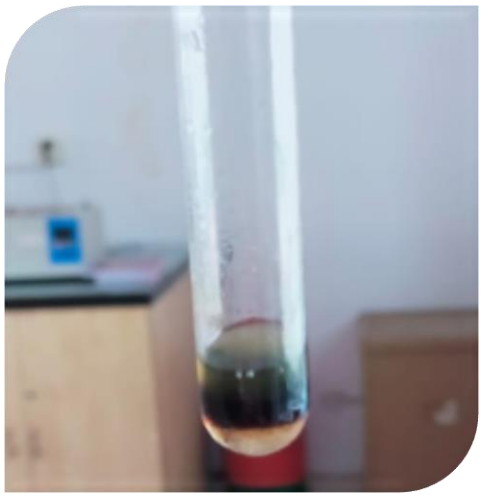


Fig 13: Tannins test

CHAPTER SIX: PHARMACOLOGICAL ACTIVITY, DRUG DESIGN AND ITS WORKING PROCEDURE

PHARMACOLOGICAL ACTIVITY.

6.1 SGPT TEST:

The SGPT test, also called the Serum Glutamic Pyruvic Transaminase test, is a diagnostic tool used to measure the levels of the enzyme GPT in the bloodstream. GPT is primarily produced by key organs such as the liver, heart, kidneys, and muscles. While this test is commonly utilized to monitor liver health, it is a minimally invasive procedure with no adverse effects, aside from the brief discomfort of a needle or prick during blood collection. To maintain optimal liver health, it is imperative to undergo regular SGPT testing, especially if you have a history of liver ailments such as Hepatitis A, B, and C. Even if you have not experienced any liver issues in the past, it is highly recommended to undergo this test biannually to ensure the well-being of your liver. [55][56]

The SGPT blood test is a valuable tool for identifying potential ailments in the heart, kidney, liver, or muscle groups. Primarily utilized for detecting liver issues, this test is commonly recommended by physicians for regular monitoring of liver health in patients.[57] While temporary elevation of SGPT levels may not manifest as physical discomfort, it should be viewed as a significant concern due to its association with potentially serious liver conditions such as cirrhosis or viral infections like Hepatitis. Additionally, it is worth noting that certain medications, such as statins used to manage cholesterol levels, can also contribute to elevated SGPT levels in the bloodstream.[58] SGPT serves as an important biomarker in determining the health of your liver. It is critical to maintain normal SGPT levels for your liver to function properly. Certain lifestyle changes, such as maintaining a healthy weight, getting enough vitamin D, exercising regularly, and avoiding smoking and alcohol, can help improve liver function and lower enzyme levels. [59]

6.2 HYPERLIPIDEMIA:

Hyperlipidemia is when there is an abnormally high level of fats, cholesterol, and triglycerides in the blood, which can lead to serious complications.[60] Complications often arise due to the development of atherosclerosis and its associated conditions, including heart disease, peripheral artery disease, strokes, and brain strokes.[61] The build-up of lipids, particularly cholesterol, in the walls of arteries leads to narrowing of the vessels and reduced blood flow.[62] The development of atherosclerosis is shown in Figure 1, and rates of morbidity and mortality are higher when other diseases such as hypertension, diabetes mellitus, and renal disorders are also present.[63][64]

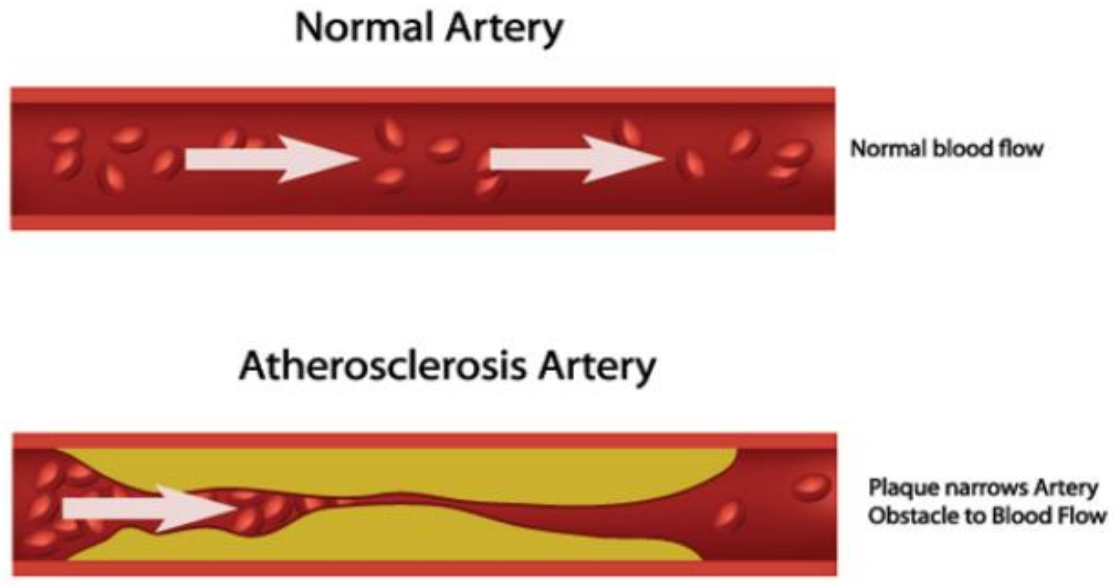


Fig 14: Fat deposit artery vs normal artery

https://st.depositphotos.com/1067125/3671/v/950/depositphotos_36719139-stock-illustration-atherosclerosis-illustration.jpg

In 1990, there were 14.4 million deaths worldwide due to cardiovascular diseases. This number increased to 17.5 million in 2005 and is expected to reach 20 million in 2015.[65] Dyslipidemia is considered the main risk factor for these diseases and deaths. Hyperlipidemia is classified into primary and secondary categories, with primary being caused by genetic abnormalities and secondary resulting from underlying causes. Another classification system is based on the type of elevated lipids, such as high cholesterol, high triglycerides, and low lipoproteins.[66]

6..3 CHOLESTEROL TEST:

A complete cholesterol test is a special blood test that can tell us how much cholesterol and triglycerides are in your blood. A cholesterol test can check if you have too much fat in your blood that can stick to your arteries and make them narrower or blocked. This can cause problems in your body.[67]

Total Cholesterol: This is the total of your blood's cholesterol content.

The NHLBI recommends that individuals should have their cholesterol levels checked for the first time when they are between 9 and 11 years old, and then have subsequent screenings every five years. The NHLBI suggests that men aged 45 to 65 and women aged 55 to 65 should have cholesterol screenings every 1 to 2 years, while individuals over 65 should get tested annually. If you have abnormal initial test results or coronary artery disease, are on cholesterol-lowering medications, or are at a higher risk for coronary artery disease, you may need to undergo more frequent testing. Are overweight, physically inactive, Eat an unhealthy diet, Smoke cigarettes.[68] [69]

6.4 LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL.

This is a type of cholesterol that can be harmful to your body. When there is too much of it in your blood, it can make fatty deposits in your arteries. These deposits can make it harder for blood to flow through your body. Sometimes, these deposits can break and cause a heart attack or stroke.[70]

6.5 HIGH-DENSITY LIPOPROTEIN (HDL) CHOLESTEROL:

This is like having a superhero inside your body that helps to keep your blood flowing smoothly. It's called "good" cholesterol because it helps to remove another type of cholesterol that can clog up your arteries. When your arteries are clear, it's easier for your blood to move through them and keep you healthy.[71]

6..6 TRIGLYCERIDE:

Triglycerides are like little bits of fat in your blood. When you eat too much food, your body turns the extra calories into triglycerides, which get stored in your body. If you have too many triglycerides, it can be because you eat too much sugar, drink too much alcohol, don't exercise, or have certain health problems like diabetes.[72]

6.7 BLOOD GLUCOSE TEST;

Blood glucose tests are also known as blood sugar tests. Glucose, a basic sugar that fuels the body, is measured through blood glucose tests at a pathology center to determine its levels in the bloodstream. A blood sugar test may be conducted. After not eating for several hours, you can have a random blood glucose test without any specific preparation. Alternatively, you can also have an oral glucose tolerance test as part of the examination.[73] Blood glucose tests are typically employed to detect or oversee diabetes. Your doctor may recommend this test if you: You may be at risk of developing diabetes if you have symptoms or test results indicating the possibility of the condition. Your doctor may recommend further tests, like the HbA1c test, to confirm a diabetes diagnosis. If you have symptoms that could be caused by low blood glucose levels, your healthcare provider may suggest getting a blood glucose test.[74]

An oral glucose tolerance test is recommended in certain situations.

An oral glucose tolerance test is recommended to check for type 2 diabetes and pre-diabetes. Pre-diabetes is when the sugar in your blood is a little too high, but not high enough to be called diabetes. Doctors often suggest a test called an OGTT to see if a pregnant woman has gestational diabetes, which is a type of diabetes that can happen during pregnancy.[75] During an oral glucose tolerance test, a person is given a sugary drink and their blood sugar levels are monitored over a certain period to evaluate how their body processes glucose.[76] Having too much sugar in your blood is usually because of diabetes, pre-diabetes, or gestational diabetes. But there are also other reasons why your blood sugar levels can be too high or too low. [77][78]

6.7.1 TEST MATERIAL

Dried methanolic extract of *moringa oleifera* bark and root.

Reagent: Water, Blood, Anesthetic

Apparatus

SI NO	Name
1.	Electronic balance
2.	Beaker
3.	Nose masks and hand glove
4.	Eppendorf
5.	Cotton
6.	Tissue paper
7.	Test tubes
8.	Glass road
9.	Syringe filter
10.	Syringe
11.	Measuring cylinder
12.	Animal feeder
13.	Vortex mixture

6.7.2 SAMPLE PREPARATION:

Test samples were measured to prepare a solution at dosages of 400 mg/kg per body weight. For two samples. A tiny amount of water was added to the extract to create a volume of 2 ml, causing it to be triturated unidirectionally. To prepare 0.098 mg of Silymarin at a dose of 140 mg/kg of body and weight and 0.0002 mg of atorvastatin 10mg/kg body weight was consumed.

6.7.3 EXPERIMENTAL ANIMAL:

Twenty albino Wistar mice weighing 35-45 g were obtained from the animal house at the University of Daffodil. They were allowed to acclimate for a week in well-ventilated cages with controlled environmental conditions before being used for research. The study protocol followed NIH guidelines for animal care and was approved by the University of Calabar's Animal Ethics Committee.[79]

6.7.4 EXPERIMENTAL DESIGN:

The study involved 20 mice divided into 4 groups, each with 4 non-diabetic mice. The doses administered were determined based on previous research on LD50 values.

6.7.5 EXTRACT AND DRUG ADMINISTRATION:

The extracts were mixed with distilled water and given orally at a single dose of 400mg/kg body weight before being administered. The Standard drug is used for two samples is required for 120mg and 10mg/kg body weight. The animals received 2ml of sample solution and drug.

6.7.6 WORKING PROCEDURE:

We had five groups of mice, named Group I, Group II, Group III, and Group IV, Group V Each group had four mice in it. We gave different treatments to each group, like a special extract, a positive control, or just a regular control. We made sure to weigh each mouse carefully and adjust the doses of the test samples and control materials accordingly.

Standards: In mice, they were given a certain amount of Atorvastatin and silymarin based on their body weight by mouth.

Control: The group of mice was fed are high-fat diet and normal food.

Sample: 400mg/kg body weight extract is put on the oral route.

6.7.7 COLLECTION OF SAMPLES FOR ANALYSIS: Following a period of fasting and access to water, the mice were gently euthanized and sacrificed using chloroform vapor. Whole blood samples were collected via cardiac puncture with sterile equipment, allowed to clot, and then centrifuged to separate the serum for further analysis. The serum was carefully stored and frozen until needed for biochemical testing.

Fig 15: Blood collection

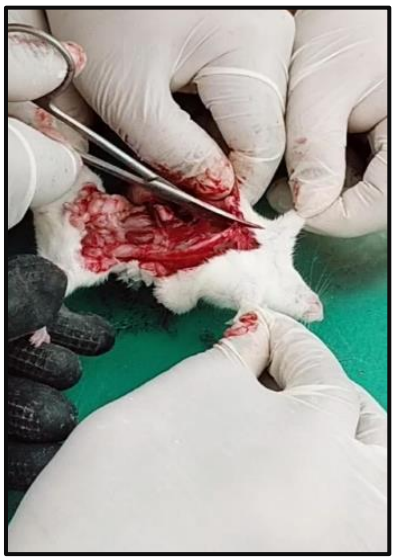


Fig 16: Fat deposited

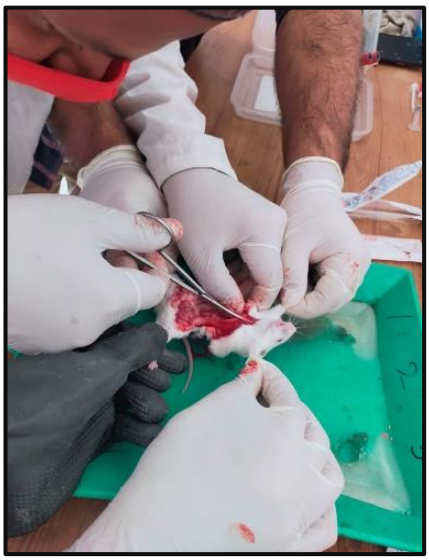


Fig 17: Control mice

6.7.8 ESTIMATION OF SERUM LIPID PROFILE LEVEL:

The serum lipid profile, including triacylglycerol, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, was meticulously assessed using a colorimetric assay kit method at the esteemed Department of Pathology at EMCH. This sophisticated analysis was conducted by the highly skilled Consultant Pathologist, Prof. Dr. Md Mamun Ali Biswas, who holds multiple prestigious degrees in Applied Laboratory Sciences and Pathology from BSMMU. As the Lab in Charge at Labtech Diagnostic Services, Prof. Dr. Biswas ensures the utmost accuracy and precision in lipid profile testing.

6.7.9 STATISTICAL ANALYSIS:

Elegant and persuasive statistical analysis was conducted utilizing one-way analysis of variance (ANOVA) followed by the student "t" test. The data presented are depicted as mean ± standard error, with a sample size of 4 mice in each group. Significance was determined by p values less than or equal to 0.05.

CHAPTER SEVEN: RESULT AND DISCUSSION

7.1 PHYTOCHEMICAL SCREENING:

PHYTOCHEMICAL CONSTITUENTS	RESULT
Alkaloid test for (Moringa bark and root)	+
Mayer's Test Dragendroff's Test	
Glycoside test for (bark and root)	+
Gum test for (bark and root)	+
Steroids test for (bark and root)	+
Flavonoid for (bark and root)	+
Saponins for (bark and root)	-
Tannins test for (bark and root)	+

Table 2: Qualitative analysis of Methanolic extract of leaves of *moringa oleifera* bark and root. (+) indicates presence, (-) indicates absence

7.2 LIPID PROFILE

Groups for Lipid profile	T cholesterol (mg/dl (Mean±SEM)	Triglyceride(mg/dl) (Mean±SEM)	HDL (Mean±SEM)	LDL (Mean±SEM)
HF Control	125.54±1.74	142.56± 6.92	53.66±6.43	18±3.46
HF Standard Atorvastatin	118.66± 4.37	121.33± 6.43	66.22±9.07	17±3.78
HF Sample(bark)	89.24± 4.58 *	91.33±6.48 *	60.44±4.61***	14±3.21**
HF Sample (Root)	109.66± 3.92 **	90.12± 10.26 *	68.66±11.56**	25±4.16

Table 3: Data were analyzed by one-way ANOVA following the Bonferroni post hoc test. Values were presented as Mean±SEM, n=4. ($p< 0.05$) = Significant*, ($p< 0.01$) = Less significant**, ($p< 0.001$) = Very less significant as compared to high fat control.

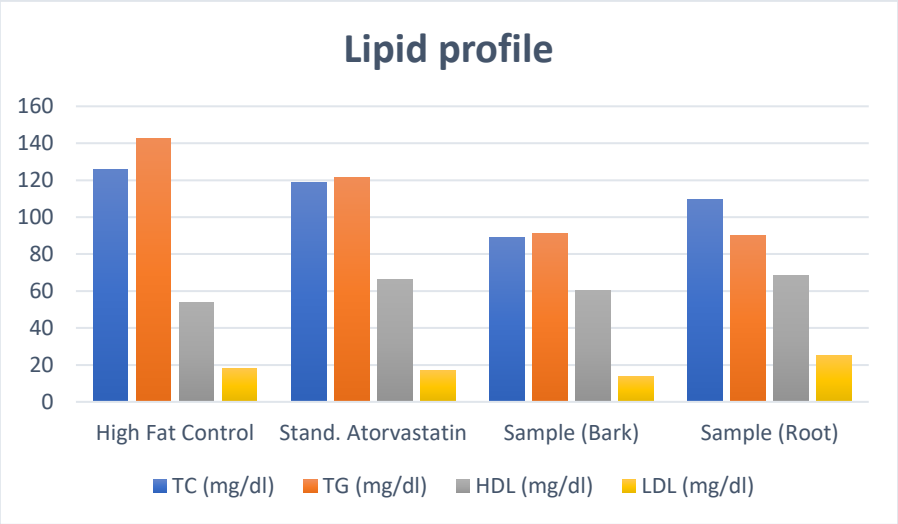


Fig 18: Lipid profile graph

7.3 HEPATOPROTECTIVE TEST:

Groups for SGPT	SGPT (Mean±SEM)
HF Control	142.66±5.92
HF Standard Silymarin	115.66±7.53
HF Sample(bark)	72.33±4.58 *
HF Sample (Root)	74.33±11.85*

Table 4: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=4. ($p < 0.05$) = Significant *, ($p < 0.01$) = Less significant**, ($p < 0.001$) = Very less significant as compared to High Fat control.

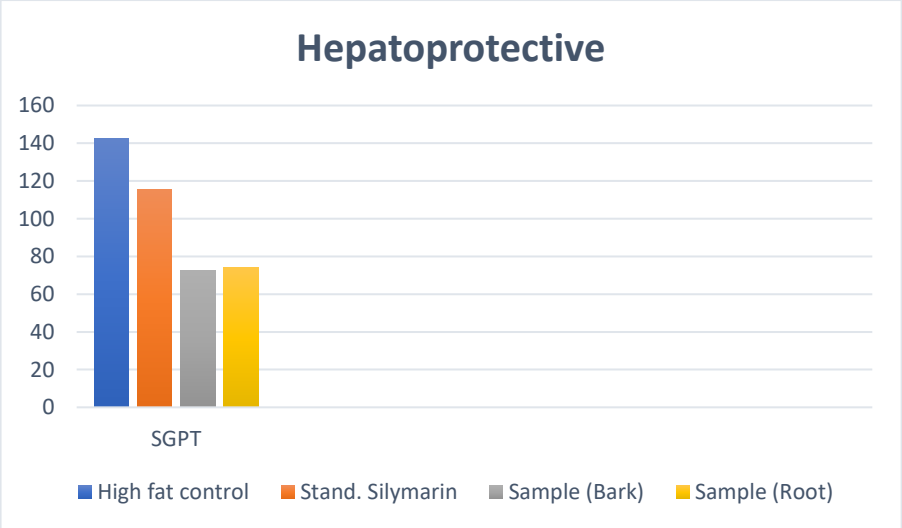


Fig 19: SGPT profile graph

7.4 GLUCOSE TEST:

GROUPS FOR BLOOD GLUCOSE TEST	Glucose TEST
HF Control	5.66±0.33
HF Standard Silymarin	3.83±0.32
HF Sample(bark)	4.93± 0.42**
HF Sample (Root)	3.23±0.18**

Table 5: Data were analyzed by one-way ANOVA following the Bonferroni post hoc test. Values were presented as Mean±SEM, n=4. ($p< 0.05$) = Significant *, ($p< 0.01$) = Less significant**, ($p< 0.001$) = Very less significant as compared toa high-fat diet and standard as silymarin.

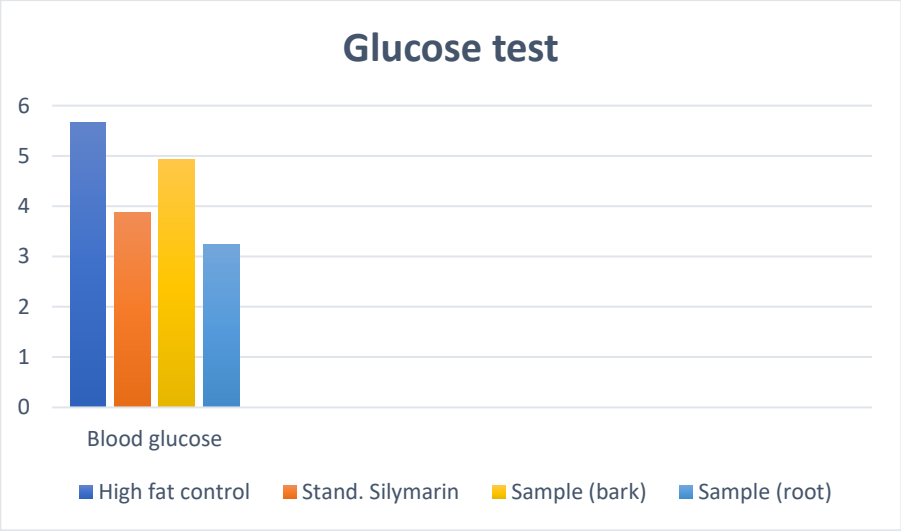


Fig 20: Glycemic control graph

7.5 DISCUSSION:

Moringa oleifera is a significant plant that has a strong ability to lower lipid levels in the body. [77] Alternative medicine is a different way to treat diabetes and protect the liver. Different types of studies have shown that having high levels of cholesterol in the blood can lead to heart disease. [77] [78], Cholesterol is usually thought of as the main problem, but other fats in the blood may also be important. [79] The results of this investigation indicated that there was a significant decrease and increase in TG and TC levels in all the groups treated with samples from both the root and bark, compared to the control group that received a high-fat diet.

The study investigated the protective effects of Moringa oleifera stem bark and root extracts on the liver in mice with high-fat-induced liver damage. The extracts significantly reduced levels of SGPT enzymes in the blood, indicating protection and restoration of liver health. This was attributed to the non-toxic and tissue-protective properties of Moringa oleifera against harmful metabolites.

The study evaluated the hypoglycemic effect of the aqueous extract of Moringa oleifera stem bark and root on mice with high fat-induced diabetes. The extract from both the bark and root of M. oleifera showed a significant decrease in blood glucose levels compared to the high-fat control group, although it was more effective than silymarin, which was used as a standard comparison.

CHAPTER EIGHT: CONCLUSION

8.1 CONCLUSION:

The findings of the study highlight the considerable potential of Moringa oleifera bark and root bark extracts to have beneficial effects on the body. Specifically, these extracts have been shown to have strong abilities to protect the liver, decrease lipid levels in the blood, and regulate blood sugar levels. These results offer exciting possibilities for using Moringa oleifera as a natural solution for liver ailments, abnormalities in lipid metabolism, and the management of diabetes. However, to fully understand how these extracts work and maximize their therapeutic benefits, further research and investigation are crucial.

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