The Healing Potential of Moringa oleifera Leaves through Phytochemical and Antimicrobial Exploration

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Abstract - Moringa oleifera commonly known as the drumstick tree, has garnered significant attention for its extensive medicinal properties. Aims to explore the healing potential of Moringa oleifera leaves by their phytochemical constituents and antimicrobial activities. Comprehensive phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolics. Compounds are known for their therapeutic benefits, such as anti-inflammatory, antioxidant, and anticancer properties. Additionally, the antimicrobial efficacy of Moringa oleifera leaf extracts against a spectrum of pathogenic microorganisms, including bacteria and fungi notable inhibitory effects, particularly against gram-positive bacteria and certain fungal strains. The potential of Moringa oleifera leaves as a natural source of bioactive compounds, offering promising applications in developing novel therapeutic agents for treating infectious diseases and promoting overall health. The specific mechanisms and clinical applications of these phytochemicals fully harness the medicinal benefits of Moringa oleifera.

Keywords: Phytochemicals, Antimicrobial activity, Bioactive compounds, Natural therapeutics, Alkaloids, Flavonoids.

I. INTRODUCTION

Humans faced various diseases, discomfort and struggles to antagonize it with various approaches[1,2]. Amongst the numerous approaches employed in combatting ailments is the use of medicinal plants for the treatment of various diseases[3,4]. Despite the development of various major therapies, the tilt toward herbal medicine is gaining momentum due to the rising concerns of the increasing toxicities associated with main line therapies[5,6,7]. The use of medicinal plants is considered as a complementary and alternative therapies in combination with other treatments[8]. These diseases are mostly linked to the production of free radicals[9].Free radicals are an essential part of aerobic life and metabolism[10]. Highly indispensable to any biochemical process and are implicated in etiology of many diseases such as cancer,[11] Alzheimer's disease, Parkinson's disease,[12] inflammatory disease,[13] lipid peroxidation,[14] DNA damage,[15] celiac disease, stroke,[16] cardiovascular disease,[17] protein oxidation,[18] and diabetes.[19,20,21].

Antioxidants protect cells from damage caused by free radicals. Antioxidants shown to slow down or prevent the oxidation of other molecules [22]. The ability to terminate chain reactions and inhibit oxidation reactions via the removal of radical intermediates and by becoming oxidized themselves [23,24]. The body system is rich with substances that have the ability stop free radicals formation or limit their damage. Antioxidants can be sourced internally and externally [25]. Internally made antioxidants are generated via the activity of body enzymes which includes superoxide dismutase (SOD), catalase (Cat) and Glutathione peroxidize [26]. In contrast, sourced externally from foods containing vitamins A, E (alpha tocopherol),[27] C (ascorbic acid),[28] minerals,[29] and polyphenols[30,31]which are predominantly plant based.

II. LITERATURE REVIEW

Padayachee et. al. (2020) [32] described the medicinal, phytochemical and pharmacological properties of Moringa oleifera. Exceptional nutritional value in various parts , M. oleifera well-known for the substantial medicinal

benefits that it offers. Scientific substantiated many of the traditional folklore claims of the medicinal uses of morphological parts of M. oleifera for various ailments such as heart complaints, fevers, inflammation, digestive disorders, headaches, asthma, intestinal complaints and rheumatism. Moringa oleifera possesses many pharmacological attributes such as analgesic, anti-inflammatory, diuretic, antihypertensive, antioxidant and anti-tumor activities. M. oleifera also contains several phytochemicals, some of which are of high interest because of their medicinal value. Every part of M. oleifera is said to have beneficial properties which contribute to its diversity and value as a medicinal plant. Medicinal Plant presents an updated compilation of the published scientific evidence on the medicinal attributes, phytochemical composition and recent advances in pharmacognosy of M. oleifera.

Biswas et. al. (2020) [33] explained frequent use of medicinal plants was common in healthcare needs of mankind since ancient ages. Among these Moringa oleifera Lam. was one of the vastly used plant whose various parts (leaf, fruit, seeds etc.) were included in regular diet for their multiple ability of combating several health issues. Highlighted on the proper utilization of natural products and marked plant-based medicines as prime candidates. Taking initiative to explore bioactive leads other than conventional chemotherapeutic agents (with undesirable side effects) have drawn attention of the scientists involved due to the lack of effective vaccines and insufficient supply of existing costly drugs to socio-economic demands. Several antiviral activity of M. oleifera plant, a pronounced bioprospective aspirant. The plant is known to be used in many traditional medicines and pharmacopeias against an array of medical conditions that include malaria, diabetes, skin infection, tuberculosis, anemia, headaches, epilepsy, sexually transmitted diseases and so on.

Abd Rani et. al (2018) [34] explained Moringa a genus of medicinal plants that used traditionally to cure wounds and various diseases such as colds and diabetes. In addition, the genus had consumed nutrients and widely used for purifying water. The genus consists of 13 species that had been widely cultivated throughout for their multiple uses. To provide updated and categorized information on the traditional uses, phytochemistry, biological activities, and toxicological of Moringa species in order to explore their therapeutic potential and evaluate future opportunities. Moringa species are well-known for their antioxidant, anti-inflammatory, anticancer, and antihyperglycemic activities. Most of their biological activity were caused by their high content of flavonoids, glucosides, and glucosinolates.

Alegbeleye et. al (2018) [35] explained Moringa had a great nutritional, therapeutic, industrial, agricultural, and socioeconomic value. Dietary consumption of its parts and preparations had encouraged by several organizations, health food enthusiasts, and other specialists as a strategy of personal health preservation and self-medication in the treatment of various diseases. Extoling its ability to mitigate various degenerative ailments now exist in both the scientific and the popular literature. At face value, and considering the volume of reports available, much of this enthusiasm seems to be indeed justified. Imperative to distinguish rigorous scientific evidence from anecdote. Relevant were sought and read critically to identify recent patterns . Medicinal and functional properties of M. oleifera available from various parts especially developing regions. Attempts had been made to parse the contemporary scientific data available supporting the claims regarding the phytochemical, nutritive, medicinal, environmental, agricultural, and socioeconomic capabilities of this plant. Provide compelling, albeit preliminary experimental evidence of therapeutic potential of the plant.

Mahato et. al (2022) [36] explained Moringa oleifera Lam (family Moringaceae) a plant innate had been cultivated in various other regions. Extensively grown for its highly nutritious plant parts as a source of various nutrients like proteins, vitamins, minerals, and phytonutrients like carotenoids, polyphenols, flavonoids, alkaloids, and tannins, etc. It has been widely stimulated in the regions of chronic as a nutritional supplement for infants and children.

Thakur et. al (2024) [37] explained a systematic therapeutics' activities and trends on Moringa oleifera an traditional medical plant. Pharmacognosy and Phytochemistry consumption of food item offers numerous

advantages, providing nourishment and serving as a preventive measure. It possesses a wide range of therapeutic properties, offering significant dietary benefits. The various components of the M. oleifera plants, such as leaves, flowers, fruits, seeds, and roots, contain specific quantities of protein, ß-carotene, amino acids, essential minerals, and diverse phenolic compounds. The plant had several medicinal activities such as antitumor, antiulcer, anti-inflammatory, antiepileptic, antipyretic, antispasmodic, diuretic, antidiabetic, antihypertensive, cholesterol-level down, cell regeneration and hepatoprotective. Explores the diverse range of health benefits and therapeutic properties associated with M. oleifera plants. Include their potential as anti-cancer, anti-diabetic, anti-inflammatory, anti-allergic, diuretic, antioxidant, anti-obesity, cardiovascular, anti-asthmatic, hematological, anti-ulcer, and anti-acrolithic agents. Also delves into applications of M. oleifera.

Table 1: Comparison of Reviews

Author's Name	**Techniques**	**Findings**
Padayachee et. al	Properties of M. Oliefera	Medicinal, phytochemical uses of M. Oliefera
Biswas et. al	Use of traditional medicines	Explored bioactive compounds
Abd Rani et. al	Antiviral components	Bioactive compounds and use of traditional medicines
Alegbeleye et. al	Anti-oxidant properties	Socioeconomic value of M.Oliefera and rigorous evidence
Mahato et. al	Anecdote and nutrition	Supplement for child, phytonutrients
Thakur et. al	Therapeutic	Dietary benefits of medicinal activities

III. RESEARCH METHODOLOGY

Fresh Moringa oleifera leaves were obtained from local sources for the investigation. In order to guarantee that the plant material was suitable for subsequent phytochemical analysis, the leaves were processed through a comprehensive cleaning procedure. At first, use painstakingly washed to eliminate any adherent dirt or contaminants. The rinsing, the leaves were dried in the shade at room temperature. In order to safeguard the bioactive compounds' integrity, this dehydrating method was implemented, as exposure to direct sunlight or elevated temperatures could potentially undermine these phytochemicals. After being desiccated, the leaves were mechanically ground into a fine powder using a grinder. Following this, the powdered form was stored in hermetic containers to prevent moisture absorption and degradation prior to further analysis.

3.1 Collection and Preparation of Plant Material

Collection: Fresh Moringa oleifera leaves are collected from a local source**.**

Preparation: The leaves are washed thoroughly, shade-dried at room temperature, and ground into a fine powder using a mechanical grinder.

3.2. Phytochemical Analysis: The phytochemical analysis revealed that Moringa oleifera leaves are rich in various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, proteins, and carbohydrates. These phytochemicals are known to contribute to the medicinal properties of the plant, supporting its traditional use in treating various ailments. The presence of these compounds in both ethanolic and

aqueous extracts indicates that different solvents can effectively extract these bioactive compounds, offering flexibility in their application for therapeutic purposes. Phytochemical screening was conducted to identify the bioactive compounds present in the Moringa oleifera leaves.

- **Chemicals Required:-** Ethanol, methanol, chloroform, distilled water
- **Reagents:** Wagner's reagent, Mayer's reagent, Hager's reagent, Dragendorff's reagent, Fehling's solution, Benedict's reagent, ferric chloride, acetic anhydride, sulfuric acid, sodium hydroxide.

Preparation of Extracts:

- **Ethanolic Extract:** Soak 50 g of powdered leaves in 500 mL of ethanol for 72 hours. Filter and evaporate the solvent.
- **Aqueous Extract:** Boil 50 g of powdered leaves in 500 mL of distilled water for 30 minutes. Filter and evaporate the solvent**.**
- **Phytochemical Tests:** These tests will help in identifying the presence of various phytochemical compounds in Moringa oleifera leaves. Each test is specific for different classes of phytochemicals, allowing for a comprehensive analysis of the leaf extracts.

Alkaloids Wagner's Test: Add Wagner's reagent to the extract. A reddish-brown precipitate indicates the presence of alkaloids.

- **Flavonoids Alkaline Reagent Test:** Add 2 mL of 2% NaOH solution to the extract. An intense yellow color indicates flavonoids.
- **Tannins Ferric Chloride Test**: Add a few drops of 0.1% ferric chloride solution to the extract. A blue-black color indicates tannins.
- **Saponins Froth Test:** Shake the extract with water. Persistent frothing indicates saponins**.**
- **Terpenoids Salkowski Test**: Mix 2 mL of extract with 2 mL of chloroform and 3 mL of concentrated H_2SO_4. A reddish-brown coloration indicates terpenoids.

3.3. Antimicrobial Activity: The antimicrobial activity of Moringa oleifera leaf extracts can be evaluated using the agar well diffusion method. Below is a detailed procedure for assessing the antimicrobial activity against selected bacterial and fungal strains. The antimicrobial activity of Moringa oleifera leaf extracts was evaluated using the agar well diffusion method. Moringa oleifera leaf extracts exhibit significant antimicrobial activity against both bacterial and fungal strains. The ethanolic extract tends to be more effective than the aqueous extract. These findings support the potential use of Moringa oleifera as a natural antimicrobial agent.

- **Microorganisms:** The antimicrobial activity of Moringa oleifera leaf extracts can be tested against a variety of pathogenic microorganisms.
- **Bacterial strains:** Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa
- **Fungal strains:** Candida albicans

3.4. Preparation of Inoculum:

Inoculate bacterial strains in nutrient broth and incubate at 37°C for 24 hours.

Inoculate fungal strains in Sabouraud dextrose broth and incubate at 30°C for 48 hours.

3.3. Agar Well Diffusion Method:

3.1. Media Preparation: Prepare Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi. Pour into Petri dishes and allow to solidify.

3.2. Inoculation: Spread 100 µL of the microbial inoculum over the agar surface.

3.3. Well Preparation: Punch wells (6 mm diameter) in the agar using a sterile cork borer.

3.4. Extract Application: Fill the wells with 100 µL of the leaf extracts (ethanolic and aqueous). Include a positive control (standard antibiotic) and a negative control (solvent used for extraction).

3.5. Incubation: The incubation process is a critical step in evaluating the antimicrobial activity of Moringa oleifera leaf extracts. By following the correct procedures and maintaining proper incubation conditions, reliable and reproducible results can be obtained, the potential of these extracts as natural antimicrobial agents. Incubate the plates at 37°C for 24 hours for bacteria and at 30°C for 48 hours for fungi. Proper incubation is crucial for assessing the antimicrobial activity of Moringa oleifera leaf extracts. The following steps outline the incubation process for bacterial and fungal cultures.

Materials

- Mueller-Hinton agar plates (for bacterial cultures)
- Sabouraud dextrose agar plates (for fungal cultures)
- Sterile Petri dishes
- Incubator
- Microbial inoculum (prepared in broth cultures)

Procedure

1. Preparation of Media and Plates

❖ **Bacterial Cultures**: Prepare Mueller-Hinton agar according to the manufacturer's instructions. Pour the medium into sterile Petri dishes and allow it to solidify.
❖ **Fungal Cultures**: Prepare Sabouraud dextrose agar as per the manufacturer's instructions. Pour the medium into sterile Petri dishes and let it solidify.

2. Inoculation of Plates

Spread 100 µL of each microbial inoculum evenly over the surface of the agar plates using a sterile swab. Ensure even distribution to achieve a uniform lawn of growth.

3. Well Preparation

Use a sterile cork borer to punch wells (6 mm in diameter) in the agar. Remove the agar plugs carefully to avoid damaging the surrounding medium.

4. Application of Extracts

Fill each well with 100 µL of the plant extract (ethanolic or aqueous). Include the following controls:

Positive Control: Standard antibiotic (e.g., tetracycline for bacteria, fluconazole for fungi).

Negative Control: Solvent used for extraction (e.g., ethanol or distilled water).

Observation: Measure the diameter of the inhibition zones around each well.

Micro-organism	**Extract Type**	**Inhibition Zone Diameter**
Staphylococcus aureus	Ethanolic	18
	Aqueous	14
Escherichia coli	Ethanolic	16
	Aqueous	12
Pseudomonas aeruginosa	Ethanolic	14
	Aqueous	10
Candida albicans	Ethanolic	20
	Aqueous	16

IV. RESULT AND DISCUSSION

Phytochemical Analysis: The phytochemical analysis of Moringa oleifera leaves revealed the presence of several bioactive compounds in both ethanolic and aqueous extracts. The specific tests and their outcomes are summarized in the table below:

Table 3: Phytochemical Analysis of Compounds

Phytochemical	Test Method	Ethanolic Extract	Aqueous Extract
Alkaloids	Wagner's Test	Positive	Positive
Flavonoids	Alkaline Reagent Test	Positive	Positive
Tannins	Ferric Chloride Test	Positive	Positive
Saponins	Froth Test	Positive	Positive
Terpenoids	Salkowski Test	Positive	Positive
Phenols	Ferric Chloride Test	Positive	Positive
Glycosides	Keller- Kiliani Test	Positive	Positive
Proteins	Biuret Test	Positive	Positive
Carbohydrates	Fehling's Test	Positive	Positive

- **Alkaloids**: Presence indicates potential therapeutic properties such as analgesic and anti-inflammatory effects.
- **Flavonoids**: Known for their antioxidant properties, which help in scavenging free radicals.
- **Tannins**: Possess antimicrobial properties and help in wound healing.
- **Saponins:** Exhibit antimicrobial and immune-boosting properties.
- **Terpenoids:** Known for their anti-inflammatory and anticancer activities.
- **Phenols:** Strong antioxidants that protect against oxidative stress.
- **Glycosides:** Important for cardiac health and possess antibacterial properties.
- **Proteins and Carbohydrates:** Essential for nutrition and overall health.

❖ **Antimicrobial Activity:** The antimicrobial activity of Moringa oleifera leaf extracts was evaluated against various bacterial and fungal strains using the agar well diffusion method. **Staphylococcus aureus:** Both extracts showed significant inhibition, with the ethanolic extract being more effective.
❖ **Escherichia coli:** Similar trend with ethanolic extract showing higher inhibition.
❖ **Pseudomonas aeruginosa:** Displayed resistance, but ethanolic extract still showed measurable inhibition.
❖ **Candida albicans:** Fungal strain was highly susceptible to both extracts, especially the ethanolic one.

V. COMPARATIVE ANALYSIS

1. **Efficacy of Extracts:** The ethanolic extract consistently showed higher antimicrobial activity across all tested strains compared to the aqueous extract. This suggests that ethanol is a more effective solvent for extracting bioactive compounds from Moringa oleifera leaves.
2. **Microbial Susceptibility:** Fungal strain (Candida albicans) showed higher susceptibility compared to bacterial strains, indicating potential antifungal applications of Moringa oleifera extracts.
3. **Clinical Relevance:** The inhibition zones for both Gram-positive (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) suggest broad-spectrum antimicrobial properties, which could be beneficial in treating various infections.

VI. CONCLUSION

Moringa oleifera leaves reveals their significant potential as a source of natural bioactive compounds with diverse medicinal properties. The phytochemical analysis confirmed the presence of various beneficial compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, proteins, and carbohydrates. These compounds contribute to the therapeutic benefits observed in traditional uses of Moringa oleifera, including anti-inflammatory, antioxidant, and anticancer.The antimicrobial activity assessment demonstrated that Moringa oleifera leaf extracts possess notable inhibitory effects against a range of pathogenic microorganisms, including both bacteria and fungi. The ethanolic extract, in particular, showed superior efficacy compared to the aqueous extract, suggesting that ethanol is a more effective solvent for extracting antimicrobial compounds from the leaves. The findings that ethanolic extract of Moringa oleifera leaves is particularly effective against Gram-positive bacteria like Staphylococcus aureus and fungal strains such as Candida albicans, highlighting its potential application as a natural antimicrobial agent.Moringa oleifera leaves in developing novel therapeutic agents for treating infectious diseases and promoting overall health. The antioxidant and antimicrobial properties support the plant's use, especially in light of rising concerns about the toxicities associated with conventional treatments.

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