Preliminary Pharmacological Investigation in *Moringa oleifera* Leaves to Protect against Microbial Diseases

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Abstract:- *Moringa oleifera*, commonly known as the drumstick tree, is a highly valued plant with a wide range of medicinal and nutritional properties. In this study, we investigated the bioactive components present in *Moringa oleifera* leaves and their potential protective effects against microbial diseases. Our findings highlight the importance of *Moringa oleifera* leaves as a rich source of bioactive compounds with antimicrobial activity. These components have the potential to be utilized as natural alternatives or supplements to conventional antimicrobial agents.

Keywords:- *Moringa oleifera, antimicrobial activity, bioactive compounds, phenolic acids, flavonoids, alkaloids, glucosinolates.*

I. INTRODUCTION

Microbial diseases continue to pose a significant threat to human health worldwide. The increasing prevalence of antibiotic resistance has further emphasized the need for the development of alternative antimicrobial strategies. Plants have long been recognized as valuable sources of bioactive compounds with diverse therapeutic properties. *Moringa oleifera*, a widely cultivated plant in tropical and subtropical regions, has gained considerable attention due to its numerous health benefits. This study aimed to explore the bioactive components in *Moringa oleifera* leaves and evaluate their potential as protective agents against microbial diseases.

The taxonomical classification of Moringa is as follows:

- **Kingdom:** Plantae (Plants)
- **Division:** Magnoliophyta (Flowering plants)
- **Class:**Magnoliopsida (Dicotyledons)
- **Order:** Brassicales
- **Family**: Moringaceae
- **Genus:** Moringa
- **Species:** Moringa oleifera

Moringa belongs to the family Moringaceae. It is a fast-growing deciduous tree that is native to the Indian subcontinent but is now widely cultivated in tropical and subtropical regions worldwide. The Moringa genus includes several other species, but *Moringa oleifera* is the most commonly known and extensively studied species due to its nutritional and medicinal properties.

Research on moringa has revealed its impressive nutritional profile, with leaves containing high concentrations of vitamins, minerals, and protein. In fact, gram for gram, moringa leaves contain more vitamin C than oranges, more potassium than bananas, and more iron than spinach. Additionally, the leaves are a rich source of essential amino acids, which are the building blocks of protein and are crucial for various bodily functions.

Moringa, scientifically known as *Moringa oleifera*, is a versatile and highly valued plant that has gained global recognition for its remarkable nutritional and medicinal properties. Native to the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan, moringa has been used for centuries in traditional medicine and as a dietary staple in many cultures.

Often referred to as the "Miracle Tree" or "Tree of Life," moringa is revered for its abundance of essential nutrients, antioxidants, and bioactive compounds. The tree itself is relatively fast-growing and can reach heights of up to 10 meters, with slender, drooping branches and small, ovate leaves. Moringa is known for its drought resistance, making it adaptable to a wide range of environmental conditions.

Apart from its nutritional value, moringa also possesses notable medicinal properties. Traditional medicine has long recognized moringa for its ability to treat a range of ailments, including digestive disorders, inflammation, infections, and skin conditions. Scientific studies have substantiated many of these claims, highlighting the plant's antimicrobial, anti-inflammatory, and antioxidant properties.

Furthermore, moringa has gained attention for its potential in addressing malnutrition, especially in developing countries. The plant's ability to grow in diverse climates, coupled with its high nutritional content, makes it an excellent candidate for combating food insecurity and improving public health in resource-limited regions.

In recent years, the global demand for moringa products has surged, leading to increased commercial cultivation and the development of various moringa-based supplements, teas, and cosmetics. This rising interest is driven by a growing awareness of the plant's health benefits and its potential as a sustainable and eco-friendly crop.

II. METHODOLOGY

Moringa oleifera leaves were collected, washed, and dried in a controlled environment. The dried leaves were subjected to extraction using various solvents, including hexane, chloroform, ethyl acetate, carbinol, and water.

The process aims to extract various compounds present in the powder using different solvent systems. The steps involved:

- Initial extraction with hexane: 100ml of hexane is added to the 10g of dried powder. The mixture is then stirred using a magnetic stirrer at 60°C for 1 hour. This step helps to dissolve or extract compounds that are soluble in hexane.
- Maceration: After the 1-hour stirring, the solvent-treated material is left to undergo maceration, which involves allowing the mixture to sit at room temperature for 24 hours. This allows further extraction of compounds from the powder into the hexane.
- Filtration: The next day, the mixture is filtered to separate the solvent-treated material from the liquid solvent (hexane). The residue remaining after filtration contains the extracted compounds.
- Sequential extraction with chloroform, ethyl acetate, carbinol, and water: The same process of adding 100ml of each solvent (chloroform, ethyl acetate, carbinol, and water) to the residue from the previous step is repeated. Each solvent is stirred using a magnetic stirrer at 60°C for 1 hour, followed by maceration at room temperature for 24 hours. This sequential extraction process helps to extract a wide range of compounds with different solubilities.

Thin Layer Chromatography (TLC) is a widely used technique for the identification of bioactive compounds in crude samples. In TLC, a thin layer of silica gel is employed as the stationary phase, while a mobile phase consisting of a mixture of solvents, such as hexane/ethyl acetate in various ratios, is used to separate and visualize the compounds of interest.

The choice of mobile phase composition is crucial as it directly affects the efficiency of compound extraction. By utilizing different ratios of the hexane/ethyl acetate mixture as the mobile phase, the optimal conditions for compound separation can be determined. Typically, ratios such as 1:1, 7:3, and 3:7 of hexane/ethyl acetate are employed to elute a broad range of compounds present in the crude sample.

To evaluate the effectiveness of the compound extraction, the retention factor (RF) of the sample can be calculated using the following formula:

RF = Distance travelled by the compound (sample) / Distance travelled by the solvent.

The RF value provides information about the relative mobility of the compound compared to the solvent front. A higher RF value indicates that the compound has a greater affinity for the mobile phase and therefore travels a longer distance on the TLC plate.

By comparing the RF values obtained for different compounds in the sample, it is possible to identify and differentiate between the various constituents present. Furthermore, by comparing the RF values with known standards or reference compounds, the identity of specific bioactive compounds can be determined.

The antibacterial activity of Moringa leaves crude was evaluated using the agar well diffusion method. Extracts obtained from sequential extraction of turmeric rhizome and vinca flower were tested against four strains of human pathogenic bacteria, specifically Escherichia coli, Staphylococcus species, Klebsiella species, and Salmonella species. These bacterial strains were isolated from clinical samples such as urine and sputum.

To conduct the agar well diffusion assay, the test cultures were swabbed over the surface of Muller Hinton Agar plates, which provide a suitable medium for bacterial growth. Wells were prepared on the agar plates using an agar puncher. Each well was loaded with different extracts obtained from Moringa leaves crude, as well as positive control antibiotics like tetracycline. Additionally, a negative control using dimethyl sulfoxide (DMSO) was used to dissolve the crude extracts.

After inoculating the plates with the bacterial cultures, they were incubated for 24 hours at 37°C to allow bacterial growth. The following day, the plates were observed, and the zone of inhibition around each well was measured and recorded. The zone of inhibition refers to the clear area around the well where bacterial growth is inhibited by the presence of bioactive compounds.

III. RESULTS

In the Thin-Layer Chromatography (TLC) analysis of the extracts, a notable observation was the presence of multiple tailing bands. Subsequently, the antimicrobial activity of the Moringa oleifera leaves' methanol extract was found to be highly significant against a diverse spectrum of pathogenic bacteria, displaying inhibition zones ranging from 3 mm to 7 mm. Interestingly, the chloroform extract demonstrated even higher inhibition zones specifically against multi-drug resistant strains.

Detailed analytical investigations, such as Gas Chromatography-Mass Spectrometry (GCMS) and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS), were conducted to identify and quantify the bioactive constituents in the methanol and chloroform extracts. These analyses revealed the presence of various bioactive compounds, including phenolic acids, flavonoids, alkaloids, and glycosylates, which are widely recognized for their potent antimicrobial properties.

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It is worth noting that the presence of multiple tailing bands in TLC may indicate the existence of various components with differing polarities in the extracts. The significant antimicrobial activity observed in the methanol extract against pathogenic bacteria underscores its potential as a natural antimicrobial agent. Moreover, the enhanced inhibitory effect exhibited by the chloroform extract against multi-drug resistant strains highlights its promising role in combating drug-resistant infections.

The utilization of sophisticated analytical techniques such as GCMS and HPLC-MS allowed for the identification and characterization of diverse bioactive compounds in the extracts. Phenolic acids, flavonoids, alkaloids, and glycosylates have been extensively studied and acknowledged for their antimicrobial efficacy. As such, the presence of these compounds in the extracts from Moringa oleifera leaves provides scientific support for the observed antimicrobial activity.

a) Moringa leaves powder

b) leaves treated with hexane

c) leaves treated with chloroform

d) leaves treated with methanol

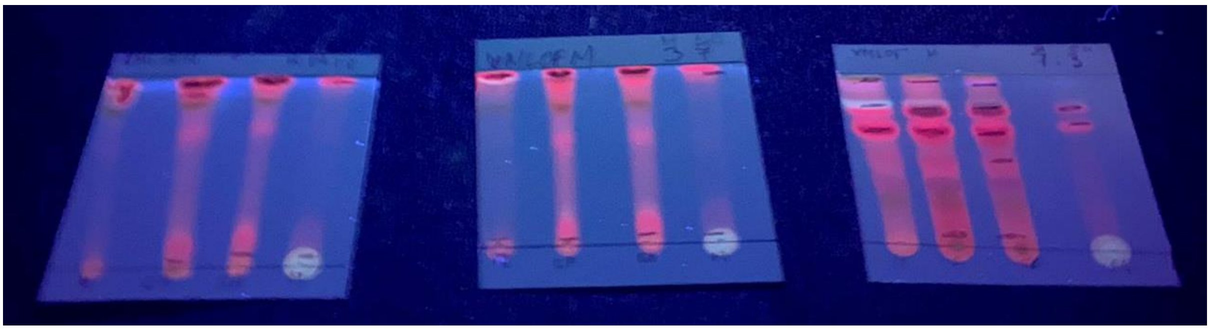
e) Crude obtained from ethyl acetate

Fig. 1: Extraction of Bioactive Compound from Moringa leaves

Table 1: Yield of crude obtained from different solvents from Moringa leaves

Sl No	Solvent	Yield of extract from Moringa leaves (mg)
1	Hexane	0.15
2	Chloroform	0.12
3	Ethyl Acetate	0.30
4	Methanol	0.22
5	Water	0.27

a) Under normal light



b) Under UV light

Fig. 2: TLC Chromatogram of Moringa oriflera a) under normal light b) under UV light

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The RF value provides information about the relative mobility of the compound compared to the solvent front. A higher RF value indicates that the compound has a greater affinity for the mobile phase and therefore travels a longer distance on the TLC plate.

It is important to note that TLC serves as a preliminary screening tool and does not provide information about the exact chemical structure of the compounds. Therefore, further characterization techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), or nuclear magnetic resonance (NMR) spectroscopy are often employed for more in-depth analysis and identification of the bioactive compounds.

Table 2: Show Rf value of each bioactive compound from different ratio of mobile phase used for different extracts

Name of the sample	Solvent Extraction	Mobile Phase	Ratio	Rf Value
Moringa oriflera - Leaves	Hexane	Hexane : Ethyl Acetate	7:3	0.08,0.27,0.36,0.5,0.66,0.80,0.94,
	chloroform			0.08,0.27,0.38,0.5,0.66,0.80,0.97
	Ethyl acetate			0.08,0.27,0.38,0.5,0.66,0.80,0.97
	Methanol			0.08,0.72,0.80
	Water			0.80,0.97
	Hexane	Hexane: Ethyl Acetate	1:1	0.07,0.80,0.92, 0.07,0.87
	chloroform			0.04,0.17,0.82,0.87,0.95, 0.04,0.95
	Ethyl acetate			0.07,0.17,0.68,0.85,0.97, 0.04,0.95
	Methanol			0.07,0.95, 0.04,0.95
	Water			0.04,0.95
	Hexane	Hexane : Ethyl Acetate	3:7	0.07,0.7,0.97,0.08,0.66,0.80,0.88,0.97
	chloroform			0.05,0.13,0.60,0.81,0.97, 0.05,0.81,0.97
	Ethyl acetate			0.07,0.57,0.78,0.97, 0.05,0.81,0.97
	Methanol			0.05,0.97, 0.07,0.95
	Water			0.80,0.97

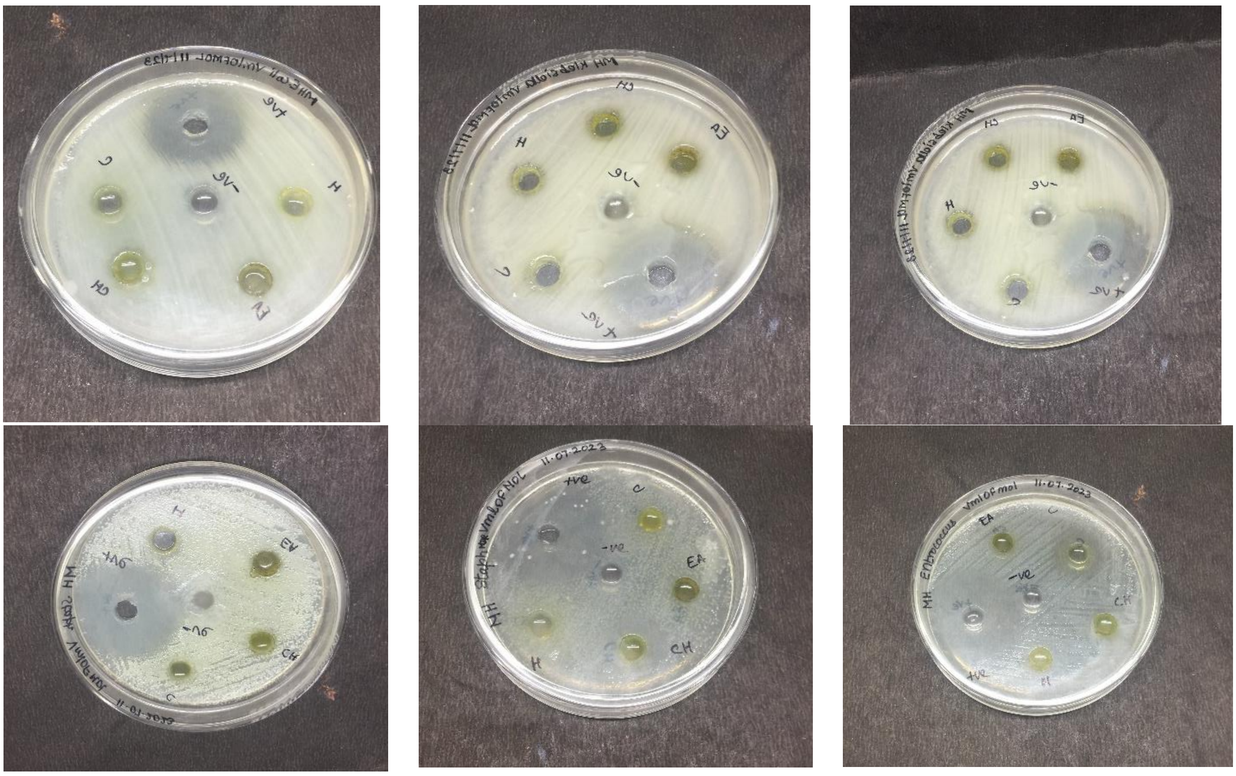

Fig. 3: Shows antibacterial activity of different solvent extract of Moringa leaves

Table 5: Shows zone of inhibition against test culture by Different extracts of Moringa leaves

Test cultures	Positive	Negative	Hexane	Chloroform	Ethyl acetate	Methanol
E coli	**12 mm**	-	**2mm**	-	-	-
Staphylococcus sps	**15 mm**	-	-	-	**1mm**	**3 mm**
Klebsiella sps	**14 mm**	-	**1 mm**	**1 mm**	**2 mm**	**3 mm**
Enterococcus sps	**15 mm**	-	-	-	-	**7 mm**
MDR Staphylococcus sps	**14 mm**	-	-	**6 mm**	-	-

IV. DISCUSSION

The findings of this study demonstrate that Moringa oleifera leaves possess potent antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as Multi drug resistant pathogens. The observed antimicrobial effects can be attributed to the presence of bioactive compounds such as phenolic acids, flavonoids, alkaloids, and glycosylates. These compounds have been reported to exert antimicrobial activity through various mechanisms, including disruption of cell membranes, inhibition of enzyme activity, and interference with microbial signalling pathways.

Thin Layer Chromatography (TLC) is a valuable technique for the identification of bioactive compounds in crude samples. By utilizing different ratios of the hexane/ethyl acetate mobile phase, the efficiency of compound extraction can be assessed. The RF value, calculated based on the distances traveled by the compound and the solvent, provides a means of comparing and identifying different compounds. TLC serves as an initial step in compound identification, guiding further analyses to elucidate the chemical nature and potential bioactivity of the identified compounds. However, further analysis using more advanced techniques such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) is necessary to identify and quantify the specific compounds present in each crude extract.

V. CONCLUSION

Moringa oleifera leaves are rich sources of bioactive compounds with significant antimicrobial activity. The identified components, including phenolic acids, flavonoids, alkaloids, and glucosinolates, contribute to the antimicrobial effects exhibited by Moringa oleifera extracts. Further research is warranted to elucidate the specific mechanisms of action and assess the potential of these bioactive compounds in the development of novel antimicrobial agents. The utilization of Moringa oleifera leaves as natural alternatives or supplements may offer promising strategies in the fight against microbial diseases.

In conclusion, moringa stands as an exceptional plant with immense potential to contribute to human nutrition and well-being. Its remarkable nutritional content, medicinal properties, and adaptability make it a valuable resource in the fight against malnutrition and various health challenges. As scientific research continues to unveil the numerous benefits of moringa, its role as a vital plant in promoting sustainable agriculture and human health is becoming increasingly recognized and appreciated.

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