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Phytochemical and Pharmacological Analysis of Moringa Oleifera Leaves

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ABSTRACT

The use of medicinal plant extract-mediated treatment was found to be very effective in controlling bacterial infection. The plant moringa oleifera leaves was evaluated against gram negative pathogenic bacteria S. aureus, E. coli, S. typhi, P. aeruginosa. The methanolic extract of moringa oleifera leaves also possesses moderate antifungal activity against candida albicans and candida tropical The MeOH.Ext and Aq Ext of moringa oleiferawere screened for insecticidal activity, against three different insect species i.e. Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis, revealed that MeOH.Ext and Aq Ext of the plant, showed significant activity against the tested insects.. The methanolic extract showed significant result in growth inhibition at concentrations of 1000 and 100 µg/ml, respectively. While no phytotoxic activity was observed at concentration 10 µg/ml. The MeOH Ext and Aq Ext ofmoringa oleifera show no hemagglutination activity against human red blood cell. The search for plant-based antimicrobials is growing due to rising resistance, high costs, and reduced efficacy of conventional medicines. Moringa oleifera has shown potential as a natural antimicrobial agent, though its active compounds were not fully assessed. This study identified inhibitory plant compounds against tested pathogens, suggesting their promising role in pharmaceutical applications. These findings highlight M. oleifera as a potential source of novel antimicrobials for controlling pathogenic bacteria effectively.

INTRODUCTION

A word Moringa comes from the Tamil word (Murungai) stand for "twisted pod", referring to the new fruit. This plant belongs to the monogeneric family called moringaceae which consists of 13 species [1]. *M. oleifera* is known as "miraculous tree" due to its excessive nutritive and pharmacologic value [2]. Dietary addition of *M. oleifera* showed to improve antioxidant capability, growth performance, health status, milk production and meat quality in numerous livestock SPP [3].

The common specie of Moringa genus is *M. oleifera* having resources of many phytochemical substances has antibacterial activity [4]. The species is used in the medicine globally due to its pharmacological activities and extensive therapeutic compounds. Due to these pharmacological activity this plant is designated as miraculous tree and has been placed among high value plants. *Moringa* has been used as a drug by many ayurvedic practitioners for the treatment of asthma and

its methanolic extract showed anthelmintic activity against adult ethopian earthworm's pheretima posithuma the word at various dose [5].

M. oleifera Leaves have been frequently cooked and eaten as vegetable and its leaf powder can also be used for soups making sauce supplement. Likewise, its fruit and immature pod are used as a highly nutritive vegetables and flower can be dried and steeped as tea in various parts of Africa and southern Asia [6]. Furthermore, it is nutrient dense and has been used variety of items including oils, meals, sauces, and medicine [7].

The human realized its preventive and curative properties and has been used as a medicine since thousands of years. It has also been reported that this tree kept substantial antibacterial, antifungal, anthelminthic as well as anti-leishmanial activities [8]. Wonderfully, Methanol extracts of Moringa oleifera leaves exhibited radioprotection effect against damaged produced by high

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doses of ionizing radioactivity showing the versatility and potential uses of this plant [9].

M. oleifera is ancient treatment of several skin diseases such as stimulant in paralytic conditions, epilepsy, and hysteria. The root of this plant is having ability to exhibit anti-inflammatory activity. But its other parts such as leaves, stem and seeds also had demonstrated various therapeutic properties [10]. M. oleifera in traditional popular medicine is a good curative plant. Numerous pharmacological studies have revealed the ability of this plant to exhibit antiantipyretic, inflammatory, analgesic, antioxidant. anticancer, nootropic, hepatoprotective, gastroprotective, cardiovascular, anti-ulcer, anti-obesity, antiepileptic, antiasthmatic, antidiabetic, urolithiatic, diuretic, local anesthetic, anthelmintic, antiimmunomodulatory, allergic. wound healing. antibacterial, and antidiarrheal properties. Moringa oleifera has pharmacological and wide traditional uses in various [11].

MATERIAL AND METHODS

The experimental plant M. oleifera was procured from Faisalabad. The healthy and uninfected leaves of the said plant were thoroughly washed with taped water followed by distilled water. The plant sample was then air dried at room temperature and grinded in to powder form [13]. Afterward, 150g of the powdered leaves were extracted with 600 ml of methanol. The extract was separately filtered using Whitman's filter paper No. 1. Then the extract was concentrated by rotatory evaporator at 40 C⁰. The leftover methanol in the extract was removed by placing it at room temperature overnight to obtained dried extract.

Phytochemical Screening of M. Oleifera Leaves **Extract**

Phytochemical analysis of *M. oleifera* leaves was carried out using standard procedures to identify the Qualitative constituents, Alkaloids, Flavonoids, Tannins and Phenols.

Test for Alkaloids

The MeOH Ext (1 mg) of M. oleifera was taken in test tube containing 1% of HCL and heat it for 20 minutes. Now shake the test tube gently and left to cool. Take 1 ml of the extract and added few drops of Wagner's reagent notice a creamy brown indicate the presence of Alkaloids.

Test for Flavonoids

About 3 ml of *M. oleifera* extract was added to 10ml of distilled water and mix it well notice a yellow color indicate the presence of Flavonoid.

Test for Tannins

Added the 2ml of *M.oleifera* Ext and put it in test tube and gently heat it for 2min add 3 drops of Ferric chloride notice orange color indicate the presence of Tannin.

Test for Phenols

About 3ml of M. oleifera extract added to 5ml distilled water then add few drops of 5% Ferric chloride notice dark green indicate the presence of phenols.

Antibacterial Activity Bacterial strains

The bacterial strains i.e. Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa were obtained from Sudais Hospital Charsadda. The strains were stored at -20 °C in ultra-low freezer at Bacha Khan University Charsadda.

Materials

The organic solvents i.e. methanol and dimethyl sulfoxide (DMSO) used in the experiments were of analytical grade. Others materials include nutrient ager. sterile cork-borers, petri dishes (14 cm), micropipettes, autoclave, laminar flow unit and incubator.

Procedure

The crude methanol and aqueous extract were screened for antibacterial activity against the above-mentioned pathogens. The antibacterial susceptibility test was performed by well diffusion method as recommended procedure (reference). Initially, nutrient agar (2.8g/ 100 mL) media was prepared to obtain the subculture of each bacterial strains on a separate Petri plates. The bacterial subculture was later inoculated in 20 ml nutrient broth (1.3g/100 ml) using sterile conical flasks and then incubated for 24 hours at 37 °C. Afterward, the test organisms were re-inoculated in 7 mL sterile nutrient broth and incubated for 24 hours at 37 °C. Nutrient agar medium was prepared on the same day of the experiment and poured into sterile petri dishes. The plates were incubated overnight for sterility test at 37 °C. After sterility test of the medium, test cultures (18-24 hours old) from the nutrient broth were transferred and evenly distributed over the nutrient agar plates using laminar flow unit. A 6 mm wells were then made with a sterile cork-borer in the respective agar medium plates. The methanol and aqueous extracts (10 µl) at concentration of 3 mg/mL were introduced into their respective wells. The organic solvent i.e. DMSO along with standard antibiotic Ampicillin were also introduced into the separate well as negative and positive control using micropipette. All the plates were incubated at 37 °C for 24 hours and the results were expressed in centimeter.

Insecticidal Activity Materials

The test insects viz: Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis, organic solvent (methanol), growth chamber, test sample, standard insecticidal drug (Permethrin), petri dishes (9 cm diameter), micropipette (1000 µl), filter paper, glass vials and brush.



Preparation of Test Sample

The stock solution. Plant's methanolic extracts and Aq Ext were dissolved in 3 ml 200 of volatile solvent for preparation of stock solution.

Rearing Technique

The stored grain pests were reared in plastic bottles containing breeding media (sterile) under controlled conditions required for the rearing of pests. The insects, selected for the experimental work, were of uniform size and age.

Procedure

The contact toxicity assay [13] was performed as following on first day, the filter paper was cut, equal in size and placed it in petri dishes of about 9 cm or 90 mm in size. Sample solutions of the crude methanolic extracts and Aq Ext were loaded to the petri dishes using a sterile micropipette. The plates were left overnight to evaporate the organic solvents from the samples completely. After complete evaporation of organic solvents from the petri dishes, on second day, 10 healthy insects small in size from each species were selected and transferred with the help of a clean brush to each plate including test samples and control. The plates containing samples and insects were incubated for 24 hours at 27°C in growth chamber with 50% relative humidity. After incubation, the results were recorded by counting the number of survived insects in each plate [15].

Antifungal Bioassay Materials Test Fungi

Saccharomyces cerevisiae, Candida albicans and Candida tropicalis. Sabouraud Dextrose Agar (SDA), autoclave, incubator, micropipettes, magnetic stirrer, dimethyl sulfoxide (DMSO), screw test tubes, standard antifungal drugs such as Miconazole and test samples (Cr. Ext. and Aq Ext).

Procedure

The anti-fungal activity of the test samples was performed as per our reported procedure. Stock solutions (24 mg/ml) of Cr. Ext. and Aq Ext were prepared in DMSO. Sabouraud dextrose agar (SDA) medium, for the growth of fungal specie, was prepared as per our reported procedure. Stock solution were transferred to screw test tubes that were sterilized using moist steam sterilization method. The test tubes were allowed to cool in slanting position. The final concentration became 400 µg/ml of SDA. Fresh culture of each specie was inoculated to test tubes. Other test tubes supplemented with DMSO and standard drugs were used as negative and positive control, respectively. All the test tubes were incubated for 3-7 days at 27±1°C. On day 7th, the visible non-mycelial linear growths (mm) microorganisms were measured and percent linear growth inhibitions of fungal strains were calculated by comparison with reference drug [16].

Phytotoxic Activity Materials

The test samples (MeOH. Ext. and Aq Ext) were tested for phytotoxic bioassay against Lemna minor. Micropipettes, flasks, growth chamber, test samples and organic solvent (methanol) were used in the experiments.

Procedure

The plant extracts were screened for phytotoxic activity. The experiment was performed by following steps. Stock solutions (20 mg/ml) of the test samples were prepared in methanol. Medium was prepared for the growth of L. minor from the stock solution, test sample at concentration of 10, 100 and 1000 μ g/ml were poured to flasks and left at room temperature to evaporate the organic solvent. After evaporation of the organic solvent, 20 ml of the E-media was added to all flasks sixteen healthy L. minor plants were selected, with a rosette of three fronds and put in respective flasks. The flasks containing L. minor and E-media were cubated at 27 ± 1 oC in growth chamber for seven days. Results were noted after seven days of incubation. [17].

Hemagglutination

Materials

All blood group samples, test tubes, centrifuge, incubator and phosphate buffer (pH 7.0).

Procedure

Hemagglutination activity of test samples was performed according to procedure. Phosphate buffer (pH 7.0) was prepared each in 50 ml of distilled water. The ratio of 3:7 (V/V). Stock solution (1 mg/ml of DMSO) was prepared and different dilutions i.e. 1:2, 1:4, 1:8 and 1:16 were made in phosphate buffer, from the stock solution. Fresh blood samples, from healthy persons, were collected on the same day of the experiment and centrifuged, the plasma was discarded and red blood cells were collected for the next steps. 2% RBC's suspension was prepared in the phosphate buffer. From each dilution 1 ml of sample was taken in a test tube and then adds 1 ml of the RBC's suspension to the sample. Incubate the test tubes for 30 minutes at 37oC. After incubation the test tubes were examined for the button formation, indicating negative results [18].

RESULT AND DISCUSSION

Phytochemical screening of the sequential extract of *M. oleifera* leaves showed the presence of various bioactive components which are phenol, alkaloids, flavonoids and tannin are the most prominent components and the result of phytochemical test is presented in Table 1. Among these phytochemical tests, methanol extract were found to contain maximum of alkaloids, flavonoids, tannin and phenol in comparison with other solvents.

Table 1 *Phytochemical Analysis of M.oleifera Leaves*

S. No.	TEST	Results	Remarks
1	Alkaloids	++	Positive
2	Flavonoids	+	Positive
3	Saponins	+	Positive
4	Tannins	+	Positive

Antifungal Activity

Antifungal activity of *M.oleifera* was study against several fungi namely Saccharomyces cerevisiae, Candida albicans and Candida tropicalis. The MeOH leaf significant Ext showed ctivity {80} Saccharomyces cerevisiaeas and Candida tropicalis. The Ag Ext of *M.oleifera*leaves show good activity {52} Candida tropicalis and Saccharomyces agnist cerevisiaeas. The MeOH Ext and Aq Extract of the plant ware inactive against Candida albicans The leaves of M.oleiferaresult show low antifungal agents against Candida albican And show good result against Saccharomyces cerevisiaeas and Candida tropicalis.

Table 2Antifungal activity of crude methanol and aqueous extracts of Moringa oleifera

Extracts	S. cerevisiae	C. tropicalis	C. albicans
MeOH	20±	5	0
AQ	13	4	0
+Control	25	9	6

Figure 1
Antifungal activity of crude methanol and aqueous extracts of Moringa oleifera

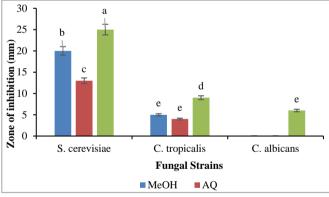


Figure 2 SDA media preparation



Figure 3Slant Preparation

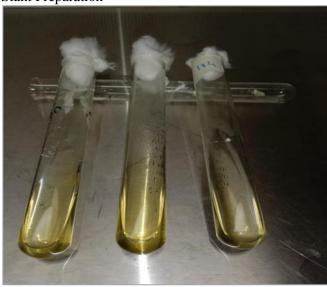


Figure 4Result of antifungal activity in slant



Antibacterial Activity

Crude Meth extract and Aq Ext of *M.oleifera* leaves were screened against the tested pathogens for possible antibacterial activity. Antibacterial activity of the crude MeOHExt and Aq Ext against bacterial species. The MeOH Ext presented significant activity against E. coli (55%), moderate against P. aeruginosa (40%), while low activity against S. typhi (30%) The Aq Ext of the plant show good result against *E.coli* (33%) moderate activity against *S. typhi* (20%) and the plant are inactive against *P. aeruginosa* (0%) The above results indicated that the MeOH Extof the plant showed significant activity up to (55%) as compared to the standard, which revealed that the plant contains potent antibacterial constituents.

Table 3Antibacterialactivity of crude methanol and aqueous extracts of M.oleifera

Extracts	E. coli	S. typhi	P. aeruginosa
MeOH	55	33	40
AQ	35	20	0

Figure 5
Antibacterial activity of crude methanol and aqueous extracts of Moringa oleifera

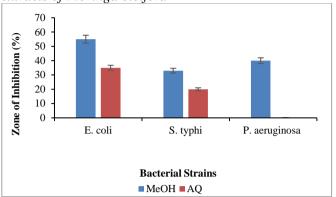


Figure 6 SDA media preparation



Figure 7 Plates prepare for activity



Figure 8Antibacterial activity in MeOH Ext



Insecticidal Activity

The crude methanol and Aq extracts of *M.oleifera* leaves were screened for insecticidal activity, using impregnated filter paper method. Methanolic and Aqueous Ext showed insecticidal activity against T. castaneum, Callosobruchus analis, R. dominica Permethrin (capex) was used as positive control while the organic solvent (DMSO) was treated as negative control. The positive control shows 100 % mortality while the mortality rate was 0% in case of negative control. From the results it may be concluded that *M.oleifera* leaves shows significant insecticidal property.

Table 4 *Insecticidal activity of crude MeOH and AQ extractsof M.oleifera*

Insects names	Days	MeOH Ext	AQ Ext	+Control
	1st	8	5	16
T. castaneum	2nd	11	14	16
	3rd	16	16	16
	1st	14	10	16
R. dominica	2nd	16	12	16
	3rd	16	15	16
	1st	6	7	16
C. analis	2nd	14	16	16
	3rd	16	16	16

Figure 9
Insecticidal activity of crude MeOH and AQ extractsof
M. oleifera

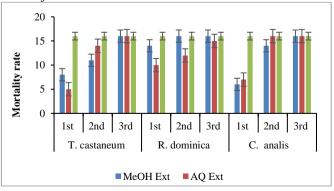


Figure 10 10 µl in Me OH Ext



Figure 11 100 μl inMe OH



Figure 12 1000 µl in Ext



Figure 13 1000 µl in DMSO Solution



Phytotoxic Activity

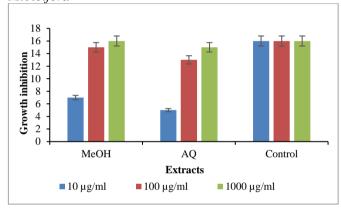
Herbicides of the *M. oleifera* origin are environmentally friendly that necessitates for their search. The phytotoxic results of crude extract and Aq Ext of *M. oleifera* leaves against Lemna minor. The MeOH Ext and Aq Ext growth inhibition at concentrations of 1000 and 100 μ g/ml, respectively. While no phytotoxic activity was observed at concentration 10 μ g/ml. Paraquat was used as standard plant growth inhibitor.

Table 5

Phytotoxic activity of crude methanol and aqueous extracts of Moringaoleifera

Extracts	Concentration (µg/ml)			
	10	100	1000	
MeOH	7	15	16	
AQ	5	13	15	
Control	16	16	16	

Figure 14
Phytotoxic activity of crude MeOHExtand AqExt of M.oleifera



Hemagglutination Activity

Crude extract and Aq Ext of *M.oliefera* at different dilutions were screened for possible hemagglutination activity against human erythrocytes of all blood groups (ABO). The results showed that crude MeOH Etx and Aq Ext show negative hemagglutination effect against human RBCs of all blood group.

Table 6Hemagglutination activity of crude MeOH and AqExt of M.oleifera

Blood group	1:2	1:4	1:8	1:16
AB-	-	-	-	-
B-	-	-	-	-
A-	-	-	-	-
B+	-	-	-	-
A+	-	-	-	-
AB+	-	-	-	-
O+	-	-	-	-
O-	-	-	-	-

CONCLUSIONS AND RECOMMENDATIONS

In recent times the use of plants as a source of novel compounds to combat microbialinfections has gained prominence. The necessity to search for plant based antimicrobial isincreasing due to high cost, reduced

efficacy and increased resistance to conventionalmedicines. Various findings suggest a new pathway in elucidating a potent antimicrobial agent from M. olifera. Generally though the active principle of the plant were notassessed. Hence, under this research the result indicated that the active principle of the plantwhich were inhibitory in the growth of the tested pathogens could become the promisingnatural antimicrobial agents with the potential applications in

pharmaceutical industries for controlling the pathogenic bacteria. In the contrary the powder form of the plantextract which was derived from fresh leave juice lacks those active principles which areof inhibitory for the growth of the tested pathogens, but the nutritive value of the extractwas safe for the growth of the tested bacteria which brings to an idea of the medium maydo have replace role of the synthetic general purpose medium.

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