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Antioxidant, antimicrobial activity and mineral profile of *Moringa oleifera* leaves extracts

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Abstract

This study was shaped to discover the antimicrobial, antioxidant and mineral profile of *Moringa oleifera* leaves extracts. The leaves crude extract was fractionated into different fractions using solvents of varying polarity. DPPH and FRAP methods were used to evaluate the antioxidant capacities of the extract fractions. DPPH scavenging activities were found in the range of 44.6 to 62.04% whereas the reducing power was in the range of 1.4 to 1.85mg/ml for ether and methanolic extracts respectively. Total phenolic contents (TPC) and total flavonoid contents (TFC) were also calculated and were found 284.66 ppm and 85.12 for methanolic and ethanolic extracts respectively. Antimicrobial analysis was completed against two fungal strains *Aspergillus niger* and *Aspergillus flavus* and bacterial strains *Escherichia coli* and *Bacillus subtilis* using Rifampicin and Terbinafine as control. ZID and MIC values were calculated. The analysis of mineral profile showed rich amounts of important minerals, in which the value for potassium was found to be greatest (17.12 ppm). The results of antioxidant and antimicrobial assays revealed that the plant leaves exhibited potent use to sustain healthy life.

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Introduction

Phytochemicals have the influence to inhibit oxidative damage brought on by the free radicals, oxygen anions, and hinder the oxidation process by reacting with the free radicals, chelating catalytic metals, and by acting as oxygen scavengers. Reactive oxygen species (ROS) are recognized as the reactive derivatives of the oxygen, which include hydrogen peroxide, hydroxyl radicals, superoxide anions or singlet of oxygen and are repeatedly created during the metabolic reactions occurring in the body. The reactive species are deactivated in the body by the antioxidants present in the body like catalase, superoxide dismutase and glutathione peroxidase. The excess production of the ROS is an unhealthy process specially when there is an improper protection by the antioxidants. Oxidative stress may damage the important molecules present in the body like proteins, lipoproteins, membrane lipids, DNA and RNA etc. (Patra *et al.,* 2008). Free radicals are molecular species proficient of self-regulating survival that surrounds one or more unpaired electrons that make them relatively more reactive, and must be transformed to some non-toxic shape to keep away from causing injury to the cell and ultimately the whole tissue may be in trouble (Bridges *et al.,* 1993).

Antioxidants are the materials which neutralize the dangerous outcomes of the ROS and are very efficient to donate their own electron to the free radicals. By taking free electrons from the antioxidants, they have not the energy to damage the cell and so the chain reaction ceased (Dekkers *et al.,* 1996). Antioxidants are broadly used in nutritional enhancement for the avoidance of altitude sickness, neurological problems, coronary heart disease, and cancer. These are also used to prevent food worsening during processing, circulation and storage. Artificial antioxidants such as butylated hydroxytoulen (BHT), butylated hydroxyanisol (BHA), tertiary butylhydroquinoe (TBHQ) and propyl gallate (PG) have been routinely used to prevent from oxidation. These antioxidants have lethal and carcinogenic effects and so its uses are therefore avoided. Thus, the requirement of natural antioxidants is increasing day by day that's why the concentration is spotlight on the growth of new, safe and inexpensive antioxidants of natural plant origin in the food industry and in preventive drug (Shanab *et al.,* 2007).

Plants contain therapeutic compounds like unsaturated long chain fatty acids, peptides, flavonoids, alkaloids, essential oils, phenols and aldehydes. These compounds are essential in remedial application against human and animal pathogens, including bacteria, fungi and viruses. These secondary metabolites shaped by plants are organic chemicals of high importance, which participate in variety of diverse purposes together with bacteriostatic, bactericidal, chemotherapeutic and antimicrobial functions and are used in nutraceutical industry (Patra *et al.,* 2008).

Moringa oleifera Lam (usually named as "The Miracle Tree," "Horseradish-tree," or "Ben oil tree") is the well-known and most extensively circulated species of *Moringaceae* family, having an inspiring range of therapeutic uses with elevated dietary worth all over the world. Native to Western and sub-Himalayan tracts, Pakistan, India, Asia, and Africa, this plant is well distributed in the Philippines, Cambodia, America, and the Caribbean Islands (Morton *et al.,* 1991). Associations such as Trees for Life, Educational Concerns and Church World Service have advocated *Moringa* as "Natural Nutrition for the Tropics" in different divisions of the world. Approximately each part of this extremely valued tree has long been consumed by humans and used for a variety of household functions as animal feed, for alley cropping, domestic cleaning agent, biogas, fertilizer, blue dye, green manure, foliar nutrient, gum, honey and sugar cane juice-cleaner, biopesticide, ornamental plantings, pulp, rope, tannin for tanning hides, water purification, machine lubrication, hair care products and manufacture of perfume (Palada *et al.,* 1996).

The leaves have own extraordinary dietary and curative traits. They hold elevated quantity of vitamin C, which battle a host of diseases including colds and flu; vitamin A, which acts as a protect against eye ailment, skin diseases, diarrhea, heart ailments etc.;

Calcium, which manufacture well-built bones and teeth and assist to avert osteoporosis; Potassium, which is crucial for the performance of the brain and nerves, and Proteins, the basic structure blocks of all our body cells. An additional significant point is that *Moringa* leaves hold all of the essential amino acids in an excellent amount, which are the building blocks of proteins. These leaves could be an immense benefit to people who do not acquire protein from meat. *Moringa* even includes argenine and histidine two amino acids particularly imperative for newborns, which are not capable to make sufficient protein for their development necessities (Mishra *et al.,* 2011). The micro-nutrient are more in dried leaves; (ten times the vitamin A of carrots), (17 times the calcium of milk), (15 times the potassium of bananas), (25 times the iron of spinach) and (nine times the protein of yogurt) (Manzoor *et al.,* 2007).

Keeping in view the above-mentioned facts it has been assumed that medicinal plants are very precious resources gifted by the nature and have gigantic effects for the human race and are called therapeutic goldmines. In the ongoing studies *M. oleifera* possessing abundant industrial and medicinal uses was pharmacologically investigated to explore its hidden medicinal potential.

Materials and methods

The leaves of *M. oleifera* were collected from District Nankana Sahib Punjab, Pakistan. The plant was identified by the Botany Department, University of Agriculture Faisalabad, Pakistan.

Preparation of leaves crude extracts

The crude extracts of the leaves of *M. oleifera* were prepared individually using 80% solution of five solvents of different polarity like *n*-hexane, ether, ethanol, methanol and acetone using distilled water as described below. The leaves of the collected plant were instantly transported to the laboratory, independently washed with distilled water and spread over newspaper for air exposure under shadow. After total dryness, the leaves of the plant were powdered using blender. A known quantity (10g) powdered of the plant leaves were taken in 250ml conical flasks and added with 100ml of the 80% solution of each solvent separately. The solvent-leaf powdered mixture was reserved for 24hrs on orbital shaker. After 24hrs, the extract of each flask solution was filtered using whattman filter paper to leave out the leaf powder remainders. The solvents were then removed using rotary evaporator. A semisolid material obtained were transported to screw-cap bottles, tagged and preserved in refrigerator at 4°C till further use.

Antioxidant activity

DPPH scavenging method

The 0.004% (w/v) of the DPPH was prepared in methanol. To the DPPH solution (1 ml) of each crude extract fraction were added to make the total volume 3ml and shake for 30 minutes. Color of the above solution was changed from purple (violet) to yellowish. Then measured the absorbance of each solution at 515nm. Same procedure was repeated by using ascorbic acid as standard. Percent inhibition was measured by using formula: $[(A_o\text{-}A_s/A_o) \times 100]$, where A_o is the absorbance of the standard and A_s is the absorbance of the sample (Mahmoudi *et al.,* 2009).

FRAP method

The 2.5ml leave extract was taken. Added 2.5 mL of the 0.2 M phosphate buffer maintained at pH 6.6 and 2.5mL of 0.1% potassium ferricyanide $[K_3Fe(CN)_6]$. Incubated for 20 minutes at 50°C and then 2.5ml of trichloro acetic acid (10%) was then added. The solution was then centrifuged for 10 minutes at 3000 rpm. After that 2.5ml of the upper layer was diluted with 2.5ml distilled water and then added the 0.5ml of the 0.1% ferric chloride. Color was changed from yellowish to bluish green. The absorbance was noted at 700 nm and Vitamin C was used as standard (Mahmoudi *et al.,* 2009).

Total phenolic contents (TPC)

TPC were measured by using Folin-Ciolcalteu method. Plant leave extract 0.25 mL was diluted with 3.5ml of the distilled water. Then in the above solution 0.25 ml of the Folin-Ciolcalteu reagent was added. 1ml of Na_2CO_3 (20%) was then added in the above solution. Solution was incubated at 40°C for 45 minutes and blue color was formed.

Absorbance of the above solution was measured at 685 nm. Standard was prepared by using methanol (80%) instead of the plant sample. Standard curve (abs. vs conc.) was prepared by using gallic acid. The TPC were then measured for each plant extract sample from the graph. The results were displayed as gallic acid equilent (GAE) (Singleton *et al.,* 1965).

Total flavonoid contents (TFC)

Leaves extract sample (2.5ml) was taken. Then added to it 150µL of the sodium nitrite ($NaNO_2$) followed by addition of 10% $AlCl_3$ (150µL) after five minutes. After 1 minute 1ml of the 1M NaOH was added. A blank solution was prepared by using 80% methanol instead of the leaves extract sample. Standard curve was constructed by using catechin as standard. Absorbance was noted at 510nm. TFC were calculated by using standard curve (Zhishen *et al.,* 1999).

Antimicrobial activity

Minimum Inhibitory Concentration (MIC) values for leaves extracts

Table 1. Material required.

Muller hinton medium
Nutrient broth
10 µl inoculums
100 µl plant sample
10 µl of Resazurin which indicate the cell growth
96 well plates

Preparation of the bacterial and fungal culture

A solitary colony was put into the bottles (100ml) of the isosensitest test broth as explained in the aseptic procedure. The bottles were placed in the incubator for 24 hours at 28°C and 37°C for fungus and bacteria respectively. A clean sample of the bacteria and fungus were obtained by centrifuging. The broth was spinned at 4000 rpm in a centrifuge for a period of 5 minutes. The supernatant was worthless and discarded. Normal saline (20ml) was used and the pellet was again rotated at 4000 rpm in a centrifuge for a period of 5 minutes. This procedure sustained until a clear solution was obtained. In a normal saline (20ml) the pellet was suspended and labeled for recognition. The optical density (OD) was calculated at 500 nm and dilute constantly until the OD reached between 0.5 and 1.0. The number of colony forming units was calculated by the viability graph.

Sample preparation for mineral profile

The sample was prepared according to the instructions given in the method described by AOAC (1990). I g of sample was taken in a beaker cleaned with distilled water. Then added 5ml of nitric acid and heated. Per chloric acid (5ml) was then added to improve the digestion. Then heated continuously until the solution remained 1-2mL. Then the volume of the solution was increased to 25mL by adding distilled water. The turbidity, if any could be removed by adding some drops of nitric acid.

Results and discussion

Table 2. Percent yield obtained from *M. oleifera* plant leave extracts.

S. No.	Solvent used for extraction	Yield (%)
1	Ethanol	3.52
2	Methanol	3.88
3	Acetone	3.45
4	Ether	1.58
5	*n*.Hexane	1.84

Total phenolic and Total flavonoid contents

The total phenolic contents (TPC) and total flavonoid contents (TFC) extracted from *M. oleifera* leaves by different solvents are presented in the (Fig 1). The range of TPC contents was 176.22 to 284.66mg GAE/g of Dw whereas TFC were in the range of 45.48 to 85.12mg CE/g of Dw. The maximum TPC contents were extracted by ethanolic extracts while minimum was by ether extracts, whereas maximum TFC contents were showed by methanolic solution and lowest by ether solution. The order of increasing TPC contents extracted by different solvents is; methanol > ethanol > acetone > *n*.Hexane > ether. The order of increasing TFC is given as Methanol > ethanol > *n*.Hexane > acetone > ether.

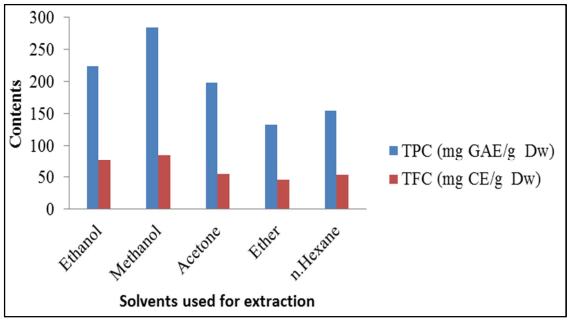

Fig. 1. Total Phenolic and Flavonoid Contents of *M. oleifera* plant leave extracts.

Antioxidant activity

The scavenging activities were in the range of 38.44 to 62.04%. The greatest potential was proved by methanolic solution (62.04%) and the least from *n*-hexane solution (38.44%). The order of increasing DPPH capacities are given as; methanol > ethanol > acetone > ether > *n*-hexane. The reducing power obtained was 1.40 to 1.92mg/ml. The uppermost reducing power was obtained by methanolic solution and lowest by acetonic solution. The order of increasing reducing power is given as methanol > ethanol > *n*-hexane > ether > acetone. (Table 3).

Antimicrobial activity

It was cleared from the results that the leaves extract has very good antifungal activities. The greatest MIC values was shown by methanolic extract (9.9mm) and lowest from ether extract (4.1mm) against *A. niger* and in the same way methanolic extract (9.6 mm) and ether extract (3.7mm) were the values of MIC for *A. flavus*. The order of the increasing antifungal activity performed by different solvents against *A. niger* and *A. flavus* is given as; methanolic > ethanolic > acetone > *n*-hexane > ether and methanolic > ethanolic > *n*-hexane > acetone > ether respectively (Fig. 2). The highest MIC value was showed by methanolic extract (18.8 mm) and the lowest by ether extract (9.1mm) against *E. coli,* while that for *B. subtilis* the greatest value was also for methanolic extract (17.1mm) and lowest from ether extract (11.4mm). The order of increasing MIC value for *E. coli* and *B. subtilis* is given as methanolic > acetone > ethanolic > *n*-hexane > ether and methanolic > ethanolic > acetone > *n*-hexane > ether respectively (Fig. 3).

Table 3. DPPH scavenging capacity (%) of the *M. oleifera* plant leave extracts.

S. No	Leave Extracts (80% soln.)	DPPH scavenging capacity (%)	Reducing Power (mg/mL)
1	Ethanol	52.43	1.85
2	Methanol	62.04	1.92
3	Acetone	48.18	1.40
4	Ether	44.60	1.64
5	*n*-hexane	38.44	1.82

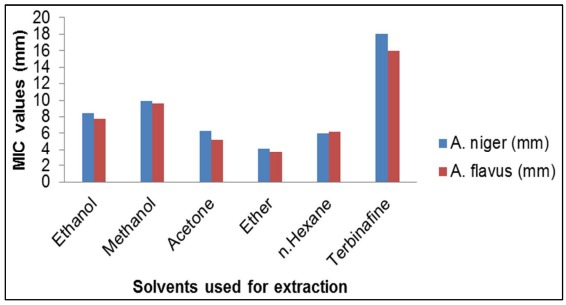

Fig. 2. Antifungal activity of *M. oleifera* plant leave extracts by Minimum Inhibitory Concentration (MIC) against standard strain.

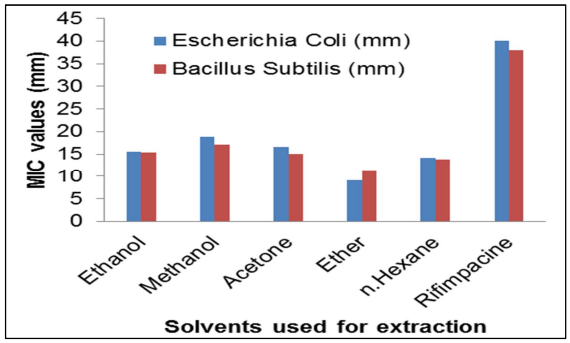

Fig. 3. Antibacterial activity of *M. oleifera* plant leave extracts by Minimum Inhibitory Concentration (MIC).

Table 4. Mineral Composition of *M. oleifera* leaves.

S. No.	Quantified Minerals	Concentration (mg/100g of DW)
1	Zinc	0.26
2	Copper	0.08
3	Iron	2.63
4	Manganese	0.77
5	Potassium	17.12
6	Phosphorous	12.35
7	Sodium	12.93

Mineral profile

By using AAS and following the procedure described in AOAC (1990), seven different minerals were analyzed. The results obtained were in the range of 0.08 to 17.12 ppm. It was cleared from the results that *M. oleifera* plant leave contain abundance of

important minerals which are necessary for the normal growth and working of the human body. The order of increasing concentration is as; Potassium> Sodium> Phosphorous> Iron> Manganese> Zinc> Copper (Table 4).

Conclusion

As the present research was designed to reveal the medicinal importance of *M. oleifera* plant, the results and discussion clearly emphasize the purpose of the study. The plant leaves were extracted for the bioactive material using ethanol, methanol, acetone, ether and *n*-hexane. 80% solution was prepared of each solvent for the extraction. Then, the obtained extracts were used to measure the antioxidant, antibacterial capacities and mineral profile. The results clearly indicate its potential against these microbes. Four parameters were examined to confirm the antioxidant capacities of the plant including DPPH scavenging capacities, reducing power, TPC and TFC and all of them showed promising results with reference to the standards. The antibacterial and antifungal activities were tested using two microorganisms for each against standard antibacterial and antifungal agent and the performance of the extracts was pleasingly good. The mineral profile also revealed that the plant leaves contain some very important minerals to sustain the healthy life like potassium, sodium, phosphorous, zinc, iron, manganese and copper.

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