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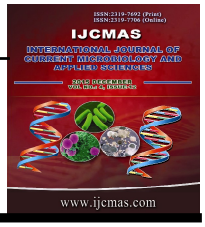

Original Research Article

Comparison of the Antimicrobial Activities of the Leaves-Crude Extracts of *Moringa peregrina* and *Moringa oleifera* in Saudi Arabia

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Keywords

M. peregrina, Moringa oleifera, antimicrobial activities, leave-crude extracts

A B S T R A C T

The present study investigated the potential antimicrobial activities of the leave-crude extracts of the moringa species, ***M. peregrina*** in comparison to those of *M. oleifera***.** Most of the previous studies on antimicrobial, antifungal, and antioxidant activities were concentrated on (*M. oleifera*) because of its prevalence in the poor areas in Africa and Asia where most of the people in the rural areas search for edible natural food resources. However, ***M. peregrina***, the tree that grown as a wild plant in the Arabian desert has received less focus and no details studies were traced on its chemical composition and biological activity. Results indicated that, ***M. peregrina*** proved to have antimicrobial activities against five bacterial species but this activity was less than that observed by *M. oleifera.* The genetic relations between the three Moringa species (***M. peregrina, M. oleifera and M. ovalifolia*** were studied using RAPD analysis. ***M. peregrina*** showed higher genetic similarity with *M. oleifera (49%)* than that with *M. ovalifolia (44%).* The results of this study indicated the possibility of using the leave extract of the Arabian moringa (***M. peregrina***) as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens.

Introduction

Moringa tree (also is known as the "miracle plant) belongs to the flowering plant family Moringaceae that contains 13 species from tropical and subtropical climates that range in size from tiny herbs to massive trees. However, the most widely cultivated species is *Moringa oleifera* and *M. peregrina. Moringa oleifera* Lam is the best known and most widely distributed species of Moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world (Tahany et al.,

2010). Almost every part of this highly esteemed tree have long been consumed by humans and used for various domestic purposes as for alley cropping, animal forage, biogas, domestic cleaning agent, blue dye, fertilizer, foliar nutrient, green manure, gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), ornamental plantings, biopesticide, pulp, rope, tannin for tanning hides, water purification, machine lubrication (oil), manufacture of perfume, and hair care products (Tahany et al., 2010). In general, *Moringa peregrina* tree belongs to the Moringa family, commonly known as a drumstick tree that is native to tropical widely naturalized and cultivated in many countries including Malaysia (Okuda *et al*., 2001). A literature survey indicated that the presence of quercetin flavonoids (Selvakumar and Natarajan, 2008), sterols (Yammuenart *et al*., 2008), tocopherols (γ and α), β -carotene and other antioxidants (Anwar *et al*., 2007) have been reported from the plant. The different extracts of the plant were also screened for In vitro anti-inflammatory and antioxidant activities (Anwar *et al*., 2007). The main product derived from *Moringa peregrina* is seed oil, called 'ben oil'. The use of the oil goes back to antiquity and is already referred to in old Egyptian texts and the Bible. The oil is used for cooking, in cosmetics and in medicine. In southern Sudan and Yemen *Moringa peregrina* is a bee plant and its leaves are used as fodder. The seeds are used in medicine in the Middle East and Sudan. The plant is grown as ornamental in Saudi Arabia and the Middle East.

Most of the previous studies on Moringa concentrated on (*M. oleifera*) because of its prevalence in the poor areas in Africa and Asia where most of the people in the rural areas search for edible natural food resources to support their living and fill the stomach in respective of the nutritive value of these resources. However, *M. peregrina*, the tree that grown as a wild plant in the Arabian desert and known as the Arabian tree of moringa has received less focus and no details studies were traced on its chemical composition and biological activity. Bukar *et al*., 2010, reported that a very important step in the screening of a plant material for sanitizing/preservative activity is to evaluate its antimicrobial activity against food – borne microorganisms. The determination of a plant's antimicrobial profile against food – borne microorganisms may promote the plant to further tests geared towards its evaluation as a sanitizer or preservative in foods.

Accordingly, the present study was proposed to investigate the potential antimicrobial activities of the leave-crud extracts of the *M. peregrina* in comparison to those of *M. oleifera*. In vitro studies using diffusion plate method were used to evaluate the antimicrobial activities. In addition, the fingerprint of the two moringa species was obtained using RAPD markers.

Materials and Methods

Collection of Plant Material

Leaves and seeds of *Moringa oleifera and M. peregrina* (family Moringaceae) were obtained from as a kindly gifted from Taibah University, Medina, Saudi Arabia. In addition, the seeds and leaves of *M. peregrina* were obtained from their original growing places in Egypt by the Desert research center (Fig.1). The species were identified and authenticated to the genus and specie level by specialized botany taxonomist. The collected leaves were air-dried at room temperature for 2 weeks, then ground and stored at -20°C. The collected

seeds were planted in soils (soil and sand 1:1) and grown in green house to obtain seedling and whole plants for DNA extraction and finger print studies.

Microorganisms (Bacterial strains)

Five food-borne bacterial species were used in this study (*Escherichia coli, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aerognosa* and *Enterococcus sp.*) to evaluate the antibacterial activity. The bacteria were obtained as kindly gift from the microbial genetics Lab., Biotechnology and Genetic Engineering Unit, Scientific Research Center, Taif University, KSA.

Preparation of leave extracts and Antimicrobial activity

The agar-well diffusion method was employed for determination of antibacterial activities (Cakir *et al*., 2004). All bacteria were suspended in sterile water and diluted to~10^6 CFU/ml. The suspension (100 µl) was spread onto the surface of NA medium. Wells (4.6 mm in diameter) were cut from the agar with a sterile borer. The concentrations of the leaves extract (8 mg /ml) were applied. Negative controls were prepared using water.

The artificial Streptomycin with a concentration of (12 mg/ml) was used as positive reference standards to determine the sensitivity of each microbial species tested and to compare the relative percent of antibacterial activity.

The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicate.

Isolation and Purification of Genomic DNA

Fresh young healthy leaves were collected from the mother and in vitro regenerated plants and grounded to powder with liquid N_2 using a mortar and pestle. Genomic DNA was isolated from leaf samples using the procedure described by the plant isolation kit (Biospin Plant Genomic DNA Extraction Kit, Japan).

RAPD Analysis

A mongo the total of 20 random primers that were used to detect the polymorphism, the sequence of the 12 primers that produce a clear scoriable and reproducible banding pattern is illustrated in (**Table 2).** The amplification reactions were performed in a 25 µl reaction volume containing about 30 ng genomic DNA, 2mM of each primers (Operon Technologies Inc.), 12.5 µL of Promega master mix (2X) and the final volume was adjusted to 25 µL with PCR water. The PCR reactions were applied using the Bio-Rad C1000 thermal cycler (Germany). An initial step of 5 min at 94°C was performed and followed by 40 cycles of 60 s at 94°C, an annealing step of 1 min at 37°C and an elongation step of 1 min at 72°C; and finally a 7 min extension at 72°C. The amplification products were resolved by electrophoresis in a 1,5% agarose gel containing ethidium bromide (0.5 µg mL^{-1}) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

Results and Discussion

To compare the antimicrobial activity of the two *Moringa* spicies (*Moringa oleifera* and *M. peregrina*) using leaves extract, the agar-

well diffusion method was used. This method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms (Bezerra *et al.,* 2004). The antimicrobial activity as a clear inhibition zone (in mm diameter) of methanol and ethanol extracts of *Moringa oleifera* and *M. peregrina* at the concentrations of 8 and 16 mg/ml were investigated. The two extracts were used against five pathogenic organisms, *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Pseudomonas sp.* and *Bacillus sp*. The results are presented in Table.1 and showed that the *Moringa oleifera* extract at final concentrations of 8 mg/ml were active against the five types of microorganism, while, at the same concentration for *M. peregrina* extract was not efficient to kill any types of treated microorganism (Fig. 2). On the other hand, the final concentration of 16 mg/ml of *Moringa oleifera* extract was effective to kill the treated microorganism (Fig. 2).

Study of Genetic Relationship Between Moringa Species

To study the genetic relations between the two Moringa species (*M. Peregrina, M. Oleifera,* another species *(M. Ovalifolia)* was used to perform the RAPD analysis. Among the 12 random primers used the profile of 8 primers that produced clear and reproducible bands is illustrated in Figure (3).

Seven primers produce polymorphic band pattern and only one primer (OP-A01 showed monomorphic band pattern. A total of 128 bands were obtained among with 91 polymorphic bands with polymorphism ratio of 75.8%) (Table.2). Primer OP-A03 produced the heights number of bands (18), while, primer OP-A10 produced the lowest number of bands (11).

Genetic Relationships as Revealed by RAPD

To examine the genetic relationships of the three Moring spices, the data scored from the RAPD markers were compiled and analyzed using the Dice similarity coefficient. The genetic similarity matrices based on the Dice coefficients was illustrated in Table 3. *M. Pregrena* showed higher genetic similarity with *M. Oleifera (49%)* than that with *M. Ovalifolia (44%).*

UPGMA clustering dendrogram based on DICE similarity index was obtained (Fig. 3). The dendrogram cluster diagram classified the evaluated genotypes in two major clusters. Both *M. peregrina and M. Oleifera* were located in the same cluster while *M. Ovalifolia* was in another cluster.

Recently, plants as an excellent source of medicine due to their high contents of different kinds of biologically active compounds played an important role in the control of many diseases. Searching and discovering new drugs from plants as antioxidant, antimalarials, anticancer, antimicrobials, antischistosomals etc have been continued by many researchers (Heinrich *et al.,* 2004). In this study the agar well diffusion method showed more clear results comparing with the disc well diffusion method when they used to assess the antimicrobial activity of two *Moringa* cultivars (*Moringa oleifera* and *M. peregrina*) using leaves extract (Fig. 2). This method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms (Omenka and Osuoha, 2000). Using agar well diffusion method, the antimicrobial activity as a clear inhibition zone (in mm diameter) of methanol and ethanol extracts of *Moringa oleifera* and *M. peregrina* at the concentrations of 8 and 16 mg/ml.

Table.1 Diameter of Inhibition Zone (DIZ) in mm of Five Bacterial Strains which Caused by Leaves Extract of *M. oleifera and M. peregrina*

Isolates	DIZ of Strep.	DIZ of *Moringa oleifera* extract		DIZ of *Moringa peregrina* extract	
		M	E	M	E
E. coli	**17**	**9**	**11**	**6**	**7**
S. aureus	**18**	**9**	**10**	**6**	**6**
Enterococcus sp.	**18**	**9**	**11**	**8**	**8**
A. hydrophila	**17**	**12**	**13**	**6**	**7**
P. aerognosa	**13**	**11**	**13**	**7**	**7**

Table.2 Total Bands, Polymorphic Bands and Percentage of Polymorphism of each Genetic Primers in the Three Moringa Species

Primers	Total bands	No. of Monomorphic bands	No. Polymorphic bands	% Monomorphic bands	% Polymorphic bands
OP-A01	13	0	13	00.0	100
OP-A02	17	4	13	23.5	76.5
OP-A03	18	7	11	39.0	61.0
OP-A08	13	1	12	7.60	92.4
OP-A10	11	1	10	9.00	91.0
OP-B06	16	2	14	12.5	87.5
OP-B08	17	8	9	47.1	52.9
OP-C02	15	6	9	40.0	60.0
Total	120	29	91		

Table.3 Similarity Matrix Table Showing the Genetic Dissimilarity Between the Three *Moringa* Species

	M. peregrine	***M. oleifera***	***M. ovalifolia***
M.Pregrena	0.0000		
M. Oleifera	0.51	0.00	
M.Ovalifolia	0.56	0.65	0.00

Fig.1 Seeds of Three Moringa Species, (A) *M. peregrina*, (B) *M.oleifera* and (C) is *M. Ovalifolia* (M. sp)

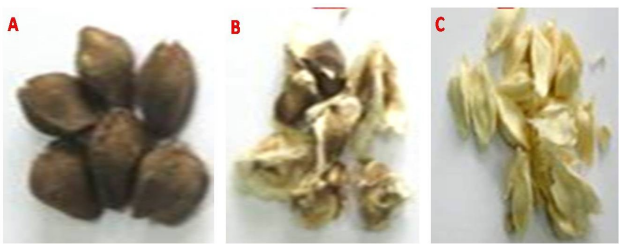
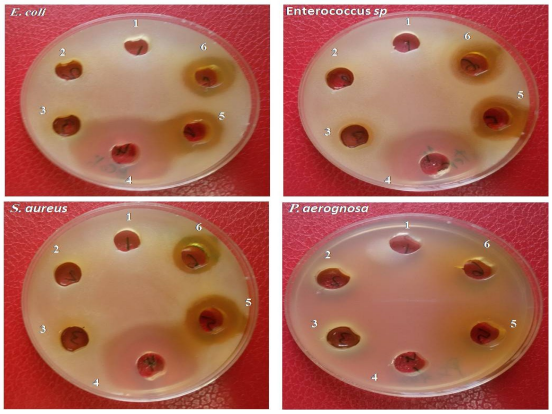
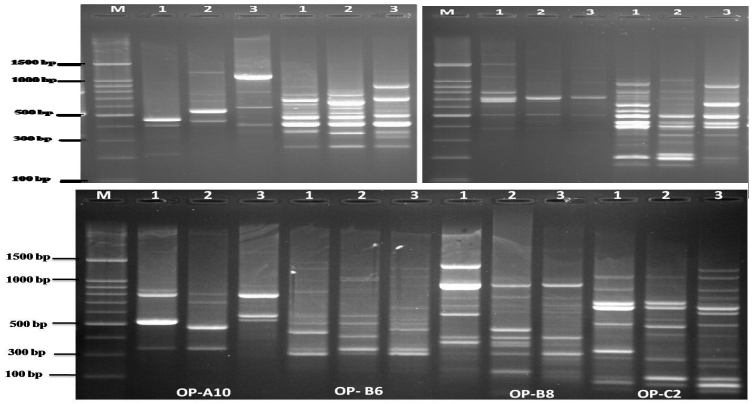


Fig.2 The Antimicrobial Activity of the Ethanol Extract of the Leaves of *M. pregrena* and *M. oleifera* against Four Bacterial Species using Agar Diffusion Method



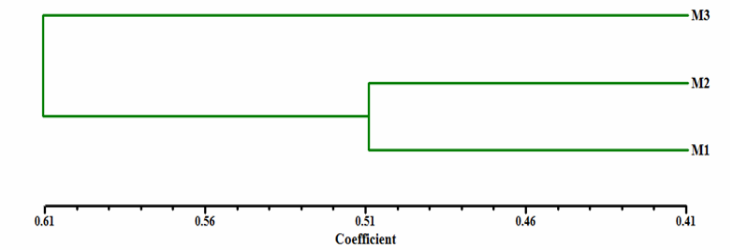
1= water, 2, 3 = *M. pregrena* methanol extract (8 mg/ ml) and (16 mg/ml), respectively, 4 = Streptomycin (15 mg/ml), 5, 6 = *M. oleifera* methanol extract (8 mg/ ml) and (16 mg/ml), respectively

Fig.3 PCR-RAPD Profile of the Three Moringa Species using 8 Random Primers



The primers are OP-A1, OP-AT, OP-A3, OP-A8, OP-A10, OP-B6, OP-B8 and OP-C2. Lan1 is *M. Oleifera*, lane 2 is *M. Pregrena* and lane 3 is *M. Ovalifolia*. M is the DNA marker, 100 ladder

Fig.4 RAPD Phylogenetic Analysis Showing the Genetic Relations Between the Three Moringa Species



M1 is *M. Pregrena*, M2 is *M. Oleifera* and M3 is *M. Ovalifolia*

The results indicated that the ethanol was the best solvent for extracting antimicrobial substances from the leaves of these plants compared to methanol. In a comparison, *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, and *Bacillus sp*. were mostly susceptible to *Moringa oleifera* extract than *M. peregrina* extract. *Pseudomonas sp* was the most resistant organisms to the concentrations of 16 mg/ml of the ethanol extract. Maximum activity of ethanol extract for *Moringa oleifera* was seen against *Aeromonas hydrophila* (13 mm).While, poor inhibitory effect was detected against *Staphylococcus aureus*.

Guevara *et al*., 1999 sugessted that the antimicrobial activity of *M. oleifera* seed is due to the presence of an array of phytochemiacals, but most importantly due to the activity of a short polypeptide named 4 (ά– L – rhamnosyloxy) benzyl-isothiocyanate. This peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Suarez *et al*., 2003). The antibacterial properties of the leaf of *M. oleifera* as shown in the present study corroborate the earlier claims by Aktar *et al*.,. 2006 and Foidl *et al*., 2001 who reported on the antibacterial properties of *M. oleifera* seed and leaf. In addition, various authors have reported antimicrobial activities of plant extracts on food – borne pathogens (Afolabi, 2007; Atiqur Rahman and Sun, 2009; Moreira *et al*., 2005; Kotzekidou *et al*., 2007), which indicates the vigorous pursuit in the search for more candidates of plant derived sanitizers and preservatives.

The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as *E. coli*, *S. aureus*, *A. hydrophila*, and *Bacillus sp* is encouraging as these organisms are pathogenic organisms liable to cause food – borne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically synthesized sanitizers/ preservatives with "naturally derived" ones (Jancxsen *et al*., 2002; Lanciotti *et al*., 2003).

RAPD analysis was used for finger printing of three Moringa species and to study the genetic relationship between them. The obtained 120 markers could clearly discriminate between the three Moringa species. Phylogentic analysis revealed that the Arabian Mornga (*M. pregrina)* is genetically more related to *M, oleifera* than *M. Ovalifolia*. This may be explained as it grown in North-Eastern Africa Ethiopia, Eritrea, Djibouti, Israel, Jordan, Somalia, Sudan, Syria, Yemen and some in Iran and Pakistan (mild-east) and *M. oleifera* is grown and distributed in near these area (in most of African countries), while *M. Ovalifolia* is grown only in Namibia and South Western Angola.

In conclusion, the antimicrobial activates of the Arabian moringa (*M. peregrina*) were compared with *M. oleifera* using the methanolic and ethanolic extract of the leaves. *M. peregrina* proved to have antimicrobial activities against five bacterial species but this activity was less than that observed by *M. oleifera.* In addition, ethanol was the best solvent comparing with water and methanol for moringa leaves as its extract showed the best antimicrobial activity. This finding indicates the possibility of using the leave extract of the Arabian moringa (*M. peregrina*) as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens or as sanitizers/preservatives against some food – borne microorganisms often implicated in the

spoilage of foods and food – borne illnesses. Further research should be conducted to test the sanitizing and preservative effect of the extracts on some foods.

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