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Antimicrobial Potential of Moringa peregrina Against Some Causative Agents of Urogenital Infections

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Abstract

Aim: To evaluate and compare the antimicrobial effectiveness of aqueous and methanol extracts from *Moringa peregrina* leaves against standard strains of microorganisms and clinical isolates.

Study Design: An experimental study which was carried out at the microbiology laboratory, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, during the period from September 2012 to March 2013.

Methodology: Aqueous and methanol extracts of *Moringa peregrina* at concentration of 100 mg/ml were subjected to antimicrobial screening against six standard strains include *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 7596) and 133 clinical isolates including bacterial and fungal species. Screening for antimicrobial activity of extracts, standard antibiotics and antifungal drug were detected by the agar well diffusion method. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) for the tested extracts were determined by using the broth dilution method. The MDIZs of clinical isolates were analyzed statistically using One-Way analysis of variance.

Results: Methanol extract of *Moringa peregrina* leaves revealed prominent activity against standard strains compared to the aqueous extract of the same plant where the diameter of inhibition zone (DIZ) was ranged from 18 mm to 21 mm. The aqueous extract exhibited moderate activity which was ranged from 16 mm to 17 mm. The MIC of leaves methanol extract of *Moringa*

peregrina for standard bacterial strains was ranged from <6.25 to 12.50 mg/ml, while the MBC was ranged from 6.25 mg/ml to 50 mg/ml. Standard strain and clinical isolates of *Candida albicans* was inhibited by 25 mg/ml from methanol extract, while the MFC was 50 mg/ml. The highest MDIZs obtained by the leaves methanol extract of *Moringa peregrina* on the clinical isolates was 18.93±3.07 mm for *Escherichia coli*, 18.50±2.07 mm for *Pseudomonas aeruginosa*, 18.62±2.72 mm for *Proteus mirabilis* and 18.11±2.47 mm for *Staphylococcus saprophyticus*. The antifungal activity of leaves methanol extract of *Moringa peregrina* on clinical isolates of *Candida albicans* was 16.56±1.62 mm which was lower than activity of Candizole 5 mg/ml. *Moringa peregrina* leaves extract showed varied MICs values towards clinical isolates which was ranged from 6.25 mg/ml to 25 mg/ml, while the MBC of the same isolates was from 25 mg/ml to 50 mg/ml.

Finally, Methanol extract of *Moringa peregrina* was found to be effective against both the standard strains and clinical isolates compared to the aqueous extract.

Keywords: Antimicrobial activity; *Moringa peregrina*; Methanol extract; Clinical isolates; aqueous extract; Antifungal activity.

Introduction:

Developed antimicrobial resistance is a worldwide problem. The situation in developing countries, however, is especially serious for the following reasons. In many countries, antimicrobials can be obtained outside of the recognized treatment centers, and taken without medical authorization or supervision. These lead to inappropriate use of antimicrobials and their being taken at wrong dosages and for an insufficient length of time [1, 2]. In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents [3]. Plant-derived products contain a great diversity of phytochemicals and other small compounds [4]. These compounds possess numerous health- related effects such as antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities [5]. Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing has no undesired side effects. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants [6]. *Moringa peregrina* (Forssk.) belonging to family Moringaceae, it is a small tree up to 10 meters tall [7]. Leaves alternate, in bunches at the ends of branches [8]. Adult trees produce leaves with a full complement

of tiny leaflets, only to drop them as the leaf matures [9, 10]. In tropical Africa it is reported from Sudan, Ethiopia, Eritrea, Djibouti and Somali [8, 11]. *Moringa peregrina* tree has a numerous uses; for cooking, in cosmetics and in medicine and to purify water [12]. In southern Sudan and Yemen it is a bee plant and its leaves used as fodder and it has claimed used to treat urinary tract infection [13]. The seeds used in medicine in the Middle East and Sudan [14]. Pterygospermin and related compounds (isothiocyanates), found in various parts of the Moringa plant, have been shown to have antibiotic and fungicidal properties. Investigation of the aerial parts of *M. peregrina* yielded arrays of biologically active compounds which include lupeol acetate, β-amyrin, α-amyrin, β-sitosterol, β-sitosterol-3-O-glucoside, apigenin , rhamnetin , neochlorogenic acid and methoxy-acacetin-β-glucoside [15, 16, 17]. The aim of this study was to assess the antimicrobial potential of aqueous and methanol extracts of the medicinal plant *Moringa peregrina*.

2. Materials and Methods

2.1 Plant material identification

The fresh dried leaves of *Moringa peregrina* was purchased from Omdurman local market, Omdurman, Sudan. The botanical identification of the plant was kindly made by Professor Hatil Hashim El-Kamali, Department of Botany-Faculty of Science and Technology, Omdurman Islamic University (OIU), Omdurman, Sudan. The Voucher specimens of the plant (HHK 378) have been deposited at Faculty of Sciences and Technology. Botany Department Herbarium, OIU. The microbiological investigation has been carried out in the microbiology laboratory, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan.

2.2 Preparation of plant extracts and standard antimicrobial agents

2.2.1 Preparation of aqueous extract

Aqueous extract was prepared according to the method described by EL-Kamali and Awad ELKarim, [18]. Exactly 100 g of the plant powder poured in 1 liter (1000 Milliliters) of hot sterile distilled water and left for 24 hours at room temperature. The mother liquor was filtered through What-man No.2 filter paper (Sigma Aldrish, Inc. USA). Extract was kept in deep freezer for 48 hours, then introduced in freeze dryer till completely dried. The dried plant extract was crushed from the flask using a spatula. The residue was weighed and the yield percentage was determined. The crude extract was stored in dark dry sterile containers in the microbiology laboratory, MAPRI, Khartoum, Sudan, until use for antimicrobial screening. At the time of testing, the aqueous residue (2 g) was dissolved in sterile distilled water 20 ml (con. 100 milligram/milliliter), and kept in refrigerator until used.

2.2.2 Preparation of methanol extract

The leaves were washed with distilled water, dried at 60°C overnight, then cut into small pieces and crushed in a mechanical mortar to a coarse powder. Powder sample (100 g) was soaked in 200 ml of 98 % methanol (Chem. Lab. Ltd. Belgium) for three days at room temperature and then filtered through What-Man No.2 filter paper. The methanol extract was filtered and evaporated under reduced pressure again using Rotary Evaporator to complete dryness. The residue was weighed and the yield percentage was determined. Then, the residue was stored dry in sterile containers in the microbiology laboratory, (MAPRI), until use for antimicrobial testing [18].

2.2.3 Preparation of standard antibiotic and antifungal drug

Gentamicin (Lunik Pharma Pvt. Ltd., India) and Candizole (The United Pharmaceutical. Ltd. Jordan) were prepared immediately before used by diluting them in sterile de-ionized water. A series of concentrations were done by double fold dilution method from the original concentration to get 40 µg /ml, 20 µg /ml, 10 µg /ml and 5 µg /ml for standard antibiotic, whereas the antifungal drug (Candizole) was prepared at concentration of 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml.

2.3 Preparation of culture media and inoculum

Bacteria were grown in enriched and selective culture media. Mueller-Hinton agar (Oxoid, Ltd, England) was used as base medium for screening of antibacterial activity. Sabouraud's dextrose agar (Oxoid, Ltd, England) was used as a medium for identification and it is used for screening of antifungal activity [19].

2.3.1 Preparation of McFarland standard

0.5 McFarland standard was prepared by mixing 9.95 ml of 1% Sulphuric acid (Shree Pushkar Chemicals & Fertilizers Ltd. India) with 0.05 ml of 1% anhydrous barium chloride in distilled water in order to estimate the approximate bacterial density [20]. The tube was use for comparison of bacterial suspension with the standard inoculum whenever required.

2.3.2 Preparation of bacterial suspension

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto Mueller-Hinton agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml of sterile normal saline, to produce a suspension containing approximately 10^7 CFU/ ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [21]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried Mueller-Hinton

agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°Cfor 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [22].

2.3.3 Preparation of fungal suspension

The inoculum of *C. albicans* was prepared according to method previously described by Cormican and Pfaller, [23]. Briefly, *C. albicans* was grown on Sabouraud's Dextrose Agar (SDA) plates for 24 hours. For each isolate, five colonies were grown until their diameters were at least 1 mm. Then, the colonies were picked off and suspended in 0.85% saline solution. The suspension was adjusted to the turbidity of a 0.5 McFarland standard at a wavelength of 530 nm. Quantitative colony plate counts were determined on SDA to verify the inoculum size. The final inoculum concentration was standardized to approximately 1×10^6 CFU/ml. The suspension was stored in the refrigerator at 4°C until used [24].

2.4 Purification of cultures by streaking plate method

The bacteriological and fungal techniques followed in the present work were described by Mackie and McCartney, [19], Koneman *et al*. [20], Kavanagh, [22]; Cruickshank *et al*. [25]; Arvidson *et al*. [26]; Cheesbrough, [27]. Once the primary inoculum from the clinical specimens was made, a wire loop was use to spread the material into the four quadrants of the plate, as described by Cheesbrough, [27] and Koneman *et al*. [20]. The wire loop was sterilized between each successive quadrant streak. The inoculated plates were incubated at 37°C for 24 hours. The purpose of this technique is to dilute the inoculum on the surface of the Agar medium, so that single isolated colonies of bacteria and fungi, known as colony forming units, can be isolated. This method was used for sub-culturing the standard strains and obtained clinical isolates on Nutrient agar, MacConkey's Agar, Mannitol salt agar, Blood Agar, Chocolate Agar, Nutrient Agar and Sabouraud's dextrose agar (Oxoid Limited, England).

2.5 Sources and maintenance of examined microorganisms

2.5.1 Sources of standard microorganisms

Five standard strains of bacteria and one species of fungi were obtained from American Type Culture Collection (ATCC) Rockville, Maryland, USA. Those reference strains include *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 7596).The strains were activated and sub-cultured three successive times and stored at 4°C.

2.5.2 Sources of clinical isolates

The clinical isolates were obtained from urogenital infections specimens of Sudanese patients (urines, urethral discharges and high-vaginal swabs). These isolates were collected from different General and Private Hospitals of Khartoum State, Sudan. After explaining the purpose of the study, verbal approval from the directors of these hospitals has been taken. Clinical isolates were maintained frozen in Trypton Soya Agar TSA, (Oxoid Ltd. England).

2.5.3 Identification of clinical isolates

Each clinical isolate was inoculated on a suitable culture media and incubated aerobically and the other an aerobically. The obtained isolates were then purified by streaking on plates containing the appropriate selective and differential culture media that mentioned above. The purified isolates were then identified by microscopic examination, cultural characters and biochemical tests and stored in a refrigerator until they were used.

2.6 In vitro screening for antimicrobial activity

2.6.1 Testing of antimicrobial activity of standard antibiotics and antifungal against standard microorganisms.

The agar well diffusion method was followed to determine the antimicrobial activity of standard antibiotic and antifungal drug against standard microorganisms. 20 ml of two culture media; Mueller-Hinton agar and Sabouraud's dextrose agar were poured on a glass Petri-dish of same size and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with the standard strain. Agar plate was punched with a sterile cork borer (No.4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each antibiotic using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured [19, 25].

2.6.2 Determination of antibacterial activity of plant extracts

The agar well diffusion method [19] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^7CFU/ ml were thoroughly mixed with 100ml of cooled molten sterile Mueller-Hinton agar which was maintained at 45ºC.20 ml aliquots of the inoculated Mueller-Hinton agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile Cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated.

2.6.3 Determination of antifungal activity of plant extracts

Testing of antifungal activity of extracts was performed by the agar well diffusion method with minor modifications. Two ml of the standardized *Candida albicans* stock suspension 10^6 CFU/ ml were thoroughly mixed with 100 ml of cooled molten sterile Sabouraud's Dextrose agar which was maintained at 45ºC. 20 ml aliquots of the inoculated Sabouraud's Dextrose agar were distributed into sterile Petri-dishes. The agar was left to solidify and in each of these plates 4 cups was cut using a sterile cork borer (No. 4) and the agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 24 - 48 hours. Two replicates were carried out for each extract against each of the test fungus. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated [28].

2.6.4 Determination of minimum inhibitory concentration (MIC) for standard microorganisms and clinical isolates

The active plant extract was later tested to determine the MIC values for each strain. Mueller-Hinton broth medium was used to test the inhibitory effect of tested extracts on standard bacteria and clinical isolates, while Sabouraud's dextrose broth was used to determine the MFC of extracts on the standard strains and clinical isolates of *Candida albicans*. MIC was determined using broth dilution method as described by Mackie and McCartney [19]. Tubes were prepared in the series of increasing concentrations of the plant extracts. The extracts were double-fold diluted to give the final concentrations of 3.125, 6.25, 12.50, 25, and 50 mg/ml. The organisms tested were

growing in broth over night to contain 10^7CFU/ml. A loop-full of diluted culture was spots with a standard wire loop that delivers 0.001 ml of inoculum and inoculated in tubes with equal volume of Mueller Hinton broth, Sabouraud's dextrose broth and the plant extracts. The tubes were incubated aerobically at 35°C for 24 to 48 hours. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hours when compared with the control tubes was considered as initial MIC.

2.6.5 Determination of minimum bactericidal and minimum fungicidal concentrations (MIC&MFC) for standard microorganisms and clinical isolates

The Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) determine the lowest concentrations that completely kill bacteria and fungi after 48 hours of incubation at 35°C. MBC and MFC were determined for each standard organism and clinical isolate after determination of MICs of microorganisms has completed. The streaks were taken from the two lowest concentrations of the plant extract plates that exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto the Mueller-Hinton agar plates (for bacteria) and Sabouraud's dextrose agar plates (for fungi). The plates were incubated at 35°C for 24 hours then examined for bacterial and fungal growth in corresponding to plant extract concentration. MBC and MFC were taken as the concentration of the plant extract that did not exhibiting any bacterial or fungal growth on the freshly inoculated agar plates [20].

2.7 Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences, SPSS Version 20 (SPSS, IBM, Corporation, New York, USA). The diameters of inhibition zones of tested organisms were expressed as mean ±SD. The data were subjected to one-way analysis of variance (ANOVA). The significance of differences between means was assessed, where appropriate. A two-tailed P-value of less than 0.05 was considered an evidence of statistical significance.

2.8 Ethical consideration

Approval was obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan to conduct the laboratory work.

Results and Discussion

In this study the aqueous and methanol extracts of *Moringa peregrina* leaves at concentration of 100 mg/ml were screened for their antimicrobial properties against six standard strains of microorganisms. As well as, the methanol extract was tested against 133 clinical isolates recovered

from urogenital infections of Sudanese patients. The work was also directed to compare the performance of *Moringa peregrina* extracts relative to selected standard antimicrobial drugs; gentamicin and Candizole. Based on the results of identification tests, it was found that the 133 clinical isolates were dispersed as follows 29 *Escherichia coli*, 18 *Pseudomonas aeruginosa*,16 *Proteus mirabilis*, 14 Staphylococcus *saprophyticus*, 13 *Neisseria gonorrhoeae*, 12 *Klebsiella pneumoniae*, 11 *Enterococcus* spp. and 20 *Candida albicans* (Table 1).

Table (1): Biochemical properties of the tested clinical isolates

Characterestics	**Tested clinical isolates**							
	E.c.	P.a.	P.m.	S.sa.	N.g.	K.p.	E.sp.	C.a.
Gram stain	G-ve	G-ve	G-ve	G+ve	G-ve	G-ve	G+ve	ND
Aerobic growth	+	+	+	+	+	+	+	+
Motility Test	+	+	+	-	-	-	-	ND
Catalase Test	+	+	+	+	+	+	-	ND
Coagulase Test	ND	ND	ND	-	ND	ND	ND	ND
Indole Test	+	-	-	-	-	-	-	ND
Methyl red Test	+	-	-	+	-	-	-	ND
Voges Proskauer	-	-	+/-	-	ND	+	+	ND
Oxidase Test	-	+	-	-	+	-	-	-
Urease Test	-	-	+	+	-	+	-	-
Citrate Test	-	+	+/-	-	ND	+	-	-
DNase Test	-	-	-	-	-	-	-	-
Nitrate Test	+	+	+	-	-	+	+	-
Germ tube Test	ND	ND	ND	ND	ND	ND	ND	+
Novobiocin(5µg)	S	ND	ND	R	ND	S	S	ND
Bacitracin	ND	ND	ND	ND	ND	R	ND	ND
Mannitol	+	+	-	+	-	+	-	-
Sucrose	+/-	-	-	+	-	+	-	+
Lactose	+	-	-	+	-	+	+	-
Acid from glucose	-	+	+	+	+	+	+	+
Gas from glucose	+	-	+	-	+	+	-	-

Key: E.c.: *Escherichia coli*; P.a.:*Pseudomonas aeruginosa*; P.m.: *Proteus mirabilis*; S.sa.: *Staphylococcus saprophyticus*; N.g.: *Neisseria gonorrhoeae* K.p.: *Klebsiella pneumoniae*; E.sp.:

Enterococcus spp.; C.a.: *Candida albicans*; +: more than 80% of isolates had positive reaction. **;** - : more than 80% of isolates had negative reaction. +/-: variable character. ; ND: not determined**;** R: Resistant S: Sensitive; G-ve: Gram negative reaction. ; G +ve: Gram positive reaction.

3.1 The antimicrobial potency of *Moringa peregrina* leaves extracts on the standard strains:

The yield percentages of *Moringa peregrina* leaves methanol extract was 5.4%, while the yield of the aqueous extract was 2.6% which was lower compared to the yield percentages of methanol extract. The results of susceptibility were interpreted as active (>18 mm), moderate active (14-18 mm), and inactive (< 14 mm) [20, 29, 30]. Methanol extract of *Moringa peregrina* leaves was found active against standard strains compared to the aqueous extract (Table 2). The highest diameter of inhibition zone obtained by *Staphylococcus aureus* was 21 mm. Standard strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* have got a diameter of inhibition zone which was 20 mm for each. The antifungal activity of methanol extract of *Moringa peregrina* towards standard strain of *Candida albicans* was found to be active where the diameter of inhibition zone was 19 mm. The leaves aqueous extract was moderately active against *Escherichia coli* (16 mm), *Staphylococcus aureus* (17 mm) and *Proteus vulgaris* (16 mm), whereas it was inactivate against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* (Table 2). These results are similar to those findings by Eilert *et al*. [31]; Abdel-Rahman *et al*. [16] and Lalas *et al*., [32]. Simultaneously, it was noted that the methanol extract of *Moringa peregrina* leaves affects Gram positive and Gram negative bacteria, as well as the tested fungus. The results were supported that, the patterns of inhibition not varied with plant part only, but also the solvent system and method of extraction may affect both the yielded extract and antimicrobial activity on the tested organism. These findings are in agreement with Abdel-Rahman *et al*. [16] and Hajar and Nehad, [33]

Table (2): The antimicrobial activity of *Moringa peregrina* extracts against standard strains.

Plant name & Part used	Solvent system	Yield %	Standard strains DIZ/mm of 100 mg /ml.					
			E.c	S.a	P.a	K.P	p.v	C.a
Moringa peregrine leaves	Methanol	5.4	20	21	18	20	20	19
	Aqueous	2.6	16	17	-	-	16	-

Key :(-) No growth. E.c. = *Escherichia coli* (ATCC 25922), Ps.a.= *Pseudomonas aeruginosa* (ATCC 27853), K.p.=*Klebsiella pneumoniae* (ATTCC 53657), P.v. = *Proteus vulgaris* (ATCC 6380), S.a. = *Staphylococcus aureus* (ATCC 25923) and C. a. = *Candida albicans* (ATCC 7596).

3.2 Effects of standard antibiotics versus methanol extract of *Moringa peregrina* on the standard strain

Analysis of variance showed that there are statistical differences between effects of Gentamicin 40 µg/ml, Gentamicin 20 µg/ml and Gentamicin 5 µg/ml and effect of 100 mg/ml of leaves methanol extract of *Moringa peregrina* on the tested standard bacterial strains, however these differences are highly significant (P=0.000). On the other hand, there is no statistical significant differences between effects of Gentamicin 10 µg/ml and 100 mg/ml of methanol extract of *Moringa peregrina* (P=0.997) (Table 3, Fig. 1). Regarding antifungal activity 100 mg/ml of leaves methanol extract of *Moringa peregrina* showed high antifungal activity towards standard strain of *Candida albicans* which was approximately nearest to the activity of Candizole 5 mg/ml (Table 4, Fig. 2)

Table (3) Antibacterial activity of standard antibiotics against the standard bacterial strain

Antibiotic	**Concentrations (µg /ml)**	**Tested standard strains/ DIZ mm**				
		E.c.	**S.au.**	**Ps.a.**	**K.p.**	**P.v.**
Gentamicin	**40**	28	28	21	23	24
	20	26	26	19	21	22
	10	16	24	18	20	20
	5	-	20	17	18	18

Key: E. c. = Escherichia coli (ATCC 25922), S. a. =Staphylococcus aureus (ATCC 25923), Ps. a. = Pseudomonas aeruginosa (ATCC 27853), K. p. = Klebsiella pneumoniae (ATCC 53657), P. v. = Proteus vulgaris (ATCC 6380) and **DIZ/ mm: Diameter of Inhibition Zone in Millimeters. µg /ml= microgram/milliliter

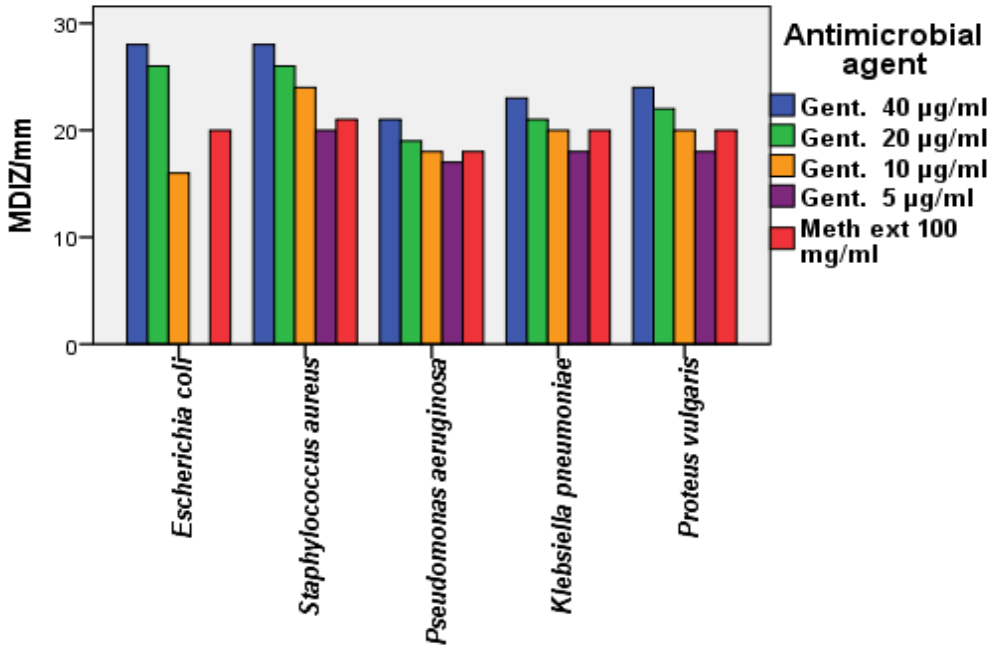


Fig. (1): Antibacterial activity of standard antibiotic and tested extract versus standard bacterial strains

Table (4) Antifungal activity against standard *Candida albicans*

Antifungal drug	**Concentrations (**mg /ml**)**	***Candida albicans*/ DIZ mm**
Candizole	40	26
	20	25
	10	23
	5	20

Key: *Candida albicans* (ATCC 7596). DIZ/ mm: Diameter of Inhibition Zone in Millimeters. mg / ml= milligram/milliliter

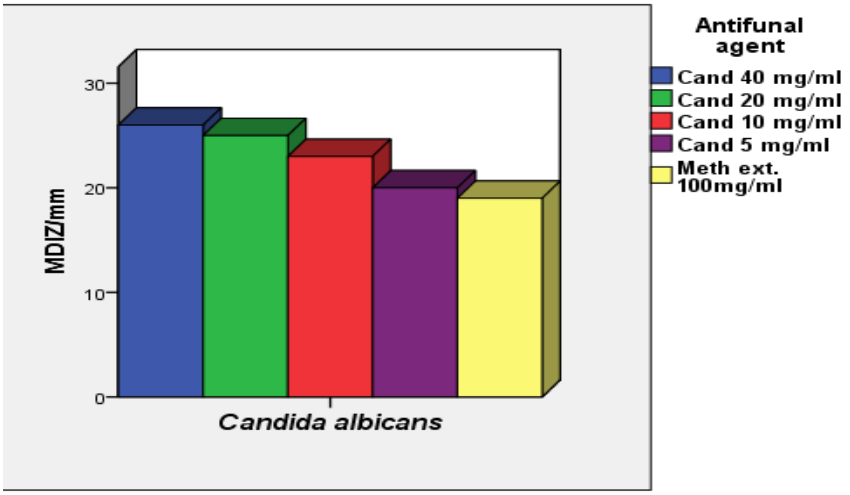


Fig. (2): Activity of standard antifungal drug and tested extract versus standard fungus

3.3 The antimicrobial activity of *Moringa peregrina* methanol extract on the clinical isolates:

The methanol extract of leaves of *Moringa peregrina* was only assessed against clinical isolates because the results of antimicrobial activity of the aqueous extract on the standard strains was lower and not encouraging to continue in measuring further antibacterial activity screening for the aqueous extracts. The antimicrobial activity of extracts on the clinical isolates was expressed as MDIZ/ mm ± SD. The highest MDIZs obtained by the leaves methanol extract of *Moringa peregrina* on the clinical isolates was 18.93±3.07 mm for *Escherichia coli*, 18.50±2.07 mm for *Pseudomonas aeruginosa*, 18.62±2.72 mm for *Proteus mirabilis* and 18.11±2.47 mm for *Staphylococcus saprophyticus*. On the other hand, the rest of clinical isolates exhibited lower susceptibility, where the clinical isolates of *Neisseria gonorrhoeae*, *Klebsiella pneumoniae* and *Enterococcus* sp. were revealed MDIZ of 17.91±2.70 mm, 17.78±1.79 mm and 17.88±1.96 mm for each respectively (Table 5). The antifungal activity of leaves methanol extract of *Moringa peregrina* on the clinical isolates of *Candida albicans* was 16.56±1.62 mm which was lower than activity of Candizole 5 mg/ml (Table 6).

Table (5): Antibacterial activity of methanol extract of *Moringa peregrina* versus standard antibiotic on clinical isolates

No. of clinical isolates	MDIZ/mm of tested extract and standard antibiotic				
	Meth. ext.100 mg/ml	Gentamicin/ concentration (µg /ml)			
		40	20	10	5
E. c.(29)	18.93±3.07	28	25	22	18
Ps. a.(18)	18.50±2.07	21	19	17	17
P. m.(16)	18.62±2.72	24	22	20	18
S. s.(14)	18.11±2.47	28	24	21	18
N. g.13	17.91±2.70	21.85	18.50	17	16
K. p.12	17.78±1.79	23	21	19	17
E. spp. 11	17.88±1.96	21.36	19.5	18	17
Total 133					

Key: E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa. P.m = Proteus mirabilis; S.s. = Staphylococcus saprophyticus; N.g.= Neisseria gonorrhoeae.; K.p.= Klebsiella pneumoniae, E.spp.=Enterococcus sp. MDIZ/ mm: Mean Diameter of Inhibition Zone ± SD: Standard Deviation; Meth ext: Methanol extract; Gent. : Gentamicin.

Table (6) Antifungal activity of methanol extract of *Moringa peregrina* versus standard antifungal drug on clinical isolates

No. of clinical isolates	MDIZ/mm of tested extract and standard antifungal drug				
	Meth. ext.100 mg/ml	Candizole concentration (mg/ml)			
		40	20	10	5
Candida albicans (20)	16.56±1.62	26	25	23	20

Key: E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa. P.m = Proteus mirabilis; S.s. = Staphylococcus saprophyticus; N.g.= Neisseria gonorrhoeae.; K.p.= Klebsiella pneumoniae, E.spp.=Enterococcus sp.

3.4 MICs, MBCs and MFC values of methanol extract of *Moringa peregrina* versus standard strains

Moringa peregrina leaves methanol extract exhibited MICs values which was about 6.25 mg/ml, for *E. coli* and *Pseudomonas aeruginosa*, 12.25 mg/ml for *Proteus vulgaris* and *Staphylococcus aureus* and it was 25 mg/ml for *Klebsiella pneumoniae* (Table 7). The MBC of *Moringa peregrina* leaves methanol extract on the standard strains was ranged from 6.25 to 50 mg/ml. Standard strain of *Candida albicans* was inhibited by 25 mg/ml, while the MFC of it was 50 mg/ml. A glance at Table 3 reveals that the antimicrobial activity of the methanol extract of *Moringa peregrina* is parallel to the activity of 5 to 10 µg/ml gentamicin, while the antifungal effect of the same plant found to be less than activity of 5 mg/ml candizole. There are some studies reported the antimicrobial potential of *Moringa oleifera*, however, little studies evaluate this activity on *Moringa peregrina* [34, 16, 32, 33].

Table (7): MICs, MBCs and MFC values of methanol extract of *Moringa peregrina* versus standard strains.

Standard organisms	**MICs, MBCs and MFC values per mg /ml**											
	E.c		Ps.a		P.v		S.a		K.p		C.a	
Leaves methanol extract	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
	<6.25	6.25	6.25	12.5	12.5	25	12.5	25	25	50	25	50

Key: MICs: Minimum inhibitory concentrations, MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration; E.c. = *Escherichia coli;* S.a. = *Staphylococcus* aureus; Ps.a. = *Pseudomonas aeruginosa;* K.p.= *Klebsiella pneumonia;* P.v. = *Proteus vulgaris ;*C. a. = *Candida albicans*

3.5 MICs of *Moringa peregrina* methanol extract against clinical isolates:

Moringa peregrina leaves extract showed varied MICs values towards clinical isolates, as the MIC value was about 12.50 mg/ml for *Escherichia coli*, *Staphylococcus saprophyticus*, *Neisseria gonorrhoeae* and *Enterococcus* spp. On the other hand, it was 25 mg/ml for *Proteus mirabilis*, *Klebsiella pneumoniae* and *Candida albicans*, while the same extract revealed MIC value > 6.25 mg/ml towards isolates of *Pseudomonas aeruginosa* (Table 8 and Table 9). The MBC of the

leaves methanol extract of *Moringa peregrina* against isolates was ranged from 6.25 to 25 mg/ml. Clinical isolates of *Candida albicans* was inhibited by 25 mg/ml, while the MFC of it was 50 mg/ml. This activity could be due to presence of β- amyrin, α- amyrin, β-sitosterol and terepenoids that have been proved as antimicrobial agents (Abdel-Rahman *et al*. 2010). In this study, *Moringa peregrina* found to be active almost against all tested isolates. These findings corresponding to Abdel-Rahman *et al*. [16].

Table (8): MICs, MBCs and MFC values of methanol extract of *Moringa peregrina* versus standard strains.

Clinical isolates	**MICs, MBCs and MFC values per mg /ml**													
	E.c		**Ps.a**		**P.m**		**S.sap**		**N.g**		**K.p**		**E.sp**	
Leaves methanol extract	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	12.5	25	6.25	12.5	25	50	12.5	25	12.5	25	25	50	12.5	50

Key: MICs: Minimum inhibitory concentrations. ; E.c. = *Escherichia coli*, Ps.a. = *Pseudomonas aeruginosa*. P.m = *Proteus mirabilis*; S.sap. = *Staphylococcus* saprophyticus; N.g. = *Neisseria gonorrhoeae*; K.p.= *Klebsiella pneumoniae* E.spp.= *Enterococcus* sp.; C. a. = *Candida albicans*; (-): Not determined.

Table (9): MIC and MFC values of methanol extract of *Moringa peregrina* versus clinical isolates of Candida albicans per mg /ml.

Plant part	MIC and MFC values of clinical isolates of Candida albicans per mg /ml	
	MIC	MFC
Leaves methanol extract	25	50

Conclusion

Methanol extract of *Moringa peregrina* leaves, exhibited various extents of antimicrobial activity on the standard strains and clinical isolates observed in this study. Methanol extracts *Moringa peregrina* leaves found more effective on the tested microorganisms compared to the aqueous extracts of the same plant. Most of Gram positive and Gram negative bacteria which screened in this study were found to be sensitive to methanol extracts of *Moringa peregrina* by various degrees. On the other hand, the clinical isolates of *Candida albicans* inhibited by the leaves methanol extract of *Moringa peregrina*. An interesting finding of this work is that the clinical isolates of known resistant Gram negative bacteria *Pseudomonas aeruginosa* were highly susceptible to methanol extract of *Moringa peregrina*. Moreover, the investigated plant exhibited unique antimicrobial property that may equal or exceed the activity of 5 to 10 µg/ml gentamicin and 5 mg/ml Candizole. Consequently, this plant revealed promising biological activity and could form a good basis for its selection for further investigation in order to develop a new natural bioactive compounds.

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Competing Interests

Author has declared that no competing interests exist.

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