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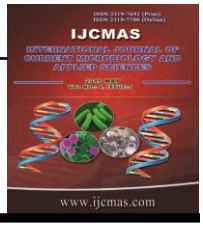

Original Research Article

Effect of *Moringa oleifera* Seed Oil on Antimicrobial Activity of some Antibiotics against some Pathogenic Gram Negative Bacteria

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Keywords

M. oleifera, Gram negative, resistance, imipenem, chloramphenicol

A B S T R A C T

Several reports had focused on the antimicrobial activity of *Moringa oleifera* oil against pathogenic microorganisms but none of these reports had studied the antimicrobial activity of combinations between *M. oleifera* oil and antibiotic against Gram negative bacteria. In the present study, antimicrobial efficacy of *M. oleifera* oil alone and combined with antibiotic is studied by agar diffusion method. The results revealed that the antibacterial activity of *M. oleifera* oil against *E. coil*, *Klebsiella* sp. *Pseudomonas* sp. and *Proteus* sp. is weak or not existed. In addition, the sensitivity of tested bacteria to some tested antibiotics had increased in medium contained *M. oleifera* oil depending on tested bacteria and antibiotic, as well as the concentration of *M. oleifera* oil. In addition, the sensitivity of all tested bacteria to imipenem (IMP) had significantly increasedin medium contained *M. oleifera* oil. While, the sensitivity of *Klebsiella* sp. and *Proteus* sp. to chloramphenicol (C) had significantly decreased in medium contained *M. oleifera* oil, but it had significantly increased with *E. coli*. Moreover, addition of *M. oleifera* oil to the medium had significantly increased the sensitivity of *E. coli* & *Klebsiella*; *E. coil* & *Pseudomonas* and *E. coil* & *Proteus* tomeropenem (MEM), cefixime (CFM) and ertapenem (ETP) & doxycycline (DO), respectively. Furthermore, the antimicrobial activity of amikacin (AK) & gentamicin (EN) in medium contained *M. oleifera* oil had significantly increased only against *Proteus & Pseudomonas*. Thus, *M. oleifera* oil could be used as antibiotic resistant modifying agent against multi-drug resistant Gram negative bacteria.

Introduction

During the last decades, the limit of microbial diseases and infections has been exceeded dramatically. A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment.

The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes (Ang *et al.*, 2004; Sokovi *et al.*, 2010; Bajpai *et al.*, 2013).

Natural products isolated from various medicinal plants have traditionally been the most common source of drugs and still represent more than 30% of the current pharmaceutical markets (Jabar and Al-Mossawi, 2007; Fakurazi *et al.,* 2012 and Kumar *et al.*, 2012). *Moringa oliefera* is an ancient tree that is historically known to possess numerous medicinal qualities (Posmontier, 2011) and it's a native to the sub-Himalayan parts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing drumstick tree was utilized ancient Romans, Greeks and Egyptians and has become widely cultivated and naturalized in many locations in the tropics and sub tropics, West, East and South Africa, Latin America, the Caribbean, Florida and the Pacific Islands. Recently, many investigations pointed to the antimicrobial properties of the various parts of *M. oleifera* roots, flowers, bark, stem and seeds against various pathogenic microorganisms, especially Gram negative bacteria (Lockett *et al.,* 2000; Ghebremichael *et al.*, 2005; Anwar and Rashid, 2007; Rahman *et al.*, 2009; Walter *et al.*, 2011). Present study was planned to detect the effect of *M. oleifera* oil on antimicrobial activity of some antibiotics against pathogenic Gram negative bacteria.

Materials and Methods

Microorganisms and plant material

Clinical strains of Gram negative bacteria including *Escherichia coli*, *Klebsiella* sp. *Proteus* sp. and *Pseudomonas aeruginosa* were obtained from Al Borg Laboratories, Mohandeseen, Giza, Egypt during November, 2013. Tested strains were confirmed their identification before study using the key proposed by Barrow and Feltham (2003). Tested bacterial cultures were maintained on nutrient agar slants at 4^{o}C throughout the study and used as stock cultures. *M. oleifera* oil was purchased from Pure Life Company for Agricultural Investment, Giza, Egypt.

Media and antimicrobial agents

Muller-Hinton agar medium (MHA), Nutrient agar medium (NA), antimicrobial agent disks including: Ampicillin (AMP)10µg, Cefepime (FEP) 30µg, Cefixime (CFM) 5µg, Ceftriaxone (CRO) 30µg, Ertapenem (ETP) 10µg, Imipenem (IPM) 10µg, Meropenem (MEM)10µg, Amikacin (AK) 30µg, Gentamicin (EN) 10µg, Doxycycline (DO) 30 µg, Ciprofloxacin (CIP) 5µg, Levofloxacin (LVX) 5µg, Norfloxacin (NOR) 10µg, Nalidixic acid (NA) 30 µg and Chloramphenicol (C) 30µg/disk were purchased from Oxoid Ltd. Co. and Tween 20 was purchased from Sigma Chemicals Company (St. Louis, Mo, USA).

Preparation of bacterial inoculum

Bacterial suspension of various tested clinical strains was prepared by direct colony suspension method as follow: appropriate number of separated colonies were picked up from NA fresh culture plate (previously inoculated with single colony of tested strain and incubated for 24h at 37^{o}C), suspended with sterile saline solution and adjusted their inoculum to a turbidity equivalent to 0.5 McFarland standard.

Antibacterial activity of *M. oleifera* oil

Antibacterial activity of *M. oleifera* oil against various tested clinical bacterial isolates was studied by agar well diffusion method according to Parez *et al.* (1990) using 200µL of *M. oleifera* oil for each well. After 24h of incubation at 37ºC, all plates were observed for zones of growth inhibition, and the diameter of these zones

was measured in millimeters. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

Detection of synergetic interaction between *M. oleifera* oil and antibiotics

Sterile Mueller-Hinton agar plates containing 0.125,0.25, 0.5, 1.0 and 2.0ml/100 ml of *M. oleifera* oil were prepared by adding *M. oleifera* oil to melted MHA, cold to 45–55^{o}C and supplemented with 0.5% (v/v) Tween 20. In addition, the same previous agar plates without *M. oleifera* oil were used in the present study as control.

A sterile cotton wool swab dipped into the bacterial suspension was spread evenly on the surface of previous MHA plates. The inoculated plates were allowed to dry before placing the diffusion antibiotic disks. Susceptibility of 4 tested isolates to various tested antibiotics was performed by disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI, 2011). Using commercially available antibiotic disks containing AMP (10µg),FEP (30µg), CFM (5µg), CRO (30µg), ETP (10µg), IPM (10µg), MEM (10µg), AK (30µg), EN (10µg), DO (30µg), CIP (5µg), LVX (5µg), NOR (10µg), NA (30µg) and C (30µg) were placed on the surface of the inoculated MHA plates with *Escherichia coli*, *Klebsiella* sp. or *Proteus* sp. while FEP (30µg), IPM (10µg), MEM (10µg), AK (30µg), EN (10µg), CIP (5µg), LVX (5µg) and NOR (10µg) were placed on the surface of the inoculated MHA plates with *P. aeruginosa*. The inoculated plates were then incubated at 37 °C for 24 h. Inhibition zone diameters were measured inclusive of the diameter of the disks (three replicates were applied for each test). Results were expressed as sensitive, intermediate and resistant according to CLSI, (2011). Data were subjected to analysis of variance (ANOVA) using SPSS at 5% level of significance and means values were compared using a least significant difference (LSD).

Result and Discussion

Antibacterial of *M. oleifera* oil against various clinical tested strains was weak or not existed against various tested strains. The efficacy of *M. oleifera* oil combined with antibiotics was studied by agar diffusion method. Data presented in Table 1 showed that the effect of *Moringa oleifera* oilon antibacterial activity of various tested antibiotics against *E. coli* was divers depending on the antibiotic used and the concentration of *M. oleifera* oil. In the case of beta-lactam and cephlosporin antibiotics, the antibacterial activity of variuos tested beta-lactam and cephlosporin antibiotics had significantly increased with in mediumcontaned *M. oleifera*oil, except AMP and CFM antibiotics. In addition, 0.125% (v/v) of *M. oleifera* oil was the most suitable concentration for significant increasing of antimicrobial activity of CRO and IPM antibiotics against tested strain compared to control (Table 1), which increased their antimicrobial activities to 25.7 and 6%, respectively.

Also, 1.0 % (v/v) of the tested oil was the most suitable concentration for significant increasing of antimicrobial activity of ETP and MEM antibiotics against tested strain compared to control, which exceeded the antimicrobial activity of control by 20.3 and 8.6%, respectively. In addition, 2.0% (v/v) of the tested oil was the most suitable concentration for significant increasing of antibacterial activity of FEP antibiotic against tested strain compared to control,

which exceeded the antimicrobial activity of control by 29.5% (Table 1 and Fig. 2).

In the case of chloramphenicol and doxycycline, the antimicrobial activity of each tested antibiotics had significantly increased in medium contained 0.5 and 1.0% (v/v) of *M. oleifera* oil, respectively, which exceeded the antimicrobial activity of control by 41.9 and 13.41%, respectively. On the other hand, addition of *M. oleifera* oil in tested medium did not give any significant change on antimicrobial activity of tested aminoglycoside (AK and EN) or fluoroquinolone antibiotics (CIP, LVX, NOR and NA) compared to control (Table 1 and Fig. 1).

From previous results, it could be concluded that the sensitivity of *E. coli* to chloramphenicol, doxycycline and most of cephalosporin antibiotics had significantly increased in medium contained *M. oleifera* oil, but the level of sensitivity was influenced by the type of tested antibiotic and the concentration *M. oleifera* oil. In contrast, the sensitivity of *E. coli* to aminoglycoside, fluoroquinolone and some beta lactam antibiotics (AMP and CFM) had not significantly changed in medium contained *M. oleifera* oil compared to control.

Data presented in Table 2 detect that the effect of *M. oleifera* oil on antibacterial activity of various tested antibiotics against *Klebsiella* sp. was divers depending on the antibiotic used and the concentration of *M. oleifera* oil. In the case of beta-lactam and cephalosporin antibiotics, the antibacterial activity of MEM and IMP against tested strain had significantly increased in medium contained 1.0 and 2.0 % (v/v), respectively, which exceeds the antimicrobial activity of control by 33.34 and 32.23%, respectively (Fig. 2). While, the antimicrobial activity of other tested beta-lactam and cephalosporin antibiotics against tested strain had not changed in medium contained *M. oleifera* oil.

In the case of aminoglycoside antibiotics, addition of *M. oleifera* oil 1% (v/v) in tested medium gave a significant increase of antibacterial activity against tested strain with AK, which exceeded the antimicrobial activity of control by 18.68%. While, the antimicrobial activity of EN against tested strain had not changed in medium contained *M. oleifera* oil (Table 2 and Fig. 2).

In the case of fluoroquinolone antibiotics, addition of *M. oleifera* oil (0.125, 1.0 and 2.0 %, v/v) to the medium gave a significant increase in the antimicrobial activity against tested strain with LVX, NA and NOR, respectively, which exceeded the antimicrobial activity of control by 31.46, 35.46 and 46.65%, respectively. On the other hand, the sensitivity of tested strain to chloramphenicol had significantly decreased in medium contained 0.5% (v/v) of *M. oleifera*, which reduced the antimicrobial activity to 16.1 % compared to control. While, the antimicrobial activity of DO against tested strain had not changed in medium contained *M. oleifera* oil (Table 2 and Fig. 2).

From previous results, it could be summarized that the sensitivity of *Klebsiella sp.* to most tested antibiotics in medium contained *M. oleifera* oil was not changed. While, the sensitivity of tested strain to fluoroquinolone (except CIP) and some cephalosporins including MEM and IMP had significantly increased in medium contained *M. oleifera* oil. Furthermore, addition of *M. oleifera* oil to the medium had significantly reduced the sensitivity of tested strain to chloramphenicol.

Data recorded in Table 3 showed that the change of antibacterial activity of various tested antibiotics against *Proteus sp*. was divers depending on the antibiotic used and the concentration of *M. oleifera* oil. In the case of beta-lactam and cephalosporin antibiotics, the antibacterial activity of IMP and ETP against tested strain had significantly increased in medium contained 0.125 and 2.0 % (v/v), respectively, which exceeded the antimicrobial activity of control by 97.80 and 54.19%, respectively (Fig. 3). While, the antimicrobial activity of other tested beta-lactam and cephalosporin antibiotics against tested strain had not changed in medium contained *M. oleifera* oil.

In the case of aminoglycoside antibiotics, addition of *M. oleifera* oil (1.0 and 2.0 %, v/v) in tested medium gave a significant increasing of bacterial sensitivity to EN and AK, respectively, which exceeded the antibacterial activity of control by 4.84 and 56.25%, respectively. Also, addition of 1.0ml of *M. oleifera* oil to the medium had significantly increased the bacterial sensitivity to doxycycline against tested strain, which exceeded the antibacterial activity of control by17.17% (Table 3 and Fig. 3). On the other hand, the sensitivity of tested strain to chloramphenicol had significantly decrease in medium contained 0.125 % (v/v) *M. oleifera*, which reduced the antibacterial activity to 12.48 % compared to control. While, the antibacterial activity of fluoroquinolone antibiotics against tested strain had not changed in medium contained *M. oleifera* oil (Table 3 and Fig. 3).

From previous data, it could be concluded that the addition of *M. oleifera* to the medium had increased the sensitivity of *Proteus sp*. to tested aminoglycosides, doxycycline and some cephalosporins (IMP and ETP). While, the sensitivity of tested bacteria to fluoroquinolones, most of beta-lactam and cephalosporin antibiotics had not changed. Furthermore, addition of *M. oleifera* oil to the medium had significantly reduced the sensitivity of tested strain to chloramphenicol.

Data current in Table 4 show that the sensitivity of *Pseudomonas sp.* to most tested antibiotics had significantly increased but with different levels based on the type of antibiotic used and the concentration of *M. oleifera* oil. In case of cephalosporin antibiotics, the sensitivity of tested bacteria to IPM and FEP had significantly increased in medium contained 0.125 and 1.0% (v/v), which increased the antimicrobial activities of tested antibiotics against tested strain to 46.31 and 77.4%, respectively (Fig. 4).While, the antimicrobial activity of MEM against tested strain had not changed in medium contained *M. oleifera* oil.

In the case of aminoglycoside antibiotics, the sensitivity of tested bacteria to AK and EN had significantly increased in medium contained 0.25 and 1% (v/v), which increased the antimicrobial activities of tested antibiotics to 46.31 and 77.4%, respectively, the sensitivity of tested bacteria to NOR had significantly increased in medium contained 0.5% (v/v), which increased the antimicrobial activity of tested antibiotics to 77.4%. While, the antimicrobial activity of CIP and LVX against tested strain had not changed in medium contained *M. oleifera* oil (Table 4 and Fig. 4).

From all abovementioned results, it could be concluded that the sensitivity of tested bacteria to various tested antibiotics might be changed in medium contained *M. oleifera* oil depending on tested bacteria and antibiotic, as well as the concentration of *M.*

oleifera oil. In addition, the sensitivity of all tested bacteria to IPM had significantly increased in medium contained *M. oleifera* oil. Also, the sensitivity of *Klebsiella* and *Proteus* to chloramphenicol has significantly decreased in medium contained *M. oleifera* oil, but it has significantly increased with *E. coli*.

In addition, addition of *M. oleifera* oil to the medium has significantly increased the sensitivity of *E. coli & Klebsiella*; *E. coil* & *Pseudomonas and E. coil* & *Proteus* to MEM, FEB and ETP & DO, respectively. Furthermore, the sensitivity to AK & EN in medium contained *M. oleifera* oil had significantly increased only against *Proteus & Pseudomonas*.

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phyto-therapy, spices and nutrition. This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects (Prashith Kekuda *et al.*, 2010).

Table.1 Antibacterial1 response to combinations between antibiotics and *M. oleifera* oil against *E. coli*

Antibiotic disks (Concentration)	*M. oleifera* oil concentrations % (v/v)					
	Control[2]	0.125	0.25	0.5	1.0	2.0
	Inhibition zone means[3] ±SD (mm)					
AMP(10µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
FEP(30µg)	20.33±0.6[a]	20.33±0.6[a]	20.33±1.5[a]	20.00±1.0[a]	20.67±0.6[a]	26.33±0.6[b]
CFM(5µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
CRO(30µg)	24.67±0.6[a]	29.67±1.7[b]	31.00±0.0 [b]	30.67±1.2[b]	29.00±1.7[b]	29.00±0.6[b]
ETP(10µg)	24.67±0.6[a]	25.33±1.2 [a]	25.33±0.6 [a]	25.67±1.2 [a]	29.67±0.6[ab]	30.67±0.6 [ab]
IPM (10µg)	33.33±1.5 [a]	35.33±0.6 [b]	35.33±0.6 [b]	35.33±0.6 [b]	35.33±0.6[b]	35.33±0.6[b]
MEM(10µg)	31.00±1.7[a]	30.67±1.0[a]	31.67±0.6[a]	31.00±1.7[a]	33.67±1.2[b]	34.00±1.2[b]
AK(30µg)	21.67±1.5[a]	21.33±1.7[a]	21.33±0.6 [a]	21.33±1.5 [a]	22.00±0.6[a]	22.00±0.6[a]
EN(10µg)	10.33±0.6[a]	10.00±0.6[a]	10.00±0.6 [a]	10.00±0.00 [a]	10.33±0.0[a]	10.33±0.0[a]
DO(30 µg)	22.33±0.6[a]	21.33±0.6[a]	21.00±0.6[a]	23.33±0.6[a]	24.67±1.7[b]	25.33±0.6[b]
CIP(5µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
LVX(5µg)	10.67±0.6[a]	9.67±0.6[a]	9.67±1.2[a]	10.33±0.6[a]	10.67±0.6[a]	10.33±0.6[a]
NOR(10µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0 [a]	0.00±0.0[a]	0.00±0.0[a]
NA (30 µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
C (30µg)	20.67±1.2[a]	25.00±1.2[b]	24.67±1.2[b]	29.33±0.6[c]	29.33±0.6[c]	29.33±1.0[c]

1: Studied by disk diffusion method (CLSI, 2011) using Mueller Hinton agar supplement with M. oleifera oil and Tween 20 (0.5%, v/v), 2: without M. oleifera, 3: Values in the same raw followed by same letter are not significantly different according to ANOVA (L.S.D. $p \leq 0.5$), SD: Standard division.

Table.2 Antibacterial1 response to combinations between antibiotics and *M. oleifera* oil against *Klebsiella* sp

Antibiotic disks (Concentration)	*M. oleifera* oil concentrations % (v/v)					
	Control[2]	0.125	0.25	0.5	1.0	2.0
	Inhibition zone mean[3] ±SD (mm)					
AMP(10µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
FEP(30µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
CFM(5µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
CRO(30µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
ETP(10µg)	20.2±0.6[a]	20.2±1.2[a]	19.33±0.0[a]	19.67±0.6[a]	20.00±1.5[a]	19.33±0.6[a]
IPM (10µg)	30.00±1.0[a]	33.67±0.6[b]	33.67±1.5[b]	33.33±1.5[b]	33.67±0.6[b]	39.67±0.6[c]
MEM(10µg)	29.00±1.0[a]	32.67±1.0[b]	32.33±0.6[b]	34±1.7[b]	38.67±0.6[c]	38.00±0.6[c]
AK(30µg)	25.00±1.0[a]	24.33±1.0[a]	23.67±0.6[a]	24.00±1.0[a]	29.67±0.6[b]	28.00±1.2[b]
EN(10µg)	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]	0.00±0.0[a]
DO(30 µg)	13.67±1.2[a]	13.67±1.0[a]	14.67±0.6[a]	15.00±0.0[a]	14.33±0.6[a]	13.00±0.6[a]
CIP(5µg)	20.67±0.6[a]	21.67±1.2[a]	20.33±0.6[a]	20.67±1.2[a]	20.67±0.6[a]	20.67±0.6[a]
LVX(5µg)	23.33±0.6[a]	30.67±0.6[b]	29.67±0.6[b]	29.33±0.6[b]	29.67±0.6[b]	29.33±0.6[b]
NOR(10µg)	20.00±1.0[a]	24.33±1.23[b]	25.33±1.23[c]	26.23±1.0[d]	26.33±0.58[a]	29.33±1.15[a]
NA (30 µg)	20.6±1.2[a]	20.67±1.2[a]	21.00±1.7[a]	21.33±1.5[a]	28.00±1.7[b]	27.67±1.2[b]
C (30µg)	29.00±1.0[a]	30.00±0.6[a]	30.33±1.2[a]	26.00±1.7[b]	24.33±1.5[b]	25.67±1.7[b]

1: Studied by disk diffusion method (CLSI, 2011) using Mueller Hinton agar supplement with *M. oleifera* oil and Tween 20 (0.5%, v/v), 2: without *M. oleifera*, 3: Values in the same raw followed by same letter are not significantly different according to ANOVA (L.S.D. $p \leq 0.5$). SD: Standard division.

Table.3 Antibacterial response to combinations between antibiotics and *M. oleifera* oil against *Proteus* sp

Antibiotic disks (Concentration)	*M. oleifera* oil concentrations % (v/v)					
	Control[2]	0.125	0.25	0.5	1.0	2.0
	Inhibition zone mean[3] ±SD (mm)					
AMP(10µg)	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	19.33±0.0	0.00±0.0 [a]
FEP(30µg)	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
CFM(5µg)	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
CRO(30µg)	0.00±0.0[a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
ETP(10µg)	16.00±1.0 [a]	24.67±1.0[b]	24.67±0.6 [b]	24.33±1.2 [b]	24.67±0.6 [b]	26.00±0.6 [c]
IPM (10µg)	15.0±0.0 [a]	29.67±0.6 [b]	29.33±0.6 [b]	29.33±1.2 [b]	29.67±1.2[b]	28.67±0.6 [b]
MEM(10µg)	29.33±1.2 [a]	29.33±0.6 [a]	29.33±1.2 [a]	29.67±o.6 [a]	29.33±1.2 [a]	29.33±1.2 [a]
AK(30µg)	16.00±1.0 [a]	16.67±1.0 [a]	19.00±1.2 [b]	19.33±1.2 [b]	19.33±1.7 [b]	25.00±1.5[c]
EN(10µg)	20.67±1.2[a]	20.67±0.0[a]	20.67±1.5[a]	20.33±0.6[a]	21.67±0.6[b]	21.00±1.2 [b]
DO(30 µg)	9.67±0.6[a]	10.33±1.5 [a]	10.33±0.6 [a]	10.33±0.6 [a]	11.33±0.6 [b]	11.67±0.6 [b]
CIP(5µg)	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
LVX(5µg)	9.67±0.6 [a]	10.33±0.6 [a]	9.67±0.6 [a]	9.67±o.6 [a]	9.67±0.6 [a]	9.67±0.6 [a]
NOR(10µg)	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
NA (30 µg)	0.00±0.0[a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]	0.00±0.0 [a]
C (30µg)	29.33±1.2[a]	25.67±0.6[b]	25.67±1.0[b]	25.33±o.6[b]	25.33±1.2[b]	25.33±1.2[b]

1: Studied by disk diffusion method (CLSI, 2011) using Mueller Hinton agar supplement with *M. oleifera* and Tween 20 (0.5%, v/v), 2: without *M. oleifera*, 3: Values in the same raw followed by same letter are not significantly different according to ANOVA (L.S.D. $p \leq 0.5$). SD: Standard division.

Table.4 Antibacterial response to combinations between antibiotics and *M. oleifera* oil against *Pseudomonas* sp

Antibiotic disks (Concentration)	*M. oleifera* oil concentrations % (v/v)					
	Control[2]	0.125	0.25	0.5	1.0	2.0
	Inhibition zone mean ±SD (mm)					
FEP (30µg)	10.33±0.0[a]	10.33±0.0[a]	10.33±0.0[a]	10.33±0.0[a]	10.33±0.0[a]	18.33±0.0[b]
IPM(10µg)	22.33±1.2[a]	32.67±1.5[b]	32.67±1.5[b]	31.67±1.5[b]	31.33±0.6[b]	31.67±0.6[b]
MEM(10µg)	29.67±0.6[a]	30.67±1.2[a]	30.33±1.7[a]	30.67±0.6[a]	31.00±1.5[a]	31.33±1.2[a]
AK(30µg)	21.67±1.5[a]	22.33±1.2[a]	24.67±1.0[b]	24.00±1.2[b]	24.00±0.6[b]	24.33±1.5[b]
EN(10µg)	18.33±1.5[a]	19.33±0.6[a]	19.33±1.2[a]	21.00±1.7[b]	35.33±1.2c	35.67±1.2c
CIP(5µg)	28.33±0.6[a]	28.67±1.2[a]	28.67±1.2[a]	29.33±0.6[a]	29.33±0.6[a]	29.33±0.6[a]
LVX(5µg)	30.67±1.5[a]	31.00±1.2[a]	30.33±1.5[a]	30.33±0.6[a]	31.67±1.2[a]	30.67±1.7[a]
NOR(10µg)	27.00±0.0[a]	27.67±1.2[a]	29.33±1.5[a]	32.67±0.6[b]	32.67±1.2[b]	32.33±1.2[b]

1: Studied by disk diffusion method (CLSI, 2011) using Mueller Hinton agar supplement with *M. oleifera* and Tween 20 (0.5%, v/v), 2: without *M. oleifera*, 3: Values in the same raw followed by same letter are not significantly different according to ANOVA (L.S.D. $p \leq 0.5$). SD: Standard division.

Fig.1 Effect of *M. oleifera* oil on antimicrobial activity of some antibiotic against *E. coli*

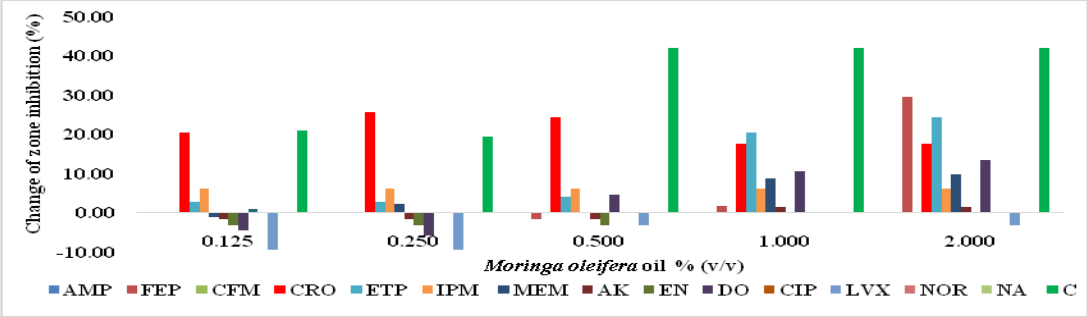


Fig.2 Effect of *Moringa oleifera* oil on antimicrobial activity of some antibiotic against *Klebsiella* sp

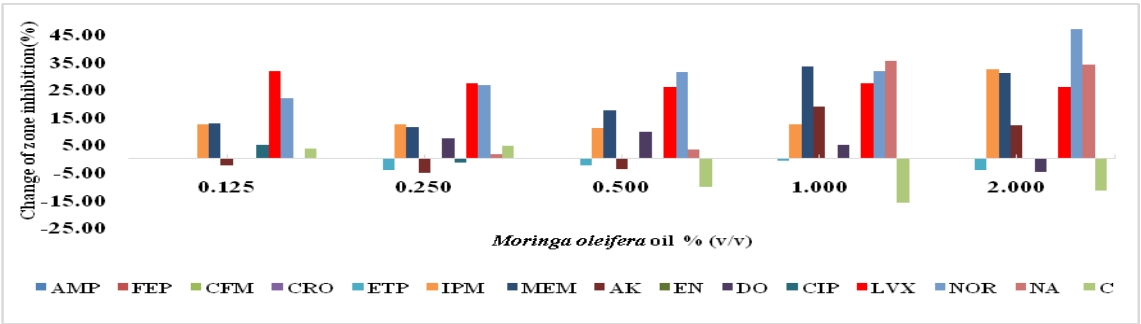


Fig.3 Effect of *M. oleifera* oil on antimicrobial activity of some antibiotic against *Proteus* sp.

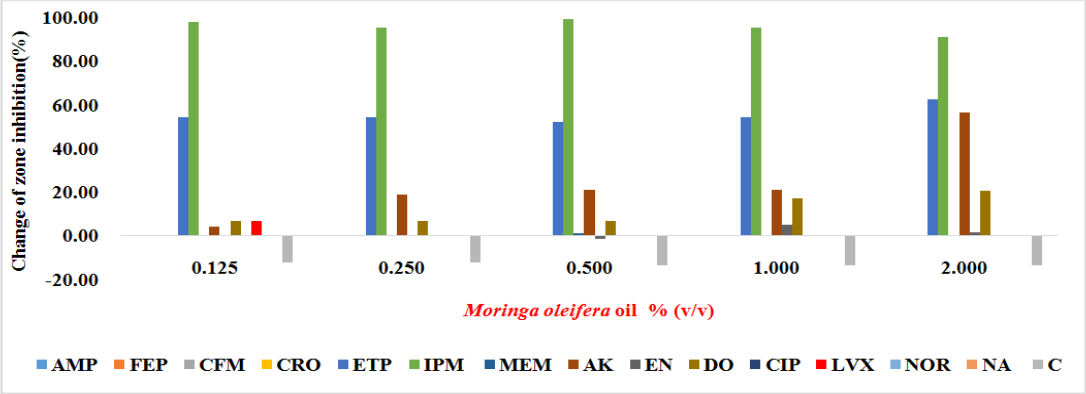
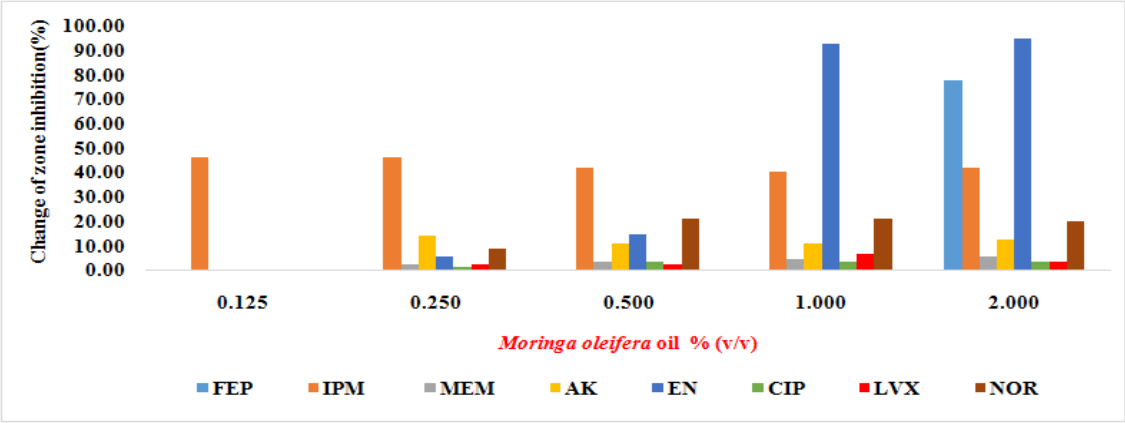


Fig.4 Effect of *M. oleifera* oil on antimicrobial activity of some antibiotic against *Pseudomonas* sp.



Although, there are many investigations revealed the antimicrobial activity of *M. oleifera* oil against bacteria and fungi (Prashith Kekuda *et al.*, 2010 and Marrufo *et al.*, 2013), but in the present study the antimicrobial activity of *M. oleifera* oil against various tested clinical strains is weak or not existed and that may be due to the highly resistant of tested strains to the contents of *M. oleifera* oil, while the sensitivity of various tested bacteria to some antibiotics was increased in medium contained *M. oleifera* oil compared to control. Obtained results revealed that antibacterial activity of antibiotics against some pathogenic bacteria could be increased in case it combined with other materiel even it has antibacterial activity or not.

Marrufo *et al.* (2013) revealed that the antimicrobial effectiveness of most essential oil against Gram negative is due to the phenol compounds. In addition, the composition of outer membrane of gram negative bacteria, essential oil can alter not only such structures but penetrate within the cell, leading to those alterations, such as the denaturation of proteins and enzymes, the "unbalance" of the K + and H + ion concentration, until the modification of the entire cell morphology, which can lead to the death of the microorganisms.

Furthermore, The molecular mechanism of action of the essential oil of *M. oleifera* is unknown, but the essential oil can probably inhibit the generation of adenosine triphosphate from dextrose and disrupt the cell membrane (Gill and Holley, 2004). Thus, combination between *M. oleifera* oil and antibiotics could increase the antibiotic activity against resistant bacteria.

This is the first report concerning the synergistic effects of *M. oleifera* oil in combination with different traditional antibiotics against most common pathogenic Gram negative bacteria, which has emphasized that *M. oleifera* oil is one of the most promising natural compounds that can be used as antibiotic resistance modifying agent in microorganisms. Further studies are focused on the active phytochemicals of *M. oleifera* oil and their interaction with IMP and C antibiotics against resistant pathogenic Gram negative bacteria.

M. oleifera oil could be used as antibiotic resistant modifying agent against multi-drug resistant Gram negative bacteria, which can contribute in some way to overcome of bacterial resistance to many traditional antibiotics and therefore can be reused again especially in developing countries, such as Egypt.

References

Ang, J.Y., Ezike, E., Asmar, B.I. 2004. Antibacterial resistance. *Indian J. Pediatr*, 71: 229–239.

Anwar, F., Rashid, U. 2007. Physico-chemical characteristics of *Moringa oleifera* seed and oil from a wild provenance of Pakistan. *Pak. J. Bot.*, 39(5): 1443–1453.

Bajpai, V.K., Shukla, S., Sharma, A. 2013. Essential oils as antimicrobial agents. *Natural Prod.*, Pp. 3975–3988.

Barrow, G.I., Feltham, R.K.A. 2003. Cowan and steel's manual for the identification of medical bacteria. Cambridge University Press, Cambridge. Pp. 50–150.

CLSI (Clinical And Laboratory Standards Institute), 2011. Performance standards for antimicrobial susceptibility testing, twenty-first informational supplement. CLSI document M100-S21. Vol. 31, No.1. Wayne, Pennsylvania, USA. Pp. 42–87.

Fakurazi, S., Sharifudin, S.A., Arulselvan, P. 2012. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules,* 17: 8334–8350.

Ghebremichael, K.A., Gunaratna, K.R., Henriksson, H., Brumer, H., Dalhammar, G.A. 2005. Simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Res.*, 39(11): 2338–44.

Gill, A.O., Holley, R.A. 2004. Mechanisms of bactericidal action of cinnamaldehyde against Listeria. monocytogenes and of eugenol against L. monocytogenes and *Lactobacillus sakei*. *Appl. Environm. Microbiol.*, 70: 5750–5755.

Jabar, M.A., Al-Mossawi, A. 2007. Susceptibility of some multiple resistant bacteria to garlic extract. *Afr. J. Biotechnol.*, 6(6): 771–776.

Kumar, V., Pandey, N., Mohan, N., Singh, R.P. 2012. Antibacterial & antioxidant activity of different extract of *Moringa oleifera* leaves- an *in vitro* study. *Int. J. Pharma. Sci. Rev. & Res.*, 12(1): 89–94.

Lockett, C.T., Calvert, C.C., Grivetti, L.E. 2000. Energy and micronutrient composition of dietary and medicinal

wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. *Int. J. Food Sci. Nutr.*, 51(3): 195–208.

Marrufo, T., Nazzaro, F., Mancini, E., Fratianni, F., Coppola, R., De Martino, L., Bela Agostinho, A., De Feo, V. 2013. Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules,* 18: 10989–11000.

Parez, C., Paul, M., Bazerque, P. 1990. Antibiotic assay by agar well diffusion method. *Acta. Biol. Med. Exp.*, 15: 113–115.

Posmontier, B. 2011. The medicinal qualities of *Moringa oleifera*. *Holistic. Nurs. Pract.*, 25(2): 80–87.

Prashith Kekuda, T.R., Mallikarjun, N., Swathi, D., Nayana, K. V., Aiyar, M.B., Rohini, T.R. 2010. Antibacterial and antifungal efficacy of steam distillate of *Moringa oleifera* Lam. *J. Pharm. Sci. & Res.*, 2(1): 34–37.

Rahman, M.M., Sheikh, M.M., Shamima, A.S., Islam, M.S., Rahman, M.A., Rahman, M.M., Alam, M.F. 2009. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. Against some human pathogenic bacteria. *CMU. J. Nat. Sci.*, 8(2): 219.

Sokovi, M., Glamoèlija, J., Marin, P.D., Brki, D., Van Griensven, L.J.L.D. 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules*, 15: 7532–7546.

Walter, A., Samuel, W., Peter, A. Joseph, O. 2011. Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *Afri. J. Microbiol. Res.*, 5(2): 153–157.