Egypt. J. Microbiol. **55,** pp. 45-61 (2020)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Antimicrobial Efficiency of *Moringa oleifera* Leave Extracts against Some Multidrug Resistant Pathogen

Hala Abd Elmonem Ahmed[#], Zeinab Hassan Kheiralla, Doaa Abd ELrazk Shehata Ali

Department of Botany, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

THE MEDICINAL plants have vital sources of natural antimicrobial substances for human health avoiding side effects precipitated by using synthetic chemical. The decreasing effectiveness of traditional antibiotics towards multi drug resistant microorganism is a global public health. It was found that the mixtures between different plant extract and commercial antibiotics increased the effectiveness of antibiotics multi drug resistant bacteria. The combinations between different plant extract and commercial antibiotics increased the effectiveness of antibiotics against multi drug resistant strains. Extracts of *Moringa oleifera* leaves showed remarkable antimicrobial activity against the growth of the gram positive and negative bacteria drug resistant clinical strains (*Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29737 and *Staph epidermidis* ATCC 12228, *Bacillus pumilis* ATCC 14884, *Micrococcus luteus* ATCC 10240, *Bordetella* sp, *Bacillus subtilis* and *Klebsiella* sp . Antimicrobial activity of methanol extract of *Moringa oleifera* leaves (MML) using well diffusion method was higher (30mm inhibition zone) than ethanolic and aqueous extract. The minimum inhibition concentration (MIC) of the selected pathogenic bacteria ranged between 23 -800 µg/ml. The interactions between different extract of *Moringa oleifera* leaves and different antibiotic groups of Ampicillin (Am10), Amoxicillin (AMC30), Amikacin (AK30) , Gentamycin (CN10), Tetracycline (TE30), Erythromycin (E15), Bacitracin (B10) were conducted using agar diffusion method against the selected pathogenic bacteria. Methanol extract of *Moringa oleifera* leaves could be used as a source for resistance-modifying agents against infectious multi-drug resistant bacteria.

Keywords: Antibiotics, Antibacterial assay, *Moringa oleifera*, Synergistic effect.

Introduction

Moringa oleifera generally referred to as moringa is viewed as one of the most famous and valuable trees in the world. It has many different names over all the world (Thilza et al., 2010). Moringaceae is a fast-growing, drought -resistant flowering plant family which is treasured by Ancient Egyptians, the Greeks and the Romans for its nutritional and medicinal aspects. It is found mostly in semi-arid, tropical, and subtropical areas in the Horn of Africa, Madagascar, Southwestern Africa and tropical Asia to westward in Egypt and grows in almost all the phytogeographical regions (Mridha, 2015).

All parts of Moringa tree are useful (leaves, flower and pods, many studies from around the world published that Moringa are often used for medicinal purposes as well as for human nutrition. Moringa rich in antioxidants and other nutrients such as vitamins, minerals and amino acids, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins (Leone et al., 2015). The leaves have been used to combat malnutrition, mostly among pregnant woman, nursing moms and kiddies (Anwar et al., 2007). They are great variants amongst the nutritional values of *Moringa* which depends on elements such as genetic background, environment and cultivation method (Moyo et al., 2011). Plant extracts assigned with their

[#]Corresponding author email: hala.elsagher@women.asu.edu.eg
Received 13/6/ 2020; Accepted 31/8/ 2020
DOI: 10.21608/ejm.2020.32559.1162
©2020 National Information and Documentation Center (NIDOC)

antimicrobial properties due to compounds synthesized in the secondary metabolism of the plant this explains the reason for its vast use in the treatment of human diseases (Anwar et al., 2007). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic protypes (Afolayan, 2003; Martín et al., 2013).

Infectious diseases are the leading cause of death worldwide. Excessive and irrational use of antibiotics is the first cause of distribution of multidrug resistant microbes over the world, this issue is continuously increasing at an alarming rate especially in developing countries where there are increases in the indiscriminate use of wide broad spectrum antibiotic as a result of self-medication, immunosuppressive agent and outbreak of epidemics. Therefore, there are decrease in the effectiveness of many typical antibiotics (Alo et al., 2012). Hence it is necessary to search for new infection controlling strategies to fight microbial infection. Several food and medicinal plants were documented as potential candidate to fight multidrug resistant microbes (MDR) (Fankam et al., 2011). The beneficial effects of medicinal plants for humanity as it is containing a great number of bioactive compounds as they are the cheapest and safer alternative sources of antimicrobials (Martín et al., 2013).

Recent studies demonstrated that Moringa oleifera leave extracts contain a group of chemical compounds which are known for its antibacterial activity against new multidrug resistant strains (Idris & Abubakar, 2016). Furthermore, others investigated the synergistic action of the combination between *Moringa oleifera* leave extracts and known antibiotics against some clinical pathogen as it improve these antimicrobial effectiveness (Dzotam et al., 2016).

The emergence and spread of MDR microorganisms become a serious problem threat now-a-days to public health so this study aimed to examined the synergistic activities of *Moringa oleifera* leave extracts alone and in combination with some antibiotics against some clinical pathogen thus providing a new natural, safer, and cheaper therapeutic alternative to antibiotics (Bina et al., 2010).

Materials and Method

Collection of samples

Moringa oleifera leaves was purchased from the Botanical garden at Department of Botany, Faculty of Women for Arts, Science and Education, Ain Shams University ,Cairo Egypt. The collected fresh plant leaves were cleaned, washed, shade dried, and homogenized to a fine powder and stored in an airtight bottle.

Extraction

Three solvents (hot and cold aqueous, ethanol and methanol) were used in the extraction of the antimicrobial component of the leaves powder. One hundred grams of ground leaves were extracted separately in 100ml conical flasks with 50mL of each of aqueous, ethanol and methanol. The flasks containing plant leaves powder and solvent were shaken at 120rpm for one hour then filtered off using sterile filter paper (Whitman no. 1) into a clean conical flask. The different extracts concentrated at 45^0C using rotary evaporator, then filtered again using syringe filter 0.22µm to obtain the sterile extract then left until completely evaporation. The concentrated extracts were kept in sterile glass bottle at -20^0C then dissolved in Dimethyl Sulphoxide (DMSO, 10%w/v) prior to use (Onyekaba et al., 2013).

Phytochemical analysis

The phytochemical screening was qualitative analyzed to determine the secondary metabolites present in each extract. Each extract was, to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel (TLC-grade; Merck India) according to the method described by Talukdar et al. (2010).

Microbial strains

Nine tested strains of pure bacteria overnight-grown culture of bacteria was used for the evaluation of the antimicrobial potential of the leaves extracts including both Gram-positive and Gram -negative strains. *Escherichia Coli* ATCC10536, *Pseudomonas aeruginosa* ATCC9027, *Staphylococcus aureus* ATCC 29737, *Staph Epidermidis* ATCC 12228, *Bacillus Pumilis* ATCC 14884,*Micrococcus Luteus* ATCC 10240 were obtained from were obtained from American Type Culture Collection(ATCC,US) , while the other strains, *Bordetella* sp*, Bacillus Subtilis* and *klebsiella* sp were kindly provided from Microbial Lab of Microbiology Department of Botany

department, Faculty of Women for Arts, Science and Education, Ain Shams University.

Assessment of the antibacterial activity of different plant extracts

The antimicrobial activity of different *Moringa oleifera* leaf extracts was evaluated using well-diffusion method. Nutrient agar plates were inoculated with different strains of bacteria separately using 0.2 ml of inoculum containing 10^6 test organisms was inoculated on the plates of solidified agar and spread uniformly (Meenal et al., 2010). A lawn of the test organism was made on nutrient agar plate using sterile swabs.

The inoculum of each bacterium was developed by growing the organism overnight in nutrient broth medium at 37 ^{0}C. Turbidity of each suspension was adjusted to match 0.1 optical density at 600nm (approximately 2×10^6 CFUml**)** (CLSI, 2006) by diluting with 0.9% NaCl solution.

Using sterile corkborer to create well (6mm in diameter) on the agar surface onto which the extracts were placed then filled with 100µl of each extract and then the plates were left at refrigerator for 3hrs to allow diffusion of the test sample, sterile distilled water**,** methanol and ethanol as a blank were individually tested against studied bacteria. The bacterial plates were incubated at 37°C for 24hrs (Sachin et al., 2012). Afterwards, antibacterial activity was determined by measurement of the inhibition zone.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antimicrobial effects of different *Moringa oleifera* leaf extracts against different bacterial pathogens were determined according to Srinivasan et al. (2001). Percent of the bacterial growth after an overnight incubation in comparison with the negative control (Abbaszadegan et al., 2015). The antimicrobial activity of plant extract was examined using the standard broth dilution method (CLSI, 2012). The MIC was determined in nutrient broth using serial two-fold dilutions of plant extract in concentrations ranging from (10^{-1} to 10^{-10}) with adjusted bacterial suspensions concentration (0.1 at 600nm). One milliliter of each bacterial suspension that matches 0.1 OD at 600 nm was then inoculated into the resultant serial dilution series and incubated at 37°C for 24hrs. Positive and negative control tubes are included for every tested bacterial pathogen. The negative control tube did not contain bacterial inoculum, whereas the positive control tube was free from plant extract. Minimum inhibitory concentration was examined visually by checking the turbidity of the tubes. MBC was detected after the MIC determination of the plant extract aliquots of 50µl from serial dilution test tubes which showed no visible bacterial growth were spread on nutrient agar plates were incubated for 24hrs at 37°C. The MBC were observed for presence or absence of bacterial growth in agar plates after incubation (Krishnaraj et al., 2010).

Antibiotic susceptibility test

Antibiotic resistance patterns of nine tested bacterial strains were observed using the agar disk diffusion method. Bacteria were grown until they reached a final inoculum of 5×10^5 CFU/ml as compared to 0.5 McFarland standard then streaked on Mueller-Hinton agar (Difco). Antimicrobial disks (µg/ disc) (Oxoid, UK) were saturated with each of Ampicillin (AM10), Amoxicillin (AMC30), Amikacin (AK30), Gentamycin (CN10), Tetracycline (TE30), Erythromycin (E15), Bacitracin (B10) as standard antibacterial agents. Test was performed according to the interpretive standards for inhibition zone diameter provided by the Fernández et al. (2010), CLSI (2012).

The antibiotic discs were applied to plates of Mueller-Hinton agar previously inoculated with the different tested bacterial strains using a sterile swab to produce a confluent lawn of the growth. The appropriate discs were placed on the surface of the inoculated plate suitably spaced (25mm from disc to disc and 15 mm from the rim). The plates allowed to pre-diffuse at 4^{o}C for two hours. Plates were incubated at 37^{o}C for 24hrs, and then examined for the presence of inhibition zones of bacterial growth around antibiotic discs (Fernández et al., 2010; CLSI, 2012).

Evaluation of synergistic effect interaction of antibiotics and different Moringa oleifera leave extracts

Disk diffusion was used to assess the antibacterial potential of the combination between antibiotics and the MICs of different leave extracts of *Moringa oleifera* against all selected bacterial strains on nutrient agar plates. This method was a modification of the agar disk diffusion method described in the (CLSI, 2006). In order to determining the synergistic effects each standard antibiotic disk was impregnated

with 15µl of the freshly prepared of plant extract (x % concentration). Disk containing 15µl sterile water was used as negative controls. Plates were labeled carefully and incubated at 37^0C for 24hrs to check the activity. Antibacterial activity was expressed as the diameter of the zone of inhibition (millimeters) and evaluated by the increase in fold area (Shahverdi et al., 2007; Kheiralla et al., 2014). Experiments were conducted in triplicates, and average inhibitory zone diameter with its standard deviation was determined.

Increase in fold area was assessed by calculating the mean diameter of the inhibition zone of each antibiotc by the equation $(y^2-x^2)/x^2$, where 'x' are the inhibition zones for antibiotic and 'y' antibiotic + extract of *Moringa oleifera* (Birla et al., 2009).

Statistical analysis

After testing the data for normality, the differences in the *Moring aoleifera* different extracts and tested antibiotics against tested bacterial pathogen were tested using one-way analysis of variance (ANOVA1) according to SPSS software (SPSS, 2006). A post –hoc test (LSD) was applied when differences were significant.

Results and Discussion

The results of qualitative phytochemical screening of *Moringa oleifera* plant aqueous, ethanol or methanol extract. Table 1 showed that the presence of flavonoids, saponins, alkaloids, Phenol, tannins and terpenoids and in using different solvent extracts whereas saponins and flavonoids was not discovered in water.

Different solvents have different extraction capacities and different spectra of solubility for the phytoconstituents (Srinivasan et al., 2001). Ethanol and methanol were found the most effective solvents that extracted all phytochemicals except alkaloids. Various studies showed that ethanol extract has capability to extract maximum number of compounds (Bennett et al., 2003; Tijjan et al., 2009). Our results do not agree with the finding of Bukar et al. (2010) who isolated saponins and flavonoids from ethanol extract but failed to separate tannins and alkaloids from it. The absence of later phytochemicals was also reported by Bukar et al. (2010) and Kasolo et al. (2010) who also reported the absence of alkaloid in *M. oleifera* extract, from ethanol extract, this could be as a result of different climatic environment at which it was planted or the physiological and its maturity at the stage of harvesting (Taylor et al., 2001).

Quantitatively, phenols and tannins were present in very higher amount in ethanol, methanol and water. Tannins are a group of polymeric phenolic substances capable of killing microorganisms (Cowan, 1999; Hausteen, 2005). Tannins has been reported to have anti-cancer, ant-atherosclerotic, anti-inflammatory and anti-hepatoxic properties (Adedapo et al., 2015).

The result of the preliminary phytochemical screening of leaves *Moringa oleifera* shows that alkaloids, phenol, tannins and terpenoids were present in aqueous extract, but unable to detect flavonoids and saponins which contradicted with the study of (Kwaghe & Ambali, 2009; Kasolo et al., 2010).

Apart from determination of nutritional value of the plant, Schneider & Wolfhing (2004) have reported the therapeutic effect of some phytochemical constituent such as tannins, cardiac glycoside against cardiovascular disease and digestive problems. It is important also to note that phytochemical constituent is an important factor that determines the antimicrobial properties of the leave extract. This explain why medicinal plants are used as antimicrobial drugs, several authors have linked the presence of bioactive compound to the antimicrobial properties of the plant extract. Flavonoids as one of the bioactive constituents is known to inhibit or kill many bacterial strains, it also inhibits viral enzymes such as reverse transcriptase and protease (Afolabi et al., 2007).

TABLE 1. Qualitative Phytochemical Screening of leaf extracts by different solvent.

Solvent	Flavonoids	Saponins	Alklaoids	Phenol	Tannins	Terpenoids
Ethanol	++	++	+	+++	+++	+
Methanol	+++	++	+	+++	+++	+
Water	-	-	++	+++	+++	+

Absent = ─, Trace = +, moderately present = ++, highly present = +++.

Antimicrobial substance is a tool for controlling undesirable microorganism specially in infection control or preservation. Different solvents have different extraction capacities and different spectra of solubility for the phytoconstituents (Srinivasan et al., 2001).

The preliminary screening of antimicrobial activity showed that different leaf extracts of *Moringa oleifera* possess significant different antimicrobial activity against both Gram- positive and Gram- negative bacteria with inhibition zone range of 15-30mm (Table 2). Methanol and ethanol showed maximum antimicrobial activity followed by hot water extraction however weak potency recorded when cold water extraction was tested through measuring diameter of zones of inhibition of the tested bacterial growth.

In the current work, it was shown that methanol extract of *M. oleifera* leaves had a broad spectrum of antimicrobial activity against some human pathogenic Gram-positive bacteria more than Gram-negative bacteria. The highest zone of inhibition (30mm) was showed on *Micrococcus luteus* ATCC 10240 in hot aqueous, methanol and ethanol extract, also on *Bacillus pumilis* ATCC 14884 in hot aqueous and *Staphylococcus aureus* ATCC 29737 using methanol leaf extract. The extracts yielded from *Moringa oleifera* aqueous leaf extract was (7%), *Moringa oleifera* ethanol leaf extract was (3.8%) and *Moringa oleifera* methanol leaf extract was (19.7%). *Moringa oleifera* leaves extract have been reported to have antimicrobial properties against a wide range of bacteria (Ofokansi et al., 2013).

These results are in agree with other findings (Doughari et al., 2007; Bukar et al., 2010; Porte, 2014). The differences in sensitivity between Gram-positive and Gram-negative bacteria may be attributed to their morphological variations; an outer phospholipidic membrane of Gram-negative bacteria carrying the structural lipopolysaccharide components led to cell wall impermeability to lipophilic solutes, whereas porinsporins represent a selective barrier to the hydrophilic solutes.

The antimicrobial activity of each extract is qualified by measuring MIC value which serve as a guide for treatment of most infection (Ofokansi et al., 2013).

The MIC values of aqueous, methanolic and ethanolic extract of *Moringa oleifera* leaves against the selected clinical pathogen are presented in Table 3. The MIC was variable according to the type of bacteria and extract (range from 23.0µg/mL (*Escherichia coli* ATCC10536) using Aqueous extract to 800µg/ml (*Pseudomonas aeruginosa* ATCC9027) using ethanolic extract. Furthermore, MBC was equal to the minimum inhibitory concentration for all strains. The present study showed that MIC of the plant extract were higher and acts as a bacteriostatic at lower concentrations and as a bactericidal at higher concentrations, these findings are just in line with the observations of (Rahman et al., 2009).

TABLE 2. Antimicrobial activity of different extracts of *Moringa oleifera* leaves.

Inhibition zone (mm) Concentrations 100µg/mL.					
Bacteria	**Hot aqueous**	**Cold aqueous**	**Ethanol**	**Methanol**	**LSD at 0.5**
Micrococcus luteus ATCC 10240	30	15	30	30	8.2
Staphylococcus aureus ATCC 29737	20	0.0	25	30	4.4
Bacillus pumilis ATCC 14884	30	0.0	0.0	15	6.5
Staphylococcus epidermidis ATCC 12228	20	0.0	20	20	12
Bacillus subtilis sp	0.0	0.0	25	20	3.8
Escherichia coli ATCC10536	20	0.0	25	20	3.6
Bordetella sp	20	15	20	25	4.0
Pseudomonas aeruginosaATCC9027	0.0	0.0	20	25	4.7
Klebsiella sp	0.0	0.0	0.0	15	0.62
LSD at 0.5	7.5	3.4	4.2	0.45	

TABLE 3. Determination of the MIC of *Moringa oleifera* of different extracts against pathogenic bacteria µg/ml.

Extract / Microorganism	Ethanol extract	Methanol extract	Aqueous extract
Micrococcus luteus ATCC 10240	100	250	187.5
Staphylococcus aureus ATCC 29737	250	125	187.5
Bacillus pumilis ATCC 14884	0.0	250	187.5
Staphylococcus epidermidis ATCC 12228	500	250	187.5
Bacillus subtilis sp.	500	200	0.0
Escherichia coli ATCC10536	100	250	23.5
Bordetella sp.	400	125	23.0
Pseudomonas aeruginosa ATCC9027	800	250	0.0
Klebsiella sp.	0.0	200	0.0

In vitro antibiotic susceptibility test was performed against nine tested bacterial pathogens was summarized in Fig 1. All selected bacterial pathogen was resistance to ampicillin and amoxicillin except *Staphylococcus epidermidis*. On the other hand, most of selected bacterial pathogen showed higher susceptibility to amikacin and gentamycin except *Klebsiella* sp., while *Bacillus subtilis and Escherichia coli* were resistant to gentamycin (Fig. 1).

All gram negative and gram-positive bacteria were resistance to tetracycline except *Staphylococcus epidermidis* and *Bacillus subtilis*. Furthermore, *Micrococcus luteus*, *Bacillus pumilis*, *Staphylococcus epidermidis* and *Bordetella* sp. showed higher susceptibility to erythromycin. All tested bacteria showed complete resistance to bacteriocin.

Statistical analysis showed significant difference between antibiotics in case of *Staphylococcus epidermidis*. No significant difference was observed between antibiotics in case of *Klebsiella* sp. and between all selected bacteria in case of bacitracin this result was in agreement of several authors as they found that there were increasing prevalence of resistance for many pathogens over the years in different regions of the world including developing countries (Babayi et al., 2004 , Trookman et al., 2011; Yusha et al., 2010). The development of drug resistant organisms has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, and societal and technological changes (Bajpai et al., 2005; Mbengui et al., 2013).

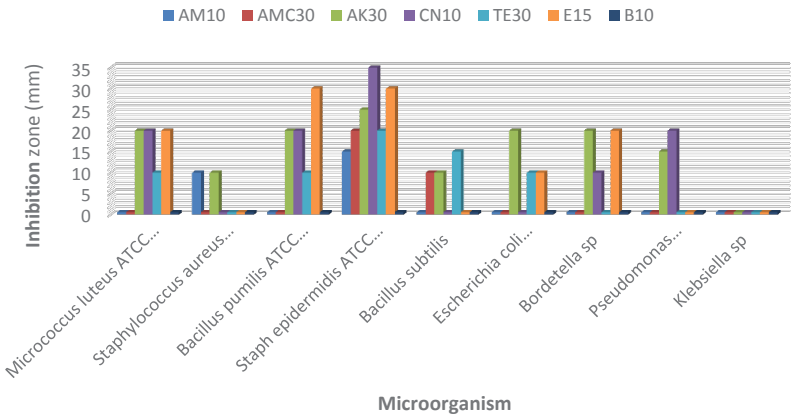

Fig. 1. Antibacterial activity of standard antibiotics against pathogenic bacteria.

In the present study, using ampicillin and amoxicillin antibiotics are a group of beta-lactam antibiotics which is part of the amino penicillin family like other penicillin-like antibiotics great effect on all tested gram positive bacterial pathogen as it act in a bactericidal manner by inhibiting and preventing the production of the major cell wall component peptidoglycan the bacterial cell ultimately undergoes cell lysis due to the inhibition of cell wall synthesis (Rafailidis et al., 2007) while *Pseudomonas aeruginosa* and *Klebsiella* sp. gram-negative bacteria resistant to β-lactam antibiotics as ampicillin and amoxicillin (Alekshun & Levy, 2007).

Gentamicin and Tetracycline is a bactericidal antibiotic that works by irreversibly binding the 30S subunit of the bacterial ribosome, interruptin g protein tetracycline group kills the bacteria by binding to the bacterial 30S ribosomal subunit near the A site, which consequently inhibits protein synthesis through the prevention t-RNA docking (Zakeri & Wright, 2008). This mechanism of action is similar to other aminoglycosides .

Tetracycline from tetracycline's group kills the bacteria by binding to the bacterial 30S ribosomal subunit near the A site, which consequently inhibits protein synthesis through the prevention tRNA docking (Zakeri & Wright, 2008).

Erythromycin is in the macrolide family and it interferes with aminoacyl translocation, preventing the transfer of the tRNA synthesis. This mechanism of action is similar to other aminoglycosides, tetracycline from bound at the A site of the rRNA. Many *P. aeruginosa* isolates are resistant.

The present study was conducted to obtain preliminary information on the interaction between *Moringa oleifera* and different antibiotics against some clinical bacteria by disk diffusion method. Table 4 showed the *in vitro* combined activities of hot aqueous extract of *Moringa oleifera* and the conventional antibiotics against tested Gram positive and Gram negative bacterial pathogen, the combinations produced varying zones of inhibition. Gram negative bacteria showed significant sensitivity toward ampicillin which showed the highest percentage of synergism in three of the tested organisms; *Escherichia coli* and *Bordetella* sp. at 20mm. Amoxicillin shows an increase in the bioactivity was recorded against *Escherichia coli* at 20mm and moderate synergistic action was observed against *Bordetella* sp and *Klebsiella* sp at 15 mm. Highly synergistic interactions with amikacin against *Escherichia coli* and *Bordetella* sp at 25 mm and *Klebsiella* sp at 20 mm. Gentamicin with highly synergistic action was observed against *Klebsiella* sp at 25 mm, *Escherichia coli* and *Bordetella* sp at 20 mm.

Tetracycline and erythromycin had no effect on tested bacteria except *Bordetella* sp. at 20mm with Tetracycline but with erythromycin at 25mm. Moderate synergistic action with bacitracin against *Bordetella* sp. at 15mm. Combinations of antibiotics and *Moringa oleifera* hot aqueous extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 5.25-10.1 for ampicillin and amoxicillin, 0.56-10.1 for Amikacin, 3-16.4 for gentamycin, 10.1 for tetracycline, 0.56 for erythromycin and 5.25 for bacitracin. The highest value in the increase fold area was about 16.4% for Gentamycin indicating significantly enhancement in the presence of *Moringa oleifera* in case *Klebsiella* sp. followed by 10.1% against Ampicillin, Amoxicillin also Gentamycin in case of *Escherichia coli*.

The combination in case of Gram positive bacterial pathogen also produced varying zones of inhibition. Ampicillin had highly synergistic action was observed against *Micrococcus luteus*, *Bacillus pumilis* and *Staphylococcus epidermidis* at 20mm but moderate synergistic action with *Staphylococcus aureus* and *Bacillus subtilis* at 15mm. Synergetic action between amoxacillin and *Moringa* hot aqueous extract against *Staphylococcus epidermidis* at 25mm and moderate synergistic against the other tested Gram positive bacteria at 15mm.

Amikacin showed Synergistic interactions against all tested bacterial pathogen at 20mm except *Staphylococcus epidermidis* with highly synergistic interactions at 25mm. Maximum zone of inhibition were observed for *Bacillus pumilis* . Gentamycin and bacitracin showed synergistic action for *Staphylococcus aureus* and *Staphylococcus epidermidis* at 25mm, *Micrococcus luteus* and *Bacillus subtilis* at 20mm highly synergistic action was observed for tetracycline against *Staphylococcus epidermidis* at 35mm, *Bacillus pumilis* at 20mm and *Micrococcus luteus* at 15mm.

TABLE 4.Antibacterial effect of hot aqueous extract of Moringa oleifera leaf and different antibiotic against different pathogenic G+ve and G-ve bacterial pathogen.

	Inhibition zone (mm)								
M.O / Different treatments	Gram positive					Gram negative			
	Escherichia coli ATCC10536	*Bordetella* sp	*Pseudomonas aeruginosa* ATCC9027	*Klebsiella* sp.	*Micrococcus luteus* ATCC 10240	Staphylococcus ylococcus aureus ATCC 29737	*Bacillus pumilis* ATCC 14884	*Staphylococcus epidermidis* ATCC 12228	*Bacillus subtilis*
Moringa oleifera extract	20	20	0.0	0.0	30	20	30	20	0.0
(x) AM10	0.0	0.0	0.0	0.0	0.0	10	0.0	15	0.0
(y) AM10_+Mo	20	20	0.0	15	20	15	20	20	15
Increase in fold (b)area	10.1	10.1	0.0	5.25	10.1	1.25	10.1	0.8	5.25
AMC30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20	10
AMC30+Mo	20	15	0.0	15	15	15	15	25	15
Increase in fold area	10.1	5.25	0.0	5.25	5.25	5.25	5.25	0.56	1.25
Ak30	20	20	15	0.0	20	10	20	25	10
Ak30+ Mo	25	25	0.0	20	20	20	20	25	20
Increase in fold area	0.56	0.56	-0.84	10.1	0.0	3	0.0	0.0	3
CN10	0.0	10	20	0.0	20	0.0	20	35	0.0
CN10+ Mo	20	20	0.0	25	20	25	40	25	20
Increase in fold area	10.1	3.0	0.0	16.4	0.0	16.4	3	-0.49	10.1
TE30	10	0.0	0.0	0.0	10	0.0	10	20	15
TE30+ Mo	0.0	20	0.0	0.0	15	0.0	20	35	0.0
Increase in fold area	0	10.1	0.0	0.0	1.25	0.0	3	2.1	0.0
E15	10	20	0.0	0.0	20	0.0	30	30	0.0
E15+Mo	0.0	25	0.0	0.0	25	0.0	20	25	0.0
Increase in fold area	0.0	0.56	0.0	0.0	0.56	0.0	0.0	-0.3	0.0
B10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B10+Mo	0.0	15	0.0	0.0	15	0.0	40	20	0.0
Increase in fold area	0.0	5.25	0.0	0.0	5.25	0.0	43.5	10.1	0.0

- (Am10) Ampicillin, (AMC30) Amoxacillin, (AK30) Amikacin, (CN10) Gentamycin, (Te30) Tetracycline, (E15) Erythromycin, (B10) Bacitracin and Mo *Moringa oleifera.*

- Increase in fold area was calculated as = (y2 - x2)/x2, where 'x' and 'y' are the mean inhibition zones for antibiotic and antibiotic + M extract, respectively in the absence of bacterial growth inhibition zones, the disk's diameter (6mm).

Combination of erythromycin with *Moringa oleifera* extract had synergistic action was observed against *Micrococcus luteus* at 25mm. Synergistic action for bacitracin was observed against *Bacillus pumilis* at 40mm and *Staphylococcus epidermidis* at 20mm and *Micrococcus luteus* at 15mm.tetracycline,erythromycin and bacitracin, but when combination with *Mo* hot aqueous extract were not effect on *Staphylococcus aureus* and *Bacillus subtilis*.

Combinations of antibiotics and *Moringa oleifera* hot aqueous extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 0.8-10.1 for ampicillin and amoxicillin, 3.0 for amikacin, 3-16.4 for gentamycin,1.25-3.0 for tetracycline,0.56 for erythromycin and 5.25-43.5 for bacitracin . This result indicating that the bio-active compounds of this extract are not the substrates of bacterial efflux pumps as in case of Gram-negative bacteria over-express efflux pumps (Tran et al., 2010).

Table 5 showed the combined effect of *Moringa oleifera* cold aqueous extract and the conventional antibiotics against tested Gram-negative and Gram-positive bacterial pathogen. This combination showed different activity, it was obvious Ampicillin, Amikacin and Amoxicillin have moderate synergism with *Escherichia coli* and *Bordetella* sp. Antagonistic interaction of *Moringa oleifera* cold aqueous extract with all used antibiotics was observed against *Pseudomonas aeruginosa*. The same result was obtained in case of *Klebsiella* sp. except with Gentamycin and Amoxacillin synergistic action was observed.

Tetracyclin and erythromycin have no effect in such combination on tested bacteria except *Escherichia coli* at 30mm and *Bordetella* sp. with erythromycin at 25mm. No synergistic action with bacitracin against tested bacteria except *Escherichia coli* at 20mm.

Combinations of antibiotics and *Moringa oleifera* cold aqueous extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 1.8-5.25 for ampicillin and amoxicillin,5.25 for amikacin,1.8-10.1 for gentamycin, 1.8-8 for tetracycline, 0.56-8 for erythromycin and 1.8-10.1 for bacitracin. Typically, maximum resulted in average fold-area increases in antibacterial activity (zone of inhibition) of *Escherichia coli* with gentamycin + Mo cold aqueous extract and bacitracin with *Moringa oleifera* cold aqueous extract at 10.1.

Combination between antibiotic and medicinal plant extracts became a useful tool in fighting emerging drug resistance microorganisms but we must be approached with care since the combination may increase the antagonistic rather than synergistic. Recently, the health benefits of Moringa oleifera leaves as a dietary supplement were investigated by many researchers (Rahman et al., 2009; Mensah et al., 2012; Okechukwu et al., 2014).

The combined activities of cold aqueous extract from *Moringa oleifera* and the conventional antibiotics against tested Gram-positive bacterial pathogen produced varying zones of inhibition. Ampicillin had observed synergistic action against *Micrococcus luteus*, *Staph epidermidis* and *Bacillus subtilis*. Amikacin also have synergistic action against *Bacillus pumilis* and *Staph epidermidis* and highest value noticed in case of *Staphylococcus aureus*. In case of Gentamycin only the synergistic action was observed in *Staphylococcus aureus* and *Bacillus subtilis*.

Table 6 showed the results of the synergistic interaction of the combinations of the ethanol *Moringa oleifera* extract and antibacterial drugs against selected Gram positive and Gram negative bacterial pathogen combinations produced great variation in zones of bacterial sensitivity toward antibiotics. In gram negative selected pathogens, ampicillin and amoxicillin when combined with the ethanol extract were not produced synergistic effects against *Pseudomonas aeruginosa* and *Klebsiella* sp. Gentamycin in combination had highest synergism was observed against *Escherichia coli* and *Bordetella* sp., On the other hand, antagonistic interaction against *Pseudomonas aeruginosa* .

Tetracycline which was most active antibacterial in combination against both *Escherichia coli* and *Bordetella* sp. while maximum synergistic action was observed with erythromycin against *Bordetella* sp. Bactricin and erythromycin had not synergistic action against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. Combinations of antibiotics and *Moringa oleifera* ethanol extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 1.8 for ampicillin and amoxicillin, 0.8-1.8 for amikacin, 3-16.4 for gentamycin, 3-10.1 for tetracycline, 1.25 for erythromycin and 1.8 for bacitracin.

While in case of gram positive selected pathogens, Ampicillin had highly synergistic action was observed against *Micrococcus luteus* and *Staphylococcus epidermidis*. While the antagonistic interactions against *Bacillus subtilis* were detected. Moreover, amoxicillin had highly synergistic action against *Bacillus pumilis* and *Micrococcus luteus* and *Bacillus subtilis* at 20mm. Amikacin synergistic action was observed

against *Micrococcus luteus*, *Staphylococcus epidermidis* and *Bacillus pumilis*. Gentamycin showed synergistic interactions in combination only on *Micrococcus luteus*. Combination between tetracycline with *Moringa oleifera* ethanol extract showed synergistic interactions against tested bacteria except *Staphylococcus aureus*. While erythromycin and tetracycline showed maximum inhibition, zone was observed against *Staphylococcus epidermidis* at 35mm. Combination of bacitracin with *Moringa oleifera* ethanol extract had synergistic action was observed against *Micrococcus luteus* and *Staphylococcus epidermidis* at 20mm. (Dzotam et al., 2016).

TABLE 5. Antibacterial effect of cold aqueous extract of Moringa oleifera leaf and different antibiotic against different pathogenic G+ve and G-ve bacterial pathogen.

	Inhibition zone (mm)								
	Gram positive				Gram negative				
M.O / Different treatments	*Escherichia coli* ATCC10536	*Bordetella* sp.	*Pseudomonas aeruginosa* ATCC9027	*Klebsiella* sp.	*Micrococcus luteus* ATCC 10240	*Staphylococcus aureus* ATCC 29737	*Bacillus pumilis* ATCC 14884	*Staphylococcus epidermidis* ATCC 12228	*Bacillus subtilis*
Moringa oleifera extract	0.0	15	0.0	0.0	15	0.0	0.0	0.0	0.0
(x) AM10	0.0	0.0	0.0	0.0	0.0	10	0.0	15	0.0
(y)AM10_+Mo	15	10	0.0	0.0	10	10	0.0	20	10
Increase in fold (b)area	5.25	1.8	0.0	0.0	1.8	0.0	0.0	0.78	1.8
AMC30	0.0	0	0.0	0.0	0.0	0.0	0.0	20	10
AMC30+Mo	15	10	0.0	0.0	10	10	10	20	10
Increase in fold area	5.25	1.8	0.0	0.0	1.8	1.8	1.8	0.0	0.0
Ak30	20	20	15	0.0	20	10	20	25	10
Ak30+ Mo	20	20	10	5	20	20	30	30	20
Increase in fold area	0.0	0.0	0.0	5.25	0.0	3	1.25	0.44	3
CN10	0.0	10	20	0.0	20	0.0	20	35	0.0
CN10+ Mo	20	20	10	10	20	20	20	30	15
Increase in fold area	10.1	3	-0.75	1.8	0.0	10.1	0.0	-0.27	5.25
TE30	10	0.0	0.0	0.0	10	0.0	10	20	15
TE30+ Mo	30	10	0.0	0.0	15	0.0	10	35	15
Increase in fold area	8	1.8	0.0	0.0	1.25	0.0	0.0	2.06	0.0
E15	10	20	0.0	0.0	20	0.0	30	30	0.0
E15+Mo	30	25	0.0	0.0	20	0.0	20	35	0.0
Increase in fold area	8	0.56	0.0	0.0	0.0	0.0	-0.55	0.36	0.0
B10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B10+Mo	20	10	0.0	0.0	15	0.0	10	20	0.0
Increase in fold area	10.1	1.8	0.0	0.0	5.25	0.0	1.8	10.1	0.0

- (Am10) Ampicillin, (AMC30) Amoxacillin, (AK30) Amikacin, (CN10) Gentamycin, (Te30) Tetracycline, (E15) Erythromycin, (B10) Bacitracin and Mo *Moringa oleifera.*

- Increase in fold area was calculated as = (y2 - x2)/x2, where 'x' and 'y' are the mean inhibition zones for antibiotic and antibiotic + M extract, respectively in the absence of bacterial growth inhibition zones, the disk's diameter (6mm).

TABLE 6. The combined antibacterial effect of *Moringa oleifera* leaf ethanol extract and different antibiotic against different pathogenic G+ve and G-ve bacterial pathogen.

	Inhibition zone (mm)								
	Gram positive				Gram negative				
M.O / Different treatments	*Escherichia coli* ATCC10536	*Bordetella* sp.	*Pseudomonas aeruginosa* ATCC9027	*Klebsiella* sp.	*Micrococcus luteus* ATCC 10240	*Staphylococcus ylococcus* aureus ATCC 29737	*Bacillus pumilis* ATCC 14884	*Staphylococcus epidermidis* ATCC 12228	*Bacillus subtilis*
Moringa oleifera extract	25	20	20	0.0	30	25	0.0	20	25
(x) AM10	0.0	0.0	0.0	0.0	0.0	10	0.0	15	0.0
(y)AM10_+Mo	10	10	0.0	0.0	20	0.0	10	20	0.0
Increase in fold (b)area	1.8	1.8	0.0	0.0	10.1	-0.64	1.8	0.78	0.0
AMC30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20	10
AMC30+Mo	10	10	0.0	0.0	20	0.0	30	10	20
Increase in fold area	1.8	1.8	0.0	0.0	10.1	0.0	24	-0.75	3
Ak30	20	20	15	0.0	20	10	20	25	10
Ak30+ Mo	30	30	20	10	35	10	20	30	10
Increase in fold area	1.25	1.25	0.8	1.8	2.06	0.0	0.0	0.44	0.0
CN10	0.0	10	20	0.0	20	0.0	20	35	0.0
CN10+ Mo	25	20	10	0.0	30	0.0	20	20	0.0
Increase in fold area	16.4	3	-0.75	0.0	1.25	0.0	0.0	-0.67	0.0
TE30	10	0.0	0.0	0.0	10	0.0	10	20	15
TE30+ Mo	20	20	0.0	0.0	20	0.0	20	30	25
Increase in fold area	3	10.1	0.0	0.0	3	0.0	3	1.25	1.8
E15	10	20	0.0	0.0	20	0.0	30	30	0.0
E15+Mo	0.0	30	0.0	0.0	30	0.0	30	35	15
Increase in fold area	0.0	1.25	0.0	0.0	1.25	0.0	0.0	0.36	5.25
B10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B10+Mo	0.0	10	0.0	0.0	20	0.0	0.0	20	0.0
Increase in fold area	0.0	1.8	0.0	0.0	10.1	0.0	0.0	-0.67	0.0

- (Am10) Ampicillin, (AMC30) Amoxacillin, (AK30) Amikacin, (CN10) Gentamycin, (Te30) Tetracycline, (E15) Erythromycin, (B10) Bacitracin and Mo *Moringa oleifera.*

- Increase in fold area was calculated as = (y2 - x2)/x2, where 'x' and 'y' are the mean inhibition zones for antibiotic and antibiotic + M extract, respectively in the absence of bacterial growth inhibition zones, the disk's diameter (6mm).

Combinations of antibiotics and *Moringa oleifera* ethanol extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 0.44-10.1 for ampicillin ,0.75-24 for amoxicillin, 0.44-2.06 for amikacin, 1.25 for gentamycin, 1.25-3.0 for tetracycline, 0.36-1.25 for erythromycin and 10.1 for bacitracin. The maximum resulted in average fold-area increases in antibacterial activity of *Bacillus pumilis* was with amoxicillin.

The combined effect *Moringa oleifera* methanol extract and the conventional antibiotics against tested Gram negative bacterial pathogen produced varying zones of inhibition (Table 7).

TABLE 7. The combined antibacterial effect of Moringa oleifera leaf methanol extract and different antibiotic against different pathogenic G+ve and G-ve bacterial pathogen

	Inhibition zone (mm)								
M.O / Different treatments	Gram positive				Gram negative				
	Escherichia coli ATCC10536	*Bordetella* sp.	*Pseudomonas aeruginosa* ATCC9027	*Klebsiella* sp.	*Micrococcus luteus* ATCC 10240	*Staphylococcus ylococcus* aureus ATCC 29737	*Bacillus pumilis* ATCC 14884	*Staphylococcus epidermidis* ATCC 12228	*Bacillus subtilis*
Moringa oleifera extract	20	25	25	15	30	30	15	20	20
(x) AM10	0.0	0.0	0.0	0.0	0	10	0.0	15	0.0
(y)AM10_+Mo	15	20	0.0	20	15	15	30	20	15
Increase in fold (b)area	5.25	5.25	0.0	10.1	5.25	1.25	24	0.78	8
AMC30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20	10
AMC30+Mo	10	20	0.0	10	20	15	20	20	20
Increase in fold area	1.8	10.1	0.0	1.8	10.1	5.25	10.1	0.0	3
Ak30	20	20	15	0.0	20	10	20	25	10
Ak30+ Mo	25	40	20	20	30	20	25	30	20
Increase in fold area	0.56	3	0.8	10.1	1.25	3	0.56	0.44	3
CN10	0.0	10	20	0.0	20	0.0	20	35	0.0
CN10+ Mo	20	20	30	15	30	20	20	35	25
Increase in fold area	10.1	1.8	1.25	5.25	1.25	10.1	0.0	0.0	16.4
TE30	10	0.0	0.0	0.0	10	0.0	10	20	15
TE30+ Mo	15	20	10	20	20	0.0	20	30	20
Increase in fold area	1.25	10.1	1.8	10.1	3	0.0	3	1.25	0.78
E15	10	20	0.0	0.0	20	0.0	30	30	0.0
E15+Mo	25	20	10	25	20	20	30	40	0.0
Increase in fold area	5.25	0.0	1.8	16.4	0.0	10.1	0.0	0.78	0.0
B10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B10+Mo	15	15	0.0	15	15	10	20	15	15
Increase in fold area	5.25	5.25	0.0	5.25	5.25	1.8	10.1	5.25	5.25

- (Am10) Ampicillin, (AMC30) Amoxacillin, (AK30) Amikacin, (CN10) Gentamycin, (Te30) Tetracycline, (E15) Erythromycin, (B10) Bacitracin and Mo *Moringa oleifera.*

- Increase in fold area was calculated as = (y2 - x2)/x2, where 'x' and 'y' are the mean inhibition zones for antibiotic and antibiotic + M extract, respectively in the absence of bacterial growth inhibition zones, the disk's diameter (6mm).

Ampicillin had synergistic effects against *Bordetella* sp., *Escherichia coli* and *Klebsiella* sp. In case of *Pseudomonas aeruginosa*, the antagonistic interaction was observed with Ampicillin, Amoxicillin and Bacitracin. Maximum synergetic action for amikacin against *Bordetella* sp. at 40mm, *Escherichia coli at* 25mm, *Pseudomonas aeruginosa* and *Klebsiella sp.* at 20mm. Gentamicin had highly synergistic action against *Pseudomonas aeruginosa* at 30mm, *Escherichia coli*, *Bordetella s*p. at 20mm and moderate synergetic action against *Klebsiella* sp. at 15mm. Tetracycline when combined with *Mo* methanol extract was effect on *Bordetella* sp*., Klebsiella* sp. at 20mm and moderate synergetic action against *Escherichia coli* at

15mm. Synergistic action was observed with erythromycin against *Escherichia coli, Klebsiella* sp. at 25mm and *Bordetella* sp. at 20mm. Moderate synergistic action for bacitracin against tested bacteria at 15mm except *Pseudomonas aeruginosa*.

Combinations of antibiotics and *Moringa oleifera* methanol extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 1.8-5.25 for ampicillin and amoxicillin, 1.8-5.25 for amikacin, 1.25-5.25 for gentamycin, 1.25-10.1 for tetracycline, 1.8-16.4 for erythromycin and 1.8-5.25 for bacitracin. Typically, maximum resulted in average fold-area increases in antibacterial activity (zone of inhibition) of *Klebsiella* sp. with erythromycin *Mo* methanol extract at 16.4. (Singh et al ., 2011). The maximum synergistic interaction between all antibiotic when combined with the methanol extract was observed against all tested bacterial pathogen.

No interaction between erythromycin when combined with *Moringa oleifera* methanol extract was observed against *Micrococcus luteus, Bacillus pumilis* and *Bacillus subtilis*. On the other hand tetracycline showed synergistic interactions against all tested bacterial pathogen except *Staphylococcus aureus*. Amoxicillin had highly synergistic action was observed against tested bacteria at 20mm except *Staphylococcus epidermidis* at 15mm. This synergistic action provides scientific bases for the use of concoctions traditionally in the treatment of diseases. This result is in agreement with Dzotam et al. (2016) who assessed a synergistic combination of biologically active components from wild *Moringa* against different pathogens, indicating the high efficacy of combination therapy over mono therapy.

Synergistic interactions of Amikacin against *Micrococcus luteus* at 30mm, *Staphylococcus epidermidis* at 30mm was recorded. Synergistic action was observed for *Bacillus pumilis* by combination between gentamycin and *Mo* methanol extract at 25mm. Erythromycin showed great effect on all tested bacterial pathogen except *Bacillus subtilis.* Combination of bacitracin with *Mo* methanol extract had synergistic action was observed against tested bacteria except *Staphylococcus aureus*. Combinations of antibiotics and *Moringa oleifera* methanol extract resulted in average fold-area increases in antibacterial activity (zone of inhibition) of 0.78-24 for ampicillin, 3.0-10.1 for amoxicillin, 0.44-3 for amikacin, 1.25-16.4 for gentamycin, 0.78-3.0 for tetracycline, 0.78-10.1 for erythromycin and 1.8-5.25 for bacitracin.

Typically, maximum resulted in average fold-area increases in antibacterial activity (zone of inhibition) of *Bacillus pumilis* with ampicillin +*Mo* methanol extract at 24. The methanol extract showed additive effect along with gentamicin and ampicillin against *E.coli* and *S. aureus* (Mahesh & Satish, 2008). Gram-positive bacteria are more susceptible because of the presence of an outer peptidoglycan layer, which is not an efficient Permeability.

Our result in the agreement with Olufunmiso & Afolayan (2011), they found that the combination of Moringa methanol/ amoxicillin gave different interactions against pathogenic bacteria.

The results of this work are very important taking in account the medicinal importance of the tested activity of *Moringa oleifera* remain unchanged, indicating that the bioactive compounds of this extract are not the substrates of bacterial efflux pumps, as the tested MDR bacteria. Over-express efflux pumps**.** *M. oleifera* leaf extract showed higher antimicrobial activity than that of tetracycline (Kalpana et al., 2013).

The prediction of synergy between the synthetic antibiotic and a natural product is very crucial issue. This result is in agreement with Tahany et al. (2010) who assessed a synergistic combination of biologically active components from wild *Moringa peregrine* against different pathogens, indicating the high efficacy of combination therapy over monotherapy **.** So, this plant extracts having good healing properties without side effects when compared with synthetic antibiotics (Manikandan et al., 2016).

Conclusion

Moringa oleifera extracts having good healing properties without side effects when compared with synthetic antibiotics. Combination between *Moringa oleifer*a leave extract and chemotherapy antibiotics appears greater effectiveness on pathogenic bacteria and *Moringa oleifera* considered as a promising

source of phytochemicals, which could increase the effectiveness of many traditional antibiotics against bacteria. The antagonist properties of some combinations against some Gram negative and /or Gram positive bacterial pathogen in the current study revealed that the using of *Moringa* leaves as dietary supplement needs some precautions, because it may decrease the effectiveness of antibiotics against disease causing bacteria. The result of this study correlates with previous reports on the effect of *Moringa* leaves extracts combination with antibiotics and recommend the high efficacy of combination therapy over mono therapy as a new green ecofriendly drug.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions: All the authors have contributed to the work by participating in reviewing the literature, designing and writing the manuscript**.**

References

Abbaszadegan, A., Nabavizadeh, M., Gholami, A., Aleyasin, Z.S., Dorostkar, S., Saliminasab, M., Ghasemi, Y., Hemmateenejad, B., Sharghi, H. (2015) Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: A promising disinfectant in root canal treatment. *International Endodontic Journal*, **48**(8), 790–800.

Adedapo, A.A., Falayi, O.O., Oyagbemi, A.A. (2015) Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. *Journal of Basic and Clinical Physiology and Pharmacology*, **26**(5), 491–499.

Afolayan, A.J. (2003) Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi. *Pharmaceutical Biology*, **41**(1), 22-25.

Afolabi, C.A., Ibukun, E.O., Afor, E., Obuofor, E.M., Farombi, E.O. (2007) Phytochemical constituent and antioxidant activity of extracts from the leaves of *Ocimum gratissimum*. *Science ResearchesEssay,* **2**, 163-166.

Alekshun, M.N., Levy, S.B. (2007) Bacterial drug resistance: Response to survival threats. In: "*Bacterial Stress Responses*", Storz, G., Heugge-Aronis, R. (Eds.), pp. 323-366. Washington, DC: ASM Press; 2000.

Alo, M.N., Anyim, C., Elom, M. (2012) Coagulation and antimicrobial activities of *Moringa oleifera* seed storage at 3°C temperature in turbid water. *Advances in Applied Science Research*, **3**(2), 887–894.

Anwar, F., Latif, S., Ashraf, M., Gilani, A.H. (2007) *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy research: PTR*, **21**(1), 17–25.

Babayi, H., Kolo, L., Akogun, J.I., Ijah, U.J.J. (2004) The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri Iournal*, **16**, 106-111.

Bajpai, M., Pande, A., Tewari, S.K., Prakash, D. (2005) Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition*, **56**(4), 287–291.

Bennett, R.F., Mellon, J., Pratt, M., Dupont, L., Kroon, P. (2003) Profiling glucosinolates and phenolics in vegetative and reproductive tissues of multi-purpose trees *Moringa oleifera* horseradish tree. *Journal of Agriculture and Food Chemistry,* **51**(2), 3546-5553.

Bina, B., Mehdinejad, M., Dalhammer, G., Nikaeen, G., Attar, H. (2010) Effectiveness of *Moringa oleifera* coagulant protein as natural coagulant aid in removal of turbidity and bacteria from turbid waters. *International Journal of Environmental and Ecological Engineering*, **4**(7), 261-263.

Birla, S.S , Tiwari, V.V., Gade, A.K., Ingle A.P., Yadav, A.P., Rai, M.K. (2009) Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Lett. Appl. Microbiol.* **48**(2), 173-179.

Bukar, A., Uba, A., Oyeyi, T.I. (2010) Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food borne microorganisms. *Bayero Iournal of Pure and Applied Science,* **3**, 43-48.

CLSI (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.

Clinical and Laboratory Standards Institute (CLSI) Document M07-A7. Wayne, Pa. USA.

CLSI (2012) Clinical and Laboratory Standards Instituteperformance Standards for Antimicrobial Disk Susceptibility Tests. CLSI document M100-S17 Pennsylvania, USA.

Cowan, M.M. (1999) Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews,* **12**(4), 564-582.

Doughari, J.H., Pukuma, M.S., De, N. (2007) Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi. African Journal Biotechnology*, **6**, 2212-2215.

Dzotam, J., Touani, F., Kuete, V. (2016) Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosoma mafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria. *BMC Complementary and Alternative Medicine* ,**16**, 9 DOI 10.1186/s12906-016-0990-7

Fankam, A.G., Kuete, V., Voukeng, I.K., Kuiate, J.R., Pages, J.M. (2011) Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. *BMC Complementary and Alternative Medicine*, **11**, 104.

Fernández, L., Gooderham, W.J., Bains, M., McPhee, J.B., Wiegand, I., Hancock, R.E. (2010) Adaptive resistance to the "last hope" antibiotics polymyxin B and colistin in Pseudomonas aeruginosa is mediated by the novel two-component regulatory system ParR-ParS. *Antimicrobial Agents and Chemotherapy*, **54**(8), 3372–3382.

Hausteen, B.H. (2005) The biochemistry and medical significance of the flavonoids. P*harmacology Therapeutics Journal*, 96, 67-202.

Idris, A., Abubakar, U. (2016) Phytochemical and antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, **8**(5) 28-33.

Kalpana, S, Moorthi, S., Kumara, S. (2013) Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gram-negative bacteria. *International Journal of Current Microbiology and Applied Sciences*, **2**(12), 514-518.

Kasolo, J.N., Bimenya, G.S., Lonzy, O. (2010) Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *Journal of Medical Plants Research*, **4**(9), 753-757.

Kheiralla, Z.M.H., Rushdy, A.A., Betiha, M.A., Yakob, N.A. (2014) High-performance antibacterial of montmorillonite decorated with silver nanoparticles using microwave assisted method. *Journal of Nanoparticles Rresearch,* **16**, 2-14.

Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T., Mohan, N. (2010) Synthesis of silver nanoparticles using *acalyphaindica* leaf extracts and its antibacterial activity against water borne pathogens . *Colloids and Surfaces B Journal,* **76**, 50-56.

Kwaghe, A., Ambali, A.G. (2009) Preliminary phytochemical screening of fresh and dried *Moringa oleifera* leaves and that of chloroform, ethylacetate and N-butanol fractions. *Sahel Journal of Veterinary Science*, **21**, 1-24.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., Bertoli, S. (2015) Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Sciences*, **16**(6), 12791–12835.

Mahesh, B., Satish, S. (2007) Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences*, **4**, 839-843.

Manikandan, P.A., Gnanasekaran1, P., Julikarthika, I., Arvind Prasanth, D. (2016) Antibacterial efficacy of *Moringa oleifera* leaf against medically important clinical Pathogens. *International Journal of Current Microbiology and Applied Sciences*, **5**(4), 109-116.

Martín, C., Martín, G., García, A., Fernández, T., Hernández, E., Puls, J. (2013) Potential applications of *Moringa oleifera*. A critical review. *Pastos y Forrajes,* **36**(2) 137-149.

Mbengui, R.D., Guessennd, N.K., M'boh, G.M., Golly, J.K., Okou, C.O., Nguessan, J.D., Dosso, M.,

Djaman, J.A. (2013) Physiochemical screening and study of comparative antibacterial activity of aqueous and alcoholic extracts of the leaves and barks of *Terminalia catappa* on multiresistant strains. *Journal of Applied Biosciences*, **66**, 5040-5048.

Meenal, S.K., Khadabadi, S.S., Saboo, S.S., Ghorpade, D.S., Modi, A.J. (2010) *In vitro* antimicrobial activity of the crude extracts of *Moringa oleifera* leaves (Araceae). *International Journal of Pharmaceutical Sciences and Research*, **8**, 88-91.

Mensah, J.K., Ikhajiagbe, B., Emokhor, J. (2012) Phytochemical, nutritional and antibacterial properties of dried leaf powder of *Moringa oleifera* from Edo north province, Nigeria. *Journal of Natural Product and Plant Resources*, **2**(1), 107-112.

Moyo, B., Masika, P.J., Hugo, A., Muchenje, V. (2011) Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, **10**(60), 12925-12933.

Mridha, M. (2015) Prospects of Moringa cultivation in Saudi Arabia. *Journal of Applied Environmental and Biological Sciences*, **5**(3), 39-46.

Ofokansi, K.C., Attama, A.A., Uzor, P.F., Ovri, M.O. (2013) Evaluation of the combined antimicrobial activity of the leaf extract of *phyllantus* muellerianus with ciprofloxacin. *Journal of Pharmaceutical Technology & Drug Research*, **2**, 16.

Okechukwu David, B., Kenechukwu, F., Chioma, A.O. (2014) *Moringa oleifera* leaf extract potentiates anti-pseudomonal activity of ciprofloxacin. *African Journal of Biotechnology*, **13**(31), 3516-3521.

Olufunmiso, O.O., Afolayan, A.J. (2011) *In vitro* antibacterial activities of the methanol extract of *Ziziphus mucronata* Willd. subsp. Mucronata Willd *Journal of Medicinal Plant Research*, **5**(16), 3791-3795.

Onyekaba, T.U., Chinedu, O.G., Fred, A.C. (2013) Phytochemical screening and investigations of antibacterial activities of various fractions of the ethanol leaves extract of *Moringa oleifera* LAM (*Moringaceae*). *International Journal of Pharmaceutical, Chemical and Biological Sciences,* **3**, 962-973.

Porte, S.M. (2014) Overview of folk medicine user for typhoid in India. *International Journal of Research in Ayurveda and Pharmacy*, **5**(2), 219- 224.

Rafailidis, P.I., Ioannidou, E.N., Falagas, M.E. (2007) Ampicillin/sulbactam: current status in severe bacterial infections. *Drugs*, **67**(13), 1829–1849.

Rahman, M., Sheikh, M.I., Sharmin, S.A., Islam, S., Rahman, A., Rahman, M., Alam, M.F. (2009) Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against some Human Pathogenic Bacteria. *Journal of Natural Science*, **8**(2), 219-227.

Sachin, K., Singh, K., Gupta, R.K. (2012) Coupled higgsfield equation and hamiltonianamplitude equations and lie classical approach and (G'/G) expansion method. *Pramana– Journal of Physics,* **79**, 41-60.

Schneider, G., Wolfhing, J. (2004) "*Sherries Medical Microbiology: An Introduction to Infectious Disease*". 4th ed. Jenson Books Inc, 4, pp. 261-273.

Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R., Minaian, S. (2007) Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine: Nanotechnology, Biology, and Medicine*, **3**(2), 168–171.

Singh, M., Manikandan, S., Kumaraguru, A.K. (2011) Nanoparticles: A new technology with wide applications. *Research Journal of Nanoscience and Nanotechnology*, **1**(1), 1-11.

Srinivasan, D., Nathan, S., Suresh, T., Lakshmana Perumalsamy, P. (2001) Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of ethnopharmacology*, **74**(3), 217-220.

Tahany, M.A.A.R., Hegazy, A., Sayed, M.A., Kabiel, H., El-Alfy, T., El-Komy, S. (2010) Study on combined antimicrobial activity of some biologically active constituents from wild *Moringa peregrine* Forssk. *Yeast and Fungal Research*, **1**, 15-24.

Talukdar, A.D., Choudhary, M.D., Chakraborty, M., Dutta, B.K. (2010) Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantea*

(Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana.* Wall. ex. Hook (Cl. & Bak.). *Assam University Journal of Science & Technology*: *Biological and Environmental Sciences*, **5**(1), 70-74.

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jäger, A.K., van Staden, J. (2001) Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulation*, **34,** 23-37.

Thilza, I.B., Sanni, S., Zakari, A.F.S., Sanni, M.T., Musa, B.J. (2010) *In vitro* antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. *Academia Arena*, **2**, 80-82.

Tijjani, M.B., Bello, I.A., Aliyu, A.B., Olurishe, T., Maidawa, S.M., Habila, J.D., Balogun, E.O. (2009) Phytochemical and antibacterial studies of root extract of *cochlospermum tinctorium* A. Rich. (Cochlospermaceae). *Research Journal of Medicinal Plant*, **3**(1), 16-22.

Tran, Q.T., Mahendran, K.R., Hajjar, E., Ceccarelli, M., Davin-Regli, A., Winterhalter, M., Weingart, H., Pagès, J.M. (2010) Implication of porins in beta-lactam resistance of *Providencia stuartii*. *The Journal of Biological Chemistry*, **285**(42), 32273–32281.

Trookman, N.S, Fadd, M.D., Rizer, R.L., Weber, T. (2011) Treatment of minor wounds from dermatologic procedures and a comparison of three topical wound care ointments using a laser wound model. *Journal of the American Academy of Dermatology*, **64**(3), 8-15.

Yusha, M., Hamza, M.M., Abdullahi, N. (2010) Antibacterial activity of adansoniadigitata stem bark extracts on some clinical bacterial isolates. *International Journal of Biomedical and Health Sciences*, **6**, 129-135.

Zakeri, B., Wright, G.D. (2008) Chemical biology of tetracycline antibiotics. *Biochimie et Biologie Cellulaire*, **86**(2), 124-136.

كفاءة تاثير مستخلصات أوراق المورينجا أوليفيرا كمضادات الميكروبات ضد بعض مسببات الأمراض المقاومة للأدوية المتعددة

هالة عبد المنعم احمد ابراهيم، زينب محمد حسن خير الله، دعاء عبد الرازق شحاته على
قسم النبات- كلية البنات للعلوم والآداب والتربية - جامعة عين شمس - القاهرة - مصر.

تعتبر النباتات الطبية من اهم المصادر حيوية للمواد الطبيعية المضادة للميكروبات والامنه لصحة الإنسان وذلك لتجنب الآثار الجانبية الناتجة عن استخدام المواد الكيميائية الاصطناعية. وقد اثرت قله فعالية المضادات الحيوية التقليدية تجاه الكائنات الحية الدقيقة المقاومة للأدوية المتعددة على الصحة العامة العالمية. وجد أن الخلطات بين المستخلصات النباتية المختلفة والمضادات الحيوية المعروفه تجاريا يزيد من فعالية المضادات الحيوية تجاه البكتيريا المقاومة للأدوية المتعددة. وقد وجد ان التوليفات بين المستخلصات النباتية المختلفة والمضادات الحيوية التجارية إلى زيادة فعالية المضادات الحيوية ضد السلالات المقاومة للأدوية المتعددة.

أظهرت مستخلصات أوراق المورينجا نشاطًا مضادًا للميكروبات ضد نمو مجموعه من السلالات البكتيريا الموجبة والسالبة لصبغه جرام والمقاومة ايضا للأدوية وهم: (*Escherichia coli* ATCC 10536 و *Pseudomonas aeruginosa* ATCC 9027 و *Staphylococcus aureus* ATCC 29737 و *Staph epidermidis*