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Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection

Nagwa M. Atef[1], Sanaa M. Shanab[1], Sahar I. Negm[2*] and Yasmeen A. Abbas[3]

Abstract

Background: Due to rapid development of microbial resistance against chemotherapeutic agents (mostly antibiotics), it has become essential currently to screen effective, safe, cheap, and available therapeutics from various medicinal plants—like herbs—for their potential antimicrobial effect.

Aim: To estimate the antibacterial activity of aqueous, ethanol, and methanol extracts of each of *Moringa oleifera* L. leaves and *Matricaria recutita* L. flowers against antibiotic-resistant and sensitive bacterial strains isolated from patients having wound infections.

Results: In the present study, a total of one hundred clinical samples were obtained from different cases of infected wounds. Forty isolates (40%) of pure bacterial cultures were detected. *Pseudomonas aeruginosa* was found to be the predominant agent isolated from the wound infections (32.5%) followed by *Staphylococcus* spp. (25%), *E. coli* (20%), *Klebsiella* spp. (20%), and *Proteus mirabilis* (2.5%). Sensitivity of the bacterial isolates was tested against antibiotic discs: piperacillin, ampicillin, oxacillin, pinicillin, gentamicin, tobramycin, amikacin, streptomycin, ceftriaxone , ceftazidime, cefoxitin, cefoperazone, cefuroxime, cefepime, cefotaxime, ciprofloxacin, oflaxacin, levofloxacin, lomefloxacin, moxifloxacin, tetracycline, aztreonam, azithromycin, erythromycin, imipenem, piperacillin-tazobactum, ampicillin-sulbactam**,** linezolid, teicoplanin, trimethoprim-sulphamethoxazole , chloramphenicol, and clindamycin. Out of the 40 bacterial strains studied, 20 isolates were multidrug-resistant (MDR), 7 extensively drug-resistant (XDR) and 3 were pan drug-resistant (PDR). The in vitro susceptibility test showed that the water, ethanol (95%), and methanol (80%) extracts of *Moringa oleifera* L.(leaves) and *Matricaria recutita* L.(flowers) produced an inhibitory effect against 12 resistant MDR, XDR, and PDR test isolates, with minimum inhibitory concentration (MIC) ranging from 7.8–62.5 mg/ml. Water and methanol extracts of both plants represented good activity against most of the sensitive and resistant isolates whereas ethanol extract of both plants showed a lesser activity against nearly all of the isolates

Conclusion: This study had the potential value to develop antibacterial agents against resistant (MDR, XDR, and PDR) and susceptible bacteria supporting the significant use of plant extracts in treating wound infections related to bacteria and these active extracts will provide useful information for discovering new compounds with better activity and more effective against resistant (MDR, XDR, and PDR) and susceptible bacteria responsible for wound infections than currently available antibiotic agents.

Keywords: Clinical samples, Wound infections, Bacteria, Antibiotic sensitivity, Plant extracts, Antibacterial activity, MIC, MBC

* Correspondence: saharnegm1@hotmail.com
[2]Microbiology Department, Research Institute of Ophthalmology (RIO), 2 El haram street, Giza, Egypt
Full list of author information is available at the end of the article

Background

Surgical site infection is the most common hospital-acquired infections in developing countries. Other wound infections include burn wound infection, diabetic foot ulcer infection, bite wound infection, acute soft tissue infection, and pressure ulcer infection (Bhalchandra *et al.*, 2018). Gram-positive cocci such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp. and Gram-negative bacilli such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* species are the most common pathogenic bacteria isolated from wounds (Pallavali *et al.,*2017).

Antibiotic resistance among bacterial strains is a serious situation. It may be so rapid that the effectiveness of common antibiotics may be lost within a span of 5 years due to genetic changes (Chandra *et al.*, 2017). *Pseudomonas aeruginosa* is responsible for burns and wound infections; it is also an important cause of wound infections in diabetic individuals and infected wounds following surgeries (Bassetti *et al.*,2018). *Staphylococcus aureus* is the most common cause of hospital-acquired wound infections. *Klebsiella* spp. and *E. coli* as predominant bacteria are also associated with burn wounds. Multidrug-resistant (MDR) was defined as such because of their in vitro resistance to more than one antimicrobial agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories). To characterize a bacterium as pan drug-resistant (PDR), it must be tested and found to be resistant to all approved and useful antimicrobials (Magiorakos *et al.*, 2012).

One of the surveys conducted by the World Health Organization (WHO) reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases. Forced with the growing resistance of MDR microbe strains to antibiotics and other drugs, the search for alternatives is serious (WHO, 2005; Chandra *et al.*, 2017 and Dan *et al.*, 2018).

There are numerous plants and natural products which have antibacterial, antifungal, and antiprotozoal effect that could be used either systemically or locally. Medicinal properties of plants have also been preferred throughout the world, due to their potent pharmacological activities, low toxicity, and economic viability, when compared with synthetic drugs. Medicinal plants are rich in a wide variety of bioactive secondary metabolites such as tannins, terpenpoids, alkaloids, saponins, flavonoids, and phenolic compounds that can produce a definite physiological action on the human body (Shakya, 2016).

Moringa oleifera L. is one of the best known, widely distributed, and grown species of a monogeneric family Moringaceae. The plant has been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases. Significant medicinal properties of the plant include anti-inflammatory, antibacterial, and antifungal effects (Abalaka *et al.*,2012 and Tirado-Torres *et al.*,2019). *Matricaria recutita* L. *or Matricaria chamomilla* L., commonly known as chamomile, is an annual plant of the composite family Asteraceae. It is widely used and well-documented medicinal plants. It was included in the pharmacopeia of 26 countries. It has been used as a medicinal plant for external wounds, eczema, skin irritations, leg ulcers, diaper rash, inflammation of the skin, bacterial skin diseases, and many others (e.g., Ali and Alattar, 2018).

The present study is an attempt to estimate the antibacterial activity of aqueous, ethanol, and methanol extracts of *Moringa oleifera* L. leaves and *Matricaria recutita* L. flowers against antibiotic-resistant and sensitive bacterial strains isolated from patients having wound infections.

Material and methods

Plant collection

Fresh flowers of *Matricaria recutita* L. (Family: Asteraceae) (German chamomile) and fresh leaves of *Moringa oleifera* L. (Family:Moringaceae) were collected in June to September 2018 from a farm in El-Fayoum governorate, Egypt. They were identified as *Matricaria recutita* (German Chamomile) and *Moringa oleifera* by specialists in the Horticulture Research Institute, Agriculture Research Center in EL-Dokky,Giza, Egypt, and in the Herbarium of Cairo University ,Cairo, Egypt. Fresh plant materials were air-dried for 2 weeks and grinded into fine powdered form, by using a grinder, kept in plastic bags, and subjected later to extraction.

Bacterial samples

One hundred bacterial samples were collected from patients with different wound infections. All of these samples were obtained from the Kasr El Aini Hospital, Cairo, Egypt. Wound surface was cleansed with sterile normal saline, then samples were collected using a sterile cotton swabs; the inner surface of the infected area was swabbed gently; swabs were inserted immediately into a tube containing nutrient broth media then transferred to the Microbiology Laboratory unit at Research Institute of Ophthalmology, Giza, Egypt as soon as possible for further investigations (Manikandan and Amsath, 2013).

Antibiotic discs (Oxoid)

For Gram-negative bacteria, the following antibiotic discs were used: penicillins group: piperacillin (PRL), ampicillin (AMP); the aminoglycoside group: gentamicin (CN), amikacin (AK), streptomycin(S), tobramycin (TOB); cephems including the cephalosporin group: ceftriaxone (CRO), ceftazidime (CAZ), cefoxitin (FOX), cefoperazone (CFP),

cefuroxime (CXM), cefepime (FEP), cefotaxime (CTX); the fluoroquinolone group: ciprofloxacin (CIP), oflaxacin (OFX), levofloxacin (LEV), lomefloxacin (LOM), tetracycline (TE); monobactams: aztreonam (ATM); the macrolide group: azithromycin (AZM); the carbapenem group: imipenem (IMP); β-lactamase inhibitor combinations: piperacillin-tazobactam (TZP), ampicillin-sulbactam (SAM); and the lipopeptide group: polymyxin B (PB). The antibiotic discs tested for Gram-positive bacteria were as follows: the aminoglycoside group: gentamicin (CN), amikacin (AK); the fluoroquinolone group: ciprofloxacin (CIP), ofloxacin (OFX), moxifloxacin (MFX), tetracycline (TE); the oxazolidinone group: linezolid (LZD), glycopeptides, teicoplanin (TEC); the macrolide group: erythromycin (E), azithromycin (AZM); folate pathway inhibitor: trimethoprim-sulphamethoxazole (SXT); the phenicol group; chloramphenicol (C), lincosamides ; clindamycin (DA); penicillinase-stable penicillins: oxacillin (OX); and penicillinase-labile penicillins: penicillin(P).

Preparation of plant extracts

Water extract

The extraction procedure was performed according to Rahman *et al.* (2009). Known weight (100 g) air-dried powder from each plant was extracted with 400 ml distilled water by percolation with occasional shaking for 7 days, then filtered and lyophilized under reduced pressure and 200 mg of the dry extracts was dissolved in 0.4 ml of dimethyl sulfoxide (DMSO) to give 500 mg/ml concentration. The dried extract was kept at − 4 °C for antibacterial activity assay.

Organic solvent extraction

Ethanol and methanol extracts: 100 g of air-dried plant powder was extracted by ethanol (95%) and methanol (80% v/v). The residue (the mixture) was transferred into a percolator with overnight maceration; the extract was filtered and evaporated to dryness at 40 °C in a water bath. Finally, the dry extract was weighted and the concentration of each extract was calculated. The obtained extracts were stored at − 20 °C for antibacterial activity assay. (There is no difference between the extraction process of ethanol and methanol.)

Identification of bacterial isolates

Identification of the collected Gram-positive and Gram-negative isolates was carried out according to Bergey's Manual of Systematic Bacteriology (1989) and Cheesbrough (1984).

Preparation of inoculums

Inoculums were standardized to give a density of 10^6 colony-forming units (CFU)/ml. A loopful of the test organism was inoculated into 5.0 ml of nutrient broth and incubated at 3 °C for 24 h. 0.2 ml from the 24-h culture of the organism was dispensed into 20 ml sterile nutrient broth and incubated for 3–5 h to standardize the culture to 10^6 CFU/ml (corresponding to 0.5 McFarland standards). Plates were inoculated within 15 min of standardizing the inoculum, to avoid changes in inoculum density (Abalaka *et al.*, 2012).

Antibiotic sensitivity test

The sensitivities of the isolated bacterial species against different antibiotics were tested based on the disc diffusion (Kirby–Bauer) technique (Bauer *et al.*, 1966) as described by Saif *et al.* (2017).

Antibacterial assay

It was carried out by agar well diffusion method as described by Das *et al.* (2013). One hundred microliters (10^6 CFU /ml) fresh microbial culture was spread on a Muller Hilton agar plate with non-toxic swab. Four wells of 6-mm diameter were punched off into the agar medium with sterile cork-borer (6 mm) and filled with 100 μl (500 mg/ml) of plant extract by using a micropipette in each well under aseptic conditions. DMSO was used as a negative control. The plates were allowed to stand for 1 h to allow for pre-diffusion of the extract into the medium. The plates were incubated aerobically in an upright position at 37 ± 2 °C for 24–48 h. The antibacterial screening was evaluated by measuring the zone of inhibition (mm).

Determination of minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of the drug which will inhibit growth as measured by observed turbidity in the test tube (CLSI, 2016). The MIC was determined for the antibacterial most efficient extracts, using the method of Greenwood (1989) as described by Usman *et al.*(2014) .Six sterile test tubes were arranged in four rows, each extract in one row. Each potential extract was determined by micro-broth dilution technique. One hundred microliters of sterile nutrient broth was pipetted into all the tubes.

Two hundred milligrams of dried extract was added to 0.4 ml of dimethyl sulfoxide DMSO to obtain a concentration of 500 mg/ml. Then, 100 μl was used containing 250 mg. Thereafter, there was a serial dilution of the extract in each tube to obtain concentrations of 125, 62.5, 31.25, 15.62, and 7.81 mg ml^{-1}, respectively. One hundred microliters of 10^6 CFU/ml of each of the tested bacterium were pipette into each test tube and incubated at 37 °C for 24 h. Two control tubes were used: nutrient broth inoculated with bacteria was used as a positive control and nutrient broth containing the plant extract was used as a negative control.

The lowest concentration that kills the organisms completely, where no bacterial growth is observed (MBC) CLSI (2016), was determined by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 °C for 24 h and observed for bacterial growth (Usman *et al.*, 2014).

Results

From one hundred specimens collected, 40 (40%) yielded pure bacterial culture. The distribution of bacterial isolates from the wounds was as follows: 10 (25%) Gram positive cocci (GPC) *(Staphylococcus* spp.) and 30 (75%) Gram-negative bacilli (GNB) (Fig 1). The detected organisms were 13 (32.5%) *Pseudomonas aeruginosa*, 10 (25%) Staphylococcus spp, 8 (20%) *E. coli*, 8 (20%) *Klebsiella* spp., and only one (2.5%) isolate *Proteus mirabilis* (Table 1). *P. aeruginosa* strains showed high sensitivity to levofloxacin (92.3%), polymyxin B (84.6%), and ciprofloxacin (69.23%), and were mostly resistant to aztreonam and ceftazidime (53.8%) and aminoglycosides (Table 2). In the same table, *P. aeruginosa* isolates: Ps 5, 7, 9 and Ps 10 were variant (sensitive or resistant) against fluoroquinolones (CIP, OFX, and LEVO). The previously tested isolates were also resistant against cephems (CAZ) and monobactams (ATM).

E. coli were mostly sensitive to imipenem, cefoxitin, ceftazidime, aztreonam, and amikacin (87.5%) followed by gentamycin, ceftriaxone and azithromycin (75%), while 100% resistant to cefuroxime, and (87.5%) to ciprofloxacin, levofloxacin, lomefloxacin, oflaxacin, ampicillin- sulbactam, ampicillin, piperacillin, tetracyclin, and cefotaxime (Table 3).

Table 3 also shows that *Klebsiella* spp. were very sensitive to imipenem and cefoxitin (75%) followed by tobramycin (62.5%), but (100%) resistant to azithromycin

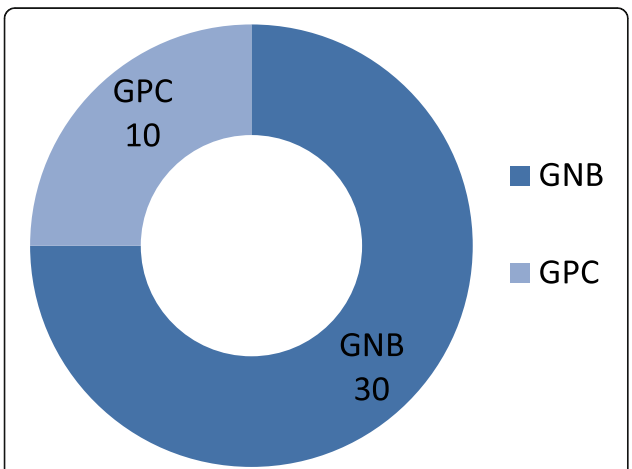

Fig. 1 Incidence of the number of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) causing wound infection ($n = 40$)

Table 1 Distribution of the bacterial isolates from wound infections

Bacterial isolates	Number of isolates	% positive of total number
P. aeruginosa	13	32.5
Staphylococcus spp.	10	25
Klebsiella spp.	8	20
E. coli	8	20
Proteus mirabilis	1	2.5

followed by cefuroxime, ampicillin-sulbactam, tetracycline (87.5%) and showed (75%) resistance against cefotaxime, fluoroquinolones, ampicillin, and Piperacillin. *Proteus mirabilis* showed (100%) sensitivity to cefoxitin, ceftazidime, ceftriaxone, cefotaxime, amikacin, tobramycin, ciprofloxacin, levofloxacin, lomefloxacin, and oflaxacin and aztreonam, on the other hand, 100% resistant against tetracycline, azithromycin, and cefuroxime.

Staphylococcus spp. isolates exhibited 100%, 80%, and 60% resistance against penicillin, oxacillin, and tetracyclin respectively. These strains showed 80% sensitivity to chloramphenicol, trimethoprim-sulphamethoxazole, amikacin, ciprofloxacin, oflaxacin, and linezolid and also exhibited 70–60% sensitivity to moxifloxacin, teicoplanin, azithromycin, and clindamycin (Table 4).

Out of the 40 bacterial strains, 20 (50%) were MDR, 7 (17.5%) were XDR, and 3 (7.5 %) were PDR (Fig. 2). Two *P. aeruginosa* strains were MDR, and 5 were XDR. Six *E. coli* strains were MDR and one responded as PDR. Out of the 8 *Klebsiella* spp., 4 strains were MDR, 2 XDR, and 2 were PDR. *Proteus mirabilis* strain reacted as an MDR, and finally, 7/10 isolates of the Gram-positive *Staph.*spp. were MDR (Fig. 3).

Water and methanol extracts represented good activity against most of the sensitive and resistant isolates while the ethanol extract of both plants showed a lesser activity against nearly all of the isolates (Table 5). Table 6 shows the MIC and MBC of 12 resistant isolates for water and methanol extracts of *M. oleifera* leaves and *Metricaria recutita* flowers. High MIC value was recorded for *Klebsiella* spp. with 15.6 to 62.5 mg/ml and *S. aureus* and *E. coli* with 7.8 to 62.5 mg/ml. On the other hand, lower MIC values were observed for *P. aeurogenosa* and *Proteus mirabilis* with 7.8 to 31.25 mg/ml and 15.6 to 31.25 mg/ml respectively. The extracts showed the same bacteriostatic activity against *S. aureus and E. coli* in the range of 15.6 mg/mL to 125 mg/ml. The MBC of the extracts presented 31.25 mg/ml to 125 mg/ml against *Klebsiella* spp., 15.6 to 62.5 mg/ml, and 31.25 to 62.5 mg/ml against *P. aeurogenosa and Proteus mirabilis* respectively.

Discussion

From one hundred specimens collected, 40 (40%) yielded pure bacterial culture. The distribution of bacterial isolates

Table 2 Susceptibility test of the studied *Pseudomonas aeruginosa* (Ps) isolates against standard antibiotics

Bacterial samples	Antibiotic											
	CIP	LEV	OFX	CN	TOB	AK	PRL	TZP	IPM	CAZ	ATM	PB
Ps 1	S	S	S	S	S	S	S	S	S	S	S	R
Ps 2	S	S	S	S	S	S	S	S	S	S	I	S
Ps 3	S	S	S	S	S	S	S	S	S	S	S	I
Ps 4	S	S	S	S	S	S	S	S	S	S	S	S
Ps 5	I	S	R	R	R	R	R	R	R	R	R	S
Ps 6	S	S	S	S	S	S	S	S	S	R	R	S
Ps 7	I	R	R	R	R	R	R	R	R	R	R	S
Ps 8	S	S	S	S	S	S	S	S	S	S	S	S
Ps 9	S	S	R	R	R	R	R	R	R	R	R	S
Ps 10	S	S	R	R	R	R	R	R	R	R	R	S
Ps 11	R	S	S	R	S	S	S	R	S	R	S	S
Ps 12	R	S	S	R	S	S	S	S	S	I	R	S
Ps 13	S	S	R	R	R	R	R	R	R	R	R	S

The diameters of the inhibition zones were interpreted according to CLSI (2016), and the examined isolates were reported as *R* resistant, *I* intermediate, *S* sensitive, CN gentamycin, *TOB* tobramycin, *AK* amikacin, *IMP* imipenem, CAZ ceftazidime, *CIP* ciprofloxacin, *OFX* oflaxacin, *LEV* levofloxacin, *TZP* piperacillin-tazobactum, *PRL* piperacillin, *ATM* aztreonam, *PB* polymyxin B

Table 3 Susceptibility test of the studied *KLebsiell a*spp.(K),*E. coli* and *Proteus mirabilis* (P) isolates against standard antibiotics

Bacterial	Antibiotic																					
Samples	CIP	LEV	LOM	OFX	CN	TOB	S	AK	SAM	TZP	IMP	CFP	CXM	FOX	CRO	CTX	FEP	CAZ	ATM	TE	AZM	AMP	PRL
																							E. coli
E1	R	R	R	R	S	S	S	S	R	I	S	I	R	S	S	R	S	S	S	R	R	R	R
E2	R	R	R	R	I	R	R	I	R	R	S	I	R	S	S	R	R	S	S	R	S	R	R
E3	R	R	R	R	S	I	I	S	R	I	S	I	R	S	S	R	S	S	S	R	S	R	R
E4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E5	S	S	S	S	S	S	S	S	I	S	S	S	R	S	I	I	S	S	S	S	S	S	S
E6	R	R	R	R	S	S	S	S	R	S	S	S	R	S	S	R	S	S	S	R	S	R	R
E7	R	R	R	R	S	S	S	S	R	S	S	S	R	S	S	R	S	S	S	R	S	R	R
E8	R	R	R	R	S	S	S	S	R	S	S	I	R	S	I	R	S	S	S	R	S	R	R
																							Klebsiella spp.
K1	R	R	R	R	R	R	R	R	R	I	S	I	R	S	I	R	S	S	R	R	R	R	R
K2	R	R	R	R	S	S	S	S	R	I	S	S	R	S	S	R	S	S	S	R	R	R	R
K3	S	S	S	S	S	S	S	S	R	S	S	I	R	S	R	R	I	R	R	R	R	R	I
K4	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K5	R	R	R	R	R	S	S	R	R	S	S	R	R	S	R	R	R	I	I	R	R	I	R
K6	R	R	R	R	S	S	S	S	R	S	S	S	R	S	S	I	S	R	S	R	R	I	R
K7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K8	S	S	S	S	S	S	R	S	S	S	S	S	I	S	S	S	S	S	S	I	R	R	I
																							Proteus mirabilis
P1	S	S	S	S	R	S	I	S	R	I	R	R	I	S	S	S	I	S	S	R	R	S	R

The diameters of the inhibition zones were interpreted according to CLSI (2016), and the examined isolates were reported as *R* resistant, *I* intermediate, *S* sensitive. CN gentamycin, *TOB* tobramycin, *AK* amikacin, *S* streptomycin, *IMP* imipenem, *CIP* ciprofloxacin, *OFX* ofloxacin, *LEV* levofloxacin, *LOM* lomefloxacin, *TZP* piperacillin-tazobactam, *SAM* ampicillin-sulbactam, *PRL* piperacillin, *AMP* ampicillin, *ATM* aztreonam, *TE* tetracycline, *CAZ* ceftazidime, *CFP* cefoperazone, *CXM* cefuroxime, *FOX* cefoxitin, *CRO* ceftriaxone, *CTX* cefotaxime, *FEP* cefepime, *AZM* azithromycin

Table 4 Susceptibility test of the studied *Staphylococcus* spp. isolates against standard antibiotics

Bacterial samples	Antibiotic														
	CIP	MFX	OFX	CN	AK	P	OX	TEC	SXT	AZM	E	DA	TE	C	LZD
S. aureus															
Sa 1	S	S	S	R	S	R	R	I	S	S	S	R	I	S	S
Sa 2	S	S	S	R	S	R	R	R	S	S	S	S	S	S	S
Sa 3	R	R	R	S	S	R	R	R	R	R	R	R	R	S	R
Sa 4	S	S	S	I	I	R	R	S	S	I	I	I	R	I	R
Sa 5	S	S	S	S	S	R	R	S	R	I	I	S	R	R	S
Sa 6	S	I	S	S	S	R	R	S	S	I	I	S	R	S	S
Sa 7	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Sa 8	S	S	S	R	R	R	R	S	S	S	I	R	R	S	S
Sa 9	R	R	R	S	S	R	R	S	S	S	R	S	S	S	S
S. epidermidis															
S1	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S

The diameters of the inhibition zones were interpreted according to CLSI (2016), and the examined isolates were reported as *S* susceptible, *I* intermediate, or *R* resistant to the antibiotic under test. *CN* gentamycin, *AK* amikacin, *CIP* ciprofloxacin, *OFX* ofloxacin, *MFX* moxifloxacin, *P* penicillin, *OX* oxacillin, *TEC* teicoplanin, *SXT* trimethoprim-sulphamethoxazole, *AZM* azithromycin, *E* erythromycin, *DA* clindamycin, *C* chloramphenicol, *TE* tetracycline, *LZD* linezolid

from the wounds were as follows: 10 (25%) bacterial strains were Gram-positive cocci (GPC) and 30 (75%) were Gram-negative bacilli (GNB)(Fig. 1). These findings are in line with those of previous studies in Asia and other African settings (Osariemen *et al.*,2013). This might be due to the high antibiotic resistance of Gram-negative bacteria .

Most Gram-positive bacteria are surrounded by a coarse peptidoglycan cell wall. This structure, although mechanically strong, appears to offer little resistance to the diffusion of small molecules such as antibiotics (Nikolaidis *et al.*, 2014). *Escherichia coli*, in contrast, as Gram-negative bacteria, surround themselves with a second membrane, the outer membrane, which functions as an effective barrier.

The high incidence of *P. aeruginosa* infection recorded in this work is in agreement with other reports by Aljanaby and Aljanaby (2018). Other studies by Sultana *et al.* (2015) and Mohammed *et al.*(2017) reported that *S. aureus* was the most common bacteria isolated from wound infections. These variations could be attributed to numerous factors including the nature of the wound site, and the type of prophylactic antibiotics used for treatment. The data outlined in (Table 2) indicated that *P. aeruginosa* strains were highly sensitive to levofloxacin (92.3%) followed by polymyxin B (84.6%) and ciprofloxacin (69.23%), however showed resistance against aztreonam and ceftazidime (53.8%). This was explained by Aldred *et al.* (2014) that the individual members of fluoroquinolones demonstrate different spectra of activity and pharmacokinetic profiles, fluoroquinolones target two essential bacterial enzymes, DNA gyrase (topoisomerase II) and DNA topoisomerase IV. During this process, the drugs trap a reaction intermediate containing quinolone enzyme and

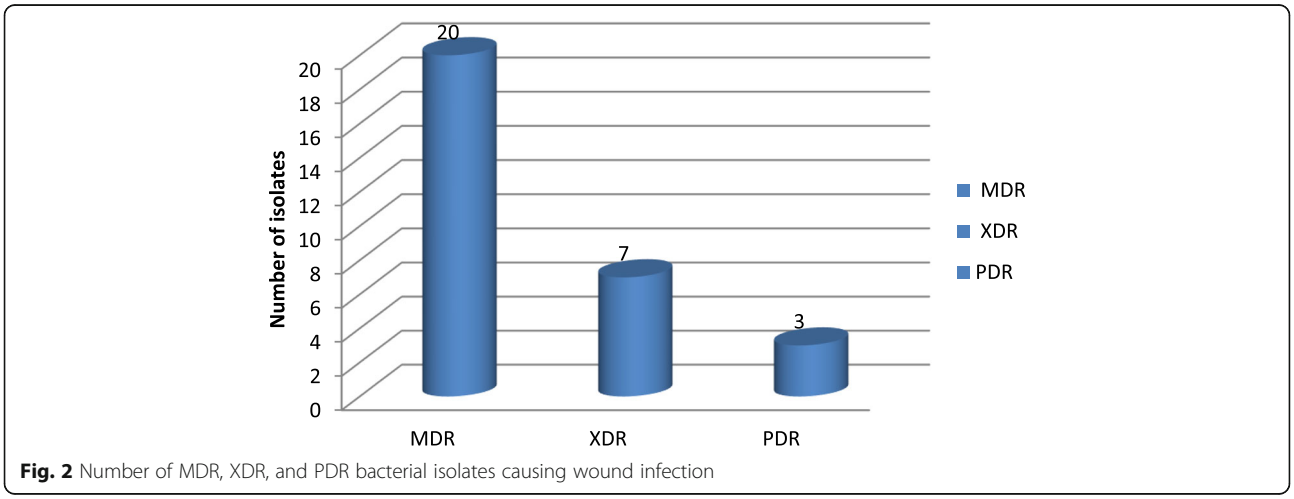

Fig. 2 Number of MDR, XDR, and PDR bacterial isolates causing wound infection

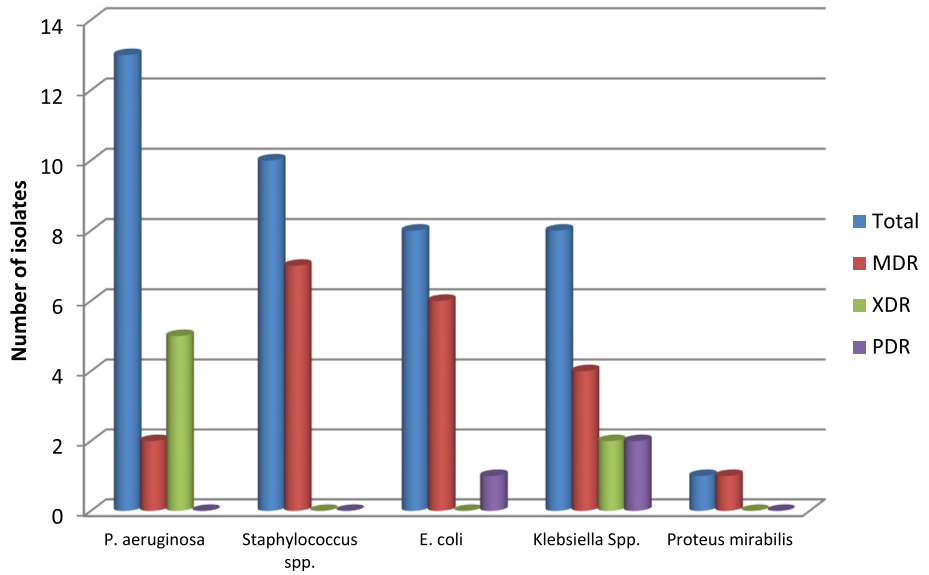

Fig. 3 Incidence of MDR, XDR, and PDR of the Gram-negative bacillus and Gram-positive coccus isolates

broken DNA, which leads to the blockage of DNA replication, and for some bacteria, death occurs within hours. Furthermore, polymyxins bind to the cell membrane and alter its structure. The result of this process causes an increase in the permeability of the cell envelope consisting of the cell wall and the cytoplasmic membrane, leakage of cell contents, and, subsequently, cell death (Parija, 2012). In the same table, *P. aeruginosa* isolates: Ps 5, 7, 9 and Ps 10 were variant (sensitive or resistant) against fluoroquinolones (CIP, OFX, and LEVO). Resistance is due to changes in DNA gyrase enzyme and/or the topoisomerase enzyme(s) or by the defective function of porine channels (Hooper and Jacoby,2015). The abovementioned strains showed resistance against aminoglycosides (CN, AK, TOB). Aminoglycoside-inactivating enzymes reduced the uptake of the aminoglycoside into bacteria. The previously tested isolates were also resistant against cephems (CAZ) and monobactams (ATM). This resistance is due to β-lactamase production (extended-spectrum *beta lactamases*) that hydrolyze these drugs (Palzkill, 2018).

Earlier studies by Saha *et al.* (2017) and Bhalchandra *et al.*(2018) reported that *P. aeruginosa* strains were sensitive to polymyxinb, levofloxacin, and ciprofloxacin and showed high resistance against Aztreonam and ceftazidime. Yakha *et al.* (2015) and Perim*et al.* (2015) described high resistance against ceftazidime and polymyxin B.

In the present study, Table 3 presents that *E.coli* strains showed high sensitivity to imipenem, cefoxitin, ceftazidime, aztreonam, and amikacin reached 87.5% followed by gentamycin, ceftriaxone, and azithromycin (75%) . These strains exhibited 100% resistance against cefuroxime and (87.5%) against ciprofloxacin, levofloxacin, lomefloxacin, oflaxacin, beta-lactamase inhibitor combination (ampicillin-sulbactam), ampicillin, piperacillin, tetracycline, and cefotaxime. Similar observations have been reported by Yakha *et al.*(2015); Gomatheswari and Jeyamurugan (2017), and Saha*et al.*(2017) that imipenem, amikacin, and gentamycin were very effective drugs against *E. coli* isolates but presented resistance against cefuroxime,ciprofloxacin , ofloxacin ,cefotaxime, and ampicillin.

Table 3 also shows that *Klebsiella* spp. strains were very sensitive to imipenem and cefoxitin (75%) followed by Tobramycin (62.5%). On the other hand, these strains were (100%) resistant to azithromycin followed by cefuroxime, ampicillin-sulbactam, and tetracycline (87.5%) and showed (75%) resistance against cefotaxime, fluoroquinolones, ampicillin, and piperacillin. These results go with the report by Gomatheswari and Jeyamurugan (2017).

In the same table, *Proteus mirabilis* showed (100%) sensitivity to cefoxitin, ceftazidime, ceftriaxone, cefotaxime, amikacin, tobramycin, ciprofloxacin, levofloxacin, lomefloxacin, and oflaxacin and aztreonam, on the other hand, 100% resistant against tetracycline, azithromycin and cefuroxime. Kassam *et al.* (2017) stated that *Proteus* spp. was sensitive to cefoxitin, ceftazidime, ceftriaxone, amikacin, and ciprofloxacin and resistant against tetracycline.

The results in Table 4 illustrated that *Staphylococcus* spp. isolates exhibited 100%, 80%, and 60% resistance against penicillin,oxacillin, and tetracyclin respectively. These strains showed 80% sensitivity to chloramphenicol, trimethoprim-sulphamethoxazole, amikacin, ciprofloxacin, oflaxacin, and linezolid. These species also exhibited 70–60% sensitivity to moxifloxacin, teicoplanin, azithromycin, and clindamycin. Our study agrees with the findings of Etok *et al.* (2012) who stated that large numbers of *S. aureus* are sensitive to quinolones and aminoglycosides.

Table 5 Antimicrobial activity (in mm) of plant extracts against tested bacterial isolates

Bacterial isolates	Diameter of inhibition zone in mm					
	Moringa oleifera extracts			*Metricaria recutita* extracts		
	Aqueous	Ethanol 95%	Methanol 80 %	Aqueous	Ethanol 95%	Methanol 80%
Escherichia coli						
E 1	19	15	10	15	10	10
E 2	16	13	10	13	6	6
E 3	13	12	11	12	10	10
E 4	15	15	14	11	12	14
E 5	15	10	14	11	15	14
E 6	19	13	15	19	12	13
E 7	16	12	15	16	10	13
E 8	16	13	16	15	12	15
Klebsiella spp.						
K 1	19	12	12	11	12	6
K 2	16	6	10	15	18	20
K 3	12	6	6	22	14	15
K 4	14	12	6	20	16	18
K 5	21	11	11	20	17	17
K 6	17	7	11	16	18	21
K 7	23	15	20	23	14	20
K 8	16	6	15	15	12	15
P. aeruginosa						
Ps 1	20	21	18	21	15	16
Ps 2	20	16	16	16	14	14
Ps 3	15	15	6	16	13	13
Ps 4	15	10	10	14	14	15
Ps 5	14	11	12	20	18	17
Ps 6	12	15	13	19	15	15
Ps 7	15	15	6	20	15	15
Ps 8	15	15	13	17	15	15
Ps 9	15	12	6	20	16	18
Ps 10	15	13	15	20	16	17
Ps 11	14	12	15	21	14	18
Ps 12	21	12	20	22	14	20
Ps 13	14	10	13	16	13	16
Proteus mirabilis						
P1	15	13	13	13	19	25
S. aureus						
Sa 1	15	22	25	20	27	23
Sa 2	13	15	15	23	25	30
Sa 3	17	19	15	25	24	24
Sa 4	16	23	25	30	26	30
Sa 5	16	21	25	20	26	29
Sa 6	13	12	14	15	6	20
Sa 7	20	19	25	30	24	30
Sa 8	13	12	16	20	19	24
Sa 9	20	15	25	25	20	30
S. epidermidis						
S1	30	20	27	27	26	30

Fluoroquinolones (CIP, OFX, and MFX) are bactericidal agents as previously described. Trimethoprim-sulphamethoxazole (SXT) is also a bactericidal agent against *S. aureus* and inhibits bacterial replication. Aminoglycosides (AK and CN) are the only ribosome-targeting antibiotics that are bactericidal. This is due to their unique mechanism of action in causing misreading of mRNA during translation. Linezolid is a synthetic oxazolidinone class of antimicrobial agent that binds to the ribosome and inhibits microbial protein synthesis. Studies have confirmed that linezolid has good activity against most Gram-positive bacteria, specially against MRSA (Foster 2017). Resistance to penicillin was determined at the rate of 100% due to beta-lactamase production (Rağbetli *et al.*,2016). Inactivation of oxacillin is due to β-lactamase hyperproduction. These hyperproducers of β-lactamase tend to resist oxacillin through limited hydrolysis of the antibiotic, resulting in a phenotype that, with deference to oxacillin, is called oxacillin-resistant *S. aureus* (MRSA). A second mechanism of resistance to oxacillin is due to the production of an altered penicillin-binding protein (PBP 2' or PBP 2a) which facilitates bacterial growth and cell wall synthesis at concentrations of β-lactams inhibitory to native penicillin-binding proteins. Yakha *et al.*(2015) reported that *S. aureus* showed high resistance against penicillin and oxacillin. In this study (Table 4), S.a3 and S.a9 isolates showed total resistance against CIP and MXF and OFX.

As illustrated in Fig. 2, the incidence of MDR, XDR, and PDR studied isolates were as follows: out of the 40 bacterial strains, 20 (50%) were MDR, 7 (17.5%) were XDR, and 3 (7.5 %) were PDR. The remaining 10 isolates (25%) were detected to be sensitive to most of the tested antibiotics. In the present study, Fig. 3 shows that two *P. aeruginosa* strains were MDR, and 5 were XDR, 6 *E. coli* strains were MDR, and one responded as PDR. Out of the 8 *Klebsiella*spp., 4 strains were MDR, 2 XDR, and 2 were PDR. *Proteus mirabilis* strain reacted as an MDR and finally7/10 isolates of the Gram-positive *Staph.*spp. were MDR.

WHO, (2014) reports showed that approximately 50% of *E. coli*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa*

Table 6 MIC and MBC of aqueous and methanol extracts of *M. oleifera* and *M. recutita* against clinical isolates of MDR, XDR and PDR bacteria

	Different concentrations of extracts against tested bacterial isolates with reference to MIC and MBC																								MIC				MBC			
Concentrations	250 mg/ml				125 mg/ml				62.5 mg/ml				31.25 mg/ml				15.6 mg/ml				7.8 mg/ml				MW	MM	CW	CM	MW	MM	CW	CM
Bacterial isolates	MW	MM	CW	CM	MW	MM	CW	CM	MW	MM	CW	CM	MW	MM	CW	CM	MW	MM	CW	CM	MW	MM	CW	CM								
S. aureus																																
Sa1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	7.8	7.8	–	7.8	15.6	15.6	–	15.6
Sa2	–	–	–	–	–	–	–	–	+	+	–	–	+	+	–	–	+	+	+	–	+	+	+	+	62.5	62.5	15.6	7.8	125	125	31.25	15.6
Sa3	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	+	+	+	+	+	+	+	+	25.31	62.5	15.6	15.6	62.5	125	31.25	31.25
P. aeruginosa																																
Ps 1	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+	+	+	+	+	+	25.31	15.6	15.6	15.6	62.5	31.25	31.25	31.25
Ps2	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	+	+	+	+	15.6	31.25	15.6	7.8	31.25	62.5	31.25	15.6
Ps3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	7.8	7.8	7.8	–	15.6	15.6	15.6	–
E. coli																																
E. coli1	–	–	–	–	–	–	–	–	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	62.5	62.5	15.6	25.31	125	125	31.25	62.5
E. coli2	–	–	–	–	–	–	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	+	+	62.5	62.5	7.8	7.8	125	125	15.6	15.6
Klebsiella sp.																																
k1	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+	31 25.	31 25.	15.6	15.6	62.5	62.5	31 .25	125
K2	–	–	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	31 25.	62.5	62.5	31 25.	62.5	125	125	62.5
K3	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	+	+	31 25.	15.6	15.6	31 25.	62.5	31.25	31.25	62.5
Proteus mirabilis																																
P1	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+	31 25.	31 25.	15.6	15.6	62.5	62.5	31 .25	31 .25

MW M. oleifera water extract, *MM M. oleifera* methanol extract, *CW M. recutita* water extract , *CM M. recutita* methanol extract , *MIC* minimum inhibition concentration , *MBC* minimum bacterial concentration, + showing growth, – no growth

were resistant to the majority of antibiotics, such as cephalosporin. The increasing trend in development of antibiotic resistance could be attributed to frequent, unnecessary, and abuse of antibiotics and longer duration of hospitalization.

The studied plant extracts of *M. oleifera* and *M. recutita* showed varied levels of antibacterial activity against antibiotic-sensitive and resistant *P. aeruginosa*, *Klebsiella* pp., *E. coli*, *Proteus mirabilis*, *and Staphylococcus* spp. isolates. Water and methanol extracts of both plants represented good activity against most of the sensitive and resistant isolates, whereas ethanol extract of both plants showed a lesser activity against nearly all of the isolates. Aqueous and methanol extract of both plants showed high inhibition zones against the studied bacteria isolates (Table 5).

Moreover, most people who use *M. oleifera* leaves as a traditional means of treatment of various skin ailments and other diseases make use of water-based extract of the leaf. This agreed with Dike-Ndudim *et al.*(2016) and Muhuha *et al.* (2018) who noted that the aqueous extract of *M. oleifera* leaf possesses significant antimicrobial activity against both Gram-negative and Gram-positive bacterial organisms from wounds, thus signaling its broad spectrum of antibacterial activity. Further chemical composition analysis revealed that the *M. oleifera* leaf extract with antibacterial activities contains alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, tannins, triterpenes, sterols, saponins, and some other secondary metabolites.

The experiments carried out by Daotam *et al.*(2016) confirmed that the methanol extract of *M. oleifera* leaves showed different inhibition patterns against different bacterial strains including *E. coli, E. aerogenes, K. pneumoniae* and *P. aeruginosa.* Abdalla and Abdelgadir, (2016) reported that water and methanol extracts of *M. chamomilla* showed different degrees of antibacterial activities against bacteria, including *P. aeruginosa, S. aureus, B. cereus* and *E. coli.*

Conclusion

The application of herbal products for the bio-control of diseases, as a novel emerging alternative to antimicrobial treatments leading to nontoxic and more environmental managing for virulent diseases, is a must.

Due to the rapid development of resistance against chemotherapeutic agents (mostly antibiotics), it has become essential currently to think over some substitute and effective therapeutics like herbs.

This study had the potential value to develop herbal products as antibacterial agents against resistant and susceptible bacteria supporting the significant use of plant extracts in treating wound infections related to bacteria and these active extracts will provide useful information for discovering new compounds with better activity and more effect against resistant (MDR, XDR. and PDR) and susceptible bacteria responsible for wound infections than currently available antibiotic agents.

Abbreviations

CDC: Centre for Disease Control and Prevention; CLSI: Clinical and Laboratory Standards Institute; ECDC: European Centre for Disease control; GNB: Gram-negative bacilli; GPC: Gram-positive cocci; MBC: Minimum bactericidal concentration; MDR: Multidrug-resistant; MIC: Minimum inhibitory concentration; PDR: Pan drug-resistant; WHO: World Health Organization; XDR: Extensive drug-resistant

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Authors' contributions

NA she made the main contributions to the work design; she was involved in microbiological laboratory testing of samples, revising the scientific data, and approving the final version. SS she revised drafting the clinical aspect of the study. She read the manuscript and gave her final approval to be published. SN: she has been involved in microbiological laboratory testing, microbial isolation and antibiotic sensitivity test of the samples. She was responsible for submission of the article as a corresponding author and the resubmission after making the necessary revision and correction of the manuscript. YA performed the clinical work of the study, including patient examination and obtaining the samples to the microbiology laboratory. She recorded the results and data collection.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Agreement for the study, in addition to the ethical approval, was obtained first from the Medical Research Committee of RIO and Kasr El Aini, Cairo,Egypt. All the patients who were enrolled had submitted an informed consent in advance.

Consent for publication

Not applicable (participants were fully anonymous).

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, Egypt. [2]Microbiology Department, Research Institute of Ophthalmology (RIO), 2 El haram street, Giza, Egypt. [3]Giza, Egypt.

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