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Prevalence, antibiotic and oil resistance pattern of some bacterial isolates from burns

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ABSTRACT

Infection is an important cause of mortality in patients with burns. Rapid emergence of hospital pathogens and antibiotic-resistant organisms necessitate periodic evaluation of bacterial colonization patterns and antibiogram sensitivity in burn wards. Sixty isolates from wounds of burns were collected from two hospitals in Cairo, Egypt along the period of 12 months in 2013. Antibiotic sensitivity of these isolates was assessed by single disk diffusion method. Multi drug resistance percentage and the most prevalent resistance phenotype among bacterial isolates were recorded. In addition, 19 essential oils were tested against the MDR isolates. The most potent oils were analyzed by GC-MS to determine their main chemical constituents. According to microbiological and biochemical identification method, *Pseudomonas aeruginosa* was the most dominant organism 23 (38%), followed by *Staphylococcus aureus* 16 (27%), *Klebsiella* spp. 11 (18%), *Acinetobacter* spp. 4 (7%). Three isolates of *Escherichia coli* (5%) and three isolates of *Proteus* spp. (5%). Piperacillin-tazobactam, imipenem and linezolid antibiotics were the most effective antibiotics against *Pseudomonas aeruginosa*, *Enterobacteriaceae* and *S. aureus* isolates respectively. Cinnamon and thyme essential oils were the most potent oils against the multi drug resistant burn wound isolate. Cinnamaldehyde (60.7%) and ρ-cymene (50%) were the major chemical constituents in cinnamon and thyme essential oils, respectively. It is clear that antibiotic resistance levels are high among the examined bacterial isolates of burn wounds. This study could be useful for physician to better choice of empiric therapy. Cinnamon and thyme may be used as a promising an alternative medicine for the treatment of burn wound infections.

INTRODUCTION

Burns are damage to the skin caused by variety of non-mechanical sources including chemicals, electricity, heat, sunlight or nuclear radiation. Thermal injury is a serious type of trauma required care in a specialized units. It has been estimated that approximately 2.5 million people sustain burns of which 100,000 are hospitalized and there are around 12,000 deaths per year due to thermal injuries (Mayhall, 2003). Thermal destruction of the skin barrier and concomitant depressions of local and systemic host cellular and humoral immune responses are pivotal factors contributing to infectious complications in patients with severe burns. The burn wound surface is a protein rich environment consisting of avascular necrotic tissue (eschar) that provides a favorable niche for microbial colonization and proliferation. The avascularity of the eschar results in impaired migration of host immune cells and restricts delivery of systemically administered antimicrobial agents to the area, while toxic substances released by eschar tissue impair local host immune response (Church *et al*., 2006). The cause of nosocomial infections in burn patients might be endogenous or exogenous. Endogenous infections are caused by organism present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel or medical devices (Samuel *et al*., 2010). The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Taneja *et al*., 2004).

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Essential oils are very complex natural mixtures which contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts (Bakkali *et al*., 2008).

The antibacterial properties of plant essential oils have been known for many centuries. Essential oils have been found to have great effects in disrupting the bacterial membrane. It is likely due to the presence of lipophilic compounds such as cyclic hydrocarbons, terpenes and aromatics which are abundantly found in the aromatic plants (Langeveld *et al*., 2013).

This investigation was carried was carried out to determine which bacteria are prevalent in burn wounds and to study their antibiotic resistance pattern. Also, the role of 19 essential oils in inhibition of the growth of these antibiotic resistant bacteria was investigated.

MATERIAL AND METHOD

Bacterial isolates, isolation and identification

Collection of burn wound swabs was carried out during the period of January 2013 to December 2013 from burn units of two hospitals in Cairo; Ain-Shams University (El-Demerdash Hospital) and Cairo University (El-Kasr El-Eini- Hospital). Swabs were transported to the Department of Microbiology C Lab at National Organization for Drug Control and Research (NODCAR) and cultured immediately at the same day of arrival on blood agar and MaCconkey agar.

The plates were incubated at 37°C for 24 h. Identification of bacteria were carried out according to Mahon *et al.* (2011), and Engelkirk & Duben-Engelkirk (2008). Biochemical tests were performed to identify the collected isolates.

Gram-negative rods were identified by performing a series of biochemical tests; triple sugar iron agar (TSI), Indole test, Methyl red- Voges proskauer tests, Simon's citrate agar test, oxidase test, urea test and motility test. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test.

Screening for antibiotic resistance

Antibacterial susceptibility test was performed on Muller Hinton agar by standard disk diffusion method Bauer *et al*. (1966), following recommendation of Clinical and Laboratory Standards Institute CLSI (2006).

The tested antibiotic disks were purchased from Oxoid, UK and they included ampicillin (10 µg), piperacillin (100 µg), penicillin (10 units), amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), cefepime (30 µg), cefotaxime (30 µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), amikacin (10 µg), tobramycin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), trimethoprim/ sulfamethoxazole (1.25/23.75 µg), aztreonam (30 µg), cefoxitin (30 µg), oxacillin (30 µg), teicoplanin (30 µg), erythromycin (15 µg), tetracycline (30 µg), clindamycin (2 µg), and linezolid (30 µg).

Antibacterial activity of oils by agar well diffusion method

The tested essential oils are listed in Table (1). These oils were selected according to Deans and Ritchie (1987) and Baser and buchbauer (2010). Essential oils were obtained from Phyto-Chemistry Department, National Organization for Drug Control and Research (NODCAR). Antibacterial activity of the oils against the most resistant burn wound isolates were tested by agar well diffusion method according to CLSI (2006).

Table 1: Essential oils tested

Essential oils	Binomial name	Plant family name	Plant part used
Lemon oil	*Citrus lemon*	*Rutaceae*	*Fruit*
Cinnamon bark oil	*Cinnamomum verum*	*Lauraceae*	*Bark*
Garlic oil	*Allium sativum*	*Amaryllidaceae*	*Bulb*
Caraway oil	*Carum carvi*	*Apiaceae*	*Fruit*
Peppermint oil	*Mentha piperita*	*Lamiaceae*	*leaves*
Tea tree oil	*Melaleuca alternifolia*	*Myrtaceae*	*leaves*
Geranium oil	*Pelargonium*	*Geraniaceae*	*leaves*
Thyme oil	*Thymus vulgaris*	*Lamiaceae*	*Leaves*
Fennel oil	*Foeniculum vulgare*	*Apiaceae*	*Seed*
Eucalyptus oil	*Eucalyptus globulus*	*Myrtaceae*	*Leaves*
Clove	*Syzygium aromaticum*	*Myrtaceae*	*Flower buds*
Olive oil	*Olea europaea*	*Oleaceae*	*Fruit*
Camphor oil	*Cinnamomum camphora*	*Lauraceae*	*Wood*
Anise oil	*Pimpinella anisum*	*Apiaceae*	*Seed*
Orange oil	*Citrus Sinensis*	*Rutaceae*	*Fruit*
Dill oil	*Anethium graveolens*	*Apiaceae*	*Seed*
Ginger oil	*Zingiber officinale*	*Zingiberaceae*	*Rhizomes*
Moringa oil	*Moringa oleifora*	*Moringaceae*	*Seed*
Rose marry oil	*Rosmarimus officinalis*	*Lamiaceae*	*Flowers*

GC- mass analysis of the most potent oils

The most potent essential oils were analysed by gas chromatography combined with mass spectrometry (GC/MS). The GC/ MS analysis was performed using a thermo scientific, trace ultra /isq single quadrupole ms, tg-5ms fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280^{o}C. The oven temperature was programmed at an initial temperature 40^{o}C (hold 3 min) to 280^{o}C as a final temperature at an increasing rate of 5^{o}C/ min (hold 5 min).

RESULTS AND DISSUCION

Nosocomial Infection is an important cause of mortality in burns, and is considers one of the most serious complications in burn patients. It has been estimated that 75% of all deaths following thermal injuries are related to infections (Mehta *et al.*, 2007). In this study; sixty isolates were collected from burn units at two hospitals in Cairo, Egypt. Twenty isolates from Cairo University hospital and forty isolates from Ain Shams University hospital. Bacterial identification results are shown in Figure (1). 16(26.6%) isolates were gram- positive Cocci and 44 (73.3 %) isolates were gram negative bacilli. these results are consistent with results reported by Kehinde *et al.* (2004) who reported that the rate of gram negative bacterial species isolated from burn wound was more than twice that gram positive one.

Bacterial identification results in Figure (1) shows that the most dominant bacteria was *Pseudomonas aeruginosa* 23

*(*38%*)*, followed by *Staphylococcus aureus* 16 (27%), *Klebsiella pneumoniae* 11 (18%), *Acinetobacter baumanii 4 (*7%). *Escherichia coli* 3 (5%) and *Proteus vulgaris* 3(5%) isolates were also detected.

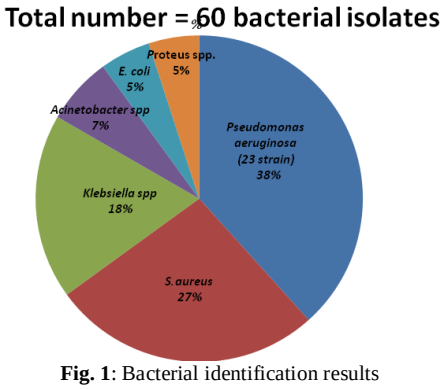

Fig. 1: Bacterial identification results

P. aeruginosa was found to be the most important resistant and dangerous organism in burn patient infection; *Pseudomonas* infection is a common complication in burn patients and contributes to their morbidity and mortality (Lari *et al.*, 2005). *P. aeruginosa* was found to be the most common pathogen isolated from burned patients and accounted for (38%) of the total isolates, which refer to that *P. aeruginosa* is a major factor in the etiology of burn wound infection. In many economically developing countries such as Zimbabwe (Igumbor *et al.*, 2001), South Korea (Song *et al.*, 2001), Jordan (Al-Akayleh, 1999), Libya (Husain *et al.*, 1989), Nigeria (Atoyebi *et al.*, 1992), India (Pandit *et al.*, 1993; Revathi *et al.*, 1998 and Kaushik *et al.*, 2001), and Turkey (Arslan *et al.*, 1999 and Oncul *et al.*, 2002), *P. aeruginosa* was reported to be the most common bacteria among burn patients. Few burn centers in Canada and the USA, (Shankowsky *et al.*, 1994) and France (Cremer *et al.*, 1996), have been reported *P. aeruginosa* as an important microorganism in burn units. Other studies reported the same result; (Nagoba *et al.*, 1999; Nasser *et al.*, 2003; Agnihotri *et al.*, 2004; Ekrami and Kalantar, 2007 and Rajput *et al.*, 2008). In contrast to our study, some reports indicated that *P. aeruginosa* was not the major causative pathogen in burn wound infections; Vindenes and Bjerknes (1995) found that *P. aeruginosa* represent only (10.9 %) out of the total number of isolates.

Staphylococcus aureus is a versatile human pathogen. It was the predominant cause of burn wound infection and still persists as an important pathogen, the second most commonest isolate in our study was *S. aureus* with incidence rate (27%), this result was similar to that of Song *et al.* (2001) who reported that *S. aureus* was the second pathogen isolated in their burn units, Altoparlak *et al.* (2004) was observed that *S. aureus* (30.4%) was the second frequent pathogen isolated in burns unit, Ramakrishnan *et al.* (2006) studied a period of 6 years infections in burn patients and found that *S. aureus* with incidence rate (37%) forming the second main organism. Rajput *et al.* (2008) observed that the second most common isolate was *S. aureus* with incidence rate (19.29%). In contrast to our results other studies found that *S. aureus* was the most common isolate in burn wound infection (Komolafe *et al.*, 2002 and Imran *et al.*, 2007).

Klebsiella pneumoniae was recovered in a frequency rate of (18%), Nagesha *et al.* (1996), found that *Klebsiella pneumoniae* was the third common pathogen with an incidence rate of (12%). Also Rajput *et al.* (2008) showed that *Klebsiella* species was the third commonest pathogen with incidence rate (11.4%). In contrast to our results; Ozumba and Jiburum (2000) observed the prevalence of *K. pneumonia* with incidence rate (26.7%).

In our study; *Escherichia coli* and *Proteus vulgaris* species were accounted for 5% of the total isolates. The low incidence of *E. coli and P. vulgaris* species is in conformity with other previous studies. (Ozumba and Jiburum, 2000; Kehinde *et al.*, 2004; Rajput *et al.*, 2008; Agnihotri *et al.*, 2004 and Guggenheim *et al.*, 2009)**.**

Antibiotic sensitivity test is important for epidemiological and clinical purposes. Incresing antimicrobial reisstance among burn wound isolates is a matter of concern, with limited treamtment options available for resistant strains (Aruna *et al.*, 2010).

Figure (2) indicates the antimicrobial resistance level of *S. aureus* isolates to different antibiotics. The obtained results clearly showed that *S. aureus* was highly resistant to the most of the tested antibiotics. *S. aureus* was completely resistant to oxacillin, penicillin, and cefoxitin with resistance rate (100%), these results are consistent with that obtained from a study conducted by Xu *et al* (2013). In the present study, all the *staphylococci* isolates were methicillin-resistant *staphylococci* (MRSA), similar studies reported the higher incidence of MRSA in burn infections (Song *et al.*, 2001 and Montazeri *et al.*, 2013), and supports the fact that there was increasing evidence that MRSA has become a significant problem.

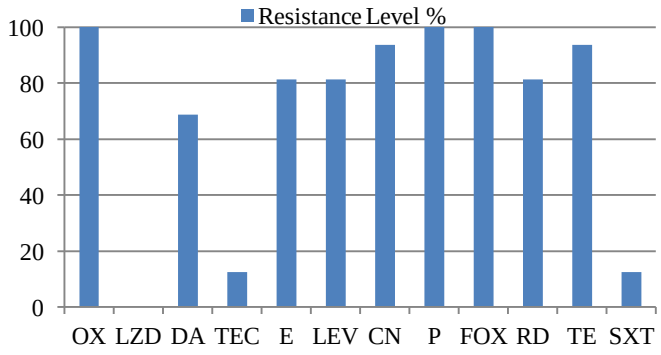

Fig. 2: Antibiotic resistance level of *S. aureus* isolates.
Keys : OX (Oxacillin), LZD (linezolid), DA (Clindamycin), TEC (Teicoplanin), E (Erythromycin), LEV (Levofloxacin), CN (Gentamicin), P (Penicillin), FOX (Cefoxitin), RD (Rifampicin), SXT (Trimethoprim-sulphamethoxazole), TE (Tetracycline).

In this study; linezolid was found to be the most effective antibiotic against *S. aureus* isolates. this finding is consistent with many other reports (Manjula *et al.*, 2007; Dash *et al.*, 2013; Vaijnath, 2013 and Xu *et al.*, 2013). Most of *S. aureus* isolates

were sensitive to trimethoprim-sulphamethoxazole and teicoplanin, a study conducted by Song *et al*. (2001) reported that trimethoprim-sulphamethoxazole was very effective against *S. aureus* isolates with resistance level (10%). In additon; Altoparlak *et al.* (2004) recorded higher susceptibility of *S. aureus* to teicoplanin with only (2.7%) of the isolates were resistant to this antibiotic.

The percentage of resistance level of gram negative isolates to antibiotics is presented in Figure (3). The most effective drugs against *P. aeruginosa* were piperacillin/tazobactam and piperacillin 21.7 %, this result is in a conformity with the result of other studies in which piperacillin/tazobactam recorded the least resistance (Dash *et al.*, 2013 and Vaijnath, 2013). Imipenem antibiotic was the second effective drug against *P. aeruginosa* isolates. Dash *et al.* (2013) reported the same results; low resistance pattern was shown towards piperacillin/tazobactam, piperacillin and imipenem. this could be attributed to its restricted use because of highr cost or limited availability. Resistance rate of *P.aeruginosa* to meropenem was 69%, in contrast to our study, Bayram *et al.* (2013) showed that merpoenem was more effective against *P. aeruginosa* with resistant rate 19%. Another study conducted with Rezaei *et al.* (2011) reported that relative frequency of resistance to meropenem antibiotic was 18.5%.

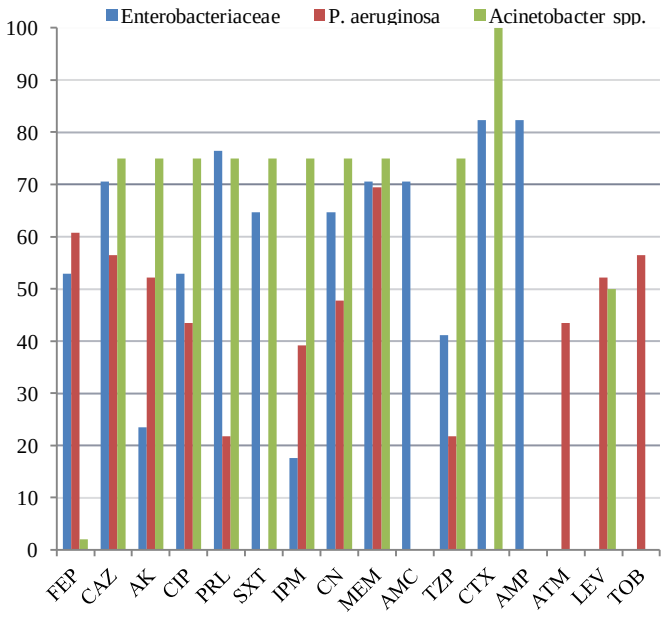

Fig. 3: Antibiotic resistance level of gram negative isolates.
Keys: FEP (cefepime), CAZ (ceftazidime), AK (Amikacin), CIP (ciprofloxacin), PRL (piperacillin), SXT (Trimethoprim-sulphamethoxazole), IPM (imipenem), CN (gentamicin), MEM (Meropenem), AMC (Amoxicillin-clavulanic acid), TZP ((Piperacillin- tazobactam), CTX (cefotaxime), AMP (ampicillin), ATM (aztreonam), LEV (levofloxacin), TOB (tobramycin).

The least effective antibiotics against *Enterobacteriaceae* were ampicillin and cefotaxime with resistance rate more than 82%, similar result was reported by another study (Mohammedaman *et al.*, 2014). Other study reported lower resistance rate for cefotaxime with *Klebsiella spp.* (59.5%) and *E. coli* (23.1%) Bhat and Vasaikar (2010). Imipenem was the most effective antibiotic against *Enterobacteriaceae* followed by amikacin, this result was in agreement with another study reported by Bhat and Vasaikar (2010).

Most of *Acinetobacter baumanii* showed high resistance level to many of tested antibiotics**.** This result was in agreement with that of (Song *et al.*, 2001; Singh *et al.*, 2003 and Rezaei *et al.*, 2011).

Other studies reported moderate resistance level to piperacillin/ tazobactam and lower resistance level to imipenem and meropenem (De Marcedo & Santos, 2005 and Guggenheim *et al.*, 2009).

The overall rate of multiple drug resistance of the isolates in this study was 82%, other previous study reported that the multi-drug resistance was found in 85% of the total burn isolates (Mohammedaman *et al.*, 2014). The high antibiotic resistance of burn wound pathogens is an alarming trend that necessitates following a strict antibiotic policy to minimize resistance (Abbas *et al.*, 2013).

Table 2: Resistance profile of *S.aureus* isolates

Resistance profile	
Antibiotics (n=12)	**Isolates no.**
Resistant to 3 antibiotics	14
OX, P, FOX	
MDR isolates	
Resistant to 6 antibiotics	33
OX, CN, P, FOX, RD, TE	
Resistant to 7 antibiotics	1
OX, LEV, CN, P, FOX, RD, TE	
OX, E, CN, P, FOX, SXT, TE	51
Resistant to 8 antibiotics	46
OX, E, LEV, CN, P, FOX, SXT, TE	
Resistant to 9 antibiotics	59, 55, 54, 53, 41, 40,
OX, DA, E, LEV, CN, P, FOX, RD, TE	39, 37, 34
Resistant to 10 antibiotics	58 , 5
OX, DA, TEC, E, LEV, CN, P, FOX, RD, TE	

Keys : OX (Oxacillin), LZD (linezolid), DA (Clindamicin), TEC (Teicoplanin), E (Erythromycin), LEV (Levofloxacin), CN (Gentamicin), P (Penicillin), FOX (Cefoxitin), RD (Rifampin), SXT (Trimethoprim-sulphamethoxazole), TE (Tetracycline)

Table (2) shows that the most prevalent resistance profile in *S. aureus* isolates is (OX, DA, E, LEV, CN, P, FOX, RD, TE) which was detected in 8 isolates, followed by (OX, DA, TEC, E, LEV, CN, P, FOX, RD, TE) which was detected in two isolates. Resistance to 10 antibiotics was exhibited by isolates no. 58 and 5.

Table (3) shows that the most prevalent resistance profile in *Enterobacteriaceae* is (CAZ, PRL, SXT, CN, MEM, AMC, CTX, AMP) which was detected in 4 isolates, followed by (FEP, CAZ, CIP, PRL, SXT, CN, MEM, AMC, TZP, CTX, AMP) which was detected in two isolates. Resistance to 12 or 13 antibiotics was detected in isolates no. 9, 10, and 35.

Table (4) showed that the most prevalent resistance profile in *P. aeruginosa* isolates is (ATM, CAZ, CIP, TOB, LEV, MEM, CN, FEP, AK) which was exhibited in 3 isolates, followed by resistance profile (IPM, TZP, ATM, PRL, CAZ, MEM, FEP, AK) which was detected by two isolates. Resistance to 11 or 12 antibiotics was detected in isolates no. 42, 28 and 12.

Table 3: Resistance profile of *Enterobacteriaceae* isolates

Resistance profile	
Antibiotics (n=13)	**Isolates no.**
MDR isolates	
Resistant to 5 antibiotics FEP, PRL, MEM, CTX, AMP	3
Resistant to 7 antibiotics FEP, CAZ, CIP, SXT, CN, CTX, AMP	2
Resistant to 8 antibiotics CAZ, PRL, SXT, CN, MEM, AMC, CTX, AMP	21, 23, 24, 26
Resistant to 9 antibiotics FEP, CAZ, CIP, PRL, SXT, CN, AMC, CTX, AMP	15
FEP, CAZ, CIP, PRL, MEM, AMC, TZP, CTX, AMP	17
Resistant to 11 antibiotics CAZ, AK, CIP, PRL, SXT, CN, MEM, AMC, TZP, CTX, AMP	49
FEP, CAZ, CIP, PRL, SXT, CN, MEM, AMC, TZP, CTX, AMP	31,32
Resistant to 12 antibiotics FEP, AK, CIP, PRL , SXT, IPM, CN, MEM, AMC, TZP, CTX, AMP	10
FEP, CAZ, AK,CIP, PRL, SXT, IPM, MEM, AMC, TZP, CTX, AMP	35
Resistant to 13 antibiotics FEP, CAZ, AK, CIP, PRL, SXT, IPM, CN, MEM, AMC,TZP,CTX, AMP	9

Keys: FEP (Cefepime), CAZ (Ceftazidime), AK (Amikacin), CIP (Ciprofloxacin), PRL (Piperacillin), SXT (Trimethoprim-sulphamethoxazole), IPM (Imipenem), CN(Gentamicin), MEM (Meropenem), AMC (Amoxicillin-clavulanic acid) ,TZP (Piperacillin-tazobactam), CTX (Cefotaxime), AMP (Ampicillin).

Table 4: Resistance profile of *Pseudomonas aeruginosa* isolates

Resistance profile	
Antibiotics (n=12)	**Isolates no.**
Resistant to 1 antibiotics CN	44
ATM	60
Resistant to 2 antibiotics IPM, MEM	57
MDR isolates	
Resistant to 3 antibiotics TOB, LEV, MEM	22
CIP, TOB, LEV	38
Resistant to 4 antibiotics CAZ, TOB, MEM, FEP	8
IPM, MEM, FEP, AK	56
Resistant to 6 antibiotics IPM, CAZ, TOB, MEM, FEP, AK	13
ATM, CAZ, TOB, LEV, MEM, FEP	36
Resistant to 7 antibiotics IPM, CIP, TOB, LEV, MEM, CN, AK	45
Resistant to 8 antibiotics ATM, CAZ, CIP, TOB, LEV, CN, FEP, AK	4
IPM, TZP, ATM, PRL, CAZ, MEM, FEP, AK	16, 20
IPM, ATM, CAZ, CIP, LEV, MEM, CN, FEP	47
Resistant to 9 antibiotics ATM, CAZ, CIP, TOB, LEV, MEM, CN, FEP, AK	11, 27, 30
Resistant to 11 antibiotics IPM, TZP, PRL, CAZ, CIP, TOB, LEV, MEM, CN, FEP, AK	28
IPM, TZP, PRL, CAZ, CIP, TOB, LEV, MEM, CN, FEP, AK	12
Resistant to 12 antibiotics IPM, TZP, ATM, PRL, CAZ, CIP, TOB, LEV, MEM, CN, FEP, AK	42

Keys: IPM (Imipenem), TZP (Piperacillin- tazobactam), ATM (Aztreonam), PRL (Piperacillin), CAZ (Ceftazidime), CIP (Ciprofloxacin), TOB (Tobramycin), LEV (Levofloxacin), MEM (Meropenem), CN (Gentamicin), FEP (Cefepime), AK(Amikacin)

Table (5) shows that the resistance profile (AK, CAZ, TZP, SXT, CN, IPM, LEV, PRL, CIP, MEM, CTX) were observed in three of four *A. baumanii* isolates; isolates no. 7, 48 and 50. Overall; multi drug resistance (MDR) was found in 49 (82%) of the isolates, (94%) of the gram positive isolates showed multi drug resistance, (77.2%) of gram negative isolates showed MDR. During the last few years, medicinal plants have attracted the attention of pharmaceutical and scientific communities, and evidence has demonstrated the promising potential of antimicrobial plant-derived substances (Nash *et al.*, 2011 and Osbourn, 1996). In this study, the antibacterial activity of different plant oil extracts were tested against the most antibiotic resistant burn wound isolates that is listed in Table (6). The obtained results revealed that thyme oil is the most effective followed by cinnamon oil and eucalyptus oil, peppermint oil and clove oil showed a moderate activity against *S. aureus* isolates, this findings are quite similar to that reported by Yousef and Tawil (1980), Janssen *et al.* (1986), Hili *et al.* (1997), Singh *et al.* (2007).

Table 5: Resistance profile of *Acinetobacter baumanii* isolates.

Resistance profile	
Antibiotics (n=11)	**Isolates no.**
Resistant to 1 antibiotic CTX	Ac_{29}
MDR strains	
Resistant to 11 antibiotics AK,CAZ,TZP,SXT,CN,IPM,LEV,PRL,CIP,MEM,CTX	Ac_{50},Ac_{48},Ac_{7}

Keys: AN (Amikacin), CAZ (Ceftazidime), TZP (Piperacillin- tazobactam), SXT (Trimethoprim-sulphamethoxazole), CN (Gentamicin), IPM (Imipenem), LEV (Levofloxacin), PRL (Piperacillin), CIP (Ciprofloxacin), MEM (Meropenem), CTX (Cefotaxime).

Table 6: Antibacterial activity of essential oils against multi drug resistant wound isolates.*

Isolates no. / **selected oils**	**5**	**58**	**9**	**10**	**31**	**32**	**49**	**11**	**12**	**27**	**28**	**30**	**7**	**48**	**50**
1.Lemon oil	18 ± 1	18 ± 1	15 ± 0	18 ± 1	15 ± 1	15 ± 1	16 ±1	13 ± 1	14 ± 1	14 ± 0	13 ± 1	13 ±1	0 ± 0	0 ± 0	0 ± 0
2.Cinnamon oil	33 ± 1	39 ± 1	25 ± 1	35 ± 2	37 ± 1	31 ± 1	24 ± 1	27 ± 1	30 ± 1	24 ± 1	31 ± 2	25 ± 1	35 ± 2	38 ± 2	35 ± 1
3.Garlic oil	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
4.Caraway oil	17 ± 0	18 ± 1	13 ± 1	13 ± 1	15 ± 1	17 ± 1	13 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14 ± 1	17 ± 1	18 ± 1
5.Peppermint oil	25 ± 1	29 ± 1	17 ± 1	20 ± 1	18 ± 1	18 ±1	18 ± 1	15 ± 1	15 ± 1	17 ± 1	16 ± 1	17 ± 1	17 ±1	17 ± 1	22 ± 1
6.Tea tree oil	24 ± 1	24 ± 1	20 ± 1	22 ± 1	20 ± 1	21 ± 1	20 ± 1	16 ± 1	16 ± 1	16 ± 1	16 ± 1	16 ± 1	19 ± 1	25 ± 1	21 ± 1
7.Geranium oil	0 ± 0	13 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
8.Thyme oil	40 ± 1	40 ± 2	21 ± 1	30 ± 1	33 ± 1	33 ± 2	22 ± 1	20 ± 1	20 ± 1	23 ± 1	29 ± 1	27 ± 1	27 ± 1	30 ± 1	29 ± 1
9.Fennel oil	14 ± 1	13 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10.Eucalyptus oil	34 ± 1	39 ± 1	20 ± 1	28 ± 1	22 ±1	22 ± 1	24 ± 1	22 ± 1	20 ± 1	20 ± 1	20 ± 1	19 ± 1	26 ± 1	30 ± 1	23 ±1
11.Clove oil	25 ± 2	25 ± 1	19 ± 1	22 ± 1	28 ± 1	25 ± 1	21 ± 1	0 ± 0	0 ± 0	11 ± 0	0 ± 0	13 ± 1	25 ± 1	32 ± 2	24 ± 1
12.Olive oil	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
13.Camphor oil	13 ± 1	18 ± 1	11 ± 1	14 ± 1	17 ± 1	14 ± 1	15 ± 1	0 ± 0	0 ± 0	15 ± 1	15 ± 1	13 ± 1	15 ± 1	16 ± 1	18 ± 1
14.Anise oil	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
15.Orange oil	14 ± 1	14 ± 0	11 ± 0	14 ± 0	17 ± 1	14 ± 1	15 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	18 ± 1	15 ± 1	13 ± 1
16.Dill oil	18 ± 1	20 ±1	13 ± 1	0 ± 0	17 ± 1	15 ± 1	15 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	20 ± 1	0 ± 0
17.Ginger oil	20 ± 1	19 ± 0	0 ± 0	0 ± 0	17 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13 ± 1	0 ± 0	0 ± 0
18.Moringa oil	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
19.Rose mary oil	14 ± 1	14 ±1	0 ± 0	0 ± 0	13 ± 0	14 ± 1	13 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16 ± 1	17 ± 1

50 µl of different essential oils were pipetted into Muller Hinton agar plates seeded with 24 h old cultures of burn wound isolates and incubated for 24 h at 37°C.
* All data represented are means of two experiments ±SE.

Also, Hili *et al.* (1997) and Singh *et al.* (2007) recorded high antibacterial activity of cinnamon and thyme oils against *E. coli*. Other studies reported moderate activity of tea trees and eucalyptus oils (Schelz *et al.*, 2006).

Table 7: chemical composition of cinnamon bark essential oil as identified by GC/MS analysis.

Peak	**Retention time**	**Compounds**	**% of relative content**
1	10.10	α-Pinene	2.99
2	10.90	Camphene	0.58
3	11.75	Sabinene	2.21
4	13.94	1,8-Cineole	9.12
5	19.01	endo-Borneol	0.44
6	19.50	Terpinen-4-ol	2.61
7	20.10	α-Terpineol	3.23
8	23.69	Cinnamaldehyde	60.78
9	26.30	α-Copaene	0.51
10	27.43	trans-α-Bergamotene	0.45
11	27.71	trans-Caryophyllene	0.71
12	28.73	Cinnamyl acetate	10.39
13	30.90	α-Muurolene	0.46
14	32.72	ë-Cadinene	0.52
15	34.42	Caryophyllene oxide	0.39
16	34.79	tau.-Muurolol	0.52
17	34.92	α-Cadinol	0.37
Total identified compounds			97.99

Cinnamon oil, thyme oil and eucalyptus oil were found to be the most active oils against *P.aeruginosa* isolates followed by peppermint oil and tea tree oil, this result was almost similar to that described by Deans and Ritchie (1987), El-Shouny and Magaam (2009) and Dahiya and Purkayasth (2012). Data presented in Table (7) shows GC/MS analysis of cinnamon bark essential oils. It indicated that cinnamaldehyde (60.78%) was the major constituent. This findigs are quite similar to that reported by El-Baroty *et al.* (2010) and Boniface *et al.* (2012). The antibacterial activity of cinnamon essential oil may be due to the presence of the carbonyl group on cinnamaldehyde that may bind to proteins and interfere with the function of bacterial amino acid decarboxylase (Wendakoon & Sakaguchi, 1993 and 1995).

Results in Table (8) shows GC/MS of thyme essential oil which identified eleven chemicals as constituents; of these ρ-cymene and thymol were the main constituents (83.1%). This result is in agreement with that reported by Burt (2004), he found that thyme oil contained of 10% - 64% thymol and 10% - 56% p-cymene. p-cymene has a high affinity for membranes and causes membrane expansion and affect the membrane potential of intact cells (Ultee *et al.*, 2002).

Thymol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP (Helander *et al.*, 1998).

Table 8: chemical composition of thyme essential oil as identified by GC/MS analysis

Peak	Retention time	Compounds	% of relative content
1	10.64	α-pinene	0.41
2	11.20	Camphene	0.43
3	14.65	p-cymene	50.14
4	15.67	c-Terpinene	5.21
5	17.28	Linalool	1.69
6	18.71	Camphor	0.86
7	19.70	1-Borneol	4.66
8	20.06	4-Terpineol	0.63
9	24.73	Thymol	32.9
10	28.27	trans-Caryophyllene	1.42
11	33.28	Caryophyllene oxide	1.28
Total identified compounds			99.63

CONCLUSION

Burn patients were most commonly infected with *P. aeruginosa* and *S. aureus*. The majority of these isolates are found to be multidrug resistant. A burn unit-specific nosocomial infection surveillance system may be introduced to reduce the incidence of multidrug resistant infections among burn patients, and for selecting appropriate antimicrobial agents. The obtained results indicate that cinnamon and thyme oils could be a promising an alternative medicine for the treatment of burn wound infections.

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