INTRODUCTION

Burn-wound infection due to Pseudomonas aeruginosa poses a significant challenge in terms of graft loss, systemic sepsis, prolonged hospital stay, and even increased mortality. Armour et al. [1] reported on 48 adult patients with gentamicin-resistant P. aeruginosa colonization compared with case-matched controls; this cohort required a two-fold increase in grafting procedures as well as an average of 15 days’ longer hospital stay. Geyik et al. [2] reported the only specific series to detail the effect of P. aeruginosa in children, and they found that 65% frequency of wound colonization, with systemic sepsis in 7.2%, when associated with bacteremia, was associated with an 80% mortality. Herbal medicines have been important sources of products for developing countries in treating common infectious diseases and the problems of resistance and side effects of the currently available antimicrobial agents [3]. The World Health Organization estimates that 80% of the people living in developing countries almost fully depend on traditional herbal medicines in Iraq known as Arabic medicine. This means that approximately 3,300 million people use medicinal plants on a regular basis. Medicinal plants used in traditional medicine should be studied for safety [4]. Hence, we use three types of plants which approved their ability as antimicrobial activity against pathogenic bacteria isolated from burn infection, especially against P. aeruginosa, onion bulbs (Allium cepa), leaves of mint (Mentha asiatica), and outer peel of pomegranate (Punica granatum).

Each one of the chosen plant extract has special active chemical compound against the specific chemical compound on/or inside pathogenic cell, and onion extract is very effective against P. aeruginosa due to flavonoids and polyphenols which has been reported to have a broad spectrum of antibacterial activity [5]. While mint extract includes monoterpenes, mainly menthol, menthone, and their derivatives [6]. Pomegranate peels reported that phenolic compounds are punicalagin isomers, ellagic acid derivatives, and anthocyanins [7].

The objective of the study was to investigate the antimicrobial activities of three solvent extracts of selected Iraqi plant against multi-resistant P. aeruginosa isolated from burns and wounds.

MATERIALS AND METHODS

P. aeruginosa isolation and identification

About 30 burn-wound swabs were taken from both male and female burned patients, and average age 9-40 years, from a burn unit of teaching Al-Hillah Hospital, Babylon Province, Iraq, during September 2016-January 2017. All specimens were inoculated on 5% blood agar, MacConkey agar, and Chocolate agar plates and incubated overnight at 37°C aerobically. The sample was also put into liquid media (Brain Heart Infusion broth) and was subcultured after overnight incubation onto blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques [8]. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method [9]. The antibiotics tested for bacterial isolates were: Ciprofloxacin (Cip10), amikacin (AK30), chloramphenicol (C30), tetracycline (T30), oxacillin (Ox1), Cip10, trimethoprim (Tm5), and doxycycline (Do30). The inhibition zone diameter (mm) of antibacterial activities was calculated.

Preparation of plant extracts

Three plant samples were used in this study: peel of pomegranate, onion bulbs, and mint leaves were purchased from the local markets.
The plants were classified by specialists in the Botanical Garden at the University of Babylon. These plants were air-dried at room temperature, ground to powder with a mechanical grinder.

**Aqueous extraction using hot water**

20 g of the weighed plant powder was soaked in 100 ml of hot boiled water for 2 h. The plant extract was separated from the plant solid residue using filter paper with vacuum, then centrifuged and the supernatant filtered using Whatman filter paper No.1. The filtrate was concentrated by a rotary evaporator (rotavapor R300) at 40°C. The concentrated extract was dried to obtain an extract powder [10]. The powdered was stored in clean containers at 4°C for further analysis.

**Alcoholic extraction**

Alcoholic extracts were prepared using two solvents such as ethanol and methanol. The plant powder (50 g) was soaked in 250 ml of 99.9% for each ethanol or methanol at room temperature for 24 h. The extracts were separated from the other solid plant residues using filter paper with vacuum, and then, it was centrifuged, and the supernatants were filtered using Whatman filter paper No.1. The filtrates were concentrated using a rotary evaporator (rotavapor R300) at 40°C. The concentrated extracts were dried to obtain the extract powders [10,11]. These powders were stored in clean containers at 4°C for further analysis.

**Preparation of the stock solutions and dilutions**

A 15 g of each plant extract powder was dissolved in 50 ml sterile hot-distilled water to prepare the stock solution (200 mg/ml). While the ethanol extract was firstly dissolved in 1 ml of 99.9% ethanol due to it was insoluble in water and it was oily nature. Then, diluted in sterile distilled water to give an ethanolic stock solution. All stock solutions were filtrated by Whatman filter paper No.1 and then by Millipore filter membrane (0.45 µm) for sterilization. Different dilution frequencies (50, 100, and 150 mg/ml) were made with sterile distilled water.

**Antibacterial activity determination**

The antibacterial activity of the crude extracts against *P. aeruginosa* was determined by the agar-well diffusion method [12,13] using Muller-Hinton agar plates. Bacterial suspension (10⁶ CFU/ml) was made, and then, the Muller-Hinton plates were streaked with a bacterial suspension using sterile swab. After that, the wells were made with a diameter of 6 mm by punched aseptically with a sterile cork borer (No. 6). Approximately 50 µl of the crude extract at different concentrations of 50, 100, and 150 mg/ml were loaded into the wells, and the negative control was used a sterile distilled water, in triplicate. One an hour pre-diffusion time was allowed, after that, the plates were incubated at 37°C for 18 h. The diameter of inhibition zones was measured in millimeter and calculated the mean of triplicate results [14,15].

**RESULT AND DISCUSSION**

**Bacterial isolation and identification**

The bacterial isolates (9) were recovered from only 9 burn-wound swabs out of 30 swabs that indicate 30% of examined burn patients had invasive burn-wound infections. These results are consistent with the previous studies [16-18] who showed that the burn-wound infections are one of the most important and potentially serious issues that occur in the acute period following injury. Furthermore, Raja and Singh [9] demonstrated that the infectious complications are considered a major cause of morbidity and mortality and the type and amount of microorganisms onto and into the injured tissues influence wound healing. In the present study, the most commonly isolated organisms from burning patients were *P. aeruginosa* identified by conventional biochemical methods according to standard microbiological techniques [8] and using the specific chromagar for *P. aeruginosa* identification.

**Antimicrobial susceptibility testing**

Antibacterial susceptible of pseudomonad isolates was detected to determine the multidrug-resistant *P. aeruginosa* isolates. The test was performed by disc diffusion method with different antibiotic discs. The results showed that all *Pseudomonas* isolates appeared multidrug resistant for all used antibiotics, including Ox1, C30, T30, Tmp5, and Do30. However, it had moderate resistance to AK30 and Gip10 as shown in Fig. 1. These results were consistent with the previous studies [17,19]. Increasing resistance to various anti-Pseudomonas agents has been reported worldwide, and this poses a serious problem in therapeutic management of the bacterial infections [17,20]. Furthermore, our results explained that most of the isolates were resistant to many antibiotics.

**Antibacterial activity of plant extracts against *P. aeruginosa***

Agar well diffusion method was used for antibacterial activity determination of aqueous, ethanolic, and methanolic extracts of plants, including outer peel of pomegranate (*P. granatum*), onion bulbs (*A. cepa*), and mint leaves (*M. asiatica*). Quantitative evaluation of this activity was carried out against *P. aeruginosa* by measuring of inhibition zone surrounded the wells containing the extract.

As shown in Table 1, plant extracts had antibacterial activity against *P. aeruginosa* with clear differentiation among the extracts depending on the concentration of the extract, type of solvent, and type of plant. The results show high activity to all ethanolic extract of three plant extracts than methanolic and water extracts as shown in Tables 1-3 due to the ability of ethanol to solve solid organic compounds and liberate all chemical components rather than other solvent used in the present study. Peel of *P. granatum* (Table 1) showed the highest anti-pseudomonad activity than other plants in all of the concentrations. The highest concentration of peel of *P. granatum* extract (200 mg/ml) appeared highest inhibition zones for all types of extraction methods (30 mm for ethanolic extract, 36 mm for methanolic extract and 22 mm for the water extract). Peel of *P. granatum* contains substantial amounts of polyphenolics such as ellagic tannins, ellagic acid, and gallic acid, which have antimicrobial activity [21]. These results were in agreement with Hayrapetyan et al. [22] who reported that the presence of two pure compounds commonly found in the pomegranate-peel extract, namely, ellagic acid and gallic acid. While other study revealed that the phenolic compounds of pomegranate juice are punicalagin isomers, ellagic acid derivatives, and anthocyanins [23]. Ahmad and Beg [24] reported that the phytochemical compounds found in the alcoholic extract of pomegranate are alkaloid, flavonoid, glycoside, phenol, and tannin. The results of antimicrobial activity of the peelethanolic extract of *P. granatum* were in agreement with the study of Oskay et al. [25] who recorded 16 mm inhibition zone diameter against *P. aeruginosa*.

The methanolic extract showed lower action than the ethanolic extract as antibacterial agents. This may be due to little diffusion properties of the extract in the agar or because fresh plants contain
active substances which may be affected, insoluble, or attributed by the used solvent [5].

Water extract of pomegranate even when it less active than alcoholic solvent still gives obvious 22 mm inhibition zone diameter that means the extract seems to be thermostable because of using hot-water in plant metabolite extraction. These results were consistent with Al-Zoreky who found the inhibition zone diameters were ranged from 13 to 17 mm [7], whereas, the other study [23] found that the inhibition zone diameter was lower (10-40 mm).

The water extract, in the present study, showed lower activity than other extracts that may be due to water extracted less phenolic contents where the phenolic groups are the active component against microbial growth [26]. The effect of the lowest concentration (50 mg/ml) of P. granatum of all extractions solvent were still higher than other plant extracts that may be referred to the P. granatum extract containing high amounts of active phenolic contents against microbial growth [27].

The antibacterial activity of onion (A. cepa) extract (Table 2) can be attributed to the presence of flavonoids and polyphenols which has been reported to have a broad spectrum of antibacterial activity [28]. Furthermore, the polyphenols of plants have been reported to have antibacterial activity [29]. In the present study, the onion ethanolic extract showed inhibition zone about 30 mm at 200 mg/ml concentration. These zones gradually reduced with decreasing of concentration until 50 mg/ml which showed no inhibition zone. While the methanolic extract showed high activity than ethanolic one, with inhibition zone was 29, 25, and 18 mm at concentrations 1000, 500, 200, and 100 mg/ml, respectively. That means the methanol solvent is the best solvent for phenolic content extraction, which has antibacterial activity as reported in the study of Hendrich [29]. These results were in agreement with the previous studies [30] which reported the inhibition zone was 29, 26, 25, and 24 mm at concentrations 1000, 500, 200, and 100 mg/ml, respectively. Furthermore, it found the antibacterial activity against pseudomonad bacteria of the onion-water extract was 23, 18, 16, and 14 mm at concentrations 1000, 500, 200, and 100 mg/ml, respectively.

In the present study, we showed different results which were lower than ethanolic and methanolic extracts with 20 and 13 mm at 200 and 150 mg/ml concentration, respectively. However, they showed no inhibition zone at concentrations 100 and 50 mg/ml. That is because of water is more polarity than alcoholic solvent, so low level of phenolic and alkaloid compounds was extracted which disruption of the cell membrane [31-35].

M. asiatica leaves extract (Table 3) also shows most antimicrobial activity in the ethanol extract than methanol and water extract with a range of inhibition zones 36, 30, 25, and 18 mm at concentrations 200, 150, 100, and 50 mg/ml, respectively. These results agreed with the findings of El-Taweel [5] but less activity (3.3 and 7.3 mm inhibition zone at 200 and 100 mg/ml concentration, respectively). The principle active components of peppermint M. asiatica are monoterpenes, mainly menthol, menthone, and their derivatives (e.g., isomenthone, neomenthol, acetyl menthol, and pulegone). These essential oils dilate blood vessels and inhibit bacteria. Especially, menthol has a broad-spectrum antibacterial activity [36].

Also, the methanolic peppermint extract was higher antibacterial activity against test organisms than water extract with 30 mm inhibition zone at 200 mg/ml concentration and 19 mm at 50 mg/ml. The results of the present study were higher than other study [5] which used chloroform as extraction solvent instead methanol to solve peppermint that showed 9.2 mm inhibition zone at 150 mg/ml. The results of the present study were higher than other study [5] which used chloroform as extraction solvent instead methanol to solve peppermint that showed 9.2 mm inhibition zone at 150 mg/ml and 14.2 mm at 100 mg/ml of chloroform or methanol increased the suspended higher compounds.

Water extract of M. asiatica also has moderately active against P. aeruginosa where alcoholic solvents are more effective, and results show an inhibition zone range 21, 19, 13, and 9 mm at concentrations 200, 150, 100, and 50 mg/ml, respectively, which was the lowest effect on P. aeruginosa than other plants.
The result of the study revealed that all solvents, actively effect against the P. aeruginosa that are a common cause of infections. M. asiatica shows significant activity as because of their leaves contain many potent compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone [37].

**CONCLUSION**

From the study, all three studied plants had antibacterial activity against multidrug-resistant P. aeruginosa isolated from burn wound. P. granatum showed the highest antibacterial activity, followed by...
A. cepa, and finally, M. asiatica. It is a recommendation that natural products can be used as therapeutic agents which will probably not elicit resistance in bacteria. More research must continue to isolate and purify the active components and applied in experimental animal models.

REFERENCES

1. Armour AD, Shankowsky HA, Swanson T, Lee J, Tredget EE. He impact of nosocomially-acquired resistant Pseudomonas aeruginosainfection in a burn unit. J Trauma 2007;63(1):164-71.
2. Geyik MF, Aldemir M, Hosoglu S, Tacyildiz HH. Epidemiology of burn unit infections in children. Am J Infect Control 2003;31:342-6.
3. Kianbakht S, Jahaniani F. Evaluation of antibacterial activity of Tribulusterristris L. growing in Iran. Iran J Pharm Ther 2003;2:22-4.
4. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol 1998;60(1):1-8.
5. El-Taweil M. Assessment of antimicrobial activity of onion extract (Alliumcepa) on Staphylococcus aureus; In vitro study. Int Conf Chem Agric Med Sci 2014;29(30):16-7.
6. Hayyan I, Al-Taweil M. Antimicrobial effect of mint essential oils on some pathogenic bacteria. Int J Life Sci Res 2014;2(4):90-3.
7. Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int J Food Microbiol 2009;134(3):244-8.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott Diagnostic Microbiology. 10th ed. London: Mosby; 1998.
9. Rajas, Singh NN. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosain a tertiary care hospital. J Microbiol Immunol Infect 2007;40(1):45-9.
10. Okigbo RN, Mnekca EK. Antimicrobial effects of three tropical plant extracts on Staphylococcus aureus, Escherichia coli and Candida albicans. Afr J Tradit Complement Altern Med 2008;5:226-9.
11. Sama Fonkeng L, Mouokeu RS, Tume C, Njateng GS, Kamcthueng MO, Ndounke NJ, et al. Anti - Staphylococcus aureus activity of methanol plants used in Cameroonnian folk medicine. BMC Res Notes 2015;8:710.
12. Irobi ON, Moom-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extracts of Bridelia ferruginea (Euphorbiaceae). J Ethnopharmacol 1994;43:185-90.
13. Omran R. Production of antimicrobial and anticancer from feather-keratinolytic Nocardopsis sp. 29ROR as a novel strain using feather meal medium. Int J Pharm Pharm Sci 2017;9(3):175-9.
14. Al-Marzook FA, Omran R. Cytotoxic activity of alkaloid extracts of different plants against breast cancer cell line. Asian J Pharm Clin Res 2017;10(7):168-71.
15. Omran R, Al-Tae ZM, Hashim HO, AL-Jassani MJ. Extraction of phenolic compounds as antioxidant from some plants and their cytotoxic activity against breast cancer cell line. Asian J Pharm Clin Res 2017;10(7):168-71.
16. Ani V, Varadaj M, Naidu K. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (Cuminum rigorum L.). Eur Food Res Technol 2006;224(1):109-15.
17. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp 1990;15:113-5.
18. Al-Marzook FA, Omran R. Cytotoxic activity of alkaloid extracts from three Iraqi plants against breast cancer cell line. Asian J Pharm Clin Res 2017;10(9):78-81.
19. Adebayo OL, Edwin BA. Solvent extract of different plants of Phyllanthus niruri show potent growth inhibition against six human pathogenic bacteria in vitro. Int J Pharm Pharm Sci 2014;6(7):272-7.
20. Skay M, Oskay D, Kalioncu E. Activity of some plant extracts against multi-drug resistant human pathogens. Iran J Pharm Res 2009;8(4):293-300.
21. Naumsetti T, Dechayuenyong P, Tantiapibulut S. Antibacterial activity of pomegranate fruit peels and arils. Sci Asia 2012;38:319-22.
22. Voravuthikunchai S, Lortheeranuwat A, Jeeu W, Srisirat T, Phongpaichit S, Supawira T. Effective medicinal plants against enterohaemorrhagic Escherichia coli O157:H7. J Ethnopharmacol 2004;94(1):49-54.
23. Grover A, Bhandari B, Nishant R. Antimicrobial activity of medicinal plants - Azadirachta indica A. Juss, Allium cepa L. and Aloe vera L. Int J Pharm Tech Res 2011;3(2):1059-65.
24. Hendrich A. Flavonoid-membrane interactions: Possible consequences for biological effects of some polyphenolic compounds. Acta Pharmocol Sin 2006;27(1):27-40.
25. Ani V, Varadaj M, Naidu K. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (Cuminum rigorum L.). Eur Food Res Technol 2006;224(1):109-15.
26. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp 1990;15:113-5.