Original research article

Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from Egyptian flora


Abstract

Plants offer unlimited source of bioactive compounds that have tremendous applications in pharmaceutical industry. To find new sources of antioxidants and antimicrobial agents against pan-drug resistant (PDR) pathogens of skin burn infections, methanolic extracts of nine Egyptian plants were evaluated. Total phenolic content varied from 19.48 to 65.48 mg GAE/g dry weight (dw). Total flavonoid content varied from 2.90 to 11.09 mg QE/g dw, while total alkaloid content varied from 18 to 60 mg/g dw. Suaeda vermiculata, Vartemia candicans and Arthrocnemum glaucum showed the highest content of phenolics, flavonoids and alkaloids, respectively. The results of antioxidant (DPPH assay) activity were varied to a great extent and Vartemia candicans (90.6%) showed the highest antioxidant activities. Antimicrobial activity was assessed against four PDR bacterial and fungal strains; namely Staphylococcus aureus, Klebsiella pneumo- niae, Candida albicans and Aspergillus flavus. Among all examined extracts, Salsola vermiculata and Sueda vermiculata showed significant antimicrobial activity. The fatty acid composition of Salsola vermiculata and Sueda vermiculata leaf extracts was detected using GC–MS analysis. Overall, Salsola vermiculata and Sueda vermiculata could be used as an alternative source for the exploration of new antioxidant and antimicrobial agents that are potentially valued for food and biomedical applications.

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Introduction

Egyptian folk medicine has a long history of development, which is derived from many different cultures. The “old” pharaonic in conjunction with “recent” Arabic Unani medicine, represent the major and most important sources of all formulations in the current herbal market (Hamdy et al., 2017). Egyptian North West Coast region possesses a rich source of biodiversity and is considered as a major hot spot of Egypt. The wide geographical and climatic diversity of this region provides a repository of important indigenous medicinal plants for the treatment of human ailments. The most important advantage of herbal medicine is the relatively low cost compared to the synthetic medicines as well as minimal side effects (Pu et al., 2017).

World Health Organization (WHO) indicated that 70–80% of the world’s population depend on herb plants as a primary health care source (Muhammad et al., 2011). Medicinal plants are the most potent antimicrobial natural source, used as ethnomedicine in many countries (El-Shoumy et al., 2018). The medicinal importance of these plants lies in the presence of active chemical components. Plants are rich in a wide variety of phytochemical secondary metabolites, such as phenolics, alkaloids, flavonoids, and...
terpenoids which have been found in many studies to have important antimicrobial activities (Annu Ahmed et al., 2018; Kumar et al., 2015; Nayak et al., 2017). These natural metabolites provide unlimited opportunities for new drug leads.

Oxidative stress induced by free oxygen radicals is the main reason for various degenerative diseases such as gastric ulcers, cancer, atherosclerosis, and other conditions. Medicinal plants are the source of many antioxidants acting as active oxygen scavengers. Recently interest has been focused on antioxidants from natural sources to avoid drawbacks of synthetic antioxidants (Gandhia et al., 2018). In recent studies, the potent activity of antioxidants is attributed to phytochemical compounds present in considerable high amounts in the plants (Tahaa et al., 2018).

Nowadays, millions of people in the world suffer from chronic wound burns without effective solutions. Burn followed by microbial infection is a very serious complication that often results in the patients’ death (Ali et al., 2017). About 45% of mortality is recorded in burned patients as a consequence of microbial infections (Bloemstra et al., 2008). On the other hand, WHO regarding drug resistance has encouraged and promoted screening and utilization of medicinal plants as a new alternative therapy against multi-drug resistant (MDR) and pan-drug resistant (PDR) pathogens that cause severe infections and difficult-to-treat diseases (El-Shouny et al., 2018). Overall, medicinal plants offer unlimited opportunities for the discovery of novel antimicrobial agents. Most of the natural products extracted from medicinal plants and used in folk remedy have solid scientific evidence due to their antioxidant and antimicrobial activities. Recently, exploring neglected wild plants as the potential alternative biomedical source had become a great priority. Therefore, our study reports the assessment of some Egyptian plant extracts for their antioxidant and antimicrobial activities against drug-resistant clinical strains of skin burn infections.

### Materials and methods

#### Plant material

Nine plant species (Hyoscynamus muticus L., Varthenia candidans Boiss., Salsola vermiculata L., Peganum harrama L., Marrubium vulgare L., Suaeda vermiculata Forssk, Pluchea dioscoridis L., Datura stramonium L. and Arthrocnemum glaucum Delile) (Supplemental information, Fig. S1) were collected from the North West Coast region, Borg El-Arab City, Alexandrea, Egypt in the summer season (July–August 2015). The voucher specimens presented in Table 1 were maintained in the Herbarium of Botany Department, Faculty of Science, Tanta University (TANE) for further reference.

#### Extract preparation

The plants were washed with tap water, distilled water and subsequently oven-dried at 40°C for 3 days until constant weight was achieved. The dried samples were then pulverized to fine powder and stored at 4°C for further use. Five grams of each dried samples were extracted with 100 ml of 80% methanol for 6 h at 40°C using the Soxhlet apparatus. Afterward, the resulting extracts were filtered using a Whatman filter paper and centrifuged at 8000 rpm for 10 min. The supernatants obtained were concentrated using a rotary vacuum evaporator under reduced pressure at 40°C. The yield of extracts was measured and freeze-dried at –20°C. All extracts were stored at 4°C for further characterization studies.

#### Qualitative phytochemical analysis

The qualitative preliminary detection of tannins, saponin, steroids, terpenoids, phenols, alkaloids, cardiac glycosides and flavonoids was carried out following the procedure of Harborne (1989). The preliminary

### Table 1

<table>
<thead>
<tr>
<th>Voucher name</th>
<th>Scientific name</th>
<th>Family</th>
<th>Uses</th>
<th>References</th>
</tr>
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<td>Alizadeh et al. (2014)</td>
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<td>Asteraceae</td>
<td>Anti-inflammatory</td>
<td>Ahmed et al. (2013); Elhaak et al. (2014)</td>
</tr>
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<td>Asteraceae</td>
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<td>Sharma and Goyal (2011)</td>
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</table>
phytochemicals were analyzed in order to detect the presence or absence of the specific phytochemical groups.

Quantitative phytochemical analysis

Total phenolics, total flavonoids and total alkaloids were measured quantitatively according to standard methods as described in details by Zengin et al. (2014). Briefly, total phenolic content in the methanolic extracts was determined using Folin–Ciocalteu’s method (Fu et al., 2011). The absorbance of the resulting blue color solution was measured spectrophotometrically using a UV–vis spectrophotometer (Shimadzu-UV2600, Japan) at 760 nm. The total phenolic content was expressed as milligrams gallic acid equivalents per gram of the dried plant material (mg GAE/g dry weight). Total flavonoid content of each sample was measured using aluminum trichloride assay (Koolen et al., 2013). The absorbance was measured spectrophotometrically at 415 nm. The results of total flavonoid content obtained were expressed as milligrams quercetin equivalents per gram of the dried plant material (mg QE/g dry weight). Total alkaloid content was measured spectrophotometrically at 470 nm. The total alkaloid content was expressed as mg/g dry weight.

Fatty acid composition by gas chromatography–mass spectrum (GC–MS)

Fatty acid methyl esters (FAMEs) of the methanolic extracts tested were performed as we described previously (El-Shoumy et al., 2018). The compounds were identified by comparing the mass spectral data with those of the National Institute of Standards and Technology’s (NIST-05-L) mass spectral library data provided by the software of the GC–MS system.

Antioxidant activity

DPPH scavenging activity

The free radical scavenging activity of nine methanolic plant extracts was measured against 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Zengin et al. (2014). The absorbance was measured spectrophotometrically at 415 nm using a UV–vis spectrophotometer (Shimadzu-UV2600, Japan), where lower absorbance indicated higher free radical scavenging activity.

Nitric oxide (NO) scavenging activity

The NO scavenging activity was quantified by using Griess reagent (Green et al., 1982). This reagent consists of sulphanilamide (1%), phosphoric acid (2%) and naphthyl ethylenediamine (0.1%) following the procedure of Nayak et al. (2017). Scavengers of free radicals result in the reduced production of NO. Ascorbic acid was used as standard reference compound. The absorbance was measured at 546 nm and the percent inhibition activity was calculated using the formula: % NO scavenging activity = [(Ac – At)/ Ac] × 100, where Ac is the absorbance of the control reaction and At represents the absorbance of the test reaction.

Bacterial and fungal strains isolated from skin burn infections

Fifty-three swabs of skin burns were obtained from Burn Units of Tanta University Hospitals, Egypt. Isolation of bacteria and fungi was carried out at Microbiology Lab of Science Faculty, Tanta University. These isolates were identified as we described previously (Ali et al., 2017).

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolated pathogens was determined by the modified Kirby–Bauer disc diffusion method (Fig. 1) on Muller–Hinton agar plates (Bauer et al., 1966) using 22 antibacterial and antifungal agents (Oxoid, England) (Fig. 2). Isolated strains were grown on nutrient agar and sabouraud dextrose agar plates for bacteria and fungi, respectively. The turbidity was adjusted equivalent to 0.5 McFarland (approximately 107 CFU/ml for bacteria and fungi). The sensitivity was determined by measuring the zone of growth inhibition (mm) around the antibiotic discs. The results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2014). MDR is defined as resistance to at least three or more antimicrobial categories (Ali et al., 2017). However, non-susceptibility to all agents in all antimicrobial classes is defined as PDR (El-Shoumy et al., 2018; Magiorakos et al., 2011).

Fig. 1. Visual representation of zones of bacterial growth inhibition shown by antibacterial agents used in this study against PDR Staphylococcus aureus (A) and Klebsiella pneumonia (B). ATM, amterazonam; AX, amoxicillin; C, chloramphenicol; CAZ, cefazidime; CIP, ciprofloxacin; CN, gentamicin; CRO, ceftriaxone; CT, colistin sulfate; CTX, cefotaxime; FEP, cefepime; IPM, imipenem; K, kanamycin; SXT, cotrimoxazole; TE, tetracycline; TOB, tobramycin.
Antimicrobial activity

To test the antimicrobial activity, all plant extracts were dissolved in pure dimethyl sulfoxide (DMSO) to a final concentration of 100 g/l (100 mg plant extract in 1 ml of DMSO). The extracts were sterilized by filtration on 0.45 mm Millipore filters. Then, 15 μl of each extract were soaked separately into sterile filter paper discs. These discs were placed on Muller-Hinton agar plates previously swabbed with 100 ml of bacterial and fungal inoculum (approximately 10⁷ CFU/ml). All plates were then incubated for 18 h at 37°C for bacteria and at 30°C for fungi (24-48 h). Streptomycin (10 μg/ml) and fluconazole (25 μg/ml) were used as positive controls for antibacterial and antifungal activities, respectively. The DMSO added disc was taken as negative control. Antimicrobial activity was determined by measuring the zone of growth inhibition (mm) around the discs.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Microbroth dilution method was performed to determine the MIC, and MFC values of the most effective methanolic plant extracts in 96 multi-well microtitre plates as previously described (Panghal et al., 2011). MIC and MFC were defined as the lowest concentration at which the color change occurred.

Statistical analysis

Numerical data presented as mean ± standard deviation (SD) of three replicates while categorical data presented as number and percent. The statistical analyses were carried out using PC-ORD for Windows (ver. 5) for two ways hierarchical cluster analysis with Sorensen methods for distance and beta (~0.025) for group linkage, and Minitab 17.1.0.0 for Windows (Minitab Inc., 2013, Pennsylvania, USA). Data normality was checked using Shapiro-Wilk test. One-way and two-way (ANOVA) tests used to compare between more than two groups of normally distributed data, while Kruskal Wallis test was used to compare between more than two groups of non-normally distributed data. Holm Sidak and Tukey tests were used for multiple comparisons. Pearson’s correlation was calculated to assess the correlations between various study parameters. A P-value < 0.05 is considered significant.

Results and discussion

Phytochemical profiling

The preliminary phytochemical analysis is the simplest method for detecting secondary metabolites in plant extract. The clustering analyses of the phytochemical screening are presented in Fig. 3. The dendrogram was performed to detect the presence of the specific phytochemical group of diverse secondary metabolite classes in the methanolic plant extracts. These analyses were based on the visual observation of the color change or a precipitate formation after the addition of specific reagents. It was observed that steroids, alkaloids and flavonoids were found in all the methanolic extracts (Fig. 3).

The preliminary phytochemical screening of *Argemone mexicana* leaf and stem methanolic extracts showed positive results for most of...
The presence of the basic phytochemicals that were confirmed qualitatively led us for the quantitative assessment of the three most important phytochemical classes, total alkaloids, total phenolics and total flavonoids.

**Quantitative phytochemical analysis**

The total phenolic content among the nine methanolic plant extracts ranged between 19.48 ± 0.3–76.88 ± 0.1 mg GAE/g dw and *Suaeda vermiculata* extract showed the highest total phenolic content. The median of total phenolic content in *Suaeda vermiculata* leaf extract was significantly higher than that of other methanolic extracts (P = 0.001) (Fig. 4; Supplemental information, Table S1). Several reports have shown the close relationship between total phenolic content and antioxidative activity of plants (Abdille et al., 2005). Phenolics are the most abundant family of phytochemicals consisting of one or more aromatic rings with hydroxyl groups, which act as a defense mechanism against microbial pathogens. Additionally, these compounds are reported for their role in reproduction and growth (Nayak et al., 2017). It was reported that methanol was the best solvent to exhibit high phenolic content in *A. mexicana* (Apu et al., 2012). Several plant-derived phenolic extracts were previously reported as having prominent antimicrobial effects against *Staphylococcus aureus*. *Senna macranthera*, *Artemisia absinthium*, *Cymbopogon nardus* and *Baccharis dracunculifolia* extracts were markedly highlighted as effective antimicrobial agents in the management of *S. aureus* (Diaz et al., 2010). Gomes et al. (2018) also reported that the phenolic compounds of *Eucalyptus globulus* extract were responsible for the observed antibacterial activity against *S. aureus*. At the same time, Martins et al. (2015) reported that several plant-derived phenolic extracts showed promising antifungal activity against *Candida* species. Seleshe et al. (2017) investigated the antimicrobial activity of three ethanol extracts (*Rubus corchorifolius*, *Rubus parvifolius* and *Duchesnea chrysanthha*). Authors of this study reported that *R. parvifolius* extract had the highest phenolic and flavonoid contents. These extracts showed antibacterial activity against *S. aureus* and *Klebsiella pneumoniae*. However, there was no antifungal activity. The presence of the basic phytochemicals that were confirmed qualitatively led us for the quantitative assessment of the three most important phytochemical classes, total alkaloids, total phenolics and total flavonoids.

**Fig. 3.** Dendrogram of phytochemical analysis of the methanolic plant extracts. STE, steroids; ALK, alkaloids; FLA, flavonoids; TAN, tannins; TER, terpenoids; SAP, saponin; CAR, cardiac glycosides; PHE, phenols; H. m, *Hyoscyamus muticus*; V. c, *Varthemia candicans*; Sal. v, *Salsola vermiculata*; P. h, *Peganum harmala*; M. v, *Marrubium vulgare*; Sua. v, *Suaeda vermiculata*; P. d, *Pluchea dioscoridis*; D. s, *Datura stramonium*; A. g, *Arthrocnemum glaucum*.

**Fig. 4.** Quantitative analyses of total phenolics (A), total flavonoids (B), total alkaloids (C), and DPPH radical scavenging activity of crude methanolic plant extracts (D). H. m, *Hyoscyamus muticus*; V. c, *Varthemia candicans*; Sal. v, *Salsola vermiculata*; P. h, *Peganum harmala*; M. v, *Marrubium vulgare*; Sua. v, *Suaeda vermiculata*; P. d, *Pluchea dioscoridis*; D. s, *Datura stramonium*; A. g, *Arthrocnemum glaucum*. P-value < 0.05 is considered significant.
activity against Candida albicans and Aspergillus niger. Transmission electron microscope examination confirmed that a novel phenolic compound from Cedrus deodara disrupted the cell membrane of S. aureus and caused severe morphological changes, which even led to leakage of intracellular constituents (Wu et al., 2016).

The total flavonoid and alkaloid contents of our extracts ranged from 2.90 ± 0.04 to 11.09 ± 0.37 mg QE/g dw and from 18 ± 0.8 to 60 ± 1.4 mg/g, respectively. Varthemia candicans and Arthrocathedum glaucum extracts showed significantly higher median total flavonoid (P = 0.001) and median total alkaloid (P = 0.002) contents respectively compared to other methanolic extracts (Fig. 4; Supplemental information, Table S1).

Flavonoids are a diverse group of phytochemical compounds which are generally associated with providing distinct role in reducing the risk of major chronic diseases in humans such as renal, cardiovascular diseases, and diabetes diseases (Xiao et al., 2011). Flavonoids and alkaloids were reported to be most likely compounds eliciting in vitro cytotoxicity effect (Verma et al., 2010). These phytochemical compounds are also medicinally important to exhibit antioxidant, analgesic, immunomodulatory, anti-arthritic and anti-inflammatory properties (Gill et al., 2011). Flavonoid compounds from Swietenia macrophylla methanolic extract showed antibacterial activity against Escherichia coli and Bacillus cereus (Mursiti, 2017). The antibacterial activity of alkaloids and flavonoids of Aegle marmelos extracts against pathogenic bacteria; S. aureus, K. pneumonia, Pseudomonas aeruginosa and E. coli was reported previously (Jaishree and Kumar, 2016; Ramya et al., 2012). Additionally, Eichornia crassipes-derived flavonoid and alkaloid leaf extracts exhibited antimicrobial activities against E. coli, P. aeruginosa, Bacillus subtilis, Aspergillus flavus, A. niger, C. albicans and Alternaria alternate (Haggag et al., 2017). Alkaloids of the ethanol extract of Tordilla asiatica roots showed antimicrobial activity against S. aureus, K. pneumonia, P. aeruginosa, E. coli, C. albicans, C. tropicalis and Streptococcus mutans (Hu et al., 2014).

The yield of all extracts was calculated (Supplemental information, Table S1). A non-significant difference in the median yield of all tested methanolic extracts was recorded (P = 0.43).

Antioxidant activity

The DPPH scavenging activity and NO scavenging activity were used in this study to determine the antioxidant activity. Polyphenolic compounds, inherent in plant extracts, can elicit a myriad of various biological activities including their antioxidant ability (Hassan et al., 2009). Recently, several reports have highlighted the potential health benefits of polyphenols together with their pharmacological potentials which include anti-inflammatory activity, anti-diabetic, anti-carcinogenic, anti-ulcerogenic and anti-estrogenic (Unuofin et al., 2017). Additionally polyphenols rarely produce little or no toxic effect upon their ingestion (Havsteen, 2002). The secondary metabolites like polyphenols, flavonoids and alkaloids from plants have been reported to be potent free radical scavengers and reactive oxygen species (ROS). They are found in all parts of plants such as leaves, roots, fruits, bark and seeds (Banothu et al., 2017; Tiwary et al., 2015; Vijayaraghavan et al., 2018).

DPPH radical scavenging activity

The scavenging activity on DPPH radical assay is generally a basic method for screening the anti-radical activity of various plant extracts. It is one of the recognized mechanisms by which antioxidants inhibit lipid peroxidation (Halliwell and Gutteridge, 2006). The color of DPPH radical changes from violet to yellow upon reduction, which is demonstrated by the decrease in absorbance at 415 nm. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors. The median DPPH percentages of Varthemia candicans and Suaeda vermiculata were significantly higher than that of other methanolic extracts (P = 0.001). The results of this study demonstrated that the methanolic extracts tested possessed different levels of free radical scavenging activity (Fig. 4; Supplemental information, Table S1). More than 90% DPPH activity was recorded in Varthemia candicans and Suaeda vermiculata, which possessed the highest phenolic constituents (65.48 and 76.88 mg GAE/g dw, respectively). Pluchea dioscoridis maintained relatively reasonable DPPH activity (67.1%) though its elevated phenolic content recorded 54.75 mg GAE/g dw. Therefore, the antioxidant activity could be due to an array of polyphenolics contained in these plant extracts with appreciable amounts of total alkaloids and total flavonoids. Phenolics are the diverse group of molecules containing flavonoids, flavanols and tannins that are present in most of the plant leaves (Vijayaraghavan et al., 2018). Mahmoudi et al. (2016) reported that phenolics, flavonoids and alkaloids have a strong antioxidant activity by scavenging ROS which prevent potential damage to cellular components such as proteins, lipids and DNA. The phenolic compounds which have moieties of multi hydroxyl (OH) group and center of unsaturation, enable them to donate a proton to DPPH to prevent diseases originated from excessive NO production in the body (Moncada and Higgs, 1991). The results of NO scavenging ability of all methanolic extracts are presented in Table 2. Marrubium vulgare, Suaeda vermiculata, Peganum harmala and Salsola vermiculata showed a significant elevation in the NO radical scavenging activity at different concentrations compared to ascorbic acid (P < 0.001). NO scavenging power of all extracts was concentration-dependent, and the highest inhibitory effect (86%) was observed at the concentration of 100 μg/ml. On the other hand, the ascorbic acid (positive control; 100 μg/ml) had 81% inhibitory effect on scavenging of NO radical. Both ascorbic acid and the plant extracts tested showed significant NO radical scavenging activity (P < 0.001) in a concentration-dependent manner. The findings of DPPH and NO antioxidant activity were found consistent with the previously published reports on other plant species extracts (Mahmoudi et al., 2016; Nayak et al., 2017; Vijayaraghavan et al., 2018).

Incidence of microbial infections

Out of 53 clinical swabs, polymicrobial infections were recorded in 33 patients (62.3%), while the remaining cases yielded
monomicrobial infection. Out of 33 patients who had polymicrobial cultures, mixed bacteria alone were recorded in 8 (24.3%) patients, mixed fungi alone were isolated from 6 patients (18.2%), and mixed bacteria and fungi were recorded in 19 patients (57.6%).

The predominant microorganisms of the polymicrobial (bacteria and fungi) infections were S. aureus, K. pneumoniae, P. aeruginosa, and C. albicans.

Drug resistance profiles (DRPs)

DRPs of the polymicrobial strains were investigated and results are presented in Figs. 2 and 6. Of these, 15 bacterial strains were tested against 15 antibacterial agents and also 15 fungal strains were tested against seven antifungals. The bacterial and fungal strains were clustered using the two-way hierarchical dendrogram and the results revealed that 14 clusters (Fig. 2A) and 11 clusters (Fig. 2B) were obtained for bacteria and fungi, respectively. According to our results, we concluded that all strains were MDR except for SA-7, KP-5, CA-3 and AF-1, which were identified as PDR strains. Among the tested antibiotics, all bacterial strains were found to be resistant (100%) to cotrimoxazole (SXT) and tobramycin (TOB). The incidence of resistance against chloramphenicol (C) and amoxicillin (AX) was 80% and 73.3% respectively, while 66.7% of these bacterial strains exhibited resistance to imipenem (IPM), kanamycin (K), colistin sulfate (CT) and tetracycline (TE). In addition, the incidence of resistance against cefotaxime (CTX), ciprofloxacin (CIP) and gentamicin (CN) was 60%. Only 40% of the strains were resistant to ceftazidime (CAZ) and ceftriaxone (CRO) (Fig. 6A). Indeed, all fungal strains were resistant to amphotericin (AMB). Of 15 fungal strains, 80% were resistant to micafungin (MCFG), 73.3% to fluconazole (FLC), 66.7% to terbinafine (TRB), 40% to nystatin (NYT), and 40% to clotrimazole (CLT) (Fig. 6B).

Antimicrobial resistance is a worldwide problem that knows no international boundaries and can spread between continents (Rajpai et al., 2014). The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria and fungi has become a significant threat to public health, as there are fewer, or sometimes even no, effective antimicrobial agents available for infections caused by these clinical pathogens (Magiorakos et al., 2011). The prevalence of PDR among bacterial and fungal clinical isolates is disturbingly high. These findings are alarming because infections with multiply drug-resistant isolates leave clinicians with no treatment options, leading to increased morbidity and mortality. Our findings of highly resistant bacteria and fungi, isolated from burn infections, to commercially antibiotics are in agreement with our previous studies (Ali et al., 2017; El Shafay et al., 2016; El-Shouny et al., 2018; El-Zawawy and Ali, 2016a, b; Khalil et al., 2015; Morsy et al., 2017; Sonbol et al., 2015). In the face of treatment failure with the commercially used antibiotics as well as the emergence of MDR strains, evaluation of medicinal plants is encouraged as an alternative to conventional antibiotics.

Antimicrobial activity

Phenolics and flavonoids have been reported to have antimicrobial activity owing to their ability to interact with cellular enzymes, destabilize cellular membranes and to inhibit cell cycle progression (Sen and Batra, 2012). In this study, both S. aureus 7 (SA-7) and K. pneumonia 5 (KP-5) strains exhibited resistance to all antibacterial tested. In addition, C. albicans 3 (CA-3) and A. flavus 1 (AF-1) showed resistance to all antifungals tested. Therefore, the antimicrobial activities of the nine methanolic extracts were evaluated against these four PDR strains (Table 3 and Fig. 7), where to the best of the authors’ knowledge it has been no report on the antimicrobial activity of these extracts to PDR bacterial and fungal pathogens of skin burn infections, yet. A significant elevation in the inhibition zone diameter was produced by Salsola vermiculata and Suaeda vermiculata compared to other methanolic extracts on the
Table 2
Nitric oxide (NO) radical scavenging activity.

<table>
<thead>
<tr>
<th>Inhibition (%)</th>
<th>Concentration (μg/ml)</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td>61</td>
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<td>80</td>
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<td>0.2</td>
<td>57</td>
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\( ^{*} \)P-value <0.001

Two-way ANOVA with multiple comparison (Holm–Sidak test). \( P \)-value < 0.05 is considered significant.

Fig. 6. Incidence of drug resistance in the bacterial (A) and fungal (B) strains. SA, Staphylococcus aureus; KP, Klebsiella pneumonia; CA, Candida albicans; AF, Aspergillus flavus.
tested PDR strains \((P=0.001, 0.002, 0.001 \text{ and } 0.001)\) (Table 3). On the other hand, *Suaeda vermiculata* showed a better potential antimicrobial activity against four PDR strains tested compared to the *Salsola vermiculata* leaf extract, with an insignificant statistical difference \((P=0.09)\) (Fig. 7). The use of medicinal plants against human pathogens has long been practiced before the advent of antibiotics (Sumner, 2008). Phytochemical products of these plants are still used in modern medicine (Emeka et al., 2012). Our results showed that the Gram-positive strain, SA-7, was more sensitive to inhibition by leaf extracts tested than the Gram-negative strain, KP-5. This phenomenon was previously reported (Mahmoudi et al., 2016; Morsy et al., 2017). It is not known exactly why Gram-negative bacteria are less susceptible, but it may be related to the outer membrane which contains peptidoglycan and lipopolysaccharide. The presence of outer membrane endows the bacterial surface with strong hydrophilicity and acts as strong permeability barrier (Mann et al., 1997).

The MIC and MFC of *Suaeda vermiculata* and *Salsola vermiculata* methanolic leaf extracts ranged from 125 to 250 μg/ml for bacteria and from 125 to 625 μg/ml for fungi, respectively (Fig. 8). The effects of these leaf extracts on growth of the tested four PDR strains support their traditional medicinal value in folk medicine as remedies against a

### Table 3

Antimicrobial activity of nine methanolic extracts against selected four PDR strains.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Median inhibition zone diameter (mm)</th>
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<tr>
<td></td>
<td>SA-7</td>
</tr>
<tr>
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<td><em>Varthemia candicans</em></td>
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<td><em>Marrubium vulgare</em></td>
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<tr>
<td><em>Suaeda vermiculata</em></td>
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<tr>
<td><em>Pluchea dioecoides</em></td>
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<tr>
<td><em>Datura stramonium</em></td>
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<tr>
<td><em>Arthrocnemum glaucum</em></td>
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</table>

\( ^3 \text{P-value } < 0.05 \text{ is considered significant. SA, Staphylococcus aureus; KP, Klebsiella pneumonia; CA, Candida albicans; AF, Aspergillus flavus.} \)

**Fig. 7.** Median inhibition zone (IZ) diameters of methanolic plant extracts on PDR *Staphylococcus aureus* (SA), *Klebsiella pneumonia* (KP), *Candida albicans* (CA) and *Aspergillus flavus* (AF). \( P \)-value < 0.05 is considered significant. \( P_1 \)-value is for *Suaeda vermiculata*; \( P_2 \)-value is for *Salsola vermiculata*.

**Fig. 8.** Minimum inhibitory concentration (MIC) (A) and minimum fungicidal concentration (MFC) (B) values of *Salsola vermiculata* and *Suaeda vermiculata* methanolic leaf extracts against four selected PDR strains. SA, *Staphylococcus aureus*; KP, *Klebsiella pneumonia*; CA, *Candida albicans*; AF, *Aspergillus flavus*.
long list of microbial infections of the skin burns. Makinde et al. (2007) evaluated the use of some crude plant extracts, which inhibited bacterial growth. Additionally, other studies confirmed that plant extracts possess antimicrobial properties (Vieitez et al., 2018), while to the best of our knowledge this is the first report on the evaluation of antimicrobial and antioxidant activities, as well as estimation of total alkaloids, total flavonoids and total phenolics of Salsola vermiculata and Suaeda vermiculata. Therefore, the characterization of Salsola vermiculata and Suaeda vermiculata using GC–MS was further investigated in this study.

GC–MS analysis

The fatty acid composition of any plant extract determines all the chemical properties by using the GC–MS of the fatty acid methyl esters (Yeboah et al., 2017). The fatty acids detected in the Salsola vermiculata and Suaeda vermiculata extracts are known to possess several biological activities (Table 4). To the best of our knowledge, this is the first report to analyze the phytochemical composition of the methanolic extracts of Salsola vermiculata and Suaeda vermiculata. The antimicrobial activity of both extracts could be due to the fatty acids identified in these plants. According to our results, hexadecanoic acid methyl ester was the major identified compound representing 48.49 and 43.69% in Salsola vermiculata and Suaeda vermiculata, respectively. Hexadecanoic acid methyl ester (palmitic acid methyl ester) had been reported to cause autolysis of membranous structures, induce significant aortic dilation, inhibit phagocytic activity and nitric oxide production of certain cells, reduce levels of tumor necrosis factor-alpha (TNFα), prostaglandin E2 (PGE2), and interleukin-10 (IL-10) without affecting ATP levels (Lin et al., 2009; Wang et al., 2010). In addition, major uses of other compounds (8-octadecenoic acid methyl ester; 1,12-tricarboxylic acid-1-hydroxy-1,1-dimethyl ester; 7-hydroxy-octadecatrienoic acid methyl ester and 9,12,15-octadecatrienoic acid (Z,Z,Z)-methyl ester) revealed by GC–MS in Salsola vermiculata and Suaeda vermiculata were also presented in Table 4. Overall, the unique properties of Salsola vermiculata and Suaeda vermiculata methanolic extracts may be potentially applied as a promising source of antioxidant and antimicrobial agents and may be used for the management of drug-resistant pathogens of burn infections.

Conclusions

Phytochemical analysis of the methanolic extracts of nine Egyptian plants confirmed that these extracts were rich in phenolics, flavonoids, and alkaloids. The results also indicated potential antioxidants as well as antimicrobial activities against PDR S. aureus, K. pneumonia, C. albicans, A. flavus strains isolated from skin burns. Based on the results above, it can be obviously observed that Salsola vermiculata and Suaeda vermiculata leaf extracts have attractive antioxidant and antimicrobial activities. This study might provide useful information to guide the application of these leaf extracts in food and pharmaceutical fields. To increase the antioxidant and the antimicrobial potential of methanolic extracts from Salsola vermiculata and Suaeda vermiculata, further in vivo study is currently under investigation after the success of in vitro assessment of purified isolated compounds from Salsola vermiculata and Suaeda vermiculata extracts on bacterial and fungal PDR strains from polymicrobial skin burn infections for treating burns and developing other biomedical applications.

Conflict of interests

The authors have no conflict of interests to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jab.2018.08.001.

References


