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RESEARCH ARTICLE

Assessment of the antimicrobial and free radical scavenging activities of *Moluccella spinosa*, *Helichrysum sanguineum*, and *Styrax officinalis* folkloric medicinal plants from Palestine

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Abstract

The emergence of pathogenic microbes with increased resistance to established antibiotics provides a major incentive for the discovery of new antimicrobial agents. Herbals may provide valuable solutions for this global problem. In addition, the replacement of harmful synthetic antioxidants with natural ones may prevent various serious diseases. The present investigation describes for the first time the antioxidant and antimicrobial activities of the aqueous and organic extracts of *Helichrysum sanguineum*, *Moluccella spinosa* and *Styrax officinalis* plants aerial parts. The free radical scavenging activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl method, while the antimicrobial activity was evaluated against selected microbial strains from American Type Culture Collection and clinical isolates such as *Shigella sonnie*, *Staphylococcus aureus*, *Enterococcuss feacium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans, Epidermophyton floccosum* and Methicillin Resistant *Staphylococcus aureus* (MRSA) using minimum inhibitory concentration assay. A mixture of phytochemical compounds was found in all of the studied plants extracts which also showed remarkable potentials of antioxidant and antimicrobial activities. The current study provides initial data that justify the use and importance of these plants in the Palestinian traditional medicine. In addition, it provides evidence that the aqueous and organic extracts of *H. sanguineum*, *M. spinosa* and *S. officinalis* exhibited interesting antioxidant activity comparing with Trolox. Furthermore, the organic extract of *H. sanguineum* strongly exhibited bacterial growth of *S. aureus*, *E. faecium* and MRSA which suggested to be used as antibiotic alternative or as sufficient natural food preservative.

Keywords *Helichrysum sanguineum* · *Moluccella spinosa* · *Styrax officinalis* · Antibacterial · Antifungal · Antioxidant

Introduction

The use of plants as natural free radical scavenging agents in medicine and in the processed foods have become of increasing importance in the pharmaceutical and food industries as natural and safe alternatives to synthetic antioxidants. However, these natural products are claimed for their protective effects against cardiovascular diseases, certain forms of cancer and photosensitivity reactions (Griffiths et al. 2016; Zamora and Hidalgo 2016). In addition, antibiotic resistance has become a serious global concern, and the discovery of novel antimicrobial natural products may provide a valuable solution to overcome this problem (Laxminarayan et al. 2013).

Herbals can be used to solve these problems, and the traditional medicine is rich with herbal remedies which treated

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various infectious diseases and cure the oxidative damages caused by free radicals in human body.

Moluccella spinosa L., *Helichrysum sanguineum* (L.) Kostel, and *Styrax officinalis* L. are folkloric medicinal plants from Palestine and used in the treatment of some urological infectious diseases and oxidative stress (Viegas et al. 2014; Jaradat 2005).

However, *H. sanguineum* (Compositae family) is commonly known as Red Everlasting, and Blood of the Messiah. It is used in the folk medicine for treatment of various urological infectious diseases and wildly distributed in Palestine, Jordan, Lebanon, Turkey and Syria and (Aslana et al. 2006; Erolu et al. 2010; Mericli et al. 1984). The leaves, stems and flowering heads of *H. sanguineum* contain kaempferol, 5,7-dihydroxy-3,8-dimethoxy flavone, 3,5,7-trihydroxy-6,8-dimethoxy flavone, kaempferol 3-glucoside, quercetin 4′-glucoside flavonoids, pelargonidin 3-glucoside and peonidin 3-glucoside anthocyanins (Mericli et al. 1984).

While, *M. spinosa* plant belongs to the Lamiaceae family and commonly known as Spiny molucca. It is wildly distributed in Palestine and other Mediterranean regions, especially in the roadsides and fallow fields areas (Ronel et al. 2010). The main chemical composition of *M. spinosa* aerial parts essential oil collected in Sicily were α-pinene, caryophyllene oxide and β-caryophyllene. This oil showed slight antibacterial and a moderate antifungal activities (Casiglia et al. 2015).

In addition, the *S. officinalis* (family Styracaceae) used in the traditional medicine for treatment of urinary tract infectious diseases, respiratory system infections, and dermatitis. It is native to southern regions of Europe and wildly distributed in the forests of Palestine (Jaradat 2005; Danin 2004; Ali-Shtayeh et al. 2013). However, the leaves major chemical composition of essential oil were (E)-2-hexenal, octanol and geraniol (Tayoub et al. 2006).

The current investigation aimed to characterize the phytochemical components also to evaluate the antioxidant and antimicrobial effects of *H. sanguineum*, *M. spinosa* and *S. officinalis* plants aqueous and organic extracts. These plants were chosen due to their importance in the Palestinian traditional medicine. In addition, to the best of author's knowledge, the literature survey could not ascertain any antimicrobial and antioxidant studies carried out on the aerial parts of these plants.

Materials and methods

Instrumentation

Shaker incubator (Memmert, Germany), rotary evaporator (Heidolph, Germany), stir-mixer (Tuttnauer, Jerusalem), grinder (Moulinex model, Uno, China), lyophilizers (Mill rock technology, BT85, China), sterile syringe filter hydrophobic, 0.45 µm 25 mm (PTFE, China), weighing scale (Adam Equipments, USA), incubator with CO_2 (Tuttnauer, Jerusalem), balance (Rad wag, AS220/c/2, Poland), multichannel micro-pipet (Eppendorf, Germany) and multichannel micro-pipet (Thermo Fisher Scientific, India).

Chemicals

Ethanol, sodium hydroxide, *n*-hexane, and acetic acid were obtained from Lobachemie, India, and Iron (III) chloride, Millon's reagent, Benedict's reagent, Dimethyl sulfoxide (DMSO) were purchased from Riedeldehan, Germany. While Molish's reagent, sulfric acid, and iodine solution were obtained from Alfa-Aesar, England. Also, chloroform and HCl were purchased from Sigma-Aldrich, Germany. In addition, Magnesium ribbon and Ninhydrin solution were obtained from Alfa Agar, England. Moreover, the nutrient broth and MacConkey agar were brought from Himedia (India), while BBL Mueller–Hinton II broth, Difco Sabouraud Dextrose Agar, and Bacto tryptic soy broth were obtained from Dickinson and company sparks (USA).

Plants materials

The aerial parts of *H. sanguineum*, *M. spinosa* and *S. officinalis* plants were collected in May 2016 from Tulkarem region of the West-Bank Area/Palestine. The taxanomical identifications were established by the pharmacognosist Dr. Nidal Jaradat in the Pharmacognosy Laboratory, Faculty of Medicine and Health Sciences, An-Najah National University. While, the voucher specimen codes were Pharm-PCT-1170, Pharm-PCT-1598 and Pharm-PCT-2365, respectively.

The used plants parts were washed well and dried in the shade at room temperature (25 ± 2 °C), and humidity (55 ± 5 RH). After drying, the used plant materials were grounded well using a mechanical grinder and transferred into airtight containers with proper labeling for future use.

Preparation of plants extracts

A total of 25 g of the powdered plant material was weighed and then extracted by adding 100 ml of *n*-hexane and 150 ml of 50% ethanol in triple distilled water. The mixture was then shaken for 48 h at room temperature using shaker device that was set at 200 rpm. Afterwards, the mixture was filtered using suction flask and Buchner funnel. The obtained filtrate was separated individually by separatory funnel into 2 phases; a lower aqueous phase representing the first aqueous extract and the upper organic phase representing the organic extract. The remaining solid materials were re-extracted separately by 150 ml of 50% ethanol in triple distilled-water and

the re-extraction process was carried out as described above. The aqueous extract was dried using freeze dryer for 48 h. Meanwhile, organic extracts were placed in hood at 25 °C to evaporate leftover organic solvents and completely dried organic extracts till use (Jaradat et al. 2014).

The organic extracts yields of *M. spinosa*, *H. sanguineum* and *S. officinalis* were 17.88, 20.8 and 7% respectively, while the aqueous extracts yields for the same plants were 16.6, 7.25 and 12.04%, respectively.

Bacterial and fungal strains

Antibacterial activity of the aqueous and organic extracts of *M. spinosa*, *H. sanguineum* and *S. officinalis* were examined against 6 reference bacterial strains obtained from the American Type Culture Collection (ATCC), which were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecium* (ATCC 700221) and *Shegilla sonnie* (ATCC 25931) and clinical isolate Methicillin Resistance *Staphylococcus aureus.* (MRSA).In addition, the antifungal activity of aqueous and organic extracts of the same plant extracts were examined against the growth of *Candida albicans* (ATCC 90028) and *Epidermatophyton floccosum* (ATCC 52066).

Phyto-analytical qualitative tests

The *M. spinosa*, *H. sanguineum* and *S. officinalis* aqueous and organic extracts were screened for the presence of major natural phytochemical classes using standard identification analytical tests which described by Harborne (1998), Trease and Evans (1983).

Free radical scavenging assay

For evaluation of the free radical scavenging activity, a stock solution of a concentration of 100 µg/ml in methanol was firstly prepared of each plant extract and Trolox (standard reference compound). The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 µg/ml) were prepared by serial dilution method with methanol from the stock solution.

A fresh DPPH solution was prepared at a concentration of 0.002% w/v then mixed with methanol and the above prepared working concentration in a ratio of 1:1:1, respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 min at room temperature before the absorbance readings were recorded at 517 nm. The percentage of the free radical scavenging activity of the plants and the Trolox standard were calculated using the following formula:

$$\text{Percentage of inhibition of DPPH activity } (\%) = (B - A)/B \times 100\%$$

where B = Absorbance value of the control (prepared as sample solution but using methanol instead of plant extract), S = Absorbance value of the sample.

The free radical scavenging activity half maximal inhibitory concentration (IC_{50}) for the plant samples and the standard were calculated using BioDataFit edition 1.02 (data fit for biologist) (Jaradat et al. 2016).

Antimicrobial Activity

Preparation of plants extracts for antimicrobial activity assessment

Aqueous extract of plant extract was dissolved in sterile distilled water to achieve a concentration of 50 mg/ml solution. Organic extract was dissolved in 10% Dimethyl sulfoxide (DMSO) achieving a concentration of 50 mg/ml. The resulting solutions were sterilized by syringe filter with 0.45 µm pore size (Jaradat et al. 2016).

Broth microdilution method

Detection of antimicrobial activity of both organic and aqueous plant extracts were carried out using broth microdilution method. The applied procedure was similar to that of CLSI (Forbes et al. 2016; Wikler 2007). In Brief, plant extract solutions were serially diluted (twofold) 11 times (up to well 11) with Mueller–Hinton (MH) broth. Well number 11 was considered negative control of bacterial growth (bacterial isolate was not inoculated in it), while well number 12 contained MH broth only and was used as a positive control of bacterial growth. For detection of any possible antibacterial activity of DMSO in broth microdilution method conditions, a serial twofold dilution of DMSO with nutrient broth was prepared with concentration form 0.098 to 50%. The final bacterial concentration in each well (except negative control) was adjusted to 5×10^5 CFU/ml. After inoculation of bacteria, the plates were covered and incubated at 35 °C for 24 h. Each plant extract was examined in duplicate. The lowest concentration of plant extract that did not allow any visible bacterial growth in the test broth was considered minimal inhibitory concentration (MIC) (Jaradat et al. 2016).

Determination of anti-yeast activity of plant extracts

MIC of plant extracts against *C. albicans* was determined by broth microdilution similar to (Forbes et al. 2016; Klepser et al. 1997). In this procedure RPMI1640 was used instead of MHB.

RPMI1640 preparation

A weight of 1.04 g of RPMI powder was dissolved in 90 ml sterile, distilled water. MOP (3.456 g) was added to the solution. The pH of solution was adjusted to 7 at 25 °C by using 1 mol/l NaOH. Then, sterile distilled water was added up to 100 ml. The solution was sterilized by filtration by 0.45 µm syringe filter.

Broth microdilution

A volume of 100 µl of RPMI 1640 broth media was placed in each well. Then, 100 µl of plant extract was placed in first well and mixed. This was followed by 100 µl from first well to second one. This step was repeated up to wells number 11 from which 100 µl was discharged after mixing. Well number 12 contained only RPMI 1640 broth and represents the positive control of yeast growth. The concentrations of plant extract in the micro-wells were assigned to evaluate their antifungal activities ranged from 0.065 to 55 mg/ml (Wikler 2007).

Inoculum preparation

Candida albicans was sub-cultured on sabouraud dextrose agar at 37 °C for 24 h. Five colonies were placed in 5 ml 8.5% saline. Then, cell density was adjusted to 0.5 McFarland's standard (0.08–0.1 absorbance at 625 nm) to obtain a yeast concentration 1×10^6 to 5×10^6 CFU/ml. This suspension was of *C. albicans*, was diluted 1:50 and then 1:20 with broth medium (RPMI 1640 broth), which results in 1×10^3 to 5×10^3 CFU/ml, from which 100 µl was pippeted in each well except well number 11, which represented negative control of yeast growth. All of the inoculated plates were incubated at 35 °C. The incubation period was 48 h. The final concentrations of microbial cells were about $0.5–2.5\times10^3$ colony-forming unit (CFU)/ml (Jaradat et al. 2016; Wikler 2007).

Determination of anti-mold activity

Epidermatophyton floccosum mold inhibition by plant extract was evaluated by agar dilution method (Forbes et al. 2016; Falahati et al. 2005). Sabouraud dextrose agar (SDA) was placed in tubes and kept at 40 °C water bath after sterilization by autoclave. Plant extracts were serial diluted with SDA. The prepared concentrations were from 0.78 to 25 mg/ml for aqueous extracts, and organic extracts. Then, the prepared tubes were allowed to solidify in slanted position at room temperature. A suspension with turbidity similar to 0.5 McFarland standard was prepared from fresh culture of *E. floccosum*. Then 20µl of the suspension of was added to all tubes. Tubes with SDA only were used as positive control of the mold. Results were taken after 10 days of incubation at 25 °C. Minimum inhibitory concentration was the lowest concentration that completely inhibits the growth of *E. floccosum* (Forbes et al. 2016).

Statistical analysis

Free radical scavenging activity was determined in the aqueous and organic extracts from *M. Spinosa*, *H. Sanguineum* and *S. officinalis* in triplicates. Results were expressed as means ± standard deviation (SD).

Results

Phytochemical analytical tests

Phytochemical standard analytical tests of the aqueous and organic extracts of *M. Spinosa*, *H. Sanguineum* and *S. officinalis* revealed that the plants both extracts contained mixtures of primary and secondary metabolic compounds. The preliminary phytochemical screening of the aqueous extract of *M. Spinosa* revealed the presence of cardiac glycosides, saponin glycoside, phenols, volatile oils, tannins, steroids and flavonoids, while its organic extract contained saponin glycoside, phenols, tannins, steroids and flavonoids. Moreover, the aqueous extract of *H. Sanguineum* contained saponin glycoside, phenols, tannins, steroids and flavonoids, while its organic extract contained cardiac glycosides, starch, phenols, volatile oils, tannins, steroids and flavonoids. Additionally, the aqueous extract of *S. officinalis* contained alkaloids, cardiac glycosides, phenols, saponin glycoside, volatile oils, tannins, steroids and flavonoids. Moreover, its organic extract contained cardiac glycosides, starch, volatile oils, steroids and flavonoids as presented in Table 1.

Free radical scavenging activity

The free radical scavenging activity for the studied plants species was established in comparison with Trolox which considered a reference molecule for the evaluation of the antioxidant activity. The studied plants species showed potential free radical scavenging properties in comparison with the used potential reference compound Trolox which had IC_{50} value of 2.8 µg/ml. However, the aqueous extract of *M. spinosa* had the best antioxidant activity with IC_{50} value 5.6 ± 0.09 µg/ml. The *M. Spinosa*, *H. Sanguineum* and *S. officinalis* plants antioxidant activity experiments results are shown in Table 2 and Figs. 1, 2 and 3.

Table 1 Phytochemical analytical tests for the aqueous and organic extracts of the studied three plants

Phytochemical compounds	Aqueous extract			Organic extract		
	M. Spinosa	*H. Sanguineum*	*S. officinalis*	*M. Spinosa*	*H. Sanguineum*	*S. officinalis*
Cardiac glycosides	+	−	+	+	+	+
Saponin glycoside	+	+	+	−	−	−
Alkaloids	−	−	+	−	−	−
Protein	−	−	−	−	−	−
Starch	−	−	−	+	+	+
Phenols	+	+	+	−	+	−
Volatile oil	+	−	+	+	+	+
Tannin	+	+	+	−	+	−
Steroids	+	+	+	+	+	+
Flavonoid	+	+	+	−	+	+

Table 2 Antioxidant IC_{50} (µg/ml) values for each organic and aqueous extracts of three investigated plants

Plants	IC_{50} (µg/ml)	
	Organic extracts (±SD)	Aqueous extracts (±SD)
M. spinosa	11 ± 0.61	5.6 ± 0.09
H. sanguineum	8.6 ± 0.72	11.2 ± 0.45
S. officinalis	15.5 ± 0.69	11.7 ± 0.53

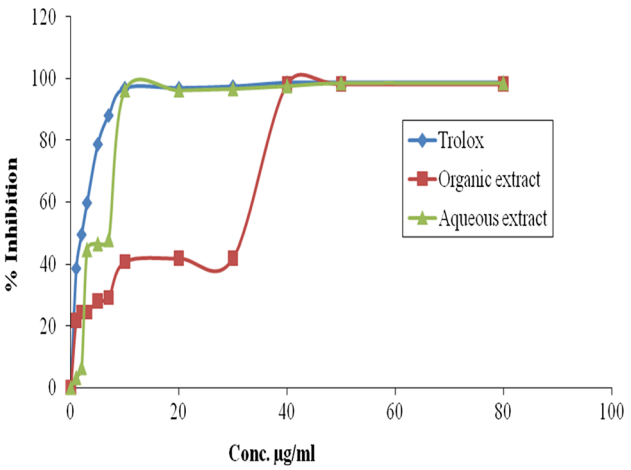

Fig. 1 Inhibition activity of Trolox, the organic and aqueous extracts of *M. Spinosa*

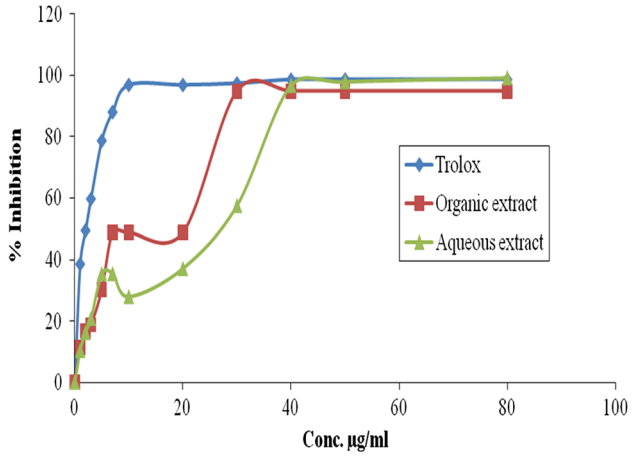

Fig. 2 Inhibition activity of Trolox, the organic and aqueous extracts of *H. Sanguineum*

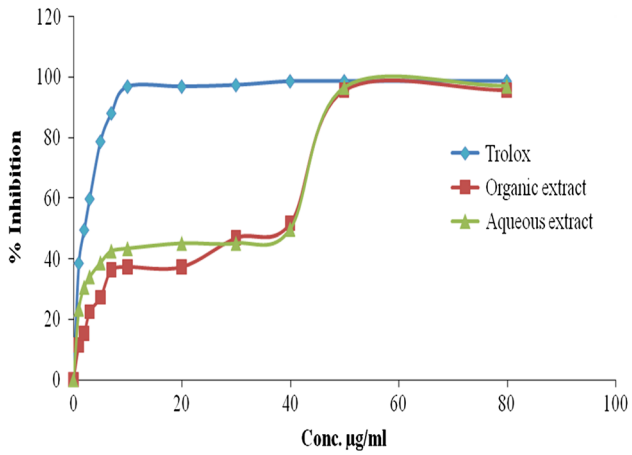

Fig. 3 Inhibition activity of Trolox, the organic and aqueous extracts of *S. officinalis*

Antimicrobial analysis

The majority of *H. sanguineum*, *M. spinosa* and *S. officinalis* aqueous and organic extracts exhibited bacterial and fungal studied isolates. The organic extract of *H. sanguineum* showed the best antibacterial effect against *E. faecium* and

MRSA with MIC values 0.39 and 0.78 mg/ml, respectively as shown in Table 3.

Discussion

Nowadays, WHO announced that the antimicrobial resistance is considered a global public health problem that threatening the effective prevention and the treatment of huge ranges of infections caused by bacteria, fungi, viruses and parasites. However, without effective antimicrobial medications, the success of cancer chemotherapy and major surgery would be compromised. In addition, the patients suffering from resistant infections, their health care costs are higher than care for patients with non-resistant infections due to additional required tests, longer duration of illness, and use of very expensive antimicrobial medications. For example, about half million patients globally developed multidrug resistant Tuberculosis each year, and drug resistance is starting to complicate the fight against malaria and, HIV as well Antimicrobial resistance [http://www.who.int/mediacentre/factsheets/fs194/en/].

Recently, there has been a great deal of attention toward the field of antioxidant molecules, since the imbalances between free radicals and antioxidants are necessary for proper healthy physiological function because free radicals adversely alter lipids, proteins, and DNA and trigger a number of physiological diseases and the consumption of antioxidant supplements can assist in reducing the oxidative stress. Meanwhile, the chemical synthetic antioxidants as butylated hydroxyanisole and butylated hydroxytoluene have recently been reported to be harmful to human health. For this reason, the investigation for non-toxic and effective natural supplements with antioxidant activity has been intensified in recent years (Lobo et al. 2010).

On the other hand, the *H. sanguineum* aqueous extract showed the best antibacterial activity against *P. aeruginosa*, *E. faecium* and MRSA with MIC's 3.125, 6.25 and 6.25 mg/ml, respectively. Furthermore, the best antibacterial results of organic extract of *M. spinosa* was against *S. aureus* with MIC 3.125 mg/ml and the best antibacterial results of aqueous extract was against *P. aeruginosa* with MIC 3.125 mg/ml followed by *E. faecium*, *S. aureus* and MRSA with MIC 6.25 mg/ml for all pathogens. Meanwhile, the organic extract of *M. spinosa* did not exhibited fungal growth of *C. albicans* and *E. floccosum*, while its aqueous extract exhibited fungal growth of *E. floccosum* and *C. albicans* with MIC values of 12.5 mg/ml and 25 mg/ml, respectively.

In addition, the antibacterial and antifungal activities of the organic *S. officinalis* extract were the best against *S. aureus*, *E. faecium* and *C. albicans* with MIC 12.5 mg/ml for all pathogens. Meanwhile, the aqueous extract of *S. officinalis* was the best against the growth of MRSA and *P. aeruginosa* with MIC 3.125 and 6.25 mg/ml, respectively.

However, the best growth inhibitor against *S. aureus* was for the organic extract of *H. sanguineum* with MIC value of 0.195 mg/ml, as well as against *E. coli* were the aqueous and organic extracts of *M. spinosa* and *H. sanguineum* with MIC 12.5 mg/ml for both plants species both extracts. In addition, the best growth inhibitors against *P. aeruginosa* were the aqueous extracts of *M. spinosa*, *H. sanguineum* and *S. officinalis* with MIC's 3.125 mg/ml, 3.125 mg/ml and 6.25 mg/ml, respectively.

It is plausible to say that the best antibacterial extracts against the growth of *S. sonnie* were *M. spinosa* and *H. sanguineum* aqueous extracts with MIC value 12.5 mg/ml for both plants extracts and the best antibacterial extract against the growth of *E. faecium* was the organic extract of *H. sanguineum* (MIC 0.39 mg/ml) also the best antibacterial extracts against the growth of MRSA was also the organic extract of *H. sanguineum* (MIC 0.78 mg/ml). Moreover, the best extract against *C. albicans* and *E. floccosum* was the organic extracts of *H. sanguineum* (MIC 6.25 mg/ml against the both studied fungi).

Table 3 Antimicrobial activity of *H. sanguineum*, *M. spinosa* and *S. officinalis* plants aqueous and organic extracts

Bacterial and fungal isolates	MIC value (mg/ml)					
	Aqueous extracts			Organic extracts		
	M. spinosa	*H. sanguineum*	*S. officinalis*	*M. spinosa*	*H. sanguineum*	*S. officinalis*
S. aureus	6.25	12.5	12.5	3.125	0.195	12.5
E. coli	12.5	12.5	25	12.5	12.5	NI
P. aeruginosa	3.125	3.125	6.25	NI	NI	NI
S. sonnie	12.5	12.5	25	NI	NI	NI
E. faecium	6.25	6.25	12.5	25	0.39	12.5
MRSA	6.25	6.25	3.125	25	0.78	NI
C. albicans	25	25	NI	NI	6.25	12.5
E. floccosum	12.5	NI	25	NI	6.25	25

NI no inhibition

Under these circumstances, from all the studied plants' species whether they were organic and aqueous extracts, the most promising antimicrobial results were obtained from the organic extract of *H. sanguineum* which strongly exhibited bacterial growth of *S. aureus*, *E. faecium* and MRSA with MIC's 0.195, 0.39 and 0.78 mg/ml, respectively. Obviously, it is also strongly exhibited fungal growth of *C. albicans* and *E. floccosum* with MIC 6.25 mg/ml in both studied fungi.

In a study conducted by Casiglia et al. (2015) on the essential oil of *M. spinosa,* found that this oil exhibited the growth of *S. aureus*, *E. coli*, *P. aeuriginosa* and *C. albicans* with MIC's 10, 5, 10 and 10 mg/ml, respectively.

Another study conducted by Lourens et al. (2004) investigated that *H. excisum*, *H. felinum* and *H. petiolare* against *Staphylococcus aureus* and had MIC values of 2 mg/ml, 0.25 mg/ml and 0.625 mg/ml, respectively.

Meanwhile, in a study which was conducted by Bertanha et al. (2013) found that *Styrax pohlii* ethanol extract exhibited the growth against *P. aeruginosa* with MIC value of 0.4 mg/ml.

Regarding the free radical scavenging properties, the results revealed that *H. sanguineum*, *M. spinosa* and *S. officinalis* organic and aqueous extracts have antioxidant potentials. The best free radical scavenging potential was for the aqueous extract (the lowest IC_{50}) of *M. Spinosa*, which had IC_{50} value 5.6 µg/ml and the organic extract of *H. Sanguineum* which had IC_{50} value 8.6 µg/ml.

Meanwhile, the organic extract of *M. spinosa* and the aqueous extracts of *H. sanguineum* and *S. officinalis* have almost the same free radical scavenging potentials with IC_{50}'s 11, 11.2 and 11.7 µg/ml, in comparison with the reference compound Trolox which had IC_{50} value of 2.8 µg/ml.

In comparison with another study which conducted by Süzgeç-Selçuk and Birteksöz (2011), on the antioxidant activity of another species of Helichrysum (*H. chasmolycicum*) found that its aqueous extract had antioxidant with IC_{50} value of 920 µg/ml. Meanwhile, another study performed by Tepe et al. (2005) found that the antioxidant activity of the *H. chionophilum* aqueous extract had an IC_{50} of 40.5 µg/ml.

Another study established by Albayrak et al.(2010), investigated that the antioxidant (IC_{50} value) of the *H. stoechas* aqueous extract was 7.95 µg/ml, also in a study which was conducted by Lourens et al. (2004) found that the methanolic extract of *H. excisum* has antioxidant activity with IC_{50} value of 13.67 µg/ml.

In comparison with the studied *H. sanguineum* extracts which has antioxidant IC_{50} value 8.6 and 11.2 µg/ml, organic and aqueous extracts, respectively. Which means that *H. sanguineum* studied species has better antioxidant potential than *H. chasmolycicum*, *H. chionophilum* and *H. excisum*. Meanwhile, *H. stoechas* has higher antioxidant activity than the studied *H. sanguineum* extracts.

Moreover, in a study established by de Almeida Silva et al. (2016), revealed that *S. camporum* and *S. ferrugineus* have antioxidant activity with IC_{50} 18.47 µg/ml and 24 µg/ml, respectively.

In brief, to the best of our knowledge, the literature survey could not ascertain any antimicrobial and antioxidant studies which were carried out on the aerial parts of *H. sanguineum*, *M. spinosa* and *S. officinalis* to approve their uses in folk medicine. Finally, the organic and aqueous extracts of *H. sanguineum*, *M. spinosa* and *S. officinalis* have promising antioxidant and antimicrobial potentials and further analytical and clinical studies required to isolate the active molecules from these plants and to evaluate their pharmacological effects clinically.

Conclusion

The present study provides evidence that the aqueous and organic extracts of *H. sanguineum*, *M. spinosa* and *S. officinalis* exhibited interesting antioxidant activity in comparison with Trolox. Because of the side effects of the chemical molecules which used as antioxidants today, there is increasing interest in the use of natural antioxidant products in the pharmaceutical and food industries. Furthermore, the organic extract of *H. sanguineum* strongly exhibited bacterial growth of *S. aureus*, *E. faecium* and MRSA which can be used as antibiotic alternative or as natural food preservative.

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Authors' contributions NJ wrote and drafted the manuscript. NJ, MM. FH, AZ, KS, FA, AS and AE carried out experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Ethical statement N/A.

Conflict of interest The authors declare that they have no competing interests.

Consent for publication All authors gave their consent for the publication of the manuscript for Nidal Jaradat to be the corresponding author.

Availability of data and materials All data generated or analyzed during this study are included in this published article.

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