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Antimicrobial activities of six plants used in Traditional Arabic Palestinian Herbal Medicine

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Ethanollic extracts of six plants: *Arum palaestinum* Bioss, *Urtica pilulifera* L., *Thymbra capitata* (L.) Cav., *Origanum syriacum* L., *Teucrium creticum* L., and *Teucrium polium* L., used in Traditional Arabic Palestinian Herbal Medicine were evaluated for their antibacterial, anti-candida, and antidermatophyte activities using well diffusion, micro-dilution and food poisoned techniques. The extracts were tested against: six bacterial strains including *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae*; five *Candida albicans* isolates, and two dermatophytes: *Microsporum canis*, and *Trichophyton rubrum*. The most active plants extracts were *T. capitata* and *O. syriacum* against the tested bacteria, while the remaining plant extracts did not express any activity or exhibited only very low activity against tested bacteria species and candida isolates. *O. syriacum* was also the most active plant against all *Candida* strains with inhibition zones that ranged from 22.5 to 29.5 mm. On the other hand, *T. capitata* extract showed the highest activity against the test dermatophytes (producing a complete inhibition at $\leq 45 \mu\text{g/mL}$).

Key words: Medicinal plants, antibacterial activity, antifungal activity, *Arum palaestinum*, *Urtica pilulifera*, *Thymbra capitata*, *Origanum syriacum*, *Teucrium creticum*, *Tecrium polium*.

INTRODUCTION

In Palestine, the screening of flora for pharmacological active compounds started in the late nineties (Ali-Shtayeh et al., 1998) and continue to provide useful means for treating ailments (Ali-Shtayeh and Jamous, 2008). Little scientific research has been carried out to investigate the plants used in Traditional Arabic Palestinian Herbal Medicine (TAPHM) (Ali-Shtayeh et al.,

1998, 2003). In the course of our investigations we found that several plants of the Palestinian ethno- medicine possess interesting biological activities, which could be of interest for all parts of the world. The activities have been selected because of their strong medicinal relevance (Saad et al., 2005; Abu-Lafi et al., 2007).

Arum palaestinum Bioss (Araceae) is considered edible

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after being soaked in salty water or dried. The plant is also used in folk medicine to treat several diseases such as stomach acidity, atherosclerosis, cancer, diabetes and food toxicity (Ali-Shtayeh et al., 1998). Phytochemical and biological investigations carried out on the plant have shown that it possesses an inhibitory effect on smooth muscle contraction in rats (Afifi et al., 1999), anticancer activity against lymphoblastic leukemia due to the presence of a pyrrole alkaloid (El-Desouky et al., 2007), and antioxidant activity (Husein et al., 2014).

Urtica pilulifera L. (Urticaceae), has long been used in traditional medicine in many countries around the world to treat various ailments including: sore joints, rheumatism, hemorrhage, renal stones and inflammation of the bladder, diabetes mellitus and other ailments (Kavalali et al., 2003; Lopatkin et al., 2005; Irshid and Mansi, 2009).

Thymbra capitata (L.) Cav. (Lamiaceae) is an aromatic plant found in Palestine and locally known under the common name Zaa'tman. The essential oil of the plant was reported to have antimicrobial activities most of which are mediated by thymol and carvacrol (Bhaskara et al., 1998). The plant is also important as a source of perfume, cosmetics, flavoring and pharmaceutical industries (Tabata et al., 1988). In traditional food the plant has been used for its flavors as refreshing drink or in cooking. It is used in folk medicine against cold, influenza and throat infection (Ali-Shtayeh and Jamous, 2008). Later on the plant was found to contain antiseptic and antimicrobial agents (Bremnes, 2002).

Origanum syriacum L. (Lamiaceae) is one of the most popular herbs among Palestinians (Ali-Shtayeh and Jamous, 2008). The green leaves of the herb are rich in essential oil, which is responsible for its characteristics of flavor and fragrance. Oil of cultivated *O. syriacum* is an important commercial product and is obtained mainly by steam distillation of the fresh leaves. Plant extract is also found to have strong biological activity, and this may be due to the presence of phenols, thymol and carvacrol as major constituents of thyme oil in the plant (Abu-Lafi et al., 2007).

Teucrium creticum L. (Lamiaceae) is found in Palestine and locally known under the common name Ja'adh. The plant is used traditionally to cure diabetes in Palestine (Ali-Shtayeh et al., 2012). No phytochemical studies were found on this plant, and this may be attributed to its limited distribution in Palestine (Saad et al., 2005).

Teucrium polium L. (Lamiaceae) is found in Palestine and locally known under the common name Ja'adhat al-sibian. The plant is well known for its diuretic, antipyretic, diaphoretic, antispasmodic, tonic, anti-inflammatory, antihypertensive, anorexic, analgesic (Saad et al., 2005; Tariq et al., 1989) antibacterial (Mansouri et al., 1999) and antidiabetic effects (Esmaeili and Yazdanparast, 2004). Hot infusion of tender parts of plant is taken for stomach and intestinal troubles (Panovska and Kulevanova, 2005). The plant shows antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*

(Lemordant et al., 1997). Plants belonging to the genus *Teucrium* have been shown to contain different classes of compounds such as fatty acid, esters, terpenes, flavonoids and polyphenolics (Harborne et al., 1986; Rizq et al., 1986).

In this study, the ethanolic extracts prepared from six Palestinian medicinal plants have been investigated for their antibacterial and antifungal activities by means of the agar diffusion, micro-dilution and food-poisoned methods. In addition, the current study reports values of minimum inhibitory concentration (MIC) for active plants extracts.

MATERIALS AND METHODS

Chemicals

The chemicals used included chloramphenicol, peptone, agar, dextrose, ethanol, Muller-Hinton (Fluka), Sabouraud dextrose agar (Difco), gentamicin, ampicilline, amphotericin B, econazole, ethanol, and DMSO. All chemicals and reagents were of analytical grade.

Plant material

Six medicinal plant species *A. palaestinum* (Voucher number BERC-C0064), *U. pilulifera* (BERC-C0066), *T. capitata* (BERC-C0245), *O. syriacum* (BERC-C0026), *T. creticum* (BERC-C0173), and *T. polium* (BERC-C0167), screened in this study were collected from April-June 2013 from Nablus region and were identified by Prof. M. S. Ali-Shtayeh from the Biodiversity and Environmental Research Center, BERC, Til Village, Nablus. Voucher specimens are deposited in the Herbarium of BERC.

Plants extracts preparation

Fifty grams of each dried plant was ground using a Molenix (Mooele Depose type 241) for a minute and the resulting powder was extracted by continuous stirring with 200 ml 70% ethanol at 24°C for 72 h. Extracts were filtered through Whatman No. 4 filter paper, and the residue was then washed with additional 50 ml ethanol. The combined ethanol extracts were dried using rotary evaporator followed by freeze drying and stored at -20°C for future use.

Microorganisms used and growth conditions

Six bacterial strains from the American Type Culture Collection (ATCC; Rockville, MD, USA) were employed. They included Gram-positive (G +ve) bacterium: *Staphylococcus aureus* (ATCC 25923), and the following Gram-negative (G -ve) bacteria: *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883).

Five *Candida albicans* strains were used, three of which were clinical isolates from cutaneous candidiasis patients (BERC-N43, BERC-N72, and BERC-N66), and the other two from CBS type cultures: CBS6589, and CBS9120.

Two dermatophytes strains: *Microsporum canis* (CBS132.88), and *Trichophyton rubrum* (BERC-EH-TR9, clinical strain from a tinea pedis patient) were also used.

Disk diffusion method

Disc diffusion method was made according to Zongo et al. (2009).

Dried plant extracts were dissolved in DMSO to a final concentration (100 mg/mL) and sterilized by filtration through a 0.45 µm membrane filter. Inoculums (10^6 bacterial cells/mL) were spread on Muller-Hinton agar plates (1 mL inoculum/ plate). Filter paper discs (6 mm in diameter) were individually impregnated with 40 µL of each plant extract, and controls, and placed onto the surface of inoculated Petri dishes. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 2 h and incubated after at 37°C for 24 h for bacteria growth. After incubation, the diameters (mm) of inhibition zones were measured including diameter of discs. The antimicrobial potentials were estimated according to the index reported by Rodriguez et al. (2007). All the experiments were done in triplicates. Gentamicin (10 mg/mL) and DMSO served as a positive and negative control, respectively.

Well diffusion method

Anti-*Candida* susceptibility testing was done using well-diffusion method to detect the antifungal activities of plant samples (Perez et al., 1990). A sterile swab was used to evenly distribute fungal culture over Muller-Hinton agar plates supplemented with glucose- methylene blue. The plates were allowed to dry for 15 min before use in the test. Wells were then created and 50 µL of the crude extract (100 mg/mL) of each plant extract were pipetted into each well. The same extract was used in each plate; with a total of two plates used for each extract including two wells for the positive and negative controls. The plates were incubated at 37°C for 24 h after which they were examined for inhibition zones (Delahaye et al., 2009). All the experiments were done in triplicates. Amphotericin B (32 µg/mL) and DMSO were used as positive and negative controls, respectively.

Micro-dilution Test

Broth micro-dilution was performed following the CLSI M27-A2 method (NCCLS, 2002) with little modifications. Muller-Hinton media (pH 7.2) was used for bacteria, while Muller-Hinton supplemented with glucose-methylene blue was used for *Candida*. Plant extracts were dissolved in DMSO and the correct volume was pipetted in the first micro-well plate with Muller-Hinton, for the concentration of each plant extract to be 25 mg/mL in that well. The cell suspension was prepared in 0.85% saline, with an optical density equivalent to 0.5 McFarland standards, and diluted 1:100 in the media to obtain a final concentration of 1×10^4 to 5×10^4 colony-forming units per milliliter (CFU/mL). This suspension was inoculated in each well of a micro-dilution plate previously prepared with the plant extracts to give concentrations from 25 mg/mL down to 0.012 mg/mL (Scorzoni et al., 2007). The plates were incubated with agitation at 37°C for 24 h for all species. The control drugs were gentamicin for bacteria strains, and amphotericin B for *Candida*, respectively. Concentrations of controls were ranged from 250-1 µg/mL for gentamicin, and from 16.0-0.125 µg/mL for amphotericin B. Value of minimum inhibitory concentration (MIC), determined by broth macro- dilution, and defined as the lowest concentration of the drug completely inhibited the growth of the isolate. For plant extracts, MIC value was defined as the lowest concentration able to inhibit any visible bacterial or candidal growth. Results were read visually and spectrophotometrically.

Antidermatophytes testing

Plants extracts were tested at different concentrations for their antidermatophytes activity against the test pathogens using a modified poisoned food technique (Dikshit and Husain, 1984). Different amounts of each extract were incorporated in pre-sterilized

SDA medium to prepare a series of concentrations of the extract (15, 30, 45, and 60 µg/mL). A mycelial agar disk of 5 mm diameter was cut out of 12 days old culture of the test dermatophytes and inoculated onto the freshly prepared agar plates. In controls, sterile distilled water was used in place of the tested sample as a negative control, while econazole (5 µg/mL) was used as the positive control. Three replicate plates were used for each treatment (concentration). The inoculated plates were incubated in the dark at 24°C and the results were recorded after 10 days.

Percentage of mycelial inhibition was calculated using the following formula:

$$\% \text{ mycelial inhibition} = (dc - ds / dc) \times 100\%$$

Where, dc is the colony diameter of the negative control and ds is colony diameter of the sample.

RESULTS

Six medicinal plant species belonging to 3 families were selected based on their uses in Traditional Arabic Palestinian Herbal Medicine (TAPHM) for the treatment of various ailments. The antibacterial, anti-*Candida* and antidermatophytes activities of ethanolic extracts of the selected plants were screened in this study.

Antibacterial activity

The ethanolic extracts of six Palestinian plants were subjected to a preliminary screening for antimicrobial activity against six human pathogenic bacteria *S. aureus*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. typhi*. The inhibition zones of the plant extracts against different types of bacteria strains and MIC values of plants extracts are presented in Table 1. Gentamicin was used as a positive control. Two plant extracts (*T. capitata*, and *O. syriacum*) inhibited the growth of all tested bacteria strains. The MIC values for *T. capitata* were 390 µg/mL for *Pr. vulgaris*, *E. coli*, and *K. pneumonia* while for *O. syriacum* it was 390 µg/mL for *K. pneumonia*. Other plants extracts revealed very low or no activity against different types of bacteria (Table 1).

Anti-candida activity

The anticandida activity of tested plants extracts was evaluated according to their mean of inhibition zone (100 mg/mL) against various candida isolates, and the MIC values of active plants extracts against the five isolates of *Candida*. Results of inhibition zone were compared with the activity of standard amphotericin B (32 µg/mL). *Origanum syriacum* and *T. capitata* exhibited strong and moderate inhibition activity against candida isolates, while the other 4 plant extracts show no activity (Table 2). The MIC value for *O. syriacum* is 150 µg/mL

Table 1. Inhibition zones (mm) of plant extract against different types of bacteria strains using the disc diffusion method.

Microorganism	Inhibition zone (mm±SD)			MIC (µg/mL)		
	<i>T. capitata</i>	<i>O. syriacum</i>	Positive control	<i>T. capitata</i>	<i>O. syriacum</i>	Positive control
<i>P. vulgaris</i>	13.38± 0.7*	13.8± 0.7	23.3± 0.7	390	780	7.8
<i>S. aureus</i>	14.3± 0.9	15.0± 1.3	23.7± 0.5	780	780	3.9
<i>E. coli</i>	15.2± 0.7	14.5± 0.5	23.8± 0.7	390	780	7.8
<i>P. aeruginosa</i>	15.2± 1.6	14.7± 1.5	24.5± 0.5	780	780	7.8
<i>S. typhi</i>	14.5± 0.8	12.8± 0.7	23.2± 0.7	780	780	7.8
<i>K. pneumoniae</i>	15.8± 1.1	15.5± 1.0	24.8± 1.6	390	390	3.9

*Values of inhibition zone diameter in mm ± SD.

Table 2. Inhibition zone (mm) and MIC values of tested extracts against *Candida albicans isolates* compared to amphotericin B.

<i>Candida albicans</i> isolates	Inhibition zone (mm)			MIC (µg/ml)		
	<i>T. capitata</i>	<i>O. syriacum</i>	Amphotericin B	<i>T. capitata</i>	<i>O. syriacum</i>	Amphotericin B
BERC-N43	13.5± 1.5*	22.5± 2.5	31.0± 1.7	310	310	8
BERC-N72	17.0± 0.0	22.5± 0.5	30.5± 0.5	310	625	8
BERC-N66	20.0± 0.0	29.5± 0.5	31.5± 0.5	310	310	8
CBS6589	19.0± 2.0	25.0± 0.5	33.5± 0.5	625	310	2
CBS9120	17.5±0.5	24.5±0.5	29.5± 0.5	310	150	2

*Values of inhibition zone diameter in mm ± SD.

Table 3. Mean of % inhibition ±SD of fungi at 45 µg/mL concentration.

Plant	% Inhibition	
	Dermatophyte	
	<i>Trichophyton rubrum</i>	<i>Microsporum canis</i>
<i>Arum palaestinum</i>	49.6± 1.4	62.6± 3.5
<i>Urtica pilulifera</i>	81.3± 1.3	88.6± 1.1
<i>Thymbra capitata</i>	100± 0.0	100± 0.0
<i>Origanum syriacum</i>	93.6± 0.4	84.3± 1.7
<i>Teucrium creticum</i>	71.6± 0.5	66.3± 0.8
<i>Teucrium polium</i>	89.6± 1.3	100± 0.0
Econazole (5 µg/ml)	100± 0.0	100± 0.0

against the type culture isolate, while it was 390 µg/mL against the clinical isolates.

Antidermatophyte activity

The antidermatophyte activity of the tested plants extracts was expressed as the means of % inhibition values of dermatophytes growth. Four different concentrations of plants extracts concentrations were tested. The percent of inhibition of the two dermatophytes ranged between 49.6-100%, with *T. capitata* exhibiting a significant % of

inhibition (100%) against the two dermatophyte species at a concentration of 45 µg/mL comparable to the positive control (Table 3).

DISCUSSION

Substances derived from plants have recently attracted much attention with regard to human health, due to their low cost, broad availability and limited toxicity. Thus, plant based antimicrobial compounds have vast therapeutically potential as they can serve the purpose

without any side effects that are often associated with synthetic drugs.

Antimicrobial activity

The primary results of bioassays for plants extracts open the possibility of finding new clinically effective antimicrobial compounds, such as the extract of *Micromeria nervosa* which is found in different locations of Palestine and are well known plant used for various medical purposes (Ali-Shtayeh et al., 1997).

Antimicrobial activity of plant materials can be classified according to their MIC values. MIC values lower than 500 indicate the strong antimicrobial activity. Furthermore, MIC values equal to 600-1500 µg/mL and higher than 1600 µg/mL indicate intermediate and weak antimicrobial activities respectively (Durate et al., 2007; Fabri et al., 2009; Michielin et al., 2009). Therefore, it was clear from the results of inhibition zone that the most pronounced activity against all test bacteria with inhibition zones ≥ 12 mm at a concentration of 100 mg/mL was shown by the ethanolic extracts of *O. syriacum* and *T. capitata*. The majority of the remaining plant extracts did not express any activity (Table 1). This tends to show that the biological activities of the plant extracts are different from one plant to another. The absence of antibacterial activity of ethanolic extracts of those plants indicates either the absence of active chemical compounds or the insolubility of the active ingredients in the solvent used.

The activity of *T. capitata* and *O. syriacum* might be attributed to the active compounds in their essential oils. *O. syriacum* is an aromatic, perennial, herbaceous plant. The plant has been reported to pose antibacterial activity (Leja and Thopil, 2007). The major volatiles and semi-volatiles of Palestinian wild *O. syriacum* are α -phelandrene, α -pinene, β -myrecene, *o*-cymene, *p*-cymene, *c*-terpinene, thymol, and carvacrol (Abu-Lafi et al., 2008). The plant has a long history of medicinal use among Palestinian (Ali-Shtayeh and Jamous, 2008) as well as by the Creks as an antidote to poisoning and snake venom, by the Romans for stomach disorders and more recently for digestive, antispasmodic and sedative properties (Evans, 2002).

T. capitata essential oil is traditionally considered to exhibit powerful antiseptic properties, thus being used to treat cutaneous infections (Palmeira-de-Oliveira et al., 2012). Essential oils of *T. capitata* were analyzed using gas chromatography (GC) in combination with retention indices (RI), gas chromatography-mass spectrometry (GC-SM) and ¹³C NMR spectroscopy. Carvacrol (68.2%-85.9%) was the major component of the plant, while the content of thymol (0.1-0.3%) was very low. Other components present in appreciable amounts were gamma-terpinene (up to 8.9%), *p*-cymene (up to 7.1%), linalool (up to 4.4%) and (E)-beta-caryophyllene (up to 4.1%) (Bakhy et al., 2013). Thus, the antimicrobial activity

of *T. capitata* can be attributed mainly to the presence of carvacrol in the plant (Ali-Shtayeh et al., 1997) such as carvacrol, thymol, *p*-cymene and terpinene.

Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of antibacterial compounds from these plants and also to determine their full spectrum of efficacy. However the present study of *in vitro* antibacterial evaluation of some extracts forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

Anti-Candida activity

The results reveal that extracts of *T. capitata* and *O. syriacum* are potent antimicrobials against all the microorganisms studied. *O. syriacum* showed inhibition zone of 29.5 \pm 0.5 mm against BERC N66, (94% of amphotericin B activity) and *T. capitata* (inhibition zone 20.0 mm) against the same genotype of *Candida*. Other extracts did not show significant activity against the studied microorganisms (Table 3). It is noteworthy to mention that plants belonging to the same family sometimes exhibited comparable anticandidal activity since these plants are expected to possess similar active constituents (*T. capitata*, and *O. syriacum* of the Family Labiateae), while some plants of the same family exhibited different effects (*T. creticum* and *T. polium* of the Family Labiateae) indicating that there might be some constituents found in one member of the family but not in the other.

Antidermatophyte activity

Only a few antifungal substances are known or available in the market as compared to antibacterial substances. Antimycotic substances are also relatively unsatisfactory in the control of dermatophytes. The discovery of active components exhibiting a broad spectrum antifungal activity may prove useful for the development of antifungal agents. Laboratory assessment showed the nature of fungi static activity encountered in plant extracts. Table 3 shows percentage of inhibition due to plant type at (45 µg/mL) concentration. Values of % inhibition increase with increasing concentration of each plant extract (Figure 1).

All tested plants revealed antimycotic activity against all two tested dermatophytes, with *T. capitata* exhibiting the highest activity; a complete inhibition by the ethanolic extract of this plant was at a concentration lower than 45 µg/mL. *O. syriacum* extract have also showed considerable antimycotic activity against the same tested dermatophytes, complete inhibition was at a concentration <60 µg/mL. The oil of *T. capitata* have been shown to exhibit antidermatophytic activity, with MIC values ranging from 0.08 to 0.32 µL/mL (Salgueiro

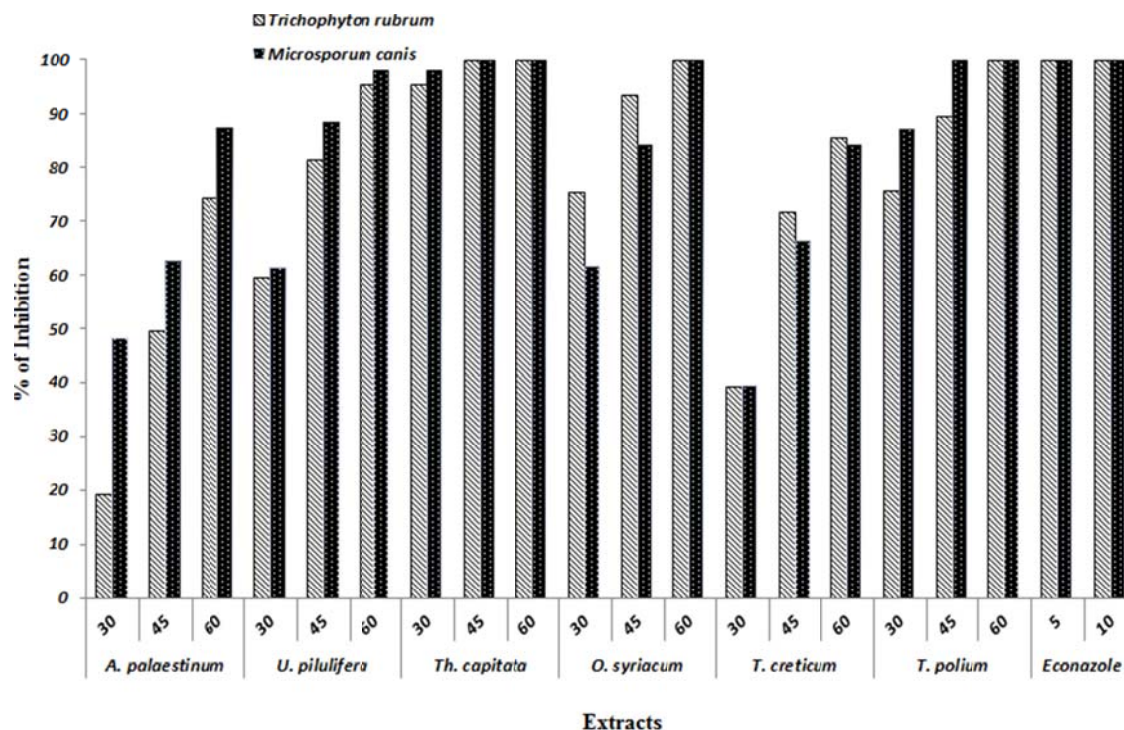


Figure 1. Percent of inhibition of plants extracts against *T. rubrum* and *M. canis* at different concentrations (30, 45, and 60 µg/mL).

et al., 2004). In addition to these plants, *A. palaestinum* showed 50% inhibition against *M. canis* at a concentration of ~45 µg/mL.

The present work has shown that most of the studied plants are potentially good source of antidermatophytes and demonstrates the importance of such plants in medicine and in assisting primary health care. However, the screened plants are among the wild edible plants consumed by Palestinian (Ali-Shtayeh et al., 2008). From those results we can suggest that plants extracts investigated here could find practical application in the introduction of highly active and safe antimicrobial agents including antidermatophytes. These results justify the usage of those plants in folk medicine (Ali-Shtayeh et al., 2008, Ali-Shtayeh and Jamous, 2008).

Conclusion

Overall, some crude extracts prepared from plants commonly grown in Palestine or commonly used by Palestinian were found to exert, *in-vitro* some antimicrobial effect. However, further work is needed to isolate and identify the active compound(s) from active plant species.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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