

Screening for Biological Activity of Eleven Medicinal Plants Used 3 in Traditional Arabic Palestinian Herbal Medicine

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Original Research Article

Screening for Biological Activity of Eleven Medicinal Plants Used in Traditional Arabic Palestinian Herbal Medicine

ABSTRACT

Aims: To evaluate eleven medicinal plants as natural sources that possesses strong antidermatophytic, antibacterial, anticandidal and antioxidant substances with potential applications in therapeutics and food industry.

Place and Duration of Study: Sample: Biodiversity and Environmental Research Center, BERC, between December 2013 and April 2014.

Methodology: Twenty methanolic extracts were prepared from different parts of eleven plants used in traditional medicine in Palestine. The plants extracts were screened for total flavonoid and phenolic content using standard procedures. The crude extract was screened against six bacterial strains (*Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Klebsiella pneumoniae*), 5 *Candida albicans* strains, and 2 dermatophytes (*Microsporum canis*, and *Trichophyton rubrum*). The antioxidant potential of the crude extract was also determined using the DPPH assay.

Results: The best free-radical scavenging was for the leaves of *Epilobium hirsutum* (IC₅₀=33 µg/ml) and *Rhus coriaria* (49 µg/ml) compared with BHA standard (9 µg/ml). The highest value of phenolics was in *Rhus coriaria* fruits (14.7 mg/g dried plant material) and for flavonoids was for *Epilobium hirsutum* leaves (1.14 mg/g). The most active extracts against bacteria was the *Rhus coriaria* leaves (% inhibition, 66.2 %) compared with gentamicin (100%) and against *Candida* were leaves of *Rhus coriaria* (100 %) and *Epilobium hirsutum* (72.4 %) compared with amphotericin B (100 %). On the other hand fruits of *Rhus coriaria* showed the best antifungal activity against all the tested dermatophytes, 97% and 86% inhibition were achieved against *Microsporum canis* and *Trichophyton rubrum*, respectively.

Conclusion: Our results introduce a natural source (*Rhus coriaria* and *Epilobium hirsutum*) that possesses strong antidermatophytic, antibacterial, anticandidal and antioxidant substances with potential applications in therapeutics and food industry.

Keywords: antioxidant, phenolics, flavonoids, antibacterial, antidermatophyte, anticandida, *Rhus coriaria*, *Epilobium hirsutum*

1. INTRODUCTION

Herbal medicine is common in developing countries, and is practiced by a large proportion of the population for the treatment of various diseases. In Palestine, many medicinal plants used in folk

14 medicine against various diseases have been documented with the ethnobotanical field surveys carried
15 out in the area, for the treatment of various diseases including cancer, injuries, and chronic diseases [1-
16 6]. Many medicinal plants and their parts have been shown to have medicinal value and can be used to
17 prevent, alleviate, or cure several human diseases. Plants contain various phytochemicals which can play
18 an important role in reducing occurrences of many diseases by boosting up various organ functions of the
19 human body [7,8].

20 A large number of medicinal plants have been investigated for their biological activities all over the world.
21 Numerous scientific studies were designed for plant species used as folk remedies. Most of research
22 results are in good agreement with the traditional utilization of the tested plants [9]. It is believed that folk
23 remedies are major sources of new materials for antimicrobial and antioxidant drugs [10]. Antioxidants
24 have many potential applications, especially in relation to human health, both in terms of prevention of
25 disease and therapy [11]. Antioxidants are considered to play an effective role in inhibiting and
26 scavenging free radicals, and are also of particular importance because they might serve as leads for the
27 development of novel drugs. The most commonly used synthetic antioxidants have side effects such as
28 liver damage and carcinogenesis [12]. Natural antioxidants either in the form of raw extracts or their
29 chemical constituents are very effective to prevent the destructive processes caused by stress [13]. In this
30 context, antioxidants especially derived from natural sources such as medicinal plants and herbal drugs
31 require special attention.

32 Drug resistance to human pathogenic bacteria and fungi has been commonly reported from all over the
33 world [14], thus the increasing prevalence of multidrug resistant strains of pathogenic microorganisms
34 and the recent appearance of strains with reduced susceptibility to antibiotics raises the need to search
35 for new sources of antimicrobial agents [15]. Human infections, particularly those involving skin and
36 mucosal surfaces constitute a serious problem. Fungal infections have increased at an alarming rate in
37 the last 20 years, mainly among immune compromised individuals [16]. New data indicate that the relative
38 proportions of organisms causing nosocomial bloodstream infections have changed over the last decade,
39 with *Candida* species now firmly established as one of the most frequent agents. Candidemia is not only
40 associated with a high mortality but also extends the length of the hospital stay and increases the costs of
41 medical care. Among human gastrointestinal tract isolates, 50-70% of total yeast isolates were identified
42 as *Candida albicans* [17,18]. Therefore the discovery of antioxidant, antimicrobial and antifungal agents
43 from plants based on the evaluation of traditional plant extracts is a very important research topic.

44 In this study 20 methanol extracts prepared from different parts of 11 Palestinian plants used in
45 Traditional Arabic Palestinian herbal medicine (TAPHM) for the treatment of various ailments were
46 evaluated for their antioxidant activity using DPPH, total flavonoid and phenolic compounds content, and
47 the biological activity of these plants extracts against bacteria, *Candida* and dermatophytes.

48

19 2. MATERIAL AND METHODS

50 2.1. Chemicals

51 2,2-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisol (BHA), and α -tocopherol were
 52 purchased from Sigma, (Sigma, Aldrich GmbH, Sternheim, Germany); pyrocatechol, quercetin, Folin-
 53 ciocalteu's reagent (FCR), peptone, agar, dextrose, Muller-Hinton agar (Fluka), sabouraud dextrose agar
 54 (Difco), dextrose, agar, gentamicin, amphotericin B and econazole, were purchased from Merck
 55 (Darmstadt, Germany). Sodium carbonate, ethanol, methanol and all other chemicals and reagents were of
 56 analytical grade.

57 2.2. Plant Material

58 Medicinal plant species screened in this study were collected from different regions of Palestine between
 59 April and August 2013. They were identified by Prof. M. S. Ali-Shanabneh from the Biodiversity and
 60 Environmental Research Center, BERC, Til Village, Nablus (Table 1). Voucher specimens are deposited
 61 in the Herbarium of BERC.

62 2.3. Extracts Preparation

63 Fresh plant parts were ground using a Molenix (Mooele Depose type 241) for a minute and the resulting
 64 powder was lyophilized and stored in at - 80°C for future use. Fifty grams of the lyophilized plant material
 65 were extracted by homogenization with 80 % methanol (10 ml g⁻¹), for 72 h then filtrated through
 66 Whatman No. 4 filter paper. The solvent was removed at 45°C under reduced pressure followed by freeze
 67 drying using freeze dryer (Alpha 1-2 LD plus). The crude extracts were stored at -20°C for further use.

68 2.4. Phytochemical Screening

69 2.4.1. Determination of total phenolic contents

70 The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent using the
 71 method of Dicko et al [19] with adaptation of the method to the 96 well-plate. Gallic acid was used as a
 72 standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentrations
 73 of 2.4, 4.87, 9.75, 19.5, 39, 78, 156 μ g/ml of gallic acid were prepared in methanol. Concentration of 2.5
 74 mg/ml of plant extract was also prepared in methanol and 20 μ l of each sample were introduced into the
 75 wells and mixed with 100 μ l of 0.2 N Folin- Ciocalteu reagent, the plate was incubated 5 min at room
 76 temperature followed by the addition of 80 μ l of 7.5% sodium carbonate. The micro-well plate was
 77 covered to protect from light and allowed to stand for 30 minutes at room temperature before the
 78 absorbance was read at 735 nm using a multi-well plate reader Biotek, USA. All determinations were
 79 performed in triplicate. The Folin-Ciocalteu reagent being sensitive to reducing compounds including
 80 polyphenols is producing a blue color upon reaction which can be measured spectrophotometrically [20].

81

82 **Table 1. Antioxidant and phytochemical analysis of the selected plants extracts**

No.	Scientific Name	Family Name	Voucher No.	Plant Part*	Antioxidant activity		Phytochemical analysis	
					DPPH		Total phenolic content (GAE mg/gm)	Total flavonoid content (QE mg/gm)
					IC50	AAI		
1.	<i>Ailanthus altissima</i> (P. Mill)	Simarubaceae	BERC-BX-C-0599	FR	>10000	0	5.85±0.11	0.43±0.02
2.				LE	286.0	0.15	2.24±1.29	0.58±0.08
3.	<i>Alcea setosa</i> (Boiss.) Alef.	Malvaceae	BERC-BX-C-0072	FL	>10000	0.01	2.01±0.16	0.52±0.03
4.	<i>Cerantonia siliqua</i> L.	Fabaceae	BERC-BX-C-0137	FR	255	0.17	7.53±0.27	0.79±0.65
5.				LE	81	0.53	1.26±0.19	0.94±0.08
6.	<i>Echinops adenocaulos</i> Boiss.	Asteraceae	BERC-BX-C-0100	FL	4429.0	0.01	3.70±0.15	0.09±0
7.	<i>Ephedra aphylla</i> Forssk.	Ephedraceae	BERC-BX-C-0140	FR	606.0	0.07	6.53±0.46	0.50±
8.				LE	1776.0	0.02	8.10±0.16	0.32±0.02
9.	<i>Epilobium hirsutum</i> L.	Onagraceae	BERC-BX-C-0250	LE	33.0	1.3	13.46±0.77	1.14±0.08
10.	<i>Eucalyptus camaldulensis</i> Dehnh.	Myrtaceae	BERC-BX-C-0039	FR	141.0	0.30	1.26±0.19	0.34±0.01
11.				LE	325.0	0.13	1.72±0.22	0.50±0.04
12.	<i>Lycium schweinfurthii</i> Dammer	Solanaceae	BERC-BX-C-0591	LE	>10000	-	1.66±0.10	0.55±0.02
13.				FR	>10000	-	0.53±0.04	0.07±0.00
14.	<i>Pistacia palaestina</i> Boiss.	Anacardiaceae	BERC-BX-C-0010	LE	131	0.33	1.35±0.30	0.82±0.02
15.				FR	143	0.30	9.70±1.44	1.11±0.10
16.	<i>Rhus coriaria</i> L.	Anacardiaceae	BERC-BX-C-0037	FR	153	0.28	14.91±0.94	0.52±0.02
17.				LE	49	0.87	0.90±0.27	0.61±0.06
18.	<i>Urginea maritima</i> (L.) Baker	Liliaceae	BERC-BX-C-0277	UG	>10000	-	7.03±0.27	0.05±0.01
19.				FR	>10000	-	6.83±0.37	0.25±0.03
20.				FL	1895	0.02	5.304±0.04	0.27±0.03
21.	Butylated hydroxyanisole				9	4.76		
22.	Gallic acid				33	1.3		
23.	Vitamin C				70	0.61		

83

84 * FL flower; LE leaves; UG Underground parts, FR Fruits

85 **2.4.2. Determination of total flavonoids contents**

86 The total flavonoids content of each plant extract was estimated by aluminium chloride colorimetric assay
 87 described by Chatatikun & Chiabchalard [21]. The reaction was carried out by mixing 25µl of the plant
 88 extract (2.5mg/ml) or standard solution of quercetin (400, 200, 100, 50, 25, 12.5, 6.1, 3.6 µg/ml) in 80 %
 89 methanol, with 10 µl of AlCl₃ solution (10%), followed by the addition of 175 µl of 100 % methanol.
 90 Methanol (80 %) was used as reagent blank. Finally 10 µl of 1M sodium acetate was added to the mixture
 91 in a 96 well plate. The reaction was mixed and incubated for 40 minutes at room temperature protected
 92 from light. The absorbance was measured at 415 nm with a Micro plate Reader (Biotek, USA.). Total
 93 flavonoid contents in the plants extracts were expressed as mg Quercetin Equivalents (QE) per gram of
 94 dry plant material. All samples were analyzed in triplicates.

95 **2.5. Determination of Antioxidant Activity Using DPPH Free Radical Scavenging**

Free radical scavenging activity of the extracts was determined using the free radical 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH). The effect of the plant extracts on DPPH radical was performed as described by Liyana-Pathirana and Shahidi [22] with minor modification. Briefly, 25µl of each plant extract (ranging from 0 to 10 mg/ml) or standard solution of ascorbic acid, BHA and Gallic acid (ranging from 0.0024 mg/ml to 0.156 mg/ml) were added to 175 µl of 0.0042% DPPH methanol solution in 96 micro-well plate. Appropriate blanks were prepared using the solvent only in addition to the same amount of DPPH reagent to overcome any inherent solvent activity. All reaction mixtures were mixed well and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm with a Microplate Reader (Piatek, USA). Experiments were done in triplicates. The ability to scavenge DPPH radical was calculated using the following equation:

$$RSA = [(Ac-As)/Ac] \times 100\%$$

Where RSA is the percentage of free radical scavenging activity, Ac is the absorbance of blank, As is the absorbance of sample. The concentration of sample required to scavenge 50% of the DPPH free radical (IC50) was determined from the curve of % of inhibitions plotted against the respective concentration.

The antioxidant activity index (AAI) was then calculated as follows:

$$AAI = [DPPH] (\mu g \text{ ml}^{-1}) / IC_{50} (\mu g \text{ ml}^{-1})$$

Where [DPPH] is final DPPH concentration.

2.6. Microbiological Studies

Antimicrobial activity of different plant extracts was evaluated by agar well diffusion method and minimum inhibitory concentration MIC. Microorganisms used in this study are listed in table 2.

Table 2. Test microorganisms.

Microrganism	Species name	Source	Notes
Bacteria	<i>Staphylococcus aureus</i>	ATCC 25923	G +ve
	<i>Proteus vulgaris</i>	ATCC 13315	G -ve
	<i>Pseudomonas aeruginosa</i>	ATCC 27853	
	<i>Salmonella typhi</i>	ATCC 14028	
	<i>Escherichia coli</i>	ATCC 25922	
	<i>Klebsiella pneumoniae</i>	ATCC 13883	
Candida	<i>Candida albicans</i>	CBS6589	Clinical Specimens
		CBS9120	
		BERC N43	
		BERC N72	
		BERC N66	
Dermatophytes	<i>Microsporum canis</i>	CBS132.88	

	<i>Trichophyton rubrum</i>	BERC-EH-TR9	Clinical Specimen
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117

118 **2.6.1. Well-diffusion method**

119 Antibacterial and anticandidal activities of the selected plants extracts were assessed using the agar well-
 120 diffusion method [23]. Muller-Hinton and Muller-Hinton supplemented with glucose-methylene blue plates
 121 were used for antibacterial and anticandidal susceptibility tests, respectively. An inoculum of 18 hour old -
 122 broth culture (turbidity adjusted to approximately 108 CFU/ml of bacterium and candida, compared with
 123 0.5 McFarland standards) [24] of respective bacterial and candida strain was uniformly spread on these
 124 media in separate plates [25]. Wells (6 mm diameter) were created in these plates, and 50 µl of plant
 125 extracts (100 mg/ml) were pipetted into the wells and allowed to diffuse at room temperature for 30 min.
 126 Plates were incubated at 37°C for 18-24 h [26]. The zone of inhibition for each extract was measured and
 127 expressed in mm [25]. The activity index (AI) and Percent Inhibition (PI) were calculated for all extracts
 128 obtained at a concentration of 100 mg/ml using the following formula:

$$129 \text{ AI} = \frac{\text{Mean zone of inhibition of each extract}}{\text{Zone of inhibition obtained for standard antibiotic}}$$

$$131 \text{ PI} = \text{AI} \times 100$$

132 All the experiments were done in triplicates. Gentamicin (10mg/mL) and amphotericin B (32µg/mL) were
 133 used as positive controls for bacteria and candida, respectively.

134 **2.6.2. Broth Micro-dilution test**

135 Broth micro-dilution was performed following the CLSI M27-A2 method [27] Plant extracts were
 136 dissolved in methanol and the correct volume was pipetted in the first micro-plate well with Muller-Hinton
 137 media (pH 7.2), for the concentration of each plant extract to be 5 mg/mL in that well. The cell suspension
 138 was prepared in 0.85% saline, with an optical density equivalent to 0.5 McFarland standards, and diluted
 139 1:100 in the media to obtain a final concentration of 1×10^4 to 5×10^4 colony-forming units per milliliter
 140 (CFU/mL). This suspension was inoculated in each well of a micro-dilution plate previously prepared
 141 with the plant extracts to give concentrations from 5 mg/mL down to 0.039 mg/mL [28]. The plates
 142 were incubated with agitation at 37°C for 24 h for all species. The control drugs were gentamicin for
 143 bacteria strains, and amphotericin B for Candida, respectively. Concentrations of controls were ranged
 144 from 250-1µg/mL for gentamicin, and from 16.0-0.125µg/mL for amphotericin B. Value of minimum
 145 inhibitory concentration (MIC), determined by broth micro- dilution, and was defined as the lowest
 146 concentration of the drug completely inhibited the growth of the isolate. For plant extracts, the lowest
 147 concentration without visible growth (visually and spectrophotometrically) was defined as MICs

148 **2.6.3. Anti-dermatophyte testing**

149 Plants extracts were tested for their anti-dermatophyte activity against two dermatophyte species using a
 150 modified poisoned food technique [29]. Each extract was incorporated in pre-sterilized SDA medium at a

concentration of (0.4mg/ml). A mycelial agar disk of 5 mm diameter was cut out of 12 days old culture of the test fungus and inoculated on to the freshly prepared SDA 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 observations were recorded 10 days after incubation. Percentage of mycelial inhibition was calculated using the following formula:

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

dc: colony diameter of the control , ds: colony diameter of the sample

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Content

Polyphenols are secondary metabolites, naturally occurring compounds found largely in plants; they are generally involved in defence against ultraviolet radiation or aggression by pathogens [30]. In food, polyphenols may contribute to the bitterness, astringency, colour, flavour, odour and oxidative stability [31]. Epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, chronic diseases, osteoporosis and neurodegenerative diseases [32, 33]. Polyphenols and other food phenolics are the subject of increasing scientific interest because of their possible beneficial effects on human health. In this study TPC was estimated using Folin-Ciocalteu's method. TPC of all extracts was found to be in the range of 0.53-14.9 mg GAE/g extract, results showed that TPC differ among different plant parts (Table 1), the highest level of TPC was found in the fruits *Rhus coriaria* (14.9 mg GAE/g extract), while it was only 0.89 in the plant leaves. Other plants with high TPC were the leaves of *Epilobium hirsutum* (13.46 mg GAE/ g), fruits of *Pistacia palaestina* (9.7 mg GAE/g), and the leaves of *Ephedra aphylla* is (8.08 mg GAE/ g).

A phytochemical analysis of the fruits of *Rhus coriaria* was conducted recently [34], a total of 211 compounds were identified in the epicarp (fruits) of the plant of which 9 compounds were phenolic acids derivatives, and 26 compounds were unusual phenolics conjugated with glycoside-malic acid [34]. Also, several *Pistacia* species are known to be rich in gallotannins and related phenolic compounds [35, 36].

3.2. Total Flavonoid Content

Flavonoids comprise the most studied group of polyphenols. More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the attractive colours of the flowers, fruits and leaves [37].

32

185 Total flavonoid content was measured using the aluminium chloride colorimetric assay. The total
186 flavonoid content of all extracts ranged between 0.05-1.14 mg QE/g extract (Table 1), the highest level
187 of flavonoid content was found in the leaves of *Epilopium hirsutum* and *Pistacia palaestina* (1.14 and
188 1.11 respectively), while the lowest flavonoid content was in the underground part extract of *Urginea*
189 *maritima* (0.05 mg QE/g extract). Other plants with high levels of flavonoid were leaves of *Lycium*
190 *schweinfurthii* (0.82 mg QE/g), and fruits of *Ceratonia siliqua* (0.789 mg QE/g). The fruit extracts of
191 *Lycium schweinfurthii* and flowers of *Echinops adenocaulos* had very low levels of flavonoid (0.07 and
192 0.089 mg QE/g, respectively).

193 Methanol plant extracts contained a higher proportion ($\geq 50\%$) of phenolics than flavonoids (Figure 1).

29

194 3.3. Antioxidant Activity

195 In this study, the antioxidant activity of plant extracts were evaluated using DPPH free radical scavenging
196 assay. Except for the fruits of *Ailanthus altissima*, flowers of *Alcea setosa*, leaves and fruits of *Lycium*
197 *schweinfurthii*, and underground parts and fruits of *Urginea maritima* extracts, all extracts showed DPPH
198 radical scavenging activity. *Epilopium hirsutum* leaves revealed the highest antioxidant activity with (AAI=
199 1.298, $IC_{50}=33 \mu g/ml$), followed by the extract of *Rhus coriaria* leaves (AAI= 0.87, $IC_{50}=49 \mu g/ml$) (Table
200 1). The activities of leaf extracts varied from ($IC_{50}=33\mu g/ml$) in *Epilopium hirsutum* to ($IC_{50} = 325\mu g/ml$) in
201 *Eucalyptus camaldulensis*. While the activity of fruit extracts varied from AAI =0.304 in *Eucalyptus*
202 *camaldulensis* to AAI= 0 in *Ailanthus altissima*, *Lycium schweinfurthii* and *Urginea maritima*. However, the
203 fruits and leaves of *Pistacia palaestina* which have AAI=0.327 and 0.3, respectively, have been shown by
204 others to possess a high antioxidant activity [38].

54

205 In our study, a weak correlation was found between radical scavenging antioxidant activity and total
206 phenolics in plant parts. Interestingly, a few of the collected plant parts with high-antioxidant activity are
207 "low" in phenolic content including the leaves of *Ceratonia siliqua* and *Rhus coriaria*. These plants may
208 serve as sources of antioxidants with new chemotypes.

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209

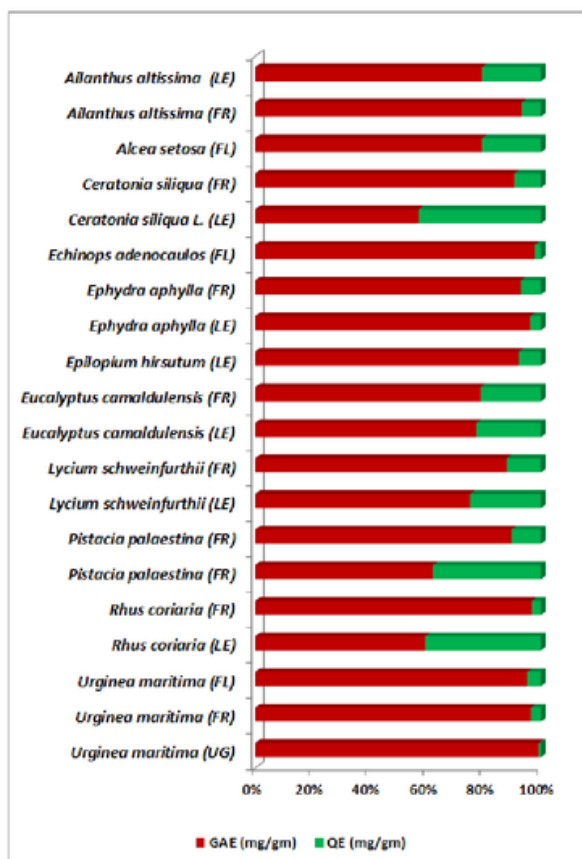


Figure 1 Proportional relation (%) of flavonoids content to phenolic acids in analysed medicinal plants.

3.4. Antibacterial Activity

In the present work the antibacterial activity of the twenty methanol extracts of plants parts were evaluated against six bacterial strains, using agar well diffusion and serial micro dilution (MIC) methods. The results of the antibacterial screening test showed that of the twenty extracts tested only seven extracts belonging to 4 plants species showed antibacterial activity (Table 3). The most active plant extract against all bacteria strains was the leaves of *Rhus coriaria*. The percent of inhibition of *Rhus coriaria* leaves extract ranged between 60.9- 76.2 against tested bacteria. However, the leaves of *Ailanthus altissima* showed moderate antibacterial activity with percent of inhibition of ranged from 41.8 to 55.8. However, the fruits of *Ailanthus altissima* and leaves of *Eucalyptus camaldulensis* showed the least activity. Our results are in accordance with previous studies in which the leaves of *Rhus coriaria* and *Ailanthus altissima* have been shown by other researchers to possess high antibacterial activity [5, 39, and 40]. Bioactive compounds produced by plants have been found to protect plants against bacteria, fungi and pests [41, 42], thus it is expected that the plants extracts were composed of antibacterial activity.

227 **Table 3. Percent inhibition and MIC (mg/mL) of plant extracts against bacterial strains**

Plant name (Part*)	<i>Salmonella typhi</i>		<i>Klebsiella pneumoniae</i>		<i>Staphylococcus aureus</i>		<i>Proteus vulgaris</i>		<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>	
	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)
<i>Ailanthus altissima</i> (FR)	42.9	2.50	0	0.00	35.9	1.25	0	0.00	0	0.00	0	0.00
<i>Ailanthus altissima</i> (LE)	55.8	2.50	41.8	2.50	45.3	1.25	46.6	5.00	45.3	2.50	48.5	5.00
<i>Eucalyptus camaldulensis</i> (LE)	0	0.00	0	0.00	48.4	2.50	0	0.00	0	0.00	48.6	2.50
<i>Pistacia palaestina</i> (FR)	0	0.00	60.8	0.30	39.1	2.50	61.9	0.63	45.3	1.25	52.6	5.00
<i>Pistacia palaestina</i> (LE)	38.6	5.00	37.3	1.25	34.4	1.25	40.5	2.50	0	0.00	40.5	2.50
<i>Rhus coriaria</i> (FR)	51.5	5.00	45.6	1.25	48.4	0.30	61.9	2.50	0	0.00	0	0.00
<i>Rhus coriaria</i> (LE.)	68.7	2.50	60.8	0.60	60.9	1.03	76.2	1.25	65.8	1.25	64.7	5.00
Gentamicin (10mg/ml)	100	0.01	100	0.01	100	0.01	100	0.01	100	0.01	100	0.01

228 * FR, fruit; LE, leaves.

229 3.5. Anti-Candida Activity

230 Of the tested plants, six species out of eleven showed anticandidal activity against all strains (Table 4).
 231 The most active plants extracts were the leaves of *Epilobium hirsutum* and *Rhus coriaria*, and the leaves
 232 and flowers of *Eucalyptus camaldulensis* with percent of inhibition ranging from 42.4 to 82.6 (Table 4). On
 233 the other hand, the fruits of *Ailanthus altissima* and *Ceratonia siliqua* were the least active plant extract
 234 with PI ranging between 0.0-36.5, and 0.0-46.1, respectively.

235 **Table 4 Percent inhibition zone and MIC (mg/mL) of plant extracts against *Candida albicans***
 236 **strains**

Plant (Part)*	BERC N43		BERC N72		BERC N66		CBS 6985		CBS 39	
	PI	MIC (mg/mL)	PI	MIC (mg/ml)	PI	MIC (mg/mL)	PI	MIC (mg/mL)	PI	MIC (mg/mL)
<i>Ailanthus altissima</i> (FR)	36.5	5.0	0	0	0	0	0	0	0	0
<i>Ailanthus altissima</i> (LE)	65.2	0.60	64.0	0.15	42.5	1.25	39.1	1.25	44.3	2.5
<i>Ceratonia siliqua</i> (FR)	46.1	0.15	38.1	0.6	39.6	0.6	36.5	0	0	0
<i>Ceratonia siliqua</i> (LE)	46.1	1.25	36.3	1.25	39.6	2.5	36.5	0.6	40.6	5.0
<i>Epilobium hirsutum</i> (LE)	80.9	0.15	75.2	0.15	72.5	0.3	66.8	0.6	66.6	0.6
<i>Eucalyptus camaldulensis</i> (FR)	80.9	0.30	54.9	0.15	56.4	1.25	51.9	2.5	63.9	2.5
<i>Eucalyptus camaldulensis</i> (LE)	82.6	0.30	58.4	0.6	57.5	5.0	52.9	5.0	42.4	5.0
<i>Pistacia palaestina</i> (LE)	46.9	0.30	55.9	0.3	42.5	1.25	39.1	1.25	46.1	1.25
<i>Rhus coriaria</i> (FR)	57.4	0.30	54.2	0.6	45	2.5	41.4	02.5	47.1	2.5
<i>Rhus coriaria</i> (LE)	81.7	0.15	71.7	1.25	67.1	5.0	61.8	0.3	64.2	5.0
Amphotericin B (32µg/mL)	100	.008	100	.008	100	.002	100	0.001	100	.001

237 * FR, fruits; LE, leaves.

238

239 3.6. Antidermatophyte Activity

240 Many effective synthetic antifungal agents are currently available and have been used for the treatment of
 241 dermatophyte infections [9]. However, these antifungal drugs tend to have serious side-effects including
 242 toxicity, drug interactions, inadequate pharmacokinetic properties and the development of resistance
 243 have been reported [43]. The discovery of natural active components exhibiting a broad spectrum of

244 antidermatophyte activity may prove useful for the development of antifungal agents. Medicinal plants
245 have been a source of wide variety of biologically active compounds for many centuries and used
246 extensively as crude material or as pure compounds for treating various disease conditions [44]. Various
247 previous researches have been conducted to evaluate the anti-dermatophytic activity of plants [9, 45-47].

248 In this study, plant extracts tested have shown considerable antidermatophytic activities at concentration
249 of 0.4 mg/ml against the two tested dermatophytes (*M. canis*, and *T. rubrum*) in comparison with the
250 positive control (econazole). The percent of mycelial inhibition at the concentration of 0.4 mg/ml plant
251 extract ranged between 13%-97% against *Microsporum canis* and 7%-86% against *Trichophyton rubrum*
252 (Figure 2). The most active plants extracts which exhibited more than 50% inhibition against both
253 dermatophytes were the leaves and fruits of *Lycium chweinfurthii*, *Eucalyptus camaldulensis* and *Rhus*
254 *coriaria* and leaves of *Epilobium hirsutum* and *Ailanthus altissima*. Of these extracts the fruits of *Rhus*
255 *coriaria* and leaves of *Ailanthus altissima* revealed the highest antidermatophyte activities with 97 % and
256 74 % mycelial inhibition against (*Microsporum canis*), respectively, and 86 % and 74% against
257 *Trichophyton rubrum* (Figure 2). Abdolmaleki et al [48] have shown that methanolic extracts of stem and
258 fruit of sumac had the highest inhibitory activity against *Fusarium oxysporum* and *Phytophthora*.
259 *drechsleri*, respectively. While the ethanolic extract of the leaves and methanolic extracts of fruit, leaf and
260 stem had the highest inhibitory activity against *Rhizoctonia solani*.

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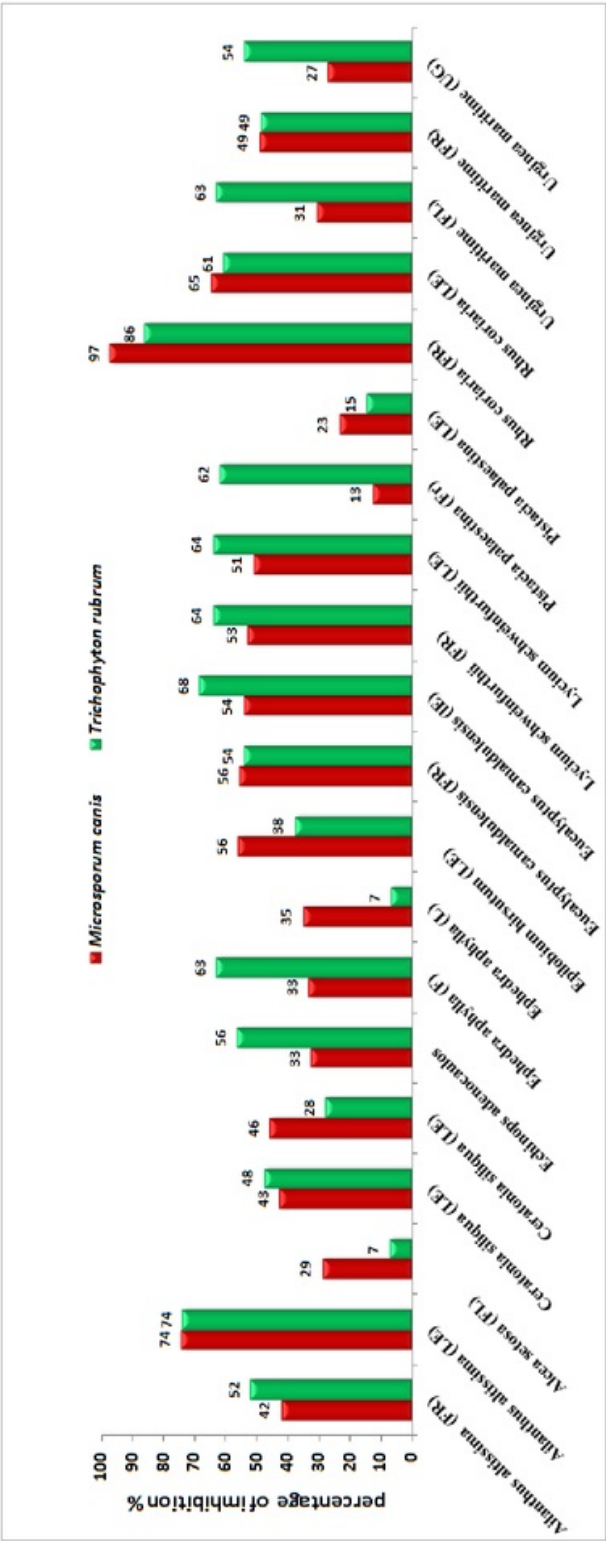


Figure 2 Antidermatophyte activity of plant Extracts (% inhibition)

5
264 Plants are rich source of thousands of new useful phytochemicals of great diversity, which have inhibitory
265 effects on all types of microorganisms *in vitro*. Although more than 600 plants have been reported for their
266 antifungal properties, however a few of them were explored for the active components [44]. In this study
267 leaves have shown to be more active than fruits of the same plant. This might be attributed either to the
268 presence of different active chemical compounds or to the different concentrations of these compounds
269 between leaves and fruits. Previous research studies reported the presence of different chemical groups
270 in plant extracts including: phenolics, flavonoids, organic acids, saponins, terpenoids and alkaloids [44,
271 49-51]. The variation between plants extracts activity might be related to the different chemical groups
272 and the variation in their concentrations in these plants. The results of the present study might suggest
273 that *Rhus coriaria* and *Eucalyptus camaldulensis* are promising and presumably possess compound(s)
274 with chemical properties against dermatophytes.

275 4. CONCLUSION

276 In conclusion, most of plants in this study could be considered as potential sources of natural antioxidant,
277 which can be used as health promoting agents. *Rhus coriaria* extracts have shown to possess promising
278 antibacterial, anticandidal and antidermatophytic activity. Other plants including *Epilobium hirsutum* and
279 *Lycium chweinfurthii* have also shown to possess good anticandidal and antidermatophytic activity,
280 respectively. Our results introduce natural sources that possesses strong antidermatophytic, antibacterial,
281 anticandidal and antioxidant substances with potential applications in therapeutics and food industry.

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