Original Research Article

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

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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~~R. *coriaria* fruits (14.7 mg/g dried plant material) and for flavonoids was for ~~Epilopium~~ Epi. *hirsutum* leaves (1.14 mg/g). The most active extracts against bacteria was the ~~Rhus~~R. *coriaria* leaves (% inhibition, 66.2 %) compared with gentamicin (100%) and against *Candida* were leaves of ~~Rhus~~R. *coriaria* (100 %) and ~~Epilopium~~Epi. *hirsutum* (72.4 %) compared with amphotericin B (100 %). On the other hand fruits *of* ~~Rhus~~R. *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~~R. *coriaria* and Epi~~lobium~~ *hirsutum*) that

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

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

Drug resistance to human pathogenic bacteria and fungi has been commonly reported from all over the world [14], thus the increasing prevalence of multidrug resistant strains of pathogenic microorganisms and the recent appearance of strains with reduced susceptibility to antibiotics raises the need to search for new sources of antimicrobial agents [15]. Human infections, particularly those involving skin and mucosal surfaces constitute a serious problem [16]. Fungal infections have increased at an alarming rate in the last ~~20~~ 2 decades~~years~~, mainly among immune compromised individuals [~~16~~17]. *Candida* species have been reported to be among the one of the most frequent ~~New data indicate that the relative proportions of~~ organisms causing nosocomial bloodstream ~~infections have changed over the last decade, with *Candida* species now firmly established as one of the most frequent agents~~ [18]. Candidemia is not only associated with a high mortality but also extends the length of the hospital stay and increases the costs of medical care. A large percentage (50-70%) of total yeast isolates recovered from ~~mong~~ human

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gastrointestinal tract ~~isolates, 50-70% of total yeast isolates~~ were identified as *Candida albicans* [~~17~~18,~~18~~19]. Therefore the discovery of antioxidant, antimicrobial and antifungal agents from plants based on the evaluation of traditional plant extracts is a very important research topic.

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

2. MATERIAL AND METHODS

2.1. Chemicals

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

2.2. Plant Material

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

2.3. Extracts Preparation

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

2.4. Phytochemical Screening

2.4.1. Determination of total phenolic contents

The total amount of ~~total~~ phenolics in plant extracts was determined with the Folin-Ciocalteu reagent ~~using~~ following the method of Dicko et al [~~19~~20] with adaptation of the method to the 96 well-plate. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentrations of 2.4, 4.87, 9.75, 19.5, 39, 78, 156 µg/ml of gallic acid were prepared in methanol. Concentration of 2.5 mg/ml of plant extract was also prepared in methanol and 20 µl of each

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sample were introduced into the wells and mixed with 100 µl of 0.2 N Folin- Ciocalteu reagent, the plate was incubated 5 min at room temperature followed by the addition of 80 µl of 7.5% sodium carbonate. The micro-well plate was covered to protect from light and allowed to stand for 30 minutes at room temperature before the absorbance was read at 735 nm using a multi-well plate reader Biotek, USA. All determinations were performed in triplicate. The Folin-Ciocalteu reagent, being sensitive to reducing compounds including polyphenols, is producing a blue ~~color~~colour upon reaction which can be measured spectrophotometerically [~~20~~21].

Table 1. Antioxidant activity 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/g~~m~~)	Total flavonoid content (QE mg/g~~m~~)
					IC50	AAI		
1.	*Ailanthus altissima* (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.	*Alcea setosa* (Boiss.) Alef.	Malvaceae	BERC-BX-C-0072	FL	>10000	0.01	2.01±0.16	0.52±0.03
4.	*Ceratonia siliqua* 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.	*Echinops adenocaulos* Boiss.	Asteraceae	BERC-BX-C-0100	FL	4429.0	0.01	3.70±0.15	0.09±0
7.	*Ephedra aphylla* 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.	*Epilobium hirsutum* L.	Onagraceae	BERC-BX-C-0250	LE	33.0	1.3	13.46±0.77	1.14±0.08
10.	*Eucalyptus camaldulensis* 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.	*Lycium schweinfurthii* 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.	*Pistacia palaestina* Boiss.	Anacardiacea	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.	*Rhus coriaria* L.	Anacardiacea	BERC-BX-C-0037	FR	153	0.28	14.91±0.94	0.52±0.02
17.				LE	49	0.87	0.90±027	0.61±0.06
18.	*Urginea maritima* (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		

* FL flower; LE leaves; UG Underground parts; FR Fruits.

2.4.2. Determination of total flavonoids contents

The total flavonoids content of each plant extract was estimated by aluminium chloride colorimetric assay described by Chatatikun & Chiabchalard [~~21~~22]. The reaction was carried out by mixing 25 µl of the plant extract (2.5 mg/ml) or standard solution of quercitin (400, 200, 100, 50, 25, 12.5, 6.1, 3.6 µg/ml) in 80 %

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methanol, with 10 µl of $AlCl_3$ solution (10%), followed by the addition of 175 µl of 100 % methanol. Methanol (80 %) was used as reagent blank. Finally 10 µl of 1M sodium acetate was added to the mixture in a 96 well plate. The reaction was mixed and incubated for 40 minutes at room temperature protected from light. The absorbance was measured at 415 nm with a Micro plate Reader (Biotek, USA.). Total flavonoid contents in the plants extracts were expressed as mg/g Quercitin Equivalents (QE) per gram of dry plant material. All samples were analyzedanalysed in triplicates.

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-diphenly-2-picrylhydrazyl-hydrate (DPPH). The effect of the plant extracts on DPPH radical was performed as described by Liyana-Pathirana and Shahidi [2223] with minor modification. Briefly, 25 µl of each plant extract (ranging from 0 to 10 mg/ml) or standard solution of ascorbic acid, BHA and Gallic 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 (Biotek, 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:

$$AAI=[DPPH]\ (\mu g/ml^{-1})/IC50\ (\mu g/ml^{-1})$$

Where [DPPH] is final DPPH concentration.

2.6. Microbiological Studies

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

Table 2. Test microorganisms.

Microrganisms	Species name	Source	Notes
Bacteria	*Staphylococcus aureus*	ATCC 25923	Gram Positive +ve
	Proteus vulgaris	ATCC 13315	Gram Negative –ve
	Pseudomonas aeruginosa	ATCC 27853	
	Salmonella typhi	ATCC 14028	

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	Escherichia coli	ATCC 25922	
	Klebsiella ~~pneumoniae~~pneumonia	ATCC 13883	
Candida	*Candida albicans*	CBS6589	
		CBS9120	
		BERC N43	Clinical Specimens
		BERC N72	
		BERC N66	
Dermatophytes	*Microsporum canis*	CBS132.88	
	Trichophyton rubrum	BERC-EH-TR9	Clinical Specimen

2.6.1. Well-diffusion method

Antibacterial and anticandidal activities of the selected plants extracts were assessed using the agar well-diffusion method [~~23~~24]. Muller-Hinton and Muller-Hinton supplemented with glucose-methylene blue plates were used for antibacterial and anticandidal susceptibility tests, respectively. An inoculum of 18 ~~hour~~ old -broth culture (turbidity adjusted to approximately 10^8 CFU/ml of bacterium and candida, compared with 0.5 McFarland standards) [~~24~~25] of respective bacterial and ~~*candida*~~ *Candida* strain was uniformly spread on these media in separate plates [~~25~~26]. Wells (6 mm diameter) were created in these plates, and 50 µl of plant extracts (100 mg/ml) were pipetted into the wells and allowed to diffuse at room temperature for 30 min. Plates were incubated at 37℃ for 18-24 h [~~26~~27]. The zone of inhibition for each extract was measured and expressed in mm [~~25~~26]. The activity index (AI) and ~~Percent~~ percent ~~Inhibition~~ inhibition (PI) were calculated for all extracts obtained at a concentration of 100 mg/ml using the following formula:

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

$$PI = AI \times 100$$

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

2.6.2. Broth Micro-dilution test

Broth micro-dilution was performed following the CLSI M27-A2 method [~~27~~28]. Plant extracts were dissolved in methanol and the correct volume was pippeted in the first micro-plate well with Muller-Hinton media (pH 7.2), for the concentration of each plant extract to be 5 mg/~~mL~~ 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~~ml). This suspension was inoculated in each well of a micro-dilution plate previously prepared with the plant extracts to give concentrations from 5 mg/~~mL~~ ml down to 0.039 mg/~~mL~~ ml

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[2829]. The plates were incubated with agitation at 37℃ 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 1-250-1 µg/mL ml for gentamicin, and from 0.125-16.0- 0.125µg/mL ml for amphotericin B. Value of minimum inhibitory concentration (MIC), determined by broth micro- dilution, and was defined as the lowest concentration of the drug completely inhibited the growth of the isolate. For plant extracts, the lowest concentration without visible growth (visually and spectrophotometerically) was defined as MICs

2.6.3. Anti-dermatophyte testing

Plants extracts were tested for their anti-dermatophyte activity against two dermatophyte species (*Microsporum canis* and *Trichophyton rubrum*) using a modified poisoned food technique [2930]. Each extract was incorporated in pre-sterilized SDA medium at a concentration of (0.4 mg/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/mLml) 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:

% mycelial inhibition = (dc- ds / dc) x100%

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 [3031]. In food, polyphenols may contribute to the bitterness, astringency, colour, flavour, odour and oxidative stability [3132]. Epidemiological studies and associated meta-analyses strongly have suggested that long term consumption of diets rich in plant polyphenols offered provide some protection against diseases including development of cancers, chronic diseases, osteoporosis and neurodegenerative diseases [3233, 3334]. Polyphenols and other food phenolics are the subject of increasing scientific interest because of their possible beneficial effects on human health. In this study total phenolic content (TPC) was estimated using Folin-Ciocalteu's method. TPC of all extracts was found to be in the range of 0.53-14.91 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 *RhusR. coriaria* (14.91 mg GAE/g extract), while it was only 0.89 in the plant leaves.

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Other plants with high TPC were the leaves of *Epilobium Epi. hirsutum* (13.46 mg GAE/ g), fruits of *PistaciaP. palaestina* (9.7 mg GAE/g), and the leaves of *EphedraEph. aphylla* is (8.08 mg GAE/ g).

A phytochemical analysis of the fruits of *R.hus coriaria* was conducted recently [3435], 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 [3435]. Also, several *Pistacia* species are known to be rich in gallotannins and related phenolic compounds [3536, 3637].

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 [3738].

Total flavonoid content was measured using the aluminium chloride colorimetric assay. The total flavonoid content of all extracts ranged between 0.05-1.14 mg QE/g extract (Table 1), the highest level of flavonoid content was found in the leaves of *EpilopiumEpi. hirsutum* and *PistaciaP. palaestina* (1.14 and 1.11 respectively), while the lowest flavonoid content was in the underground part extract of *UrgineaU. maritima* (0.05 mg QE/g extract). Other plants with high levels of flavonoid were leaves of *LyciumL. schweinfurthii* (0.82 mg QE/g), and fruits of *CeratoniaC. siliqua* (0.789 mg QE/g). The fruit extracts of *LyciumL. schweinfurthii* and flowers of *EchinopsEch. adenocaulos* had very low levels of flavonoid (0.07 and 0.089 mg QE/g, respectively).

Methanol plant extracts contained a higher proportion (≥ 50%) of phenolics than flavonoids (Figure 1).

3.3. Antioxidant Activity

In this study, the antioxidant activity of plant extracts were evaluated using DPPH free radical scavenging assay. Except for the fruits of *Ailanthus altissima*, flowers of *AlceaAl. setosa*, leaves and fruits of *LyciumL. schweinfurthii,* and underground parts and fruits of *UrgineaU. maritima* extracts, all extracts showed DPPH radical scavenging activity. *EpilopiumEpi. hirsutum* leaves revealed the highest antioxidant activity with (AAI= 1.298, IC50=33 µg/ml), followed by the extract of *RhusR. coriaria* leaves (AAI= 0.87, IC_{50}=49 µg/ml) (Table 1). The activities of leaf extracts varied from (IC_{50}=33µg/ml) in *EpilopiumEpi. hirsutum* to (IC_{50} = 325µg/ml) in *EucalyptusE. camaldulensis*. While the activity of fruit extracts varied from AAI =0.304 in *EucalyptusE. camaldulensis* to AAI= 0 in *AilanthusA. altissima*, *LyciumL. schweinfurthii* and *UrgineaU. maritima*. However, the fruits and leaves of *PistaciaP. palaestina* which have AAI=0.327 and 0.3, respectively, have been shown by others to possess a high antioxidant activity [3839].

In our study, a weak correlation was found between radical scavenging antioxidant activity and total phenolics in plant parts. Interestingly, a few of the collected plant parts with high-antioxidant activity are

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220 ‘‘low’’ in phenolic content including the leaves of *CeratoniaC. siliqua* and *RhusR. coriaria*. These plants
221 may serve as sources of antioxidants with new chemotypes.

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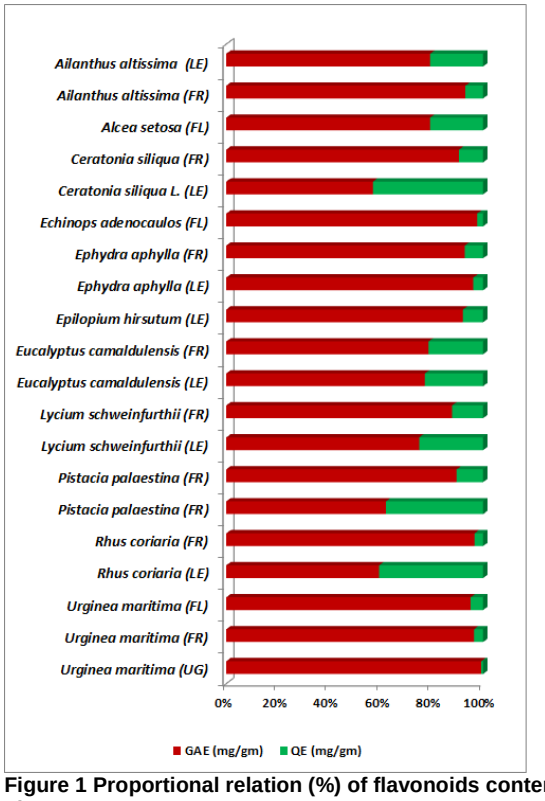

223
224 **Figure 1 Proportional relation (%) of flavonoids content to phenolic acids in analysed medicinal**
225 **plants.**

226 **3.4. Antibacterial Activity**

227 In the present work the antibacterial activity of the twenty methanol extracts of plants parts were
228 evaluated against six bacterial strains, using agar well diffusion and serial micro dilution (MIC) methods.
229 The results of the antibacterial screening test showed that of the twenty extracts tested only seven
230 extracts belonging to 4 plants species showed antibacterial activity (Table 3). The most active plant
231 extract against all bacteria strains was the leaves of *RhusR. cCoriaria*. The percent of inhibition of *RhusR.*
232 *coriaria* leaves extract ranged between 60.9-–76.2 against tested bacteria. However, the leaves of
233 *AilanthusA. altissima* showed moderate antibacterial activity with percent of inhibition of ranged from 41.8
234 to 55.8. However, the fruits of *AilanthusA. altissima* and leaves of *EucalyptusE. camaldulensis* showed
235 the least activity. Our results are in accordance with previous studies in which —the leaves of *RhusR.*

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coriaria and *Ailanthus altissima* have been shown by other researchers to possess high antibacterial activity [5, 39, and 41]. Bioactive compounds produced by plants have been found to protect plants against bacteria, fungi and pests [42, 43], thus it is expected that the plants extracts were composed of antibacterial activity.

Table 3. Percent inhibition (PI) and minimum inhibitory concentration (MIC) (mg/mL) of plant extracts against bacterial strains

Plant name (Part*)	*Salmonella typhi*		*Klebsiella pneumoniae*		*Staphylococcus aureus*		*Proteus vulgaris*		*Pseudomonas aeruginosa*		*Escherichia coli*	
	PI**	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)	PI	MIC (mg/ml)
Ailanthus altissima (FR)	42.9	2.50	0	0.00	35.9	1.25	0	0.00	0	0.00	0	0.00
Ailanthus altissima (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
Eucalyptus camaldulensis (LE)	0	0.00	0	0.00	48.4	2.50	0	0.00	0	0.00	48.6	2.50
Pistacia palaestina (FR)	0	0.00	60.8	0.30	39.1	2.50	61.9	0.63	45.3	1.25	52.6	5.00
Pistacia palaestina (LE)	38.6	5.00	37.3	1.25	34.4	1.25	40.5	2.50	0	0.00	40.5	2.50
Rhus coriaria (FR)	51.5	5.00	45.6	1.25	48.4	0.30	61.9	2.50	0	0.00	0	0.00
Rhus coriaria (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 (10 mg/ml)	100	0.01	100	0.01	100	0.01	100	0.01	100	0.01	100	0.01

* FR, fruit; LE, leaves.

3.5. Anti-Candida Activity

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

Table 4. Percent inhibition zone (PI) and minimum inhibitory concentration (MIC) (mg/mL) of plant extracts against *Candida albicans* strains

Plant (Part)*	BERC N43		BERC N72		BERC N66		CBS 6985		CBS 9120	
	PI	MIC (mg/mL)	PI	MIC (mg/ml)	PI	MIC (mg/mL)	PI	MIC (mg/mL)	PI	MIC (mg/mL)
Ailanthus altissima (FR)	36.5	5.0	0	0	0	0	0	0	0	0
Ailanthus altissima (LE)	65.2	0.60	64.0	0.15	42.5	1.25	39.1	1.25	44.3	2.5
Ceratonia siliqua (FR)	46.1	0.15	38.1	0.6	39.6	0.6	36.5	0	0	0
Ceratonia siliqua (LE)	46.1	1.25	36.3	1.25	39.6	2.5	36.5	0.6	40.6	5.0
Epilobium hirsutum (LE)	80.9	0.15	75.2	0.15	72.5	0.3	66.8	0.6	66.6	0.6
Eucalyptus camaldulensis (FR)	80.9	0.30	54.9	0.15	56.4	1.25	51.9	2.5	63.9	2.5
Eucalyptus camaldulensis (LE)	82.6	0.30	58.4	0.6	57.5	5.0	52.9	5.0	42.4	5.0
Pistacia palaestina (LE)	46.9	0.30	55.9	0.3	42.5	1.25	39.1	1.25	46.1	1.25
Rhus coriaria (FR)	57.4	0.30	54.2	0.6	45	2.5	41.4	02.5	47.1	2.5
Rhus coriaria (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	0.008	100	0.008	100	0.002	100	0.001	100	0.001

* FR, fruits; LE, leaves.

3.6. Antidermatophyte Activity

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Many effective synthetic antifungal agents are currently available and have been used for the treatment of dermatophytec infections [9]. However, these antifungal drugs tend to have serious side-effects including toxicity, drug interactions, inadequate pharmacokinetic properties and the development of resistance have been reported [43~~44~~]. The discovery of natural active components exhibiting a broad spectrum of antidermatophyte activity may prove useful for the development of antifungal agents. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [44~~45~~]. Various previous researches have been conducted to evaluate the anti-dermatophytic activity of plants [9, 45~~46-48-47~~].

In this study, plant extracts tested have shown considerable antidermatophytic activities at concentration of 0.4 mg/ml against the two tested dermatophytes (*M. canis, and T. rubrum*) in comparison with the positive control (econazole). The percent of mycelial inhibition at the concentration of 0.4 mg/ml plant extract ranged between 13%-97% against *M. ~~jcrosporum~~ canis* and 7%-86% against ~~*Trichophyton T. rubrum*~~ (Figure 2). The most active plants extracts which exhibited more than 50% inhibition against both dermatophytes were the leaves and fruits of ~~*LyciumL. chweinfurthii*~~, ~~*EucalyptusE. camaldulensis*~~ and ~~*RhusR. coriaria*~~ and leaves of ~~*EpilopiumEpi. hirsutum*~~ and ~~*AilanthusA. altissima*~~. Of these extracts the fruits of ~~*RhusR. coriaria*~~ and leaves of ~~*AilanthusA. altissima*~~ revealed the highest antidermatophyte activities with 97 % and 74 % mycelial inhibition against (*M.~~jcrosporum~~ canis*), respectively, and 86 % and 74% against *T~~richophyton~~ rubrum* (Figure 2). Abdolmaleki et al [48~~49~~] have shown that methanolic extracts of stem and fruit of sumac had the highest inhibitory activity against *Fusarium oxysporum* and *Phytophthora~~.~~ drechsleri*, respectively. While the ethanolic extract of the leaves and methanolic extracts of fruit, leaf and stem had the highest inhibitory activity against *Rhizoctonia solani*.

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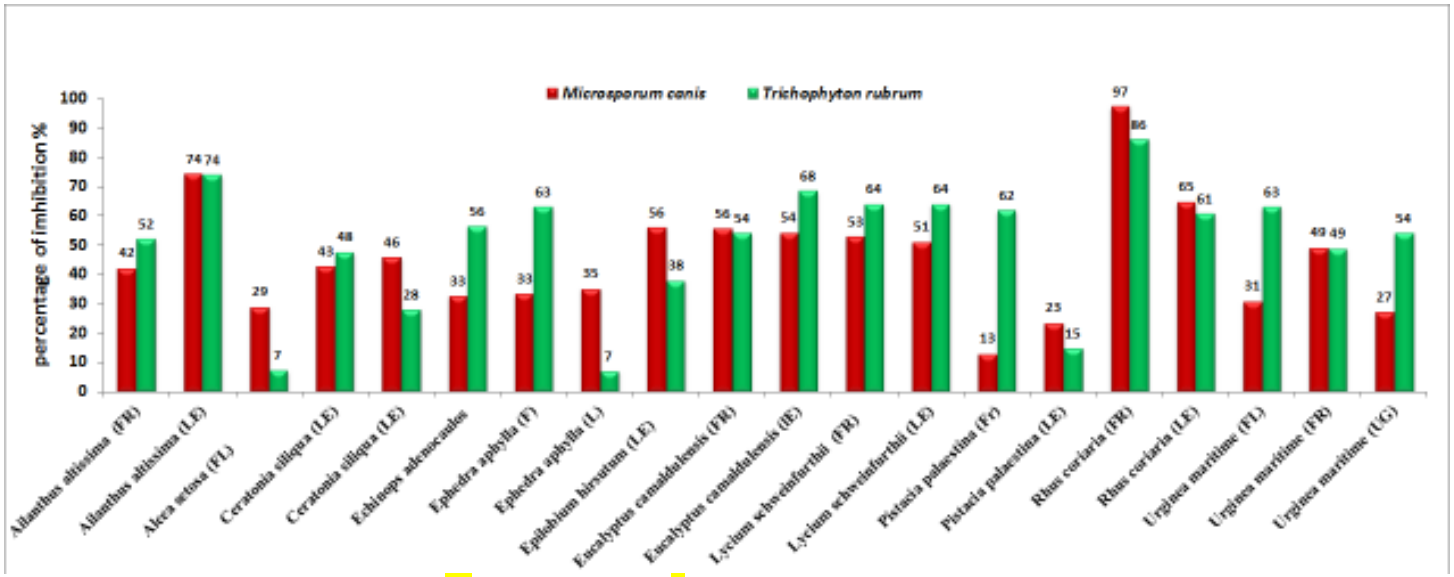

Figure 2 Antidermatophyte activity of plant eExtracts (% inhibition).

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Plants are rich source of thousands of new useful phytochemicals of great diversity, which have inhibitory effects on all types of microorganisms *in vitro*. Although more than 600 plants have been reported for their antifungal properties, however a few of them were explored for the active components [4445]. In this study leaves have shown to be more active than fruits of the same plant. This might be attributed either to the presence of different active chemical compounds or to the different concentrations of these compounds between leaves and fruits. Previous research studies reported the presence of different chemical groups in plant extracts including: phenolics, flavonoids, organic acids, saponins, terpenoids and alkaloids [4445, 4950-52-51]. The variation between plants extracts activity might be related to the different chemical groups and the variation in their concentrations in these plants. The results of the present study might suggest that *RhusR. coriaria* and *EucalyptusE. camaldulensis* are promising and presumably possess compound(s) with chemical properties against dermatophytes.

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4. CONCLUSION

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

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ACKNOWLEDGEMENTS

This research was performed with funding from the European Union under the ENPI CBC MED Programme as part of the BioXplore project (www.bioxplore.org), and is a collaborative international project ref. no. I-B/1.1/288.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

MSA-S and RMJ designed the study, and wrote the manuscript. SYA-Z, AIH, and IBYQ performed the lab work. All authors read and approved the final manuscript.

CONSENT (WHERE EVER APPLICABLE)

It is not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

It is not applicable

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