Original Research Article

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

Rana M. Jamous, Salam Y. Abu Zaitoun, Ahmad I. Husein, Iman B. Y. Qasem, Mohammed S. Ali-Shtayeh*

Biodiversity and Environmental Research Center (BERC),Til Village, Nablus, Palestine.

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 possesses strong antidermatophytic, antibacterial, anticandidal and antioxidant substances with potential applications in therapeutics and food industry.

* Tel.: +970 9 2536406; fax: +970 9 2536147.
E-mail address: msshtayeh@yahoo.com.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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 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.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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 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

Formatted: Font: Italic
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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 [~~2~~2021].

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.	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.	*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 [~~2~~2122]. 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 % 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

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Subscript

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 ~~per gram~~ of Quercitin Equivalents (QE) ~~per gram~~ of dry plant material. All samples were ~~analyzed~~analysed 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:

$$AAI=[DPPH]\ (\mu g/ml)/IC50\ (\mu g/ml)$$

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.

Microrganism	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	
	Escherichia coli	ATCC 25922	
	Klebsiella ~~pneumoniae~~pneumonia	ATCC 13883	
Candida	*Candida albicans*	CBS6589	

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

		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 [2324]. 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) [2425] of respective bacterial and candida Candida strain was uniformly spread on these media in separate plates [2526]. 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 [2627]. The zone of inhibition for each extract was measured and expressed in mm [2526]. 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 X100

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 candidaCandida, respectively.

2.6.2. Broth Micro-dilution test

Broth micro-dilution was performed following the CLSI M27-A2 method [2728]. 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 [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

Formatted: Highlight
Formatted: Highlight
Formatted: Superscript
Formatted: Highlight
Formatted: Font: Italic, Highlight
Formatted: Highlight
Formatted: Font: Italic, Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Font: Italic, Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Superscript
Formatted: Superscript
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Font: Italic
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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 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/~~mL~~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:

% 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 [~~30~~31]. In food, polyphenols may contribute to the bitterness, astringency, colour, flavour, odour and oxidative stability [~~31~~32]. 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 [~~32~~33, ~~33~~34]. 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 ~~*Rhus*~~*R. coriaria* (14.91 mg GAE/g extract), while it was only 0.89 in the plant leaves. Other plants with high TPC were the leaves of ~~*Epilobium Epi.*~~ *hirsutum* (13.46 mg GAE/ g), fruits of ~~*Pistacia*~~*P. palaestina* (9.7 mg GAE/g), and the leaves of ~~*Ephedra*~~*Eph. aphylla* is (8.08 mg GAE/ g).

A phytochemical analysis of the fruits of ~~*Rhus*~~*R. coriaria* was conducted recently [~~34~~35], 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~~35].

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

Also, several *Pistacia* species are known to be rich in gallotannins and related phenolic compounds [35, 36, 37].

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, 38].

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 *Epilopium Epi. hirsutum* and *Pistacia P. palaestina* (1.14 and 1.11 respectively), while the lowest flavonoid content was in the underground part extract of *Urginea U. maritima* (0.05 mg QE/g extract). Other plants with high levels of flavonoid were leaves of *Lycium L. schweinfurthii* (0.82 mg QE/g), and fruits of *Ceratonia C. siliqua* (0.789 mg QE/g). The fruit extracts of *Lycium L. schweinfurthii* and flowers of *Echinops Ech. 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 *Alcea Al. setosa,* leaves and fruits of *Lycium L. schweinfurthii,* and underground parts and fruits of *Urginea U. maritima* extracts, all extracts showed DPPH radical scavenging activity. *Epilopium Epi. hirsutum* leaves revealed the highest antioxidant activity with (AAI= 1.298, IC50=33 µg/ml), followed by the extract of *Rhus R. coriaria* leaves (AAI= 0.87, IC_{50}=49 µg/ml) (Table 1). The activities of leaf extracts varied from (IC_{50}=33µg/ml) in *Epilopium Epi. hirsutum* to (IC_{50} = 325µg/ml) in *Eucalyptus E. camaldulensis*. While the activity of fruit extracts varied from AAI =0.304 in *Eucalyptus E. camaldulensis* to AAI= 0 in *Ailanthus A. altissima*, *Lycium L. schweinfurthii* and *Urginea U. maritima*. However, the fruits and leaves of *Pistacia P. palaestina* which have AAI=0.327 and 0.3, respectively, have been shown by others to possess a high antioxidant activity [38, 39].

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 ‘‘low’’ in phenolic content including the leaves of *Ceratonia C. siliqua* and *Rhus R. coriaria*. These plants may serve as sources of antioxidants with new chemotypes.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

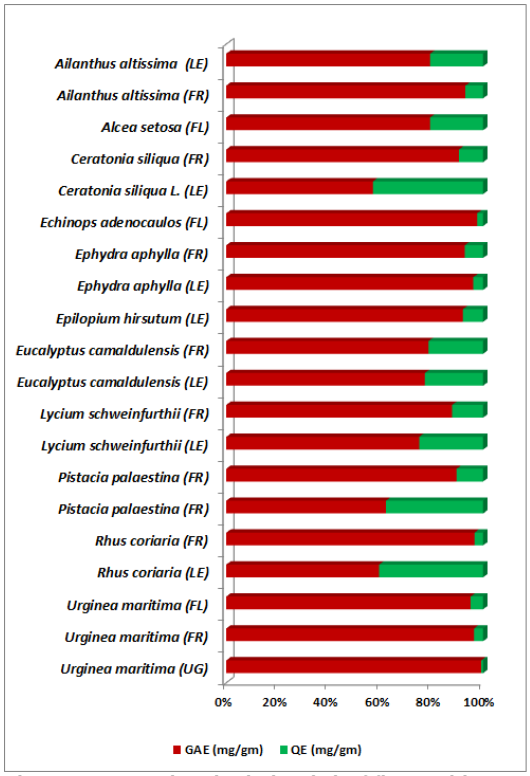

Figure 1 Proportional relation (%) of flavonoids content to phenolic acids in analysed medicinal plants.

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 *RhusR. cCoriaria*. The percent of inhibition of *RhusR. coriaria* leaves extract ranged between 60.9-–76.2 against tested bacteria. However, the leaves of *AilanthusA. altissima* showed moderate antibacterial activity with percent of inhibition of ranged from 41.8 to 55.8. However, the fruits of *AilanthusA. altissima* and leaves of *EucalyptusE. camaldulensis* showed the least activity. Our results are in accordance with previous studies in which –the leaves of *RhusR. coriaria* and *AilanthusA. altissima* have been shown by other researchers to possess high antibacterial activity [5, 3940, and 4041]. Bioactive compounds produced by plants have been found to protect plants against bacteria, fungi and pests [4142, 4243], thus it is expected that the plants extracts were composed of antibacterial activity.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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

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

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Font: Bold, Highlight
Formatted: Font: Bold
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

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 [4445]. Various previous researches have been conducted to evaluate the anti-dermatophytic activity of plants [9, 4546-48-47].

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. icrosporum 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.icrosporum canis*), respectively, and 86 % and 74% against *Trichophyton rubrum* (Figure 2). Abdolmaleki et al [4849] 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*.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

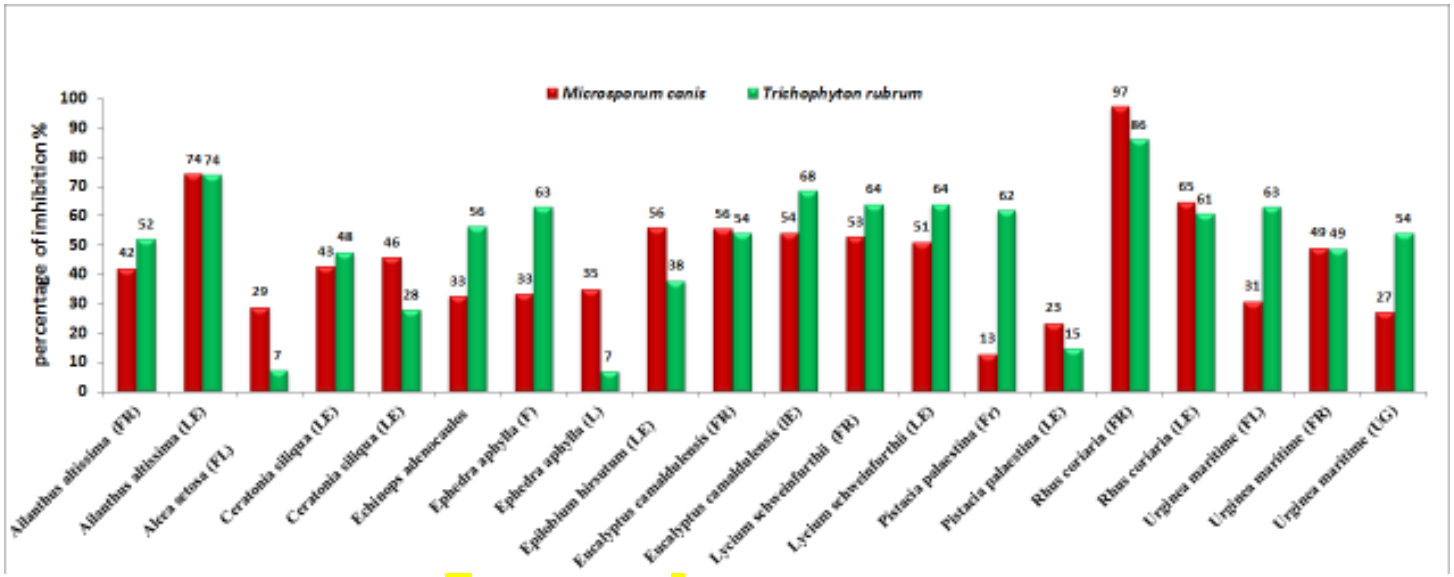

Figure 2 **Antidermatophyte activity of plant eExtracts (% inhibition).**

Formatted: Font: (Default) Arial, Not Bold, Highlight

Formatted: Highlight

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.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

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.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

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

REFERENCES

1. Ali-Shtayeh MS, Yaniv Z, Mahajna J. Ethnobotanical survey in the Palestinian area: A classification of the healing potential of medicinal plants. Journal of Ethnopharmacology. 2000;73:221-32.

2. Ali-Shtayeh MS, Jamous RM, Al-Shafie' JH, Elgharabah WA, Kherfan FA, Qarariah K, et al. 2008. Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): A comparative study. J Ethnobiol Ethnomed. 2008;4: 1-13.

3. Ali-Shtayeh MS, Jamous Rana M, Jamous Rania M. Herbal preparation use by patients suffering from cancer in Palestine. Complementary Therapies in Clinical Practice. 2011;17(4):235-240. http://dx.doi.org/10.1016/j.ctcp.2011.06.002

4. Ali-Shtayeh M.S., Jamous Rana M., Jamous Rania M. 2012. Complementary and alternative medicine use amongst Palestinian diabetic patients. Complementary therapies in clinical practice. 2012; 18(1):16-21.

5. Ali-Shtayeh MS, Al-Assali AA, Jamous RM. Antimicrobial activity of Palestinian medicinal plants against ance-inducing bacteria. African Journal of Microbiology Research. 2013;7.

6. Ali-Shatyeh MS, Jamous RM. Traditional Arabic Palestinian Herbal Medicine. Til- Nablus, Palestine: Biodiversity & Environmental Research Center; 2008.

7. Dhar U, Rawal RS, Samant SS, Airi S, Upreti J. People's participation in Himalayan biodiversity conservation: a practical approach. Current Sci. 1999;76:36–40.

8. Azam NK, Mannan A, Ahmed N. Medicinal plants Used by The Traditional Medical Practitioners of Barendra and Shamatat (Rajshahi & Khulna Division) Region in Bangladesh for treatment of Cardiovascular Disorders. Journal of Medicinal Plants Studies. 2014;2(2):9-14.

9. Orhan DD, Özçelik B, Hoşbaş S, Vural M. Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dematophytes and yeast-like fungi. Turk J Biol. 2012;36:672-686.

10. Chang LY, Crapo JD. Inhibition of airway inflammation and hyperreactivity by an antioxidant mimetic. Free Radical Biology and Medicine. 2002;33(3):379–86.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Font: Not Italic
Formatted: Highlight
Formatted: Font: Not Italic

11. Kelly SA, Havrilla CM, Brady TC, Abramo KH, Levin ED. Oxidative stress in toxicology: established mammalian and emerging Piscine Model systems. Env. Hlth. Persp. 1998;06:375–384.

12. Meenakshi S, Manicka GD, Tamil MS. Total flavonoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram coast. Global J. Pharmacol~~GJP~~. 2009;3:59-62.

13. Zengin G, Cakmak YS, Guler GO, Aktumsek A. Antioxidant Properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. Hayekiana Wagenitz. Rec Nat Pro. 2011;5:123-32.

14. Obeidat M. Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens. Journal of Medicinal Plants Research. 2011;5(16):3856-60.

15. Sieradzki K, Wu SW, Tomasz A. Inactivation of the methicillin resistance gene mecA in vancomycin-resistant *Staphylococcus aureus*. Micro Drug Resist. 1999;5(4):253–57.

16. Portillo A, Vila R, Freixa B, Adzet T, Canigueral S. Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol. 2001;76:93-98.

~~16~~17. Perea S, Patterson TF. Antifungal resistance in pathogenic fungi. Clin Infec Dis. 2002;35:1073-80.

~~17~~18. Barberino MG, Silva N, Reboucas C, Barreiro K, Alcantara AP, Martins NE. Evaluation of blood stream infections by *Candida* in three tertiary hospitals in Salvador, Brazil: a case-control study. Braz J Infect Dis. 2006;10:36-40.

~~18~~19. Colombo AL, Nucci M, Park BJ, Nouer SA, Arthington-Skaggs B, Da Matta DA. et al. Brazilian network candidemia study. Epidemiology of candidemia in Brazil: anationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbio. 2006;44:2816-23.

~~19~~20. Dicko MH, Hilhorst R, Gruppen H, Traore AS, Laane C, van Berkel WJH. et al. Comparison of content in phenolic compounds, polyphenol oxidase, and peroxidase in grains of fifty sorghum varieties from Burkina Faso. Journal of Agricultural and Food Chemistry.2002;50(13):3780-88.

Formatted: Font: 11 pt, Font color: Auto, Pattern: Clear, Highlight

Formatted: Highlight

Formatted: Font: (Default) Arial, 11 pt, Highlight

Formatted: Highlight

Formatted: Font: (Default) Arial, 11 pt, Highlight

Formatted: Highlight

Formatted: Font: (Default) Arial, 11 pt, Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Indent: Left: 0", First line: 0"

Formatted: Highlight

Formatted: Highlight

20. Savitre M, Isara P, Nitaya SL, Worapan S. Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used in Primary Health Care. Journal of Pharm. Science. 2004;9(1):32-35.

21. Chatatikun M, Chiabchalard A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn) root crude extracts. Journal of Chemical and Pharmaceutical Research. 2013;5(4):97-102.

22. Liyana-Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. Journal of Agricultural and Food Chemistry. 2005;53(7):2433-40.

23. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medecine Experimentaalis. 1990;15:113- 115.

24. Mahida Y, Mohan JSS. Screening of plants for their potential antibacterial activity against *Staphylococcus* and *Salmonella spp*. Nat. Prod. Rad. 2007;6:301-305.

25. Omoregie EH, Ibrahim I, Nneka I, Sabo AM, Koma OS, Ibumeh OJ. Broad Spectrum Antimicrobial Activity of *Psidium guajava* Linn. Leaf. Nature and Science. 2010;8(12):43-50.

26. Delahaye C, Rainford L, Nicholson A, Mitchell S, Lido J, Ahmad M. Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. Journal of Medical and biological Sciences. 2009;3(1):1-7.

27. National Committee for Clinical Laboratory Standards (NCCLS). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts—Second Edition: Approved Standard M27-A2. Wayne, PA, USA: NCCLS, 2002.

28. Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Gianinni MJ. 2007. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts Candida sp. and Cryptococcus sp. Braz J Microbiol. 2007; 38: 391-397.

29. Dikshit A, Husain A. Antifungal action of some essential oils against animal pathogens. Fitoterapia. 1984;55:171–176

30. Beckman CH. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? Physiol. Mol. Plant Pathol. 2000;57:101–110.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Font color: Black, Highlight

Formatted: Highlight

Formatted: Highlight

31. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity. 2009;2(5):270-278.

32. Graf BA, Milbury PE, Blumberg JB. Flavonols, flavonones, flavanones and human health: Epidemological evidence. J Med Food. 2005; 8:281-90.

33. Arts ICW, Hollman PCH. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr. 2005;81:317-25.

34. Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D, Segura-Carretero A. 2015. HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. Food Chemistry. 2015;166:179-191. DOI: 10.1016/j.foodchem.2014.06.011

35. Barotto MC, Tattini M–, Galardi C, Pinelli P, Romani A, Visioli F. et al. Antioxidant activity of galloyl quinic derivatives isolated from *P. lentiscus* leaves. Free Radical Res. 2003;37:405-12

36. Hou AJ, Peng LY, Liu YZ, Lin ZW, Sun HD. Gallotannins and related polyphenol from *Pistacia weinmannifolia*. Planta Medica. 2000;66:6246.

37. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol. 1998;12:249-55.

38. Alali FQ, Tawaha Kh, El-Elimat T, Syouf M, El-Fayad M, Abulaila Kh, et al. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. Natural Product Research. 2007;21(12): 1121-1131.

39. Erturk O. Antibacterial and antifungal effects of alcoholic extracts of 41 medicinal plants growing in Turky. Czech J. Food sci. 2010;28:53-60.

40. Rahman A, Kim E, Kang SCH. Antibacterial and antioxidant properties of *Ailanthus altissima* swingle leave extract to reduce foodborne pathogens and spoiling bacteria. Journal of Food Safety. 2009;29(4):499–510.

41. Patra JK, Dhal NK, Thatoi HN. In vitro bioactivity and phytochemical screening of Suaeda maritime (Dumort): A mangrove associate from Bhitarkanika, India. Asian Pacific J. of Tropical Med. 2011;4(9):727-734

42. Aboaba OO, Smith SI, Olude FO. 2006.Antimicrobial effect of edible plant extract on Escherichia coli 0157: H7. Pak J Nutr. 2006;5: 325-327.

Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Font: Not Italic
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight
Formatted: Highlight

43. Ayati A, Falahati M, Irannejad H, Emami S. Synthesis, in vitro antifungal evaluation and in silico study of 3-azolyl-4-chromanone phenylhydrazones. Daru. 2012;20:46.

44. Arif T, Mandal TK, Dabu R. Natural products: Anti-fungal agents derived from plants. In: Tiwari VK, Mishra BB, editors. Opportunity, challenge and scope of natural products in medicinal chemistry. India: Research Signpost; 2011.

45. Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. Mycoses. 1999;42:665-672.

46. Ali-Shtayeh MS, Zayed RAG, Jamous RM. Palestinian plants as a source of antimycotics. In: Rai MK, Mares D, Eds. Plant Derived Antimycotics Binghamton: The Haworth Press; 2003:399-427.

47. Husein AI, Al-Nuri MA, Zatar NA, Jondi W, Ali-Shtayeh MS, Warad I. Isolation and antifungal evaluation of *J. regia* L. extracts. IJRRAS. 2012;13(2):655-60.

48. Abdolmaleki M, Panjeke N, Bahraminejad S, Abbasi S. Antifungal activity of extracts of different sumac (*Rhus coriaria* L.) organs on four phytopathogenic fungi species. Agricultural Research. 2008;7(4A):121-31.

49. Orhan Deliorman D, Hartevioğlu A, Küpeli E, Yeşilada E. In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from Rosa canina L. fruits. J. Ethnopharmacol. 2007;112:394-400.

50. Oliveira AP, Pereira JA, Andrade PB. Targeted metabolites and biological activities of *Cydonia oblonga* Miller leaves. Food Chemistry. 2008;111:393-99.

51. Zidorn C, Schubert B, Stuppner H. Phenolics as chemosystematic markers in and for the genus *Crepis* (Asteraceae, Cichorieae). Sci. Pharm. 2008;76:743-50. doi:10.3797/scipharm.0810-25

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Font: Italic

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight