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Investigation of antibacterial & antioxidant activity for methanolic extract from different edible plant species in Palestine

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Abstract

Medicinal plants are finding their way into pharmaceutical derivatives, cosmetics and food supplements. The present study was undertaken to investigate the leave extracts of eleven plant species; Malva sherardiana, Plumbago europaea, Ephedra alata, Arum Palestinum , Centaurea dumulosa , Eruca Sativa, Teucrium polium, Bupleurum subovatum , Geranium robertianum , Ononis speciosa and Plumbago auriculata for their potential activity against human bacterial pathogens and antioxidant efficiency. The crude extract of the plant leaves and selected antibiotic was evaluated against five different bacterial pathogens : *Staphylococcus aureus*, (gram positive), *Escherichia coli*, *Proteus mirabilis*, *Klebsiella sp*., and *Pseudomonas aeroginosa* (gram negative) using agar well diffusion method. The Gram – positive bacteria *S. aureus* showed the higher susceptibility of inhibition with most plant extracts mainly *Plumbago europaea methanol* extract with zone of 12 mm, Centaurea dumulosa extract with 10 mm inhibition zone and Ononis speciosa with 6 mm inhibition zone. whereas Gram – negative bacteria; E. coli and P. aeruginosa showed some degree of susceptibility only for extracts of Centaurea dumulosa, and Klebsiella sp. Showed a little degree of inhibition with 2 mm zone for extracts of Bupleurum subovatum. In DPPH (free radical scavenging assay) for antioxidant evaluation for each plant extract ; Eruca Sativa had the lowest IC50 value which was 11.74 µg/mL the closest value to Trolox IC50 , Ephedra alata & Teucrium polium had also relatively potent antioxidant activity with 15.85 & 15.13 µg/mL IC50 value respectively. On the other hand Plumbago auriculata was considered as the weakest plant on free radical scavenging assay with 83.56 µg/mL for IC50 value. Other investigated medicinal plants showed relatively moderate free radical scavenging activity. This study support the use of these herbal plants traditionally to cure some infectious diseases, mainly caused by Gram – positive bacterium and also as antioxidant agents.

Keywords: *antibacterial; pharmaceutical; antimicrobial; traditional medicine; well diffusion method.*

1. Introduction

Infectious bacterial diseases are among the most important global health problems. Antibiotic treatment is a preferred choice to treat bacterial infections; however, emergence of antimicrobial resistance and toxicity issues subside the use of antibacterial agents [1]. For ages, people in both developed and developing countries were utilizing herbal medicines for improving their health status [2 -5]. This includes the usage of various types of phytochemicals found in plants, as they play an important role in decreasing the incidence of many human diseases [6 -8]. In addition, folk remedies are also used as a raw material of antimicrobial and antioxidant drugs [9]. In Palestine, several ethnobotanical studies and field surveys have proved that medicinal plants used in folk medicine are effectively used as a treatment of various diseases including cancers, injuries, and other chronic diseases [10, 11]. In a screening study, of some Palestinian medicinal plants for antibacterial activity, it was found that, eight plant species out of 15 showed antibacterial activity, each plant species has unique reactivity against different bacteria. The most active antibacterial plants against both gram-positive and gram-negative bacteria were *Thymus vulgaris* and *Thymus origanium*. [12]
Nowadays, the bacterial resistance of the available antimicrobial agents is becoming a growing problem. Therefore, several research projects are working on improving new antimicrobial agents that could overcome the resistance [13 - 17]. In addition, natural antioxidants have been shown to be beneficial in a variety of complications such as cancer [18], burn [19],diabetes [20] , hyper-lipidemia and amnesia [21]. The objective of this research is to evaluate the potentiality of eleven plant extracts on standard microorganism strains as well as multi-drug resistant bacteria, and to evaluate their Free radical scavenging activity compared with standard antioxidant trolox.

2. MATERIALS AND METHODS

2.1. Chemicals

Agar, dextrose, Mannitol Salt Agar (MSA), MacConkey-Agar (MAC), Methylene blue Agar (EMB), Muller–Hinton agar (MHA), ampicillin, were purchased from Merck (Darmstat, Germany). Sodium carbonate, ethanol, methanol, acetone and all other chemicals and reagents were of analytical grade

2.2. Instrumentation

Shaker device (Memmert shaking incubator, Germany), rotary evaporator (Heidolph OB2000 Heidolph VV2000, Germany), grinder (Moulinex model, Uno, China), balance (Rad wag, AS 220/c/2, Poland), filter paper (Machrery-Nagel, MN 617 and Whatman no.1

2.3. Bacterial selections and culturing media:

Five different species of bacteria were selected. Four of these species were gram negative (*Escherichia coli*, *Proteus mirabilis*, Klebsiella sp., and Pseudomonas aeroginosa) and one Gram-positive (*Staphylococcus aureus*); all were clinically isolated and identified from patients suffering of bacterial infections with relevant ones. Each species of bacteria was inoculated and maintained primarily on its corresponding media [22 - 24]. i.e. *Staphylococcus aureus* was on Mannitol Salt Agar (MAS) agar gel; *Pseudomonas aeruginosa* on MacConkey-Agar (MAC); *Escherichia coli* on Eosin Methylene blue Agar (EMB); *Proteus mirabilis* and *Klebsiella sp*. on Mueller-Hinton-Agar (MHA)

2.4. Selection of medicinal plants

Eleven different medicinal plant species; *Malva sherardiana, Plumbago europaea, Ephedra alata, Arum Palestinum , Centaurea dumulosa , Eruca Sativa, Teucrium polium, Bupleurum subovatum , Geranium robertianum , Ononis speciosa and Plumbago auriculata* were collected in March 2015 from the mountains in different regions of

Palestine. The leaves of these plants were washed and then dried in the shade at room temperature until all the plant parts became well dried. After drying, the plant materials were then powdered well by using grinder and placed into a well closed container.

2.5. Extracts Preparation

Twenty five g of the grounded plant leaves were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rpm for 72 hours at room temperature then stored in refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under vacuum on a rotary evaporator. The crude extract was stored at 4ºC for further use.
For antibacterial test, a sample from each plant extract with a concentration of 50mg/ml in 10% DMSO was prepared and used for antibacterial analysis.

2.6. Antibacterial activity screening test

The antibacterial activity screenings were tested using the well diffusion method (Perez et al., 1990) [25,26] . After adjusting the turbidity of inoculums bacterial suspension, a sterile cotton bacterial swab was dipped in the bacterial suspension and was streaked on its corresponding agar media to cover the entire surface of the plates. Plates were left few minutes to dry at room temperature and about 5 mm wells were punched in each plate, and filled with 50µl of 50 mg/ml of sample crude extracts in 10% DMSO ; added into duplicate wells, while 10 mg/ml of Ampicillin (positive control) was used as a control in each plate. Plates were allowed to stand at room temperature to let the tested derivative be diffused into the agar, and afterwards, they were incubated at 37°C for overnight. Then, plates were examined for bacterial growth and inhibition zones to be then measured in diameter.

2.7. DPPH assay for antioxidant activity evaluation

The following protocol for Free radical scavenging activity of different extracts of Tubers of was adopted. plant were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) according to the following procedure[27]: A stock solution of a concentration of 1mg/ml in methanol was firstly prepared for the plant extract and Trolox. The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/ml) were prepared by serial dilution with methanol from the stock solution. DPPH was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above prepared working concentration in a ration of 1:1:1 respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 minute at room temperature before the absorbance readings were recorded at 517nm. The percentage of antioxidant activity of the plants and the trolox standard were calculated using the following formula:
Percentage of inhibition of DPPH activity (%) = (A-B)/A ×100%
Where: A = optical density of the blank, B = optical density of the sample.

3. Results

The antibacterial activities of the methanolic extracts of selected plants against Gram – positive and Gram – negative bacteria were reported based on the observed clear zone of bacterial inhibition surrounding the well on the plate (Table 1). The result indicates that six of the plant extracts; *Malva sherardiana, Ephedra alata, Arum Palestinum* , *Eruca Sativa. Teucrium polium and Geranium robertianum* did not show any bacterial activity on gram – positive nor gram – negative bacteria at a 50µL concentration, while the extract of *Plumbago europaea* has the highest bacterial activity against *S.aureus* (Gram – positive) with a zone of inhibition of 12 mm, and has no activity for Gram

– negative bacteria, the same results obtained for extracts of *Ononis speciosa* with an inhibition zone of 6mm and *Plumbago auriculata* with 2mm inhibiotion zone against *S.aureus* only. On the other hand, the extract of *Centaurea dumulosa,* showed antibacterial activity against *S.aureus* , Gram – positive bacteria with inhibition zone of 10 mm, also it exerts some degree of sensitivity on Gram – negative bacteria; *E. coli* with inhibition zone 8 mm and *P. aeruginosa* with 6mm zone of inhibition. The only extract that showed a mild degree of sucibtiblity for (Gram – negative) bacteria *Klebsiella sp.* is *Bupleurum subovatum* with 2mm inhibition zone, in addition this extract also showed a bacterial activity against *S.aureus* (Gram – positive) with a zone of inhibition of 4 mm.

Table 1. Zone of Inhibition of eleven plant species

Compound/ plant species' extract	Zone of inhibition (mm)				
	Gram-positive **Bacteria**	Gram-negative **Bacteria**			
	S. aureus	*E. coli*	*P.mirabilis*	*P. aeruginosa*	Klebsiella sp.
Ampicillin	24	16	15	16	15
Malva sherardiana	-	-	-	-	-
Plumbago europaea	12	-	-	-	-
Ephedra alata	-	-	-	-	-
Arum Palestinum	-	-	-	-	-
Centaurea dumulosa	10	8	-	6	-
Eruca Sativa	-	-	-	-	-
Teucrium polium	-	-	-	-	-
Bupleurum subovatum	4	-	-	-	2
Geranium robertianum	-	-	-	-	-
Ononis speciosa	6	-	-	-	-
Plumbago auriculata	2	-	-	-	-

The results showed that *P.mirabilis* is insensitive for all tested plant extracts at 50µl in concentration, where as other Gram – negative bacteria; *E. coli* and *P. aeruginosa* showed some degree of susceptibility for extracts of *Centaurea dumulosa, and Klebsiella sp.* for extracts of *Bupleurum subovatum.* in contrast Gram – positive bacteria *S. aureus* showed the higher zone of inhibition in *Plumbago europaea methanol* extract with 12 mm, *Centaurea dumulosa* extract with 10 mm inhibition zone**,** *Ononis speciosa* extracts with 6mm inhibition zone and very small zones of inhibition in *Bupleurum subovatum* with 4mm and *Plumbago auriculata* with 2mm. For the part in our project the investigation of these medicinal plants in free radical scavenging ability to be considered as antioxidants table 2, showed the results for IC_{50} values in (µg/mL) for each investigated plant compared with Trolox the standard reference compound. From these resulted data in DPPH (free radical scavenging assay) for antioxidant evaluation for each plant extract ; *Eruca Sativa* had the lowest IC_{50} value which was 11.74 µg/mL the closest value to Trolox IC_{50} , *Ephedra alata & Teucrium polium* had also relatively potent antioxidant activity with 15.85 & 15.13 µg/mL IC_{50} value respectively. On the other hand *Plumbago auriculata* was considered as the weakest plant on free radical scavenging assay with 83.56 µg/mL for IC_{50} value. Other investigated medicinal plants showed relatively moderate free radical scavenging activity .the % Inhibition of DPPH was summarized in Figures 1.

Table 2 : DPPH assay for antioxidant activity evaluation for each plant extract compared to Trolox (reference standard compound)

Compound/ plant species' extract	IC_{50} (µg/mL)
Trolox (standard reference compound)	2.08
Malva sherardiana	41.68
Plumbago europaea	21.38
Ephedra alata	15.85
Arum Palestinum	45.70
Centaurea dumulosa	22.9
Eruca Sativa	11.74
Teucrium polium	15.13
Bupleurum subovatum	18.19
Geranium robertianum	64.56
Ononis speciosa	19.05
Plumbago auriculata	83.56

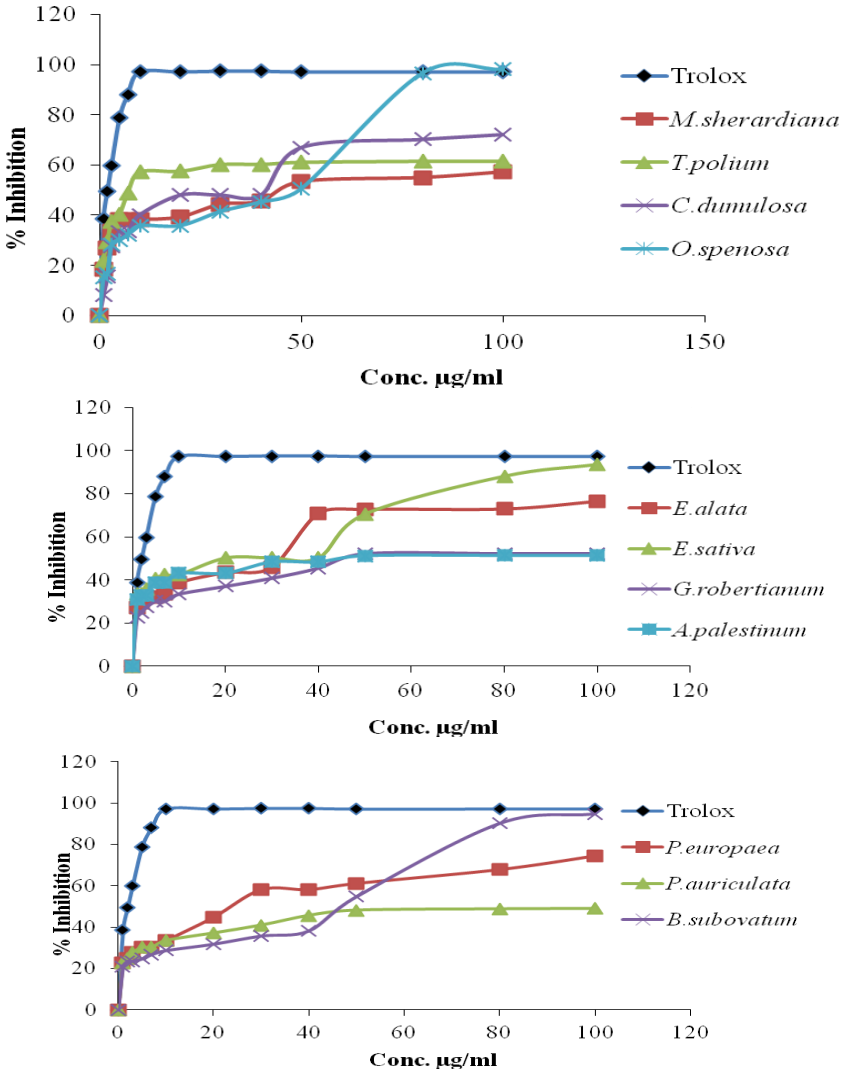

Figure 1: %Inhibition for DPPH (free radical) by Trolox standard and methanolic plant extracts

4. Discussion:

Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from plants. [28], as Plants possess antimicrobial properties due to the presence of a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc.[5]**.** In Palestine, a diverse flora of medicinal plants is grown naturally [10,11]. In the present study, we have investigated the antibacterial activity of eleven naturally growing plants: *Malva sherardiana, Plumbago europaea, Ephedra alata, Arum Palestinum , Centaurea dumulosa , Eruca Sativa, , Teucrium polium, Bupleurum subovatum , Geranium robertianum , Ononis speciosa and Plumbago auriculata,* The biological activity of these plant extracts was tested against known human bacterial pathogens.

On the basis of the results obtained in this investigation, we conclude that the methanolic extract of *Plumbago europaea , Centaurea dumulosa and Ononis speciosa* have in vitro antimicrobial activity especially on Gram – positive bacteria, but have mild or no activity in Gram – negative bacterium, possibly because of the presence of outer membrane that serves as an effective barrier in Gram – negative bacteria which prevent penetration of plant extracts. [29, 30]. However, not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms. Our finding corroborates previous study of Avancini et al., (2000), who reported that the antimicrobial actions of "carqueja" (*Baccharis trimera* Less.) decoction on gram-positive (*Staphylococcus aureus* and *Streptococcus uberis*) was more than on gram-negative (*Salmonella gallinarum* and *Escherichia coli*) bacterial strains [31]. Several studies were conducted in Palestine on the antibacterial activity of certain plant species, however, with different plant species, that already known for their antibacterial activity [12] , in our study, it is the first time to prove the microbial activity of some of the tested plant species. However, Based on these results, further studies on the pharmacological and bioactivities in *Plumbago europaea* and *Centaurea dumulosa* may be recommended.On the other hand for DPPH (free radical scavenging assay) assay to evaluate antioxidant activity for each plant extract ; *Eruca Sativa* had the lowest IC_{50} value which was 11.74 µg/mL the closest value to Trolox IC_{50} , *Ephedra alata & Teucrium polium* had also relatively potent antioxidant activity with 15.85 & 15.13 µg/mL IC_{50} value respectively. On the other hand *Plumbago auriculata* was considered as the weakest plant on free radical scavenging assay with 83.56 µg/mL for IC_{50} value. Other investigated medicinal plants showed relatively moderate free radical scavenging activity

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