

Antibacterial and Antioxidant Activities of Ethanol Extracts of Some Plants Selected from South Jordan

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ABSTRACT

Three Jordanian medicinal plants; *Peganum harmala* (*P. harmala*), *Geropogon hybridus* (*G. hybridus*), and *Salvia officinalis* (*S. officinalis*) were selected from Al-karak region in order to investigate their antibacterial and antioxidant activities. The crude extract yield of all used plants were varied from 10.6% in *Salvia officinalis* and 15.6% in *P. harmala*, to 18 % in *G. hybridus*. The antibacterial activity of these plants was carried out on four bacterial strain (*S. aureus*, *E.coli*, *E. aerogenes* and *B. subtilis*). These plant ethanol extracts have effects on the gram positive strains and gram negative bacteria except *S. officinalis* did not show activity against *E. Coli*. The minimal inhibition concentration (MIC) of *P. harmala* *S. officinalis* and *G. hybridus* (MIC) ranged from 125 µg/ml to 500 µg/ml. The ethanol crude extract of *G. hybridus* gave 10 fractions using TLC method, fraction 1,3 and 10 exhibited antibacterial activity against all bacterial strains. Their total phenolic contents were evaluated; the highest total phenol content was observed in *G. hybridus* with 410 mgGAE/g plant extract followed by *P. harmala* and *S. officinalis* with 305 and 203.7 mg/g plant extract, respectively. The antioxidant activity was estimated by using DPPH method. The highest antioxidant activity was obtained with *G. hybridus* extract and found to be 0.89 mg Trolox/g plant extract. The lowest antioxidant activity was obtained with *S. officinalis* which was 0.53 mg Trolox/g plant extract. The lowest IC₅₀ (highest antioxidant activity) was observed in *G. hybridus* with 0.83 mg/ml. Intermediate effect observed in *P. harmala* with IC₅₀ 1.1 mg/ml. The lowest antioxidant activity was obtained in *S. officinalis* with IC₅₀ 1.38 mg/ml.

Key words: Antibacterial, Antioxidant, Ethanol Extracts, *Peganum harmala*.

INTRODUCTION

A large number of chemical compounds and molecules are present in plants as a primary and secondary metabolites, primary metabolites play an important role in basic life functions such as cell division, growth, respiration, storage, and reproduction^{1,2}. Several other compounds which are not essential for the above functions are known as secondary metabolites which including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids^{2,3}.

Medicinal plants have been used in traditional medicine^{4,5}. All parts of the plant, including the seeds, root, stem, leaves, and fruit, potentially contains bioactive components used as the source of medicines. There are many advantages and benefits associated with the use of medicinal plants, the main ones being their cost-effectiveness and global availability⁶⁻⁸.

In addition to their nutritional components, medicinal plants were used in treating a wide spectrum of diseases. Moreover, they have been screened for their potential uses as alternative remedies, food preservatives, and as antioxidants to neutralize the toxic effects of oxidants^{9,10}. Jordan has a high flora diversity with wide traditional uses of medicinal plants as antibacterial, antioxidant, and antifungal agents¹¹. Moreover, random screening of plants for the active chemical compounds

is important as the screening of ethno botanical targeted species¹²⁻¹⁵.

Several medicinal plant species are widely distributed in Jordan¹⁶, plants, *P. harmala*, *S. officinalis* and *G. hybridus*, are examples of the local medicinal plants. *P. harmala* is a plant of the family Nitrariaceae belongs to Asian origin and grows in the middle east and in part of south Asia mainly in India and Pakistan¹⁷. *S. officinalis* is a perennial plant (subshrub), native to the Mediterranean region, belongs to the Lamiaceae family, its leaves are mainly used as a spices with established biological activity¹⁸. *G. hybridus*, commonly known as salsify, oyster plant, goat's beard and vegetable oyster belonging to Asteraceae family, this plant was not mentioned within the medicinal plants^{19,20}. *Tragopogon spp* which belong to the same family was mentioned to have a strong antibacterial potential²¹. The study was planned to investigate the efficiency of ethanol extract of the selected plants on pathogens obtained from clinical samples using standard microbiological procedures.

MATERIALS AND METHODS

The dried plant materials were collected from a local region in Alkarak, Jordan. The scientific, common, family names and parts of plant used in this study were presented in Table 1. Four bacterial strains were used in this study: *S. aureus* ATCC43300, *E.coli* ATCC25922, *E. aerogenes* ATCC13048 and *B. subtilis*

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Table 1: The botanical data of the four selected plant species used in this study.

#	Scientific name	Common name	Family	Parts used
1	<i>P. harmala</i>	Harmal	Nitrariaceae	Seed
2	<i>S. officinalis</i>	Maramiah	Lamiaceae	Arial
3	<i>G. hybridus</i>	Haisabon	Asteraceae	Arial

ATCC6633. These bacterial strains were obtained from research Lab, Department of Biology, Mu'tah University.

Plant processing and extraction

The dried plant materials were ground by coffee blender into fine powder. Twenty five grams of each plant powder were soaked in 250 ml Ethanol solution and kept in shaker at 150 rpm, in a dark place for three days at room temperature. The mixtures were then filtrated using Buchner funnel under vacuum. The filtrate was centrifuged at 3000 rpm for 10 minutes. The extracts were concentrated in rotary evaporator under reduced pressure at 55°C. The extracts were left in open vials in fume hood for three days at room temperature. The resulting extracts were stored in at 4°C in a glass container until use²².

Antibacterial activity of plant extracts

Antibacterial activities of the different extracts were investigated by the disc diffusion method²³. The crude plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) in addition to Methanol. Four to five well-isolated colonies of the same morphological type were selected and inoculated into tubes containing 5ml Muller-Hinton broth and incubated at 37°C with shaking at 150 rpm until the turbidity of the bacterial growth achieved. Then the bacterial cells in the broth culture were counted using hemocytometer. A volume ranged from 0.5 to 3 ml of each broth culture was added to the prepared sterile nutrient agar cooled to about 45-50°C to obtain 2×10^6 cell/ ml inoculum concentration of the organism, then poured into sterile Petri dishes and allowed to solidify for about 45- 60 min²⁴.

For each bacterial strain, three sterile 6mm antimicrobial susceptibility discs were used, the first and the second discs were loaded with 3 mg and 4 mg of plant extract, respectively. The third was loaded with 10% DMSO as a negative control. The discs were left to dry under aseptic conditions, then placed on the surface of the agar plate. Streptomycin(10µg) and Tetracycline(30µg) discs were used as positive controls. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the agar. The plates were incubated for 18-24 hrs at 37°C. The studies were done in triplicates. Growth inhibitory activity was calculated by measuring the diameter of clear zone around the disc using a ruler²⁵. 2×10^6 cell/mL was inoculated into tubes of 5 mL Muller-Hinton broth containing a serial dilution of plant extract (0-2000 µg/ mL). Cultures were incubated at 37°C for 24 hrs. The MIC was determined by inhibition of visible growth on lowest concentration of plant extract containing media as comparing with visible growth on extract free media²⁶.

Antioxidant activity of plant extracts

The DPPH radical assay was carried out as described by Tepe et al. (2005). Aliquots of various concentrations of the extracts were added to 5 ml of 0.004% methanol solution of DPPH (The final concentration of the plant extract in the solution was from 0 to 2000 µg/ml)²⁷. After incubating the samples for 30 min at room temperature, the absorbance was read against methanol at 517 nm. All determinations were done in triplicate. Inhibition of free radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = 100 \times (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}$$

Where $\text{Abs}_{\text{control}}$ is the absorbance of the control reaction (containing all reagents except the tested compound) and $\text{Abs}_{\text{sample}}$ is the absorbance of the tested compound with all other reagents. Extract concentration providing 50% inhibition (IC_{50}) was determined from a graph plotting percentage inhibition against extract concentration. Trolox[®] [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (final concentration 0 to 1.5 µg/ml) was used as a standard for the construction of the calibration curve, and the DPPH radical-scavenging activities were expressed as mg Trolox[®] equivalents per gram of plant extract²⁸.

RESULTS

Percentage yield of plant extracts

The results of ethanol crude extract yield of all used plants were varied from 10.6 to 18 % (Table 2).

Antibacterial activity of plant extracts

All the bacterial strains showed different response to the ethanol crude extract of *P. harmala*, *S. officinalis* and *G. hybridus* when used as antibacterial agent. The inhibition properties of all plants against the bacterial strains were increased with the increase of ethanol crude extract concentration (Tables 3-5).

Meanwhile, the ethanol extract of *G. hybridus* and *P. harmala* was active against *S. aureus*, *B. subtilis*, *E. aerogenes* and *E. coli* at both concentrations 1000 and 2000 µg/disc (Table 3 and 4). While, the *S. officinalis* was active only against *S. aureus*, *B. subtilis* and *E. aerogenes* sat 1000 and 2000 µg/disc, this plant was active as antibacterial agent against all of the bacterial strains with the exception of *E. coli* (Table 5).

Antioxidant activity

Antioxidant activities of ethanol plant extracts were assessed by detecting their ability to scavenge the free radicals using the DPPH method.

DPPH method

DPPH radical was used for the evaluation of free radical- scavenging properties of the three plants. In order to measure the Trolox equivalent antioxidant capacity (TEAC), a Trolox standard curve was established using ten concentrations of Trolox standard solution (0 to 1.5 µg/ml). The activity of ethanol plant extracts as a free radical scavenger was measured and expressed as TEAC [mg Trolox/g of plant extract] (Table 6). The results showed that *G. hybridus* the highest activity (0.89 mg Trolox/g of plant extract) compared with the other plants. Meanwhile, *S. officinalis* was the lowest (0.53 mg Trolox/g of plant extract) and the intermediate effect appeared *P. harmala* (0.67 mg Trolox/g of plant extract). These findings showed the same consequence of the TPCs.

The results of IC_{50} in Figure (2) showed that *G. hybridus* has the lowest IC_{50} value (0.83 mg/ml), *P. harmala* IC_{50} (1.1 mg/ml) and *S. officinalis* with the highest IC_{50} (1.38 mg/ml). These findings showed the same trend of the of the TPCs and TEAC, where, the best free radical scavenging activities reflected by the smallest IC_{50} values.

Table 2: Yield of ethanol crude plant extracts [%].

Plants	Dry weight [g]	Weight of ethanol extract [g]	Yield of ethanol extract [%]
<i>S. officinalis</i>	25	2.65	10.6%
<i>P. harmala</i>	25	3.9	15.6%
<i>G. hybridus</i>	25	4.5	18.0%

Table 3: Antibacterial activity of *G. hybridus* ethanol extract on the tested bacterial strains. Data are expressed as mean \pm SD, where n = 3.

Bacterial strains	Zone of inhibition (mm)	
	1000 μ g/disc Mean \pm SD	2000 μ g/disc Mean \pm SD
<i>S. aureus</i>	10 \pm 0.52	16 \pm 0.58
<i>B. subtilis</i>	10 \pm 0.42	12 \pm 0.43
<i>E. aerogenes</i>	10 \pm 0.32	16 \pm 0.53
<i>E. coli</i>	7 \pm 0.54	15 \pm 0.23

Table 4: Antibacterial activity of *P. harmala* ethanol extract on the tested bacterial strains. Data are expressed as mean \pm SD, where n = 3.

Bacterial strains	Zone of inhibition (mm)	
	1000 μ g/disc Mean \pm SD	2000 μ g/disc Mean \pm SD
<i>S. aureus</i>	7 \pm 0.58	12 \pm 0.21
<i>B. subtilis</i>	8 \pm 0.43	20 \pm 0.32
<i>E. aerogenes</i>	8 \pm 0.5	17 \pm 0.57
<i>E. coli</i>	7 \pm 0.22	15 \pm 0.58

Table 5: Antibacterial activity of *S. officinalis* ethanol extract on the tested bacterial strains. Mean \pm SD, where n = 3.

Bacterial strains	Zone of inhibition (mm)	
	1000 μ g/disc Mean \pm SD	2000 μ g/disc Mean \pm SD
<i>S. aureus</i>	13 \pm 0.32	21 \pm 0.35
<i>B. subtilis</i>	14 \pm 0.23	20 \pm 0.58
<i>E. aerogenes</i>	10 \pm 0.58	16 \pm 0.5
<i>E.coli</i>	-ve	-ve

Table 6: Activity of ethanol plant extracts as a free radical scavenger expressed by a Trolox equivalent antioxidant capacity [mg Trolox/g of plant extract].

Plant	TEAC _{DPPH} [mg of Trolox /g of plant extract]
<i>P. harmala</i>	0.67 \pm 0.02
<i>S. officinalis</i>	0.53 \pm 0.04
<i>G. hybridus</i>	0.89 \pm 0.1

Correlation between total phenolic content and antioxidant activity obtained by DPPH method.

A weak correlation was found between the phenolic compounds content and antioxidant activity of ethanol plant extracts obtained by DPPH method, $R^2=0.6655$.

DISCUSSION

The selected plants were tested for their activity as antibacterial and antioxidant activity. The results of ethanol crude extract yield of *P. harmala*, *S. officinalis* and *G. hybridus* were 15.6%, 10.6%, 18% respectively (table 2). The variation of yield percentages from these plants is due to species and plant parts variation since the same solvent, time and conditions of extraction were used for the three plants. These results are in accordance with other researchers²⁹.

The antimicrobial activities of ethanol extracts of these plant were used against two gram negative bacteria: *E. coli* and *E. aerogenes*, and two gram positive bacteria: *S. aureus*, and *B. subtilis*. The results demonstrated that

all these plant ethanol extract have effects on the gram positive strains and gram negative bacteria except *S. officinalis* did not show activity against *E. coli* (Tables 3-6).

The antibacterial effects of *P. harmala* was in agreement with other investigators results³⁰⁻³³. The antibacterial results of *S. officinalis* is in accordance with previous work³⁴⁻³⁶. However, our results disagree with reported research in Brazil who reported that *S. officinalis* was inactive against *S. aureus* and *Proteus spp*³⁷. The extract from *S. officinalis* showed the maximum zone of inhibition against gram positive bacteria *S. aureus* and the minimum zone of inhibition against gram negative bacteria *E. coli*³⁸. The variation between results of *S. officinalis* is organ and season dependent variation^{35,39}.

G. hybridus showed antibacterial activity against both gram positive and gram negative bacteria. This in accordance with other work on the same plant extracts of the same family^{21,40}.

Gram-negative bacteria had less susceptibility to the different plant extracts than Gram-positive bacteria. It has been previously shown

that Gram-negative bacteria are generally more resistant to common antibiotics than Gram-positive ones⁴¹. Gram-positive bacteria were found to have more susceptibility as compared to Gram-negative bacterial species. This is in agreement with earlier studies which attributed this to the differences in chemical composition and structure of cell wall of both types of microorganisms^{42,43}. The minimal inhibition concentration (MIC) of *P. harmala*, *S. officinalis* and *G. hybridus* (MIC) ranged from 125 µg/ml to 500 µg/ml (Figure 1). These MIC values are in accordance with antibacterial activity of these plants using the disc diffusion methods.

The antioxidant activity of *G. hybridus*, *P. harmala* and *S. officinalis* was evaluated using the DPPH method. A Trolox standard curve was established using ten concentration of Trolox standard solution (0 to 1.5 µg/ml). The antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC) [mg Trolox/g of plant extract] (Table 6). The TEAC for *P. harmala*, *S. officinalis* and *G. hybridus* was found 0.67, 0.53 and 0.89 mg/ml respectively (table 6). It was also found that the plant with the high TPC had the highest TEAC value (Table 6 and Figure 2). The IC₅₀ was also calculated for *P. harmala*, *S. officinalis* and *G. hybridus* and it was found 1.1, 1.38 and 0.83 mg/ml respectively. These

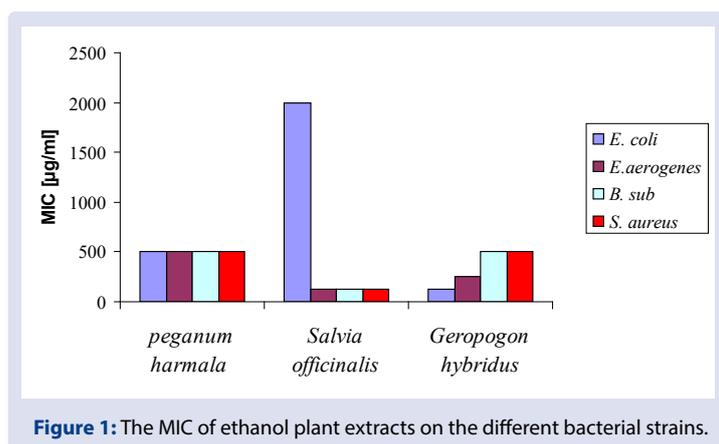


Figure 1: The MIC of ethanol plant extracts on the different bacterial strains.

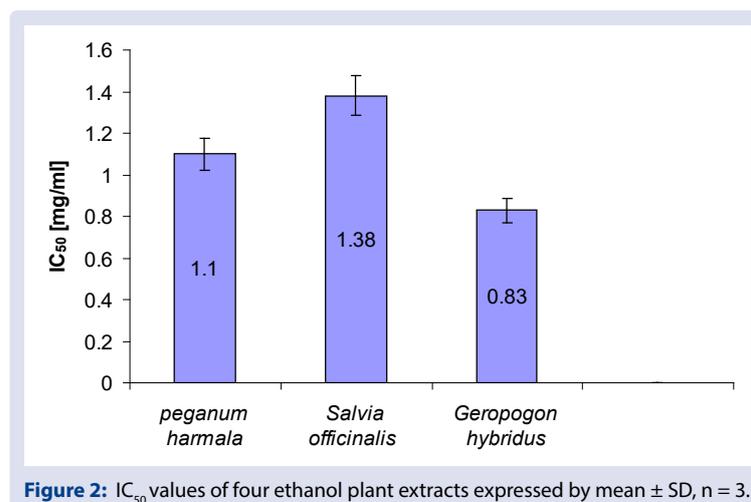


Figure 2: IC₅₀ values of four ethanol plant extracts expressed by mean ± SD, n = 3.

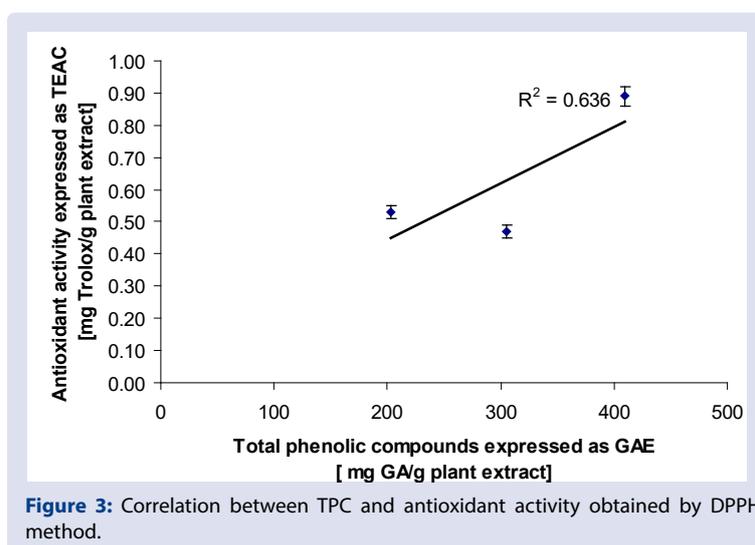


Figure 3: Correlation between TPC and antioxidant activity obtained by DPPH method.

finding are in accordance with the TPC and TEAC for each plant used in this study. Our results agree with other worker in the field^{11,21,44,45}.

Extraction of antioxidant compounds from plant material is dependent on the solubility of these compounds in various solvents. The polarity of solvent used plays a key role in increasing the solubility of these compounds^{46,47}. The correlation between TPCs and the antioxidant activity based on the DPPH assay involving the three plants was low. The weak correlation between TPCs and antioxidant activity of the plant extracts may due to the low number of plants used in this study. However, other researcher used 24 plant in their study demonstrated high correlation value between the TPC and the TEAC⁴⁸.

CONCLUSION

The antibacterial and antioxidant activities of the selected plant suggest that these plants may be source of bioactive substances with multifaceted activity, because from the present investigation, it may be concluded that ethanol extracts of the studied species possessed a potential antibacterial activity against the reference strains.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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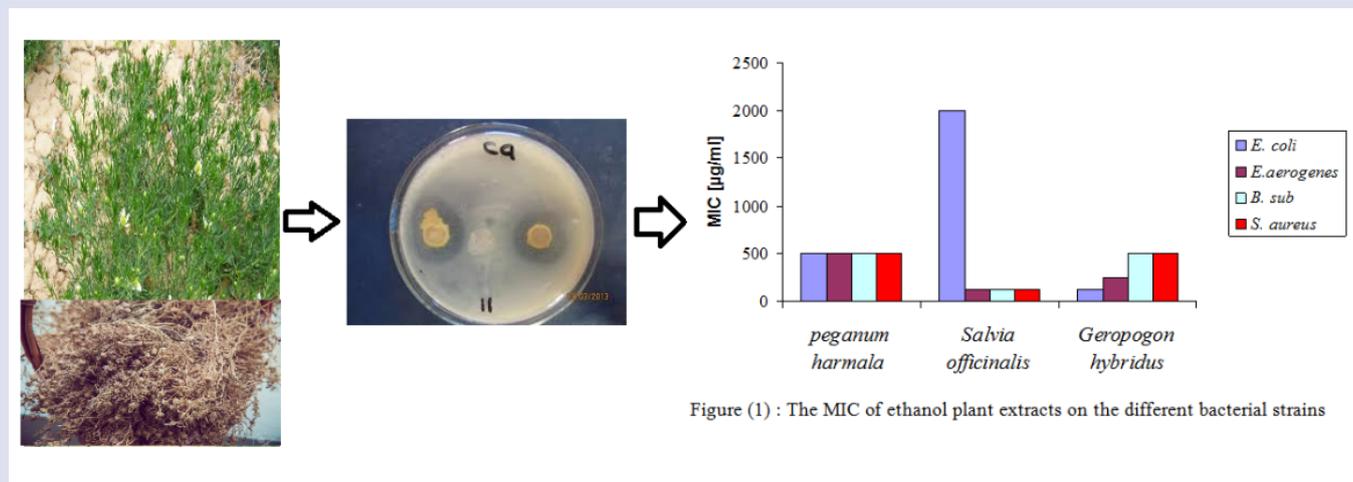
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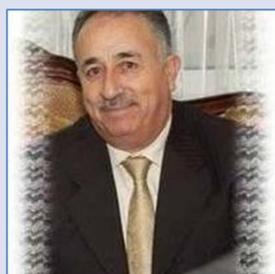
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GRAPHICAL ABSTRACT



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