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Phenol Content, Antioxidant Capacity and Antibacterial Activity of Methanolic Extracts Derived from Four Jordanian Medicinal Plants

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Abstract: This study was performed to assess the antioxidant and antibacterial properties of methanolic extracts derived from aerial parts of four Jordanian medicinal plants (*Artemisia sieberi*, *Peganum harmala*, *Rosmarinus officinalis* (Green-Flowered) and *Sarcopterium spinosium*). The possible relationship between these biological properties and the total phenolic concentrations of these extracts were also be determined. The antioxidant capacity and total phenolic concentrations were assessed by the ABTS method and Folin- Ciocalteu method, respectively. The amount of the extract required to scavenge 50% of ABTS (IC_{50}) was also measured. Broth dilution and disc diffusion assays were performed to measure the antibacterial activity of these extracts against available bacterial strains. Variations were observed among the examined plants in antioxidant and antibacterial activities as well as in their phenol contents. According to ABTS assay and IC_{50} value, the highest free radical scavenging potential was found in *Sarcopterium spinosium*, followed by *Rosmarinus officinalis*, *Peganum harmala* and *Artemisia sieberi*, respectively. Similarly, the results of antibacterial assays showed that *Sarcopterium spinosium* exhibited the highest antibacterial activity against all tested bacterial strains as compared to *Rosmarinus officinalis*, *Peganum harmala* and *Artemisia sieberi*. Moreover, *Sarcopterium spinosium* contained the highest amount of phenolic compounds followed by, *Rosmarinus officinalis*, *Artemisia sieberi* and *Peganum harmala*, respectively. In conclusion, these plants are not only interesting sources for antimicrobial agents but also have a considerable amount of antioxidants. In addition, these findings revealed that the antioxidant capacity and antibacterial activity of these plant extracts do not necessary be attributed to their total phenolic concentrations.

Key words: Antimicrobial, disc diffusion, free radicals, gallic acid, polyphenols

INTRODUCTION

Recently, interest in plant extracts has been increased due to large contributions to some important applications such as remedy for treatment of microbial infections or reducing the risk of various diseases associated with oxidative stress and utilization for preserving raw and processed food. Based on data derived from previous and recent studies, the therapeutic actions of these plant extracts are more likely related to the antioxidant and antib acterial properties of their constituents (Velioglu *et al*., 1998; Cowan, 1999; Cai *et al*., 2004; Rios and Recios, 2005; Taguri *et al*., 2006; Tawaha *et al*., 2007; Bakirel *et al*., 2008; Padam *et al*., 2012). Apparently, according to these studies, the antioxidant and antimicrobial properties exerted by various plant extracts have been related or ascribed to some extent to their phenolic constituents.

Phenol compounds are widely distributed in various plant species. It has been estimated that at least 12,000 phenolic compounds have been isolated. These phenols are mostly derivatives and isomers of flavones, phenolic acids, quinines, tannins and coumarins (Cowan, 1999; Cai *et al*., 2004; Scalbert *et al*., 2005). Importantly, a positive relationship between total phenol contents and antioxidant capacity or antibacterial activity was observed in some investigated plant extracts (Velioglu *et al*., 1998; Katalinic *et al*., 2006; Yildirim *et al*., 2001; Taguri *et al*., 2006).

The potential benefits provided by phenolic antioxidants are tremendous because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen and hydroxyl radicals as well as their ability to disrupt the free-radical chain reaction of lipid peroxidation (Cowan, 1999; Scalbert *et al*., 2005; Bakirel *et al*., 2008). Moreover, antibacterial activity of polyphenols against Gram positive and Gram negative bacteria have been reported in various plant species (Cowan, 1999; Scalbert *et al*., 2005; Taguri *et al*., 2006;

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Padam *et al*., 2012). In addition to antioxidant and antimicrobial properties, phenols can exhibit several physiological properties such as antiallergy, antifungal, antiulcer, antiinflammatory, antidiabetic, antimutagenic and antitumoral activity, estrogenic, immune-stimulating agents and activation or inactivation of certain enzymes (Cowan, 1999; Katalinic *et al*., 2006; Rios and Recios, 2005; Scalbert *et al*., 2005; Romero *et al*., 2006; Bakirel *et al*., 2008; Willoughby Sr *et al*., 2009; Bustanji *et al*., 2010).

It has been estimated that there are about 700 genera of plants in northern and southern parts of Jordan and about 485 species from 99 plant families have been claimed as medicinal plants (Khalil, 1995; Oran and Al-Eisawi, 1998; Ali-Shtayeh *et al*., 2000; Afifi and Abu-Irmaileh, 2000). However, during the last decade, limited number of studies have been carried out to screen some Jordanian plants that have been claimed to be useful in treatment of various diseases (Tawaha *et al*., 2007; Irshaid and Mansi, 2009a, b; Bustanji *et al*., 2010; Tarawneh *et al*., 2010; Irshaid *et al*., 2010, 2012; Obeidat *et al*., 2012). Moreover, as far as our literature search could ascertain, studies to determine the possible relationship between phenolic contents and the antioxidant capacity and antibacterial activities of some Jordanian medicinal plants are limited and not thoroughly documented, particularly in case of *Artemisia sieberi* (*A. sieberi*), *Peganum harmala* (*P. harmala*), *Rosmarinus officinalis* L. (*R. Officinalis*, Green-Flowered), *Sarcopterium spinosium* (*S. spinosium*). These plants are widely distributed in the semi-desert climate of Jordan and neighboring Arabic countries.

A. sieberi is well known medicinal herbs that belongs to the Asteraceae family (Ali-Shtayeh *et al*., 2000; Irshaid *et al*., 2010). This species has been shown to possess a wide range of bioactivities such as spasmolytic, vermicidal, insecticidal, antihyperglycemia, antibacterial, anticandidal and antiviral (Romero *et al*., 2006; Willoughby *et al*., 2009; Irshaid *et al*., 2012). *P. harmala* is commonly known as rue or harmal that belongs to the family of Nitrariaceae (Lamchouri *et al*., 1999; Moura *et al*., 2007; Arshad *et al*., 2008). *P. harmala* has been used for treatment of infections due to bacteria and protozoa as well as for treatment of tumors and depression (Lamchouri *et al*., 1999; Moura *et al*., 2007; Arshad *et al*., 2008). *S. spinosum* belongs to family of Rosaceae (Ali-Shtayeh *et al*., 2000; Rao *et al*., 2010). In Arabic folk medicine, *S. spinosum* can act as a tranquilizer and has been used to treat diabetes, stomachaches, toothache, gingivitis and external inflammation (Rios and Recios, 2005; Rao *et al*., 2010). *R. officinalis* (Green-flowered) is perennial plant that belongs to Lamiaceae family (Al-Sereiti *et al*., 1999; Tarawneh *et al*., 2010). This plant is well known as culinary herb, hence, addition of this herb to food and salads usually give them color and aroma. *R. officinalis* might be used to stimulate brains and aid memory as well as to treat gastrointestinal pain, acne, dandruff and eczema and has antifungal, antidiabetic and antiviral properties (Al-Sereiti *et al*., 1999; Bakirel *et al*., 2008; Bustanji *et al*., 2010).

Taken together, these data have prompted us to focuses on evaluation of antioxidant and antibacterial activities of these four important medicinal plants that commonly claimed by traditional healers or some rural people of Jordan as remedy for treatment of various illnesses and infections. In addition, the potential relationship between these biological properties and the total phenolic concentrations of these methanolic plant extracts were also be examined.

MATERIALS AND METHODS

Plant materials: *A. sieberi, P. harmala, R. officinalis* (Green-Flowered) and *S. spinosium* were collected during the months of March-June, 2011, from natural habitat at Al-Mafraq area, which is located in the northern region of Jordan. The scientific, family, common names and parts of plants used in this study are presented in Table 1. These plants were identified by a plant taxonomist from the Department of Biological Sciences, Faculty of Sciences, Mutah University, Mutah, Jordan.

Preparation of crude extracts: Fresh aerial parts were collected and separated from each selected plant species. These parts were cleaned by thoroughly washing with tap water at room temperature to remove dirt and soil particles prior to the drying process (Tarawneh *et al*., 2010). The washed aerial parts were cut into small pieces. Then, they were dried in shade and crushed into fine powder using an electric blender. The dried powders of these plants were extracted in Soxhlet apparatus with methanol. These extracts were filtered and concentrated in vacuum using a rotary evaporator. After that, the resulting plant extracts were kept in a refrigerator at 4°C in a glass container until use.

Table 1: The botanical data, local names and the parts of the four selected Jordanian medicinal plants used in this study

Scientific name	Family	Common	Part extracted	%Yield of methanolic extract
Sarcopterium spinosium	Rosaceae	Billan	Aerial parts	4.6
Peganum harmala	Nitrariaceae	Harmal	Aerial parts	6.6
Rosmarinus officinalis	Lamiaceae	Hasalban	Aerial parts	4.4
Artemisia sieberi	Asteraceae	Sheeh	Aerial parts	6.6

Antioxidant assay: The 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radical scavenging assay method was used to assess the antioxidant activity of the selected plants. This assay method was conducted as described previously (Re *et al*., 1999). Briefly, radical cation of ABTS was made by reacting ABTS stock solution (7 mM) with 2.45 mM potassium persulfate and by allowing the reaction solution to stand in the dark at room temperature for 16 hours before use. To reach an absorbance of 0.70±0.02 at 734 nm, the solution was diluted with ethanol. Then, 2 mL of different concentrations of these methanolic extracts were added to the diluted 1 mL of ABTS solution. A negative control was prepared by adding 2 mL of ethanol to 1 mL of ABTS solution. The absorbance at 734 nm was determined at 6 min after initial mixing of each sample. All experiments were repeated at least three times.

Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as a standard and calibration curve was generated. Trolox Equivalent Antioxidant Capacity (TEAC) was estimated based on standard curve of ABTS scavenging by trolox. The antioxidant capacity of each plant extract was expressed in terms of mM TEAC g^{-1} plant extract. All assays were carried out in triplicates. The IC_{50} value for each methanolic plant extract was also measured from standard curve and expressed as μg mL^{-1} extract. IC_{50} is the amounts of the extracts required to scavenge 50% of ABTS.

Screening for antibacterial activity: Four Gram-negative stains [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabills* (ATCC 426), *Enterobacter cloacae* (ATCC 29004)] and one Gram-positive [*Staphylococcus aureus* (ATCC 25923)] were used for antimicrobial activity studies. All these bacterial strains were originally purchased from the American Type Culture Collection (ATCC). All bacterial strains were cultured on Mueller-Hinton agar plates at 37°C for 18 h.

The antibacterial testing was carried out by disc diffusion and broth dilution methods. Disc diffusion method was performed as described earlier by Alzoreky and Nakahara (2003). Briefly, Mueller-Hinton agar plates were prepared. All plates were left to solidify at room temperature. A bacterial suspension containing 10^6 Colony Forming Unit (CFU) mL were placed on the agar plates and spread evenly. All extracts were dissolved in 10% dimethyl sulfioxide (DMSO) to yield concentration of 10.0 mg mL^{-1}. Then sterile discs (6.0 mm diameter) were submerge with 10, 20 or 40 μL of each extract to yield the concentration of 100, 200, 400 μg mL^{-1}, respectively and immediately placed on the inoculated plates. In addition, a blank disc for each plate was carried by adding 10% DMSO to serve as negative controls and 30 μg mL^{-1} chloramphenicol discs were also prepared and used as positive controls. The plates were incubated at 37°C for 24 h. The sensitivity of the bacterial strains to the extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs. The diameters of inhibition zones were measured in millimeters (including the 6 mm well diameter). All of the experiments were conducted in triplicate. The results were reported as the average of three experiments.

Broth dilution method was also used to determine the minimum inhibitory concentrations (MICs) as described previously (Garcia *et al*., 2002). Briefly, fresh bacterial cultures were prepared and then diluted with a sterile normal saline solution with reference to the 0.5 McFarland standards to obtain an inoculums' size of approximately 10^6 CFU mL^{-1}. A serial dilutions, ranging from 0-20 mg methanolic extract per mL, were also made. This was followed by inoculation of tubes with 20 μL of the bacterial suspension per mL nutrient broth, homogenized and incubated at 37°C. The MIC value was assessed as the minimum concentration of the methanolic extract in the broth medium that inhibited the visible growth of the test microorganism (NCCLS, 2002).

Phenolic assay: The Total Phenolic Concentration (TPC) in each plant extract was measured according to the Folin-Ciocalteu procedure modified by Miliauskas *et al*. (2004). Stock solution of each methanolic extract was made to the concentration of 1 mg mL^{-1}. One millilitter of each methanolic extract was added in presence of 5 mL of Folin-Ciocalteu reactive reagent. The mixture solution was stirred and then incubated in the dark at room temperature for 3 min. Then 5 mL of sodium carbonate solution (2%) was added to the mixture and stirred thoroughly and kept in the darkness at room temperature for 1 hour. Finally, the absorbance for each mixture was measured at 765 nm. Similarly, blank consisted of 5 mL Folin-Ciocalteu reagent, 1 mL methanol and 5 mL sodium carbonate solution was prepared and measured under the same conditions.

Gallic acid was used as a standard phenolic compound. Serial dilutions of gallic acid were prepared and a linear dose-response standard gallic acid curve was established using absorbance reading at the wavelength of 765 nm under the same condition as the extract. The TPC was calculated by comparison with the absorbance of standard gallic acid curve at different concentrations. The TPC values were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram plant extract. All measurements were repeated three times.

RESULTS

Methanolic extracts were prepared from aerial parts of four plant species (*A. sieberi, P. harmala, R. officinalis* and *S. spinosium*) grown in Al-Mafraq district. The TPC, antioxidant capacity and antibacterial activity of these four selected species were determined.

The antioxidant capacity for each of methanolic extract of the four selected plant species was calculated using ABTS *in vitro* assay and determined as TEAC. The ABTS scavenging activities of four methanol extracts from the four different species were presented in Table 2. *S. spinosium* exhibited the highest ABTS scavenging capacity (522 mmol trolox equivalent g^{-1} plant extract), followed by *R. officinalis* (499 mmol trolox equivalent g^{-1} plant extract). The lowest ABTS scavenging capacities were obtained from *P. harmala* (268 mmol trolox equivalent g^{-1} plant extract) and *A. sieberi* (200 mmol g^{-1} dry extract). The amount of the methanolic extracts required to scavenge 50% of ABTS (IC_{50}) were also estimated for these four selected plants (Table 2). The IC_{50} values were obtained from a standard curve of ABTS scavenging by Trolox. Similarly, it was observed that *S. spinosium* showed the highest antioxidant capacity when IC_{50} (20 μg mL^{-1}) was measured. This was followed by *R. officinalis* (22 μg mL^{-1}). The lowest antioxidant capacity were obtained for *P. harmala* (41 μg mL^{-1}) and *A. sieberi* (55 μg mL^{-1}).

Table 2: The trolox equivalent antioxidant capacity (TEAC) and ABTS [a] IC_{50} of methanolic extracts from the studied plants

Plant	TEAC (mmol equivalent to trolox/g plant extract)	IC_{50}[b] (μg mL^{-1})
Sarcopterium spinosium	525	20
Peganum harmala	268	41
Rosmarinus officinalis	499	22
Artemisia sieberi	200	55

[a]ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, Values are the average of triplicate experiments of plant methanolic extract at 734 nm wavelength, [b]IC_{50} is the amount of sample in μg mL^{-1} required to scavenge the ABTS free radical 100 μg mL^{-1} by 50%

In addition, two experiments were also conducted to investigate the antimicrobial activity of these selected plants extracts against some selected bacteria. The antibacterial activities of these methanolic extracts were first estimated by disc diffusion method at three different concentrations of 100, 200 and 400 μg mL^{-1} against *S. aureus, E. coli, P. aeruginosa*, *E. cloacae* and *P. mirabilis* (Table 3). Apparently, these methanolic extracts were found to posses antibacterial activity against all tested Gram positive and Gram negative bacteria in dose dependent manner. The values of inhibition zones of these methanolic extracts were found to be ranged from 8 to 30 mm. The highest values of inhibition were obtained for methanolic extract derived from *S. spinosium* against *P. mirabilis* (zone of inhibition: 30±2.5 mm), a Gram negative bacterium and *S. aureus* (zone of inhibition: 29±2.6), a Gram positive bacterium, as well as for methanolic extract derived from *R. officinalis* against *S. aureus* (zone of inhibition: 30±2.8 mm) using the extract concentration of 400 μg mL^{-1}. Conversely, weak inhibitory effects were observed against *E. coli, P. aeruginosa*, *E. cloacae* and *P. mirabilis* using methanolic extract derived from

Table 3: Antibacterial activity (zone of inhibition) of different concentrations of methanolic extract derived from four locally selected plant species on the growth of five selected bacterial strains as compared with 30 μg mL^{-1} chloramphenicol

Name of plant	Microbe	Zone of inhibition (mm)[a]		
		100 μg mL^{-1}	200 μg mL^{-1}	400 μg mL^{-1}
Sarcopterium spinosium	*S. aureus*	11±1.1	22±2.1	29±2.6
	E. coli	10±1.7	15±2.2	21±2.1
	P. aeruginosa	17±1.6	20±1.5	26±2.3
	E. cloacae	11±2.5	13±1.7	25±2.7
	P. mirabilis	17±1.6	25±1.9	30±2.5
Peganum harmala	*S. aureus*	ND	10±2.6	17±2.9
	E. coli	10±2.5	17±1.6	24±2.6
	P. aeruginosa	ND	9±1.2	12±2.0
	E. cloacae	10±1.5	13±1.9	15±2.0
	P. mirabilis	10±1.9	15±2.2	21±2.3
Rosmarinus officinalis	*S. aureus*	6±1.6	22±2.4	30±2.8
	E. coli	12±1.5	15±1.9	21±2.3
	P. aeruginosa	10±1.8	13±1.6	18±2.0
	E. cloacae	ND	11±1.6	15±2.2
	P. mirabilis	11±1.7	16±2.6	22±2.9
Artemisia sieberi	*S. aureus*	ND	ND	ND
	E. coli	ND	ND	11±2.1
	P. aeruginosa	10±1.6	12±2.5	16±2.8
	E. cloacae	10±2.4	13±1.9	15±2.4
	P. mirabilis	9±1.1	12±1.7	14±2.8

[a]Zone of Inhibition (mm) is average of triplicate experiments; Diameter of inhibition zone (mm) including disc diameter of 6 mm; ND: Not detectable; Data represent mean values of three replicates

Table 4: Antibacterial activity (minimum inhibition concentration (MIC)) of methanolic extracts derived from the four selected medicinal plant species on the growth of five selected bacterial strains

	MIC[a] ($mg\ mL^{-1}$ extract)			
Microbe	*Sarcopterium spinosium*	*Peganum harmala*	*Rosmarinus officinalis*	*Artemisia sieberi*
S. aureus	0.8	1.2	1.0	3.3
E. coli	1.2	1.0	1.2	2.8
P. aeruginosa	0.9	2.4	2.2	2.2
E. cloacae	1.0	2.0	2.4	1.5
P. mirabilis	0.9	1.4	1.6	1.8

[a]MIC values ($mg\ mL^{-1}$) are average of triplicate experiments

Table 5: Total phenolic contents of methanolic extracts derived from the four selected Jordanian medicinal plant species

Plant species	Total phenolic compounds [a] mg GAE g^{-1} plant extract
Sarcopterium spinosium	195±10.3
Peganum harmala	9±4.6
Rosmarinus officinalis	187±12.3
Artemisia sieberi	99±13.6

[a]Total phenolic concentration was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram plant extract; values are the average of triplicate experiments

A. sieberi at concentration of 400 $\mu g\ mL^{-1}$ when compared to the other tested methanolic extracts. Moreover, no zone of inhibition was detected against *S. aureus*, a Gram positive bacterium, at the highest concentration tested (400 $\mu g\ mL^{-1}$) using methanolic extract of *A. sieberi*. Chloramphenicol as a standard drug was also shown to be active against all tested bacteria using concentration of 30 $\mu g\ mL^{-1}$.

Along the same line, to investigate further the antibacterial activity of methanolic crude extracts from these four selected plants, the antibacterial effects were also assessed for these extracts by broth dilution method and were expressed as MIC (Table 4). According to broth dilution assay, the investigated methanolic plant extracts possessed the antibacterial activities in different degrees. The data of this assay revealed that the MIC for the methanolic extracts were found between 0.8-1.2 $mg\ mL^{-1}$ for *S. spinosium*, 1.2-2.2 for *R. officinalis*, 1.0-2.4 $mg\ mL^{-1}$ for *P. harmala* and 1.5-3.3 for *A. sieberi* against all tested bacterial strains. *S. spinosium* methanolic extract exhibited a strong antibacterial activity against *S. aureus* (0.8 $mg\ mL^{-1}$), *P. aeruginosa* (0.9 $mg\ mL^{-1}$) and *P. Mirabilis* (0.8 $mg\ mL^{-1}$). On other hand, the lowest inhibitory activity was shown for *A. sieberi* against *S. aureus* (3.3 $mg\ mL^{-1}$).

Furthermore, the TPCs of the methanolic extracts of *A. sieberi, P. harmala, R. officinalis* and *S. spinosium* were also measured and they are presented in Table 5. Among the plants tested, *S. spinosium* showed the highest phenolic content (195 mgGAE g^{-1} pant extract). This was followed by *R. officinalis* (187 mg GAE g^{-1} plant extract). The TPC of *A. sieberi* (99 mgGAE g^{-1} plant extract) was found to be half of that found in *R. officinalis*. Whereas, *P. harmala* had very low amount of total phenol content (9 mgGAE g^{-1} plant extract).

DISCUSSION

It is worth mentioning that and to the best of our knowledge, this is the first report on antioxidant and antibacterial screening of these four local medicinal plants (*A. sieberi, P. harmala, R. officinalis* and *S. spinosium*) from this region using methanol as solvent solution. Based on studies published to date, the most common methods of initial screening of plants for potential antimicrobial and antioxidant activities have been started with using crude alcohol extraction methods, such as ethanol or methanol extraction (Kahkonen *et al*., 1999; Yildirim *et al*., 2001; Taha *et al*., 2011; Obeidat *et al*., 2012). This is due to the ability of ethanol or methanol solution to solubilize antimicrobial and antioxidant compounds from various plant species.

To measure the scavenging ability of these methanolic extracts, ABTS assay was carried out, because it is commonly used to evaluate scavenging capacity of various water soluble and lipid soluble antioxidant compounds derived from plant extracts. According to this assay, the highest values of scavenging capacity were observed in both plants *S. spinosium* and *R. officinalis* and found to be two times higher than *P. harmala* and *A. sieberi*. Moreover, IC_{50} was determined for these plants for the first time. IC_{50} values also revealed that it is necessary to double the concentration of methanolic extracts of *P. harmala* or *A. sieberi* to get the same Trolox equivalent antioxidant capacity for *S. spinosium* or *R. officinalis*.

The results of antibacterial assays revealed that these methanolic plant extracts possessed varying degree of antibacterial activities against all tested bacterial strains. In addition, according to these tests, the methanolic extract of *S. spinosum* was more potent than that of the methanolic extracts of *R. officinalis*, *A. sieberi* or *P. harmala*, particularly against *S. Aureus*, a Gram positive bacterium and *P. Mirabilis*, a Gram negative bacterium. In addition, antibacterial activity of *R. officinalis* appeared much better than that of *A. sieberi*

or *P. harmala*. As is in the current study, previously studies have reported the antibacterial activity of various extracts of *R. officinalis* on different microorganisms (Dulger and Gonuz, 2004; Arshad *et al*., 2008; Tarawneh *et al*., 2010; Toroglu, 2011). In general, Gram positive bacteria were more susceptible to plant extracts when compared to Gram negative bacteria (Ojala *et al*., 2000; Karaman *et al*., 2003; Alzoreky and Nakahara, 2003). However, our data showed that the tested plant extracts exhibited inhibitory effects against both Gram positive and Gram negative bacteria.

According to phytochemical investigations, phenolic compounds in plant extracts can act as antioxidant and antibacterial agents and are also essentially important for their therapeutic values (Cowan, 1999; Yildirim *et al*., 2001; Cai *et al*., 2004; Katalinic *et al*., 2006; Scalbert *et al*., 2005; Taguri *et al*., 2006; Rafat *et al*., 2010). Our results indicated that the antioxidant capacity and antibacterial activity as well as the total phenol concentrations are very high in extracts of both *S. spinosum* and *R. officinalis* and low in extracts of both *A. sieberi* or *P. harmala*. Apparently, these results suggest that the antioxidant capacity or antibacterial activity of these extracts may be related to a higher amount of phenolic compounds as previously suggested by previous studies (Velioglu *et al*., 1998; Yildirim *et al*., 2001; Katalinic *et al*., 2006; Tawaha *et al*., 2007). By contrast, the phenol content of *P. harmala* extract was found to be ten-fold less than the methanolic extract of *A. sieberi*, however the antioxidant capacity and antibacterial activity of *P. harmala* were appeared to be higher than *A. sieberi*. Based on these data, there is a contradictory relationship between antioxidant capacity or antibacterial activity and the total phenol concentrations of *A. sieberi* and *P. harmala*. Thus, it is possible to suggest the antioxidant capacity and antibacterial activity of *A. sieberi* and *P. harmala* do not necessary be attributed or reliant to their phenolic concentrations. This finding was consistent with the findings of Kahkonen *et al*. (1999), Peschel *et al*. (2006), Rafat *et al*. (2010) and Padam *et al*. (2012). In fact, they noted that high antioxidant activities of some selected plant extracts does not necessarily correlated with their total phenol concentrations.

As anticipated, our studies revealed that varying values of antioxidant capacity, antibacterial activity and phenolic concentration were found in methanolic extracts of these tested plants. Furthermore, variations in the values of the antioxidant capacity, antibacterial activities and phenolic content of our methanolic plant extracts might be due to several factors, including plant species, age and vegetative cycle stage of target plant, plant organ or part, climate and soil compositions, as well as due to presence of different structural or classes of antioxidant phenols between various plant families (Kahkonen *et al*., 1999; Alzoreky and Nakahara, 2003; Cai *et al*., 2004; Katalinic *et al*., 2006; Miliauskas *et al*., 2004; Rios Recios, 2005; Taguri *et al*., 2006; Tawaha *et al*., 2007; Rafat *et al*., 2010; Tarawneh *et al*., 2010; Obeidat *et al*., 2012). Comparing and interpreting the findings of these studies to our findings can be difficult. This is due to the fact that the extraction processes, technique employed, solvents used during extraction processes and assay methods of evaluation of antioxidant and antibacterial activities were different from our study. We also can not exclude the possibility that these activities can be attributed to the presence of some non-phenolic compounds in our plant extracts. It is reported that different phenolic compounds exhibit different responses in Folin- Ciocalteu assay. Also, this assay is not specific to phenols and any interfering compounds that may present in these crude extracts might react with Folin-Ciocalteu reagent and lead to high phenolic concentrations (Katalinic *et al*., 2006; Everette *et al*., 2010). These factors might explain the observed differences in the antioxidant capacity, antibacterial activity and phenol contents between these tested methanolic plant extracts as well as other various plant extracts.

CONCLUSION

In conclusion, a considerable amounts of antioxidant capacity and antibacterial activity were observed among the methanolic extracts of these four selected Jordanian medicinal plants (*A. sieberi, P. harmala, R. officinalis* and *S. spinosium*). The relationships between the antioxidant capacity or antibacterial activity of these selected plants and their total phenolic contents are not clear or sometime contradictory. These findings suggest that promising therapeutic agents with antioxidant and antibacterial activities could be potentially isolated from these plants, particularly from *S. spinosium* and *V. officinalis*.

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