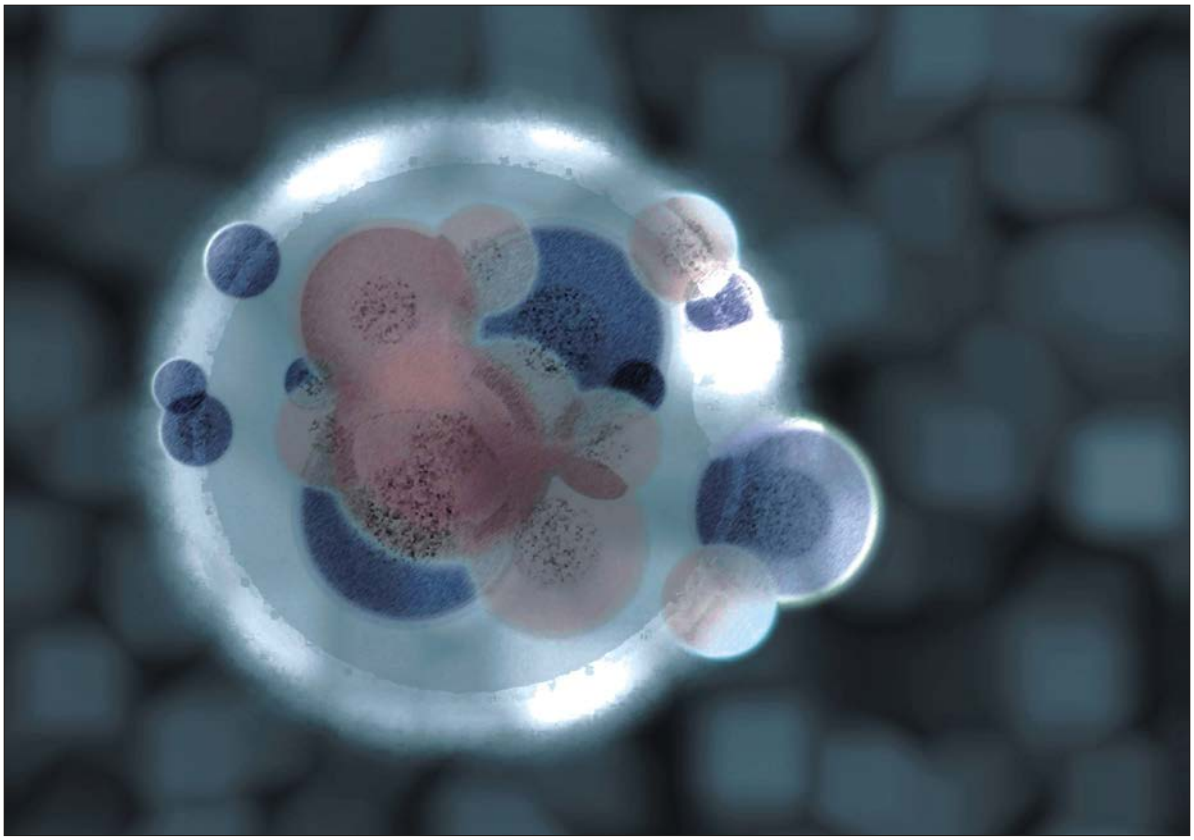

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 10 (4): 325-332, 2010
ISSN 1727-3048

Evaluation of Antibacterial and Antioxidant Activities of Methanolic Extracts of Some Medicinal Plants in Northern Part of Jordan

[1]Khaled A. Tarawneh, [2]Fawzi Irshaid, [2]Adnan S. Jaran, [2]Magda Ezealarab and [1]Khaled M. Khleifat
[1]Department of Biological Sciences, Mutah University, Mutah, Jordan
[2]Department of Biological Sciences, Al al-Bayt University,
P.O. Box 130040, Al-Mafraq 25113, Jordan

Abstract: The objective of this study was to assess the antibacterial and antioxidant activities of methanolic extracts obtained from aerial parts of four medicinal plants: *Achillea fragrantissima*, *Teucrium polium* L., *Rosmarinus officinalis* and *Alhgi graecorum*. Both broth dilution and disc diffusion methods were used to assess the antibacterial activity of these extracts against several numbers of bacterial isolates. Antioxidant activity of these extracts was measured for the first time *in vitro* by 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) method and expressed as Trolox Equivalent Antioxidant Capacity (TEAC). All extracts exhibit antibacterial activity in dose-dependent manner against Gram negative and Gram positive bacteria. MIC were found between 1.2-2.9 mg mL^{-1} for *Achillea fragrantissima*, 1.2-2.7 mg mL^{-1} for *Teucrium polium*, 1.1-1.8 mg mL^{-1} for *Alhgi graecorum* and 0.9-1.7 mg mL^{-} for *Rosmarinus officinalis* against the tested bacteria. On other hand, the *Rosmarinus officinalis* exhibited the best ABTS scavenging capacity (424 mmoL g^{-1} dry matter), followed by *Achillea fragrantissima* (192 mmol g^{-1} dry matter). The lowest ABTS scavenging capacities were obtained from *Teucrium polium* and *Alhgi graecorum*, 174 and 172 mmoL g^{-1} dry matter, respectively. Similarly, *Rosmarinus officinalis* exhibited the highest antioxidant activity when IC_{50} (21 μg mL^{-1} extract) was measured, followed by *Achillea fragrantissima* (69 μg mL^{-1} extract), *Teucrium polium* (80 μg mL^{-1} extract) and finally *Alhgi Graecorum* (103 μg mL^{-1} extract). In conclusion, our plant extracts exhibited both antibacterial and antioxidant activities with *Rosmarinus officinalis* showing the most antibacterial and antioxidant activities. Thus, our results may, in part, support the traditional use of these plant species for medicinal purposes.

Key words: Medicinal plants, methanolic extract, antioxidant, antimicrobial

INTRODUCTION

In the past century, remarkable progress in the discovery of antimicrobial drugs has been made and thus several numbers of synthetic drugs have also been synthesized. These new synthetic antimicrobial drugs play a vital role in treatment of various types of microbial infections and reducing the number of fatalities that are associated with infections caused by microbes. However, despite this remarkable progress, multiple drug resistance pathogenic microorganisms have been emerged due to indiscriminate use of the commercial available antimicrobial drugs in human (Cowan, 1999; Newman *et al*., 2003; Poole, 2005). Thus, the emergence of previously uncommon infections is considered as a serious medical problem by World Health Organization, governments, the public health and scientific communities as well as health care providers at large. This situation coupled with the undesirable side-effects of certain antibiotics has prompted scientists to search for new antimicrobial substances from various plants (Cowan, 1999; Karaman *et al*., 2003; Al-Zubairi *et al*., 2008). The use of certain plants, as a source of remedies to treat many diseases, is date back to ancient time. Medicinal plants have also been considered the traditional source of raw materials for medicine. In fact, approximately 25% of prescribed medicines in industrialized countries are obtained directly or indirectly from plant sources (Newman *et al*., 2003). In addition, according to an estimate of the World Health Organization, approximately 80 to 70% of the world populations presently use plants for medicinal purposes (Wills *et al*., 2000).

It is also worth to note that in developing countries where medicines are quite expensive, investigation on antimicrobial activities from medicinal plants may still be needed. Nonetheless, plants used in folk medicine in most

Corresponding Author: Fawzi Irshaid, Department of Biological Sciences, Faculty of Science, Al al-Bayt University, P. O. Box 130040, Al-Mafraq 25113, Jordan
Tel: 00962776948182, 962-2-6297000/2120 Fax: 0096265151261

developing are still understudied, particularly in clinical microbiology (Kirby, 1996; Wills *et al*., 2000; Pavrez *et al*., 2005; Zakaria *et al*., 2007). Recently, several numbers of plant species have been investigated for their potential antimicrobial activity using various extraction methods including water, methanol, ethanol, n-hexane, chloroform, butanol and acetone. These extracts have been assessed for their antimicrobial activity against several numbers of bacterial isolates by broth dilution, microtiter plate and/or disc diffusion methods (Scalbert *et al*., 2005; Zakaria *et al*., 2007; Lawrence *et al*., 2009). For example, Sweet Basil, stem bark of *Distemonanthus benthamianus* Baill, *Muntingia calabura* (L.) and *Dicranopteris linearis* (L.) and *Erythrophleum suaveolens* have been shown to exhibit antibacterial activity against some selected bacterial strains (Zakaria *et al*., 2007; Aiyegoro *et al*., 2007, 2008; Moghaddam *et al*., 2009). These studies demonstrated that plant extracts derived from these species could be a potential source of antibacterial agents for the treatment of normal infection caused by some bacteria such as *H. pylori* strains, *S. aureus* strains, *E. coli* and others. Additionally, several mechanisms of action have been suggested with regards to the chemical compounds which might be present in these plant extracts, particularly, flavonoids (rutin and quercetin), tannins and others (Aiyegoro *et al*., 2008).

Furthermore, previous as well as recent investigators reported that phenols, polyphenol and flavonoids are natural antioxidant plant products that have been found in various concentrations in most medicinal plants as well as other plant kingdom (Rice-Evans *et al*., 1995; Middleton *et al*., 2000; Scalbert *et al*., 2005; Kiselova *et al*., 2006; Abdel-Hameed, 2009; Borchardt *et al*., 2008; Parthasarathy *et al*., 2009). They also indicated that these compounds have been shown to possess various therapeutic values such as antibacterial, anti-mutagenicity, anti-carcinogenic and anti-inflammatory, anti-aging activities by reducing the oxidative damages or stresses that are commonly induced by free radical species. Furthermore, the growing interest in the substitution of synthetic antioxidants with natural ones has encouraged research on plant sources and screening of raw materials for identifying new ones. Plant derived antioxidants such as ascorbic acid, beta carotene, α-tocopherol, phenolic acids and flavonoids, among others, are becoming increasingly known as a primary dietary factors for humans (Ferguson, 2001; Cantuti-Castelvetri *et al*., 2000; Martin and Appel, 2010). The interest in these compounds is largely due to the growing evidence of their potential health benefits, particularly for their role in reduced risk for heart diseases, cancer and aged-related diseases as well as protection against cellular damage caused by reactive oxygen species (free-radicals) (Ferguson, 2001; Adhami and Mukhtar, 2006; Abdel-Hameed, 2009; Almeida *et al*., 2008; Martin and Appel, 2010).

Based on previous ethnobotanical studies, several numbers of medicinal plants from the northern region of Jordan have been reported (Al-Eisawi, 1982). These include *Achillea fragrantissima* (*A. fragrantissima*), *Teucrium polium* L. (*T. polium*), *Rosmarinus officinalis* (*R. officinalis*), *Alhgi graecorum* (*A. graecorum*), *Artemisia seibri*, *Peganum harmala*, *Urtica pilulifera* L., *Sarcopterum spinosium* L. and others. They are traditionally used in treatments of various aliments, including diabetes mellitus, ulcers, abdominal pain, arthritis, headache, hypertension and others (Afif and Iramileh, 2000; Irshaid and Mansi, 2009a, b). Recently, the antifungal and antioxidant activities of some plant species from south Jordan have been reported by Tarawneh *et al*. (2008). However, the antioxidant and antibacterial activities of medicinal plants from northern part of Jordan are either neglected or not explored in details. Therefore, this study was undertaken to evaluate the antibacterial and antioxidant activities of *A. fragrantissima*, *T. polium*, *R. officinalis* L. and *A. graecorum* using in vitro assay methods.

MATERIALS AND METHODS

Plant material: The fresh plant aerial parts of *A. fragrantissima*, *T. polium L.*, *R. officinalis* and *A. graecorum* were collected during the months of March-May, 2009, from Al-Mafraq area which is located in the Northern region of Jordan. The scientific, family, common name and parts of plant used in this study were presented in Table 1. The plants were identified by a taxonomist from the Department of Biological Sciences, Faculty of Sciences, Mutah University, Mutah, Jordan. The voucher specimens were deposited in the Department of Biological Sciences, Faculty of Sciences, Al al-Bayt University, Al-Mafraq, Jordan.

Plant processing: The fresh aerial parts from the selected plants were washed thoroughly with tape water at room temperature to remove dirt prior to the drying process. These washed aerial parts were dried in the shade at room

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

Scientific name	Family	English name	Part extracted
Achilla fragantissima	Compositae	Yarrow	Aerial parts
Teucrium polume L.	Labiatae	Cat Thyme	Aerial parts
Rosmarinus officinalis	Lamiaceae	Rosemary	Aerial parts
Alhagi graecorum	Fabaceae	Camel thons or manna trees	Aerial parts

temperature for seven days. Then, they were crushed into finely powdered. The powder of each plant was placed once on the Soxhlet cold extractor using 80% methanol as solvent and was kept here for three consecutive days. The extracts were concentrated to dryness in rotary evaporator under reduced pressure at 45°C. The extraction and evaporation procedures were repeated three times. Then, the resulting extracts were stored in a refrigerator at 4°C in a glass container until use.

Test microorganisms and growth media: The following Gram-positive and Gram-negative bacteria were used for antimicrobial activity studies: bacteria included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabills* (ATCC 426), *Enterobacter cloacae* (ATCC 29004) and *Staphylococcus aureus* (ATCC 25923). The bacterial strains were cultured on Mueller-Hinton agar (MHA) plates at 37°C. The stock cultures were maintained at 4°C.

Antimicrobial assay: Antibacterial activities of the different extracts were investigated by the disc diffusion method (Alzoreky and Nakahara, 2003; Beur *et al*., 1996). An inoculum size of 10^6 colony-forming units (CFU) mL^{-1} of bacteria were spread on the agar plates. The methanolic extracts were dissolved in 10% DMSO. Then discs (6.0 mm diameter) submerge with 10, 20 and 40 μL of each extract at concentration of 10.0 mg mL^{-1} were placed on the inoculated plates. Similarly, for each plate, a blank disc was carried by adding solvent alone (10% DMSO) to act as negative controls and antibiotic discs (6.0 mm diameter) of 30 μg mL^{-1} chloramphenicol were also carried out as positive controls. All the plates were incubated at 37°C for 18 to 24 h. The growth inhibition zones around the discs were estimated after 18 to 24 h of incubation at 37°C. The sensitivity of the bacterial strains to the methanolic extracts was determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the discs and values less than 8 mm were considered as not active against microorganisms. All of the experiments were conducted in triplicate. The results are reported as the average of three experiments.

Determination of Minimum Inhibitory Concentrations (MICs): The MICs of methanolic plant extracts at which the growth of the bacteria became invisible or undetectable were determined as described previously (Garcia *et al*., 2002). Briefly, 16 h cultures were prepared and then diluted with a sterile normal saline solution [0.85% (w/v) NaCl] with reference to the 0.5 McFarland standards to obtain an inoculums' size of approximately 10^6 colony forming unit mL^{-1}. A serial dilution was also prepared to give final concentrations ranging from 0-20 mg methanolic extract per ml. 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 determined as the lowest concentration of the methanolic extract in the broth medium that inhibited the visible growth of the test microorganism (NCCLS, 2002).

Antioxidant assay: The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radical scavenging assay method was used to determine the antioxidant activity of the selected plants. The enhanced technique for the generation of ABTS involves the direct production of the blue/green ABTS chromophore throughout the reaction between ABTS and potassium persulfate. This assay was performed using methods as described earlier (Re *et al*., 1999). This has absorption maximum at wavelength 734 nm. The method is applicable to the study of both water-soluble and lipid-soluble antioxidants, pure compound and food extracts. According to Re *et al*. (1999), ABTS was prepared in water as 7 mM solution. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate. This was followed by allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. Then generated ABTS solution was diluted with ethanol to reach an absorbance of 0.70±0.02 at 734 nm. Two microliter of different concentrations of different methanolic plant extracts or ethanol as solvent (blank control) were added to the diluted 1 mL ABTS solution. 2.0 mL of ethanol was added to 1 mL ABTS solution, this was used as a negative control. The decrease in absorbance at 734 nm was determined exactly at 6 min after initial mixing of each sample. All measurements were carried out at least three times. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as a standard compound. The Trolox Equivalent Antioxidant Capacity (TEAC) was determined according to standard curve of ABTS scavenging by trolox and expressed as mmol Trolox corresponding to one g dry matter of tested plant species. It is possible to calculate the concentration that would show 50% of antioxidant activity (IC_{50}). The IC_{50} value for each plant extract was also determined from standard curve and expressed as μg mL^{-1} extract.

RESULTS

Four plant species, *A. fragrantissima*, *T. polium* L., *R. officinalis* and *A. graecorum*, were collected from northern part of Jordan and their antibacterial activity and

antioxidant capacity were measured. To evaluate the antibacterial activities of these selected methanolic plant extracts, disc diffusion assay method was first employed. The antibacterial activity of these methanolic extracts against *S. aureus*, *E. coli*, *P. aeruginosa*, *E. cloacae* and *P. mirabilis* were examined at three different concentrations of 100, 200 and 400 μg mL^{-1}. At this assay, all tested methanolic extract derived from aerial parts of these selected plants exhibited antibacterial activity in dose dependent manner against both Gram positive and Gram negative bacteria (Table 2-5). The antibacterial activity of the methanolic extract from aerial parts of *R. officinalis* was presented in Table 2. Present results clearly demonstrated that the inhibition zones of this methanolic extract ranged from 7 to 25 mm. The highest zone of inhibition value was obtained against *S. aureus* (zone of inhibition: 25±2.65 mm), a Gram-positive bacterium, in comparison to *E. cloacae* (zone of inhibition: 13±1.53 mm), a Gram negative bacterium using the extract concentration of 400 μg mL^{-1}. Moderate antibacterial activity of this extract was also observed against both *P. aeruginosa* and *E. coli* followed by *P. mirabilis*.

Table 3 shows the effect of varying the amount of methanolic extract obtained from *A. fragrantissima*. This extract was found to possess maximum antibacterial activity against *E. coli* (zone of inhibition: 23±1.53 mm at concentration of 400 μg mL^{-1}), a Gram bacterium. Moderate antibacterial activity of this extract was also observed against *E. cloacae*, *P. aeruginosa* and *P. mirabilis* at the same concentration. On other hand, no zone of inhibition was observed against *S. aureus*, a Gram-positive bacterium at concentration of 400 μg mL^{-1}. Conversely, the methanol extract of *Alhagi graecorum* was found to possess maximum inhibitory effect against *S. aureus*, a Gram positive bacterium as shown in Table 4. In addition, this extract also moderately exhibited antibacterial activity against a *E. coli*, *P. aeruginosa* and *P. mirabilis*, Gram negative bacteria (zone of inhibition: ranging from 18 to 22 mm at concentration of 400 μg mL^{-1}), while minimum inhibitory effect against *E. cloacae* (zone of inhibition: 13 mm) was observed at the highest concentration tested (400 μg mL^{-1}). Further, *Teucrium polium* L. methanolic extract showed more variations in the inhibition zones against both the Gram-positive and Gram negative bacteria (Table 5). The *Teucrium polium* L. methanolic extract was also found to possess maximum antibacterial activity against *E. coli* (zone of inhibition: 26 mm), a Gram-negative bacterium in comparison to *S. aureus*, a Gram positive bacterium (zone of inhibition: 11 mm) using the extract at the highest concentration tested (400 μg mL^{-1}). In addition, this extract showed moderated antibacterial activity against *E. cloacae* (zone of inhibition: 16 mm) followed by *P. mirabilis* and *P. aeruginosa* (zone of inhibition: 13-14 mm) using the above mentioned concentration of 400 μg mL^{-1}. Similarly, the standard drug chloramphenicol was also found to be active against all tested bacteria using concentration of 30 μg mL^{-1}.

Table 2: Antibacterial activity (zone of inhibition) of *Rosmarinus officinalis* methanolic extract at different concentrations on the growth of five selected bacterial strains as compared with 30 μg mL^{-1} chloramphenicol

	Zone of inhibition (mm)[A]		
Microbe	100 (μg mL^{-1})	200 (μg mL^{-1})	400 (μg mL^{-1})
S. aureus	11±2.56	17±3.61	25±2.65
E. coli	10±1.00	14±1.53	21±1.00
P. aeruginosa	10±1.58	13±1.00	22±1.00
E. cloacae	7±0.58	9±0.58	13±1.53
P. mirabilis	10±1.00	12±1.00	19±3.21

[A]: Zone of Inhibition (mm) is average of triplicate experiments. Disc diameter = 6 mm

Table 3: Antibacterial activity (zone of inhibition) of *Achilla fragrantissima* methanolic extract at different concentrations on the growth of five selected bacterial strains as compared with 30 μg mL^{-1} chloramphenicol

	Zone of inhibition (mm)[A]		
Microbe	100 (μg mL^{-1})	200 (μg mL^{-1})	400 (μg mL^{-1})
S. aureus	ND	ND	ND
E. coli	12±2.53	17±2.52	23±1.53
P. aeruginosa	11±1.00	16±2.65	18±2.00
E. cloacae	11±2.56	14±2.56	18±3.51
P. mirabilis	10±1.00	11±0.58	17±2.56

[A]: Zone of Inhibition (mm) is average of triplicate experiments. Disc diameter = 6 mm. ND: Not detectable

Table 4: Antibacterial activity (zone of inhibition) of *Alhagi graecorum* methanolic extract at different concentrations on the growth of five selected bacterial strains as compared with 30 μg mL^{-1} chloramphenicol

	Zone of inhibition (mm)[A]		
Microbe	100 (μg mL^{-1})	200 (μg mL^{-1})	400 (μg mL^{-1})
S. aureus	13±0.90	17±1.20	22±2.52
E. coli	12±2.00	15±1.00	20±2.52
P. aeruginosa	14±0.58	16±1.00	22±4.36
E. cloacae	ND	10±1.53	13±1.53
P. mirabilis	13±1.00	15±2.28	18±4.51

[A]: Zone of Inhibition (mm) is average of triplicate experiments. Disc diameter = 6 mm. ND: Not detectable

Table 5: Antibacterial activity (zone of inhibition) of *Teucrium polium* L. methanolic extract at different concentrations on the growth of five selected bacterial strains as compared with 30 μg mL^{-1} chloramphenicol

	Zone of inhibition (mm)[A]		
Microbe	100 (μg mL^{-1})	200 (μg mL^{-1})	400 (μg mL^{-1})
S. aureus	ND	10±1.00	11±1.00
E. coli	10±1.00	14±1.53	26±2.08
P. aeruginosa	8±1.58	10±0.58	13±2.08
E. cloacae	9±1.53	11±1.53	14±1.73
P. mirabilis	10±1.58	12±1.00	16±2.65

[A]: Zone of Inhibition (mm) is average of triplicate experiments. Disc diameter = 6 mm. ND: Not detectable

Table 6: Antibacterial activity (Minimum Inhibition Concentration (MIC)) of methanolic extracts derived from four locally selected plants on the growth of five selected bacterial strains

	MIC (mg mL^{-1} extract)			
Microbe	*R. officinalis*	*A. fragrantissima*	*T. polium* L.	*A Graecorum*
S. aureus	1.2	2.9	2.2	1.2
E. coli	0.9	1.2	1.2	1.5
P. aeruginosa	1.0	1.2	2.4	1.1
E. cloacae	1.7	1.8	2.0	1.7
P. mirabilis	1.6	2.2	1.8	1.8

MIC (mg mL^{-1}) is average of triplicate experiments

To evaluate and confirm further the antibacterial activity of the selected plant methanolic extracts, we then examined the antibacterial activities of these extracts by broth dilution method. Table 6 provides the antibacterial results obtained using the diffusion broth method. According to this assay, a varying degree of antibacterial activities were observed from these methanolic extracts derived from aerial parts of these selected plants against the pathogenic bacteria in the present study. The Minimum Inhibition Concentrations (MIC) for the methanolic extracts were found between 1.2-2.9 mg mL^{-1} for *A. fragrantissima*, 1.2-2.7 for *T. polium*, 1.1-1.8 mg mL^{-1} for *A. graecorum* and 0.9-1.7 for *R. officinalis* against all tested bacterial strains. The strongest antibacterial activity was seen against *E. coli* (MIC: 0.9 mg mL^{-1}), using the methanolic extract from *R. officinalis*, followed by *P. aeruginosa* (MIC: 1.0 mg mL^{-1}) and *S. aureus* (MIC: 1.2 mg mL^{-1}). On the other hand, the weakest antimicrobial activity was seen against *P. mirabilis* (MIC: 1.7 mg mL^{-1}), followed by *E. cloacae* (MIC: 1.6 mg mL^{-1}). Along the same line, the methanolic extract of *T. polium* L. was active against all tested microorganism and the highest inhibitory activity was seen against *E. coli* (MIC: 1.2 mg mL^{-1}), while the weakest antimicrobial activity was demonstrated against *P. aeruginosa* (MIC: 2.4 mg mL^{-1}) and *S. aureus* (MIC: 2.2 mg mL^{-1}), followed by *E. cloacae* (MIC: 2.0 mg mL^{-1}) and *P. mirabilis* (MIC: 1.8 mg mL^{-1}). Conversely, the best antibacterial activity was observed against *P. aeruginosa* (MIC: 1.0 mg mL^{-1}), followed by *S. aureus* (MIC: 1.1 mg mL^{-1}) using the methanolic extract of *A. Graecorum*, whereas the lowest antibacterial activity was seen against *P. mirabilis* (MIC: 1.8 mg mL^{-1}) and *E. cloacae* (MIC: 1.7 mg mL^{-1}), followed by *E. coli* (MIC: 1.5 mg mL^{-1}). On the other hand, the methanol extract of *A. fragrantissima* showed less inhibitory activity against the tested bacteria (MIC: 1.2 to 2.9 mg mL^{-1}) than did the methanolic extracts derived from the remaining three plant methanolic extracts. The highest antimicrobial activity was seen against both *E. coli* and *P. aeruginosa* at MIC concentration of 1.2 mg mL^{-1} extract.

Table 7: The Trolox Equivalent Antioxidant Capacity (TEAC) and ABTS IC_{50} of the methanolic plant extracts

Plant	TEAC (mmol g^{-1} dry matter)	IC_{50} (μg mL^{-1} extract)
R. officinalis	424	21
A. Graecorum	172	103
T. polium L.	174	80
A. fragrantissima	191	69

Values are the average of triplicate experiments of plant methanolic extract at 734 nm wavelength. IC_{50} is the amount of sample required to scavenge 50% of ABTS

Furthermore, we also studied the antioxidant capacity of our plant methanolic extracts. The antioxidant capacities of these plant extracts from aerial parts were measured for the first time by ABTS in vitro assay and calculated as trolox equivalent antioxidant capacity (TEAC). The ABTS scavenging activities expressed as TEAC were presented in Table 7. The investigated methanolic extracts possessed the free radical scavenging properties in different degrees. According to ABTS in vitro assay, *R. officinalis* methanolic extract presented a strong antioxidant capacity (424 mmol g^{-1} dry matter), followed by *A. fragrantissima* (191 mmol g^{-1} dry matter), while the lowest TEAC value was shown for *T. polium* L. (174 mmol g^{-1} dry matter) and *A. graecorum* (172 mmol g^{-1} dry matter). Moreover, the concentrations of the extracts required to scavenge 50% of ABTS (IC_{50}) were also estimated for the first time (Table 7). The IC_{50} value on ABTS was assessed according to standard curve of ABTS scavenging by trolox. Similarily, our results revealed that *R. officinalis* exhibited the highest antioxidant activity when IC_{50} (21 μg mL^{-1} extract) was measured, followed by *A. fragrantissima* (69 μg mL^{-1} extract), *T. polium* (80 μg mL^{-1} extract) and finally *A. Graecorum* (103 μg mL^{-1} extract).

DISCUSSION

Our current study addresses the antimicrobial and antioxidants effects of four locally plants selected from Al-Mafraq area which is located in the northern part of Jordan. The selected plants were *A. fragrantissima*, *T. polium*, *R. officinalis* L. and *A. graecorum*. The selection criteria of these four species were based on their traditional uses as folk medicinal plants for treatments of various types of diseases in Jordan (Afifi and Abu Iramileh, 2000). In addition, these species are popular and widely distribute in Jordan. They usually grow under the minimum amount of rainfall and high temperature.

In this study, we decided to use methanolic extraction method. It is worth to note that various extraction methods have been employed and the antimicrobial and antioxidant activities of these extracts have been studied in several numbers of articles (Alzoreky and Nakahara, 2003; Borchardt *et al.*, 2008;

Parthasarathy *et al*., 2009). Based on these articles as well as others, methanolic extraction is the most commonly used method for commercial and therapeutic purposes when it compared to other methods of extractions such as chloroform, alkaloid, acetone and others. Thus, we utilize this methanolic extraction method as method of choice for our selected plant species.

Present results obtained from both antimicrobial assays clearly indicated that all tested plant methanolic extracts were active against all tested microorganism, except *Achilla fragrantissima*, which exhibited less or no antibacterial activity against *S. aureus*. In the present study, both assays also demonstrated that the methanolic extracts derived from the selected plants exhibited a varying degree of antibacterial activity against the tested bacteria. The antibacterial activity was also shown to be increases as the concentrations of these plant extracts increase. Present current data also showed that the methanolic extracts of *R. officinalis* exhibited the highest antibacterial activity in both broth dilution and disc diffusion assay methods. In addition, only *R. officinalis* had an effect on Gram positive bacteria at lower concentration, indicating that this plant was more effective in inhibition of Gram positive bacterial growth when compared with remaining three plant extracts. Interestingly, our results showed for the first time that Gram-negative *E. coli* was susceptible to all plant extracts. These findings are not in accordance with previous study done on *P. aeruginosa* and *E. coli* (Romero *et al*., 2005; Tarawneh *et al*., 2008). These variations have been reported previously in various plant extracts and it seems to be dependent on factors such as climate, soil composition, plant organ, age and vegetative cycle stage and others (Scalbert, 1991; Kirby, 1996; Cichewicz and Thorpe, 1996; Romero *et al*., 2005; Aiyegoro and Okoh, 2009). Taken together, these results suggest possible use of these plant extracts in the treatment of antibacterial infections, particularly *R. officinalis*. Additional studies is, therefore, need to assess their antibacterial activity *in vivo*.

The antioxidant activities of these methanolic plant extracts on removal of free radicals were also measured by ABTS *in vitro* assay for the first time in this study. Moreover, their trolox equivalent antioxidant capacities (TEAC) have also been determined and the concentration of the extract required to scavenge 50% of ABTS (IC_{50}) were also estimated for the first time. It is well known that TEAC is a quantification of the effective antioxidant activity of the sample; hence a higher TEAC value would indicate greater protective action. According to ABTS in vitro assay, *R. officinalis* methanolic extract presented a strong antioxidant capacity due to the scavenging activity towards ABTS free radicals, indicating that this plant may offer more protection from naturally or synthetic oxidant compounds. Similarly, our current data also reveal that *R. officinalis* exhibited the highest antioxidant activity when IC_{50} was measured. The IC_{50} for these selected plant extracts was found in complete accordance with its antioxidant capacity. It is possible to suggest that the higher radical scavenging activity of *R. officinalis* sample may be attributed to the high amount of antioxidant compounds which might be found in its methanolic extract. The other three plants were found to have less antioxidant activities than *R. officinalis* by two fold. Interestingly, our data also revealed that *R. officinalis* showed the most antibacterial and antioxidant activities when compared to the remaining three plant species.

The measurement of the *in vitro* antioxidant activities of these selected plant extracts might form part of the indirect evaluation of their role in removal of highly reactive free radicals. Furthermore, a positive correlation has been shown between antioxidant activity in both ABTS scavenging and DPPH assays and total phenol and flavonoid contents, indicating that these compounds are more likely to contribute to the antioxidant potential of the investigated plant extracts (Choi *et al*., 2002; Miliauskas *et al*., 2004; Sakanaka *et al*., 2005; Parthasarathy *et al*., 2009). However, the total phenol contents in our investigated plant methanolic extracts have not been yet analyzed. As mentioned above, several numbers of investigators have reported that plant phenol compounds are commonly distributed in the plant kingdom and that they are present in high amounts (Rice-Evans *et al*., 1995; Middleton *et al*., 2000; Scalbert *et al*., 2005; Kiselova *et al*., 2006; Abdel-Hameed, 2009; Borchardt *et al*., 2008; Parthasarathy *et al*., 2009). It has been reported that oxidative stress induced by reactive oxygen species may cause different subcellular damages such as lipid peroxidation. Consequently, these highly reactive free radicals have been implicated in pathology of different number of diseases in humans such as diabetes mellitus, atherosclerosis, cancer and Parkinson disease and other neurodegenerative disorders. It also reported that antioxidant compounds such as phenols and others play a vital role in removing free radicals and in inhibition of lipid peroxidation formation (Middleton *et al*., 2000; Adhami and Mukhtar, 2006; Almeida and Mukhtar, 2008). Thus, there is an inverse relationship between antioxidant activities and the level of lipid peroxidation production in animal tissues. Therefore, it is possible to suggest that our methanolic plant extracts may serve as a potential economically viable source of

natural antibacterial and antioxidant compounds such as polyphenols, flavonoids and others that might be capable of protecting biological systems from various microbes and oxidative process and therefore, limit the risk of various infection as well as degenerative diseases. Thus, our data may, in part, support the traditional use of these plant species for medicinal purposes.

In conclusion, all tested methanolic extracts from *A. fragrantissima*, *T. polium* L., *R. officinalis* and *A. graecorum*, exhibit both antibacterial and antioxidant activities in vitro assay systems with different degrees. Present data also revealed that there were variations in the antibacterial and antioxidant activities among these methanolic plant extracts. Moreover, *R. officinalis* showed the most antibacterial and antioxidant activities. Thus, it is possible to speculate that the therapeutic values of our selected plant extracts might be associated with the antioxidant activity of their constituents. We believe that this study will be useful in providing the knowledge that aerial parts from these selected plants may serve as potential potent sources of antibacterial and antioxidant compounds which might be useful for providing protections against various kinds of human diseases. Further purification of the active compounds and *in vivo* evaluation of antioxidant and antimicrobial activity along with toxicity studies of the extract from *R. officinalis* must be, therefore, carried out in experimental animal models to confirm our above speculation.

ACKNOWLEDGMENTS

Authors would like to thank the Deanship of graduate study, Al al-Bayt University, Al-Mafraq, Jordan, for the financial help. The technical assistance from Department of Biological Sciences, Mutah University, Mutah, Jordan is also gratefully acknowledged.

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