Antibacterial activity of some medicinal plants grown in Jordan

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Abstract: In the present study, we evaluated the antimicrobial activity of 16 Jordanian medicinal plant extracts against four reference bacteria; *Staphylococcus aureus, Enterobacter faecalis, Escherichia coli,* and *Salmonella typhi*. For that purpose, whole plants were extracted and antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) were determined. Ethanolic extracts of most medicinal plants exerted a dose-dependent cytotoxiciy against different reference bacteria. *Origanum syriaca, Varthemia iphionoides, Psidium guajava, Sarcopoterium spinosa* plant extracts were most active against *S. aureus* (MIC; 70 µg/mL), *E. faecalis* (MIC; 130 µg/mL), E. coli (MIC; 153 µg/mL), and S. typhi (MIC; 110 µg/mL), respectively. Results indicate that medicinal plants grown in Jordan might be a valuable source of starting materials for the extraction and/or isolation of new antibacterial agents.

Keywords: *E. coli, E. faecalis,* plant extracts, *S. typhi, S. aureus.*

INTRODUCTION

The health hazards of bacterial infections have been drastically reduced by the discovery and therapeutic use of antibiotics at the early nineteenth century. However, with wide spread use of antibiotics, bacterial resistance to antibiotics has been increasing at an alarming rate (Ahmad *et al*., 1998). The emergence of bacterial resistance, adverse effects of antibiotics and the increased cost of combination treatment, indicates a strong need for new treatment regimens with similar or more favorable therapeutic properties, but fewer adverse effects than antibiotics (Di Mario *et al*., 2006). The rational testing of bioactive products from traditional medicine is an established strategy for the discovery of novel compounds with potent and therapeutically useful antimicrobial activities.

In the present study, we evaluated the antimicrobial activity of 16 Jordanian medicinal plant extracts against four reference bacteria; *S. aureus*, *E. coli*, *E. faecalis, and Salmonella*. Selection of plants for this study was based on their earlier medicinal use for the management of various infectious diseases (Al-jarwani and Khalifeh OStamatis, 1936, Jabour, 1983, Karim and Quraan, 1986). Results indicate that some of the extracts were more effective to inhibit bacterial growth than conventionally used agents.

MATERIALS AND METHODS

Plant materials used in the current study were collected from various locations of the North of Jordan. Taxonomic identification of the plants was confirmed by Professor Jammel Lahaam, Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid-Jordan. A voucher specimen from each plant was deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid-Jordan. The plant materials were shade-dried and ground in a Wiley grinder (Model 5657 HAAN, Germany) with a 2 mm diameter mesh. Powdered plant material (50 g) was percolated in EtOH (95%). The combined ethanolic extracts were concentrated in vacuum to give a dried extract, which was subjected to further fractionation.

S. aureus (ATCC 2940), *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212), and *S. typhi* (ATCC 19430) were cultured in nutrient broth (Hi-media, M002) at 37°C and stored in nutrient agar slants at 4°C. Sterile; 5mm diameter filter paper discs were impregnated with 60-100µg of the tested plant extract in dimethylsulfoxide (DMSO) and placed in duplicates onto Mueller-Hinton agar. The surface was then spread with 0.2 ml of microorganism culture (ca. 10^8 cells/ml) and the plates were incubated for 24 hr at 37°C. The experiments were done in duplicate. The results (mean of 3 independent experiments) were the zones of growth inhibition surrounding the discs. Negative control discs contained only DMSO. The following antibiotics were tested as positive controls: Amoxicillin (10 µg/disc), tetracycline (30 µg/disc), metronidazole (5 µg/disc), and clarithro-mycin (20µg/disc).

The minimum inhibitory concentrations (MIC) were determined by serial dilution method as described in (Dickson *et al*., 2006). Briefly, stock solutions of all extracts were passed through a pyrogenic filter, then, they were serially diluted to a range of concentrations. To

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prepare the 96-well plates, 100 µl of appropriate medium, test extract and 20 µl of the innoculum were dispensed into each well. To assay bacteria, a standard nutrient Mueller-Hinton broth (MHB) was used. Turbidity indicated growth of the microorganisms, whereas clear wells indicated no bacterial growth. In each experiment, there was a sterility check (50% DMSO and medium), a negative control (50% DMSO, medium and innoculum), and a positive control (50% DMSO, medium, innoculum and clarithromycin). Plates were incubated for 24 hr at 37°C. They were, then, examined for growth in daylight. The lowest concentration that completely inhibited visible growth in the medium was considered as the MIC. All experiments were carried out in triplicate.

RESULTS

Results shown in table 1 indicate that 11 ethanolic extracts of medicinal plants induced a dose-dependent cytotoxicity of the reference bacteria. *S. aureus* growth was inhibited to variable degrees with different plant extracts. The highest bacterial growth inhibition was with *O. syriaca*, followed by *V. iphionoides, I. viscosa,* and *P. guajava.* Low growth inhibition was with *P. argentea, C. aronia, A. inculata, S. spinosa, A. triphylla, L. sativum, and A. graveolens.* Fascinatingly, ethanolic extracts from *O. syriaca* were comparable in inhibiting proliferation of *S. aureus* to some of the conventional antibacterial agents.

Antibacterial activity induced by plant extracts from *V. iphionoides and from P. guajava* against *E. faecalis* were comparable to that induced by clarithromycin and less than the activity of amoxicillin (table 1). Additionally, none of the plant extracts, except those from *P. guajava*, was effective against *E. coli* (table 1). A significant antibacterial activity against *Salmonella* was induced by extracts from *P. guajava* and *S. spinosa*. Notably, the later induced more cytotoxicity against *Salmonella* compared to the conventional antibacterial agents (table 1). The MIC of the most effective plant extracts such as *O. syriaca, V. iphionoides, P. guajava,* and *S. spinosa* against *S. aureus, E. faecalis, E. coli,* and *Salmonella*, are shown in table 2.

DISCUSSION

In this study, the antibacterial activities of several ethanolic extracts of medicinal plants are reported. Notably, the extract ethanolic extracts from *O. syriaca* were more effective to inhibit proliferation of *S. aureus* than any of the conventional antibacterial agents. These results are consistent with the previous report (Hammer *et al*., 1999) in that essential oils from *O. syriaca* such as thymol, carvacrol, and estragole have antimicrobial activity against a variety of food barn, human, and plant pathogens. Moreover, these results might explain *O. syriaca* use in earlier medicine for the treatment of abscesses, septic wounds, gastroenteritis, and chest infections associated with productive cough (Jabour, 1983, Karim and Quraan, 1986).

The extracts from *V. iphionoides and from P. guajava* showed comparable antibacterial activity against *E. faecalis* to that induced by clarithromycin and less than the activity of amoxicillin. It has been reported elsewhere that aqueous extract of *V. iphionoides* has a high content of phenolic compounds and flavonoids (Al-Dabbas *et al*., 2006). This might explain its antibacterial activity and emphasize their earlier use for treatment of gastroenteritis accompanied by abdominal spasm (Jabour, 1983, Karim and Quraan, 1986).

The extract of *P. guajava* was effective against *E. coli.* Phytochemical evaluation of the leaf has shown the presence of flavonoids, tannins, saponins, phenol lectins, triterpenes, and carotenoids (Geidam *et al*., 2007). The biological and antimicrobial activity of leaf extract was largely attributed to flavonoids and another two chemicals namely guajaverin and psidiolic acid (Berdy *et al*., 1982, Jaiarj *et al*., 1999).

Results of this study show limited of activity of tested plant extracts against gram-negative bacteria. It has been previously shown that gram-negative bacteria are generally more resistant to common antibiotics than gram-positive ones (Cos *et al*., 2006), which is related to the existence of an outer membrane in the cell wall of gram-negative bacteria that acts as a barrier for foreign materials including antibiotics (Palombo and Semple, 2001). Thus, the limited activity of tested plant extracts against gram-negative bacteria may be related to their superior resistant barrier properties. An alternative explanation of this limited activity of some extracts might be related to the method of extracts preparation. It is possible that the separation of a group of ingredients that are acting synergistically, lead to loss of their desired therapeutic activity. Moreover, limited activity of an extracts *in vitro* to a certain microorganism, does not necessarily imply that limited *in vivo* activity (e.g. (Garcia *et al*., 2003). In fact, some drugs undergo metabolic transformation in the human body rendering them much more potent *in vivo*, and plant extracts are no exception to such possibility. Thus, it is possible that these plant extracts are metabolized *in vivo* into highly active intermediates that carry significant antimicrobial activity.

Collectedly, results in the present study indicate that extracts from medicinal plants grown in Jordan such as *O. syriaca, V. iphionoides, P. guajava, S. spinosa* might be a valuable source for extraction and/or isolation of new antibacterial drugs acting against *S. aureus, E. faecalis, E. coli*, and *S. typhi*.

Table 1: Antibacterial activity of sixteen ethanolic plants extracts with different concentrations against *S aureus, E. faecium, E.coli, and S. typhi.*

Plant/ Concentration in µg /ml	Diameter of zone of inhibition (mm)																				
	S. aurus ATCC 2940							*E. faecium* ATCC 29212							*E. coli* ATCC 35821			*S. typhi* ATCC 19430			
	Stock	C1	C2	C3	C4	C5	C6	Stock	C1	C2	C3	C4	C5	C6	Stock	C1	C2 - 6	Stock	C1	C2	C3 - 6
Passiflora incarnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paronychia argentea	17 ± 3.1	14 ± 3.3	13 ± 3.1	11 ± 1.1	0	0	0	13 ± 0	11 ± 1	0	0	0	0	0	0	0	0	0	0	0	0
Crataegus aronia	16 ± 2.2	14 ± 2.3	12 ± 2.6	0	0	0	0	16 ± 2	14 ± 2.1	12 ± 1	0	0	0	0	0	0	0	0	0	0	0
Artemisia inculata	17 ± 2.5	15 ± 2.3	14 ± 2.7	10 ± .2.1	0	0	0	17 ± 3	15	13 ± 2	11 ± 1	0	0	0	0	0	0	0	0	0	0
Capparis spinosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Varthemia iphionoides	20 ± 3.5	18 ± 2.5	17 ± 2.8	15 ± 2.8	13 ± 2.3	11 ± 0.9	0	20 ± 4	18 ± 2	17 ± 2	15 ± 3	13 ± 21	11 ± 1	0	0	0	0	0	0	0	0
Inula viscosa	19 ± 3.6	17 ± 3.4	16 ± 3.1	15 ± 3.1	14 ± 3.1	12 ± 1	0	17 ± 2	16 ± 1	14 ± 2	11 ± 2	0	0	0	0	0	0	0	0	0	0
Sarcopoterium spinosum	16 ± 2.2	14 ± 1.9	12 ± 2.5	11 ± 2.1	0	0	0	15 ± 2	12 ± 1	0	0	0	0	0	0	0	0	26 ± 4.1	22 ± 3.6	16 ± 2.8	0
Psidium guajava	20 ± 3.4	18 ± 2.5	16 ± 3.1	14 ± 3.1	12 ± 1.8	10 ± 1	0	19 ± 4	18 ± 0	14 ± 2	12 ± 2	0	0	0	12 ± 2.1	10 ± 2	0	19 ± 3.3	15 ± 3.1	13 ± 3	0
Lavandula officinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aloysia triphylla	16 ± 2.1	12 ± 2.0	0	0	0	0	0	12 ± 2	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidium sativum	14 ± 2.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Origanum syriaca	32 ± 4.5	25 ± 4.3	18 ± 1.8	14 ± 2.5	0	0	0	17 ± 2	14 ± 1	0	0	0	0	0	0	0	0	0	0	0	0
Sesamum indicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urtica urens	0	0	0	0	0	0	0	14 ± 3	10 ± 0	0	0	0	0	0	0	0	0	0	0	0	0
Anethum graveolens	14 ± 3.1	12 ± 3.2	0	0	0	0	0	12 ± 0	11 ± 0	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin	32 ± 4.1							27 ± 3.5							16 ± 2.5			0			
Clarithromycin	9 ± 2.1							18 ± 3.3							18 ± 3.1			20 ± 3			
Metronidazole	0							0							0			0			
Tetracycline	9 ± 1.1							12 ± 1.4							12 ± 2.1			13 ± 2.4			

Stock = 100000 µg /ml, C1= 50000 µg /ml, C2= 25000 µg /ml, C3= 12500 µg /ml, C4= 6250 µg /ml, C5= 3125 µg /ml, C6= 1582.5 µg /ml, Amoxicillin = 10 µg/disc, Metronidazole = 5 µg/disc, Clarithromycin = 20 µg/disc, and Tetracycline = 30 µg/disc. The data shown are the mean ± SE of three individual experiments.

Table 2: MICs of plant extracts against reference bacteria

Bacteria	Plant extract	MIC (µg/ml)
S. aureus	*Origanum syriaca*	70±8.33
E. faecium	*Varthemia iphionoides*	130±11.25
E. coli	*Psidium guajava*	153±19.25
Salmonella	*Sarcopoterium spinosum*	110±9.54

REFERENCES

Ahmad I, Mehmood Z and Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.,* **62**: 183-193.

Al-Dabbas MM, Suganuma T, Kitahara K, Hou DX and Fujii M (2006). Cytotoxic, antioxidant and antibacterial activities of Varthemia iphionoides Boiss. extracts. *J. Ethnopharmacol.,* **108**: 287-293.

Al-jarwani M and Khalifeh OStamatis G (1936). Tathkirat Daoud Al-Antaki. Iben Shakroun library, Egypt. pp.154, 182.

Berdy J, Aszlos A, Bostian M and Mcnitt KL (1982). Hand book of antibiotic compounds. CRC Press, New York, p.410.

Cos P, Vlietinck AJ, Berghe DV and Maes L (2006). Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *J. Ethnopharmacol.,* **106**: 290-302.

Di Mario F, Cavallaro LG and Scarpignato C (2006). 'Rescue' therapies for the management of *Helicobacter pylori* infection. *Dig. Dis.,* **24**: 113-130.

Dickson RA, Houghton PJ, Hylands PJ and Gibbons S (2006). Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* Baill., *Securinega virosa* Roxb. & Wlld. and *Microglossa pyrifolia* Lam. *Phytother. Res.,* **20**: 41-45.

Garcia D, Leiro J, Delgado R, Sanmartin ML and Ubeira FM (2003). *Mangifera indica* L. extract (Vimang) and mangiferin modulate mouse humoral immune responses. *Phytother. Res.,* **17**: 1182-1187.

Geidam YA, Usman H, Abubaker MB, Ibrahim B and Carnat AP (2007). Effects of aqueous leaf extracts of Psidium guajava on bacteria isolated from noval of day-old chicks. *Res. J. Microbiol.,* **2**: 960-965.

Hammer KA, Carson CF and Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.,* **86**: 985-990.

Jabour J (1983). Al-kanoon in medicine of Avicenna. Al-Maaref Library, Beirut. pp.113, 224, 233-234, 271 and 285.

Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML and Ruangsomboon O (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *J. Ethnopharmacol.,* **67**: 203-212.

Karim FM and Quraan SA (1986). Medicinal plants of Jordan. Yarmouk University, Irbid-Jordan, pp.49-61.

Palombo EA and Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. *J. Ethnopharmacol.,* **77**: 151-157.