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ANTIBACTERIAL ACTIVITY OF JORDANIAN MEDICINAL PLANTS

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ABSTRACT

The antibacterial activity of ethanol extracts of 15 plant species used in the traditional medicine in Jordan and other Middle East countries were tested. Extracts of certain parts of these plants were tested in vitro *against 14 pathogenic bacterial species and strains using the agar diffusion method. Results evaluated as the diameter of inhibition zone of bacterial growth showed that 25 mg/well of 12 plant extracts have antibacterial activity on one or more of the tested bacteria. Three plants exhibited broad spectrum antibacterial activity:* Punica granatum *L.,* Quercus infectoria *Olive., and* Rhus coriaria *L. The most susceptible bacteria were* Pseudomonas aeruginosa, Bacillus cereus *and* Streptococcus pyogenes *(ATCC 12351), and the most resistant species were* Escherichia coli *(ATCC 25922 and clinical isolates),* Klebsiella pneumoniae, Shigella dysentriae *(ATCC 49345), and* Yersinia enterocolitica *(ATCC 9610). The minimum inhibitory concentrations (MIC) of active extracts ranged from 4–32 mg/ml while the minimum bactericidal concentrations (MBC) were exerted at higher doses 8–62 mg/ml.*

INTRODUCTION

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances (Alamagboul et al., 1985; Alkofahi et al., 1990; Grayer & Harborne, 1994; Olukoya et al., 1993).

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The substances that can either inhibit the growth of microorganisms or kill them are considered candidates for developing new drugs for treatment of various infectious diseases. The use of medicinal plants as a traditional medicine is well-known in the rural areas of many developing countries (Catalano et al., 1998; Martinez et al., 1996; Sundar, 1996; Taylor et al., 1996). Jordan has a great flora and a tradition in using medicinal plants for treating infectious as well as chronic diseases (Al Dejawi, 1991; Al Genaidy, 1993; Karim, 1986). Herbal medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products.

This study reports the antibacterial activity of ethanol extracts of selected parts of 15 medicinal plants, most of which are used in folklore remedies. There has been no previous reports on screening for antimicrobial activities of the selected plants in Jordan except for one study which reported on the antibacterial activity of three of the plant species used in this study using different fractionation methods (Alkofahi et al., 1996).

Keywords: Antibacterial activity, medicinal plants, plant extracts.

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MATERIALS AND METHODS

Plant Extracts

Most of the plants used in this study were collected from different locations in Jordan, while the others were purchased from grocery stores. The taxonomic

identity of the plants was confirmed by us. Voucher specimens were deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology. Certain parts of the selected plants were used in this study because of their use in traditional medicine. These plant parts were shade-dried and then ground in a Wiley grinder with a 2 mm diameter mesh. Fifty grams of the ground material were extracted by cold percolation with 95% ethanol. The ethanolic extract was concentrated under vacuum, weighed and the residue used for the tests. The 15 selected plants and their traditional use in Jordan are shown in Table 1 (Al Genaidy, 1993; Karim, 1986).

Test Bacteria

A total of 14 bacterial species strains were tested. The Gram-positive species were *Bacillus cereus, Staphylococcus aureus* (ATCC 8095), *Staphylococcus epidermidis, Streptococcus pyogenes* (ATCC 12351) and *Enterococcus faecalis*. The Gram-negative species were *Shigella dysentriae* (ATCC 49345), *Yersinia enterocolitica* (ATCC 9610), four strains of *Escherichia coli* (ATCC 25922, clinical isolates *E. coli* B, 0111, and 2759), *Klebsiella pneumoniae, Proteus vulgaris*, and *Pseudomonas aeruginosa*. The species that were not purchased were originally isolated from clinical material collected from patients; they were identified using standard biochemical tests.

Cultures of Bacteria

All bacteria were cultured on nutrient agar plates, except for *Streptococcus pyogenes,* which was cultured on blood agar plates, and were incubated for 24 h at 37°C. Few colonies from these cultures were inoculate onto brain-heart infusion broth and incubated at 37°C for 24 h. This broth was diluted 1/10 before use.

Antibacterial Susceptibility Testing

The crude extract was diluted in distilled water at different concentrations starting with 0.5 g/ml, filter sterilized using a 0.45 µm millipore filter, and tested for antibacterial activity against the above bacterial species. The well and disk diffusion methods were used (Lennette, 1985). In the first method, wells of 6 mm in diameter were made in Mueller-Hinton agar under aseptic conditions. One hundred µl of bacterial suspension of 10^6 cells/ml were spread onto the surface of Mueller-Hinton agar plates. Plant extracts were added to the wells, a concentration of 25 mg/well. Triplicates of each concentration for each bacterial species were prepared. The diameter of the inhibition zones were measured for each plate and the average reading of the three replicates for each bacterial species are shown in Table 2. The 95% ethanol solvent was used as a negative control with each bacteria tested. In the disk diffusion method, sterile filter paper disks of 6 mm were impregnated with 50 µl of plant extracts (25 mg/disks). These disks were aseptically placed onto the surface of Mueller-Hinton agar, plates were placed in a refrigerator for few hours to allow diffusion of plant extracts before they were incubated at 37°C for 24 h. Disks containing different concentrations of eight antibiotics (ampicillin/clavulanic acid [augmentin] 30 µg, erythromycin 15 µg, doxiciline 30 µg, lincomycin 2 µg, novecin 5 µg, penicillin 10 IU, tetracycline 30 µg, and tobramycin 10 µg) served as positive controls. Disks impregnated with 95% of ethanol was also included to test if it has any effect on the results obtained with the plant extracts.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts for the plants with broad antibacterial activity was determined using the broth dilution method (NCCLS M26-p, 1987; NCCLS M7-t2, 1988). The reconstituted extract was diluted in vials containing brain-heart infusion broth except for *Streptococcus* species where Todd-Hewitt broth was used to give a final concentration of 0.49, 0.98, 1.95, 3.9, 7.81, 15.63, 31.25, 62.5, 125, and 250 mg/ml. Using a micropipette, 50 µl of the standard microbial broth culture were introduced into the vials. Another set of vials containing only the growth medium and each of the test bacteria with no extracts was set up separately as a control. The vials were incubated for 24 h at 37°C. The MIC was regarded as the lowest concentration of the plant extract in the series of dilutions which did not permit the growth of the susceptible bacteria.

The MBC of the extract was determined by a modification of the methods previously described (Irobi & Banso, 1994; Rotimi et al., 1987). Subcultures made from 50 µl diluted (1:10) samples, obtained from vials which did not yield any visible turbidity (growth) in the MIC assays, were streaked on freshly prepared brain-heart infusion agar plates. After 24 h incubation at 37°C, the MBC was regarded as the lowest concentration of the plant extract that allowed less than 0.1% of the original inoculum to survive and grow on the surface of the medium used. This criteria was selected since even bactericidal drugs do not always totally sterilize a bacterial population.

Table 1. Information about medicinal plants tested in this study.

Plant species Family	Parts extracted	Traditional uses	Active constituents
Artimesia herba-alba Asso. Compositae	aerial parts	antihelminthic, hypoglycemic, regulates heartbeat and activates blood circulation	essential oil, resin, absinthol, pinene, cadinene, tannin
Ceratonia siliuqa L. Leguminosae	dry pods	for constipation, diuretic action, neutralize stomach acidity, absorbs toxins, used to treat rheumatism and venereal diseases	essential oil, tannin, mucilage, anthraquinone glycoside
Ficus carcia L. Moraceae	fruits	laxative, constipation and wounds	pectin, resin, gum, latex
Foeniculum vulgare Mill. Umbelliferae	seeds	bronchodilator, antitussive, mouth infections, stimulate production of milk, inflammation of skin, indigestion, urinary tract infections	essential oil, anethole, anisic acid, other acids, and fixed oil
Glycyrrhiza glabra L. Leguminosae	roots and rhizomes	expectorant, antitussive, included in cough medications, useful for peptic ulcer, anti-inflammatory, laxative	glycyrrhizin, asparagine, liquirtin, coumarin, sugar, and tannin
Malva parviflora L. Malvaceae	leaves and seeds	relief teeth pain, laxative, expectorant, antitussive	malvin, tannin, and mucilage
Matricaria chamomilla L. Compositae	flowers	treatment of eczema, cold, eye inflammations, rheumatism, tonsillitis, antispasmodic, sedative	essential oil, vitamin C, coumarin, and apigenin
Nerium oleander L. Apocyanceae	roots and rhizomes	toxic but the boiled leaves are used to treat bloating and to strengthen the tooth gums, from the bark of the roots an oil extract is used to treat psoriasis	neriatine, olean, neriodorin, essential oil
Punica granatum L. Punicaceae	bark and the rind of the fruit	anti-dysentery, antispasmic, anthelminthic, skin diseases such as small pox and scabies	punicine, granatonine, granatin, the alkaloid pelletiarine and tannin
Quercus infectoria Olive. Fagaceae	nuts	astringent	tannin, resin, and acids (gallic and ellagic)
Rhus coriaria L. Anacardiaceae	fruits	astringent, anti-dysentery, stops bleeding	myricetin, tannin, and oxyquercetin
Ricinus communis L. Euphorbiaceae	seeds	purgative, respiratory tract infections, ointment base and for skin ulcerations	ricinine, resin, gum, and castor oil
Salvia triloba L. Labiatae	leaves	for stomach disturbances, antiseptic, sedative, and wound healing	essential oil, tannin, camphodar cineol, borneol, pinene, and resin
Thymus capitatus L. Labiatae	leaves	antispasmodic, sedative, antitussive, used to treat cold, bronchitis, and eczema	essential oil, cymol, thymol, and tannin
Trigonella foenum-graeceum Leguminosae	seeds	externally for boils and abscesses, for juvenile diabetes, antimicrobial activity in blood, stimulate production of milk in lactating women	trigonellin, choline, tannin, and essential oil

RESULTS

The antibacterial activity of the extracts was quantitatively assessed by the presence or absence of inhibition zone and by measuring the diameter of the inhibition zone around the wells or disks.

The results of antimicrobial activity of the plant extracts are presented in Table 2. Results showed the antibacterial activity of 12/15 plant extracts tested against various bacterial species.

Three plants, *Punica granatum* L., *Quercus infectoria* Olive., and *Rhus coriaria* L. (plants number 9, 10

Table 2. The antibacterial activity of fifteen plant extracts measured by the diameter of the inhibition zone (mm).

Bacterial spp.	AMC	E	Dox	LN	Off	P	TE	TM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bacillus cereus	14	16	25	14	25	R	24	18	11	15	R	R	15	R	13	13	24	25	26	R	R	35	R
E. coli B	12	R	16	R	18	R	15	14	R	R	R	10	R	R	R	R	12	20	14	R	R	R	R
E. coli 0111	R	R	18	R	22	R	17	12	R	R	R	R	R	R	R	R	8	18	10	R	R	R	R
E. coli 2759	8	R	14	R	10	R	20	12	R	R	R	R	R	R	R	R	10	14	20	R	R	R	R
E. coli 25922	0.8	R	0.9	17	18	R	20	18	R	R	R	R	R	R	R	R	8	18	20	R	R	R	R
Klebsiella pneumoniae	8	R	15	R	15	R	18	12	R	R	R	R	R	R	R	R	12	15	18	R	R	R	R
Proteus vulgaris	14	R	20	R	35	R	20	20	R	R	R	R	R	R	R	R	20	25	25	R	12	16	R
Pseudomonas aeruginosa	R	R	R	R	17	R	R	18	11	20	R	16	25	R	14	18	12	18	18	R	22	18	28
Shigella dysentariae	15	13	20	R	24	R	21	20	R	R	R	R	R	R	R	R	7	29	20	R	R	11	R
Staphylococcus aureus	22	17	20	16	20	7	20	17	R	R	R	R	14	R	R	R	18	15	10	R	7	15	R
Staph. epidermidis	R	18	18	18	22	R	22	17	R	R	R	R	14	R	11	R	20	18	18	R	14	16	R
Streptococcus pyogenes	12	25	35	18	30	R	22	25	R	14	R	R	18	R	11	R	26	25	20	R	15	32	R
Enterococcus faecalis	*11*	*24*	*25*	*18*	*28*	*R*	*25*	*24*	*R*	*14*	*R*	*R*	*16*	*R*	*10*	*R*	*14*	*27*	*22*	*R*	*16*	*32*	*R*
Yersinia enterocoltica	8	R	21	R	20	R	22	16	R	R	R	R	R	R	R	R	11	10	22	R	R	14	R

AMC, Augmentin 30 ug; Dox, doxicilline 30 ug; E, erythromycin 15 ug; LN, lincomycin 2 ug; Off, novecin 5 ug; P, penicillin 10 IU; R, resistant; TE, tetracycline 30 ug; TM, tobramicin 10 ug; 1, *Artemisia herba-alba*; 2, *Certonia siliqua*; 3, *Ficus carcia*; 4, *Foeniculum vulgare*; 5, *Glycyrrhiza glabra*; 6, *Malva parviflora*; 7, *Matricaria chamomilla*; 8, *Nerium oleander*; 9, *Punica granatum*; 10, *Quercus infectoria*; 11, *Rhus coriaria*; 12, *Ricinus communis*; 13, *Salvia triloba*; 14, *Thymus capitatus*; 15, *Trigonella foenum-graecum*.

and 11 in Table 2) showed significant antibacterial activities against all tested species in the concentration used. Their antimicrobial activity decreased when used in lower concentrations. *Thymus capitatus* (L.) Hof. showed antimicrobial activity against 9/14 bacteria tested; it was not active against *E. coli* (four strains), or *K. pneumoniae*. *Glycyrrhiza glabra* showed activity against 6/14 including all Gram-positive bacteria, and a remarkable inhibition of *Pseudomonas aeruginosa* growth, but no effect on the growth of the other Gram-negative species. The other plant extracts demonstrated variable antimicrobial activity against the tested bacterial species. Considering all the 12 extracts that showed activity on one or more of the tested bacteria, 12/12 (100%) inhibited Gram-negative bacteria compared to 10/12 (83%) that inhibited the growth of the Gram-positive bacteria. Three plants, namely *Ficus carcia* L., *Malva parviflora* L., and *Ricinus communis* L., showed no antimicrobial activities, even in the highest concentrations used.

Results showed that the most susceptible organisms were *Pseudomonas aeruginosa* which was sensitive to 12 extracts; *Bacillus cereus* was sensitive to 9 plant extracts and *Streptococcus pyogenes* and *E. faecalis* were sensitive to 8 plants. The most resistant species were *E. coli* (all strains), *Klebsiella pneumoniae*, and *Shigella dysentriae*, which were sensitive to 3 plants. *Yersinia enterocolitica* was sensitive to 4 plants.

The MIC and MBC were determined for the three highly active plants that showed antimicrobial activity against all bacterial species. The range of the MIC for *Quercus infectoria*, *Punica granatum* and *Rhus coriaria* were 0.98–31.25, 3.9–31.25 and 1.95–31.25 mg/ml, respectively. The range of the MBC for *Quercus infectoria*, *Punica granatum* and *Rhus coriaria* were 1.95–62.5 7.81–62.5, and 3.9–62.5 mg/ml, respectively. The lowest MIC and MBC were those observed with *Streptococcus pyogenes*, and the highest were those exerted an effect on *Yersinia enterocolitica*. Ethanol impregnated disks or wells containing 95% ethanol did not have a zone of inhibition probably due to the volatile nature of ethanol, so it was not considered as a factor that might affect the results.

DISCUSSION

With the increase in resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be that alternative because most of them are safe with little side effects if any, cost less, and affect a wide range of antibiotic resistant microorganisms.

The results of this study showed that 12 out of the 15 plant extracts tested inhibited the growth of various species of Gram-positive and Gram-negative bacteria. Three of them, *Punica granatum*, *Quercus infectoria*, and *Rhus coriaria*, showed a broad range of activity by inhibiting the growth of all Gram-positive and Gram-negative species tested. *Thymus capitatus* inhibited the growth of 9 bacterial species and *Glycyrrhiza glabra*

showed activity against 6 bacterial species. The results obtained in our study concerning the antibacterial activity of *Thymus capitatus* against the Gram- positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* were in agreement with another study (Kandil et al., 1994), but different results were obtained with the Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. The results of this study concerning three plant extracts, *Quercus infectoria, Punica granatum* and *Salvia triloba*, were in agreement with a previous study (Alkofahi et al., 1996) with respect to the activity of *Q. infectoria* against three bacterial species, *Staphylococcus aureus, Proteus vulgaris*, and *Pseudomonas aeruginosa*, but not against *Escherichia coli*. For *Punica granatum* the same results were obtained only with *S. aureus* and *P. vulgaris*. For *Salvia triloba* the same results were obtained only for *E. coli* and *P. vulgaris*. The differences in results obtained could be due to the difference in the season when plants were collected, or the difference in concentrations and methods used in each study (Kumar et al., 1997). Tannins were found to be a component of all plants that showed antibacterial activity (Table 1). Tannins could be one of the components responsible for the antibacterial activity since it was reported by other studies that tested different plants (Al Genaidy, 1993; Tanaka et al., 1991). The diameter of inhibition zone around the most active extracts are comparable with those of the standard antibiotics used as a positive control. One exception is the remarkable sensitivity of *P. aeruginosa* to all the active plants, while the same bacterium was resistant to 6/8 standard antibiotics used. This may indicate the potent effect of these extracts since the MIC (4–32 mg/ml) and MBC (8–64 mg/ml) of these plants on *P. aeruginosa* were relatively low.

Antimicrobial assays on plant extracts are valuable in screening and detecting the presence of antimicrobial activities. However, such assays do not provide true quantitative measure of the activities of some components present in the extract such as the polar and large molecules which have lower mobility in the water-agar medium (Kumar et al., 1997). The biologically active components in the tested plants are not known and needs further analysis. Based on the results of this study, we will further investigate the plants that showed broad antibacterial activities *in vivo* to uncover their potential as a source of antibiotics against selected human pathogens. The active plant extracts could also be considered for use as disinfectants or antiseptics.

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