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Short communication

Screening of some Palestinian medicinal plants for antibacterial activity

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Abstract

Antibacterial activity of organic and aqueous extracts of 15 Palestinian medicinal plants were carried against eight different species of bacteria: *Bacillus subtilis*, two *Escherichia coli* species, *Staphylococcus aureus* (methicillin resistant), two *S. aureus* (methicillin sensitive) species, *Pseudomonas aeruginosa*, and *Enterococcus fecalis*. Of the 15 plants tested, eight showed antibacterial activity. Each plant species has unique against different bacteria. The most active antibacterial plants against both gram-positive and gram-negative bacteria were *Thymus vulgaris* and *Thymus origanium*. The organic and aqueous extract from the same plants showed different activities; the organic extract showed the same or greater activity than the aqueous extract. Finally, the hole–plate diffusion method showed larger activity than the disc diffusion method.

Keywords: Medicinal plants; Aqueous extracts; Organic extract; Antibacterial activity

1. Introduction

In many parts of the world there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases. These infections may be locally within the dermis and some can subsequently become generalised as a blood infection (Brantner and Grein, 1994).

Because of the side effect and the resistance that pathogenic micro-organisms build against the antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants may offer a new source of antibacterial agents for use. In many parts of the world medicinal plants are used for antibacterial, antifungal, and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, and gastrointestinal disorders (Caceres et al., 1990) and skin infections such as herpes simplex virus type 1 (Meyer et al., 1996). In the West Bank and Gaza Strip (Palestine) herbal medicine

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Table 1
Ethnobotanical data of studied plants

Species/voucher specimens	Part used	Popular uses
Artemisia herbal (408)	Aerial	Activates the function of the liver Heals rash and joints inflammations Helps in rheumatoid arthritis Acts as antiseptic Helps people with diabetes
Nigella sativa (412)	Seeds	Used for cough, especially whooping cough Treats asthma and cold Treats stomach disorders and headaches Treats skin infection Treats leprosy
Matricaria chamomilla (413)	Aerial	Used as antifungal and antibacterial Activates the circulatory system Acts as antiseptic Provides relief from cold and tonsillitis, and reduces fever Treats inflammations of the urinary tract system
Pimpinella anisum (402)	Seeds	Helps in headaches and cold Used as insecticides Decreases coughing and chest pain
Inula viscosa (411)	Aerial	Stated in literature that they are used in herbal medicine, but no specification
Thymus vulgaris (416)	Aerial	Whooping cough Emphysema Intestinal diseases Treats ulcers of the stomach and the duodenum
Thymus origanium (445)	Aerial	Whooping cough Emphysema Intestinal diseases Treats ulcers of the stomach and the duodenum
Salvia officinalis (401)	Aerial	Treatment for stomach pains Helps in pulmonary inflammations Used for hepatitis Treats intestinal infections Shows an antigermal effect, especially the leaves of the plant Helps patients suffering from tuberculosis
Rosmarinus officinalis (407)	Aerial	Acts as antirheumatic Increases blood outflow in the menstrual cycle
Teucrium polium (444)	Aerial	Leaves decoctions used for stomach pains
Foeniculum vulgare (432)	Aerial	Treatment of soreness of the eyes
	Seeds	Treatment of whooping cough and asthma Treats gastrointestinal disorders Treats urinary tract infections
Commiphora opobalsamum (405)	Aerial	Used to reduce pain sensation and increases stool excretion and urine outflow
Calamintha officinalis (430)	Aerial	Settles gas and indigestion An expectorant, good for cough and cold remedy Heals respiratory infections
Malva sylvestris (422)	Aerial	Treats cutaneous abscesses Treats inflammation of tonsils and oropharynx Treats asthma, diarrhoea
Majorana syriaca (403)	Aerial	Heals pulmonary inflammation Used for whooping cough Removes pain and heals infection in the stomach

is used to treat various diseases including gastrointestinal diseases, urinary tract infections, infertility, and cutaneous abscesses (Roweha, 1983). In this study, 15 plants which had been described in herbal books and medicinal folklore were screened for their antibacterial activity (Table 1).

Aqueous and organic extracts of different plant parts (leaves, flowers, seeds, fruits, and roots) were investigated.

2. Materials and methods

2.1. Plant materials

The plant parts were collected from different sites in the Ramallah and Jerusalem areas in the Palestinian territories during the months of April–June of 1996. Following identification, voucher specimen of the plants was deposited in the herbarium of the Biology Department at Birzeit University (see Table 1). The plant parts were air-dried and finally ground to powder. Dried seeds were purchased from (Souk Al-Attarine) old city Jerusalem, and ground to powder.

2.2. Preparation of extract

The dried, ground material was covered by a 1:1 mixture of methylene chloride:methanol overnight at room temperature. After draining the solvent, the marc was covered with 100% methanol for 20 min and drained into the same flask. The solvent was then removed by rotary evaporation. The concentrate was dried and stored at −20°C for further analysis. The marc was then covered with pure water and allowed to steep overnight at room temperature. The aqueous extract was drained and immediately frozen. After lyophilization, the dried materials were stored at −20°C (McCloud et al., 1988).

2.3. Microorganisms tested

The following bacterial cultures were used: *Bacillus subtilis* (as an aerobic spore-forming bacterium), *Escherichia coli*, *Staphylococcus aureus* (methicillin sensitive), and *S. aureus* (methicillin resistant). These organisms are stored at the Bierzeit University Biology and Biochemistry department collections. The following strains of bacteria were used as test organisms: *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterococcus faecalis* (ATCC 29212).

The bacteria were cultured overnight at 35°C in nutrient agar.

2.4. Antibacterial testing

Antibacterial activity of the crude organic and aqueous extracts of different plants and seeds were determined by the disc diffusion method (Lennette, 1985) and the hole–plate diffusion method (Brantner et al., 1994). The different organic extracts of the plants were dissolved in dimethylsulfoxide (DMSO), 1 g/1 ml, and aqueous extracts were dissolved in water 1 g/1 ml. Both extracts were sterilised by filtration through a 0.45 μm membrane filter.

2.4.1. Disc diffusion method

The micro-organisms were cultured overnight at 35°C in nutrient agar. Suspensions of the bacterial strains with an optical density of McFarlend 0.5 were made in isotonic sodium chloride solution. Petri dishes with 60 ml of sterile Mueller-Hinton agar (Difco, Detroit MI) were seeded with the appropriate bacterial suspension. Sterile, 6 mm diameter filter paper disc were impregnated with the extract, gently tapped to remove excess liquid, and positioned on seeded plates. Two other sterile blank discs — one impregnated with water and one in DMSO, were used as negative controls. After incubation for 24 h at 35°C, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimetres.

Additionally, and for comparative purposes, standard vancomycin (30 μg/disc), gentamicin (10 μg/disc) and cephalothin (30 mcg/disc) (Difco Detroit MI) were included in the test as positive controls. Results obtained in this study are presented in (Table 2).

2.4.2. Hole–plate diffusion method

Sterile Muller-Hinton agar 60 ml was poured

Table 2
Results[a] of the antibacterial screening of eight plant extracts by means of the disc diffusion method (DDM) and the hole–plate diffusion method (HPD)

Plant species	Used part	Extr.		Micro-organism				Inhibition zone diameter (mm)											
				S. aureaus		*S. aureaus*		*E. coli*		*E. coli* ATCC		*E. Feacalis* ATCC		*P. aeurag* ATCC		Bacillus		MRSA	
				DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDF	DDM	HDF	DDM	HDF
Saliva officinalis	AP	Org.	1:1	17.5	24.0	15.0	22.0	0.0	0.0	15.0	0.0	13.0	21.0	0.0	23.0	15.0	23.0	19.0	25.0
			se	(1.15)	(1.15)	(1.15)	(1.15)	(0)	(0)	(1.15)	(0)	(0.57)	(1.15)	(0)	(1.73)	(0.57)	(1.73)	(1.15)	(1.15)
		.	1:2	14.0	22.0	11.0	20.0	0.0	0.0	12.0	0.0	10.0	19.0	0.0	17.0	13.0	22.0	17.0	23.0
			se	(1.15)	(1.73)	(1.15)	(1.73)	(0)	(0)	(1.73)	(0)	(0.57)	(1.73)	(0)	(1.73)	(1.15)	(1.15)	(1.15)	(1.15)
			1:4	10.0	20.0	10.0	18.0	0.0	0.0	8.0	0.0	0.0	16.0	0.0	15.0	11.0	22.0	14.5	21.0
			se	(1.15)	(1.73)	(1.73)	(1.73)	(0)	(0)	(1.15)	(0))	(0)	(1.73)	(0)	(1.15)	(1.73)	(2.30)	(1.15)	(1.73)
		Aq.	1:1	13.0	25.0	0.0	10.0	0.0	0.0	17.0	0.0	0.0	0.0	0.0	19.0	0.0	18.5	14.0	23.0
			se	(1.15)	(1.15)	(0)	(1.15)	(0)	(0)	(1.73)	(0)	(0)	(0)	(0)	(1.73)	(0)	(1.15)	(0.57)	(1.15)
			1:2	0.0	23.0	0.0	0.0	0.0	0.0	13.0	0.0	0.0	0.0	0.0	14.0	0.0	14	11.5	21.0
			se	(0)	(1.15)	(0)	(0)	(0)	(0)	(1.15)	(0)	(0)	(0)	(0)	(1.73)	(0)	(1.15)	(1.15)	(1.15)
			1:4	0.0	21.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	11.0	0.0	11.0	9.0	19.0
			se	(0)	(1.73)	(0)	0	(0)	(0)	(1.73)	(0)	(0)	(0)	(0)	(1.73)	(0)	(1.15)	(1.15)	(1.73)
Thymus origanium	AP	Org.	1:1	22.0	43.0	19.0	25.0	19.0	23.0	17.0	25.0	17.0	25.0	0.0	40.0	21.0	30.0	30.0	40.0
			se	(1.73)	(1.73)	(1.73)	(2.30)	(1.15)	(1.73)	(1.15)	(1.73)	(1.73)	(1.15)	(0)	(1.73)	(1.73)	(1.73)	(0.57)	(1.15)
			1:2	19.0	34.0	17.0	23.0	15.0	16.0	15.0	23.5	15.0	23.0	0.0	33.0	18.5	29.0	27.5	35.0
			se	(1.15)	(1.73)	(2.30)	(1.15)	(2.30)	(2.30)	(1.73)	(1.15)	(1.15)	(1.15)	(0)	(1.73)	(1.15)	(1.15)	(1.73)	(1.73)
			1:4	13.0	31.0	15.0	21.0	8.0	11.0	13.0	21.0	11.0	22.0	0.0	30.0	15.0	27.5	22.0	33.5
			se	(1.15)	(1.73)	(1.73)	(1.15)	(1.73)	(1.73)	(1.73)	(2.30)	(1.15)	(2.30)	(0)	(1.73)	(1.15)	(1.15)	(1.15)	(1.15)
		Aq.	1:1	14.0	21.0	14.0	22.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	19.0	17.5	30.0
			se	(1.73)	(1.15)	(1.73)	(1.73)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0)	(1.15)	(1.15)	(1.15)
			1:2	12.0	18.0	13.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.0	0.0	17.0	15.0	27.0
			se	(1.15)	(1.73)	(1.15)	(1.73)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.73)	(0)	(1.73)	(1.15)	(0.57)
			1:4	11.0	15.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	13.0	13.0	24.0
			se	(1.15)	(1.15)	(1.15)	(1.15)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.73)	(0)	(0.57)	(1.15)	(1.73)
Thymus vulgaris	AP	Org.	1:1	30.0	25.0	22.0	32.5	18.5	24.5	0.0	16.0	10.0	21.5	0.0	18.0	17.5	29.5	23.5	34.5
			se	(0)	(1.15)	(0.57)	(1.15)	(1.15)	(1.15)	(0)	(0.86)	(1.57)	(1.15)	(0)	(1.15)	(1.15)	(1.15)	(1.15)	(1.73)
			1:2	21.5	22.0	17.5	28.0	10.5	22.0	0.0	14.0	0.0	18.0	0.0	16.5	15.5	25.5	19.0	29.5
			se	(0.57)	(1.15)	(0.57)	(1.15)	(0.57)	(1.15)	(0)	(1.15)	(0)	(0.86)	(0)	(0.57)	(1.73)	(1.73)	(0.86)	(1.73)
			1:4	16.5	19.0	12.5	20.5	0.0	19.0	0.0	12.0	0.0	16.0	0.0	14.0	13.0	22.5	13.5	27.0
			se	(0.57)	(1.73)	(0.57)	(0.57)	(0)	(1.15)	(0)	(0.57)	(0)	(1.73)	(0)	(0.57)	(1.15)	(0.57)	(1.15)	(1.15)
		Aq.	1:1	14.0	19.0	13.0	21.0	0.0	14.5	0.0	0.0	0.0	22.0	27.5	15.5	0.0	18.0	15.5	23.0
			se	(0.57)	(0.57)	(1.15)	(0.57)	(0)	(0.57)	(0)	(0)	(0)	(1.15)	(1.73)	(1.15)	(0)	(1.73)	(1.73)	(1.15)
			1:2	12.0	17.0	10.0	19.0	0.0	11.0	0.0	0.0	0.0	17.0	25.0	12.0	0.0	14.5	13.5	21.0
			se	(1.15)	(1.15)	(1.15)	(1.15)	(0)	(1.15)	(0)	(0)	(0)	(0.57)	(1.15)	(0.57)	(0)	(1.15)	(1.15)	(1.15)
			1:4	10.0	14.5	0.0	16.0	0.0	0.0	0.0	0.0	0.0	14.0	15.0	8.0	0.0	11.0	10.0	17.5
			se	(1.15)	(0.57)	(0)	(0.57)	(0)	(0)	(0)	(0)	(0)	(0.57)	(1.15)	(1.15)	(0)	(1.15)	(1.15)	(1.15)
Teucrium polium	AP	Org.	1:1	13.0	22.0	12.5	17.5	0.0	0.0	0.0	0.0	0.0	0.0	24.5	17.0	0.0	20.5	15.0	26.5
			se	(1.57)	(0.57)	(0.57)	(0.57)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)	(1.15)	(0)	(1.73)	(1.15)	(1.15)
			1:2	10.0	20.0	10.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	17.0	15.0	0.0	18.0	13.0	23.0
			se	(1.15)	(1.15)	(1.15)	(0.57)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(1.73)	(0)	(1.15)	(0.57)	(0.57)
			1:4	0.0	17.0	0.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	10.0	0.0	15.5	10.5	19.0
			se	(0)	(1.15)	(0)	(1.15)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(1.15)	(0)	(1.15)	(0.57)	(0.28)
		Aq.	1:1	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	17.5
			se	(0)	(0.57)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0)	(0)	(0)	(0)	(1.15)
			1:2	0.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.0
			se	(0)	(1.15)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)
			1:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)

Table 2 (*Continued*)

Plant species	Used part	Extr.		Micro-organism				Inhibition zone diameter (mm)											
				S. aureaus		*S. aureaus*		*E. coli*		*E. coli* ATCC		*E. Feacalis* ATCC		*P. aeurag* ATCC		Bacillus		MRSA	
				DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDP	DDM	HDF	DDM	HDF	DDM	HDF
Majorana syriaca	AP	Org.	1:1	20.0	28.0	27.0	29.0	13.0	21.0	14.0	22.0	15.0	20.0	14.0	21.0	18.5	>46	24.0	24.5
			se	(0.86)	(0.86)	(1.44)	(1.15)	(1.15)	(0.57)	(0.57)	(0.57)	(0.57)	(0.86)	(1.15)	(0.86)	(0.57)	(0)	(0.86)	
			1:2	16.0	24.0	17.0	23.0	9.0	19.0	10.0	20.0	13.0	18.0	11.0	19.0	15.5	>46	22.5	21.0
			se	(1.73)	(0.57)	(0.57)	(0.86)	(0.86)	(1.15)	(0.57)	(0.28)	(0.86)	(0.28)	(0.57)	(0.86)	(1.15)	(0)	(0.57)	(1.15)
			1:4	13.0	20.0	14.0	19.0	8.0	15.0	8.0	18.0	10.0.	15.0	10.0	17.0	11.0	40.0	14.0	18.0
			se	(1.15)	(0.57)	(0.86)	(1.44)	(0.57)	(1.15)	(0.57)	(0.57)	(0.57)	(1.15)	(0.86)	(0.57)	(0.86)	(0.86)	(1.15)	(0.86)
		Aq.	1:1	14.0	27.0	15.0	29.0	11.5	17.0	10.5	11.0	12.0	15.0	11.0	14.0	0.0	14.0	12.0	13.0
			se	(0.86)	(1.15)	(1.15)	(0.86)	(0.57)	(1.73)	(0.57)	(1.15)	(0.57)	(0.57)	(1.15)	(0.57)	(0)	(0.28)	(0.86)	(0.86)
			1:2	12.0	24.0	10.0	23.5	0.0	10.5	0.0	9.0	10.0	13.0	9.0	11.0	0.0	10.0	8.0	9.0
			se	(0.57)	(0.86)	(0.86)	(1.15)	(0)	(0.57)	(0)	(0.57)	(0.86)	(0.57)	(0.86)	(0.57)	(0)	(0.28)	(0.57)	(0.86)
			1:4	8.0	17.0	0.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			se	(1.15)	(0.57)	(0)	(0.57)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Commiphora opobalsamum	AP	Org.	1:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.5	22.5	29.5	0.0	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)	(0.57)	(1.15)	(0)	(0)	(0)	(0)	(0)
			1:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.5	16.5	24.5	0.0	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0.57)	(0.57)	(0)	(0)	(0)	(0)	(0)
			1:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	12.0	20.0	0.0	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0.86)	(0.57)	(0)	(0)	(0)	(0)	(0)
		Aq.	1:1	0.0	0.0	0.0	21.5	0.0	0.0	0.0	0.0	0.0	0.0	33.0	24.0	19.0	20.0	0.0	0.0
			se	(0)	(0)	(0)	(1.15)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0.86)	(0.86)	(0.86)	(0)	(0)
			1:2	0.0	0.0	0.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	23.0	21.0	14.0	17.5	0.0	0.0
			se	(0)	(0)	(0)	(0.86)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)	(0.86)	(0.86)	(0.57)	(0)	(0)
			1:4	0.0	0.0	0.0	14.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	16.5	10.0	12.0	0.0	0.0
			se	(0)	(0)	(0)	(0.86)	(0)	(0)	(0)	(0)	(0)	(0)	(0.28)	(0.57)	(0.57)	(0.57)	(0)	(0)
Foeniculum vulgare	AP	Org.	1:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.5	22.5	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(1.15)	(0)	(0)	(0)	(0)
			1:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	19.0	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)	(0.28)	(0)	(0)	(0)	(0)
			1:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	14.0	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)	(0.86)	(0)	(0)	(0)	(0)
		Aq.	1:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.0	24.0	0.0	17.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)	(0.86)	(0)	(1.15)	(0)	(0)
			1:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0.	17.0	0.0	13.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)	(1.15)	(0)	(0.57)	(0)	(0)
			1:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.0	14.5	0.0	9.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)	(0.57)	(0)	(1.15)	(0)	(0)
Rosmarinus officinalis	AP	Org.	1:1	19.5	24.0	17.0	24.0	0.0	0.0	0.0	0.0	15.0	19.5	14.0	22.0	15.5	21.5	21.5	24.0
			se	(1.15)	(1.15)	(0.86)	(0.86)	(0)	(0)	(0)	(0)	(0.86)	(0.81)	(0.86)	(0.57)	(1.15)	(1.15)	(1.15)	(0.86)
			1:2	15.5	21.0	15.0	21.0	0.0	0.0	0.0	0.0	13.5	16.0	11.0	20.0	13.0	19.0	18.0	21.0
			se	(0.57)	(0.86)	(0.86)	(0.57)	(0)	(0)	(0)	(0)	(0.57)	(1.15)	(0.86)	(0.86)	(0.57)	(1.15)	(0.57)	(1.15)
			1:4	13.0	17.0	12.0	19.0	0.0	0.0	0.0	0.0	11.0	10.0	9.0	17.0	10.0	16.0	15.0	18.0
			se	(0.28)	(1.15)	(0.57)	(0.57)	(0)	(0)	(0)	(0)	(0.86)	(0.57)	(1.15)	(1.15)	(0.57)	(0.57)	(0.86)	(0.57)
		Aq.	1:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.5	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.15)	(0)	(0)	(0)	(0)
			1:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.5	0.0	0.0	0.0	0.0
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.57)	(0)	(0)	(0)	(0)
			1:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.0	0.0	0.0	0.0	(0)
			se	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.86)	(0)	(0)	(0)	(0)

[a] AP, aerial part; Aq., water extract; Org., methylene chloride/methanol; se, standard error of the mean; disc diameter: 6 mm.

into each petri dish. Plates were seeded as in the disc diffusion method. A 10 mm core of agar was removed from the seeded agar, and the hole was sealed with sterile agar solution against the dish bottom. Holes were aseptically filled with 150 μg of each plant extract by means of a pipetter. Two other holes were aseptically filled — one with water and one with DMSO — as negative control. Two sets of control were used. In the first set, the organism was used which consisted of a seeded petri dish with no plant material. In the second set, plant material was introduced in the holes of unseeded petri dishes to check for sterility. After incubation for 24 h at 35°C, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimetres. In accordance with preliminary investigations (Brantner et al., 1994), this method turned out to be the most suitable method. Results obtained in this study are presented in Table 2. Each test was carried out in triplicate.

3. Results and discussion

Determination of the inhibition zones by means of the disc diffusion method and the hole–plate diffusion method (Table 2) showed that some of the 15 plant extracts tested exhibited an antibacterial effect against some of the eight, tested bacteria. The success of the ethnobotanical approach to drug discovery can no longer be questioned. Historical and current discoveries demonstrate its power (Cox, 1994). Medicinal plants are the backbone of traditional medicine (Farnsworth, 1994).

Of the 15 crude plants and seeds extracts tested, eight showed antibacterial activity: *Salvia officinalis*, *Teucrium polium*, *Majorana syriaca*, *Thymus origanium*, *Thymus vulgaris*, *Commiphora opobalsamum*, *Foeniculum vulgare*, and *Rosmarinus officinalis* (aerial parts). All these extracts were active against both gram-positive and gram-negative bacteria. However, the plants differ significantly in their activity against test micro-organisms. Some of the antibacterial inhibition zone diameters were more than 46 mm (*Majorana syriaca*). These differences may be attributed to the fact that the cell wall in gram-positive bacteria consists of a single layer, whereas the gram-negative cell wall is a multi-layered structure and quite complex (Yao and Moellering, 1995). Most of the extracts showed anti-microbial activity against *S. aureus* and *S. aureus* methicillin-resistant MRSA, except *Commiphora opobalsamum*, *F. vulgare* and the aqueous extract of the *R. officinalis*. The other eight extracts (*Calamintha incana*, *Inula viscosa*, *Artemisia herba*, *Matricaria chamomile*, *Malva sylvestris* [aerial parts], *Pimpinella anisum*, *Nigella sativa*, and *F. vulgare* [seeds]) did not show any antibacterial activity.

The aqueous and organic extracts from the same plants showed different activities. There are no common rules for this, but in most cases, the organic extracts showed the same or greater activity than the aqueous extracts (Olano et al., 1996). The hole–plate diffusion method showed larger activity than the disc diffusion method. Most likely, this is because larger amounts of the solvent may influence bacterial growth, thereby demonstrating that larger doses are required (Brantner et al., 1994).

Since the medicinal plants studied appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy (Olukoya et al., 1993). The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but to the combined action of different compounds originally in the plant (Bai, 1990). Seeds (*Pimpinella anisum*, *F. vulgare*, and *Nigella sativa*) showed no antibacterial activity. Their activity might be in the aerial parts, as shown in the aerial parts, of *F. vulgare*.

In literature, it has been indicated that the antibacterial activity is due to different chemical agents in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, which are classified as active anti-microbial compounds (Rojas et al., 1992). A complete study conducted with the purpose of finding these chemicals is worthwhile.

From this study we can conclude that these are promising plants, mainly the plants that showed anti-microbial activity against the MRSA. The extracts exhibited a broad spectrum of activity against both gram-positive and gram-negative

bacteria, and the results confirm the use of these plants in traditional medicine for the treatment of infections.

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