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Antibacterial Activities of Some Plant Extracts Utilized in Popular Medicine in Palestine

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Abstract: The antibacterial activities of hot water, methanol and ethanol extracts of 5 plant extracts utilized in Palestine in popular medicine were studied. The dried extracts of *Syzyium aromaticum* (Myrtaceae) (seed), *Cinnamomum cassia* (Lauraceae) (cassia bark, Chinese cinnamon) (bark), *Salvia officinalis* (Lamiaceaea) (leaf), *Thymus vulgaris* (Lamiaceaea) (leaf) and *Rosmarinus officinalis* (Labiatae) (leaf) were tested in vitro against 4 bacterial species by disk diffusion and micro-dilution. The patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* ATCC 6633 were the most inhibited microorganisms. *S. aromaticum* extract was the most active against multi-drug resistant *Pseudomonas aeruginosa* and enterohemorrhagic *Escherichia coli* O157 EHEC. The combinations of ethanolic extracts of *S. officinalis* with *R. officinalis* and of *R. officinalis* with *T. vulgaris* on bacterial species tested exhibited a higher effect than that of any individual extract. Results of this kind herald the interesting promise of designing a potentially active antibacterial synergized agent of plant origin.

Key Words: Antibacterial activities, plant extracts, enterohemorrhagic *Escherichia coli* O157, methicillin-resistant *Staphylococcus aureus*, multi-drug resistant *Pseudomonas aeruginosa*

Introduction

Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity (1-5) but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity. The objectives of this study were: 1) to examine the antibacterial effect of 5 plant extracts utilized in Palestine in popular medicine, and 2) to determine the effectiveness of some combinations of these extracts on 4 different species of Gram-positive and Gram-negative bacteria. In Palestine, this is necessary because traditional plant medicines often come in multi-component preparations aimed at curing several diseases simultaneously.

Plant materials and preparation of extracts

The plant materials used in this study consisted of *Syzyium aromaticum* (seed), *Cinnamomum cassia* (bark), *Salvia officinalis* (leaf), *Thymus vulgaris* (leaf) and *Rosmarinus officinalis* (leaf), which were collected from Palestinian markets. The air-dried plant materials were ground into fine powder and extracted with hot water, and 80% methanol or 80% ethanol. After filtration of total extracts, the extracts were evaporated to dryness in vacuo and weighed.

Test Microorganisms

Three clinical strains were used in the study: methicillin-resistant *Staphylococcus aureus*, multi-drug resistant *Pseudomonas aeruginosa* (i.e. resistant to ampicillin, cefuroxime, cefotaxime, gentamicin, amikacin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid) and enterohemorrhagic *Escherichia coli* O157 EHEC. A reference strain (*Bacillus subtilis* ATCC 6633) was also tested.

Antibacterial activity

Antibacterial activity was determined by the well diffusion method according to the NCCLS (6). Petri plates containing 20 ml of Mueller Hinton agar medium were seeded with a 24 h culture of the bacterial strains. Wells (6 mm diameter) were cut into the agar and 50 µl of the plant extracts were tested in a concentration of 100 mg/ml. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony-forming units (CFU)/ml. Incubation was performed at 37 °C for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well. A standard 30 µg tetracycline disk was used as a positive control.

Minimum inhibitory concentration (MIC) was determined by the micro-dilution method using serially diluted (2-fold) plant extracts according to the NCCLS (7). A final concentration from 6.25 to 0.781 mg/ml was used for each plant sample. The following ethanol extracts were tested: *S. officinalis*, *S. aromaticum*, *C. cassia*, *R. officinalis*, and *T. vulgaris*, and combinations of *S. officinalis* with *R. officinalis* and of *R. officinalis* with *T. vulgaris*. Bacteria inocula were adjusted to contain approximately 10^5 CFU/ml. The test plates were incubated at 37 ºC for 18 h.

Results

The antibacterial activities of the extracts obtained from the plants under study by the diffusion method are shown in Table 1. The water extracts of all the plants screened showed various inhibitory effects (10-13 mm/50 µl inhibition zone) against MRSA and *B. subtilus*. No effects were detected for EHEC or *P. aeruginosa*. The largest zone of inhibition was observed from ethanolic and methanolic extracts against MRSA and *B. subtilus*. EHEC and *P. aeruginosa* were more resistant to most extracts used in this study. The growth of EHEC was only inhibited by the methanolic extract of *S. aromaticum*. However, *P. aeruginosa* was inhibited by both methanolic and ethanolic extracts of *S. aromaticum*.

The MIC of the ethanol extracts fell in the range of 0.781 to 3.125 mg/ml for MRSA and of 0.781 to 6.25 mg/ml for *B. subtilis* (Table 2). *S. aromaticum* extract showed the most potent inhibition for *P. aeruginosa* (MIC 0.781 mg/ml).

Table 2 also shows that the combinations of ethanol extracts of *S. officinali* with *R. officinalis* and of *R. officinalis* with *T. vulgaris* not only maintained their original spectrum antibacterial activity against MRSA and *B. subtilis*, but that their synergistic effects also improved activity down to a range of 0.781 to 1.5 mg/ml. In addition, EHEC and *P. aeruginosa* were notably inhibited.

Table 1. Inhibitory properties (inhibition zone diameter in mm) of plant extracts on different bacteria.

Test agents	Bacterial species											
	B. subtilis ATCC 6633			MRSA			EHEC			*P. aeruginosa*		
	A	B	C	A	B	C	A	B	C	A	B	C
S. officinalis	12	19	20	10	18	22	6	6	9	6	6	6
S. aromaticum	13	20	30	13	25	38	6	14	6	6	19	30
C. verum	10	12	16	10	10	16	6	6	6	6	6	6
R. officinalis	10	12	18	12	17	22	6	6	6	6	6	6
T. vulgaris	10	13	16	12	18	20	6	6	6	6	6	6
Tetracycline (30 µg)		14			30			38			12	

Extraction solvent (50 µl). A: Water extract; B: Methanol extract; C: Ethanol extract
Includes diameter of well (6 mm)

Table 2. Antibacterial activity (MIC in mg/ml) of the ethanol extracts and their combinations.

Test agent	Bacterial species			
	B. subtilis ATCC 6633	MRSA	EHEC	*P. aeruginosa*
S. officinalis	3.125	1.5	na	na
S. aromaticum	0.781	0.781	na	0.781
C. verum	na	na	na	na
R. officinalis	3.125	1.5	na	na
T. vulgaris	6.25	3.125	na	na
S. officinali/R. officinalis	1.5	0.781	0.781	0.781
R. officinalis/T. vulgaris	0.781	0.781	0.781	0.781

a: Minimum inhibitory concentration

b: Not active at concentration range from 6.25 to 0.781 mg/ml

Discussion

The results indicated that the crude extracts of all the species studied showed antibacterial activities towards the Gram-positive bacteria (*B. subtilis* and MRSA). These results are consistent with previous reports on related plants regarding Gram-positive bacteria (3). The resistance of Gram-negative bacteria (EHEC and *P. aeruginosa*) to plant extracts was not unexpected as, in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (8).

Infections caused by *P. aeruginosa*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics (9). In our study, the growth of *P. aeruginosa* was remarkably inhibited by the ethanol extract of *S. aromaticum* (MIC 0.781 mg/ml). It seems very likely, therefore, that the antibacterial compound extracted from *S. aromaticum* may inhibit bacteria by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains.

EHEC are increasingly isolated from severe diarrheal disease and constitute a serious medical problem for many patients (10-12). Infection with these organisms may result in life-threatening complications such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (10). Our results demonstrate that the methanol extract of *S. aromaticum* displayed antimicrobial activity against EHEC (14 mm/ 50 µl inhibition zone). The plant may thus be a source that could be useful in the treatment of infections caused by this organism.

It appears that overall the microorganisms were not as sensitive to the water extract compared with the other extracts as determined by diffusion. The reasons for this could be that all of the identified components from plants active against microorganisms, aromatic or saturated organic compounds, are most often obtained through initial ethanol or methanol extraction (3).

The main conclusion drawn from our data is that the antibacterial enhancement (synergistic effects) of *S. officinali* with *R. officinalis* and of *R. officinalis* with *T. vulgaris* as detected in this study may partly explain the use of these combinations traditional plant medicines in Palestine against a number of infections for generations. Results of this kind herald an interesting promise of designing a potentially active antibacterial synergized agent of plant origin.

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