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Antibacterial Effects of Nutraceutical Plants Growing in Palestine on *Pseudomonas aeruginosa*

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Abstract: The antibacterial effects of single and combined plant extracts of water, ethanol and methanol for two nutraceuticals utilized in Palestine were studied against multiple drug resistances *Pseudomonas aeruginosa* using well diffusion method. These plants are Rhus coriaria and Thymus vulgaris. Combinations of these extracts showed an additive action against this pathogen.

Key Words: *Rhus coriaria*, Thymus vulgaris, nutraceutical plants, medicinal plants, combination effect, *Pseudomonas aeruginosa*, Palestine

Introduction

Medicinal plants are important elements of traditional medicine in virtually all cultures. The idea that certain plants had healing potential was known long before human being discovered the existence of pathogens. Medicinal plants which have been used by human being to treat common infectious diseases are important elements of traditional medicine. During the last years, traditional medicine has not been limited to specific culture. It has been used in developing countries as well as its using extended to developed countries (1).

Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including viral infections (2). Single and Poly herbal preparations have been used numerously through out history for the treatment of various diseases. Many studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity (3).

The green part of *Thymus vulgaris* is a most popular herbal medicine and spice used world wide. Thym phytochemicals have been used as antioxidant, antibacterial agent, antifungal agent, treatment for respiratory disease, wound healing, a stomachic, carminative, diuretic, urinary disinfectant, and vermifuge (4-8). While phytochemicals in *R. coriaria* have been used as antibacterial, antidiarrheic, antidysenteric, antihepatoxic, antiseptic, antispasmodic, antiviral, antihyperglycimic agent, astringent, candidicide, hepatoprotective, hepatotonic, protisticide, analgesic, antigastric, anti-inflammatory, antioxidant, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitor due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid (9-12).

In Palestine medicinal plants are important elements of indigenous medical systems as well as in other developing countries (3,13). *Rhus coriaria* is a well-known spice, popular and has been utilized extensively in many different meals such as in zater (dukka) which is a blend of dried ripe berries of sumac, dried leaves of *Thymus vulgaris* and citric acid with seasame seeds; almusakhan which is composed from fragmented chicken, small fragments of onions and sumac, as well as in salads and others. Also the acidic tasty of *R. coriaria* fruits is made into a condiment and sour drink in the Middle East dishes (13). Because these two plants are used in combination in (dukka) formula. This study aims to determine the antibacterial effectiveness of combinations of these plant extracts on *Pseudomonas aeruginosa*.

Materials and Methods

The plant materials used in this study consisted of *R. coriaria* (seed) and *T. vulgaris* (leaf) which are growing in Palestine. Hot water, methanol and ethanol extracts were prepared as described previously (3,13). Plant materials were dried in an open air protected from direct exposure to sunlight, and 30 g of dried plant materials were separately powdered and extracted with hot water, 80% methanol and 80% ethanol; the extracts were filtered through Whatman No. 2 filter paper under suction. Extracts were concentrated to dryness in vacuum. Then, 100 mg of the dry residue was dissolved in 1 ml of sterile distilled water.

Multi-drug resistant of clinical strain *P. aeruginosa* (i.e. resistant to different antibiotics as ampicillin, cefuroxime, cefotaxime, gentamicin, amikacin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid) has been used in this study. A reference strain [*Bacillus subtilis* ATCC6633] was also tested.

Antibacterial activity was measured using a well diffusion method according to the NCCLS (14). Briefly, Petri plates containing 20 ml of Mueller Hinton agar medium were inoculated with a 24 h culture of the bacterial strains. Wells (6mm diameter) were punched in the agar and filled with 50 µl of plant extracts (in case of combination 25 µl of each) in a concentration of 100 mg/ml. Triplicates of each single extract or combination has been done. The plates were incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. The average of three replicates for each extract has been calculated. A standard antibiotic 30 µg tetracycline disk was used as a positive control and negative control also included.

Results

The antibacterial activities of water, ethanol, and methanol single and combined extracts obtained from the *R. coriaria* (seed) and *T. vulgaris* (leaf) against clinical isolate of *P. aeruginosa* and a reference strain *B. subtilis* have been studied by well diffusion method. The average of three replicates for each extract has been shown in Table 1 and 2. All combinations of ethanol extracts and/or methanol extracts with *T. vulgaris* and *R. coriaria* or *R. coriaria* and *R. coriaria* showed an additive action.

Discussion

Clinically significant infections with *P. aeruginosa* should not be treated with single antibiotic, due to that bacteria can rapidly develop resistance when such a single antibiotics are used. According to different reports , multiple drug resistances to *P. aeruginosa* are spreading hazards in the world and making the therapeutic management of these patients more problematic (15-18). An alternative way to combat the problem of microbial resistance is development of new antibacterial agents for substitution with ineffective ones. In plants, secondary products can have a variety of functions; some

Table 1. Inhibitory properties (inhibition zone diameter in mm) of single plant extracts against clinical isolate of *P. aeruginosa* and a reference strain *B. subtilis.*

Test agents	Bacterial species					
	B. subtilis			*P. aeruginosa*		
	W	M	E	W	M	E
T. vulgaris	10	13	16	6	6	6
R. coriaria	16	22	23	10	15	16
*Sterile distilled H2O	6	6	6	6	6	6
Tetracycline (30 µg)	14			12		

Abb.: W: Water extract; M: Methanol extract; E: Ethanol extract
* Negative control: Includes diameter of well (6 mm)

Table 2. Inhibitory properties (inhibition zone diameter in mm) of combined plant extracts against clinical isolate of P. *aeruginosa* and a reference strain *B. subtilis.*

		Bacterial species	
Test agents	Combined extract	*B. subtilis*	*P. aeruginosa*
T. vulgaris / R. coriaria	E/E	20	21
T. vulgaris / R. coriaria	M/M	19	21
T. vulgaris / R. coriaria	E/M	19	20
R. coriaria / R. coriaria	E/M	19	21
*Sterile distilled H2O		6	6
Tetracycline (30 µg)		14	12

Abb.: M: Methanol extract; E: Ethanol extract
*Negative control: Includes diameter of well (6 mm)

may have bearing on potential medicinal effects for humans. Recently, different reports from different countries using different pathogens were published showing the antimicrobial activities of medicinal plants (19). Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites (20).

In our study, the data showed the effect of combinations of these plants have antibacterial enhancement (additive effect) against *P. aeruginosa*. These results may explain the traditional use of these plants *R. coriaria* and *T. vulgaris* in combination during food. Both these plants can be considered as nutraceuticals which have a nutritional role in the diet and phytochemical constituents of these plants can have long term health promoting or medicinal qualities due to long term use in the diet (3,13). While many medicinal plants exert their medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems (20). Results of this kind herald an interesting promise of constructing a potentially active antibacterial additive agent of plant origin.

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