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Antibacterial activity of an aqueous extract of *Petroselinum crispum* leaves against pathogenic bacteria isolated from patients with burns infections in Al-najaf Governorate, Iraq

Ahmed Abdul Jabbar Jaloob Aljanaby

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Abstract The purpose of this study was to investigate, by use of the agar well diffusion method, the ability of cold-water and hot-water extracts of *Petroselinum crispum* leaves to inhibit bacteria isolated from patients with burns infections. The results revealed that 250 mg/ml of the hot-water extract was the more effective inhibitor of the growth of *P. aeruginosa*. The inhibition zone diameter of 29.667 mm was significantly different ($P < 0.05$) from that for nitrofurantoin, chosen as positive control. From the overall results obtained it is evident that the plant screened has anti-bacterial activity against some bacteria associated with burns infections.

Keywords Aqueous extraction · Parsley · *P. aeruginosa* · *S. aureus* · *S. pyogenes*

Introduction

Pharmacologists' and herbalists' interest in the use of alternative medical treatment has increased in the past decade, and the antibacterial activity of many plants has been reported [1–3]. Historically, plants have been a source of novel drugs, and plant-derived medicine has contributed to human health and wellbeing [4]. Many authors have reported that plants contain useful compounds, for example butanol, unsaturated long-chain aldehydes, peptides, essential oils, alkaloids, phenols, and water, ethanol, chloroform, and methanol [5–7]. Such plants have furnished compounds with potentially significant therapeutic application against *P. aeruginosa, S. pyogenes*, and *S. aureus*, fungi, and viruses [8, 9].

The common bacteria *P. aeruginosa*, *S. pyogenes*, *S. aureus*, and *C. perfringens*, among others, in combination with factors such as trauma, primary skin disease,

A. A. J. J. Aljanaby (✉)
Department of Microbiology, College of Science, University of Kufa, Najaf, Iraq
e-mail: ahmadajjanabi@yahoo.com

hypoglycemia, immune deficiency disease, and topical use of steroids [10], have been identified as contributing to skin infections. Parsley, *Petroselinum crispum* (*Apiaceae*), the most important medicinal plant used for traditional treatment of skin diseases [11], is an aromatic biennial herb with yellowish green flowers in compound umbels, oblong leaves with a dentate margin, and crescent shaped fruits [12, 13]. Leaves of *P. crispum* contain psoralen, isopimpinellin, and imperatorin, and are used for treatment of uterine troubles, including dysmenorrhea, and have antipyretic, diuretic, antiscorbutic, and carminative activity [14].

Since their introduction to medical use, bacterial resistance to many antibiotics, for example ampicillin, penicillin, amoxicillin, and gentamicin, has become extremely common, according to Sahm et al. [15]. Furthermore, antibiotic resistance genes can be derived from the industrial microbes used for production of antibiotics [16]. This study was therefore conducted to evaluate the antibacterial activity of aqueous extracts (obtained with cold and hot water) of *P. crispum* leaves against bacteria isolated from patients with burn infections in Al-najaf Governorate, Iraq.

Materials and methods

Collection and identification of the plant

The plant was obtained from a local commercial supplier in Al-najaf city, Iraq, in 2011. It was identified by the Department of Botany, College of Science, University of Kufa. The leaves of the plant were freed from foreign materials, washed with water to remove dust, dried, and ground to a powder.

Preparation of plant extract

Leaf powder (50 g) was extracted with 500 ml cold or boiling distilled water for 24 h. The aqueous solutions were then filtered and evaporated to dryness at 45 °C. [17]. The extracts were kept in amber glass bottles and stored at 4 °C.

Collection and treatment of specimens

Specimens were collected with sterile swabs from patients with burn infections in Al-najaf Governorate, Iraq. Blood and manitol salt agar plates were inoculated with the specimens. All plates were immediately incubated aerobically for 24 h. Emergent colonies were identified by standard bacteriological methods [18]. The disc susceptibility test was performed in accordance with the National Committee for Clinical Laboratory Standards [19].

Assay of extracts activity

The antibacterial activity of the crude extracts was assayed by use of the agar well diffusion method [20]. Four wells (diameter 5 mm) were made in each agar plate by use of a sterile cork borer. Plates were then inoculated with 10^5 CFU/ml of the test

Table 1 Evaluation of the activity of antibiotics against pathogenic bacteria isolated from patients with burn infections in Al-najaf Governorate, Iraq

Antibiotic	*P. aeruginosa*; mean (mm) ± SE $R = 3$	*S. aureus*; mean (mm) ± SE $R = 3$	*S. pyogenes*; mean (mm) ± SE $R = 3$
Nitrofurantoin (25 μg)	24.950 ± 0.22141	25.200 ± 0.23094	22.120 ± 0.20153
Trimetheprim (30 μg)	1.0582 ± 0.43024	1.1067 ± 0.57159	1.0325 ± 0.32131
Ciprofloxacin (20 μg)	1.6201 ± 0.21243	1.8333 ± 0.37565	1.4152 ± 0.14121
Rifampin (25 μg)	1.8231 ± 0.23145	1.9234 ± 0.62154	2.2564 ± 0.22143
Cefotaxim (20 μg)	Resistance	Resistance	Resistance
Ampicillin (25 μg)	Resistance	Resistance	Resistance
Penicillin (10 μg)	Resistance	Resistance	Resistance
Amikacin (30 μg)	3.11485 ± 0.51247	4.33254 ± 0.21577	2.52147 ± 0.23247
Augmentin (25 μg)	5.25410 ± 0.35245	6.41350 ± 0.23214	4.12546 ± 0.82154
Amoxicillin (25 μg)	Resistance	Resistance	Resistance

R, number of replicates; SE, standard error; mean (mm), diameter of inhibition zone

bacteria. The crude plant extracts were serially diluted to yield dilutions of 100, 150, 200, and 250 mg/ml, and 40 μl of each dilution was transferred to each well, containing Mueller–Hinton agar, and left for 4 h at 20–25 °C to enable diffusion of the extract across the surface. The plates were then incubated at 37 °C, for 24 h [21]. The inhibition zone around each well was measured in millimeters. The assay was performed three times for each extract.

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 4 software by use of the *t* test. A P value <0.05 was regarded as indicative of statistical significance.

Results and discussion

The results of this study proved that nitrofurantoin (25 μg) was the best of the antibiotics tested; it resulted in of inhibition zones of diameter 24.950, 25.200, and 22.120 mm against *P. aeruginosa*, *S. aureus*, and *S. pyogenes* respectively. Nitrofurantoin (25 μg) was therefore used as positive control (Table 1). The study also demonstrated that both cold-water and hot-water extracts of parsley leaves had inhibitory activity against *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, with the hot-water extract at 250 mg/ml resulting in the largest inhibition zones (29.667, 28.233, and 27.661 mm, respectively; Table 2). The results were indicative of a significant difference $P < (0.05)$ between nitrofurantoin (25 μg) and the cold-water and hot-water extracts at different concentrations (Tables 3, 4).

Both cold-water and hot-water extracts had inhibitory activity against *S. aureus*, *P. aeruginosa*, and *S. pyogenes*, with the hot-water extract producing larger inhibition zones. This is an indication that hot water is a better solvent for extracting the anti-bacterial agent. These findings were indicative of the presence of active

Table 2 Evaluation of the antibacterial activity of cold-water and hot-water extracts of *Petroselinum crispum* leaves against pathogenic bacteria isolated from patients with burn infections in Al-najaf Governorate, Iraq

Cold-water extract				
Bacteria	100 mg/ml $R = 3$; mean (mm) ± SE	150 mg/ml $R = 3$; mean (mm) ± SE	200 mg/ml $R = 3$; mean (mm) ± SE	250 mg/ml $R = 3$; mean (mm) ± SE
P. aeruginosa	10.667 ± 0.6666	11.333 ± 0.66667	11.967 ± 0.3333	14.000 ± 1.000*
S. aureus	9.6667 ± 0.3333	11.000 ± 0.57735	11.167 ± 0.3333	12.667 ± 0.666*
S. pyogenes	9.5415 ± 0.3333	10.333 ± 0.66667	11.242 ± 0.3521	12.661 ± 0.633*
Hot-water extract				
Bacteria	100 mg/ml $R = 3$; mean (mm) ± SE	150 mg/ml $R = 3$; mean (mm) ± SE	200 mg/ml $R = 3$; mean (mm) ± SE	250 mg/ml $R = 3$; mean (mm) ± SE
P. aeruginosa	13.800 ± 0.9865	15.533 ± 0.35277	18.533 ± 0.8950	29.667 ± 0.60*
S. aureus	13.500 ± 0.7549	14.233 ± 0.18559	17.100 ± 0.6350	28.233 ± 0.90*
S. pyogenes	13.400 ± 0.5542	14.333 ± 0.18491	18.442 ± 0.7921	27.661 ± 0.70*

R, number of replicates; SE, standard error; Mean (mm), diameter of inhibition zone

*$P < 0.05$

Table 3 Comparison between nitrofurantoin (25 μg) and cold-water extract of *Petroselinum crispum* leaves (200 and 250 mg/ml) against pathogenic bacteria isolated from patients with burn infections in Al-najaf Governorate, Iraq

Bacteria	Cold-water extract		Antibiotic
	200 mg/ml. $R = 3$; mean (mm) ± SE	250 mg/ml. $R = 3$; mean (mm) ± SE	Nitrofurantoin (25 μg) (positive control). $R = 3$; mean (mm) ± SE
P. aeruginosa	11.667 ± 0.3333	14.000 ± 1.0000	24.950 ± 0.22141*
S. aureus	11.667 ± 0.3333	12.667 ± 0.6666	25.200 ± 0.23094*
S. pyogenes	11.242 ± 0.3521	12.661 ± 0.6333	22.120 ± 0.20153*

R, number of replicates; SE, standard error; Mean (mm), diameter of inhibition zone

*$P < 0.05$

Table 4 Comparison between nitrofurantoin (25 μg) and hot-water extract of *Petroselinum crispum* leaves (200 and 250 mg/ml) against pathogenic bacteria isolated from patients with burn infections in Al-najaf Governorate, Iraq

Bacteria	Hot-water extract		Antibiotic
	200 mg/ml. $R = 3$; mean (mm) ± SE	250 mg/ml. $R = 3$; mean (mm) ± SE	Nitrofurantoin (25 μg) (positive control). $R = 3$; mean (mm) ± SE
P. aeruginosa	18.533 ± 0.8950	29.667 ± 0.600*	24.950 ± 0.22141
S. aureus	17.100 ± 0.6350	28.233 ± 0.902*	25.200 ± 0.23094
S. pyogenes	18.442 ± 0.7921	27.661 ± 0.700*	22.120 ± 0.20153

R, number of replicates; SE, standard error; Mean (mm), diameter of inhibition zone

*$P < 0.05$

antibacterial agents in this plant, thus justifying its use for treatment of microbial infections. Phytochemical analysis of the plant extracts revealed the presence of tannins, saponins, anthraquinones, alkaloids, and phenols [10]. A previous report by Okogun [22] implied these and other chemicals were responsible for the curative properties of many medicinal plants. These results suggest either good antibacterial potency of the hot water extract or a high concentration of an active principle in the extracts of *P. crispum*; essential oils or flavonoids might also be responsible for this activity [23]. In previous studies antibacterial effects of xanthotoxin, herniarin, umbelliferone, and scopoletin, and antifungal activity of umbelliferone, scopoletin, and coumarin have been observed [24, 25].

Conclusion

On the basis of the results of this study *Petroselinum crispum* obtained locally can be regarded as a potential antibacterial agent against *P. aeruginosa*, *S. aureus*, and *S. pyogenes* associated with skin infections in Iraq.

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