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Antimicrobial Activity of *Petroselinum sativum* and *Coriandrum sativum* Seeds

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Abstract: Petroleum ether, ethanolic and water extracts of *Petroselinum sativum* and *Coriandrum sativum* were screened for antibacterial activity against one standard gram-positive bacterium (*Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*). Petroleum ether extract from *C. sativum* in different concentrations (25-100%) did not show any antibacterial activity against the test bacteria while the petroleum ether extract from *C. sativum* was active against only *Pseudomonas aeruginosa*. The ethanol extracts of the two plants were active against all test bacteria (*S. aureus*, *E. coli*, *Ps. aeruginosa* and *S. typhi*), but that from *P. sativum* was inactive against *E. coli*. The water extract from *P. sativum* was active against all bacteria while that from *C. sativum* was active against *S. aureus*, *Ps. aeruginosa* and *S. typhi* but inactive against *E. coli*.

Key words: *Petroselinum sativum*, *Coriandrum sativum*, Antimicrobial activity

Introduction

A sizable number of plants are used in different parts of the world for the treatment of various ailments. The World Health Organization (WHO) estimated that more than 4 billion inhabitants of the world rely mainly on traditional medicine for health care needs. A major part of traditional medicine involves the use of plant extracts and/or their derived active principles (Aizen-Mon, 1978). Therefore, there are urgent needs to carry out a systemic scientific evaluation of the biological activity and to isolate and identify the bioactive agents in medicinal plants (Al Magboul, 1992).

Plants may represent a potential source of antibiotics as evidenced by huge number of studies that have dealt with antimicrobial action of medicinal plants.

Coriandrum sativum L. Coriander is an umbelliferous annual plant of the parsley family. As a medicinal plant, coriander is a commonly used domestic remedy, valued especially for its effect on the digestive system, treating flatulence, diarrhoea and colic. It settles spasms in the gut and counteracts the effects of nervous tension (Chevallier, 1996). The seeds are ground into a paste for application to skin and mouth ulcers.

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Petroselinum sativum, Parsley has three cultivated varieties, which, in part, differ in their chemistry. *Var. latifolium* (broad-leaved) and *var. crispum* (curly-leaved) are grown for their leaves and *var. tuberosum* is grown for its root.

Parsley's traditional use for menstruation, stimulates contractions of the uterus (Tyler, 1994). A tea made from the fruits or seeds of parsley, is also a traditional remedy for colic, indigestion and intestinal gas (Gruenwald *et al.*, 1998).

A test tube study evaluated parsley extract as topical antibiotic, finding that the extract has a weak effect against *Staphylococcus* bacteria (Ross *et al.*, 1980). Parsley oil induced significant anti-inflammatory activities, inhibited a powerful antibacterial activity against all the bacteria tested except *Salmonella typhi* (Afifi *et al.*, 1994).

The present study was to investigate the possible inhibitory activity of *Coriandrum sativum* and *Petroselinum sativum* seed extracts (petroleum ether, ethanol and water) against a gram-positive bacterium (*Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*).

Materials and Methods

Plant Materials

Seeds of *Coriandrum sativum* and *Petroselinum sativum* plants were purchased from herbalist at Omdurman local market (AL Tyman Herbal Store), shade - dried and separately ground into powder.

Standard Microorganisms

The test microorganisms utilized in the present study were kindly provided by scientists at the National Health Institute, Khartoum and designated as follows:

- *Staphylococcus aureus*- NCTC 25953- Gram + ve cocci.
 - *Escherichia coli*- NCTC 25922-Gram- ve rod.
 - *Salmonella typhi*-NCTC 25936-Gram-ve rod.
 - *Pseudomonas aeruginosa*- NCTC 27853-Gram-ve rod.
- NCTC: National Collection Type Culture, London UK.

Antibiotics

Gentamicin (Oxoid Ltd., England)

Culture Media

Standard media, nutrient agar and nutrient broth.

Methods

Preparation of Crude Extracts

Twenty grams of powdered of *C. sativum* seeds and leaves of *P. sativum* were extracted successively with petroleum ether 90% at 37°C and ethanol 95% at 37°C using Soxhlet apparatus extractor for 3 h. The extracts were evaporated under reduced pressure, air-dried and yields were recorded. The aqueous extract was dried by freeze dryer and weighed. The extracts from each plant were reconstituted at the time of testing in concentrations of 100, 50, 25, 10 and 2.5%.

Preparation of Stock Extracts Solutions

One gram of each extract was dissolved in 1 mL of the same solvent used for extraction.

Preparation of the Test Organisms

The properties at the standard bacteria are summarized in (Table 1).

Table 1: Differential characteristics of test bacteria

Characteristics	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>Ps. aeruginosa</i>
Gram stain	+ ve	- ve	- ve	-ve
Motility	+ ve	+ve	+ ve	+ ve
Catalase	+ ve	+ve	(-)	+ ve
Nitrate	+ ve	+ve	(-)	+ ve
Arginine	+ ve	+ve	(-)	+ ve
Indol	-ve	+ve	-ve	-ve
Methyl red	-ve	+ve	+ve	+ ve
Vagues proskures	+ ve	-ve	-ve	-ve
Gas production from glucose	- ve	+ve	-ve	+ ve
Acid production from glucose	+ ve	+ve	+ve	-ve
Acid production from sucrose	+ ve	+ve	+ve	+ ve
Acid production from lactose	+ ve	+ve	-ve	-ve
Oxidase	- ve	+ve	-ve	+ ve
Urease	+ ve	-ve	-ve	+ ve

+ ve= 90% or more of strain had positive reactions, - ve= 90% or more of strain had negative reactions, (-) = None determined

Staphylococcus Aureus

Gram-positive organism 0.5 mm. in diameter occurring singly, in pairs or in irregular clusters, non-motile and non-spore forming, many strains form a golden yellow pigment on colonies of good growth on ordinary media, aerobic, facultatively anaerobic, catalase and urease positive, oxidase and indole negative.

E. coli

Gram- negative rod , 1.1-1.5 mm. wide and 2.0-6 mm long , with rounded ends and shape varying from coccoid to rod, motile, aerobic, facultatively anaerobic, oxidase and urease negative and citrate can not be used as a sole carbon source, most strains are fermenters of methyl red positive and VP negative, catalase positive and indol positive.

Salmonella typhi

Gram-negative, straight rod, aerobic, facultatively anaerobic, motile with peritrichus flagella, no acid and gas formation from glucose and mannitol, formation of H₂S, indole and urease negative and V.P negative.

Pseudomonas aeruginosa

Gram-negative rod, motile, aerobic, catalase positive, oxidase positive, attacks sugar by oxidation, non-sporing and non capsulated, grows on wide variety of laboratory media, most of the *Pseudomonas* sp. are urease positive and indole negative.

Preparation of Culture Media

Nutrient broth forms the basis of most bacteria used in microbiological studies. Nutrient agar was used to prepare enriched culture media.

Nutrient broth (Oxoid Ltd., London, UK)

Composition

Peptone 5 g, sodium chloride 5 g, beef extract 1 g and yeast extract 2 g at pH 7.4.

Procedure

Thirteen gram of nutrient broth powder were dissolved in a liter of distilled water and autoclaved at 121°C for 15 min.

Nutrient agar

(Oxoid Ltd. London)

Composition

Peptone 5 g, sodium chloride 8 g, beef extract 3 g and agar 12 g

Procedure

All ingredients, except agar, were dissolved in distilled water and pH was adjusted 7.6 Agar was added and medium autoclaved at 121 °C for 15 min.

Nutrient agar was used to obtain excellent colonies for evaluation of antimicrobial activity of plant extracts.

Evaluation of Antibacterial Activity of Plant Extracts

Antibacterial activity was assessed by the agar well diffusion method (Kinsbury and Wagner, 1990). The nutrient agar medium was properly inoculated with the standard organisms, separately at 27.5×10^9 cfu mL⁻¹ to achieve confluent growth and allowed to dry at room temperature. On each inoculated plate, 10 mm-diameter wells (4 wells at equal distances in one plate) were bored in the agar using sterile cork borer. Concentrations at 100, 50 and 25% of each extract was added to each well by a sterile Pasteur pipette and allowed to diffuse for 1 h. before incubating the plates for 18 h at 37°C.

The diameter of the inhibition zone resulting from the activity of the extracts was measured in mm, two replicates were made from each concentration and comparative activity was recorded. The antibacterial activity of the plant extract against the standard micro-organisms was evaluated and compared with that of the antibiotic, gentamicin (Cip-oxoid London).

Results and Discussion

The petroleum ether and ethanol extracts of *C. sativum* seed and *P. sativum* yielded (2.4 and 3 g) and (1 and 7.5 g), respectively, after evaporation dryness and water extract yielded (2 and 3.5 g).

Petroleum ether and ethanol extracts in different concentration of (25-100%) or (2.5-10%) respectively, in addition to water extract from the plants *C. sativum* and *P. sativum* were tested for their antibacterial activity against both gram-positive bacterium (*S. aureus*) and gram-negative bacteria (*E. coli*, *Ps. aeruginosa* and *S. typhi*). The growth inhibition zones (mm) were presented in Table 2-4.

C. sativum seed extracts showed no growth inhibition zone to the microorganisms tested, but Baratta *et al.* (1998), Elgayyar *et al.* (2001) and Larran *et al.* (2001) studied *Coriandrum sativum* antibacterial activity and observed that the essential oil of Coriander, inhibited microorganisms also with extracts of ethyl acetate, alcohol, chloroform and acetone when tested *in vitro* against 13 bacterial species and strains by the agar-diffusion method including *Ps. aeruginosa* and *S. aureus*.

Table 2: Evaluation of antibacterial activity of *C. sativum* and *P. sativum* petroleum ether extract

Organism	Conc. of <i>C. sativum</i> (%)	Inhibition zone of <i>C. sativum</i> (mm)	Conc. of <i>P. sativum</i> (%)	Inhibition zone of <i>P. sativum</i> (mm)
<i>S. aureus</i>	100	(-)	10	(-)
	50	(-)	5	(-)
	25	(-)	2.5	(-)
<i>E. coli</i>	100	(-)	10	(-)
	50	(-)	5	(-)
	25	(-)	2.5	(-)
<i>Ps. aeruginosa</i>	100	(-)	10	11
	50	(-)	5	9
	25	(-)	2.5	6
<i>S. typhi</i>	100	(-)	10	(-)
	50	(-)	5	(-)
	25	(-)	2.5	(-)

(-) No inhibition zone observed

Table 3: Evaluation of antibacterial activity of *C. sativum* and *P. sativum* ethanol extract

Organism	Conc. (%)	Inhibition zone of <i>P. sativum</i> (mm)	Inhibition zone of <i>C. sativum</i> (mm)
<i>S. aureus</i>	100	(-)	13
	50	12	13
	10	12	11
<i>E. coli</i>	100	(-)	13
	50	(-)	(-)
	10	(-)	(-)
<i>Ps. aeruginosa</i>	100	(-)	9
	50	13	7
	10	12	7
<i>S.typhi</i>	100	(-)	9
	50	13	7
	10	11	7

(-) No inhibition zone observed

Table 4: Evaluation of antibacterial activity of *C. sativum* and *P. sativum* water extract in concentration of 100%

Organism	Inhibition zone of <i>C. sativum</i> (mm)	Inhibition zone of <i>P. sativum</i> (mm)
<i>S. aureus</i>	7	17
<i>E. coli</i>	(-)	13
<i>Ps. aeruginosa</i>	9	9
<i>S.typhi</i>	7	14

(-) No inhibition zone observed

Table 5: Evaluation of antibacterial activity of gentamicin

Gentamicin 10 mg

Organism	Inhibition zone (mm)
<i>S. aureus</i>	22
<i>E. coli</i>	22
<i>Ps. aeruginosa</i>	13
<i>S. typhi</i>	23

The petroleum ether extract from *C. sativum* caused no growth inhibition zone against either gram-positive or gram-negative bacteria and that from *P. sativum* at 2.5-10% only caused growth inhibition of *Ps. aeruginosa* (gram -ve bacterium) at (11, 9 and 7 mm), respectively, but the extracts did not produce inhibition zone against *S. aureus* (gram +ve) and *E. coli* and *S. typhi* (gram -ve) as presented in Table 2.

The ethanol extract of *C. sativum* when used in different concentrations (100, 50 and 10%) caused inhibition zone (13, 13 and 11 mm) in *S. aureus* (Fig. 1), *E. coli* (13 mm) (Fig. 2), *Ps. aeruginosa* (9, 7 and 7 mm) and *S. typhi*, (9, 7 and 7mm), respectively. The ethanol extract of *P. sativum* used in 100% concentrations had no antibacterial activity but when used in 50 and 10% concentrations inhibition zone occurred (13 and 12 mm) in *Ps. aeruginosa*, *S. aureus*, (Fig. 3) and *S. typhi* (Fig. 4), respectively. These effects were given in Table 3.

Baratta *et al.* (1998) found that *E. coli* was more affected by the ethanolic extract of parsley which did not elicit pronounced effect on the tested Gram - positive organisms. The commercial oils of sage, thyme and parsley displayed no antimicrobial activity against *E. coli*, *Proteus mirabilis* or *Salmonella typhi*.

The activity of water extract of *C. sativum* produced inhibition zone at 9, 7 and 7 mm when tested for *Ps. aeruginosa*, *S. typhi* and *S. aureus*, respectively, but had no antibacterial activity against *E. coli*. The activity of water extract of *P. sativum* caused inhibition zone at 17, 13, 14 and 9 mm when tested for *S. aureus*, *E. coli*, *S. typhi* and *Ps. aeruginosa*, respectively (Table 4).

Antibacterial activity of gentamicin was evidenced by inhibition zone at 22, 22, 13 and 23 mm against *S. aureus*, *E. coli*, *S. typhi* and *Ps. aeruginosa*, respectively (Table 5).

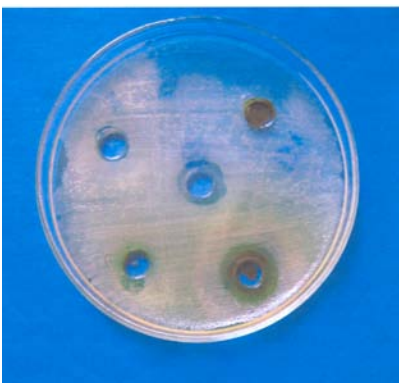


Fig. 1: Inhibition zones (13, 13 and 11 mm) produced by ethanolic extract of *C. sativum* against *S. aureus*



Fig. 2: Inhibition zones (13 mm) produced by ethanolic extract of *C. sativum* against *E. coli*

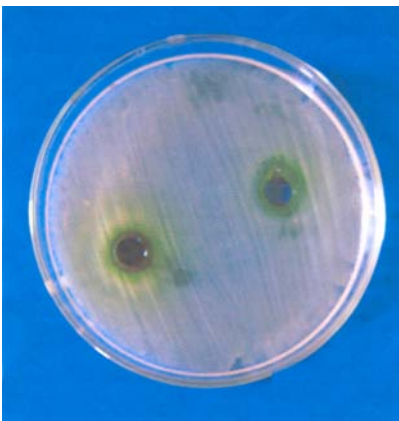


Fig. 3: Inhibition zones (13 and 12 mm) produced by ethanolic extract of *P. sativum* against *S. aureus*

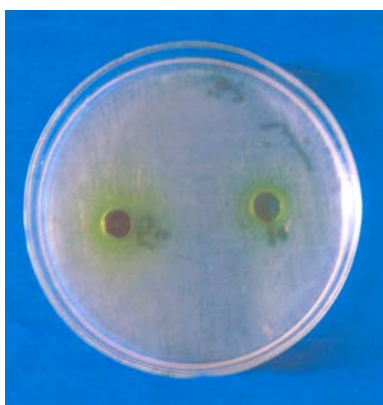


Fig. 4: Inhibition zones (13, 12 mm) produced by Ethanolic extract of *P. sativum* against *S. typhi*

In conclusion, this preliminary investigation of the antibacterial activity against four pathogenic organisms *S. aureus*, *E. coli*, *Ps. aeruginosa* and *S. typhi* of the extract from *C. sativum* and *P. sativum* may justify the use of these plants in local traditional medicine. Detailed *in vitro* studies utilizing the characterized active principles of the two plants are necessary for assessing these antibacterial constituents.

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