Antimicrobial Activity of the Essential Oil from the Leaves and Seeds of *Coriandrum sativum* toward Food-borne Pathogens

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ABSTRACT

The increasing incidence of drug-resistant pathogens and toxicity of existing antibacterial compounds has drawn attention toward the antimicrobial activity of natural products. The purpose of this study is to evaluate the antimicrobial activity of the essential oil of the leaves and seeds of Coriandrum sativum. *The five strains of bacteria comprising* Escherichia coli, Staphylococcus aureus, Yersinia enterocolitica, Salmonella enterica *and* Vibrio cholerae *were used for the antibacterial tests. In this study, antimicrobial effects of the essential oil from the leaves and seeds of* Coriandrum sativum *are evaluated by determining the minimum inhibitory concentration (MIC), the inhibition zone and minimum bactericidal concentration (MBC). The essential oil from* Coriandrum sativum *was extracted by steam distillation. The results indicate that the antimicrobial activities against the five pathogens were in the range of 2.5–320 µg/mL. Increase in essential oil concentration caused significant increase in inhibitory feature. The essential oil of the leaves and seeds of* Coriandrum sativum *showed antimicrobial activity against the food-borne pathogenic bacteria. Thus, its oil can be used as an alternative to synthetic food preservative without toxic effects. Also, it can be used in biotechnological fields as ingredients in antibiotics and the pharmaceutical industry. These results suggest that the essential oil of* C sativum *leaves and seeds may have potential use in pharmaceutical and food industries for preservatives or antimicrobial agents.*

Keywords: *Coriandrum sativum*, essential oil, food-borne pathogen, food safety

La Actividad Antimicrobiana del Aceite Esencial de las Hojas y Semillas del *Coriandrum sativum* Hacia los Agentes Patógenos Transmitidos por los Alimentos

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RESUMEN

La creciente incidencia de patógenos resistentes a los medicamentos y la toxicidad de los compuestos antibacterianos existentes, han atraído la atención sobre la actividad antimicrobiana de los productos naturales. El propósito de este estudio es evaluar la actividad antimicrobiana del aceite esencial de las hojas y las semillas del Coriandrum sativum *(conocido popularmente como cilantro). Cinco cepas de bactericida – las cuales abarcaron* Staphylococcus aureus, Yersinia enterocolítica, Salmonella enterica *y* Vibrio cholerae *– fueron utilizadas para las pruebas antibacterianas. En este estudio, los efectos antimicrobianos del aceite esencial de las hojas y las semillas del* Coriandrum sativum, *fueron evaluados determinando la concentración mínima inhibitoria (CIM), la zona de inhibición y la concentración bactericida mínima (CBM). El aceite esencial del* Coriandrum sativum *se extrajo por destilación al vapor. Los resultados indican que la actividad antimicrobiana contra los cinco patógenos estuvieron en el rango de 2.5–320 µg/mL. El aumento de la concentración del aceite esencial produjo un aumento significa-tivo de la función inhibitoria. El aceite esencial de las hojas y semillas del* Coriandrum sativum *demos-tró poseer actividad antimicrobiana contra las bacterias patógenas transmitidas por los alimentos. Por lo tanto, este aceite puede utilizarse como alternativa a los preservativos sintéticos de los alimentos, sin efectos tóxicos. También puede usarse en la industria farmacéutica y en el campo biotecnológico como ingrediente de los antibióticos. Estos resultados sugieren que el aceite esencial de las hojas y semillas*

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DOI: 10.7727/wimj.2014.162

del Coriandrum sativum *puede tener un uso potencial en las industrias farmacéutica y alimentaria como agente preservativo o antimicrobiano.*

Palabras claves: *Coriandrum sativum,* aceite esencial, patógenos transmitidos por los alimentos, seguridad alimentaria

West Indian Med J 2016; 65 (1): 9

INTRODUCTION

Food-borne disease resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. *Salmonella, Staphylococcus, E coli, Vibrio cholerae* and *Yersinia enterocolitica* are responsible for severe food-borne illnesses. These diseases are transmitted through various foods (fish, dairy products, cured or processed meat, egg, poultry, seafood, salad, fruits and vegetables). To increase food safety and reduce economic losses due to food-borne pathogens, the use of natural products (*ie* medicinal plants) as antimicrobial compounds seems to be an important way to control the presence of pathogenic bacteria and to extend the shelf life of processed food (1). Preservatives are designed to prevent food spoilage by pathogens and to increase the storage shelf life of foods. At the moment, many food additives *ie* benzoic, ascorbic and sorbic acid are used in the food industry. Although these synthetic preservatives are effective, they can be harmful to human health and consequently, an increasing number of consumers choose food products which are preservative-free or contain only trace amounts (2). Because of an increase in the antibiotic-resistant micro-organisms and residual and adverse effects of chemical antibiotics, there has been an increasing interest in the discovery of new, natural antimicrobials (3). Essential oils are compounds obtained from spices, aromatic herbs, leaves and seeds, and flowers and are characterized by their aroma (4). Essential oils have many applications in traditional medicine and also in food preservation (5). Coriander (*Coriandrum sativum L*) is an annual herb that belongs to the family Apiaceae (synonymous with Umbelliferae). Coriander is considered both a herb and a spice since both its leaves and its seeds are used as a seasoning condiment.

Coriander seeds have a health supporting reputation that is high on the list of healing spices. It has traditionally been referred to as antidiabetic (6), anti-inflammatory, cholesterol lowering (6), carminative, diuretic, stimulant, stomachic, refrigerant, aphrodisiac and analgesic (7). The oil has a characteristic odour of linalool and a mild, sweet, warm, aromatic flavour. In food technology, coriander oil is used as a flavouring agent and flavour enhancer. Coriander oil is approved for food uses by the Food and Drug Administration (FDA), the Federal Emergency Management Agency (FEMA) and the Council of Europe (CoE). Coriander is used for cooking and for children's digestive upset and diarrhoea. The Greeks and Romans also used coriander to flavour beverages and as a medicine (8). The use of coriander to accelerate childbirth has been cited in manuscript illustrations (from the early 13th century) on medieval midwifery (9). The leaves and seeds (dried) have been in use for almost 7000 years (10). The essential oil has been used as a food and aroma ingredient since the 1900s (11). The antimicrobial activity of medicinal plants and their extracts have been identified since antiquity. Due to the increased resistance of pathogens and the need for new food preservatives, the potential use of essential oils as antimicrobial agents has been the subject of new investigations. Studies also suggest that the volatile oils found in the leaves of *C sativum* plant may have antimicrobial activity against food-borne pathogens such as *Salmonella* species (12). Antimicrobial activity has been reported from the essential oil of *Coriandrum sativum* leaves and seeds against different species of Candida, Gram-positive/negative bacteria and fungi (13–15). Essential oil of *Coriandum sativum* shows pronounced antibacterial and antifungal effects (16). Wong and Kitts (2006) reported antimicrobial activity from the ethanolic and aqueous extracts of *C sativum* against *Bacillus subtilis* and *E coli* (17). The purpose of this study is to evaluate the antimicrobial activities of the essential oil extracted from Iranian *C sativum* leaves and seeds against Gram-positive and Gam-negative food-borne pathogens.

MATERIALS AND METHODS

The leaves and seeds of *Coriandrum sativum* were collected from Arak, Iran, during May 2013. The specimen was identified by the herbarium of medicinal plants, Tehran University of Medical Sciences.

The essential oils of the leaves and seeds of coriander (*C sativum* L) were obtained by hydro distillation using a Clevenger apparatus; 300 g of leaves and seeds were placed with sufficient distilled water to cover the material. Extraction continued for three consecutive hours after the water had begun to boil. The percentage of essential oil extraction was 5.3% by this method.

Antimicrobial activity test

Disk diffusion test

The essential oils were tested against *Staphylococcus aureus* ATCC 25913, *Escherichia coli* ATCC 8739, *Salmonella enterica* PTCC 1709, *Vibrio cholerae* PTCC 1611 and *Yersinia enterocolitica* PTCC (1477). The bacteria were obtained from the Microbiology Reference Laboratory (BoAli Hospital, Tehran).

The bacteria were cultured in brain heart infusion (BHI) for 18 hours at 37 °C, and resuspended in 0.5 Mac Farland Standard (5×10^8 CFU/mL) and inoculated directly in boards with Mueller-Hinton Agar (Merck). After the inoculation of each micro-organism, the agar diffusion method was used, putting 10 µL of essential oil on paper disks (6 mm in diameter) at 37 °C/24 hours, after which time the halos of inhibition were measured (18).

Determination of MIC and MBC on culture media

Stock solution of coriander essential oil [EO] (100 000 µg/mL) in 10% dimethyl sulfoxide (DMSO) was prepared. Then two-fold serial dilutions of EO (2.5 µl/mL to 320 µl/mL) were prepared. At first, 180 µL of sterile broth was added to each well of a 96-well microtitre-plate. Then 20 µL of the microbial suspension and 20 µL of each EO concentrations were added to the designed wells. Thus, the achieved EO concentrations were 2.5 µl/mL to 320 µl/mL. For every experiment, two growth controls consisting of BHI broth without essential oil and BHI broth containing DMSO inoculated with the diluted medium culture and one sterility control containing essential oil were run in each plate. The plates were finally incubated at 37 ˚C for 24 hours. The minimum inhibitory concentrations (MICs) were chosen as the least concentrations of the EO resulting in perfect inhibition of visible growth in the broth medium.

To evaluate the minimum bactericidal concentrations (MBCs) of the EO, 0.1 mL from non-turbid wells were subcultured on BHI agar and incubated at 37 ˚C for 24 hours. Then the lowest concentrations of EO that allowed less than 0.1% of the original inoculum to survive was considered as MBCs (19).

Statistical analyses

All experiments were done in triplicate. Statistical analysis was performed using SPSS software. The results showing $p < 0.05$ were considered as significant.

RESULTS

Coriander essential oil and its components are known to exhibit widespread antimicrobial activity (16, 20). Data for coriander essential oil susceptibility testing by broth micro-dilution are shown in Table 1.

All bacterial strains studied were inhibited by oil from the leaves and seeds of coriander, with different degrees of inhibition. The MIC values of coriander seed essential oil were as follows: *E coli* and *Salmonella enterica* were 160 µg/mL, *Staphylococcus aureus* and *Vibrio cholerae* were 20 µg/mL and for *Yersinia enterocolitica,* 80 µg/mL. Also, MBCs for the mentioned bacteria were 160 µg/mL, 320 µg/mL, 40 µg/mL, 80 µg/mL and 320 µg/mL, respectively. So, according to these findings, *S aureus* and *Vibrio cholerae* were more sensitive than others to the essential oil (Table 1). The MIC levels of the oil from the leaves of coriander against the bacterial strains were 5 µg/mL, 5 µg/mL, 2.5 µg/mL, 40 µg/mL and 80 µg/mL for *S aureus, Vibrio cholerae, Yersinia enterocolitica, E coli and Salmonella,* respectively. Meanwhile, the MBC of coriander leaf oil was 5 µg/mL, 10 µg/mL, 10 µg/mL, 80 µg/mL and 80 µg/mL, respectively (Table 1). There was no significant difference ($p > 0.05$) between bacterial strains in terms of in-hibitory halo diameter. But there was a statistically significant difference between the inhibitory halo diameter of the essential oil of the leaves and seeds of *C sativum.*

The results of other methods showed that the essential oil of the seeds and leaves of coriander (*Coriandrum sativum* L) showed antimicrobial activity against all of the bacterial strains used in this study: *Staphylococcus aureus* (Gram-positive), with an inhibitory zone of 12.5 mm and 13.3 mm, *Salmonella enterica* (Gram-negative), with an inhibitory zone of 8.16 mm and 10.6 mm, *E coli* (Gram -), with an inhibitory zones of 8.5 mm and 11 mm, *Vibrio cholerae* (Gram -), with an inhibitory zone of 10.16 mm and 12.17 mm and *Yersinia enterocolitica* (Gram +), with an inhibitory zone of 10.33 mm and 11.33 mm, respectively (Table 2).

Table 1: Determination of MIC and MBC value (µg/mL) for essential oil from the leaves and seeds of *Coriandrum sativum* against pathogenic bacterial strains

	Staphylococcus aureus		***Vibrio cholerae***		***Yersinia enterocolitica***		***Escherichia coli***		***Salmonella***	
Test	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**	**Seed**	**Leaf**
MIC	20	5	20	5	80	2.5	160	40	160	80
MBC	40	5	80	10	320	10	160	80	320	80

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

Table 2: Comparison of average inhibitory halo diameter (mm) of various bacterial strains for essential oil of *Coriandrum sativum* seeds and leaves

		Seed		**Leaf**	
Bacterial strain	**Samples**	**Range**	**Average ± SD**	**Range**	**Average ± SD**
Staphylococcus aureus	3	11–13.5	12.5 ± 1.32^a	13–14	13.3 ± 0.57^b
Escherichia coli	3	7–10	8.5 ± 1.5^a	10–11.5	11 ± 0.87^b
Vibrio cholerae	3	9–11.5	10.16 ± 1.25^a	11–13	12.17 ± 1.04^b
Yersinia enterocolitica	3	9–12	10.33 ± 1.52^a	11–12	11.33 ± 0.58^b
Salmonella	3	7–9.5	8.16 ± 1.25^a	9.5–11.5	10.6 ± 1.04^b

DISCUSSION

Our results showed that higher concentrations of the essence from the leaves and seeds of coriander increased the antibacterial effect. Antibacterial susceptibility was evaluated using classical microbiological techniques, disk diffusion, MIC and MBC determination. Our results showed that essential oil from the leaves and seeds of coriander has an effective antimicrobial activity against all bacteria tested. Begnami *et al* surveyed the antimicrobial effect of the essence from the leaves of *Coriandrum sativum* L against different Candida species through MIC from 125 µg/mL (*C parapsilosis* CBS 604) to 500 µg/mL [*C albicans* CBS 562] (21). Another study obtained MIC for *Coriandrum sativum* L essence at concentration from 0.2 to 0.05% against Candida species (22). Coriander essential oil is reported to possess antimicrobial activity against pathogenic and saprophytic micro-organisms, indicating that it may be useful as a disinfectant (13, 23). Also, Coriander essential oil has been reported to inhibit a broad spectrum of micro-organisms (15, 23). The coriander essential oil, at concentrations of 500 ppm, was effective against *Saccharomyces ludwigii, Zygosaccharomycesbailii, Salmonella enteriditis and Listeria innocua* (24). The MICs for coriander essential oil against different bacteria were as follows: *E coli O157:H7*, 0.23%; *Listeria monocytogenes*, 0.47%; *S aureus*, 0.4% and *S cerevisiae,* 0.13% (25). The comparison of our results to those obtained by Silva *et al* (2011) showed that all strains studied (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Pseudomonas aeruginosa, Acinetobacter baumannii, B cereus, Staphylococcus aureus* and *Enterococcus faecalis*) were inhibited by coriander essential oil (26). The essential oil from the leaf of *Coriandrum sativum* (1%, 5%, 10% and 20%) showed antimicrobial activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Klebsiella pneumonia* (27). Saeed and Tariq showed that all tested isolates were found resistant to aqueous infusion and decoction of *C sativum* (28). Another study showed that the essential oil from *Coriandrum sativum* seeds was relatively more toxic against *C maculatus* than *T confusum* (29). Also, Chaudhry and Tariq found that decoction of *C sativum* does not have antibacterial potential against Gram-positive and Gram-negative bacteria (7). Aqueous decoction of coriander was found to have no antimicrobial activity against *Helicobacter pylori* (30). In another study, some researchers have found that *C sativum* has excellent antibacterial activity against both Gram-positive and Gram-negative bacteria (31). In a similar study on food-borne pathogens, allicin and lysozyme conjugated nanocellulose had good antifungal and antibacterial effects against standard strains of *Candida albicans*, *Aspergillus niger*, *Staphylococcus aureus* and *Escherichia coli* (32). The differences between our results and that of other studies are probably due to the genotype difference of *C sativum* used in the study, along with the growth ecosystem (habitat, temperature, altitude) and experimental condition (pH and temperature). Growth and performance of the plants in the ecosystems are under the effect of many factors such as type, habitat, soil, altitude and geographical position. Each one of the factors may be considerably effective on quality and quantity of the result.

CONCLUSIONS

This study emphasizes antimicrobial activity of the essential oils from the leaves and seeds of coriander against food-borne pathogenic bacteria. It has been observed that the essential oils possess both bacteriostatic and bactericidal activity when tested *in vitro*. This essential oil may be effective on other Gram-negative and Gram-positive bacteria. In conclusion, coriander essential oil can effectively kill food pathogenic bacteria, but further investigations are required to better evaluate the suitability of coriander essential oil use for practical applications and to increase shelf life of food.

ACKNOWLEDGMENTS

This study was approved and supported by the Research and Ethics committees of Tehran University of Medical Sciences. The authors are grateful to the Tehran University of Medical Sciences (Students' Scientific Research Center) for financial support through Project no. 92-02-61-21900. The authors declare that they have no competing interests.

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