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RESEARCH ARTICLE

In Vitro Antibacterial Activity of Essential oil and Ethanolic Extract of Ajowan *(Carum Copticum)* against some Food-Borne Pathogens

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Abstract

The present study aimed at evaluating the in vitro antibacterial activity of essential oil and ethanolic extract of ajowan *(Carum copticum)* against some food-borne pathogens.The micro-dilution method was used to study the antibacterial activity of ajowan *(Carum copticum)* essential oil and ethanolic extracts against some food-borne pathogens (*Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*). The ethanolic leaf extract of ajowan against *Salmonella typhimurium* strain showed the best antibacterial activity, with the lowest minimal inhibitory concentration (MIC) of 50 mg ml^{-1} and MBC was 100 mg ml^{-1} for this bacterium and the results of essential oil on all bacteria tested showed that gram-negative bacteria were sensitively. MIC and MBC of essential oil for *Salmonella typhimurium* were 16 and 32 mg ml^{-1} respectively and MIC for *Escherichia coli* was 32 mg ml^{-1}. According to results, the essential oil and ethanolic extract of this plant has antibacterial activity and therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Keywords: *Ajowan (Carum Copticum), Essential oil, Ethanolic extract, Antibacterial activity.*

Introduction

Food-borne diseases caused by consumption of food contaminated with pathogenic bacteria are a great problem in public health (1). Thus, food industry at present uses chemical preservatives to prevent the growth of food spoiling microbes (2).

In the recent years, consumers have become more concerned about the processed food they eat. Synthetic preservatives, which have been used in foods for decades, may lead to negative health consequences (3).

Increased use of antibiotics, along with over-prescribing and/or poor patient compliance, has led to development of bacterial resistance to antibiotics. Therefore, there is growing interest in the development of new types of effective and nontoxic antimicrobial compounds.

Research into more effective antimicrobial food agents, in particular natural antimicrobials, such as essential oils (Eos), has received attention in the past decade.

Herbal and botanical products, such as essential oils and plant extracts, have been studied for their antimicrobial activities (4).

The EOs and plant extracts are generally obtained by hydro distillation (HD) and solvent extraction (SE) (5). Essential oils are aromatic oily liquids obtained from different plant parts (4) such as flower, buds, seed, leaves and fruits and roots (6, 7) and have broad activities (4).

Essential oils derived from plants and their components represent a source of natural antioxidant and antimicrobial substances and have the potential to be used in the food industry to increase the shelf life of food products without any side effects (1). They are mainly composed of a mixture of volatile low-molecular weight mono-and sesquiterpenes and other isoprenses (6).

Carum copticum (Ajowain) is a flowering plant in the family Apiaceae. The medicinal part of this plant is a strongly aromatic seed-like fruit (1). *C. copticum* is a grassy, annual plant of Umbelliferae family with a white flower and small, brownish seeds which commonly grows in Iran, India, Egypt and Europe. The seeds have been used for their flavour and spice in food industry (8).

The stearoptene is known as ajowan-ka-phul (crude thymol). A phenolic glucoside has been isolated from the seeds and identified as 2-methyl-3- glucosyloxy-5-isopropylphenol. Thymol crystallizes easily from the oil extracted from seeds of Carum copticum and the remainder consists of p-cymene, b-pinene, dipentene ß-terpinene and carvacrol.

This plant has been mentioned in Iranian traditional literature to have therapeutic effect on flatulence, indigestion, colic, dyspepsia and diarrhea. It is also applied to eradicate worms and to relieve urticaria, rheumatic and neuralgic pain such as joint pains and headache (9).

The aim of the present study was to investigate the chemical composition and antibacterial effects of essential oil and ethanolic extract of *C. copticum*.

Material and Methods

Chemicals and Plant Materials

Muller Hinton agar (Merck, Germany), BHI (Brain-heart infusion broth) (Merck, Germany), ethanol and Dimethyl sulfoxide (DMSO) (Merck, Germany) were purchased. Leaves of the *C. copticum* plant were collected in 2015 from Khorasan-Razavi Province (the northeast of Iran). The plant confirmed by Medicinal Plants Institute, Ferdowsi University, Mashhad, Iran.

Extraction of Extract

The plant samples were then dried at room temperature under shade (10), finely ground with a hammer mill, and the powdered air-dried sample from each plant was extracted with ethanol for 48 h at room temperature (11). The extracts were filtered through filter paper, dried in vacuum at 40°C, and weighed and were kept at 4°Cuntil further uses (12, 13).

Extraction of Essential Oil

In this study 150 g of dried material (leaves of plant) were finely ground in a blender and submitted to our Hydrodistillation facility. Hydrodistillation was done in 4 h, using a Clevenger-type apparatus. The EOs obtained were separated from water and dried over anhydrous Na_2SO_4 and stored in dark glass bottles at 4°C prior to use (4, 14).

Organisms and Inoculation Conditions

The ethanolic extract and essential oil were screened for antimicrobial activity against the gram-positive bacteria *Bacillus cereus* (ATCC 11778) and *Staphylococcus aureus* (ATCC 25923); and the gram-negative bacteria *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 25922). Finally, suspensions were adjusted to 0.5 Mc-Farland standard turbidity. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml (15).

Antimicrobial Assay

Minimum Inhibitory Concentration (MIC) Test

The micro-dilution method was used to determine the minimum inhibitory concentrations (MIC) of the plant extract and essential oil (16). The 96-well plates were prepared by dispensing into each well

95 µl of BHI broth (Brain Heart Infusion broth) (17-20). One hundred microliter aliquot from the stock solutions of the EOs and their serial dilutions initially prepared was transferred into wells. The final volume in each well was 200 µl and 5 µl of the inoculum (standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectrophotometer) added to the serial dilution wells. The plates were covered with sterile plate sealer and then incubated at 37 °C for 24 h (21, 22). Afterward the ethanolic extract was serially diluted in DMSO in a range from 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.75, 0.375 and 0.1875 mg ml^{-1} (12, 23).

Then 100 µl of each dilution and 95 µl from BHI added to wells. Now, the 5 µl of bacterial suspension of 0.5 McFarland standard was added to the serial dilution wells and followed by shaking for 30 s (24). Micro titer plates were then incubated at 37°C for 24 hours. After incubation, wells were examined for microbial growth (25). MIC was defined as the lowest concentration of the EO or extract in the medium in which there was no visible growth after incubation (no red color signifying live growth) (17).

Moreover, the microorganism growth inhibition was evaluated by measuring absorbance at 630 nm, using an ELISA reader (anthos2020, Switzerland) (4). Control flasks without *C. copticum* were tested in the same way. Gentamicin was used as antibacterial standards against all pathogens. Experiments were performed in triplicate, but at three different times.

Minimum Bactericidal Concentration (MBC) Test

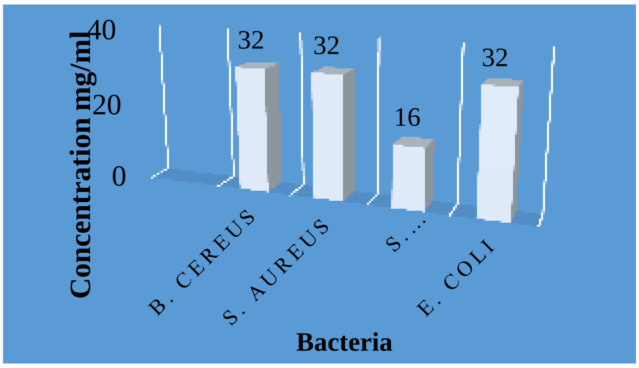
To determine MBC, 10 µl broth was taken from each well and inoculated in MHB for 24 h at 30 or 37ºC for bacteria. The MBC is defined as the lowest concentration the methanol extracts at which inoculated microorganism was completely killed (99.99%) (26).

Results

The in vitro antibacterial activity of the *C. copticum* essential oil and extract were assessed by the micro-dilution method against major bacterial food-borne pathogens. Antibacterial activities of essential oil were expressed as MIC values (table 1).

Table 1: MIC (mg ml^{-1}) of essential oil from *C. copticum*.

Bacteria	MIC
B. cereus	32
S. aureus	32
S. typhimurium	16
E. coli	32

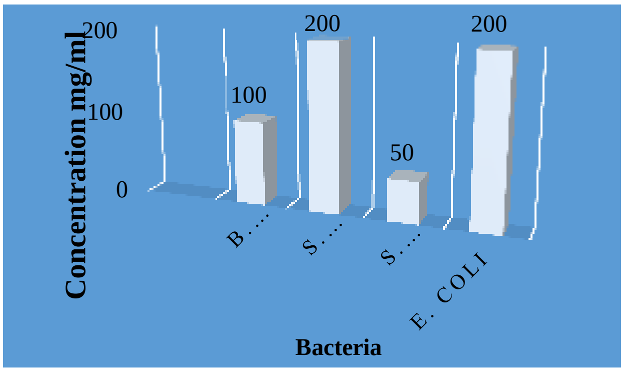


Figures 1: Results of MIC (mg ml^{-1}) of essential oil from *C. copticum*.

Eos exhibited varying levels of antibacterial activity against the bacteria investigated. The MIC of Eos was within concentration ranges 16-32 mg ml^{-1} and the results of MIC values for ethanolic extract is in table 2. The ranges of MIC of ethanolic extract was 50-200 mg ml^{-1}.

Table 2: MIC (mg ml^{-1}) of ethanolic extract from *C. copticum*.

Bacteria	MIC
B. cereus	100
S. aureus	200
S. typhimurium	50
E. coli	200



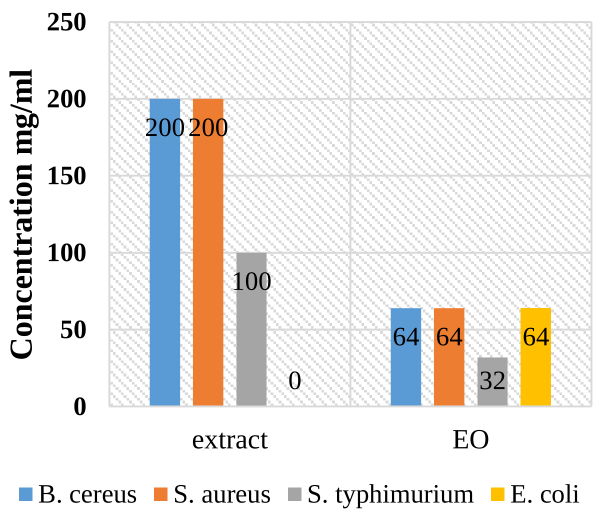
Figures 2: Results of MIC (mg ml^{-1}) of ethanolic extracts from *C. copticum*.

The results showed that EO of *C. copticum* was more active against all pathogenic bacteria than ethanolic extract. In addition, the MIC of EO from *C. copticum* on gram-negative bacteria such as *S. typhimurium* was lower than gram-positive bacteria and this bacteria was sensitively to EO and ethanolic extract.

The strongest bacteria to EO were *B. cereus, S. aureus* and *E. coli* (MIC=32) and for ethanolic extract were *S. aureus* and *E. coli* (MIC=200).The MBC values are summarized in Table 3. Results show that the essential oil of *C. copticum* was stronger than the ethanolic extract and *S. typhimurium* was sensitive bacteria (MBC=32 mg ml^{-1}).

Table 3: MBC (mg ml^{-1}) of ethanolic extract and essential oil from *C. copticum*.

MBC	Bacteria			
	B. cereus	***S. aureus***	***S. typhimurium***	***E. coli***
extract	200	200	100	>200
Essential oil	64	64	32	64



Figures 3: Results of MBC (mg ml^{-1}) of ethanolic extracts and essentialoil from *C. copticum*.

Figures of 1, 2 and 3 also confirm these results.

Discussion

It seems reasonable to assume that their antimicrobial effect of ethanolic extract and essential oils might be related to the phenolic compounds. Most of the studies on the mechanism of phenolic compounds have focused on their effects on cellular membranes. Phenolic compounds not only attack cell walls and cell membranes. Thereby effecting their permeability and release of intracellular constituents. But they also interfere with the membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of bacterial pathogens (27).

The use of natural antimicrobials such as organic acids, essential oils, plant extracts, and bacteriocins could be a good alternative to ensure food safety (28).

Plants are considered as unique sources of useful metabolites. The plant essential oils received major considerations with regard to possessing a wide range of antimicrobial effects against different groups of pathogenic organisms. So, essential oils with antimicrobial activity are potential candidates, as natural antimicrobial preservatives, that can be used in controlling microbial food contaminations (29).

Thymol, a monoterpenoid is the major component in *C. copticum* essential oil (30). Antibacterial compounds such as thymol, eugenol, and carvacrol have been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of microorganisms (28).

The present study demonstrates that plants of these one Apiaceae species have noticeable antibacterial activity and potential application in the food industry. This observation highlights that, among the essential oil and ethanolic extract of *C. copticum*, EO had the strongest antibacterial activity against tested food-borne bacteria.

The results also provide more scientific bases for antibacterial effects of the plants in folk medicine, particularly combinatory usage of various essential oil recommended in traditional practice. According to our results, we suggest of the essential oil of *C. copticum* in vitro, have activities against gram-negative bacteria.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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