Evaluation of Zenian and Avishan-e Shirazi Antibacterial Activity against Vibrio cholerae Strains

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**ABSTRACT**

**Background:** The aim of this study was to determine the antibacterial activity of Zataria multiflora Boiss (Avishan-e Shirazi) and Carum copticum (Zenian) extracts on Vibrio cholerae American Type Culture Collection (ATCC14035) and V. cholerae Persian Type Culture Collection (PTCC1611) strains.

**Methods:** Antimicrobial effects of the extracts were assayed by disc diffusion and broth microdilution methods.

**Results:** Using susceptibility tests, it was shown that Carum copticum methanolic extract had the highest antibacterial effect on V. cholerae standard strains at 6.25 mg/ml concentration.

**Conclusions:** Other evaluations considering herbal extracts as an antibacterial agent as well as in vivo examination of these extracts is needed to provide a natural, cost effective and strong alternative for traditionally less effective antibiotics normally used.


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Introduction

The Gram-negative bacterium *Vibrio cholerae*, the causative agent of the severe diarrheal disease cholerae, is responsible for the deaths of approximately 120,000 people annually (1, 2). Humans are the only known vertebrate host for *V. cholerae* and following ingestion, the bacteria must survive passage through the gastric barrier of the stomach (2). Cholerae is a diarrheal disease that remains a major global health problem with several hundreds of thousands of detected cases each year (3). This disease is contracted by ingestion of contaminated food or water and is therefore associated with inadequate sanitation and poverty (2). The investigation of medicinal properties of different plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities, convenience to users, low toxicity and economic viability (4).

*Zatariamulti flora Boiss* which belongs to Labiatae family grows in countries such as Pakistan, Afghanistan and Iran. Traditionally it has been used as a diuretic, an antiseptic, a flavoring, a carminative, an antispasmodic agent as well as for premenstrual pain, edema, sore throat, jaundice, chronic catharsis and asthma treatment. *Z. multiflora* has been reported to have applicable medical properties including pain-relieving, immune-stimulant, antibacterial, anti candidal, antifungal, antioxidant, anti-nociceptive, and anti-inflammatory effects (5). *Carum copticum* grows in Egypt, East of India and Iran with bright flowers and brownish seeds which have thymol like odour. Its essential oil contains $\alpha$-pinene, paracymene, terpinene, $\beta$-pinene and other components such as thymol and carvacrol. The seeds can be used as a diuretic anti-vomiting, analgesic, anti-asthma, anti-dyspnea, also have a wonderful effect on skin, neural and urinary tract disorders (6, 7). The aim of this study is the evaluation of *Zatariamulti flora Boiss* and *Carum copticum* methanolic, acetonic and chloroformic extracts on *Vibrio. cholerae* ATCC14035 and *Vibrio cholerae* PTCC 1611 strains.

Methods

Extraction Method

Leaves of *Zatariamulti flora Boiss* and seeds of *Carum copticum* were collected from Fars province in Iran, during 2012. Leaves of the *Zatariamulti flora Boiss* and seeds of *Carum copticum* (400 gr) were dried at 25$^\circ$C and then powdered using a mechanical grinder. Ten gram of dried plant was soaked in 100 ml methanol (96% v/v), acetone (99%) and chloroform (99.4% purity) (Merck, Germany) for a period of 48 hours without any heating procedure. Each extract was first filtered through Whatman No. 1 filter paper and then through a 0.45 µm membrane filter. The filtrate was evaporated under reduced pressure in vacuum evaporator and stored at 4$^\circ$C. After drying, extracts were preserved at -20ºC.

Disk diffusion method for plant extracts

The microbial growth inhibitory potential of the each extracts was determined using the agar disk diffusion method as described by CLSI. The extracts were diluted to concentrations ranging from 25 to 0.19 mg/ml and 10 microliter of the plant extracts were transferred onto blank disks (Mast Group, UK). Each Mueller-Hinton agar plate was uniformly seeded by sterile swab dipped in *V. cholerae* ATCC14035 and *V. cholerae* PTCC1611 suspension and then streaked on the agar plate surface and then blank disks were placed on Mueller-Hinton agar plate.
surface. The plates were then incubated at 37 ºC for 24 hours under aerobic condition then zones of inhibition were measured (8).

Figure 1. Disc diffusion method results. A: 12.5 mg/ml, B: 6.25 mg/ml, C: 3.125 mg/ml, D: 1.56 mg/ml, E: 0.78 mg/ml, F: 0.39 mg/ml of Avishan-e Shirazi extract.

**Result**

Antibacterial potency of *Carum coticum* and *Zataria multiflora* extracts were evaluated by agar disc diffusion method as described by CLSI against *V. cholerae* ATCC14035 and *V. cholerae* PTCC 1611 strains (8). It was evident from the measurement of the respective zones of inhibition (Table 1) that *Carum coticum* methanolic extract exhibited stronger inhibitory effect (Figure 1). MIC and MBC (mg/ml) results of *Carum coticum* and *Zataria multiflora* against *V. cholerae* ATCC14035 and *V. cholerae* PTCC 1611 strains are shown in Table 2.

**Discussion**

Diarrhea caused by *V. cholerae* is treatable, primarily by rehydration and antibiotic therapy. The most important treatment is to replace the fluids and electrolytes that have been lost due to diarrhea (9). This is done either through oral fluid rehydration or, in severe cases, intravenous fluid rehydration. In many cases, antibiotics are used to hasten the recovery, but they do not take the place of early and appropriate rehydration therapy. Increase in the number of multidrug resistant pathogens and the accompanied rise in case fatality rate has hampered the treatment of many infectious diseases including cholera (10). The antimicrobial activity of *Carum coticum* and *Zataria multiflora* have been proved in many other studies but antibacterial effects of these plants against *V. cholerae* strains has not been studied before (5, 11). The present study supports the idea that *Carum coticum* and *Zataria multiflora* extracts might be useful as antibacterial agent against *V. cholerae* strains.
The acetonic, methanolic and chloroformic of Zataria multiflora and Carum copticum methanolic extract had promising MIC value against all V. cholerae strains at concentrations 6.25 mg/ml and 12.5 mg/ml. In this study, the antibacterial activity of the two plant extracts against V. cholerae strains were evaluated for the first time. In 2010, Saei-Dehkordi and colleagues reported that P. aeruginosa growth can be inhibited by the use of 2-8 mg/ml of Zataria multiflora Boiss essential oil (11).

Sharififar and colleagues haves known that essential oil and methanol extract of Zataria multiflora Boiss have an inhibitory effect on S. aureus, E. coli, K. pneumoniae, S.epidermidis, E. faecalis, B. subtilis, S.typhi, S.marcescens and S.flexneri. Furthermore, Mahboubi and colleagues have shown that staphylococcus growth can be inhibited by 0.5-1 µl/ml of Zataria multiflora Boiss essential oils (5).
Conclusion

Methanolic extract of *Zataria multiflora Boiss* and *Carum copticum* had better effect on *V. cholerae* standard strains than other extract types. Other investigations considering the effects of herbal extracts as antibacterial agents as well as in vivo examination of these extracts are needed to provide a natural, cost effective and strong alternative for traditionally less effective antibiotics.

Conflict of interest

None declared conflicts of interest.

References
