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 α-pinene, paracymene, terpinene, β-pinene and other components such as thymol and carvacrol. The seeds can be used as a diureticanti-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 25oC 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 4oC. 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).

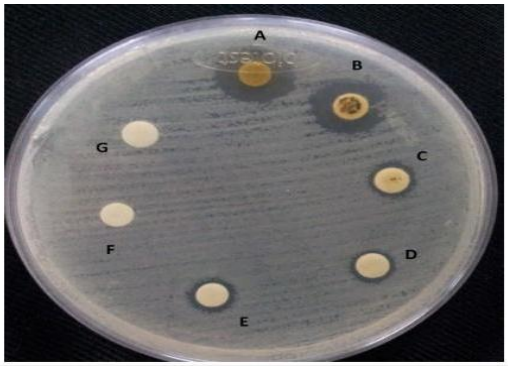

Figure 1. Disc diffusion method results. A; 12.5 mg/ml, B; 6.25 mg/ml,C;3.125 mg/ml, D;1.56 mg/ml, E;0.78 mg/ml, F,0.39 mg/ml of Avishan-e Shirazi extract.

Determination of MIC and MBC values by broth Microdilution assay

The minimum inhibitory concentration (MIC) of the extracts was determined according to the method described by CLSI (8). *Zataria multiflora Boiss* and *Carum copticum* extracts were diluted with 2% DMSO to yield concentrations ranging from 25 to 0.19 mg/ml. Cation-adjusted Muller Hinton broth was used as broth medium. After shaking, 0.1ml of each extract plus Muller Hinton broth was added to one well of 96-well microtiter plates. *V. cholerae* ATCC14035 and *V. cholerae* PTCC 1611 suspensions were adjusted to 0.5 McFarland and diluted 1:20. Then 0.01 ml of the each bacterial suspension was added to each well. Microplates were incubated aerobically at 37 ºC for 18-24 hours. The lowest concentration of the extracts that produced no visible bacterial growth was reported as the MIC. MBC values were determined according to the method described by CLSI.

Result

Antibacterial potency of *Carum copticum* and *Zatariamulti flora* 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 copticum* methanolic extract exhibited stronger inhibitory effect (Figure 1). MIC and MBC (mg/ml) results of *Carum copticum* and *Zataria multiflora* gainst *V. cholera* 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 cholerae (10). The antimicrobial activity of *Carum copticum* and *Zatariamulti flora* 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 copticum* and *Zataria multiflora* extracts might be useful as as antibacterial agent against *V. cholerae* strains.

Table 1. The comparison of *Carum copticum* and *Zataria multiflora* extracts and carbapenem antimicrobial activity against *V. cholerae* strains

Strain	Carum copticum									Zataria multiflora								
	Methanol			Acetone			Chloroform			Methanol…			Acetone…			Chloroform		
	12.5	6.25	3.12	12.5	6.25	3.12	12.5	6.25	3.12	12.5	6.25	3.12	12.5	6.25	3.12	12.5	6.25	3.12
Vibrio cholerae PTCC 1611	10	8	10	12	-	-	-	-	-	10	-	-	16	5	10	12	9	-
Vibrio cholerae ATCC 14035	15	13	8	10	-	-	-	-	-	10	8	-	15	12	11	14	11	9

The acetonic , methanolic and chloroformic of *Zataria multi flora* 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).

Table 2. Minimum inhibitory concentration (MIC mg/ml) of *Carum copticum* and *Zataria multiflora* extracts for *Vibrio cholerae* strains

Strain	*Carum copticum*						*Zataria multi flora*					
	Methanol		Acetone		Chloroform		Methanol		Acetone		Chloroform	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
***V .cholerae* PTCC 1611**	6.25	6.25	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
***V .cholerae* ATCC 14035**	6.25	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

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.

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