

## Full Length Research Paper

# Inhibitory activity of Iranian endemic medicinal plants against *Vibrio parahaemolyticus* and *Vibrio harveyi*

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Accepted 4 November, 2011

Antibacterial activity of six medicinal herbs and aromatic plants used in cooking was investigated with two *Vibrio* species. Essential oils of *Thymus daenensis* Celak., *Satureja bachtiarica* Bunge., *Satureja khuzestanica* Jamzad., *Zataria multiflora* Boiss., *Achillea kellalensis* Boiss. and *Cuminum cyminum* L. were screened against *Vibrio parahaemolyticus* and *Vibrio harveyi* local isolated from infected fishes and shrimps by serial dilution assay. The essential oils of *S. bachtiarica*, *S. khuzestanica*, *T. daenensis* and *Z. multiflora* showed antibacterial activities against *V. parahaemolyticus* and *V. harveyi*. The highest level of antibacterial activity against *V. parahaemolyticus* was demonstrated by the essential oil of *T. daenensis* (minimal inhibitory concentration (MIC) = 7 µg/ml), and the highest level of antibacterial activity against *V. harveyi* was demonstrated by the essential oil of *S. bachtiarica* (MIC = 15 µg/ml). The minimal bactericidal concentration (MBC) for *V. parahaemolyticus* and *V. harveyi* was demonstrated by the essential oil of *S. bachtiarica* (MBC = 31 µg/ml). This is the first report of an inhibitory activity of Iranian medicinal plants, on strains of *V. parahaemolyticus* and *V. harveyi*.

**Key words:** Iranian medicinal plants, antibacterial activity, *Vibrio parahaemolyticus*, *Vibrio harveyi*.

## INTRODUCTION

The biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. Among the groups of microorganisms that cause serious losses in shrimp culture, the best known are bacteria because of the devastating economic effects they have on affected farms. Vibriosis is one of the major disease problems in shellfish and finfish aquaculture. Vibriosis is a bacterial disease responsible for mortality of cultured shrimp worldwide (Lightner and Lewis, 1975).

Vibriosis is caused by a number of *Vibrio* species of bacteria, including: *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio alginolyticus* and *Vibrio penaeicida* (Brock and Lightner, 1990; Ishimaru et al., 1995). There have been occasional reports of vibriosis caused by *Vibrio damsela*, *Vibrio fluvialis* and other undefined *Vibrio* species (Lightner, 1996). *Vibrio* species

are ubiquitous in the aquatic environment. They appear at particularly high densities in marine organisms including corals, fish, molluscs, sea grass, sponges, shrimps and zooplankton (Thompson et al., 1997). Among the major diseases caused by *Vibrio* species is cholera, which occurs when *Vibrio cholerae* colonizes the small intestine and releases an enterotoxin (Gopal et al., 2005).

*V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus* are also known to cause seafood-borne infections such as septicemia and wound infections, and *V. vulnificus* has been reported to be responsible for 95% of seafood-related deaths (Todar, 2010).

Extra intestinal *Vibrio* infections often result in serious disability or death (Whitman and Griffin, 1993). Infections by *V. vulnificus*, *V. parahaemolyticus*, and possibly *V. cholerae* non-01 are more likely to cause primary septicemia in persons with pre-existing liver disease such as chronic hepatitis, cirrhosis, iron-storage diseases and compromised immune system from conditions like chronic renal insufficiency, cancer, or diabetes (Angulo and Swerdlow, 1995).

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*V. parahaemolyticus* is gram negative, non-sporeforming bacterium normally found in marine environments. It is the most likely *Vibrio* species to be implicated in foodborne disease. *V. parahaemolyticus* is found mainly in foods of marine origin, and studies carried out in the united state found that 60 to 100% of seafood samples were contaminated with the organism. The strains of *V. parahaemolyticus* produce a heat stable haemolysin, which can be performed in food. This haemolysin is thought to be responsible for the illness although other toxins could also be involved (Sakazaki et al., 2005; Nair et al., 2006).

*V. harveyi*, a gram-negative, luminous bacterium, is one of the important etiologic agents of mass mortalities of *Penaeus monodon* larval rearing systems. A large number of shrimp hatcheries (over 280) along the coastline of our country involved in shrimp seed production often suffer setbacks due to luminescent bacterial disease and suffer enormous economic losses. Among the *V. harveyi* isolates, some are virulent and some are not, suggesting a great deal of molecular and genetic variation in this group of bacteria. Luminescent *V. harveyi* appears to release exotoxins (Liu et al., 1996) and may cause 80 to 100% mortality in *P. monodon* hatcheries (Harris, 1995).

Bacterial diseases in aquaculture are mainly controlled by antibiotics. However, continuous intensive use of antibiotics is undesirable as this leads to the development of drug resistance and thereby to a reduced efficacy of the drugs. In the public health context, antibiotic resistance can be transferred to environmental and human pathogenic bacteria (Alderman and Hastings, 1998; MacMillan, 2001). In addition, antibiotics accumulate in the environment and fish, posing a potential risk to consumers and to the environment in general. Antibiotics (such as oxytetracycline, erythromycin and tetracycline) are widely used to prevent bacterial disease in fish and shrimp.

*Vibrio* species are not an exception when it comes to antibiotic resistant strains. Several studies (Ottaviani et al., 2001; Jun et al., 2001; Adeleye et al., 2008; Okoh and Igbinsosa, 2010) have reported the emergence of such strains. The development of antibiotic resistance outpaces the development of new drugs such that it has become a worldwide problem with deleterious long-term effects (Planta, 2007).

In developing countries, factors such as inadequate access to effective drugs, unregulated dispensing and manufacture of antibiotics and truncated antibiotic therapy because of cost are contributing to the development of multi-drug resistant organisms (Planta, 2007). Traditional medicines represented mainly by plants have become an alternative as they are considered synthetic antibiotics. Hence, the need to increase the relatively safer and more affordable when compared to body of knowledge on the antimicrobial activities of some traditional medicinal plants towards

curbing the effects of antibiotic resistance in *Vibrio* species becomes imperative.

The medicinal herbs contain physiologically active principles that over the years exploited in traditional medicine for the treatment of various ailments as they have anti-microbial properties (Kelmanson et al., 2000; Srinivasan et al., 2001). Antimicrobial properties of herbs have been documented in ancient literature and the interest continues to the present.

However, few of these are investigated for their antimicrobial. Several of these spices and their essential oils reported to possess antimicrobial activities including garlic, savory, basil, laurel, mint, cumin, onion, sumac and thyme (Arora and Kaur, 1999; Delgado et al., 2004; El-Khateib and Abd El- Rahman, 1987; Nasar-Abbas and Kadir, 2004; Ozcan and Erkmen, 2001; Shelef, 1983).

The results a study showed that the methanolic extract of *Garcinia kola* seeds had anti-*Vibrio* activities against 50 *Vibrio* isolates and the percentages of cells killed during the rate of kill experiment varied from 48.8 to 78.1% (Penduka et al., 2011). Also, they reported that the rate of kill of the selected test *Vibrio* species by the methanol extract proved to be generally concentration and time dependent, with the rate of kill increasing with increasing concentration of the extract and times of exposures (Penduka et al., 2011).

In this paper, therefore we report on the anti-*Vibrio* potentials of essential oils of some medicinal plants. Six Iranian medicinal plants, including *Thymus daenensis*, *Satureja bachtiarica*, *Satureja khuzestanica*, *Zataria multiflora*, *Achillea kellalensis* and *Cuminum cyminum* have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari in Iran (Ghasemi Pirbalouti, 2009a). This paper describes the use of these Iranian medicinal plants as treatment against vibriosis disease caused by *Vibrio* in fish and shrimp.

## MATERIALS AND METHODS

### Plant materials

*T. daenensis*, *S. bachtiarica* and *A. kellalensis* from Zagross mountains, Chaharmahal va Bakhtiari province, *S. khuzestanica* from north Khuzestan province, *C. cyminum* from Khorasan province, *Z. multiflora* from Fars province, Iran were collected during May to June, 2011 (Table 1). Their identity was confirmed using monographs by Ghahraman (1987 to 1989), Mozaffarian (2007), Rechinger (1963 to 1998), and voucher specimens were deposited at the Research Centre of Medicinal Plants, Islamic Azad University, Shahrekord Branch, Iran.

### Extract preparation

The aerial parts of *T. daenensis*, *Z. multiflora*, *S. bachtiarica*, *S. khuzestanica* and fruits of *C. cyminum* and flowers of *A. kellalensis* were air-dried, and then ground into fine powder using grinder and stored at room temperature. Dried plant materials were powdered (200 g) and subjected to hydro-distillation (in 2000 ml distilled

**Table 1.** Six medicinal herbs and aromatic plants used an anti-*Vibrio* activity.

Scientific name	Family name	Local name	Habit	Parts used
<i>Satureja bachtiarica</i> Bunge.	Lamiaceae	Marzeh e Koohi	Herb	Aerial plant
<i>Satureja khuzestanica</i> Jamzad	Lamiaceae	Marzeh e Khuzestani	Herb	Aerial plant
<i>Thymus daenensis</i> Celak.	Lamiaceae	Avishan e Denaei	Herb	Aerial plant
<i>Achillea kellalensis</i> Boiss. and Hauskn	Asteraceae	Golberenjazz	Herb	Flowers
<i>Zataria multiflora</i> Boiss.	Lamiaceae	Avishan e Shirazi	Herb	Aerial parts
<i>Cuminum cyminum</i> L.	Apiaceae	Zireh e Sabz	Herb	Fruits

**Table 2.** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of essential oils against *V. harveyi* and *V. parahaemolyticus*.

Essential oil	<i>Vibrio parahaemolyticus</i>		<i>Vibrio harveyi</i>	
	MIC ( $\mu\text{g/ml}$ )	MBC ( $\mu\text{g/ml}$ )	MIC ( $\mu\text{g/ml}$ )	MBC ( $\mu\text{g/ml}$ )
<i>Satureja bachtiarica</i> Bunge.	31	31	15	31
<i>Satureja khuzestanica</i> Jamzad	31	125	31	125
<i>Thymus daenensis</i> Celak.	7	31	31	125
<i>Achillea kellalensis</i> Boiss. and Hauskn	> 500	> 500	> 500	> 500
<i>Zataria multiflora</i> Boiss.	31	125	31	125
<i>Cuminum cyminum</i> L.	> 500	> 500	> 500	> 500
Gentamicin	7	31	31	125
Tetracycline	31	125	31	125

water) for 4 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1988). The essential oil samples were stored in universal bottles and refrigerated at 4°C prior to use.

#### Bacterial strain

Local strains of *V. parahaemolyticus* and *V. harveyi* were isolated from the infected fish and shrimp at the Research Centre of Aquaculture, Islamic Azad University, Shahrekord Branch, Iran. The isolates were identified as *Vibrio* using conventional morphological as well as biochemical tests. The bacteria were kept frozen in 15% glycerol and 85% saline solution. The density of bacteria culture required for the test was adjusted to 0.5 McFarland standards, ( $1.0 \times 10^7$  CFU/ml) measured using the spectrophotometer (Eppendorf, AG, Germany). Absorbance at 600 nm of known bacterial densities was determined to obtain a standard calibration curve. Subsequent dilutions were made from the previous suspension, which were then used in tests.

#### Antimicrobial test

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) values were determined by serial dilution assay (Iennette, 1985). The MIC was the lowest concentration at which bacteria failed to grow in liquid media, but viable when 100  $\mu\text{l}$  samples were plated on agar media. The MBC was the lowest concentration at which bacteria failed to grow in liquid media, but were not cultured after 100  $\mu\text{l}$  samples were plated on agar media (Smith-Palmer et al., 1998).

All essential oils were initially tested at 500  $\mu\text{g/ml}$  and serially diluted to 7  $\mu\text{g/ml}$ . Pepton water (Merck, Germany) was used to

prepare the culture medium (pH = 7.2) and autoclaved at 121°C for 15 min. Each tube was inoculated with 5 ml of bacterial suspension ( $1 \times 10^7$  CFU/ml) and incubated at 37°C for 72 h. The growth of microorganisms was observed as turbidity, determined by the measure optical density at 600 nm, by spectrophotometer. Gentamicin and tetracycline were included as positive control (at 500  $\mu\text{g/ml}$  and serially diluted to 7  $\mu\text{g/ml}$ ). Extract-free solution was used as a negative control. Control tubes were incubated under the same condition. All assays were carried out in triplicate.

## RESULTS AND DISCUSSION

The results of the anti-*Vibrio* activities of essential oils of Iranian medicinal plants are shown in Table 2. The MIC values for active essential oils ranged between 500 and 7  $\mu\text{g/ml}$ . The essential oils of aerial parts of *S. bachtiarica*, *T. daenensis*, *S. khuzestanica* and *Z. multiflora* showed the best antibacterial activities against *V. harveyi* and *V. parahaemolyticus* (Table 2). But, other essential oils such as *A. kellalensis* and *C. cyminum* showed only a slight inhibition of the tested microorganisms.

The highest level of antibacterial activity against *V. parahaemolyticus* was demonstrated by the essential oil prepared from the aerial parts of *T. daenensis* (MIC = 7  $\mu\text{g/ml}$ ), and the highest level of antibacterial activity against *V. harveyi* was demonstrated by the essential oil prepared from the aerial parts of *S. bachtiarica* (MIC = 15  $\mu\text{g/ml}$ ). The minimum bactericidal concentration for *V. harveyi* and *V. parahaemolyticus* was demonstrated by

the essential oil prepared from the aerial parts of *S. bachtiarica* (MBC = 31 µg/ml).

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Cowan, 1999). Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases (Chariandy et al., 1999). Brul and Coote (1999) have previously discussed the mechanisms of how natural compounds in herbs exert their function. Many compounds are responsible for plant flavor, and humans to season the food, and serve as useful medicinal compounds use some of the same herbs and spices. Some studies claim that the phenolic compounds present in spices and herbs play a major role in their antimicrobial effects (Hara-Kudo et al., 2004).

The essential oil and extract of some aromatic plants with a higher percentage of carvacrol and thymol (e.g. the mint family, Lamiaceae), have a higher efficacy against the bacterial strains (Rasooli et al., 2006). For example, the essential oils of *T. daenensis* and *S. bachtiarica* contained high levels of phenolics (thymol and carvacrol), and exhibited antibacterial activity (Ghasemi, 2009b, 2011a). In present study, the essential oils of *T. daenensis* and *S. bachtiarica* showed the best antibacterial activities against *V. harveyi* and *V. parahaemolyticus*. Previous studies results showed that the essential oils of *S. bachtiarica* and *T. daenensis* exhibited antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes*, *Candida albicans* and *Klebsiella pneumonia* (Ghasemi et al., 2010, 2011b). Also, the essential oil of *S. bachtiarica* had best antibacterial activity against *Streptococcus iniae* (MIC = 39 µg/ml) isolated from rainbow trout (Ghasemi et al., 2011c), and the essential oil of *S. bachtiarica* exhibited antifungal activity against *Saprolegnia parasitica* from cutaneous lesions of *Oncorhynchus mykiss* eggs (Ghasemi et al., 2009).

The compounds from essential oil *S. bachtiarica* isolated including 20% carvacrol and 19% thymol before flowering and 26% carvacrol and 5% thymol at full flowering stage, as main components (Sefidkon and Jamzad, 2000). Sefidkon et al., 2007 reported that the anti-bacterial effect of *S. bachtiarica* oil on five gram positive bacteria including: *B. subtilis*, *B. cereus*, *Micrococcus luteus*, *Staphylococcus* sp. and *S. aureus*; three gram negative bacteria including: *K. pneumonia*, *Klebsiella oxytoca* and *P. aeruginosa* was stronger before flowering stage, because of more percentage of phenolic compounds (thymol and carvacrol).

In a research, thirteen popular Thai condiments were screened for inhibitory activity against the pandemic strain of *V. parahaemolyticus* PSU 335 (Vuddhakul et al., 2007). They reported that freshly squeezed extracts of

three condiments, galangal (MIC = 1:16 and MBC = 1:16), garlic and lemon showed inhibitory activity. Velmurugan and Citarasu (2010) found antibacterial activities of methanolic extracts of *Murraya koenigi*, *Psoralea corylifolia* and *Quercus infectoria* against the bacterial pathogens such as *V. harveyi*, *P. aeruginosa* and *S. aureus* isolated from infected Indian white shrimp (*Fenneropenaeus indicus*). The results of other study (Penduka et al., 2011) showed that the methanolic and aqueous extracts of *G. kola* seeds had anti-*Vibrio* activities against 50 *Vibrio* isolates obtained from wastewater in the Eastern Cape Province, South Africa.

The present results showed that the essential oil of *C. cyminum* had no effect on *V. harveyi* and *V. parahaemolyticus*. But, previous studies showed that the essential oil of *C. cyminum* exhibited antimicrobial activities against *V. cholerae*, *S. aureus* and *E. coli* (Derakhshn et al., 2011).

## Conclusion

Medicinal and aromatic plants with antimicrobial activities have become more interesting because some of them are part of the arsenal of modern antimicrobial drugs and many people are aware of problems associated with the over-prescription and misuse of traditional antibiotics. In this study, the anti-*Vibrio* activity of endemic Iranian plants was investigated. We found the essential oils of *S. bachtiarica*, *T. daenensis*, *S. khuzestanica* and *Zataria multiflora* showed anti-*Vibrio* activity against *V. harveyi* and *V. parahaemolyticus*. The present study suggests that the essential oils of these plants are a potential source of natural antibacterial against *Vibrio*. The low MIC values observed for some of essential oils are good starting points for further research that can lead to the isolation, purification and characterization of active compounds for new anti-*Vibrio* drug development purposes.

In the present study, we demonstrated the potent antibacterial activity of *S. bachtiarica* essential oil against foodborn pathogens strains, which justifies the large use of this plant in traditional medicine. However, further research is needed to evaluate the effectiveness of *S. bachtiarica* essential oil and extract in food ecosystems to establish their utility as natural antimicrobial agents in food preservation and safety.

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