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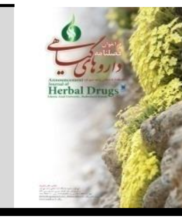

Antibacterial activity of the essential oils from five endemic herbs (Lamiaceae)

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ABSTRACT

Background & Aim: The major aim of this study was to determine the antibacterial activity of the essential oils from five medicinal plants.
Experimental: The hydro-distillation essential oils from five plant species, including *Satureja bachtiarica*, *Zataria multiflora*, *Thymus daenensis*, *Thymus carmanicus*, and *Ziziphora tenuior* (Lamiaceae) which are endemic in Iran. *In-vitro* antibacterial test was done by agar disc diffusion and serial dilution assays.
Results & Discussion: Most of the essential oils indicated relatively antibacterial activity against the tested bacteria with the diameter of inhibition zone ranging between 7 and 33 mm.
Recommended applications/industries: The essential oils from Iranian herbs could be used as natural antimicrobial agents in food preservation.

1. Introduction

There is worldwide concern about the appearance and the rise of bacterial resistance to commonly used antibiotics. In this regard, programs for monitoring resistance have been implemented in many countries for the purpose of protecting the health of humans as well as animals (Cizman, 2003; Aarestrup, 2004). In recent years there is increasing incidence of multiple resistances in human and animal pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Westh *et al*., 2004). This has forced scientist to search for new antimicrobial substances from various sources like medicinal plants. Search for new antibacterial agents should be continued by the screening of many plant families (Parekh & Chanda, 2006).

Plant substances play a major role in primary health care as therapeutic remedies in many developing countries until today (Zakaria, 1991). Medicinal herbs contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments as they contain antimicrobial properties (Kelmanson *et al*., 2000; Srinivasan *et al*., 2001).

Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents. Use of essential oils as antimicrobial agents in food systems may be considered as an additional intrinsic determinant to increase the safety and shelf life of foods (Nejad Ebrahimi *et al*., 2008). Chemical medicines, because of their harmful and irreversible effects on people, are slowly being replaced by active substances of plants (Banerjee, 2002).

Iranian medicinal plants, including *Satureja bachtiarica*, *Zataria multiflora*, *Thymus daenensis*, *Thymus carmanicus*, and *Ziziphora tenuior* have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari in Iran (Ghasemi Pirbalouti, 2009). The aim of the present study is to reveal the antibacterial properties of listed medicinal plants through *in vitro* investigation.

2. Materials and Methods

2.1. Plant material

Plants were selected for this study based on their medicinal usages in Iran. Their identity was confirmed and voucher specimens were deposited at the Research Center for Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch, Iran.

2.2. Essential oil extraction

The aerial parts of plants were powdered (200 g) and subjected to hydro-distillation (2000 ml distilled water) for 4 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The oils were stored in universal bottles and refrigerated at 4 °C prior to use.

2.3. Bacterial strains

Six bacterial strains used in this study, including *Bacillus cereus*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Proteus vulgaris* were obtained from the culture collection at Persian Institute of Industrial and Scientific Research (Persian Type Culture Collection). Cultures of bacteria were grown on Blood agar (Merck, Germany).

2.4. Sensitivity testing

The sensitivity testing of the essential oils were determined using agar well diffusion method (Irobi *et al.*, 1996). The MIC of the oils was also determined using a two-fold dilutions method. The bacterial isolates were first grown in nutrient broth for 18 h before use. The inoculum suspensions were standardized and then tested against the effect of the two plant oils at a concentration of 20 µg/ml each in DST medium. The plates were later incubated at 37°C ± 0.5°C for 24 h after which they were observed for zones of inhibition.

3. Results and Discussion

In the present study, five the essential oils from folklore medicinal plants have been tested for their antibacterial effect against six microbial strains were recorded (Table 1). Most of the oils indicated relatively antimicrobial activity against the tested bacteria with the diameter of inhibition zone ranging between 7 and 33 mm. The antimicrobial activities of herbal oils varied related to the test organism. Among the essential oils, *Satureja bachtiarica* and *Thymus daenensis* showed the best antimicrobial activity. On the other hand, concentrations of 8, 16, and 32 µg/ml of *Ziziphora tenuior* extracts had no effect on bacterial growth. The most active of the concentration was 250 µg/ml that inhibiting the growth of bacteria. The results obtained appeared to confirm the antibacterial potential of the plants investigated.

During the last years, the antimicrobial effect of medicinal plants is well documented all over the world. In Pakistan, the methanolic, hot water and cold water extracts of *Pistacia integerrima* (gall), *Polygonum Bistorta* (root), *Swetia charita* (stem), and *Zingiber officinale* (root) were screened against gram positive bacteria, such as *Bacillus subtilis* (ATCC6633), *Entereococcus faecalis* (ATCC14506) and *Staphylococcus aureus* (ATCC6538), and gram negative bacteria like *Pseudomonas aeruginosa* (ATCC27853) and *Salmonella typhi* (ATCC14028) for their antibacterial efficiency. The maximum zone of inhibition of 19 mm of methanolic extract of *Zingiber officinale* was observed against *Staphylococcus aureus*, while the maximum zone of inhibition of 15 mm of cold water extract of *Zingiber officinale* against *Pseudomonas aeruginosa* and 15 mm of cold water extract of *Swetia charita* against *Bacillus subtilis* were observed (Khalid *et al.*, 2011). The antimicrobial activities of the extracts of eight Iranian traditional plants, including *Hypericum scabrum*, *Myrtus communis*, *Pistachia atlantica*, *Arnebia euchroma*, *Salvia hydrangea*, *Satureja bachtiarica*, *Thymus daenensis* and *Kelussia odoratissima*, were investigated against *Escherichia coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes* and *Candida albicans* by agar disc diffusion and serial dilution assays (Ghasemi Pirbalouti *et al.*, 2010). Most of the extracts showed a relatively high antimicrobial activity against all the tested bacteria and fungi (Ghasemi Pirbalouti *et al.*, 2010). According to a report of Rasooli et al. (2006), various concentrations of essential oils from *Thymus eriocalyx* and *Thymus x-porlock* tested on agar plates and in broth tubes showed very strong anti-listeria properties. Also, they reported that *Thymus x-porlock* was a stronger bactericidal agent than *Thymus eriocalyx* oil.

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 antibacterial activity of endemic Iranian plants was investigated.

4. Conclusion

In the present study, we demonstrated the potent antibacterial activity of Iranian herbs oils against food-borne pathogens strains, which justifies the large use of this plant in traditional medicine. However, further research is needed to evaluate the effectiveness of these extract in food ecosystems to establish their utility as natural antimicrobial agents in food preservation and safety.

Table 1. Antibacterial activity of Iranian herbal extracts on six bacterial strain.

Bacterial strain			***S. bachtiarica***			
	8 (µg/ml)	**16 (µg/ml)**	**32 (µg/ml)**	**64 (µg/ml)**	**128 (µg/ml)**	**256 (µg/ml)**
Bacillus cereus	12[a]	15	23	26	29	33
Streptococcus agalactiae	9	13	17	19	22	27
Staphylococcus aureus	12	13	14	17	16	19
Listeria monocytogenes	10	12	24	27	29	31
Salmonella typhimurium	17	19	20	22	22	26
Proteus vulgaris	10	12	13	16	19	24
				Z. multiflora		
Bacillus cereus	9	11	13	18	19	23
Streptococcus agalactiae	10	11	11	17	22	27
Staphylococcus aureus	-[b]	-	13	16	18	19
Listeria monocytogenes	-	-	10	18	23	25
Salmonella typhimurium	-	12	13	16	23	26
Proteus vulgaris	12	12	12	15	23	25
				T. daenensis		
Bacillus cereus	13	18	19	21	25	27
Streptococcus agalactiae	14	16	18	21	24	27
Staphylococcus aureus	11	14	16	19	23	27
Listeria monocytogenes	13	17	19	23	25	26
Salmonella typhimurium	11	15	18	23	25	29
Proteus vulgaris	15	18	23	25	29	33
				T. carmanicus		
Bacillus cereus	11	17	19	22	24	28
Streptococcus agalactiae	17	19	21	24	26	27
Staphylococcus aureus	16	19	21	24	26	29
Listeria monocytogenes	15	21	24	25	28	30
Salmonella typhimurium	10	15	17	18	22	25
Proteus vulgaris	15	14	17	27	28	30
				Z. tenuior		
Bacillus cereus	-	-	-	-	7	17
Streptococcus agalactiae	-	-	-	10	13	16
Staphylococcus aureus	-	-	-	-	10	15
Listeria monocytogenes	-	-	-	7	12	14
Salmonella typhimurium	-	11	12	12	13	15
Proteus vulgaris	-	-	-	-	16	16

[a] Zone of inhibition (mm)
[b] No inhibition zone

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