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Original article

Antimicrobial effect of Satureja bachtiarica extracts aqueous, ethanol, methanol and glycerin on streptococcus pyogenes, pseudomonas aeruginosa and staphylococcus epidermidis

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ABSTRACT

The Iranian medicinal plants, such as *Satureja bachtiarica* have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari in Iran. In this study, *Satureja bachtiarica* were dried in suitable condition (in shadow) after extraction with watery, ethanol 96 %, methanol 96% and 20% glycerin antimicrobial effect of extract were determined by "screening antimicrobial activity" and "disk agar diffusion test" in 10, 20, 30 and 40 mg/ml concentration of the extract against *Streptococcus pyogenes* PTCC 1447, *Pseudomonas aeruginosa* PTCC 1310 and *Staphylococcus epidermidis* PTCC 1435. The results showed that aqueous, ethanol 96%, methanol 96% and 20% glycerin extracts were quite effective in 2 mg/ml concentration on *Streptococcus pyogenes* and *Staphylococcus epidermidis* and were prevented from growth them on medium, while extracts have no certain antimicrobial effect on *Pseudomonas aeruginosa*. In "disk agar diffusion method", 10, 20, 30 and 40 mg/ml aqueous, ethanol 96%, methanol 96% and 20% glycerin extract concentrations, was inhibited effect on *Streptococcus pyogenes* and *Staphylococcus epidermidis*, but 40 mg/ml aqueous and 30 and 40 mg/ml ethanol 96%, methanol 96% and 20% glycerin extract concentrations, has inhibited effect on *Pseudomonas aeruginosa* prevent them growing. The results indicate that alcoholic and aqueous extracts of *Satureja bachtiarica* have the greatest effect on gram positive bacterium *Streptococcus pyogenes*. As a result,

aqueous and alcoholic extracts of *Satureja bachtiarica*, have been strong antimicrobial activity against many food pathogen bacteria.

1. Introduction

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes (Reiner, 1984).

Diseases caused by bacteria are widespread worldwide. The treatment of these infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (Ahmad and Beg., 2001). In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions (Babayi *et al*., 2004). Therefore, there is a need to develop alternative antibacterial drugs for the treatment of infectious diseases from various sources such as medicinal plants. Undoubtedly, medicinal plants are the prime source of drugs in both developing and developed nations, as drugs or herbal extracts for various chemotherapeutic purposes. There are about 2000 plant species known to possess medicinal value in the traditional Asian system of medicine (Bauer *et al*., 1996).

In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes *et al*., 2007). Plant derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds (Cowan, 1999). These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities (Bidlack *et al*., 2000).

The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Shiba *et al*., 2005; Gangoue *et al*., 2008). Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Abu Shanab *et al*., 2004; Shiota *et al*., 2004).

Satureja bachtiarica Bunge, a member of Lamiaceae family, is an endemic plant that is greatly distributed in southern regions in Iran. It is well known for its medical uses in folk medicine. Satureja bachtiarica has a relatively wide distribution in Iran and has been collected from West, Central and Southwest provinces of Iran. There are about 30 species of Satureja in the world that Satureja bachtiarica is an endemic species of this genus in Iran. Satureja is carminative and tonic and it is effective to boost sexual power. It is used to relieve dental pain and if is taken with the water of fig is beneficial for cough and shortness of breath and brilliant of the face. A Satureja treat the diarrhea. It is very useful. The Satureja can be use to removal of state weakness and gastric torsion. It can also be use to exploited digestive and intestinal fermentation and flatulence.

The aim of this study was evaluation of Satureja bachtiarica antimicrobial effect on Streptococcus pyogenes, Pseudomonasaeruginosa and Staphylococcus epidermidis.

2. Materials and methods

2.1. Preparation plant

Satureja bachtiarica was collected from Shahrekord (Chaharmahal va Bakhtiari, Iran). Taxonomic identification was performed by the Faculty of Science Herbarium, Ferdowsi University of Mashhad, Iran.

2.2. Extract preparation

Aqueous**,** ethanol and methanol extracts of the samples were obtained by the following procedure. The extract was prepared by maceration 1 g sample was extracted with 50 mL ethanol 96˚or Methanol 96˚ for 20 h. The mixture was filtered through Whatman No.1 and the filtrate was evaporated to dryness under vacuum at 40 °C. The dry extract was weighed and the yield was calculated. 20% glycerin solution has been used as a solvent. 50 grams of fine powder of the leaves was added to 200 ml of prepared glycerin solvent and heated for 20 minutes. The extract was then filtered using paper filters and then centrifuged in 9000g for 15 minutes (Ahmad and Beg, 2001).

2.3. Source of microorganisms

Three strains were chosen for investigation of which one was reference bacteria: Gram positive (*Streptococcus pyogenes* PTCC 1447, *Staphylococcus epidermidis* PTCC 1435) and Gram-negative (*Pseudomonas aeruginosa* PTCC 1310) all organisms were stored at –70˚C in glycerol Mueller Hinton broth. Fresh subcultures were used for each experiment.

2.4. Preparation of microbial suspension

To preparing microbial suspensions, requires 24 hours culture from each microorganism. So, 24 hours before experiments, microorganisms were inoculated from storage medium to nutrient agar medium slope. After 24 hours, the cultures were washed by Ringer solution and microbial suspensions were prepared. Then some of the bacterial suspension was poured in sterile tubes containing Ringer solution and its turbidity, was measured by spectrophotometer at 530 nm wavelength. It was diluted by Ringer solution until the solution turbidity equalizes with 0.5 McFarland standard solutions. Suspension should have contains 1.5×10^8 CFU / ml (Valero and Salmeron, 2003).

2.5. Evaluation of antimicrobial activity

Adding extracts to the culture medium “according of the method of Collins *et al.* (1995)” and “disk agar diffusion method” were done and to evaluated the antimicrobial effects of aqueous and alcoholic *Satureja bachtiarica* extracts. Then 0.2 gram of aqueous and ethanol extract, were added to 5 ml of sterile distilled water. Then it was stirred with vortex system to assist being steady. Then 1 ml of this solution was added to sterile plates. The final concentration of the extract was 2 mg/ml (Babayi *et al*., 2004). In the next step, Mueller Hinton agar (Merck-Germany) medium were sterile and used for bacteria, were added to the plates, and placed at room temperature, so the medium was prepared. One loop of each standard strain culture media was cultured inoculums on these medium. The plates were incubated for 48 hours at 37°C. The culture with extract and without bacteria was used as control (Babayi *et al*., 2004). The disk agar diffusion method, at first a loop of each standard strain culture media was cultured on the plates, and then paper discs (from Whatman filter with 6 mm diameter) placed on Mueller Hinton (Merck) plates were saturated with 100 µl of the test compound allowed to dry and was introduced on the upper layer of the seeded agar plate with 10, 20, 30 and 40 mg/ml concentrations, were prepared in distilled water and was treated with *Satureja bachtiarica* extract and placed in culture medium by Sterile loop. Then it was fixed on the media with a light little pressure. After incubation time, the diameter of free zone was measured exactly by using a ruler in millimeters. All experiments were performed with 3 replicates (Bauer *et al*., 1966).

2.6. Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analysed using one way analysis of variance (ANOVA) using SPSS version 18.

3. Results

The results of the antimicrobial effects of extracts, by “using the method of screening antimicrobial activity” were show on in Tables 1, 2, 3 and 4. The results of the antimicrobial effects of extracts, by “the agar diffusion method” were presented in Tables 5, 6, 7 and 8.

Table 1

Antimicrobial effects of 2mg/ml ethanolic *Satureja bachtiarica* extract concentrations, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.*

Microorganism	**Satureja bachtiarica**
P. aeruginosa	-
S. pyogenes	++
S. epidermidis	+

(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of ethanolic *Satureja bachtiarica* extract

(+) in Table showed no bacterial growth on culture and antibacterial activity of ethanolic *Satureja bachtiarica* extract

(++) in Table showed no bacterial growth on culture and strong antibacterial activity of ethanolic *Satureja bachtiarica* extract

Table 2

Antimicrobial effects of 2mg/ml aqueous Satureja bachtiarica extract concentrations, on Streptococcus pyogenes, Pseudomonas aeruginosa and Staphylococcus epidermidis

Microorganism	**Satureja bachtiarica**
P. aeruginosa	-
S. pyogenes	+
S. epidermidis	+

(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous Satureja bachtiarica extract

(+) in Table showed no bacterial growth on culture and antibacterial activity of aqueous Satureja bachtiarica extract

Table 3

Antimicrobial effects of 2mg/ml methanolic *Satureja bachtiarica* extract concentrations, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.*

Microorganism	***Satureja bachtiarica***
P. aeruginosa	-
S. pyogenes	++
S. epidermidis	+

(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of methanolic *Satureja bachtiarica* extract

(+) in Table showed no bacterial growth on culture and antibacterial activity of methanolic *Satureja bachtiarica* extract

(++) in Table showed no bacterial growth on culture and strong antibacterial activity of methanolic *Satureja bachtiarica* extract

Table 4

Antimicrobial effects of 2mg/ml glycerin *Satureja bachtiarica* extract concentrations, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.*

Microorganism	***Satureja bachtiarica***
P. aeruginosa	-
S. pyogenes	+
S. epidermidis	+

(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of glycerin *Satureja bachtiarica* extract

(+) in Table showed no bacterial growth on culture and antibacterial activity of glycerin *Satureja bachtiarica* extract

Table 5
Average diameter (mm) of microbial free zone area of aqueous *Satureja bachtiarica* extract, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (disk agar diffusion method).

Microorganism			***P. aeruginosa***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	-	-	-	6.4 ±0/52
Microorganism			***S. pyogenes***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	11.4±0/57	13.7±0/50	15 ±0/25	18.3±0/25
Microorganism			***S. epidermidis***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	10.2±0/57	11.9±0/57	13.6±0/52	15.8±0/28

[a]Values are means ± standard deviations, n=3.
(-) in Table showed no inhibitory effects was shown

Table 6
Average diameter (mm) of microbial free zone area of ethanolic *Satureja bachtiarica* extract, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (disk agar diffusion method).

Microorganism			***P. aeruginosa***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	-	-	6.7 ±0/57	8.8±0/28
Microorganism			***S. pyogenes***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	15±0/76	17.9±0/50	19.6 ±0/50	21.9±0/57
Microorganism			***S. epidermidis***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	13.8±0/28	14.9±0/50	16.6 ±0/76	19.1±0/57

[a]Values are means ± standard deviations, n=3.
(-) in Table showed no inhibitory effects was shown

4. Discussion

The use of plant derived natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. The curative properties of medicinal plants are due to

the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan *et al.,* 2009; Ongsakul *et al*., 2009). They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids, and essential oils. Over 50% of all modern clinical drugs are of natural origin and play an important role in development of drugs (Cordell, 1995).

Table 7

Average diameter (mm) of microbial free zone area of methanolic *Satureja bachtiarica* extract, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (disk agar diffusion method).

Microorganism			***P. aeruginosa***		
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	-	-	6.4±0/50	7.2 ±0/50
Microorganism			***S. pyogenes***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	13.3±0/57	15.3±0/50	17 ±0/28	19.4±0/28
Microorganism			***S. epidermidis***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	12.2±0/57	13.8±0/57	14.6±0/50	16.6±0/28

[a]Values are means ± standard deviations, n=3.

(-) in Table showed no inhibitory effects was shown

Table 8

Average diameter (mm) of microbial free zone area of glycerin *Satureja bachtiarica* extract, on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (disk agar diffusion method).

Microorganism			***P. aeruginosa***		
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	-	-	6.3±0/52	6.9 ±0/52
Microorganism			***S. pyogenes***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	12.3±0/57	14.1±0/50	16 ±0/28	18.9±0/28
Microorganism			***S. epidermidis***	
Satureja bachtiarica concentration (mg/ml)	10	20	30	40
Average diameter (mm) of microbial free zone area	10.9±0/57	12.5±0/57	13.9±0/50	15.9±0/28

[a]Values are means ± standard deviations, n=3.

(-) in Table showed no inhibitory effects was shown

Many herbs have been used for treating disease caused by microorganisms such as cholera, diarrhea, dysentery, Typhoid and bacterial enteritis (Cowan, 1999). Moreover, Huge economy is invested in the imports of drugs especially antibiotics from different parts of the world. Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative antibacterial regimens. The results showed that aqueous, ethanol 96%, methanol 96% and 20% glycerin extracts were quite effective in 2 mg/ml concentration on *Streptococcus pyogenes* and *Staphylococcus epidermidis* and were prevented from growth them on medium, while extracts have no certain antimicrobial effect on *Pseudomonas aeruginosa*. Based on the results ethanolic extract of *Satureja bachtiarica* in this study have significant antimicrobial activity on the studied microorganisms. The results show that *Satureja bachtiarica* aqueous, ethanol 96%, methanol 96% and 20% glycerin extracted at all concentrations (10, 20, 30 and 40 mg/ml) had the inhibitory effect on *Streptococcus pyogenes* and *Staphylococcus epidermidis* .The results show that *Satureja bachtiarica* ethanol 96%, methanol 96% and 20% glycerin extracted at concentrations (30 and 40%) had the inhibitory effect on *Pseudomonas aeruginosa*, However, 10 and 20% concentration extracts, have no significant antimicrobial effect on *Pseudomonas aeruginosa* and it is not able to prevent the growth of bacteria on culture. gram-positive bacteria are more sensitive than gram negative bacteria to *Satureja bachtiarica* extract, due to differences in cell structure of gram negative and gram positive bacteria, because gram positive bacteria have more mucopeptide in their cell wall composition while gram negative bacteria have only a thin layer of mucopeptide and most of their cell structure is lipoprotein and lipo-polysaccharides. Thus, gram-negative bacteria are more resistant (Tassou and Nychas., 1995; Ghalem and Mohamed., 2008). These points were consistent with the results obtained in this study. (Alizadeh Behbahani *et al*., 2012) report that, ethanol extract compared to the aqueous extract was more effective and have a greater deterrent. The reason of these phenomena may be extracting more effective materials extracted by ethanol from *Avicennia marina*. These points were consistent with the results obtained in this study. The results of a study (Turker *et al*., 2009) showed that the ethanol extract of Vinca minor leaves exhibited strong antibacterial activity against L. garvieae in comparison with other alcoholic and aqueous extracts from 21 species of herbs from Bolu (Turkey).

Ghasemi et al (2010) reported that essential oils of *Myrtus communis* L., *Thymus daenensis* and *Satureja bachtiarica* exhibited antimicrobial activities against Bacillus cereus, *E. coli* O157:H7, *Candida albicans* and *Listeria monocytogenes*.

5. Conclusion

In conclusion, it can suggest that *Satureja bachtiarica* extract "*in vitro*", have considerable antimicrobial ability over the studied strains. In addition, more studies are needed "*in Situ*" be done, to identifying the effective dose of the extract on the microorganisms, and finally introduce the extract as a natural and novel antimicrobial compound.

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