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Antimicrobial effect of *Satureja bachtiarica* extracts aqueous and ethanolic on *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

According to biologically active compounds and traditional use of the *Satureja bachtiarica*, seem that this plant has significant antimicrobial effects. In this study antimicrobial effect of extracts evaluated by two methods, “Collins method” and “disk agar diffusion method” on *Escherichia coli* PTCC 1330 and *Staphylococcus aureus* PTCC 1337 microorganisms. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for both species determined by using a dilution method. Statistical analysis was carried out by analysis of variance (ANOVA). All ethanolic extract concentrations had an inhibitory effect on the disk agar diffusion method. In “Collins method” ethanolic extract in 2000 µg/ml concentration, could prevent the growth of both strains on medium. The aqueous extract, had no antimicrobial significant effect in 2000 µg/ml concentration. The ethanolic extract MIC of *Satureja bachtiarica* for *Staphylococcus aureus* was 8 mg/ml, and for *Escherichia coli* was 16 mg/ml. But the aqueous extracts MIC of *Satureja bachtiarica* for *Staphylococcus aureus* was 32 mg/ml and for *Escherichia coli* was 64 mg/ml. The ethanolic extract MBC of *Satureja bachtiarica* for *Staphylococcus aureus* was 16 mg/ml, and for *Escherichia coli* was 32 mg/ml. But the aqueous extracts MBC of *Satureja bachtiarica* for *Staphylococcus aureus* was 64 mg/ml and for *Escherichia coli* was 256 mg/ml. The *Satureja bachtiarica* extract presented the more effective impact on the growth of *Staphylococcus aureus* PTCC 1337 than *Escherichia coli* PTCC 1330

($p<0.05$). The results indicate that ethanolic extract of *Satureja bachtiarica* has the greatest effect on gram positive bacterium *Staphylococcus aureus* PTCC 1337

1. Introduction

During the last several decades, natural products with antimicrobial effect are investigated in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health. Aromatic plants are known for a very long time and they are used in phototherapy and food preservation (Matan et al., 2006).

In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. Aromatic plants have been known about for a very long time and owing to their aromatic and antiseptic properties they are used as spices and natural food preservatives, in the perfume industry, for aromatherapy and for different medical purposes. Savory species produce antimicrobial secondary metabolites, essential oils, either as a part of their normal program of growth and development or in response to pathogens' attack or stress (Mihajilov-Krstev *et al*., 2010).

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 it 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 used for removal of state weakness and gastric torsion. It can also be use to exploit digestive and intestinal fermentation and flatulence (Sefidkon *et al*., 2009).

Due to the presence of secondary metabolites such as flavonoids, steroids, terpenoids and tannins they are known for their healing properties for a long time and have been used as traditional folk remedies to treat various ailments such as cramps, muscle pains, nausea indigestion, diarrhea and infectious diseases (Bezic *et al*., 2009). Present in the medicinal plants provides the bountiful resource of active compounds for the pharmaceutical, cosmetics and food industries, and more recently in agriculture for pest control (Rice, 1995). Herbal products from medicinal plants are preferred because of less testing time, higher safety, efficiency, cultural acceptability and lesser side effects. The chemical compounds present in herbal products are a part of the physiological functions of living organisms, and hence they are believed to have better compatibility with the human body (Khanna *et al*., 1986).

About 80 percent of the population of relies on traditional medicine because western-trained medical personnel are limited or not really accepted by the community, and traditional healers are easily consulted, living in the same community. That points to the demand for Traditional Medicine Practitioners (TMPs) for medicinal plants and the fact that the majority of the people, rural and urban alike, depend largely on herbal medicines for treating a variety of diseases. This reliance is mainly due to the high cost of conventional medicine and inaccessibility of modern health care facilities in most areas WHO (2002-2005).

The aim of this study was evaluated of antimicrobial effect of aqueous and ethanolic extracts of *Satureja bachtiarica* against *Escherichia coli* and *Staphylococcus aureus* of the important food pathogen.

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

Maceration method was used to prepare extracts. The amount 50 gram of *Satureja bachtiarica* powder was added to 250 ml ethanol 96˚ or water. The ethanolic and aqueous extract mixture was preserved at laboratory temperature for 24 hours. The extract was then filtered using paper filters and then centrifuged in 9000g for 15 minutes (Ahmad and beg, 2001).

2.3. Determination dry weight of alcoholic and aqueous Satureja bachtiarica extracts

At first the weight of a tube were measured, and then 1ml of alcoholic and aqueous extracts were poured in it. The contents of the tube were dried at room temperature. After drying the extract, the tubes were weighed again. Weight differences are equivalent weight of 1ml alcohol extract. Average of three replicates, was calculated as the dry weight of the extract (Sattari *et al*, 2005).

2.4. Source of microorganisms

The bacterial strain used was *Escherichia coli* PTCC 1330 and *Staphylococcus aureus* PTCC 1337 for each test, to evaluating the antimicrobial effects, fresh medium was prepared.

2.5. 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).

2.6. 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 alcoholic *Satureja bachtiarica* extracts. Then 0.2 gram of 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 2000 µg/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), 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 20, 40, 60 and 80 mg/ml extract 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*., 1996).

2.7. Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined according to agar dilution method (Benger *et al*., 2004). Various concentrations (20, 40, 80, 160, 320, 640, 1280, 2560 mg/ml) of extract was prepared in 10 cm experimental tubes containing muller Hilton broth for bacteria. Each tube contains 9 ml of muller Hilton for bacteria was sterilized by autoclaving. On cooling, 1 ml of each extract (watery and etanoli) concentration were added to each tube, to make the final concentrations of 2, 4, 8, 16, 32, 64, 128, 256 mg/ml. The mixture of muller Hilton and extract was poured into plates aseptically in a laminar flow cabinet. On solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated 35 C° for bacteria (Yazdani *et al*., 2009). The muller Hilton without any herbal extract served as control. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 12 days of incubation (compared with control).

2.8. Minimum bactericidal concentration (MBC)

MBC was determined according to agar dilution method (Espinel-Ingroff *et al*., 2002) with slight modifications. The MBC were determined by incorporating various concentrations of extracts (2-256 mg/ml) in muller Hilton broth for bacteria.The tubes which showed no visible growth after 2 days incubation were subculture on extract free muller Hilton plates and incubated at 27°C for 3days (Yazdani *et al*., 2009). The MBC was regarded as the lowest concentration of the extract that prevented the growth of any bacteria colony on the solid medium.

2.9. 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 ethanolic and aqueous extract, by "using the method of Collins *et al*., (1995)" were show on in (Tables 1, 2 and Figure 1).

Table 1
Antimicrobial effects of 2000 µg/ml ethanolic and aqueous *Satureja bachtiarica* extracts concentrations, on *E.coli* (using the method of Collins *et al*., (1995))

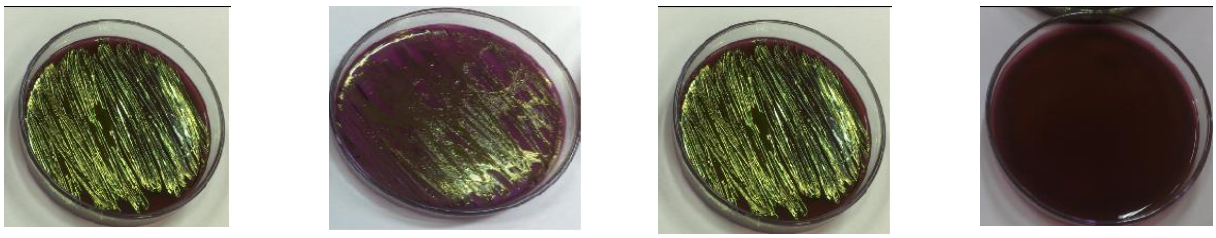
Extract	***E.coli***
Ethanolic	+
Aqueous	_

(+) in Table showed no bacterial growth on culture and antibacterial activity of ethanolic *Satureja bachtiarica* extract.
(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous *Satureja bachtiarica* extract.

Table 2
Antimicrobial effects of 2000 µg/ml ethanolic and aqueous *Satureja bachtiarica* extracts concentrations, on *S. aureus* (using the method of Collins *et al*., (1995))

Extract	***S. aureus***
Ethanolic	+
Aqueous	_

(+) in Table showed no bacterial growth on culture and antibacterial activity of ethanolic *Satureja bachtiarica* extract.
(-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous *Satureja bachtiarica* extract.



A: Control *Escherichia coli* | B: Antimicrobial effects of 2000µg/ml *Satureja bachtiarica* aqueous extract concentrations | C: Control *Escherichia coli* | D: Antimicrobial effects of 2000µg/ml *Satureja bachtiarica* ethanolic extract concentrations

Fig. 1. Antimicrobial activity of ethanolic and aqueous *Satureja bachtiarica* extract on *Escherichia coli* (using the method of Collins *et al*., (1995)).

The results showed 2000 µg/ml concentration of ethanolic extract, were quite effective on reduce of growth *E.coli* and *S. aureus* and were had prevent growth over the medium. The aqueous extract, had no antimicrobial significant effect in 2000 µg/ml concentration.

The results the antimicrobial effects of ethanolic and aqueous *Satureja bachtiarica* extracts, by "the agar diffusion method" were presented in Tables 3 and 4.

The results the MIC and MBC of ethanolic and aqueous *Satureja bachtiarica* extracts were presented in Tables 5 and 6.

Tables 3

Average diameter (mm) of microbial free zone area of by ethanolic Satureja bachtiarica extract, on *Escherichia coli* and *Staphylococcus* aureus (disk agar diffusion method).

Microorganism	**concentration (mg/ml)**			
Ethanolic *Escherichia coli*	20	40	60	80
Average diameter (mm) of microbial free zone area	6.3±0/28	7.9±0/57	9.1±0/57	11.7 ±0/28
Microorganism	**concentration (mg/ml)**			
Ethanolic *Staphylococcus aureus*	20	40	60	80
Average diameter (mm) of microbial free zone area	7.3±0/57	8.7±0/50	11 ±0/50	14.4±0/28

(-) in Table showed no inhibitory effects was shown

Tables 4

Average diameter (mm) of microbial free zone area of by aqueous Satureja bachtiarica extract, on *Escherichia coli* and *Staphylococcus* aureus (disk agar diffusion method).

Microorganism	***concentration* (mg/ml)**			
Aqueous *Escherichia coli*	20	40	60	80
Average diameter (mm) of microbial free zone area	-	-	6.6±0/57	8.6 ±0/28
Microorganism	***concentration* (mg/ml)**			
aqueous *Staphylococcus aureus*	20	40	60	80
Average diameter (mm) of microbial free zone area	6.3±0/57	7.7±0/50	10 ±0/50	11.7±0/28

(-) in Table showed no inhibitory effects was shown

4. Discussion

The plants are a reserve of biologically active substances. Essential oils can be a significant source of a great diversity of chemical species equipped with antimicrobial capacity, the *Satureja bachtiarica* can have application in therapy of the infectious diseases is like substituent of certain antibiotics or like complementary agents used in synergy with the synthesis substances. Essential oils can also have application in food industries not only like aromatizing but also like preservative of foodstuffs.

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* ethanolic extracted at all concentrations (in 20, 40, 60 and 80 mg/ml) had the inhibitory effect on *S. aureus* and *E. coli*. The results show that *Satureja bachtiarica* aqueous extracted at concentrations (in 60 and 80 mg/ml) had the inhibitory effect on *E. coli*, However, 20 and 40 mg/ml concentration extracts, have no significant antimicrobial effect on *E. coli* and it is not able to prevent the growth of bacteria on culture. The results show that *Satureja bachtiarica* aqueous extracted at all concentrations (in 20, 40, 60 and 80 mg/ml) had the inhibitory effect on *S. aureus*.

The ethanolic extract MIC of *Satureja bachtiarica* for *Staphylococcus aureus* was 8 mg/ml, and for *Escherichia coli* was 16 mg/ml. But the aqueous extracts MIC of *Satureja bachtiarica* for *Staphylococcus aureus* was 32 mg/ml and for *Escherichia coli* was 64 mg/ml. The ethanolic extract MBC of *Satureja bachtiarica* for *Staphylococcus aureus*

was 16 mg/ml, and for *Escherichia coli* was 32 mg/ml. But the aqueous extracts MBC of *Satureja bachtiarica* for *Staphylococcus aureus* was 64 mg/ml and for *Escherichia coli* was 256 mg/ml.

Table 5
Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous *Satureja bachtiarica* extracts on *Escherichia coli* and *Staphylococcus* aureus.

	concentration (mg/ml)						
Bacteria species	64	32	16	8	4	2	control
Ethanolic *Escherichia coli*	+	+	+	-	-	-	-
Ethanolic *Staphylococcus aureus*	+	+	+	+	-	-	-
Aqueous *Escherichia coli*	+	-	-	-	-	-	-
Aqueous *Staphylococcus aureus*	+	+	-	-	-	-	-

+: Positive inhibition - : Negative inhibition, n=3.

Table 6
Minimum bactericidal concentration (MBC) of ethanolic and aqueous Satureja bachtiarica extracts on *Escherichia coli* and *Staphylococcus* aureus.

	concentration (mg/ml)								
Bacteria species	**256**	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**control**
Ethanolic *Escherichia coli*	+	+	+	+	-	-	-	-	-
Ethanolic *Staphylococcus aureus*	+	+	+	+	+	-	-	-	-
Aqueous *Escherichia coli*	+	-	-	-	-	-	-	-	-
Aqueous *Staphylococcus aureus*	+	+	+	-	-	-	-	-	-

+: Positive inhibition - : Negative inhibition, n=3.

Antimicrobial effect of the extracts was different, depending on the type of microorganisms, thus, the gram positive bacterium *Staphylococcus aureus*, was higher sensitivity compared to gram negative bacteria *E. coli* (Table 3, 4). In general, the gram positive bacteria strains tested seem to be affected by the essential oil to the same extent as the gram negative bacteria strains, while previous works showed that the gram positive bacteria are more sensitive to plant oil and extracts than the gram negative ones, 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 *et al*., 2008). These points were similar with the results obtained in this study.

Also, ethanolic extract compared to the aqueous extract was more effective and has a greater deterrent. (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.

(Boroujeni *et al*., 2012) report the extracts from *S. bachtiarica* and *T. daenensis* exhibited inhibitory effect on fungal growth, suggesting that the studied plant extracts are potentially a safe and natural source of antifungal agents. Some of these plants were more effective than traditional antimicrobial to combat the pathogenic microorganisms.

(Sefidkon *et al*., 2009) showed the high antimicrobial effect of these oils. It seems the presence of thymol, carvacrol, P-cymene and gamma-terpinene in these oils caused the strong anti microbial effect of them. Several mechanisms are discussed to explain the antimicrobial effect. Several studies have been performed concerning the antimicrobial activity of essential oils or extracts of other Satureja species. Many of the previous studies demonstrated that the members of the genus Satureja show a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors (Adiguzel *et al*., 2007; Gulluce *et al*. 2003; Sahin *et al*. 2003).

In conclusion, it can suggest that *Satureja bachtiarica* extract in "*in vitro*" have considerable antimicrobial ability over the studied strains. In addition, more studies are needed in "*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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