

Original Article

Investigating antibacterial activity of *Satureja bachtiarica* against coagulase-negative staphylococcus strains isolated from hospitalized patients in ICU, Tehran-Iran

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Abstract

Given the inappropriate use of antibiotics and prevalence of resistant bacteria, there is urgent need for antibacterial drugs that have fewer side effects than antibiotics. *Satureja bachtiarica* is a medicinal plant which had many uses in traditional medicine. In this research, the ethanol extract of leave of *Satureja bachtiarica* is tested on coagulase-negative staphylococcus strains. **Materials and Methods**

In this experimental study, after collecting and drying the plant extract, the ethanolic extract of the plant was extracted by soxhlet method. Then, the antibiotic resistance pattern of coagulase-negative staphylococcus strains to Cefoxitin, Tobramycin, Kanamycin, Amikacin, Gentamicin, was assessed. To evaluate the antibacterial activity, the minimum inhibitory concentration was assessed by using micro dilution broth method.

The results of this study showed that the antimicrobial effect of *Satureja* extract is concentration dependent. The results of the antimicrobial activity of extracts, indicate that alcoholic extract in all dilutions have an antimicrobial effect on the coagulase-negative staphylococcus strains. The results show that coagulase-negative staphylococcus strains have highest resistance against Cefoxitin (0.39 mg/ml) and highest sensitivity to Amikacin (0.035 µl/ml). However, in 200 and 100 mg/ml concentrations the extract has higher antibacterial effect.

According to the results of this study, ethanol extracts of leave of *Satureja bachtiarica* had growth inhibitory effect on coagulase-negative staphylococcus strains. Therefore this plant has the potential to be evaluated as an alternative or adjunct to antibiotics to treat coagulase-negative staphylococcus infections.

Keywords: *Satureja Bachtiarica*, Coagulase-negative Staphylococci, Antimicrobial resistance

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INTRODUCTION

Coagulase-negative staphylococcus strains (CoNs) in normal skin flora and mucous membranes have recently got attention as an important hospital pathogen, specifically for nosocomial bacteremia. Currently, 15% of HAI (Hospital Acquired Infections) infections are due to CoNs (Dallal et al. 2014). Recently, these bacteria have started to gain resistance to widely used antibiotics and there is a significant increase in the methicillin-resistant staphylococci infections. Treatment options are limited for this type of infection and the available antibiotics have limited bactericidal effect on resistance bacteria (Doi et al. 2016). From morphological viewpoint, CoNs are spherical gram-positive bacteria, prevailingly non-capsulated cocci. CoNs have been steadily spreading worldwide during the last decade (Morad Asaad et al. 2015). Bacterial adaptation to antibiotics has been very successful and over the past sixty years, misuse of antibiotics has resulted in wide resistance for most available bactericidal drugs. In fact, due to this successful adaptive evolution, certain bacterial infections are almost untreatable with available antibiotics (Shree et al. 2013). Accordingly, because of antibiotic resistance the tendency of researchers and pharmaceutical companies to make antibiotic drugs has decreased. So finding new sources of antibiotics to treat the infection, is one of the most important priorities in the field of medicine. Development and production of new antibacterial drugs are very costly and time consuming (Guilhelmelli et al. 2013). Herbal medicine is one of the most important sources of antibiotic compounds and because of their antibacterial potential and less side effects, The extract of medicinal plants are one of the best candidate for development and production of novel antibiotics (Habeeb et al. 2007). In general, the genus of *Satureja* consists of 30 species and *Satureja bachtiarica* (*S. bachtiarica*) is one of the endemic species of this genus in Iran

(Soodi et al. 2015). *S. bachtiarica* species has a relatively wide distribution in Iran and widely distributed in the southern and southwest parts of country. The genus *Satureja* is well known for its therapeutic values. The chemical composition of essential oil was analyzed by GC and GC-MS. Carvacrol (45.5%) and thymol (27.9%) were the primary constituents of oil, followed by p-cymene (4.4%), and γ -terpinene (4.0%). During the last two decades scientists have become aware of its high medicinal potential (Pirbalouti & Dadfar, 2013). Thus, to implement rational strategies to minimize resistance development we need to identify new resources of antibacterial compound. Therefore, the aim of the present study was to examine antibacterial effect of *S. bachtiarica* against CoNs.

MATERIAL AND METHODS

S. bachtiarica was collected from Kohgiluyeh and Boyer-Ahmad Province. Taxonomic identification was performed by the Faculty of Science Herbarium, Shahid Beheshti University total of 40 CoNs strains were isolated from samples of patients who were hospitalized in Tehran hospitals and in Intensive Care Units (ICUs) of Shahid Beheshti University of Medical Sciences from September 2014 to October 2015. The extract of *S. bachtiarica* was prepared using Soxhlet apparatus for alcoholic extract. The amount 100 gram of *S. bachtiarica* powder was added to 300 ml ethanol 96°. The ethanolic extract was stored at 37°C temperature for 24 hours. The bactericidal activities of extract and the minimal inhibitory concentration (MIC) were assessed by using micro dilution broth method. The microplates were prepared by dispensing 100 μ l of Mueller–Hinton broth for CoNs, into each well. A 100 μ l from the stock solution of extracts was added into the first row of the plate. Then, serial dilutions were performed by using a micropipette. The

obtained concentration range was from 100 to 0.8 mg/ml, and then added 10 μ l of inocula to each well except for positive control. The test plates were incubated at 37o C for 24 h. MIC was defined as the lowest sample concentration exhibited complete growth inhibition (Performance standards for antimicrobial susceptibility testing, 2014). In addition, we compared the antibacterial effect of plant with five common antibiotic that have been using for treating CoNs infections, the antibiotics include; Cefoxitin, Tobramycin, Kanamycin, Amikacin and Gentamicin.

RESULTS

The results of this study showed that the antimicrobial effect of leave extract of *S. bachtiarica* is concentration dependent and also with increasing concentration, the growth inhibitory effect of extract was increased. The

antibacterial activity at different concentrations (12.5, 6.25, 3.125, 1.56, 0.78, 0.39 mg/ml) was determined by broth microdilution method. In addition, we evaluate the antibacterial potency of extract based on percentage; percent of bacterial colonies that grows inhibited. As demonstrated in Table 1 the results of the antimicrobial effects of extracts, indicate that alcoholic extract in all dilutions have an antimicrobial effect on the CoNs bacteria. However, in 37.50% and 20% concentration, the ethanolic extract showed significant antibacterial effect. Minimum Bactericidal Concentration (MBC), in regard to extract concentration is also depicted in the table 1. As can be seen MBC order is concentration dependent. Along with increasing extract concentration, the bactericidal effect of ethanolic extract fortified. Also, in comparing to normal group, number of suppressed bacterial colonies showed in following table. Highest inhibitory of extract belong to highest Satureja concentration (37.50% mg/ml).

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of alcoholic extract of *S. bachtiarica*

MBC(mg/ml)	MIC(mg/ml)	Number	Percentage
0.78	0.39	15	37.50%
1.56	0.78	8	20%
3.125	1.56	5	12.50%
6.25	3.125	4	10.00%
12.5	6.25	7	17.50%
50	12.5	1	2.50%

The results showed that ethanolic extract was quite effective in 12.5, 6.25 mg/mL concentrations on CoNS and had preventive characteristics on bacterial growth, while extracts have less antimicrobial effect on CoNS in 3.125 and 1.56 mg/ml. The results of comparing antibacterial effect of *Satureja bachtiarica* extract with tested antibiotics demonstrated that bactericidal effect of the extract was

significant and have similar properties like tested antibiotic. The results showed in Table 2. As depicted in following table, antibacterial effect of *Satureja bachtiarica* extract compared with common antibiotics. The results show that CoNS have highest resistance against Cefoxitin and highest sensitivity to Gentamicin.

Table 2: Comparing antibacterial effect of *S. bachtiarica* extract with tested antibiotics

Antibiotic	Intermediate	Sensitive	Resistant
Cefoxitin		11(27.5%)	29(72.5%)
Tobramycin	1(2.5%)	22(55%)	17(42.5%)
Kanamycin	2(5%)	17(42.5%)	21(52.5%)
Amikacin	1(2.5%)	33(82.5%)	6(15%)
Gentamicin		21(52.5%)	19(47.5%)

DISCUSSION

The plants are important sources of biologically active substances. Essential oils can be major source of variety of antibiotic compound species equipped with antimicrobial capacity, that can be used in treatment of the infectious diseases is like substituent of certain antibiotics or like complementary agents used in synergy with the synthesis substances (Kremer et al. 2015). Herbal active substances can also have application in food industries as foodstuffs preservative. According to the results of this study, ethanolic extract of *S. bachtiarica* have significant antimicrobial effect on the studied microorganisms. The results indicate that *S. bachtiarica* ethanolic extracted at all concentrations (in 12.5 to 0.39 mg/ml) had the inhibitory effect on Staphylococci coagulase negative bacteria. However, plant extract dose-dependently suppress the bacteria. In this regard, cumulative evidence showing that *Satureja bachtiarica* has great antimicrobial potency against various microorganisms especially against gram positive bacteria. Several mechanisms are discussed to explain the bactericidal effect. Amiri (2013) investigate antibacterial effects of *Satureja* essence against some common nosocomial pathogenic bacteria. In that study, anti-bacterial effects *Satureja* were investigated using antimicrobial analysis with NCCLS and Kirby bauer method. The agar dilution method results revealed the *Satureja* had strong inhibitory effects against common nosocomial bacteria (*Staphylococcus aureus*-MIC: 5mg/ml). The obtained results showed

the antibacterial effect of *Satureja* essence can be a new disinfectants alternative to control the nosocomial resistant bacteria. The anti-microbial effect of *S. bachtiarica* can be attributed the presence of carvacrol, thymol, P-cymene and gamma trepanned. Falsafi et al. investigate Chemical composition and anti-*Helicobacter pylori* effect of *S. bachtiarica* essential oil. *S. bachtiarica* essential oil showed strong antibacterial activity against clinical isolates of *H. pylori* (17.6 ± 1.1 mm and 0.035 ± 0.13 μ l/ml) (Falsafi et al. 2015). Numerous studies have showed that the essential oil of thyme is one of the most potent essential oils with regard to antimicrobial properties. Thymol and carvacrol were among the most active components against multiple pathogens. Heidari Sureshjani et al, investigate antimicrobial effect of aqueous and ethanolic extract of *S. bachtiarica* on *Staphylococcus aureus* and *Escherichia coli* isolates. Based on results, the aqueous extract had no significant antimicrobial effect on tested bacteria. But, ethanolic extract had antibacterial potency such that MIC for *S. aureus* was 32 mg/ml and for *Escherichia coli* was 16 mg/ml (Heidari Sureshjani et al. 2013). In our study we evaluate ethanolic extract of *S. bachtiarica* and comparing to Sureshjani study the ethanolic extract showed significant bactericidal effects (MIC: 0.39 mg/ml). The reason of these phenomena may be more active substance extracted by ethanol from *S. bachtiarica*. The result of our study, were confirmed by Mohammad pour et al, showed

that, ethanolic extract was more bactericidal and have a greater antibacterial effect compared to the aqueous extract. In their study, oil extract of *S. bachtiarica* comparing to thyme genus have greater antifungal and antibacterial effect, such that the MIC value against *E. coli* was significantly lower than other tested plants (0.8 mg/ml) (Mohammadpour et al. 2012). Evidently, more studies are needed on the antimicrobial properties of essential oils and their compounds before they can be used for drug synthesis and food preservatives. In conclusion, it can suggest that *S. bachtiarica* extract in “in vitro” have considerable antibacterial efficacy on the studied strains. However, more studies are needed be done, to evaluating the effective dose of the extract on the microorganisms, to introducing the extract as an effective and novel antimicrobial compound.

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