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DETERMINATION THE BACTERICIDAL AND BACTERIOSTATIC EFFECT OF HYDRO - ALCOHOLIC EXTRACTS OF THE AERIAL PARTS OF SATUREJA BACHTIARICA BUNGE ON STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS FAECALIS IN VITRO

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ABSTRACT

Introduction: As a result of excessive use of antimicrobial drugs in the treatment of infectious diseases, bacterial resistance against many antibiotics has increased also use of medicinal plants with antimicrobial effect has found its place in traditional medicine. According to the local sources of traditional medicine the medicinal plants offer a rich source of new antimicrobial agents. The effect of ethanol extracts of the aerial parts of (*S. bachtiarica* Bung) indigenous Weed of Chaharmahal and Bkhtiari on *Staphylococcus aureus* and *Enterococcus faecalis* bacteria has been studied. **Method:** In This laboratory research of Bakhtiari savory the aerial parts of ethanol extracts concentrations in the range 5 / 0-10 milligrams per ml was obtained by maceration method. Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) was performed using the binary dilution method. For statistical analysis SPSS version 18 was used, a significant level of $P \leq 0.05$ is intended and Vancomycin From antibiotics were used as a reference. **Results:** regards to the findings, over 2 and 4 mg/ml doses of MIC and 16 and 32 mg/ml doses of MBC were identified for *Staphylococcus aureus* and *Enterococcus faecalis* respectively. **Conclusion:** results of this study indicated that savory of Bakhtiari has remarkable antimicrobial effect on the bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, which seems more studies in this field would achieve an alternative to synthetic antibiotics as antimicrobial resistance is increasing day by day. Further studies recommended to use a variety of clinical isolates for in vitro and in vivo assessment.

INTRODUCTION

Confronting with agents of infectious diseases and poisoning is one of the most important health challenges because of the high outbreak and spread [1]. The indiscriminate use of antibiotics besides being expensive and not cost-effective to be produce, trigger larger problems such as antibiotic-resistant of any pathogens. On the other hand, long-term use of antibiotics and even cross-sectional, left behind side effects that are sometimes more dangerous than the disease itself. Hence, it seems essential to find new antibiotics with better performance and less side effects [2]. *Enterococcus faecalis* is facultative anaerobe, gram-positive, from streptococci and group D, which plays crucial role in nosocomial infections [3]. This bacterium is inherently resistant to many antibiotics. *Enterococcus faecalis* have been seen widely in root dental canal of teeth that have been under manipulation [4]. *Enterococcus faecalis*, would cause diseases such as endocarditic, bacteremia, urinary tract infections and meningitis in humans and has proved a variety of virulence factors [5]. Several virulence factors were describing in *Enterococcus faecalis* that includes Agg aggregation compounds, Enterococcus surface protein (Esp) and cytolysine (Cyl) that has bactericidal and hemolytic activity of gelatinize enzyme and assumed that these factors are the convergence by facilitating access to the quorum and setting active quorum-sensing and increasing the virulence. This leads to deeper tissue damage of bacterial invasion [6,7]. Recent studies have been shown that *E. faecalis* forms biofilm and quorum- sensing system controlling biofilm development in these bacteria [8]. *Staphylococcus aureus* is a gram-positive cocci that can be found in nosocomial infections as an important factor and in many diseases such as boils, toxic shock syndrome, endocarditis, osteomyelitis, etc. [9]. Due to indiscriminate use of antibiotics, pathogen resistance to available drugs is increasing [10]. In 1940 many strains of *Staphylococcus* were resistant to penicillin. After a decade the multiple resistant strains to tetracycline, chloramphenicol, and erythromycin have been reported. In 1960, Methicillin pickup an effective antibiotic for penicillin-resistant *Staphylococcus aureus* strains. But soon after, methicillin-resistant *Staphylococcus aureus* strains were observed [11].

The high prevalence of infectious pathogenic agents, antibiotic resistance and drug side effects caused today new approaches of using medicinal plants including their antimicrobial effects. As numerous studies on the antimicrobial effects of different plant extracts and essential oils are in progress [12].

Savory genus (*Satureja hortensis* L.) is one of the (Labiates) family. In Iran 14 species of annual and perennial herbaceous plant is available that grow in various areas of the country such as the provinces of Chahar Mahal and Bakhtiari, Lorestan, Khuzestan, Ilam, Kermanshah, Isfahan, northeast and some other parts. Iran endemic species are: *S. edmondi*, *S. sahandica*, *S. kallarica*, *S. Bachtiarica*, *S. Intermedia*, *S. Isophylla*, *S. Khuzestanica*, *S. Atropanata*, *S. rechingeri* and species of *S. mutica*, *S. macrantera*, *S.*

KEY WORDS

Enterococcus faecalis,
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spicigera and *S.boissieri* have been seen also in Turkmenistan, Anatolia, the Caucasus, Transcaucasia and Iraq [13].

Species *S. bachtiarica* has a large dispersion in Iran and is collected from the western provinces, central and southwestern [14]. This kind of plant branched off to a height of 30-20 cm with a wooden base, short among nodes, early gray stems, downy fluff with a very short and soft, glandular spotted, flowering branches erect, thin, cylindrical, non-branched more or less branched, pale brown flowers are in clusters has several flowers [15].

This study aimed to determine the antimicrobial effects of hydro alcoholic extract of savory against standard strains of *Staphylococcus aureus* and *Enterococcus faecalis* Bakhtiar *in vitro* condition.

MATERIALS AND METHODS

Analyzing method

This experimental study was performed in 2015 at the University of Medical Sciences. Bakhtiar savory prepared from Shirmardi village of Lordegan was recorded by Professor of Pharmacognosy at the University of Medicinal Plants and identified with code 423-A.

Extraction

Dried hydro alcoholic (30:70) extract of savory Bakhtiar was prepared by maceration method and then condensed in Rotary and finally in the oven at 37 °C were dried.

Preparation of bacterial strains

Bacterial strains of microorganisms used in this study *S. aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), was prepared in lyophilized form fungal and microbial Industry Research Center of Iran.

Determination of the lowest inhibition concentration (MIC) and minimum bactericidal concentration (MBC): The experiment of minimum inhibitory concentration was done in 96-cell sterile plate by broth dilution method (Micro broth dilution) so that 1,000 ml of the suspension was diluted 1 to 75 with a half-Mac into the pit 5/1 × 810cfu / ml Far land bacteria concentrations between 2 and 75 micrograms equivalent to 5,000 ml was added in Mueller Hinton broth. Controls were bacterial suspension in a row, and culture medium and concentrations of the extracts was poured in next row. Micro plates were incubated at 37 °C for 24 hours. Subsequently, 10 ml tubes were cultured by Müller Hinton agar and incubated at 37 °C for 24 hours to determine the MBC to MIC. Then, MBC, respectively after that results in the lowest concentration of the extract which turbidity observed resulting from bacterial growth in the target inhibitory concentration [16,17].

After collecting the data entered into SPSS software version 18 and P≤0. 05 was considered as significant level.

RESULTS

The results of the antimicrobial effect of hydro-alcoholic extract of Savory by extract distribution on the cell culture (all over) are shown in [Table 1]. These findings showed that this extract at a minimum concentration of 2 and 4 mg/ml were inhibited the growth of *Staphylococcus aureus* and *Enterococcus faecalis* respectively. It should be noted that the concentration of 2 mg/ml of the savory extract was chosen for onset of action based on the pervious researches have been done on this plant [18]. This concentration had inhibitory effect on the staph bacteria however 1 mg/ml concentration of the extract on has no considerable inhibitory effect on the growth of *Staphylococcus aureus* [Table 1].

Table 1: Minimum Inhibitory Concentration (MIC) of the extract from different concentrations of Savory Bakhtiar

Density Microorganism	1	2	4	8
<i>Staphylococcus</i>	-	+	+	+
<i>Enterococcus</i>	-	-	+	+

Sign (+) indicates lack of microorganism growth in cell culture and antimicrobial activity of ethanol extracts of savory. Sign (-) indicates bacterial growth in cell culture and absence of antimicrobial activity of savory extracts.

The findings from determination of minimal bactericidal concentration (MBC) of savory hydro alcoholic extracts on *Staphylococcus* and *Enterococcus* indicated that MBC for *Staphylococcus* and *Enterococcus* are respectively 16 and 32 milligrams per milliliter of savory hydro alcoholic extract [Table 2].

Table 2: Minimum bactericidal concentration (MBC) in different concentrations of Bakhtiari's savory extractin

Density Microorganism	2	4	8	16	32	64
<i>Staphylococcus</i>	-	-	-	+	+	+
<i>Enterococcus</i>	-	-	-	-	+	+

DISCUSSION

The aim of this study was to evaluate the antimicrobial effect hydroalcoholic extract of Bakhtiari's savory on standard strains of *Staphylococcus aureus* and *Enterococcus faecalis* in vitro. In this study, 2 mg/mL and 16 mg/ml doses were identified as MIC and MBC respectively for the *Staphylococcus aureus*. Also concentration over than 4mg/ml and 32mg/ml were presented for MIC and MBC of *Enterococcus faecalis* respectively. Regards to the results of the study, antibacterial effects of Savory extract on *Staphylococcus aureus* and *Enterococcus* was significant. In particular the most antibacterial effect was observed on *Enterococcus faecalis*.

According to study by Zarei et al. alcoholic extract of savory Bakhtiari indicated inhibitory effects on four pathogenic bacteria including: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus agalactiae* in vitro [19].

In another study conducted by Habibian et al. alcoholic extract of savory Bakhtiari were showed antibacterial effect on pathogenic bacteria in red meat, involving *Staphylococcus aureus* and *E. coli* [20]. Also Savory Bakhtiari have indicated antimicrobial effect on the bacteria that cause cancer hair crown (Radio bacteria) in study was established by Ashrafi et al. [21].

According to Heydari et al. aqueous, ethanol and methanol extract of Savory Bakhtiari have antimicrobial effect on *Staphylococcus epidermidis*, *Streptococcus progenies* and *Pseudomonas aeruginosa* [22]. In another study, also conducted by Heydari et al. antibacterial effect of savory Bakhtiari's hydroalcoholic extract was proved on *Escherichia coli* and *Staphylococcus aureus* [23]. In our study, antibacterial effects Bakhtiari Mountain Savory against *Staphylococcus aureus* approved and for the first time, the effect of this plant against *Enterococcus* was confirmed.

In the survey conducted by Ansari et al. Savory essential oil of Khuzestan (*Satureja khuzestanica*) has showed significant antimicrobial effect on *Lactococcus garvieae* [24].

The Evaluation of antimicrobial effects of essential oils of two Savory species called *S. bachtiarica* and *Satureja khuzistanica* on a number of gram-positive and gram-negative was demonstrated positive antibacterial effects of the both. In addition this effect relates to the presence of phenol compounds such as caracole and thymol in the essential oil of these plants. Also in this study, which was conducted by Ahmad et al. in 2009 found that Savory essential oil of Khuzestan in have antimicrobial effects in both pre-flowering and flowering stage and Savory Bakhtiari essential oil is effective only before flowering. According to previous researches, total phenol compounds, thymol of Savory Bakhtiari before flowering (39%) was more than the flowering stage (31%), hence its antimicrobial effect is greater than the flowering stage [25,26].

According to studies have mentioned, extract of savory Bakhtiari seemed to have antimicrobial effects. Thus this study was investigated its effect on standard strains of *Staphylococcus aureus* and *enterococcus* which didn't carried out before.

Also, in the study of Pirbaloti et al. the antimicrobial impact of several plants on *Streptococcus* has been examined. SSEO is the mountain that one of the most valid plants having antibacterial effects with low MIC (39 micrograms per ml) was introduced. Their conclusion was also confirmed by this study [27].

the study of Mohammadpur et al. were compared three genus of Thymus and Ziziphora clinopodioides of Shiraz, savory essences of Bakhtiari was showed stronger antibacterial effect. In addition, comparing the diameter of the inhibition of this essential oil has been shown that it has significant inhibitory effect on *Candida albicans* [28]. In 2003 Shahin et al. were investigated anti-fungal properties of Savory's essential oil and were gain similar conclusion about the effectiveness of this plant on fungus [29].

Savory Bakhtiari's ethanol extract has antimicrobial effect against gram-negative bacteria such as salmonella typhoid and gram-positive bacteria such as *Enterococcus faecalis* demonstrated by Behbahani et al. 2014 [30].

In a study conducted by Bezic cuneifolia et al. essential oils with anti-bacterial and anti-fungal effects was studied against microorganisms including, *Bacillus subtilis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia*, *Candida albicans*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae* [31].

In another study entitled, "Evaluation of the antimicrobial activity of ethanol extract of red peppers", amaranth and Savory against antibiotic-resistant *Staphylococcus aureus* were stated. In spite of

Staphylococcus aureus resistance to antibiotics, trimethoprim, ampicillin, erythromycin, penicillin, Cefixime and amikacin, herbs are effective against these bacteria; but compared with amaranth and Savory, red pepper extract showed higher antimicrobial activity [32].

Savory can be recommended for further studies and clinical trials as an anti-bacterial medications for control and treatment of infections.

Despite the fact that in many studies, the expression of antimicrobial effects of savory have been noted, in this study, we have investigated the effect of the plant on resistant bacteria, and the most common *Enterococcus faecalis* and among the study its effect on *S. aureus* was possibly less impressive.

Surely many plant compete with savory in the field of antimicrobial effects, but it doesn't reduce the importance of this plant (the native and the availability of savory and its success in multiple studies). According to the cited references it seems that savory could have been effective, not only in the field of bacterial infections but also fungal infections and could have been helpful in the treatment of some tumors. Antimicrobial Effects of various plant species is different thus, further investigation would appropriately identify effects of any ones.

CONFLICT OF INTEREST

There is no conflict of interest.

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