Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(1); 40-44

DOI number: 10.25258/ijpapr.v9i1.8038

ISSN: 0975-4873

Research Article

Assessment of *In Vitro* Antibacterial Properties of the Hydroalcoholic Extract of *Scrophularia striata* Against *Staphylococcus aureus (ATCC No. 25923)*

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Received: 20th Nov, 16; Revised: 12th Dec, 16; Accepted: 18th Dec,16; Available Online: 15th January, 2017

ABSTRACT

Scrophularia striata (*S. striata*) is a native plant in Iran, which the plant has been used as an antioxidant, antifungal, antiviral, and antiflammatory agent in Iran. Based on knowledge of authors, as we know, there is low documented proof on antibacterial properties of *S. striata* hydroalcoholic extract against *Staphylococcus aureus* (*S. aureus*) (ATCC No. 25923) in west of Iran. As a screen test to discover antibacterial properties of the extract, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to specify MIC. The results of agar disk and agar well diffusion tests showed *S. striata* have prevented the growth of *S. aureus* and destroyed it. Also, by increasing the concentration of *S. striata*, the inhibition zone in many of samples increased. The MIC and MBC value was 0.031 g/ml for *S. striata*. This study confirmed the antibacterial effects of the *S. striata* on *S. aureus*. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of the plant as an antibacterial agent in topical or oral applications.

Keywords: *Scrophularia striata*; Hydroalcoholic extract; Antibacterial properties, Macro-dilution method, Agar disk diffusion method, Agar well diffusion method.

INTRODUCTION

S. aureus as a gram positive bacterium has been the basic cause of serious sickness recently. This bacterium is becoming resistance to certain type of antibiotics (such as Cefalexin), so it has become a great concern for finding a sought substitution (such as plants and their derivatives) for treating it. Plants have a main divertimento of phytochemicals such as phenolic acids, flavonoids, tannins, and other small compounds. They have been screened for their potential uses as other remedies for the treatment of different bacterial diseases[1]. Some medicinal plants used in traditional Iranian medicine are efficacious in treating various ailments caused by bacterial and oxidative stress[2]. A plant extract is a substance or an active with favorable effects that is removed from the tissue of a plant, to be used for specific purposes such as treat of infectious diseases. The compounds of plant extracts contain numerous health-related properties such as antibacterial, antimutagenic, anticarcinogenic antithrombotic and vasodilator activities. Herbal extracts have antibacterial effect on a wide range of bacteria, and most of these compounds have phenolic groups in their structure[3]. The original benefit of plant extracts is that they do not increase the antibiotic resistance because they have a substantial role in the defense system of the plant to bacterial diseases due to their intrinsic antioxidative and antibacterial activities[4]. In Iranian medicine, plant extracts in the form of infusion, decoction, tincture or herbal extract are consumed by the population for the treatment of diseases such as bacterial diseases. In western states of Iran, a plant with the scientific name of *Scrophularia striata* has traditional medical usage. The genus Scrophularia of the family Scrophulariaceae comprises about 3000 species and 220 genera. The genus is concentrated in central Asia with only a few species in central Europe and North America[5]. *S. striata* is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. *S. striata* have been used since ancient times in traditional medicines to treat eczema, wounds, ulcers, cancer and microbial disease. Several extracts of the plant are traditionally used in treating bacterial diseases. Perhaps, the antibacterial properties of the plant are related to its phenolic, flavonoid, and flavonol compounds[6-8].

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The aim of the current study was evaluation antibacterial activities of hydroalcoholic extract of *S. striata* against *S. aureus* in west of Iran (in Kermanshah).

MATERIAL AND METHODS

Source of microorganisms

Bacterial specie namely *Staphylococcus aureus* (ATCC No. 25923) was procured from Iranian Research Organization for Science and Technology as lyophilized. Bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8 cfu/ml using Muller Hinton broth.

Culture media

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

Plant sample collection

In this empirical-experimental study, medicine plant collected from Ilam. The sample was cleaned from any strange, plants, dust, or any other contaminants.

Preparation of hydroalcoholic extract

Successive solvent extraction was performed for *S. striata*. Plants were washed, air dried for 7-8 days, and ground into powder before they were placed into the flask of the Soxhlet apparatus for extraction using 70% ethanol with increasing order of polarity to extract the phytoconstituents separately at 20ºC for 3-4 h (The ethanol used was HPLC grade obtained from Sigma-Aldrich, Germany). Whitman filter papers No.1 were then applied to filter the extract. After that, reduced pressure was applied to evaporate and dry the filtrates which were stored at -20ºC in labeled, sterile, screw capped bottles.

Evaluation of antimicrobial activities

Agar disk and agar well diffusion were used as screen tests to evaluate antibacterial property of *S. striata* based on standard protocol. The solution of the *S. striata* was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. Distillated water was used as negative control whereas cephalexin was used as positive control in case of *S. aureus*. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In this case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter[9].

Statistical Analysis

Antibacterial effect was determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.05$.

RESULTS

Chemical composition

13 compounds such as Oleyl Alcohol (24.81 %), Di-n-octyl Phthalate (21.24 %), Bis(2-ethylhexyl)phthalate (14.91 %), 1,2-Benzenedicarboxilic acid, bis(2-methylpropyl)ester (12.41 %), Hexadecanoic acid, methyl ester (5.10 %), 2,3,6-Tricholorobenzaldehyde (3.72 %), unknown (3.40 %), Phenol, 2,4-bis(1,1-dimethylethyl) (3.05%), 2-ethyl-butanal (2.54%), Octadecanoic acid, methyl ester (1.84%), 1,2-Benzenedicarboxilic acid, butyl cyclohexyl ester (1.79 %), 9-Octadecenoic acid (Z)-, methyl ester (1.77 %), Cyclohexene1-methyl-4-(1-methylethylidene) (1.67 %), Phenol, 4-(3,4-dihydro-2,2,4-trimethyl-2H-1-benzophyran-4-yl) (1.65 %), representing 99.92% of the total hydroalcoholic extract composition of SS were identified using mass gas-chromatograph[10]. The most substance found in *S. striata* hydroalcoholic extract was Oleyl Alcohol[10].

Agar disk diffusion test

About *S. striata*, the widest zone was seen in 0.25 g/ml concentration (The value of growth inhibition zone was 19 mm in this dilution). There was no inhibition zone in *S. aureus* due to 0.007 g/ml concentration. Growth inhibition zones due to different dilutions are listed in table 1. No inhibition zone was observed due to distillated water.

Agar well diffusion test

In regard to *S. striata*, the widest zone was seen in 0.25 g/ml concentration (The diameter of growth inhibition zone was 13 mm in this dilution). There was no inhibition zone in *S. aureus* due to 0.007 and 0.015 g/ml concentrations. No inhibition zone was observed due to distillated water. The data are discoverable in table 2.

MIC and MBC determination

In the examined bacterium, MIC and MBC values were the same and equal to 0.031 g/ml concentration.

DISCUSSION

Antibiotics are types of antibacterial drugs used in the treatment and inhibition of bacterial infections. They may either mortify or prevent the growth of bacteria. But overuse of antibiotics has become the principal factor for the exhaust and propagation of multi-drug resistant strains of different groups of microorganisms[11]. Cefalexin or cephalexin, is an antibiotic that can treat a number of bacterial infections. It annihilate gram-positive and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. But, this antibiotic like other antibiotics have many side effects. Usual side effects of cephalexin include stomach upset, diarrhea and allergic[12].

Table 1: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *S. striata*.

Dilution(g/ml)	Inhibition zone in disk diffusion (mm)
Microorganism	*S. aureus*
Positive control	23
1/4 (0.25)	19
1/8 (0.125)	10
1/16 (0.062)	9
1/32 (0.031)	8
1/64 (0.015)	8
1/128 (0.007)	0
Negative control	0

Table 2: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of *S. striata*.

Dilution(g/ml)	Inhibition zone in well diffusion (mm)
Microorganism	*S. aureus*
1/4 (0.25)	13
1/8 (0.125)	9
1/16 (0.062)	8
1/32 (0.031)	8
1/64 (0.015)	0
1/128 (0.007)	0
Negative control	0

Because of their safety and low cost as well as their effect on a great number of bacteria without any side effect, medicinal plants may have the potency to treat bacterial resistance to diverse types of antibiotics[13]. The antibacterial effects of plant extracts from a wide number of plants have been appraised and reviewed[14,15], and the mechanisms that enable the natural components of herbs and spices to resist bacteria have been considered[16]. Plant extracts-derived products have a main versatility of phytochemicals compounds. The results showed that these mechanisms of antibacterial properties of plants extracts vary greatly depending on the components of the plant[17,18]. *S. striata* is the member of flowering plants family called Scrophulariaceae. Many *S. striata* plants have long been used in Asian countries as a medicinal plant for the treatment of diseases; it has been applied for treating various inflammatory and bacterial diseases[19,20].
The main components were Oleyl alcohol (24.81%), Di-n-octyl phthalate (21.24%), Bis (2-ethylhexyl) phthalate (14.91 %), and 1, 2-Benzenedicarboxilic acid, bis (2-methylpropyl) ester (12.41 %)[10]. Among the compounds, Bis (2-ethylhexyl) phthalate has antibacterial effects. In previous study, revealed the antishigellosis activity of Bis (2-ethylhexyl) phthalate because it had better activity against Shigella shiga, Shigella sonnei and Shigella dysenteriae[21]. In a study, showed the activity of Bis (2-ethylhexyl) phthalate against *S. aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhimurium* and *Pseudomonas aurioginosa*[22]. In other study indicated Bis (2-ethylhexyl) phthalate have strong effect against a number of Gram-positive bacteria; *S. aureus* with MIC 1.47 µg/ml, *Bacillus subtilis* with MIC 3.5 µg/ml, and *Streptococcus equosemens* with MIC 2.37 µg/ml but the inhibition of Gram negative bacteria was lower; *Escherichia coli* with MIC 5.4 µg/ml, *Pseudomonas aeruginosa* with MIC 6.2 µg/ml and *Closteridium perfringens* with MIC >50 µg/ml[23]. As the table showed, the inhabitation zone in many of samples have been increased when the extract amount has increased. In agar disk diffusion test, no inhibitory effect of extract of the plant against the *S. aureus* in 0.007 g/ml concentration but in agar well diffusion test, no inhibitory effect of *S. striata* in 0.007 and 0.015 g/ml. *S. striata* in 0.031 g/ml concentration has prevented from the growth of the bacterium and has destroyed bacterium. Thus, the research represents the antibacterial effects of the medical plant on *S. aureus*. There are correspondences between this result and the similar studies. Different studies have indicated that the many species of Scropbularia contains substances that have antibacterial activities[6 24-26]. Results obtained in pervious study showed that *S. striata* extracts have selective antibacterial activity on the basis of the cell-wall differences of bacterial microorganisms[27,28]. The antimicrobial effect of the aqueous extract of *S. striata* on *S. aureus* and *Pseudomonas aeruginosa* was studied and it was concluded that the obtained aqueous extract can be used as antiseptic product in treatment of external infections resulted from these two microorganisms[29]. Also, in this study showed when the extract amount has increased from 50 to 90 µl, the inhabitation zone have been increased. In a study demonstrated that the MIC and MBC of methanol extract of *S. striata* against *S. aureus* were 3.12 and 6.25 mg/ml, respectively[30]. Also, in this study inhibition zone about *S. aureus* was 16.34 ± 0.65[30]. From the study it can be concluded that by increasing the concentration of the hydroalcoholic extract of *S. striata*, the inhibition zone in many of samples increased. Also, *S. striata* have inhibited the growth of *S. aureus* and eradicated it in 0.031 g/ml concentration. The results indicated that in tested bacterium, there was a considerable difference in terms of sensitivity to *S. striata* and the most sensitivity was observed in agar disk diffusion method. Thus, the present research indicates the antibacterial effects of the *S. striata* on *S. aureus*, offering to use as antibacterial supplement towards the development of new therapeutic agent.

AKNOWLEDGMENT

We, the authors wish to thank Medical Sciences University of Kermanshah, Iran for the financial support of this work. The core idea of this work came from Mohammad Mahdi Zangeneh and Akram Zangeneh, also the experiments, evaluation and Statistical Analysis of antimicrobial activities done by Mohammad Mahdi Zangeneh, Fariba Najafi, Reza Tahvilian, Akram Zangeneh, Rohallah Moradi.

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