Evaluation of Anti-Microbial Activity of Ethanolic and Aqueous Extract of Salvia Hypoleuca

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Summary

Salvia hypoleuca is an important medicinal plant belonging to the family of Lamiaceae. It is a well recognized plant in the traditional medicine and is used by people in rural areas. This study is aimed to evaluate the anti-microbial effects of ethanolic extract of *Salvia hypoleuca* .The antimicrobial property of the Salvia hypoleuca was studied against gram positive and gram negative microorganisms using the agar disc diffusion method, in which ethanolic extract of Salvia hypoleuca has shown bigger zone of inhibition (15 – 25 mm) than aqueous extract (08–19 mm). These results demonstrate that Salvia hypoleuca possesses anti-microbial effects and it can be a good candidate for producing of anti-microbial drugs.

Keywords: Salvia hypoleuca, Anti-microbial, Ethanolic and aqueous extract.

Introduction

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (1). The effective substances of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (2). Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (3, 4). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (5). Many plants possess antimicrobial activities and are used for the treatment of different diseases (6). Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. The genus Salvia, one of the most important genuses of Lamiaceae family, is widely used in flavouring and folk medicine all around the world. Fifty-eight species of this genus are documented in the Flora of Iran; 17 of them are endemic (7-9). The plants of the genus Salvia, which consist about 900 species are generally known for their multiple pharmacological effects such as analgesic and anti-inflammatory (10) hepatoprotective (11), hypoglycemic activities (12), and antiischemia (13, 14)**.** The current study was undertaken to evaluate the anti-microbial activity of ethanolic and aqueous extract of Salvia hypoleuca by, till now no pharmacological evaluation has been done on the anti-microbial activity of Salvia hypoleuca.

Materials and Methods

Plant material

Salvia hypoleuca was collected from Guilan province (Iran), and authenticated at Medicinal Plants & Drugs Research Institute, Shahid-Beheshti University, Tehran, Iran. Its leaves and fruits were dried, under shade and powdered.

Preparation of the ethanolic and aqueous extract of Salvia hypoleuca

Air dried Powdered plant was divided in to two equal parts (1 kg each); one part was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The percentage yield was 19.6 %, second part of powdered was cold macerated with distilled water then the same procedure was repeated as mentioned above. The percentage yield was 10.5%.

Phytochemical screening

The freshly prepared ethanol and aqueous extract of *Salvia hypoleuca* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's,Fehling's and Benedict's reagent; proteins and amino acids with Millon's, Biuret, and xanthoprotein test. Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red; Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures (15). These were identified by characteristic color changes using standard procedures. The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids + ve; Gums and mucilage + ve; Glycosides + ve; Saponins – ve; Terpenes + ve and Tannins + ve. Where + ve and – ve indicates the presence and absence of compounds.

Microorganisms

Bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, and *Proeus vulgaris*) were procured from Pastore Diagnostic Labs, Tehran, Iran.

Chemicals and drugs

Ethanol was obtained from Bidestan Co. (Tehran, Iran). Cloxicillin ,Amoxiclav, Cefuroxime, Cefixim were from Tablets Iran P.vt, Limited (Tehran, Iran) were used as reference antibiotics (RA) against bacteria. The nutrient agar was from Zist Iran (Tehran, Iran). Indomethacin was from Merck.

Anti microbial activity

Sensitivity test: agar disc diffusion assay

The disc diffusion method was followed to evaluate anti-microbial activities using a range of microorganisms. Sterile Discs (Whatman, 6 mm) were impregnated with 10µl of reconstituted crude extracts (1mg/ml) and placed on the surface of Muller–Hilton agar dispersion plates inoculated with microbes. Each extract was tested in triplicate. Control discs contained pure

DMSO (100%). Standard antibiotics, Cloxicillin, Amoxiclav, Cefuroxime, and Cefixime (30 µg disc-1), were used to eliminate variation between plates. Agar plates containing bacteria were incubated at 37°C for 24 h. Inhibition zones were recorded as the diameter of growth-free zones(IZ), including the diameter of the discs, in mm, at the end of the incubation period (16).

Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

Results

The ethanolic and aqueous extracts from Salvia hypoleuca has shown inhibition effects on the growth of all the organisms tested, but their efficiency in inhibition was varied from one organism to another. In almost all, the tested organisms' growth was inhibited by both ethanolic and aqueous extract has shown higher range of inhibition diameter (IDZ) from 08 to 19 mm, where as ethanolic extract has shown inhibition range of 15–20mm. *Staphylococcus aureus* is more sensitive and *Escherichia coli* is least sensitive to ethanolic extract.Where as aqueous extract has shown inhibition range of 08-19mm. *Bacillus subtilis* is more sensitive and *Escherichia coli* are least sensitive to aqueous extract. Cloxicillin, Amoxiclav, Cefuroxime, Cefixime ranged from 20-28 mm at a concentration of 30µg/zone. All IZD corresponding to test organisms are tabulated in Table 1.

Table 1: Antimicrobial activity ethanolic and aqueous extract of Salvia hypoleuca on different microbes and their corresponding IZD.

S. No.	Microbe	IZD (mm) ethanolic extract	aqueous extract	Standard
1	Staphylococcus aureus	26	17	27
2	Bacillus subtilis	21	18	26
3	Pseudomonas aeruginosa	19	11	23
4	Proteus vulgaris	17	13	23
5	Escherichia coli	16	09	19

Cloxicillin, Amoxiclav, Cefuroxime, and Cefixime are the standards (values are mean ± S.D).

Discussion

Control of the spread of antibiotic-resistant bacteria and the treatment of infections caused by them is a major problem worldwide. According to the World Health Organization (WHO), infectious diseases is the first cause of death worldwide with more that 50% of the death appearing in tropical countries. In the developing countries, treatment of such diseases is complicated not only because of the occurrence of resistant microorganisms to the commonly used antibiotics, but also because of the low income of the population, which drastically reduce their accessibilities to appropriate drugs. It is reported that about 80% of the world population is dependent (wholly or partially) on plant-based drugs (17). Results obtained in the present study have shown that both the ethanolic extract and aqueous extract, are activeagainst the growth of the microbes such as *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*. Both extracts possess promising anti-microbial activity.

Hence, the external application of these extracts on the wound prevented the microbes to invade through the wound, resulting protection of wound against the infections of the various microorganisms, because the presence of replicating microorganisms within a wound that cause host injury includes *Staphylococcus aureus, Beta-hemolytic Streptococcus, E. coli, Klebsiella, Pseudomonas, Acinetobacter, Stenotrophomonas*.etc. The overall result of the antimicrobial indicates that both the extracts can be useful in the development of antimicrobial drugs. This could be confirmed by the results of the IZD determination (Table 1). The presence of antimicrobially active metabolites classes such as flavonoids, phenols, terpenoids, alkaloids, glycosides might explain the wide spectrum of activity of the tested extracts. However, the isolation of the active principles will confirm this hypothesis and provide more explanation on mechanism of action of these extracts. In conclusion, our results indicate that IAF has anti-microbial and anti-inflammatory effects, which provide pharmacological evidence for folk uses of *Ipomoea aquatica* Forsk. In the treatment of various inflammatory disorders such as arthritis, sprains and injuries. Further molecular and cellular experiments are warranted to explore its action mechanisms. Identification of its active components is also warranted.

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