The Antimicrobial Activity of *Salvia pratensis* Extracts Against some microbial agent

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Abstract:

The effectiveness inhibitory to extract alcohol for the leaf and flower to plant sage *Salvia pratensis* each of *Staphylococcus aureus*, *streptococcus epidermidis*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* whom had any inhibition to aqueous extracts of the parts itself species bacterial and fungal. The study also demonstrated that the extract of plant containing compounds chemical such as *tannins, Alkaloids, Flavonoieds*, and *saponins*, which owns effectiveness of medical. The MIC, MBC and inhibition zones for crud extract were determinated for microbial agents.

Key words: Antimicrobial activity, *salvia pratensis*

Introduction

The genus *Salvia* L. belongs to the Lamiaceae family and shows about 900 species dispersed worldwide, mainly in the areas of the Mediterranean, Southeast Africa and Central and South America. It is cultivated for culinary, medicinal and ornamental purposes and hence presents ethno-pharmacological and economic importance, especially for small farmers. They are extensively used in popular medicine and many pharmacological research studies have sought to identify the compounds responsible for their therapeutic effect (1). Furthermore, the differences in biological activities of the drug could be observed, related to the different compounds present in plant material used (2).

Researchers have been interested in biologically active compounds isolated from plant pecies for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics. Plant products are also known to possess potential for food preservation, they have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants .(3)

The treatment of stomatitis, periodontosis, gingivitis, parodontal abscesses, gingival bleeding. Investigations have proved that the aqueous and ethanolic extracts of garden- sage have highly inhibitory activities against the periodontopathogenic bacterium *Porphymonas gingivalis* that acts collagenolytically and cytotoxically more than 60 kinds of diseases that might be treated by this plant are mentioned in the literature. Preparations of garden-sage are used for to the gingival
fibroblasts. Salvin from the acetone extract of the dried flowers is effective against S*taphylococcus aureus*. Due to its content of phenolic acid, garden-sage exhibits antibacterial effects and is used in the treatment of sore throat, laryngitis throat ulceration (4).

The aim of this study was to evaluate the antimicrobial activities of the crude extracts ethanolic and aqueous from flower, leaf and stem of *S. pratensis* in Bludan a Syrian town against a range of food born pathogenic and spoilage bacteria, evaluating minimal inhibitory concentration in an attempt to contribute to the use of these as alternative product for microbial control.

Material and Methods

The collection of samples of *S. pratensis* were carried out with period of March 2011 during flowering period, and anal part of *S. pratensis*, flowers, leaves and stem, taxonomic identify of plant was Confirmed at Department of Biology, Salah Aldeen University by Dr. Hussein Al-Kait. The Collected plant materials were dried in the shade, and grounded using suitable grinder.

Preparation of extracts

Alcoholic and aqueous extracts were prepared by taken 100g of dried and finely grounded aerial parts in 500ml ethanol 80%, boiled for 2 hours, and filtered twice, first by gauze, second by using filter paper then the filters were dried and concentrated (5).

In-vitro Antimicrobial activity

Stock inoculums suspension were prepared and Isolates of microorganisms were tested including;. Bacteria (*Staphylococcus aureus, Streptococcus epidermidis, Salmonella typhi, Pseudomonas aeroginosa, and Escherichia coli*,) and Molds *(Aspergillus niger, Candida albicans*). All isolates were clinically isolates and the identification confirmed by Biochemical tests.

Each bacterial isolates were inculcated in the nutrient broth incubated overnight at 37° C. These cultures were used as initial inoculums of bacteria and spore formation of the test isolate *Aspergillus niger* were induced by growing the isolates on (SDA) at 28° C , two slants were prepared.

At the end of incubation period, 5 ml of saline containing 1% tween 80 was added to each tube and the surface was rubbed with a sterile loop at dislodge conidia from the hyphen mat. The collected spore suspension was vortex for 15 min.

Then filtered once through sterile gauze to remove h hyphen inoculums quantification were performed to determine the variable number of Colony Forming Unit (CFU) per milliliter 10^6 CFU/ml and 10^4 CFU for bacterial and fungi respectively. The plates were inoculated with 0.1 ml of bacterial and fungi suspension then incubated at 37°C and 28 °C were observed for counting of colonies (5).

Disk diffusion assay

The dried plant extract were dissolved in diluted (50% DMSO) to concentration of 30 mg/ml and sterilized by filtration by 0.45 µm millipore filters. Antimicrobial tests were then carried out by the disc diffusion method using 100 µl of test isolates suspensions by using plate pour technique.

The discs (5 mm in diameter) were impregnated with 10u1 of the 30mg/ml extracts (300µg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts.

Cephazolin (30 µg/disc), ampicillin (10µg/disc), and Nystatin (100 µg/disc) were used as positive reference standard to determine the sensitivity of each isolate of microbial species tested (Kirby-Bauer Antimicrobial Susceptibility). The inoculated plates were incubated at 37° C for 24 hour (for bacteria) and 28° C for 72 hour (for fungi) Antimicrobial activity were evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated two (6).

Minimum Inhibitory Concentration (MIC)

A-For bacteria

Minimum Inhibitory Concentration values were studies for the bacterial strains sensitive to the alcoholic and/or aqueous extract in the disc diffusion assay. The inoculate of bacterial strains were prepared from 18 hour broth of S. *pratensis,* dissolved in (50%DMSO) was first diluted to the highest concentration (1000 µg/ml) to be tested , then serial 2-fold dilutions were made in order to obtain a concentration range (62.5 - 500 µg/ml) in 10 ml sterile test tube containing nutrient broth.

MIC values of *S. pratensis* against bacteria strains were determine based on serial dilution method by using liquid media. All tubes were incubated at 37° C for 24 hour. Microbial growth in each test tube was determined visually for turbidity.

The MIC values were defined as the lowest concentration of *S. pratensis* extract required for inhibition the growth of microorganisms (6).

B- for fungi

Minimum Inhibitory Concentration values for *S. pratensis* were determined by serial dilution method by using solid liquid media. The extracts

were added aseptically to sterile molted SDA media containing tween 80 (0.5% v/v) to produce the concentration range (62.5- 500 µg/ml). The plates were spot inoculated with 10^4 spore/ml of *A. niger*, incubated at 28° C for 72 hour. The plates were evaluated for the presence or absence of growth. The test replicated twice. MIC values were determinate as the lowest concentration of *S. pratensis* extract required for inhibition the growth of microorganisms (6).

Phytochemical Screening

The powder of plant was screend for the presence of flavonoid, tannins, saponins, starch, carbohydrates and alkaloids (7).

Results

Result of phytochemical study of *S. pratensis* showed that flavnoides, alkaloides and saponins present in alcoholic and aqueous extracts in leaves, flowers and stems as shown in table (1, 2, & 3). Whereas starch and carbohydrates do not present in alcoholic and aqueous extract flowers and stems.

The in vitro antimicrobial tests of alcoholic and aqueous *S. pratensis* extracts, assayed against a wide range of human microorganisms: bacteria such as *E. coli*, *Salmonella typhi*, *Psedumonas aeroginosa*, *Staphylococcus aureus*, *streptococcus epidermidis* and fungi such as *Aspergillus niger* and *Candidia albicans.*

Table (1) showed the chemical constituents of crude extract

Chemical constituents	flowers		Leaves		Stem	
	Alcoholic extract	Aqueous extract	Alcoholic extract	Aqueous extract	Alcoholic extract	Aqueous extract
Flavonoides	+++	+	++	+	+	-
Starch	-	-	-	-	-	-
Tannins	++	+	+	-	+	+
Carbohydrates	-	-	-	-	-	-
Saponins	+++	+	++	+	++	+
Alkaloides	++	+	+	+	+	+

+ : Present - : Absent

Their potency was qualititively and quantitavely assessed by evaluation of presence of inhibition zones, zone diameter and MIC values. The result of antimicrobial activity of *S. pratensis* equeous and alcoholic extracts are given in the table (2).

Table (2) Antimicrobial Activity of *S. pratensis* extracts.

Microorganism tested	Inhibition zone of *S. pratensis* extracts 'mm'								
	Agueous Extracts			Alcoholic extracts			R		
	Flowers	Leaves	Stem	Flowers	Leaves	Stem	Amp.	Cep.	Nys.
Escherichia coli	-	-	-	15	10	7	22	27	-
Staphylococcus aureaus	-	-	7	12	7	-	35	20	-
Streptococcus epidermidis	-	-	-	8	8	3	30	18	-
Pseudomonas aroginosa	-	-	-	12	8	-	26	16	-
Salmonella typhi	-	-	-	10	10	4	23	21	-
aspergillus Niger	-	-	-	7	-	-	-	-	14
Candida albicans	-	-	-	-	-	-	-	-	18

MIC and MBC values of alcoholic and aqeous extract of *S. pratensis*: as given in table (3)

The MIC value of *S. pratenses* is the minimum concentration of its extract which inhibit the microbial growth.

Table (3) values of MIC and MBC (µg/ml) of the alcoholic extract of *salvia pratensis*

Microorganism	Flower		Leaf		Stem	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	140	160	140	180	140	180
Salmonella typhi.	180	200	140	180	200	-
Pseudomonas aroginosa	140	160	160	180	160	180
Staphylococcus aureus	100	120	80	120	100	140
Streptococcus epidermidis	80	100	80	100	140	160
Candida albicans	140	160	140	160	160	180
Aspergillus niger	140	-	140	-	80	-

Discussion

The antimicrobial activity of ethanol and aqueous extracts of *S. pratensis* were examined against 5 bacteria and 2 fungal strains. The results show that the gram negative bacteria were more sensitive than gram positive bacteria and ethanol extract was more effective than the aqueous extracts.

The ethanol and aqueous extracts of *S. pratensis* have some common compounds such as flavonoides, (Table 1) tannins, saponinss and alkaloids, which have antimicrobial activity on bacteria, yeast and mould (7).

The present study indicates the *S. pratensis* extracts can be considered as alternative to traditional food preservative, eliminating or reducing the growth of important food borne pathogens and spoilage bacteria as well as provides an important basis for the use of extracts from these plants for the treatment of infections isolated compounds associated to the studies microorganisms (8).

The tested ethanolic extracts showed relatively on average level of antimicrobial activity against the tested microorganisms. Ethanol extract was more effective than the due to the ethanol extracts from the flower, leaf and stem of *S.pratensis* have some common components, such as 118-cineole, beta – caryophullene, alphacopaene, alpha- humulene and caryopyllene oxidean also, the essential oils of *S. pratensis* have antimicrobial activity (9 & 10).

The generous name is derived from the Latin salveo meaning "to heal or to be well and in good health" referring to the medicinal properties of some of the species salvia species commonly known as sage, are an important group of useful plants and used in traditional medicines all around the world, possessing antioxidant, antibacterial, antidiabetic, antitumor, antiplasmodial and anti-inflammatory activities (11&12).

The effective anti-bacterial which is related to existence of Flavonids and Alkaloids, the extract that contain the highest concentration of these components give greater efficiency and this is consistent with a number of studies applied on other plants have these components (13,14&15).

Finally, the antimicrobial activity of the crude extract from *S. pratensis* may be due to the presence of both antifungal and antibacterial compounds. The present study provides an important basis for the use of extracts from these plants for the treatment of infections associated to the studied microorganisms. The crude extract as well as the isolated compounds found active could be useful for the development of new antimicrobial drug. However, pharmacological and toxicity studies currently going on inour laboratory will be necessary to confirm this hypothesis.

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استخدام المستخلصات النباتية لنبات المريمية والمضادات الحيوية ضد العوامل الميكروبية

الخلاصة :-

بينت الدراسة الحالية الفعالية المثبطة للمستخلص الكحولي لورقة وزهرة نبات *Salvia pratensis* ضد كل من *Staphylococcus aureus*, *streptococcus epidermidis*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans* و لم يكن اي تأثير تثبيطي من مستخلص المائي للاجزاء النباتية المستخلصة ضد الانواع البكتيرية والفطرية. كما اظهرت الدراسة الحالية ان المستخلص النباتي يحتوي على مركبات كيميائية مثل تانينات، القلويدات، فلافونيدات والصابونيات والتي اعطتها الفعالية الطبية،وتم تحديد التركيز المثبط الادنى (MIC)،التركيز القاتل الادنى (MBC) ومناطق التثبيط للمستخلص للمسببات المايكروبية.