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Phytochemical Screening and Antimicrobial Activity of Various Extracts of *Salvia aegyptiaca* L.

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A B S T R A C T

The present study was conducted to asses phytochemical and antibacterial activity of various crude extracts *viz*, pet-ether, chloroform, methanol and aqueous extracts of *Salvia aegyptiaca* against bacteria and fungi such as *Staphylococcus aureus* (MTCC Code - 9886), *Pseudomonas aeruginosa* (MTCC Code - 6458), *Aspergillus niger* (MTCC Code - 872), *Aspergillus flavus* (MTCC Code - 8790) by adapting agar well diffusion method. The highest percentage of extraction yield was observed in aqueous extract followed by methanol, chloroform and petether extracts. The phytochemical test revealed that the presence of proteins, carbohydrates, lipids, alkaloids, phenols, flavonoids, steroids, glycosides, tannins, terpenoids and resins. The crude extracts of *Salvia aegyptiaca* have displayed significant to moderate and dose dependent (25, 50 and 100mg/ml) antibacterial and antifungal activity. The antibacterial activity of methanol extract shows maximum inhibition zone on Gram positive bacteria *Staphylococcus aureus* compared to Gram negative bacteria *Pseudomonas aeruginosa* at 100mg/ml concentration. Similarly, the antifungal activity of aqueous extract shows maximum inhibition zone on *Aspergillus flavus* compared to *Aspergillus niger* at 100mg/ml concentration. Overall, fungi were more sensitive than the bacterial strains in *Salvia aegyptiaca.*

Introduction

Medicinal plants are rich source of metabolites that are potential sources of drugs and essential oils. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The antimicrobial properties of plants have been investigated by a number of researchers worldwide though biological evaluation of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases (Tanaka *et al*., 2006) especially in light of the emergence of drug-resistant microorganisms. Despite the medicinal potential of plants being considerable in our country, knowledge and studies on wild growing *Salvia* species from this area are scarce; accounts for their therapeutic effects were found especially in other sources (Bagci and Koçaka, 2007; Tepe *et al*., 2007).

Saliva aegyptiaca L. (Egyptian sage) is belongs to Lamiaceae family. It is a green dwarf shrub that grows in various locations in

the world and commonly used in folk medicine. The seeds of the plant are used as demulcent for piles, and whole plant is used in diarrhea, gonorrhea and hemorrhoids, eye diseases and as an antiseptic, antispasmodic and stomachic (Rizk and El-Ghazaly, 1995). The plant is also used in cases of nervous disorders, dizziness and trembling and stopping perspiration (Al Yousuf *et al*., 2002). Salem (2004) has isolated some terpenoids and fatty acid esters from the nonvolatile matter of *S. aegyptiaca*. The extracts of higher plants can be very good source of antibiotics (Firdous *et al*., 1990) against various bacterial and fungal pathogens. The antimicrobial activities of *Salvia* species was reported by many researchers (Omidreza Firuzi *et al*., 2013; Nurcan Erbil and Metin Digrak, 2015; Ali and Aboud, 2010).

Due to its ethnomedicinal importance it is evident from the available literature that the search for crude drugs of plant origin with phytochemical and antimicrobial studies has become a central focus of research.

The main aim of the present study was to evaluate the phytochemical and antimicrobial activity against various pathogenic bacteria and fungi in whole plant crude extracts of *Salvia aegyptiaca.*

Material and Methods

Collection of Plant Materials

Salvia aegyptiaca (Lamiaceae) plant was collected in the month of September 2015 from campus of Karnataka state women's university, Vijayapura, India. The plant was identified with the help of flora 'The Presidency of Bombay' (Cooke, 1906). The voucher specimen has been deposited in the department of Botany, Karnataka state women's university, Vijayapura. The whole plant was dried in the shade at room temperature between 25-30^{o}C for 15-30 days, after drying the plant were chopped and grinded made into fine powder.

Preparation of Crude extracts

The 20gm of the powdered material was separately soaked in volumetric flask containing 100ml of different solvents of petroleum ether, chloroform, methanol, and aqueous for 24hr with occasional shaking. The extracts were filtered using Whatsman No.1 filter paper. The resulting liquid extracts were evaporated to dryness under reduced pressure. The plant extracts were stored in a clean sterile container for further use. The yields of the extracts were calculated using the following formula (Raghnathan, 1976; Usha Shome, *et al*., 1984).

$$\text{Extractive value (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Preliminary screening test for Phytochemicals

The preliminary test for the detection of the primary and secondary metabolites were carried out for all the extracts of *Salvia aegyptiaca* were separately tested by the standard methods (Harborne, 1998; Gibbs, 1974; Sadasivam and Manickam, 1992.)

Test for proteins

Biuret test: 2 ml of 10% NaoH was added to 2 ml of test solution, mixed well and 2 drops of 0.1% copper sulphate solution was added. Violet or pink colour indicates the presence of two or more peptide bonds of proteins.

Hopkins-Cole test: 2ml of glacial aectic acid was added 2ml of the test solution and mixed

well. To this 2ml of conc. H_2SO_4 was added carefully along the sides of test tube. Formation of violet ring at the junction of the two liquids indicates the presence of indole group of tryptophan.

Test for carbohydrates

Molisch's reagent test: 2 drops of molisch's reagent was added to 2 ml of test solution, mixed well. Inclined the tube and 1 ml of conc. sulphuric acid were added along the sides of the test tube.

At the junction of the two liquids a red come violet coloured ring indicates the presence of carbohydrates.

Benedict's test: 2 ml of Benedict's reagent was added to five drops of the test solution. Boiled for a minute in a water bath and cooled the solution. Yellow, red or green colour precipitate indicates the presence of reducing sugars.

Fehling's test: 1ml of Fehling's solution 'A' and 1ml of Fehling's solution 'B' were added to 1ml of test solution. The contents were mixed well and boiled for a minute. Yellow or brownish-red precipitate indicates the presence of reducing sugar.

Test for lipids

Stain test: Small quantity of extract was taken and pressed between to two whatsman No-1 filter paper. The stain on the filter paper indicates the presence of fixed oil.

Saponification test: To small quantity of various test solution and add few drops of 0.5N alcoholic potassium hydroxide and a few drops of phenolphthalein indicator and heat on water bath for 1-2 hour presence of fixed oil and fats is indicated by the formation of soap.

Tests for Alkaloids

Mayer's test: 1 ml of KI in iodine solution was added to the 2 ml of test solution. A creamy white precipitate formation indicated the presence of alkaloids.

Dragendorff's reagent: 2 ml of Dragendorff's reagent and 2 ml of dilute HCl were added to the test solution. An orange-red coloured precipitate indicates the presence of alkaloids.

Wagner's test: 2 ml of Wagner's reagent was added to 2 ml of test solution. The formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Phenols

Ferric chloride test: 0.5 ml of $FeCl_3$ (w/v) solution was added to 2 ml of test solution, formation of an intense colour indicates the presence of phenols.

Ellagic acid test: The test solution was treated with few drops of 5% (v/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turns muddy yellow, olive brown, Niger brown, deep chocolate colours depending on the amount of ellagic acid present.

Tests for Flavonoids

Pew's test: A pinch of zinc powder and about 5 drops of 5 N HCl were added to the test solution. It results deep purple red (dihydroquercetin) or cherry red (dihydrokaempferol) colours. Flavonones, dehydrochalcones and other flavonoids get at most pinkish or brownish colour.

Shinoda test: A pinch of magnesium powder and 5 N HCl were added to the test solution and a deep red or magenta colour formation indicates the presence of flavanone or

dihydroflavanol. However, dihydrocarchal-cones and other flavonoids did not react with this reagent.

Tests of steroid

Salkowski's test: A wine red colour was developed when chloroform and Conc. H_2SO_4 were added to the test solution; indicate the presence of steroidal nucleus.

Tests for glycosides

Conc. H_2SO_4 test: To the extract add Conc. H_2SO_4 and allowed to stand for few minutes, it turned into reddish colour.

Kellar Killiani test: The extract was dissolved in glacial acetic acid and after cooling 2 drops of ferric chloride solution was added to it. These content were transferred to a test tube containing 2 ml of Conc. H_2SO_4. A reddish brown ring was observed at the junction of two layers.

Tests for tannins

Gelatin test: To the extract add few drops of 1% gelatin solution containing 10% $Nacl_2$, A white colour precipitate observed.

Tests for terpenoids

Conc.H_2SO_4 test: To the extract add few drops of glacial acetic acid and after cooling add 2 ml of H_2SO_4 along the sides of the test tube. A reddish or brown or pinkish colour ring was observed at the junction of two layers.

Test for saponins

Foam test: 0.01 g of crude extract was shaken vigorously in 2 ml of distilled water. Formation of honeycomb like froth persists for a few minutes indicate the presence of saponins.

Test for Resins

To the 1 ml of the test solution, 2-3 ml of copper sulphate solution was added, the contents was mixed well for 2 minutes and then the solution was allowed to separate resins were indicated green coloured precipitate.

Antimicrobial Activity

The *in vitro* antibacterial activity and antifungal activity of *Salvia aegyptiaca* L. crude extracts were carried out by adopting the agar well diffusion method.

Preparation of crude extract for screening assay

The crude extracts (pet-ether, chloroform, methanol, aqueous) were dissolved in dimethyl sulfoxide(DMSO) and the final concentrations of the crude extract solution were obtained as 25 mg/ml, 50 mg/ml and 100 mg/ml respectively

Pathogens used

The pure axenice culture of bacteria and fungi such as *Staphylococcus aureus* (MTCC Code - 9886), *Pseuomonas aeruginosa* (MTCC Code - 6458), *Aspergillus niger* (MTCC Code - 872), *Aspergillus flavors* (MTCC Code - 8790) (Microbial type culture collection) were obtained from Institute of Microbial Technology, Chandigarh. All the Cultures were maintained on Nutrient Agar for further use.

Antibacterial Activity

The extracts were screened for their antibacterial activity in comparison with standard Streptomycin (10mg/ml) *in –vitro* by agar well diffusion method (Indian Pharmocopia, 1996). The Petri plates containing 15-20ml of Muller Hinton Agar

(MHA) medium was inoculated with 200µL of 18h old bacterial culture was evenly spread with a sterile bent glass rod. The inoculated plates are kept aside for few minutes. A sterile cork borer was then used to make four wells (8mm diameter) for different concentrations of the extract, on each of the plates containing cultures of the different test organisms. The four peripheral wells were loaded with 100 µl of pet ether, chloroform, methanol and aqueous plant extracts of the concentration 25, 50 and 100 mg/ml respectively. In the similar way, one agar plates for each microorganism were prepared for studying the activity of standard Streptomycin (10 mg/ml). For assaying antibacterial activity, plates were incubated at 37^{o}C for 24hr. The diameter of zone of inhibition (in mm) was recorded.

Antifungal Activity

The extracts were screened for their antifungal activity in comparison with standard Tetracycline (10mg/ml) in –vitro by agar well diffusion method (Indian Pharmocopia, 1996). About 15-20 ml of the Potato Dextrose Agar (PDA) medium was poured in the sterilized Petridis under aseptic conditions and allowed for solidifying. Then each plate was inoculated with 200 µl of 36 h old fungal culture and was evenly spread with a sterile bent glass rod. A sterile cork borer was then used to make four wells (8mm diameter) for different concentrations of the extract, on each of the plates containing cultures of the different test organisms. The four peripheral wells were loaded with 100 µl of pet ether, chloroform, methanol and aqueous plant extracts of the concentration 25, 50 and 100 mg/ml respectively. In the similar way, one agar plates for each microorganism were prepared for studying the activity of standard Tetracyclin (10 mg/ml). For assaying antibacterial activity, plates were incubated at 37^{o}C for 24hr. The diameter of zone of inhibition (in mm) was recorded.

Statistical analysis

The data of all measurements are means from three replications. Data and statistical significance of difference were evaluated with analysis of variance (ANOVA) using SPSS 10.0 package.

Results and Discussion

Yield of Crude extract: The extraction yield of different solvents varied from 0.6% to 1.20 and could be ranked from high to low i.e. aqueous > methanol > chloroform > pet-ether extracts (Fig-I). The percentage of extraction yield will increase with the ratio of solvents, temperature and sample extraction. The highest percentage of extraction yield was observed in aqueous extracts of *S. aegyptiaca*. Similar results are reported by Mukharjee (2002) in leaves of *Leucas lavandulaefolia*.

Phytochemical analysis: The crude extracts *viz.*, pet-ether, chloroform, methanol and aqueous extracts of whole plant of *Salvia aegyptiaca* was qualitatively screened for the occurrence of various primary and secondary metabolites by treating with various chemical reagents. The reactions with these reagents have shown the presence or absence of metabolites were recorded in the Table.1. The preliminary screening test revealed that the presence of protein, carbohydrate, lipids and also the presence of various groups of secondary metabolites such alkaloids, phenols, flavonoids, steroids, tannins, terpenoids and resins in all extracts. However the glycosides and tannins were observed only in methanol and aqueous extracts. The saponins were absent in all extracts of the *Salvia aegyptiaca*. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. In recent years these bioactive components are used in different forms such as infusions, syrups, concoctions, decoctions,

essential oils, ointments and creams (Sahu, 2014).

Antimicrobial activity: The crude extracts of *S. aegyptiaca* exhibited *in vitro* antibacterial activity against Gram positive bacteria of *Staphylococcus aureus* and Gram negative bacteria of *Pseudomonas aeruginosa*. As the drug dose increased (25, 50 and 100 mg/well) simultaneously increased the zone of inhibition of the bacterial growth (Table.2). *Staphylococcus aureus* exhibited considerable sensitivity against methanol (1.45±0.08 mm) at 100mg/ml concentration followed by chloroform (0.93±0.09 mm) and aqueous (1.2±0.5 mm) extracts. However pet-ether extract was totally resistant against *Staphylococcus aureus.* Whereas, *Pseudomonas aeruginosa* exhibited sensitivity towards the all crude extracts, among methanol extract possess the highest inhibition zone (1.36±0.5mm) at 100mg/ml concentration. However the aqueous extract is completely resistant against *Pseudomonas aeruginosa.* Similarly, Ebrahimabadi (2010) has reported the essential oil and methanol extract of *S. eremophila* showed relatively strong antimicrobial activity against Gram-positive and Gram-negative bacteria including *E. coli*, *B. subtilis*, *Staph. aureus* and *Staph. epidermis.*

Table.1 Metabolites Of Crude Extracts of *Salvia aegyptiaca*

Metabolites		**Pet-ether**	**Chloroform**	**Methanol**	**Aqueous**
Proteins	Biuret test	+	+	+	+
	Hopkin-Cole test	+	+	+	+
Carbohydrates	Molisch reagent test	+	+	+	+
	Benedict's test	+	+	+	+
	Fehling's test	+	+	+	+
Lipids	Stain test	+	+	+	+
	Saponification test	+	+	+	+
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendroff's test	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+
	Ellagic acid test	+	+	+	+
Flavonoids	Pew's test	+	+	+	+
	Shinoda test	+	+	+	+
Steroids	Salkowski's test	+	+	+	+
Glycosides	Conc. H_2SO_4 test	-	-	+	+
	Keller killiani test	-	-	+	+
Tannins	Gelatin test	-	-	+	+
Terpenoids	Conc. H_2SO_4 test	+	+	+	+
Saponins	Foam test	-	-	-	-
Resins	Copper sulphate test	+	+	+	+

Note: '+' Present, '-' Absent

Table.2 Antibacterial Activity of Various Crude Extracts of *Salvia aegyptiaca*

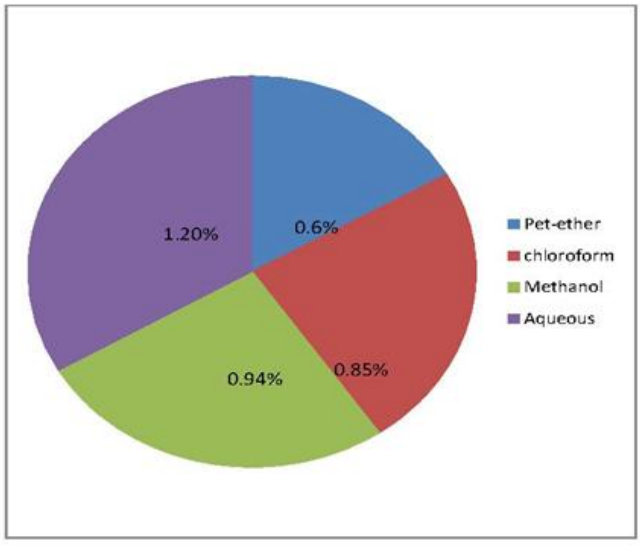
Name of Bacteria	Diameter of the Zone of inhibition (mm)					
	Conc. (mg/ml)	Pet-ether	Chloroform	Methanol	Aqueous	Streptomycin 10mg/ml
Staphylococcus aureus	**25**	-	-	0.8±0.05	0.46±0.08	5. 0±0.5
	50	-	-	1.16±0.12	0.8±0.06	
	100	-	0.93±0.09	1.45±0.08	1.2±0.5	
Pseudomaonas aeruginosa	**25**	-	0.8±0.05	0.83±0.05	-	7.0±0.2
	50	0.63±0.02	1.11±0.42	1.0±0.82	-	
	100	0.93±0.05	1.23±0.5	1.36±0.5	-	

Each value is expressed as mean ± S.D. (n=3) and statistically significant P<0.05

Table.3 Antifungal Activity of Various Crude Extracts of *Salvia aegyptiaca*

Name of fungi	Diameter of the Zone of inhibition (mm)					
	Conc. (mg/ml)	Pet-ether	Chloroform	Methanol	Aqueous	Tetracycline 10mg/ml
Aspergillus niger	**25**	-	-	-	-	4.0±0.5
	50	0.36±0.07	0.60±0.04	042±0.05	0.6±0.12	
	100	0.43±0.02	0.76±0.08	0.70±0.05	1.36±0.5	
Aspergillus flavus	**25**	0.63±0.13	-	0.56±0.06	-	6.0±0.2
	50	1.13±0.15	0.70±0.5	0.63±0.05	1.53±0.6	
	100	1.40±0.5	1.2±0.2	1.10±0.4	3.10±0.8	

Fig.1 Yield of various crude extract of *Salvia aegyptiaca* L.



Of all the bacteria tested the Gram-positive bacteria were slightly more susceptible to the extracts than the Gram-negative bacteria, therefore the present results are in agreement with earlier reports (Jigna and Sumitra, 2006; Dougharia and Manzara, 2008). The crude

extracts of *S. aegyptiaca* exhibited *in vitro* antifungal activity against *Aspergillus niger* and *Aspergillus flavus.* As the drug dose increased (25, 50 and 100 mg/well) simultaneously increased the zone of inhibition of the fungal growth (Table.3). The aqueous extract shows highest antifungal activity on both *Aspergillus niger* and *Aspergillus flavus*, among the maximum inhibition growth was observed in *Aspergillus flavus* (3.10±0.8mm) compared to *Aspergillus niger* (1.36±0.5mm) at 100mg/ml concentration. Abdulhamida *et al.,* (2013) have observed the most sensitive fungus to the esstenial oil was *Aspergillus flavus* compared to *Aspergillus niger* in *Salvia fruticosa.* It was interesting to note that bacteria was more sensitive to methanol extract whereas, fungi was more sensitive towards aqueous extracts .The difference in the observed activities of the various extract may be due to varying degree of solubility of active constituents in the solvents used. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents (Majorie, 1999). The antimicrobial activity of various crude extracts of *S. aegyptiaca* shows that the maximum inhibition zone in methanol extract against *Pseudomonas aeruginosa* (1.36±0.5mm) and aqueous extract against *Aspergillus flavus* (3.10±0.8mm). Overall, fungi were more sensitive than the bacterial strains in *Salvia aegyptiaca*. Further investigation of activity against a wider range of pathogens, identification and purification of active chemical constituents and toxicological investigation of plant extracts should be carried out with a view to developing novel drugs for human consumption.

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