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Phytochemical Screening, Anti-microbial Evaluation of Extract of Salvia officinalis L.

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ABSTRACT

The present study was conducted to asses phytochemical and anti microbial activity of extracts of Salvia officinalis L. against bacteria and fungi. This experiment was conducted to valorize Salvia officinalis post-distilled aerial parts as natural antioxidants. Total phenolic contents were determined, the phenolic constituents were determined using HPLC with UV detection S. officinalis residues revealed phenolic diterpenes as main components. The phytochemical test revealed that the presence of proteins, carbohydrates, lipids, alkaloids, phenols, flavonoids, steroids, glycosides, tannins, terpenoids and resins. The antibacterial activity of methanol extract shows maximum inhibition zone on Gram positive bacteria. These results include antioxidant, antimicrobial properties. These findings suggest that S. officinalis by-products, particularly at flowering, may be considered as an interesting source of natural antioxidants, anti microbial properties.

KEYWORDS: Medicinal applications, herbal therapies, Salvia officinalis L., anti microbial properties.

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INTRODUCTION

As per WHO Traditional medicine is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

World Health Organization define Traditional herbal medicines as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.

Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations.

There are many approaches to the search for new biologically active principles in higher plants. One can simply look for new chemical constitution and hope to find a biologist who is willing to test each substance with whatever pharmacological tests available.

A second approach is simply to collect every readily available plant, prepare extract and test each extract for one (or) more types of pharmacological activity. This random collection, broad screening method is a reasonable approach that eventually should produce useful drugs, but it is contingent as the availability of adequate findings and appropriate predictable bioassay systems.

Fig 1. Arial parts of Salvia officinalis L.

PLANT PROFILE

SALVIA OFFICINALIS L.

Salvia officinalis L. (Sage) is a perennial round shrub in the family of Labiatae/Lamiaceae. Salvia is the largest genus of this family and includes near 900 species.

Phytochemistry:

The major phytochemicals in flowers, leaves, and stem of S. officinalis are well identified. A wide range of constituents include alkaloids, carbohydrate, fatty acids, glycosidic derivatives (e.g.,cardiac glycosides, flavonoid glycosides, saponins), phenolic compounds (e.g., coumarins, flavonoids, tannins), poly acetylenes, steroids, terpenes/terpenoids(e.g., monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids), and waxes are found in S. officinalis.

MATERIAL & METHODS

PRLIMINARY INVESTIGATION

Collection of plant material:

The medicinal plant of salvia officinalis L was collected from natural habitat and authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

Preparation of plant powder:

The plant material of salvia officinalis L. was dried under shade and then powdered with mechanical grinder. The pulverized, sieved through 40 mesh to obtain a coarse powder and stored in an airtight container for further use.

Preparation of extracts:

About 250-250 gm of dried powder of salvia officinalis L. were subjected to soxhlation separately. It was first defatted with petroleum ether then exhaustively extracted with different solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.

Phytochemical Screening

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts.

Tests for carbohydrates and glycosides:

Molisch's test:

Sample was treated with 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acidwas added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Legal's test:

To the sample 1 ml of pyridine and few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test:

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Test for alkaloids:

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff"s reagent - Reddish brown ppt
- Wagner's reagent - Reddish brown ppt
- Mayer's reagent - Cream color ppt
- Hager's reagent - yellow color ppt

Test for proteins and free amino acids:

Small quantities of the sample were dissolved in few ml of water and treated with following reagents.

Million's reagent: Appearance of red color shows the presence of protein and free amino acid.

Ninhydrin reagent: Appearance of purple color shows the presence of Proteins and free amino acids

Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

Test for tannins:

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Violet color.
- 10% lead acetate solution - White precipitate

Test for flavonoids
Alkaline reagent test:
To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test:
Small quantities of the sample were dissolved in alcohol, to this piece of magnesium followed by concentrated hydrochloric acid drop wise added and heated. Appearance of magneta color shows the presence of flavonoids.

Tests for fixed oils and fats Spot test:
A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Few drops of 0.5Nalcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolpthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids:
Libermann-burchard test:
Sample was treated with few drops of acetic anhydride, boils and cooled. Then concentrated sulphuric acid was added from the side of test tube, brown ring was formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

Salkowski test:
Sample was treatedwith few drop of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilages and gums:
Small quantities of sample was added separately to 25 ml. of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

Test for waxes:
To the test solution alcoholic alkali solution was added, the waxes get saponified.

Evaluation of Antimicrobial Activity
Method of Screening of Antimicrobial Activity
A drug is considered as bacteriostatic or fungistatic when it inhibits the growth or multiplication of bacteria or fungi respectively and considered as bactericidal or fungicidal when it actually results in the death of bacteria or fungi. Drugs that are bactericidal under certain circumstances may have an apparent bacteriostatic effect at the other times. Important factors for the antimicrobial activity are size of the inoculums, metabolic state of organisms, pH, temperature and duration of interaction, concentration of the inhibitor and presence of interfering substance. In Vitro tests are used as screening procedure for new agents and for testing susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent. This method is suitable for the organisms that grow well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi such as Candida Albicans. Several forms of disc diffusion methods have been advocated. Among this Kirby Bauer method is the official method of the USA Food & Drug Administration.

Preparation of the Nutrient Media
The following broths were used in the present work. Compositions of broths are as follows:

Table 1: Composition of Nutrient broth

S. no.	Component	Amount
1	Peptone (bacteriological)	10 gm
2	Beef extract	10 gm
3	Sodium chloride	5 gm
4	Purified water	1000 ml
5	Ph	7.2 ± 0.2

Table 2: Composition of Sabouraud's broth

S. no.	Component	Amount
1	Dextrose	20 gm
2	Peptone (mycological)	10 gm
3	Agar	15 gm
4	Purified water	1000 ml
5	pH	7.2 ± 0.2

The broths were prepared by dissolving the specified quantities of the dehydrated broth (Hi media) in purified water and were distributed 4 ml quantities in to each test tube. The tubes were closed with cotton plugs and sterilized by autoclaving at 121ºC for 15 minutes.

Cultivation of Microorganisms

The bacterial cultures were aseptically inoculated into nutrient
broth and incubated under aerobic conditions at 37ºC for 24 h. Fungal cultures were inoculated into Sabouraud's broth and incubated under aerobic conditions at 25 ºC for 48 h. The following bacterial and fungal cultures were used for the study, enlisted in table 3:

Table 3: Different microbial cultures used

S. no.	Name of Microorganisms	Status
1	Bacilliu s Subtilis	Gram positive bacteria
2	Escherichia Coli	Gram negative bacteria
3	Candida Albicans	Fungi (yeast)
4	Aspergillus Niger	Fungi (mold)

The bacterial cultures were aseptically inoculated into nutrient broth and incubated under aerobic conditions at 37ºC for 24 h. fungal cultures were inoculated in to Sabouraud's broth and incubated under aerobic conditions at 25ºC for 48 h.

Determination of Antimicrobial Activity

Modified Kirby-Bauer method, one of the official methods among disc diffusion methods, was used for the evaluation of antimicrobial activity of the synthesized compounds. Circular paper disks of 6 mm diameter was impregnated with the specific amount of the test sample and were placed on a suitable nutrient/sabraud's agar medium in a petri plate which was inoculated on its surface with one of the test organisms. After incubation, the plates were observed for the growth inhibition zones around the disks. The diameter of the zone of inhibition is proportional to the antimicrobial activity of the substance. The diameters of the zone of inhibition were compared with that produced by the standard antibiotics.

Preparation of the Disks and Samples

Paper disks of 6 mm diameter and 2 mm thickness were used for the test. These disks were sterilized by autoclaving at 121 ºC (15 lb PSIG) for 15 minutes. After drying, they were used for screening the antimicrobial activity.

The sterile filter paper disc of 6mm diameter soaked with plant extract (20 to 100 µg/disk)was placed on the surface of the medium for assay and Ciprofloxacin (10 µg/disk) was taken as standard antibiotics for the comparison of the antibacterial activity of the synthesized compounds and Clotrimazole (10 µg/disk) were used as standard drugs for antifungal activity studies.

General Procedure

Each Petri plate containing nutrient/ sabouraud's agar medium was inoculated with one bacterial/ fungal culture by spreading the suspension of the organism with a sterile cotton swap. Each plate was divided into five equal portions along the diameter. Each portion was used to place one disk. Four disks of each sample were placed on four portions, one disk with standard drug and a disk impregnated with the plant extract. All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of the sample in to the refrigerator for 30 minutes to allow the diffusion of the sample into the surrounding agar medium. Then the plates inoculated with bacterial cultures were incubated at 37 ºC for 18 h and those with incubated at 25 ºC for 48 h. Diameter of the zones of inhibition wherever produced were measured and the average diameter for each sample was calculated. The diameters obtained for the test samples were compared with that produced by the standard antibiotics, ciprofloxacin for antibacterial activity and clotrimazole for antifungal activity. The results of antibacterial and antifungal activity are given in Tables and figures.

RESULTS AND DISCUSSION

Extraction

The dried powder of Salvia officinalis plants was extracted with 70% v/v hydro alcoholic solution. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of hydroalcohalic plant extract was found to be 60 %.

Phytochemical Screening

The qualitative phytochemical screening of Salvia officinalis (Linn.) in six different extracts i.e. Petroleum ether, chloroform, ethanol and hydroalcohalic (Water and ethanol in 1:1 ratio) showed that there is presence of carbohydrates, glycosides, proteins, alkaloids, saponin, flavonoids, steroids, tannins, phenolic compounds. However, steroids were totally absent in all extracts. The results of the phytochemical screening of extract of Salvia officinalis present in Table-4. Preliminary phytochemical screening was useful in prediction of nature of drugs and also useful for the detection of several constituents present in solvent. Ethanolic and Hydroalcohalic extract of Salvia officinalis (Linn.) was accounted for the presence of alkaloids, carbohydrates, glycosides, proteins, flavonoids, phenol and tannin. While chlorofor extract showed the presence of alkaloids, carbohydrates, glycosides, flavonoids and phenolic compounds. Only Petroleum ether and water extract showed the presence of mucilage & gum and wax. All the extract showed the presence of alkaloids, proteins, flavonoids, carbohydrates, glycosides and flavonoids.

Table 4: Phytochemical Screening of Salvia officinalis L Plant Extracts

S.No	Test	Petroleum ether Extract	Hydroalcohalic Extract.	Ethanol	Chloroform Extract
1.	**Alkaloids**	+ ve	+ ve	+ ve	+ ve
2.	**Flavonoids**	+ ve	+ ve	+ ve	+ ve
3.	**Steroids and Triterpenoid**	- ve	- ve	- ve	- ve
4.	**Phenolic &Tannins**	- ve	+ ve	+ ve	- ve
5.	**Saponins**	+ ve	-ve	-ve	-ve
6.	**Carbohydrate and Glycoside**	+ ve	+ ve	+ve	+ ve
7.	**Proteins and amino acid**	+ ve	+ ve	+ ve	+ ve
8.	**Mucilage and gum**	+ ve	- ve	- ve	- ve
9.	**Wax**	+ ve	- ve	- ve	- ve

Antibacterial Activity of Salvia officinalis

Antibacterial activity of different con. of plant extracts in terms of MIC is presented in Table 5 and Figure. 2 for out of 100 µg/ml concentration of plants extract exhibit any antibacterial activity. Also, the diameter of inhibition is shown in Figure. 3, its graphical representation is shown in Figure. 4.

Table 5: Anti Bacterial Activity of hydroalcohalic extract of S. officinalis

Concentration of Extract(µg/ml)	B. subtilis	E. coli
25	13(6.15)[a]	11(10.5)
50	15(13.5)	18(11.5)
100	18(22.4)	23(24.2)
Control	-	-
Ciproflaxacin	21(4.13)	20(19.2)

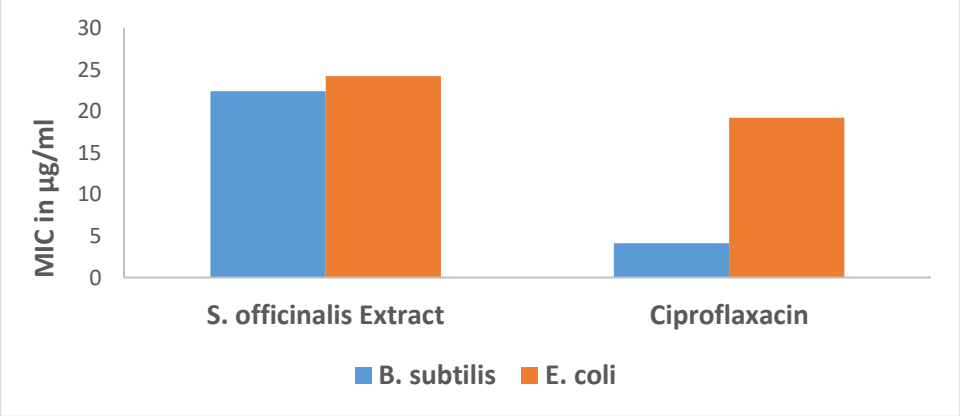

Figure 2: Minimum Inhibitory Concentration of S. officinalis compared to Std. drug

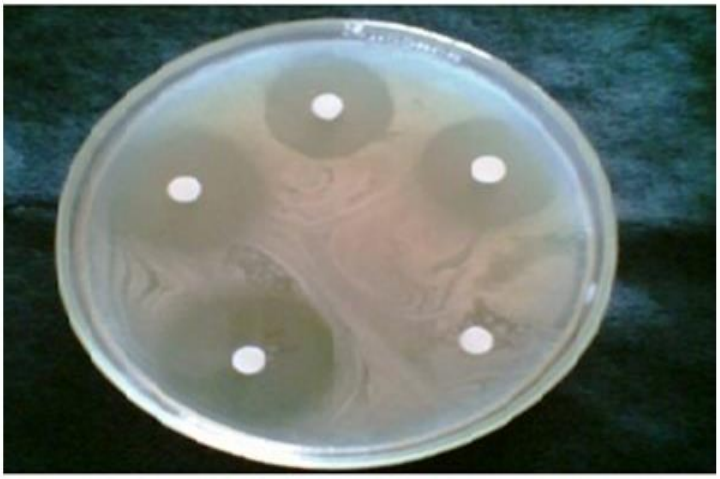

Figure 3: Antibacterial Activity of S. officinalis hydroalcohalic extract

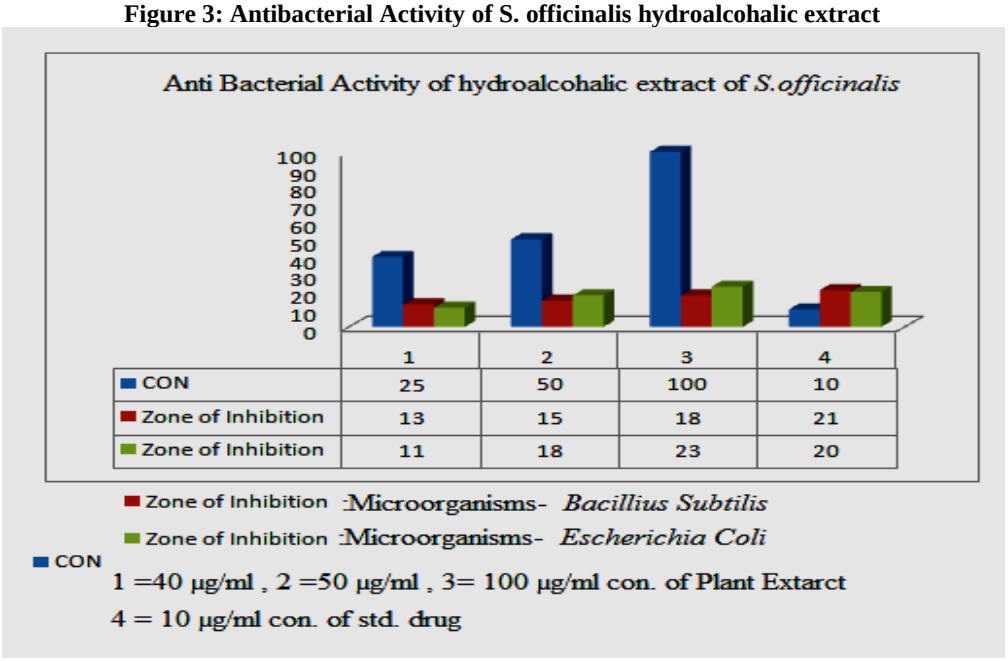

Figure 4: Anti-bacterial activity of S. officinalis Extract

Anti Fungal Activity

Antifugnal activity of different con. of plant extracts in terms of MIC is presented in Table 6 and Figure 5 for out of 100 µg/ml concentration of plants extract exhibit any antibacterial activity. Also, the diameter of inhibition is shown in Figure 6, its graphical representation is shown in Figure 7.

Table 6: Anti Fungal Activity of hydroalcohalic extract of S. officinalis

Concentration of Extract(µg/ml)	C. albicans	A. niger
25	14	18
50	16	19
100	20	21
Control	-	-
Clotrimazole	21	20

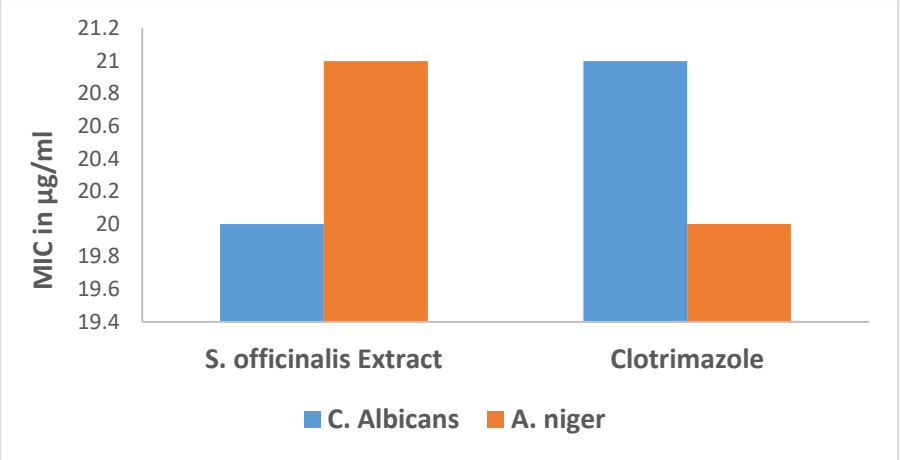

Figure 5: Minimum Inhibitory Concentration of S. officinalis compared to Std. drug

Figure 6: Antifungal Activity of S. officinalis hydroalcohalic extract

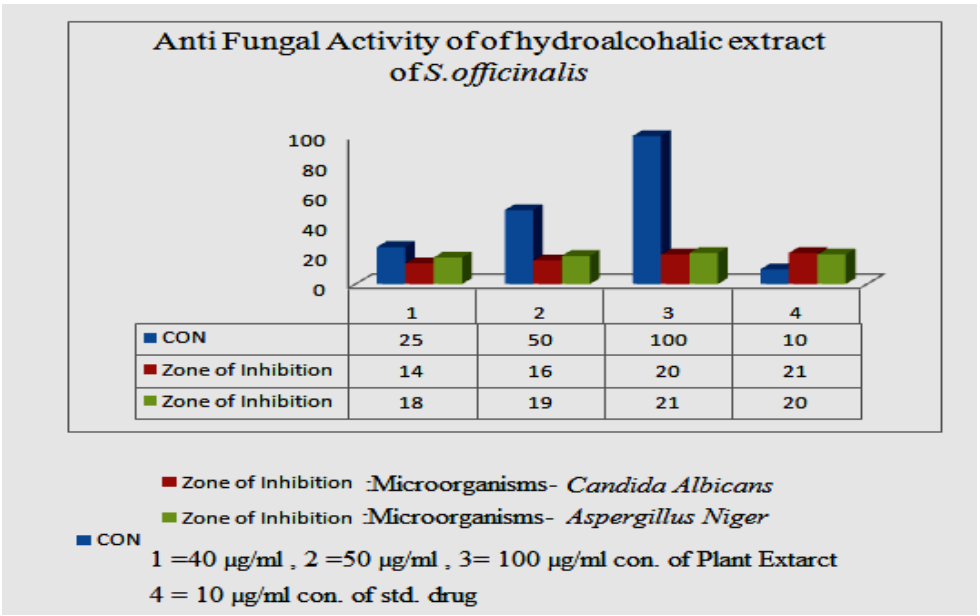

Figure 7: Anti-fungal activity of synthesize compound

S. officinalis extracts were screened for in vitro antimicrobial activity against gram-positive bacteria B. Subtilis and gram-negative bacteria and E. Coli, cutaneous fungi C. Albicans and A. Niger. Salvia officinalis plant extract at 100 µg/ml exhibited excellent antibacterial activity against E. coli in comparison to standard drug – Ciprofloxacin. and excellent antifungal activity against A. Niger in comparison to standard drug – Clotrimazole.

CONCLUSION

To conclude, the present work analyzed the extract of Salvia officinalis by studying the antioxidant, antifungal and antimicrobial activities and by performing phytochemical screnning. The results of this study show that the plant studied is rich in phenols, flavonoids, alkaloids, saponoside, sterol and tannins, which explains the important antioxidant effect of the extract, and at the same time the antimicrobial and antifungal activity against bacteria and standard yeasts used. Extracts from S. officinalis were tested for their in vitro antibacterial activity against gram-negative bacteria, E. Coli, cutaneous fungi C. albicans, and A. nigger, as well as gram-positive bacteria, B. subtilis. The plant extract of Salvia officinalis, had superior antibacterial activity against E. coli when compared to the common medication, Ciprofloxacin. significantly superior antifungal efficacy when compared to the conventional medication, Clotrimazole. This work reveled that the extract of Salvia officinalis L. having antimicrobial and antifungal activity. By studying the antioxidant, antifungal and antimicrobial activities and by performing phytochemical screening, the results of this study show that the plant can be used for various pharmacological aspects.

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