Original Article

Studying the Chemical Composition and Antimicrobial Effect of *Salvia officinalis* L.

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Abstract

Background: The therapeutic benefits of medicinal plants are well known. Sage (*Salvia officinalis* L.) is a significant medicinal herb with beneficial medical characteristics in folk medicine, such as anticancer, antioxidant, and antimicrobial properties. **Objectives:** This study was conducted to characterize and quantify the chemical composition of *S. officinalis* and to evaluate the antimicrobial activity of different plant extracts against various bacteria and fungi. **Methods and Materials:** The phytochemical content of *S. officinalis* was determined by chemical tests. The phenolic and alkaloid components of the plant were identified and measured using high-performance liquid chromatography (HPLC). The inhibitory activity of four different extracts of *S. officinalis* was evaluated against selected bacteria and fungi using the well diffusion method. **Results:** Phytochemical screening showed the presence of flavonoids, glycosides, phenolic compounds, compounds, saponin, coumarin, tannins, resins, steroids, and carbohydrates. HPLC analysis showed that *S. officinalis* contained many phenolic and alkaloid compounds. The phenolic compounds identified were chlorogenic, lignan, eugenol, cinnamaldehyde, quercitin, 4-hydroxylbenzoic acid, catechol, cinnamic, kaempferol, gallic acid, while the alkaloid compounds were caffeine, retrosine, solanum, pilocarpine, veratrum, tropane, berberine, theobromine, ethylbenzhydramine, phencyclidine, gallocatechin, benzfetamine, and amfepramone. The antimicrobial effect was tested at a concentration (100 mg/mL) for various extracts, and the ethyl acetate extract was most efficient against most tested microorganisms. The extracts demonstrated good activity against *Aspergillus* and *Penicillium* fungi. **Conclusion:** Various phenolic acids and alkaloids were identified in this investigation. *Salvia officinalis* exhibited a range of antibacterial and antifungal activity, and these plants can be used to treat illnesses caused by the test microorganisms.

Keywords: Antimicrobial activity, chemical content, HPLC, medicinal plant, sage, *Salvia officinalis L.*

Introduction

The administration of conventional therapeutic drugs is the mainstay of treatment for microbial diseases. Given the extensive use of these antimicrobial agents, pathogens have largely developed resistance to these medications.[1] The rising prevalence of antibiotic resistance in recent decades raises concerns. Herbal medicine is regarded as being one of the important area of conventional medicine in the entire world.[2,3] Medicinal herbs are employed to treat illnesses because they are widely accessible, low-risk, and cheap natural materials.[4] In traditional medicine, extracts and essential oils (EOs) from flowers and leaves are used in the belief that they may be useful to treat a variety of disorders.[5] Additionally, it is thought that medicinal plants constitute a significant source of new compounds that can be used for treatment. It has been observed that the secondary metabolites of plants are a source of a variety of phytochemicals that can be used directly as intermediates in the development of new medications.[6] The antimicrobial properties of medicinal herbs are attributed to the plant constituents of the EOs, which

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are composed of several secondary metabolites such as flavonoids, alkaloids, and tannins.[7]

Among these plants, Salvia is a significant genus that belongs to the Lamiaceae family and has over 900 species. *Salvia officinalis L.*, also known as sage, is a medicinal and aromatic plant largely utilized as a culinary herb and used in flavoring and traditional medicines.[8] It is a perennial subshrub that can reach a height of 30 cm to 1 m. It has woody stems, grayish leaves, and blue-to-purplish blooms with a powerful odor. Although it has spread throughout the world, it is native to the Mediterranean area.[9]

Salvia officials are generally known for their multiple pharmacological effects, and it is used due to its anti-inflammatory, antioxidant, and antimicrobial efficacy and for its sedative, expectorant, diuretic, and antihypertensive impacts to treat chronic bronchitis, perspiration, asthma, chronic renal failure, cirrhosis, and coronary heart disease.[10] The pharmacological properties of this plant are also well known today due to their characteristic effective compounds,[11] such as alkaloids, glycosidic derivatives (flavonoid glycosides, cardiac glycosides, and saponins), phenolic components (flavonoids, coumarins, and tannins), steroids, polyacetylenes, terpenes/ terpenoids (monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids). Over 120 constituents have been identified in the EO made from *S. officinalis* aerial parts.[12] This study aimed to determine the chemical composition of *S. officinalis* extracts and assess the antimicrobial activity of the extracts against some microbial pathogens.

Materials and Methods

The alcoholic extract was extracted according to the method adopted by AL-Mossawi and AL-Hilfi[13] in preparing plant extracts, where 100 g of sage powder was weighed and 500 ethyl alcohol 98% was added to it, mixed well, and left for 24 h at 25°C, then filtered the extract using filter paper (Whatman no. 1) and then with a Buechner funnel. Concentrate the filtrate with a rotary vacuum evaporator at a temperature of (40°C), leave the filtrate at room temperature to get rid of the solvent in general, or put it in an oven (30°C). Subsequently, the material was skimmed off and placed in airtight, dark bottles and kept in the refrigerator at 4°C until use.

Detection of resins

Five grams of dry plant powder was taken and added to 50 mL of 95% ethyl alcohol and put in a boiling water bath for 1 min at 100°C. Then, filtered the solution and about 100 mL of water (4%) hydrochloric acid was added, and the presence of resinous substances was inferred through the appearance of turbidity.[14]

Detection of glycosides

Take 5 mL of Fahlenk reagent and mix it with 5 mL of the sample aqueous extract, then leave the mixture in a boiling bath at 100°C for about 10 min. The presence of glycosides was indicated by the red precipitate. To ensure the validity of the test, 1 mL of aqueous extract was added to 5, Benedict's reagent, and the existence of glycosides was inferred by red precipitate production.[14]

Detection of flavonoids

Flavonoids were detected according to the method,[15] which included the preparation of two solutions: The first consists of dissolving 10 g of the extract for each of the samples in 5 mL of 95% ethanol, then filtering the solution.

The second solution was prepared by adding 50% ethyl alcohol to a 50% potassium hydroxide solution. The two solutions were mixed with each other in equal quantities, and the presence of flavones was inferred through the appearance of a yellow color.

Detection of phenols

The test for lead acetate was performed. Mixed about 50 mg of the plant extract with 5 mL of distilled water, and after that, added about 3 mL of lead acetate (10%) to the mixture. A bulky white precipitate revealed the phenol compounds.[16]

Detection of saponins

An aqueous solution was made from the powder of the dry sample, then put in a test tube and shaken well. Saponins were assumed to be present based on the long-lasting appearance of a thick foam.[16]

Detection of alkaloids

Hager's reagent is a saturated picric acid solution ($C_6H_3N_3O_7$). After adding a few drops of this reagent, a yellow precipitate appears, and it is positive, indicating the occurrence of alkaloids.[17]

Detection of coumarins

Dissolve 0.5 mg of plant extract in 1 mL of alcohol in a test tube, then cover the tube with a moistened filter paper with a dilute solution of NaOH and heat in a water bath on boiling for a short period of time (few minutes), after that exposed filter paper to ultraviolet light and the appearance of the yellow-green color in the filter paper evidence for the presence of coumarin.[18]

Detection of tannins

Ten gm of plant powder were boiled with 50 mL distilled water, and the solution was filtered. Then, the filtrate was left to cool and divided into two parts. To the first part, 1% lead acetate solution was added, and the appearance of a white, gel-like precipitate indicates the presence of tannins. To the second part, 1% ferric chloride was added, and the

bluish-green color appearance indicated the presence of tannins.[19]

Microbial isolates

The microorganisms used in this study included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *K. pnemoniae* bacteria, while the fungal isolates were *Aspergillus* and *Penicillium* spp. The microorganism identification was confirmed by using traditional tests and stored until used later.

Testing of antibacterial activity of plant extract

Different plant extracts' antibacterial potency was assessed using the well diffusion agar technique. This examination was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI),[20] each isolate's loop full growth was injected into the nutritional broth and incubated there for 18 h at 37°C. Normal saline was used to dilute the bacterial suspensions. The turbidity of the broth culture was calibrated using a standard tube of McFarland (number 0.5).

Bacterial seeding was performed using a swab and immersion in the bacterial suspension. The Mueller-Hinton agar plates were then seeded with the colonies, and they were allowed to dry for (5–15 min) at room temperature. Utilizing a cork borer (5 mm) diameter, five wells were made in every plate, and (0.1 mL) of the extracts were inserted in every well (the plates were processed in triplicates). The plates were left to incubate at 37°C for 24 h. After the incubation period, the results were observed by calculating the diameters of the inhibition zones.

Antifungal activity of the extracts

The antifungal efficacy of the prepared extracts of the plants was tested at a concentration of 100 mg/ml against *Aspergillus* and *Penicillium* according to the procedure by Ali and Majeed.[21] Potato dextrose agar (PDA) was prepared and sterilized, then the mentioned concentration was added to the medium. As for the control, PDA without adding any extract was used. The medium was poured into Petri dishes (9 cm) with three dishes for each extract. After solidification of the culture medium in the dishes, each dish was inoculated with a disk of the developing fungi colony at the age of 7 days, a disk with a diameter of 5 mm was placed in the center of the dish, then the process was repeated on all the dishes and transferred to the incubator. The dishes were placed in the incubator at a temperature of 25°C for 5–7 days, carried out using a completely randomized design, and after completing the growth of the fungal culture in the control group, the results were calculated by adopting the measurement of the fungal growth of the fungi colony. The measurement was taken perpendicular to the two diameters for all dishes, and the percentage inhibition was calculated according to the equation.

$$\text{Growth inhibition}\% = \frac{[\text{Growth in control} - \text{Growth in treatment}] \times 100}{\text{Growth in the control}}$$

HPLC assay

Salvia officinalis sample was analyzed using high-performance liquid chromatography (HPLC). The HPLC apparatus (Shimadzu LC-10 A, Japan) Reverse-phase chromatography investigation of phenolic components was performed according to modified methods[22] utilizing a C-18 reverse-phase column, with running conditions: mobile phase (0.1%) H3PO4 in D.W., flow rate (0.8 mL/min); and observation at (210 nm). For alkaloids compound, samples were separated according to modified methods,[23] using running conditions: mobile phase CAN: glacial acetic: tri ethyl amine 97.9:2:01, flow rate (1 mL/min), and observation at (284 nm). The samples were filtered through an ultramembrane filter (pore size 0.45 m) prior to injection in the sample loop. Chlorogenic, lignan, eugenol, cinnamaldehyde, qurcitin, 4- hydroxyl benzoic acid, catechol, cinnamic, kaempferol, galic acid were applied as flavonoid standards, while caffeine, retrosine, solanum, pilocarpine, veratrum, tropane, berberine, theobromine, ethyl benzhydramine, phencyclidine, gallocatechin, benzfetamine and amfepramon were used as alkaloid standards. The plant sample and standards were estimated using retention times gained from authentic standards run under similar conditions.

Ethical approval

The study protocol was reviewed and approved by a local ethics committee, according to document number 5250, on December 24, 2022.

Results

Phytochemical compounds determination

Phytochemical screening by the chemical tests showed the presence of flavonoids, glycoside, phenols, alkaloids, saponin, coumarin, tannins, steroids, carbohydrates, resins, and the absence of quinones in *S. officinalis* extract as shown in Table 1.

Antibacterial activity

The antimicrobial activities of the ethanolic, methanolic, acetone, and ethyl acetate extracts of *S. officinalis* at a concentration (100 mg/mL) were evaluated on the growth of selected microorganisms by the diffusion method as shown in Table 2. The different extracts of the plant showed various inhibitory effects toward the investigated microorganisms. *E. coli* and *Salmonella typhi*

Table 1: Phytochemical screening of *Saliva officinalis L.* extract

Constituent	Result
Flavonoids	+
Glycoside	+
Phenols	+
Alkaloide	+
Saponine	+
Coumarin	+
Tannins	+
Resins	+
Steroids	+
Carbohydrate	+
Quinones	–

Table 2: Inhibition zone diameters (mm) of *Salvia officinalis* extract against bacterial isolates

Bacteria	*S. officinalis* extract (100 mg/mL)			
	Ethanolic	Acetone	Methanolic	Ethyl acetate
Staphylococcus aureus	11	0	14	14
Streptococcus pyogenes	0	0	0	15
Escherichia coli	15	17	10	16
Klebsiella pnemoniae	0	0	0	12
Pseudomonas aeruginosa	13	0	0	42
Salmonella typhi	15	13	7	20

showed sensitivity to different *S.officinalis* extracts, while *Streptococcus pyogenes* and *Klebsiella pneumoniae* were resistant to most extracts except ethyl acetate extract. *Staphylococcus aureus* was found to be sensitive to all the extracts except the acetone plant extract.

Antifungal activity

The antifungal activities of the extracts obtained from the plants under study are shown in Table 3. All extracts of the plants at a concentration 100 mg/mL showed various inhibitory effects against *Aspergillus* and *Penicillium*. It was found from [Table 3] that acetone and ethyl acetate extracts had a high inhibitory activity against fungi.

Analysis of compounds of the plants by HPLC

HPLC analysis of the *S. officinalis* resulted in the separation of several components. This exploration led to the identification of 25 compounds, twelve of which were phenolic compounds and thirteen were alkaloid compounds. The plant was analyzed by interpolating the areas obtained for each compound. The presence and

Table 3: Diameter of colonies (mm) of *Aspergillus* and *Penicillium* before and after being treated with different *Salvia officinalis* extract

Fungi	*T. officinalis* extract				Control
	Ethanolic	Acetone	Methanolic	Ethyl acetate	
Aspergillus	24	27	20	26.5	70
Penicillium	23.5	29	18	30	65

Table 4: Identification of phenolic compounds of *Salvia officinalis*

Seq	Compound	Retention time (minute)	Area μ volt	Concentration
1	Chologenic	17.164	997.653	216534101
2	Lignan	17.128	1129.192	10162728
3	Eugenol	13.860	1116.296	24558512
4	Cinnamaldehyde	10.392	1439.664	214361523
5	Qurcitin	10.008	1509.964	800280
6	4- hydroxyl benzoic acid	9.872	1214.957	0.1214957
7	Catechol	8.348	495.445	9.611633
8	Cinnamic	8.188	369.139	2.620886
9	Kaempferol	5.404	439.136	1.542
10	Rutin	4.240	29.553	0.0354
11	Galic acid	3.856	72.340	0.057872
12	Pyrogallol	1.524	2997.691	2.985237

the concentration of phenolic and alkaloid compounds were simultaneously assessed in *S. officinalis* by HPLC. As for the phenolic acids, chologenic, lignan, eugenol, cinnama dehyde, qurcitin, 4- hydroxyl benzoic acid, catechol, cinnamic, kaempferol, galic acid were positively identified according to their retention in comparison with commercial standards. The phenolic profiles of the plant, as determined by HPLC, are presented in Table 4.

The findings exhibited that *S. officinalis* has a number of alkaloid compounds as shown in Table 5. The main alkaloid components identified in the plant were caffeine, retrosine, solanum, pilocarpine, veratrum, tropane, berberine, and other components were determined like theobromine, ethyl Benz hydration, phencyclidine, gallocatechin, benzfetamine and amfepramone.

Discussion

These results are consistent with previous reports on the phytochemical compounds.[19,24] The Stanojenić *et al.*[25] was referring to finding a number of effective chemical compounds in aqueous and alcoholic *S. officinalis* extract like glycosides, flavonoids, resins, alkaloids, phenols, and saponins, while giving a negative test for tannins and coumarin. Another study showed the presence of flavonoids, tannins, triterpenoids, and steroids, and the absence of alkaloids and saponins in *S. officinalis*.[26]

Table 5: Identification of alkaloid compounds of the *Salvia officinalis*

Seq	Compound	Retention time (minute)	Area μ volt	Concentration
1	Caffeine	23.828	189.155	2.005043
2	Retrosine	19.168	44.654	0.4777978
3	Solanum	18.820	83.752	0.8626456
4	Pilocarpine	18.475	68..079	0.5650557
5	Veratrum	16.868	22.211	0.0499642
6	Tropane	16.280	12.152	0.0704816
7	Berberine	14.748	7.462	0.0925288
8	Theobromine	12.544	12.005	0.163268
9	Ethyl benzhydramine	10.520	15.471	0.0247536
10	Phencyclidine	8.436	218.417	2.5336372
11	Gallocatechin	7.980	34.253	0.0729813
12	Benzfetamine	7.344	309.614	39320978
13	Amfepramone	6.740	34.626	5.2527642

Many researchers have displayed the significance of phenolics, particularly flavonoid components, as secondary metabolites responsible for the various biological properties of *Salvia* species. *Salvia officinalis* was identified and investigated as being rich in volatile oils like phenolic and terpenoid compounds that possess antioxidant, anti-inflammatory, neuroprotective, and antibacterial efficacy.[27] It is well recognized that glycosides are crucial to the survival of plants, as they play a regulatory role in the growth process and a protective role to preserve the life of the plant against some pests and insects that infect it.

The importance of tannins lies in the fact that they are a source of energy that the plant consumes in the biological processes of metabolism. They also protect the plant from harmful insects and fungi, thus helping the plant grow naturally.[19] The prevalence of these active ingredients in the stems and leaves of the sage plant indicates the importance of this plant and shows the reason for its use in medicine, both ancient and modern, as well as revealing the medicinal value of this plant.[28]

Such findings were confirmed by other researchers like Mosafa *et al.*[29] who stated that ethanol extract at 100, 400 mg/mL was shown to possess antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli*.

Bouteldja *et al.*[30] stated that methanolic and ethanolic *S. officinalis* extracts showed various inhibitory effects against *S. aureus*, *B. subtilis*, *E. coli,* and *P. aeruginosa*. Ahmed *et al.*[31] found that ethanol extracts of *S. officinalis* showed antimicrobial activity against *St. pyogenes*, *E. coli*, and *Klebsiella* spp. in which this activity was enhanced with the increasing concentrations of the extract, with no antimicrobial activity against *S. aureus*. In addition, it was recorded that *S. aureus*, *E coli* and *P. aeruginosa* showed zones of inhibition toward the study plant.[32]

The leaf extracts of *S. officinalis* have been documented to have a wide range of antimicrobial effects[33] and may be indicative of the presence of broad-spectrum effective compounds. It has been demonstrated that if solvents such as methanol, ethanol, and hexane are employed to extract plant material, the majority of them are capable of displaying an inhibitory action against microbes.[34] The reason for the effect is that it contains different active compounds like glycosides, phenols, and alkaloids that have an inhibitory effect on the growth of many pathogenic microorganisms.[35]

These findings are consistent with Yilar *et al.*,[36] who revealed that *S.officinalis* extracts showed effects against *Aspergillus niger* and *Penicullum italicum* fungi, and this effect varied depending on the extracts, extract concentration, and the species of fungus. In another study, Mocan *et al.*[27] revealed that *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus ochraceus*, *Aspergillus niger*, *Trichoderma viride*, *Penicillium funiculosum*, *Penicillium ochrochlorum*, and *Penicillium verrucosum* were found to be sensitive to *S. officinalis* extracts. It was shown by Rashidi *et al.*[37] that *S. officinalis* L. extracts have antifungal effects on *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. In contrast, Veličković *et al.*[38] reported that there was no activity of *S. officinalis* methanolic and ethanolic extracts against *A. niger*.

The variation in findings can be linked to numerous factors, including the type of extracts, methods of extraction, testing methods, isolated fungal species, and substances utilized for the test.

It was found by Gligor *et al.*[39] that quercetin, ferulic acid, caffeic acid, rutin, chlorogenic acid, catechin, cinnamic acid were the main phenolic compounds detected in *S. officinalis*, while the other phenolics syringic acid and resveratrol, are present in lower concentrations. Shoker *et al.*[40] reported that the main compounds identified in the plant were catechin, quercetin, epigallocatechin, and kaempferol. Previous reports indicated that *S. officinalis* contained Gallic acid, protocatechuic acid, catechin,

gentisic acid, chlorogenic acid, vanillic acid, benzoic acid, syringic acid, caffeic acid, epicatechin.[41] *S. officinalis* is identified by the occurrence of rosmarenic acid, cinnamic acid, methyl rosmarenate, chlorogenic acid, and quinic acid as phenolic acids, in addition to certain flavonoids like apigenin, luteolin, and quercetin.[42-46]

Conclusion

The results showed that extracts of *S. officials* have antibacterial and potent antifungal activity, which allows them to suppress growth, and this may be attributed to the phenols and alkaloid components identified by HPLC. The findings support the use of this plant as a cheap and natural agent in traditional medicine for treating infections, and it can be a source of biologically active compounds.

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Conflicts of interest

There are no conflicts of interest.

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