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Antibacterial activity of *Salvia officinalis* (sage) against *Streptococcus pyogenes* isolates from throat culture

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MAIN POINTS

- This study is the first to investigate the antibacterial effects of methanolic leaf and root extracts of *S. officinalis* against clinical *S. pyogenes* isolates.
- The MIC and MBC values were determined using the broth microdilution method, which indicated that leaf extracts had stronger antibacterial activity than root extracts.
- The results contribute to the standardization of plant-based antibacterial testing and support the potential use of *S. officinalis* as a complementary treatment against resistant bacterial strains.

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ABSTRACT

Aim: To investigate the antibacterial effects of methanolic extracts of *Salvia officinalis* (leaves and roots) against *Streptococcus pyogenes* isolates from throat cultures, based on the hypothesis that these extracts may exhibit inhibitory activity against clinical isolates, as suggested by previous reports of its antimicrobial potential.

Materials and Methods: After the plant was collected, it was separated into leaf and root parts, dried at 70°C for 24 hours, and then powdered. Dried plant samples were extracted in methanol for 24 hours. The broth microdilution method was used to determine antibacterial activity. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the extracts against the isolates were determined.

Results: MIC values of leaf and root extracts against isolates ranged from 7.8-125 µg/ml and 7.8-500 µg/ml, respectively; MBC values ranged from 7.8-125 µg/ml and 7.8-500 µg/ml, respectively. In general, lower MIC and MBC values were observed in leaf extracts than in root extracts.

Conclusion: Different methods are used in studies investigating the antibacterial effect of S. officinalis, and no standard method exists. If the safe use limits for sage are determined through cytotoxicity studies and a standard method is developed, *S. officinalis* can be used as a supplement for the treatment of infectious diseases caused by resistant strains.

Keywords: *Salvia officinalis*, Sage, *Streptococcus pyogenes*, Broth microdilution method, Antibacterial effect, Methanol extract

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INTRODUCTION

Streptococcus pyogenes is an important human pathogen that can cause both localized and life-threatening invasive infections. It usually colonizes the pharynx, anus, and genital mucosa. *S. pyogenes* is a Gram-positive bacterium that is catalase- and oxidase-negative. *S. pyogenes* grows well in 5%–10% carbon dioxide and on blood agar plates. According to the Lancefield classification, it is included in group A streptococci (GAS). The Type A antigen is a polysaccharide attached to a rhamnose polymer backbone and contains N-acetylglucosamine. The major surface protein in the cell wall of *S. pyogenes* is the M protein. Transmission routes include droplet transmission, contact with contaminated food, contaminated objects or surfaces, and skin contact with contaminated lesions [1].

S. pyogenes causes infections such as sepsis, septic arthritis, streptococcal toxic shock syndrome, scarlet fever, necrotizing fasciitis, pharyngotonsillitis, erysipelas, and impetigo. Late autoimmune reactions, such as acute rheumatic fever, rheumatic heart disease, and acute glomerulonephritis, are associated with GAS infections. According to current treatment guidelines, penicillin and β-lactams are recommended as the primary treatment options for uncomplicated GAS infections. Clindamycin is preferred in severe infections, in addition to β-lactam antibiotics. Macrolides and lincosamides are alternative options in patients with β-lactam allergy [2].

Studies report that *S. pyogenes*, which is sensitive to β-lactams (especially penicillin), is resistant to macrolides and lincosamides. Researchers attribute this situation to the increase in the number of prescribed antibiotics. [3,4]. Target

site modifications, overexpression of the efflux pump, drug inactivation, ribosomal protection are antibiotic resistance mechanisms [1].

Salvia officinalis (sage) is a perennial, shrubby plant in the family Lamiaceae. It has about 900 species and is the largest genus in the Lamiaceae family. Its prevalence extends to the Mediterranean and Middle East and occurs naturally in various countries worldwide. It is derived from the word "salvarem", which means to save or to treat. It is preferred as a spice in the food sector. In traditional medicine, its aboveground parts have been used for treatment for many years [5-7].

It has been reported in studies that secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenolic acids, terpenes and terpenoids, especially in the leaf parts of sage, have anti-inflammatory, antioxidant, antimicrobial, anticancer, antimitogenic, hypoglycemic, hypolipidemic and antinociceptive effects [5,8,9]. It is predicted that sage essential oils damage the bacterial cell wall, the cytoplasmic membrane's double-layered phospholipid layer and membrane proteins, causing increased cytoplasmic membrane permeability and loss of cellular content [10]. Although many studies have evaluated the antibacterial potential of *S. officinalis* leaves or essential oils [11-13], comparative data on leaf and root extracts against clinical *S. pyogenes* isolates are scarce. This study uniquely compares these plant parts to highlight possible variations in antibacterial potency. In addition, the chemical composition and biological activity of medicinal plants such as *Salvia officinalis* may vary depending on geographical origin, climate, soil characteristics, and harvesting conditions. Therefore, region-specific studies are important to better understand the antibacterial potential of plant extracts obtained from locally grown specimens. This study aims to contribute data from Turkey by evaluating the antibacterial activity of methanolic extracts of *S. officinalis* leaves and roots against clinical *S. pyogenes* isolates, thereby providing regionally relevant evidence to the existing literature.

MATERIALS AND METHODS

Ethical approval for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Kocaeli University Faculty of Medicine (Date: 27.03.2025, Approval number: KÜ GOKAEK-2025/07/13).

Collection of sage plant and obtaining extracts

Plant samples collected from the Nif Mountain region of Izmir Province in May 2023 were separated into leaves and roots, and dried at 70°C for 24 hours. Dried samples were powdered. Leaf powder (0.32 g) and root powder (0.40 g) were obtained. Plant powders dissolved in methanol were extracted in a soxhlet extraction device for 24 hours [14]. The obtained extracts were stored at -80°C.

Preparation of stock extract solutions

A 2 mg portion of each extract was weighed and dissolved in 1 ml of dimethyl sulfoxide (DMSO) for use in the broth microdilution test. The stock solution was obtained as 2 mg/ml [15]. All extract solutions were sterile-filtered through 0.22 µm membrane filters prior to antimicrobial testing.

Bacterial strains

A total of 18 *Streptococcus pyogenes* isolates from throat cultures processed at the Central Microbiology Laboratory of Kocaeli University Hospital between January 2023 and November 2024 were included in the study. Throat swab samples were plated on sheep blood agar (SBA). Pure colonies were identified as *S. pyogenes* using the Vitek MS system (BioMérieux, France). Bacteria were stored at −80°C until broth microdilution experiments were performed. *Escherichia coli* ATCC 25922 was used as the positive control in the broth microdilution test.

Broth microdilution test

Broth microdilution test used for determination of antibacterial activity of plant extracts was applied with modification of protocols suggested by Wijesundara et al. [13]. Sterile 96-well U-bottom microplates were used for the test. DMSO and *E. coli* ATCC 25922 were used as the negative and positive controls, respectively. 12 wells (one well for sterility control and one well for growth control) were examined for each bacterium. 100 µl of Mueller Hinton broth was added to all wells, and the extract was added on top of it. A serial dilution was performed, with an initial concentration of 1000 µg/ml and a final concentration of 1.95 µg/ml. Based on the two-fold serial dilution design, the final DMSO concentration decreased progressively across the wells and was maintained below 1% (v/v) under the assay conditions. 10 µl of the bacterial suspension, prepared in saline solution at 0.5 McFarland turbidity and diluted 1:10, was added to all wells except for the first well. Microplates were incubated at 37 °C for 18-24 hours in an atmosphere containing 5-10% CO_2. The lowest concentration with no visible growth was determined as the MIC [16]. To determine the minimum bactericidal concentration (MBC), 30 µL of the suspension containing medium, extract and bacteria in the wells where no growth was present was inoculated quantitatively to the blood agar plates [13]. The plates were incubated at 37 °C for 18-24 hours. The lowest concentration that reduced the number of bacteria in the plates after incubation by 99.9% (3 logarithms) was determined as the MBC value [17]. All experiments were performed in duplicate, and the results were consistent across replicates.

Statistical analysis

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test. Since MIC

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Table 1. Physical properties and yield percentages of different parts of *S. officinalis*.

Solvent	Plant part	Physical properties	Biomass subjected to extraction (g)	Extraction yield (g)	Percentage yield (%)
Methanol	Leaf	Honey brown powder	8.00	0.32	4.00
	Root	Honey yellow powder	9.26	0.40	4.34

Table 2. MIC and MBC values of *S. officinalis* leaf and root extracts against *S. pyogenes* isolates.

	Leaf		Root	
Isolate Number	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
SP-1	62.5	62.5	62.5	500
SP-2	31.25	62.5	125	250
SP-3	31.25	62.5	125	500
SP-4	31.25	62.5	250	250
SP-5	125	125	500	500
SP-6	62.5	62.5	125	250
SP-7	125	125	125	250
SP-8	62.5	62.5	250	250
SP-9	31.25	62.5	125	250
SP-10	62.5	62.5	125	125
SP-11	31.25	31.25	62.5	125
SP-12	7.8	15.6	7.8	7.8
SP-13	31.25	31.25	7.8	62.5
SP-14	7.8	7.8	15.6	125
SP-15	7.8	7.8	15.6	125
SP-16	31.25	31.25	31.25	62.5
SP-17	15.6	15.6	31.25	62.5
SP-18	31.25	31.25	62.5	62.5

Note: SP; Streptococcus pyogenes.

and MBC values were not normally distributed, comparisons between leaf and root extracts were conducted using the Wilcoxon signed-rank test for paired samples. A p-value <0.05 was considered statistically significant. Additionally, a post-hoc power analysis was performed based on the observed effect size at a 95% confidence level.

RESULTS

The physical properties and percentage yields of different parts of the extracts

The highest extraction yield was obtained from the leaves of the plants. Table 1 shows the physical properties and percentage yields of different parts of the extracts.

Antibacterial activity of S. officinalis extracts

According to the broth microdilution test results, the MIC and MBC values of leaf and root extracts against *S. pyogenes* isolates were determined to be in the ranges of 7.8-125 µg/ml and 7.8-500 µg/ml, respectively. Overall, leaf extracts exhibited stronger antibacterial activity than root extracts. Leaf extracts exhibited lower MIC values in 13 of 18 isolates; MIC values were equal in 4 isolates; and root extracts exhibited lower MIC values in only 1 isolate. Similarly, MBC values of leaf extracts were lower than those of root extracts in 16 out of 18 isolates, with equal values observed in 1 isolate and root extracts showing lower MBC values in 1 isolate. These findings indicate that leaf extracts have higher inhibitory and bactericidal potential than root extracts. Detailed MIC and MBC values for each isolate are presented in Table 2.

Statistical comparison of MIC and MBC values

MIC values obtained from *S. officinalis* leaf extracts were significantly lower than those obtained from root extracts (Wilcoxon signed-rank test, p=0.002). Similarly, MBC values were significantly lower in leaf extracts than in root extracts (p<0.001). Post-hoc power analysis revealed that the achieved statistical power exceeded 0.80.

DISCUSSION

S. pyogenes isolates are susceptible to penicillin. However, resistance to other antibiotics preferred for treatment is developing. The increase in antibiotic resistance results in limited treatment options, increased morbidity and mortality, and a greater burden on health services. Therefore, it leads to the discovery and research into new antibacterial agents for treatment.

The World Health Organization (WHO) has declared that medicinal plants can be a main source for drug development. Accordingly, discovering new antimicrobial agents with diverse chemical structures and modes of action is critical to combating infectious diseases. Tannins, terpenoids, alkaloids, and flavonoids are abundant secondary metabolites in plants, and these compounds have antimicrobial effects. WHO has reported that plant extracts and their active ingredients are used in traditional medicine by 80% of the world's population [18,19].

S. officinalis is a perennial woody plant with a bitter taste and strong aromatic properties. Its leaf has antioxidant and antimicrobial properties. For this reason, its extracts and essential oils are widely used in the food, cosmetics and pharmaceutical industries [20].

With increasing interest in herbal medicine, sage has also attracted attention in traditional medicine because of its anti-inflammatory properties and diverse medicinal uses. The European Pharmacopoeia has recognized that leaf extracts of *S. officinalis* are a beneficial oral solution for reducing swelling and discomfort in inflamed gum tissues [21]. The antibacterial effects observed in this study may be attributed to phenolic and terpenoid compounds, such as thujone, rosmarinic acid, and camphor, which are commonly found in

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S. officinalis. These bioactive molecules can disrupt bacterial cell membranes, interfere with energy metabolism, and inhibit protein synthesis. The higher activity of leaf extracts may result from higher concentrations of essential oils and flavonoids in leaf extracts than those in root parts, as reported in phytochemical analyses of sage species.

Antibacterial and antibiofilm effects of *S. officinalis* extracts and essential oils against clinical isolates such as *E. coli*, *Pseudomonas aeruginos*a, *Klebsiella pneumonia*e, *K. oxytoca*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus cereus*, *Mycoplasma gallisepticum* and *Pasteurella multocida* were investigated [22-25].

In dentistry, studies have investigated the antibacterial effects of *S. officinalis* extracts and essential oils against bacterial species that cause dental infections. A gargle obtained from *S. officinalis* leaves has been shown to reduce counts of *Streptococcus mutans* isolated from dental caries. [26]. In a study using the broth microdilution method, *S. officinalis* essential oil was reported to exhibit antibacterial activity against *S. mutans* and *Porphyromonas gingivalis*. Researchers emphasized that sage essential oil is a promising source of antimicrobial agents that can prevent dental caries and other oral infectious diseases [27]. In addition, antibacterial activity of sage essential oil has been reported against *S. salivarius*, *S. mitis*, and *S. sanguinis* isolated from individuals with oral disease [28,29].

Few studies have specifically investigated the antibacterial activity of *S. officinalis* against *S. pyogenes*. In a study conducted in Canada, ethanolic leaf extracts of *S. officinalis* were tested against two standard S. pyogenes strains (ATCC 19615 and ATCC 49399) and one clinical isolate using the broth microdilution method. The MIC and MBC values were reported as 62.5 µg/ml and 125 µg/ml, respectively [13]. In a related study by the same authors, sage essential oil exhibited MIC values of 250–500 µg/ml and MBC values of 500 µg/ml against standard and clinical *S. pyogenes* strains [12]. In another study, the antibacterial activity of essential oils, obtained from the aboveground parts of *S. officinalis* by hydrodistillation, was evaluated using the disk diffusion method against standard strains, including *S. pyogenes* ATCC 19615, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. The researchers reported moderate antibacterial activity of sage essential oil against *S. pyogenes* and other Gram-positive strains, while lower activity was observed against *P. aeruginosa* [11].

In this study, the antibacterial effects of methanolic extracts of *S. officinalis* leaves and roots against clinical *S. pyogenes* isolates were evaluated using the broth microdilution method, and MIC and MBC values were determined. The antibacterial activity observed is in line with previous reports demonstrating the efficacy of *S. officinalis* extracts and essential oils against a broad spectrum of bacterial species [22–29]. In several studies, Gram-positive bacteria have been reported to exhibit lower MIC and MBC values than Gram-negative bacteria, which may be attributable to differences in cell wall structure and membrane permeability. The background literature provides a contextual framework for interpreting the activity observed against *S. pyogenes* isolates in the present study.

The variation in MIC and MBC values observed among *S. pyogenes* isolates may reflect strain-specific biological differences and inherent variability in susceptibility. Such inter-isolate variability has been reported in previous studies evaluating plant-derived extracts against clinical bacterial isolates. The reliability of the results is supported by duplicate testing and standardized broth microdilution principles, based on CLSI methodology and EUCAST definitions.

In this study, the MIC values for leaf extracts ranged from 7.8 µg/ml to 125 µg/ml, and for root extracts from 7.8 µg/ml to 500 µg/ml. The MBC values in leaf extracts were determined to range from 7.8 µg/ml to 125 µg/ml, and in root extracts from 7.8 µg/ml to 500 µg/ml. While the MIC values determined in our study are similar to the study conducted by Wijesundara et al., the MBC values differ. In our study, MIC values for the isolates were lower than those reported in many other studies. These differences may be attributable to factors such as the geographical areas where the plants were collected, extraction methods, and solvent purity. Unlike most previous studies that primarily focused on leaf extracts, the inclusion of root extracts constitutes a notable strength of this study. We believe that, in our study, determining the MIC values of root extracts and observing their antibacterial activity will provide preliminary information and inform future studies. In addition, the relatively large number of clinical *S. pyogenes* isolates analyzed provides preliminary data that may contribute to future standardization of plant-based antibacterial testing.

Limitations

Limitations of this study include the lack of phytochemical profiling and cytotoxicity testing. Nevertheless, the findings provide preliminary evidence that *S. officinalis* may serve as a natural adjuvant or as a complementary therapeutic agent for mild throat infections, pending further in vivo validation. Routine antimicrobial susceptibility testing is not recommended for *S. pyogenes* due to its predictable susceptibility to penicillin, as stated in CLSI guidelines [30]. Therefore, the present study focused on evaluating the antibacterial activity of *S. officinalis* extracts rather than correlating extract activity with antibiotic resistance phenotypes.

CONCLUSION

In this study, it was observed that *S. officinalis* extracts, especially leaf extracts, could be effective against *S. pyogenes*. The pain-relieving effect of sage, which is frequently used by the public to treat throat infections, may be due to its antibacterial and anti-inflammatory properties. In addition, methods that determine the antibacterial activities of plant extracts need to be standardized, and herbal treatments cannot be used instead of medical treatment. As with other herbal products,

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uncontrolled use of *S. officinalis* should be avoided. The antibacterial effects, mechanisms of action, and cytotoxic effects of *S. officinalis* should be investigated more extensively.

Ethics Committee Approval: Ethical approval was obtained from the Kocaeli University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 27.03.2025, Approval number: KÜ GOKAEK-2025/07/13).

Informed Consent: Written informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

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