Alqalam Journal of Medical and Applied Sciences. 2026;9(8):2547-2553
https://doi.org/10.54361/ajmas.269830

Original article

Antibacterial Activity of a Laboratory-Prepared Salvia officinalis Absolute and Commercial Sage Oil against Clinical Isolates

Hudoud Taher[1]*, Sarah Fadheel[2], Manal Al-Senussi[2]

[1] *Department of Medicinal and Aromatic Plants, Faculty of Natural Resources and Environmental Sciences, Omar Al-Mukhtar University, Libya*
[2]*Faculty of Natural Resources and Environmental Sciences, Omar Al-Mukhtar University, Libya*
Corresponding Email. hudoud.tahir@omu.edu.ly

Abstract
The increasing prevalence of multidrug-resistant bacteria necessitates the exploration of alternative antimicrobial agents. Essential oils are considered promising candidates; however, product quality and concentration can substantially influence their antimicrobial efficacy. This study compared the inhibitory activity of a laboratory-prepared *Salvia officinalis* absolute extract with that of a commercially available sage oil product against several clinical bacterial isolates and evaluated the effects of concentration and bacterial species on antibacterial activity. A total of 250 bacterial isolates were recovered from 160 clinical samples collected from four hospital wards. The sage absolute extract was prepared in the laboratory using *n*-hexane extraction followed by fractional distillation under vacuum, whereas the commercial sage oil was purchased from the local market. The disc diffusion assay was performed using concentrations of 10%, 20%, and 30% for both preparations, with an additional undiluted (100%) condition for the commercial oil. Gentamicin (10 µg) served as the positive control, while DMSO and distilled water were used as negative controls. Inhibition zones were measured after 24 h of incubation at 37°C. The chemical compositions of both preparations were determined using gas chromatography–mass spectrometry (GC–MS). The laboratory-prepared sage absolute extract exhibited moderate, concentration-dependent inhibitory activity, whereas the commercial sage oil produced no inhibition zones at any tested concentration (10%, 20%, 30%, or undiluted 100%). Inhibition zones ranged from 5.2 ± 0.4 mm against *Pseudomonas aeruginosa* at 10% to 22.4 ± 1.1 mm against *Staphylococcus aureus* at 30%. GC–MS analysis revealed that 1,8-cineole (21.4%), thujone (18.7%), and camphor (14.2%) were the major constituents of the sage absolute extract, whereas these compounds were absent or present only in trace amounts in the commercial product. Gentamicin produced inhibition zones ranging from 18 to 22 mm. Differences among concentrations and bacterial species were statistically significant ($P < 0.001$ and $P < 0.01$, respectively). The laboratory-prepared sage absolute extract demonstrated moderate antibacterial activity but was less effective than the conventional antibiotic control. The tested commercial product showed no antibacterial activity, most likely due to dilution, product degradation, or storage-related deterioration. Future studies should investigate potential synergistic effects, determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using broth microdilution methods, and evaluate potential applications in controlled environmental disinfection.
Keywords: Salvia Officinalis, Sage Absolute Extract, N-Hexane Extract, Antibacterial Activity.

Introduction

Healthcare-associated infections (HAIs) remain a major global health challenge because they contribute to increased mortality, prolonged hospital stays, and higher treatment costs [1]. The increasing prevalence of antibiotic-resistant bacteria, particularly in intensive care units, has further emphasized the need to explore alternative antimicrobial agents [2,3]. Essential oils derived from medicinal plants have attracted considerable attention because their complex mixtures of phenolic and terpenoid compounds may inhibit a broad spectrum of pathogenic microorganisms [4]. Proposed mechanisms of antimicrobial action include disruption of bacterial cell membranes, inhibition of protein synthesis, and interference with essential bacterial enzymes [5].

Salvia officinalis L. (sage) is a medicinal plant rich in volatile constituents and has traditionally been used for the management of respiratory and gastrointestinal disorders. Its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* has been reported to vary according to the extraction method, storage conditions, and concentrations of bioactive constituents [6,7]. Compounds such as 1,8-cineole, α- and β-thujone, and camphor are among the major constituents considered relevant to its antimicrobial activity [8,9].

Previous studies have reported considerable variation in the inhibition-zone diameters produced by sage preparations, reflecting differences in plant origin, extraction procedures, tested microorganisms, and assay design. Commercial products available in local markets may also vary substantially in their chemical composition and quality. Therefore, the present study compared a laboratory-prepared sage absolute extract with a commercially available sage product and examined the relationship between antibacterial activity and chemical composition.

The objectives of this study were to: (1) prepare and chemically characterize a laboratory-produced sage absolute extract using gas chromatography–mass spectrometry (GC–MS); (2) evaluate its concentration-dependent inhibitory activity against clinical bacterial isolates; (3) compare its antibacterial activity with that of a locally available commercial product; and (4) provide practical recommendations for product quality assessment and future research.

Received*: 10-06-2026 -* ***Accepted****: 08-08-2026 -* ***Published****: 17-08-2026*

Materials and Methods

Collection, Isolation, and Identification of Bacterial Samples

A total of 160 clinical samples were collected from four wards of a major teaching hospital between January and March 2025: Nephrology (30 samples), Intensive Care Unit (70), Obstetrics and Gynecology (35), and Neonatal Care Unit (25). The specimens included urine, blood, wound swabs, pus, cerebrospinal fluid, and vaginal secretions.

The samples were transported in Amies transport medium and cultured on blood agar and MacConkey agar at 37°C for 24 h. Preliminary identification was based on colony morphology, Gram staining, and conventional biochemical tests, including catalase, coagulase, carbohydrate utilization tests, and API 20E for Enterobacteriaceae, according to MacFaddin [10]. Identification was confirmed using the VITEK 2 system (bioMérieux, France). Pure isolates were preserved in nutrient medium supplemented with 20% glycerol at −80°C until further use.

For the antibacterial assays, the full set of 250 recovered isolates was not tested individually. A selected subset of isolates representing the bacterial species included in Table 4 (Staphylococcus aureus, Proteus mirabilis, Escherichia coli, and Pseudomonas aeruginosa) was used for the concentration-dependent disk-diffusion assay. For each selected bacterial isolate, the assay was performed in three independent replicates (n = 3 per isolate and concentration). The MIC and MBC determinations were likewise performed in triplicate on the selected bacterial isolates.

Antibiotic susceptibility was assessed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. Among the selected isolates, 62% of *Staphylococcus aureus* isolates were methicillin-resistant (MRSA), 45% of *Escherichia coli* isolates were extended-spectrum β-lactamase (ESBL) producers, and 55% of *Pseudomonas aeruginosa* isolates were resistant to ciprofloxacin.

Laboratory Preparation of Sage Absolute Extract

A modified protocol based on Ben Farhat et al. [8] was used to obtain the sage absolute preparation. Fresh sage leaves obtained from the nursery of the Faculty of Agriculture were shade-dried at 25 ± 2°C for 8–10 days until the moisture content was below 10%. The dried plant material (500 g) was ground and macerated in 2 L of n-hexane (99.9%) at 35°C with continuous agitation at 300 rpm for 6 h. The extract was filtered, treated three times with 5% sodium hydroxide to remove fatty acids and waxes, washed until neutral, and concentrated under reduced pressure using a rotary evaporator at 40°C and 200 mbar.

The crude extract was dissolved in absolute ethanol (1:8, w/v) and maintained at −5°C for 12 h to precipitate waxes, followed by fractional distillation under vacuum (50 mbar, 30°C). The final preparation was dried over anhydrous sodium sulfate and stored in a tightly sealed amber glass vial at 4°C for no longer than two weeks before testing.

Commercial Sage Oil

Three commercial sage oil products were purchased from local pharmacies and herbal shops. Product names were anonymized for research purposes. To obtain a representative sample of products available in the local market, equal volumes of the three oils were pooled before analysis. This approach was intended to evaluate the average antibacterial performance of commercially available sage oils rather than to compare individual brands.

Packaging information for each product, including the production date, expiration date, and declared source, was recorded. The pooled commercial sample was stored at room temperature, consistent with the conditions under which the products were found in the market, and was tested at concentrations of 10%, 20%, 30%, and undiluted (100%). Although pooling reduces batch-specific variability, it may mask differences among individual commercial products and therefore represents a limitation of the present study.

GC–MS Analysis

GC–MS analysis of both preparations was performed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer. Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), with helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially maintained at 50°C for 2 min, then increased at a rate of 3°C/min to 250°C and held for 5 min. The chemical constituents were identified by comparison of their mass spectra with those in the NIST 2020 mass spectral library. Retention indices were calculated using a homologous series of C8–C40 *n*-alkane standards.

Disk Diffusion Assay

Whatman No. 1 filter paper disks (6 mm in diameter) were sterilized by dry heat at 160°C for 2 h. Bacterial suspensions adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) were evenly inoculated onto Mueller–Hinton agar plates. Each disk was loaded with 20 µL of the laboratory-prepared sage absolute extract at concentrations of 10%, 20%, or 30%, or with the commercial sage oil at the same concentrations or undiluted (100%). Gentamicin (10 µg) was used as the positive control, while DMSO and sterile distilled water served as negative controls.

The plates were incubated at 37°C for 24 h. Inhibition-zone diameters were measured to the nearest 0.1 mm. Results were expressed as mean ± standard deviation (SD) from three independent replicates for each bacterial isolate and concentration.

Received: *10-06-2026 -* ***Accepted:*** *08-08-2026 -* ***Published:*** *17-08-2026*

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the laboratory-prepared sage absolute extract were determined using the broth microdilution method in accordance with CLSI guidelines [11]. Two-fold serial dilutions of the sage absolute extract were prepared in Mueller–Hinton broth (MHB), yielding concentrations ranging from 0.625 to 40 µL/mL in sterile 96-well microtiter plates.

Each well was inoculated with the test bacterial suspension to achieve a final inoculum of approximately 5×10^5 CFU/mL and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the sage absolute extract that completely inhibited visible bacterial growth.

To determine the MBC, 10 µL aliquots from wells showing no visible growth were subcultured onto Mueller–Hinton agar plates and incubated at 37°C for 24 h. The MBC was defined as the lowest concentration resulting in ≥99.9% killing of the initial bacterial inoculum. All experiments were performed in triplicate.

Ethical Considerations and Statistical Analysis

Ethical approval was obtained from the Research Ethics Committee of the teaching hospital. Clinical specimens were handled in accordance with established biosafety guidelines and the principles of the Declaration of Helsinki.

Statistical analyses were performed using SPSS version 26.0. One-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test, was used to compare the effects of different concentrations within each bacterial species. An unpaired *t*-test was used to compare the antibacterial activity of the laboratory-prepared sage absolute extract with that of gentamicin. A *P*-value < 0.05 was considered statistically significant.

Results

Distribution of bacterial isolates

All 160 clinical samples yielded bacterial growth. Gram-negative bacteria accounted for 79.6% of the 250 isolates, with *Escherichia coli* being the most frequently isolated species (18.4%).

Table 1. Distribution of bacterial isolates recovered from clinical samples (n = 250)

Bacterial Group/Species	Number	Percentage (%)
Gram-negative bacteria	199	79.6
Escherichia coli	46	18.4
Citrobacter spp	30	. 12.0
Pseudomonas aeruginosa	25	10.0
Enterobacter aerogenes	21	8.4
Shigella flexneri	20	8.0
Proteus mirabilis	18	7.2
Klebsiella pneumoniae	16	6.4
Klebsiella aerogenes	12	4.8
Shigella dysenteriae	13	5.2
Gram-positive bacteria	49	19.6
Staphylococcus aureus (Coagulase-positive)	35	14.0
Staphylococcus spp. (Coagulase-negative)	14	5.6
Others	2	0.8
Total	250	100

Table 2. Antimicrobial-resistance patterns of selected isolates.

Bacterial Species	Resistance/Susceptibility Profile	Percentage (%)
S. aureus	MRSA (Methicillin-resistant)	62%
S. aureus	Methicillin-sensitive (MSSA)	38%
E. coli	ESBL-producing	45%
E. coli	Non-ESBL-producing	55%
P. aeruginosa	Ciprofloxacin-resistant	55%
P. aeruginosa	Ciprofloxacin-sensitive	45%
P. mirabilis	Ampicillin-resistant	40%

Chemical composition

GC–MS analysis revealed marked compositional differences between the laboratory-prepared sage absolute extract and the commercial sage oil. The six major constituents identified in the sage absolute extract accounted for 72.0% of the total composition: 1,8-cineole (21.4%), thujone (18.7%), camphor (14.2%), camphene (7.8%), α-pinene (5.6%), and borneol (4.3%). In the commercial product, the corresponding major constituents accounted for only 1.8% of the total composition.

Received: *10-06-2026 -* ***Accepted:*** *08-08-2026 -* ***Published:*** *17-08-2026*

Thujone was not detected, while 1,8-cineole and camphor were present at only 0.3% and 0.2%, respectively. Other compounds and unidentified constituents accounted for 28.4% and 69.8% of the commercial product profile, respectively.

Table 3. GC–MS compositional profile of the laboratory-prepared sage absolute extract and commercial sage oil (area %).

Compound	Sage Absolute Extract (%)	Commercial Sage Oil (%)	Retention Time (min)
1,8-Cineole (Eucalyptol)	21.4 ± 1.2	0.3 ± 0.1	9.84
Thujone	18.7 ± 1.1	ND	11.23
Camphor	14.2 ± 0.9	0.2 ± 0.1	12.07
Camphene	7.8 ± 0.6	0.5 ± 0.2	5.63
α-Pinene	5.6 ± 0.5	0.8 ± 0.3	4.87
Borneol	4.3 ± 0.4	ND	14.56
Subtotal: Major Identified Constituents	72.0	1.8	–
Other Compounds	17.0 ± 1.8	28.4 ± 1.0	–
Unidentified Constituents	11.0 ± 0.8	69.8 ± 2.1	–
Total	100.0	100.0	–

ND = Not detected (<0.05%).

Antibacterial activity

The commercial preparation produced no inhibition zones against any of the tested isolates at concentrations ranging from 10% to undiluted (100%). In contrast, the laboratory-prepared sage extract exhibited concentration-dependent antibacterial activity, with inhibition-zone diameters ranging from 5.2 ± 0.4 mm against *Pseudomonas aeruginosa* at 10% to 22.4 ± 1.1 mm against *Staphylococcus aureus* at 30%. Gentamicin produced inhibition zones ranging from 18 to 22 mm, whereas DMSO and distilled water produced no detectable inhibition.

Table 4. Effect of different concentrations of the laboratory-prepared sage extract and gentamicin on clinical bacterial isolates (mean inhibition-zone diameter in mm ± SD; n = 3 per isolate).

Bacterial Species	Sage Absolute Extract 10%	Sage Absolute Extract 20%	Sage Absolute Extract 30%	Gentamicin (10 µg)	P-value (ANOVA)
Staphylococcus aureus	14.8 ± 0.9	18.6 ± 1.0	22.4 ± 1.1	22.0 ± 0.4	<0.001
Proteus mirabilis	12.5 ± 0.8	16.2 ± 0.9	19.8 ± 1.0	20.0 ± 0.5	<0.001
Escherichia coli	8.7 ± 0.6	11.0 ± 0.8	12.9 ± 1.0	21.0 ± 0.5	<0.001
Pseudomonas aeruginosa	5.2 ± 0.4	6.8 ± 0.5	8.0 ± 0.6	18.0 ± 0.6	<0.001

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC results confirmed the concentration-dependent antibacterial activity observed in the disk diffusion assay. *Staphylococcus aureus* exhibited the highest susceptibility, with MIC and MBC values of 2.5 µL/mL and 5 µL/mL, respectively. In contrast, *Pseudomonas aeruginosa* showed the lowest susceptibility, with MIC and MBC values of 10 µL/mL and 20 µL/mL, respectively. The MIC and MBC values for both *Proteus mirabilis* and *Escherichia coli* were 5 µL/mL and 10 µL/mL, respectively. These findings are consistent with the inhibition-zone diameters reported in Table 4 and further support the differential susceptibility observed among the tested bacterial species.

Table 5. MIC and MBC values of the laboratory-prepared sage absolute extract against selected bacterial isolates

Bacterial Species	Difference between 10% and 20% (mm)	Difference between 20% and 30% (mm)	Overall Increase (10% → 30%) (mm)	*Percentage Increase (%)	Correlation Coefficient (r)
S. aureus	3.8	3.8	7.6	51.4%	1.000
P. mirabilis	3.7	3.6	7.3	58.4%	1.000
E. coli	2.3	1.9	4.2	48.3%	0.998
P. aeruginosa	1.6	1.2	2.8	53.8%	0.997

Percentage Increase (%) = [(value at 30% − value at 10%) / value at 10%] × 100. Correlation coefficient: Pearson correlation between concentration and inhibition zone.

Quantitative Relationship Between Concentration and Antibacterial Activity

A positive correlation was observed between sage absolute extract concentration and inhibition-zone diameter. The percentage increase in antibacterial activity from 10% to 30% ranged from 48.3% to 58.4% across the tested bacterial species. High correlation coefficients (*r* = 0.997–1.000) were observed, indicating a strong positive linear relationship between extract concentration and antibacterial activity within the tested concentration range.

Received: *10-06-2026 -* ***Accepted:*** *08-08-2026 -* ***Published:*** *17-08-2026*

Table 6. Linear predictive models for inhibition-zone diameter.

Bacterium	Equation	R^2
Staphylococcus aureus	y = 0.380x + 11.00	1000
Proteus mirabilis	y = 0.365x + 8.85	1.000
Escherichia coli	y = 0.210x + 6.60	0.997
Pseudomonas aeruginosa	y = 0.140x + 3.80	0.993

Note: *The intercepts were recalculated using standard linear regression to ensure internal consistency. For Proteus mirabilis, the intercept was calculated as 12.5 − (0.365 × 10) = 8.85; for Escherichia coli, as 8.7 − (0.210 × 10) = 6.60; and for Pseudomonas aeruginosa, as 5.2 − (0.140 × 10) = 3.80.*

Linear Predictive Model

A linear predictive model was developed for concentrations ranging from 10% to 30%:

Inhibition zone (mm) = (slope × concentration [%]) + intercept.

Internal consistency was verified by recalculating the intercepts directly from the observed concentration–response data reported in Table 4. MIC and MBC values of the laboratory-prepared sage absolute extract against selected bacterial isolates.

Discussion

Commercial Product

The commercial sage oil preparation failed to inhibit any of the tested bacterial isolates. This marked difference from the laboratory-prepared sage absolute extract coincided with a substantially different GC–MS profile and very low or undetectable concentrations of the major constituents typically associated with sage. Commercial oils may vary considerably in their chemical composition because of differences in source material, dilution, product age, exposure to light and heat, and oxidative degradation during processing and storage [7,12]. The high proportion of unidentified constituents detected in the commercial sample further highlights the need for more rigorous quality-control assessment and chemical characterization of commercially available sage oil products.

Laboratory-Prepared Extract and Comparison with Previous Studies

The laboratory-prepared sage absolute extract exhibited clear concentration-dependent antibacterial activity, with *Staphylococcus aureus* showing greater susceptibility than the tested Gram-negative species. These findings are generally consistent with previous reports indicating that sage preparations can inhibit bacterial growth, although the magnitude of inhibition varies considerably depending on the extraction method, phytochemical composition, bacterial strain, oil concentration, solvent system, and incubation conditions [6,9,13].

The MIC and MBC findings further supported the results obtained using the disk diffusion assay. *Staphylococcus aureus*, which exhibited the largest inhibition zones, also showed the lowest MIC and MBC values (2.5 and 5 µL/mL, respectively). In contrast, *Pseudomonas aeruginosa*, which exhibited the smallest inhibition zones, had the highest MIC and MBC values (10 and 20 µL/mL, respectively). This agreement between the two antimicrobial susceptibility approaches strengthens the evidence for species-dependent differences in susceptibility to the laboratory-prepared sage absolute extract.

Gram-Positive versus Gram-Negative Bacteria

The greater apparent susceptibility of *Staphylococcus aureus* may be partly explained by the absence of the additional outer membrane characteristic of Gram-negative bacteria. In Gram-negative organisms, this outer membrane, together with active efflux systems, can restrict the intracellular accumulation of lipophilic terpenoid compounds [4,5,14]. *Pseudomonas aeruginosa* exhibited the lowest susceptibility among the tested species, consistent with the intrinsic resistance of this organism to many antimicrobial compounds due to its relatively low outer-membrane permeability and highly efficient efflux systems.

Nevertheless, because only one Gram-positive species was included in the comparative antibacterial assays, these findings should not be generalized as evidence of a universal difference between Gram-positive and Gram-negative bacteria. Rather, they indicate species-specific differences in susceptibility among the bacterial isolates examined in the present study.

Concentration-Dependent Effect and Potential Mechanisms

Inhibition-zone diameters increased progressively with increasing concentrations of the laboratory-prepared sage absolute extract across all tested bacterial species. The percentage increase in inhibition from 10% to 30% ranged from 48.3% to 58.4%, with strong positive correlations between concentration and inhibition-zone diameter within the tested concentration range.

Terpenoids such as 1,8-cineole, thujone, and camphor are lipophilic compounds that may interact with phospholipid bilayers, alter membrane permeability, and interfere with bacterial energy metabolism [15,16]. Interactions among individual constituents may also contribute to the overall antimicrobial activity of complex plant-derived preparations [9,17]. The relatively high abundance of these compounds in the laboratory-prepared extract may therefore contribute to

Received*: 10-06-2026 -* ***Accepted****: 08-08-2026 -* ***Published****: 17-08-2026*

its observed antibacterial activity. Conversely, their absence or presence at only trace levels in the commercial preparation may partly explain its lack of detectable activity.

However, the association between chemical composition and antibacterial activity observed in the present study should be interpreted cautiously. The current findings demonstrate an association rather than direct causation, and further studies using isolated compounds and defined combinations are required to establish the contribution of individual constituents and potential synergistic interactions.

Comparison with Conventional Antibiotics

Gentamicin served as the positive control and generally produced larger inhibition zones than the laboratory-prepared sage absolute extract, particularly against the Gram-negative isolates. The laboratory-prepared extract should therefore be regarded as a natural preparation with preliminary antibacterial potential rather than as an alternative to conventional systemic antibiotic therapy.

The antibacterial activity demonstrated by the extract nevertheless warrants further investigation, particularly in combination with conventional antimicrobial agents. Future studies should evaluate potential synergistic, additive, or antagonistic interactions between sage constituents and standard antibiotics against multidrug-resistant clinical isolates. Such investigations may help determine whether sage-derived preparations have potential as adjunctive rather than replacement antimicrobial agents.

Study Limitations

Several limitations of the present study should be acknowledged. First, the *n*-hexane-derived sage absolute extract differs from a steam-distilled essential oil and may therefore possess a distinct chemical profile, limiting direct comparison with studies based exclusively on essential oils.

Second, the laboratory-prepared extract was tested within two weeks of extraction; therefore, its long-term chemical and antimicrobial stability was not evaluated.

Third, although MIC and MBC values were determined by broth microdilution in the present study, these measurements were restricted to the selected bacterial species and tested preparation. Further studies involving larger numbers of clinical isolates, independent batches, and a broader concentration range would strengthen the quantitative characterization of antibacterial activity.

Fourth, three commercial sage oil products were pooled into a single representative sample. Although this approach was intended to provide an overall assessment of products available in the local market, it prevented evaluation of inter-brand variability and may have masked differences in chemical composition and antibacterial activity among individual products. Future studies should therefore test each commercial product independently and perform separate GC–MS analyses for each product.

Finally, only antibacterial activity was investigated; potential antifungal and antiviral activities were not assessed.

Conclusions and Recommendations

The laboratory-prepared sage absolute extract demonstrated concentration-dependent antibacterial activity against the tested clinical bacterial isolates, whereas the pooled commercial sage oil preparation showed no detectable antibacterial activity under the experimental conditions used. GC–MS analysis revealed substantial differences in chemical composition between the two preparations, suggesting that variation in phytochemical composition may be associated with the observed differences in antibacterial activity.

The disk diffusion, MIC, and MBC findings collectively demonstrated species-dependent susceptibility, with *Staphylococcus aureus* showing the greatest susceptibility and *Pseudomonas aeruginosa* the lowest. Nevertheless, the laboratory-prepared preparation should not be considered a substitute for conventional antibiotics on the basis of the present findings. Further standardized investigations are required to confirm its antimicrobial potential, assess its stability and safety, and determine its possible role as an adjunctive antimicrobial or environmental disinfection agent.

Recommendations for Future Research and Quality Control

1. Chemical characterization: GC–MS characterization should be incorporated into studies evaluating essential oils and absolute extracts to facilitate meaningful correlations between chemical composition and biological activity.
2. Synergy studies: Interactions between sage preparations and conventional antibiotics should be investigated against multidrug-resistant clinical isolates. Potential synergistic effects may help determine whether these preparations could contribute to reducing the effective doses of conventional antimicrobial agents.
3. Expanded MIC and MBC assessment: Broth microdilution testing should be extended to larger numbers of clinical isolates and independent extract batches to confirm the MIC and MBC ranges observed in the present study and improve the quantitative characterization of antimicrobial activity.
4. Comparison of extraction methods: Solvent extraction and steam distillation should be directly compared, together with standardized storage-stability assessments, to determine how extraction procedures influence chemical composition and antimicrobial activity.

Received*: 10-06-2026 -* ***Accepted****: 08-08-2026 -* ***Published****: 17-08-2026*

5. Quality control of commercial products: Certificates of analysis and appropriate quality-control procedures should be encouraged for commercially available essential oil products. Establishing minimum compositional and quality standards may improve consistency and reliability in the local market.
6. Individual testing of commercial products: Future investigations of commercial sage oils should evaluate each product separately and perform independent chemical characterization by GC–MS. This approach would allow inter-brand variability to be assessed and potential sources of differences in biological activity to be identified.
7. Environmental disinfection applications: The potential use of sage-derived preparations in controlled environmental disinfection applications, particularly in healthcare settings, warrants further investigation. Such studies should include standardized efficacy, stability, material-compatibility, and safety assessments before practical application is considered.

Conflict of Interests

The authors declare no conflict of interest.

Ethical Approval

This study was approved by our university ethical committee.

Funding/Support

None

Declarations

All methods were carried out in accordance with relevant guidelines and regulations.

References

1. Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2020;377(9761):228-41. doi:10.1016/S0140-6736(10)61458-4
2. Murray CJL, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-55. doi:10.1016/S0140-6736(21)02724-0
3. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO Press; 2024.
4. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils—present status and future perspectives. Medicines. 2017;4(3):58. doi:10.3390/medicines4030058
5. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6(12):1451-74. doi:10.3390/ph6121451
6. Cutillas AB, Carrasco A, Martinez-Gutierrez R, Tomas V, Tudela J. Salvia officinalis L. essential oils from Spain: determination of composition, antioxidant capacity, antienzymatic, and antimicrobial bioactivities. Chem Biodivers. 2017;14(8):e1700148. doi:10.1002/cbdv.201700148
7. Raal A, Orav A, Ilina T, et al. Variation in the composition of the essential oil of commercial Salvia officinalis L. leaves samples from different countries. Phyton. 2024;93(8):2051-62. doi:10.32604/phyton.2024.052790
8. Ben Farhat M, Jordan MJ, Chaouech-Hamada R, Landoulsi A, Sotomayor JA. Variations in essential oil, phenolic compounds, and antioxidant activity of Tunisian cultivated Salvia officinalis L. J Agric Food Chem. 2009;57(22):10649-56. doi:10.1021/jf902542p
9. Acimovic M, Ljujic J, Vunduk J, Tesevic V, et al. Salvia officinalis L. essential oil: chemical characterization and antimicrobial activity. Processes. 2022;10(8):1608. doi:10.3390/pr10081608
10. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2000.
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI supplement M100. Wayne (PA): CLSI; 2023.
12. Hussain A, Al-Ani I, Khalil M. Quality control of essential oils in the local market: a case study from a developing country. J Appl Res Med Plants. 2022;30:100325. doi:10.1016/j.jarmap.2022.100325
13. Ünlü S, Üsküdar Güçlü A, Mirza HC, Altay Koçak A, Başustaoğlu A. In vitro effects of various essential oils on biofilm viability; their antibacterial and antibiofilm activities against clinical Staphylococcus aureus isolates. Turk J Hyg Exp Biol. 2023;80(4):491-502. doi:10.5505/TurkHijyen.2023.46504
14. Soto SM. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virulence. 2013;4(3):223-29. doi:10.4161/viru.23724
15. Sikkema J, de Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev. 1995;59(2):201-22. doi:10.1128/mr.59.2.201-222.1995
16. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 2004;94(3):223-53. doi:10.1016/j.ijfoodmicro.2004.03.022
17. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989-4006. doi:10.3390/molecules17043989

Received*: 10-06-2026 -* ***Accepted****: 08-08-2026 -* ***Published****: 17-08-2026*