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Chemical Characterization, Enantiomeric Distribution and Antibacterial Efficacy of The Essential Oils of Salvia officinalis from Nigeria

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Abstract

Purpose: This study investigated the chemical composition, enantiomeric distribution, and antibacterial activity of the essential oil of Salvia officinalis cultivated in Nigeria.

Methodology: Essential oil was extracted by hydro-distillation and analysed using GC–MS and chiral GC–MS to determine both qualitative and stereochemical profiles. Antibacterial activity was assessed against seven clinically relevant bacterial strains using the microbroth dilution method.

Results: A total of 73 constituents were identified, representing 98% of the total oil. The oil exhibited a sesquiterpene-rich chemotype, dominated by β-caryophyllene, germacrene D, trans-β-ionone, α-copaene, and δ-cadinene. Chiral analysis revealed five pairs of monoterpenoid enantiomers and three enantiomerically pure sesquiterpenoids, with β-caryophyllene, δ-cadinene, and trans-β-ionone showing 100% enantiomeric excess. The essential oil demonstrated broad-spectrum antibacterial activity, with the strongest inhibition against Streptococcus faecalis (MIC = 156.3 µg/mL) and the weakest against Escherichia coli and Pseudomonas aeruginosa (MIC = 2500 µg/mL). Major components, β-caryophyllene and germacrene D, exhibited activity comparable to the crude oil. Hierarchical cluster analysis indicated a distinct Nigerian sesquiterpene-rich chemotype, differing from the classical camphor- or α-thujone-dominant Mediterranean chemotypes.

Novelty and Contribution: This study provides first comprehensive report detailing the chemical profile, enantiomeric distribution, and antibacterial potency of Nigerian S. officinalis essential oil. The discovery of a unique β-caryophyllene/germacrene D chemotype and stereochemically pure sesquiterpenoids adds new chemotaxonomic data for the species.

Social and Practical Implications: The findings support the potential use of Nigerian S. officinalis oil as a natural antimicrobial agent and provide baseline information for pharmaceutical, nutraceutical, and quality-control applications.

Keywords: Salvia Officinalis; Essential Oil; Enantiomeric Distribution; Antibacterial Activity; Chemotype; β-Caryophyllene; Germacrene D.

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1 Introduction

Salvia officinalis L. belongs to the genus Salvia, tribe Mentheae, subfamily Nepetoideae, and mint family Lamiaceae. With almost 1000 species, Salvia is the largest genus in the Lamiaceae family. It is distributed in Central and South America, Southeast Asia, and Europe surrounding the Mediterranean (Jakovljević et al., 2019). Salvia officinalis L. (sage) has been utilised for medicinal reasons for millennia. The herb is utilised for several medical ailments, including sleeplessness, measles, seasickness, sexually transmitted infections, and parasitic worms, with a historical context tracing back to ancient Greek and Roman eras (Kačániová et al., 2021). S. officinalis is a perennial subshrub that can reach a height of up to 60 cm and exhibits outcrossing behaviour. The leaves are arranged oppositely and are simple, featuring white hairs on the lower surface and a green or greenish-grey hue on the upper surface. Stems are upright or sprawling, featuring several hairy dark green branches (Jakovljević et al., 2019). The essential oil derived from S. officinalis exhibits therapeutic properties for respiratory and digestive disorders, cardiovascular and circulatory issues, metabolic problems, and endocrine ailments. Additionally, Salvia officinalis leaves are utilised in medicine for their antibacterial and anti-inflammatory properties (Bauer et al., 2012). The plant flourishes in Nigeria, particularly in and around Vom-Jos, Plateau State, and research has been undertaken to explore its potential in treating malaria (Mokogwu et al., 2024).

In addition to standard chemical characterisation, chiral analysis of essential oils has emerged as a critical feature in phytochemical research (Sadgrove et al., 2022). Many terpenes occur as enantiomers, non-superimposable mirror copies that can differ considerably in bioactivity, toxicity, organoleptic characteristics, antibacterial potency, and metabolic routes (Chen et al., 2021). Therefore, identifying the enantiomeric distribution of chiral elements facilitates chemotaxonomic classification, improves quality control, and provides insight into probable biological pathways (Yu et al., 2022). Despite the global relevance of chiral profiling, few research have documented the enantiomeric makeup of S. officinalis essential oil (Jakovljević et al., 2019; Kačániová et al., 2021).

The usage of essential oils may provide an alternate option to combat against multidrug-resistant bacteria. Many studies have focused on active chemicals from essential oils, which contains natural bioactive substances utilised as alternative medications, especially for their significant antibacterial activity (He et al., 2020). One of these is the essential oils derived from S. officinalis, which has drawn the attention of microbiologists due to their widespread usage against pathogenic bacteria (Al-Mijalli et al., 2022). S. officinalis essential oils have been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Enterococcus faecalis, Citrobacter freundii, Escherichia coli, and Pseudomonas sp. (Aćimović et al., 2022; Lahlou et al., 2023). The essential oils of Salvia species contain a variety of bioactive chemicals, including terpenoids, steroids, flavonoids, and polyphenols. The antibacterial properties of S. officinalis are mostly due to terpens and terpenoids. It is widely recognised that pure essential oils typically have more antibacterial action than single major components, and their combinations imply that the minor components are vital to the synergistic activity, however antagonistic and additive impacts have also been observed (Badiee et al., 2012; Lahlou et al., 2023).

The paucity of region-specific information leaves a significant gap in phytochemical and pharmacological data for Nigerian S. officinalis. Given the impact of environmental influences on essential oil content, it cannot be presumed that Nigerian sage has the same chemotype or biological activity as samples from Europe or the Mediterranean (Usano-Alemany et al., 2014). Furthermore, the lack of enantiomeric data prevents a thorough knowledge of its chemical identity and any pharmacological distinctions. Evaluating the antibacterial characteristics of the Nigerian oil is also particularly essential considering the rising frequency of drug-resistant infections in Nigeria and the possibility of natural antimicrobials to supplement conventional therapy.

Therefore, this study aims to (i) characterize the chemical composition of the essential oil of Salvia officinalis cultivated in Nigeria using gas chromatography–mass spectrometry (GC–MS), (ii) determine the enantiomeric distribution of its major chiral constituents using chiral GC–MS, and (iii) assess its antibacterial activity against selected pathogenic bacteria of clinical relevance. By providing the chemical and enantiomeric fingerprint of Nigerian S. officinalis essential oil, this research contributes valuable chemotaxonomic, pharmacological, and quality-control data. The findings will support the development of sage-based natural health products, strengthen the scientific basis for its traditional use in Nigeria, and enhance its potential application as an alternative antimicrobial agent.

2 MATERIALS AND METHODS

2.1 Plant Material Collection and Identification

In June 2024, 2,000g fresh leaves of *Salvia officinalis* L. were gathered from Sabon Gari, Kaduna State, Nigeria (11.1766° N, 7.6765° E). Mr. Namadi Sunusu from the Department of Botany at Ahmadu Bello University (ABU) in Zaria, Nigeria, did the botanical authentication. A voucher specimen (ABU06639) was placed in the university herbarium so that it could be looked at again later. The leaves were dried in the shade at room temperature (25–28 °C) for 5–7 days and then ground up in an electric blender. Until it was time for analysis, the powdered sample was kept in airtight polyethylene bags.

2.2 Essential Oil Extraction

Using an all-glass Clevenger-type apparatus and the British Pharmacopoeia method, essential oil was extracted through hydro distillation. A 5 L round-bottom flask containing about 500 g of dried leaf powder was hydro distilled for four hours using distilled water. To produce a light-yellow oil, the distillate was extracted using n-hexane, dried over anhydrous sodium sulphate, filtered, and concentrated. Until chemical and biological analyses, the oil was kept in amber vials at 4 °C. Approximately 7.5 mL of oil was extracted representing a yield of 1.5% (v/w) from the 500 g of dried powder.

2.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the *S. officinalis* essential oil was analysed using a Shimadzu GCMS-QP2010 Ultra system (Shimadzu Scientific Instruments, USA), following established analytical procedures (Olubukola et al., 2024; Owolabi et al., 2020). Separation was performed on a ZB-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), with helium as the carrier gas flowing at 1.37 mL/min. The oven temperature was programmed from an initial 50 °C to 260 °C at a rate of 2 °C/min. The injector temperature was set at 250 °C, and samples prepared as 5% (w/v) solutions in dichloromethane were injected in split mode (30:1) at a volume of 0.1 µL. The mass spectrometer operated in electron impact (EI) mode at 70 eV, with a scan range of 40–400 amu. Component identification was achieved through a combination of retention index comparison using an n-alkane standard series, mass spectral matching with the FFNSC3 and NIST libraries (Satyal et al., 2013), and co-injection of authentic reference standards when available. Relative abundances of compounds were calculated based on peak area normalization.

2.4 Chiral GC–MS Analysis

A Shimadzu GCMS-QP2010S instrument fitted with a Restek B-Dex 325 chiral capillary column (30 m × 0.25 mm i.d., 0.25 µm film) was used for enantiomeric profiling of chiral terpenoids. Analytical conditions were modified from previously approved techniques (Olubukola et al., 2024; Mothana et al., 2009). Helium was used as the carrier gas, and 0.1 µL of essential oil samples (3% w/v in dichloromethane) were injected in split mode (1:45) at a rate of 1.8 mL/min. The oven temperature was set to rise from 50 °C to 120 °C at a rate of 1.5 °C per minute, then to 200 °C at a rate of 2 °C per minute, with a final hold of five minutes. The mass spectrometer scanned between 40 and 400 amu while running in EI mode at 70 eV. By comparing retention indices and mass spectra with verified Sigma-Aldrich standards, enantiomers were found. The peak area ratios of each enantiomeric pair were used to calculate the enantiomeric excess.

2.5 Antibacterial Assay

The antibacterial activity of *S. officinalis* essential oil was evaluated using the microbroth dilution method following CLSI guidelines and earlier studies (Ogundajo et al., 2021; Olubukola et al., 2024). Seven ATCC bacterial strains, *Staphylococcus aureus* 25923, *Bacillus subtilis* 6633, *Streptococcus faecalis* 9790, *Escherichia coli* 25922, *Salmonella typhi* 6539, *Proteus vulgaris* 6380, and *Pseudomonas aeruginosa* 27853 were used. Bacterial inocula were adjusted to 1.5 × 10^8 CFU/mL (0.5 McFarland standard). A 1% (v/v) essential oil stock was prepared in dimethyl sulfoxide (DMSO) and serially diluted two-fold in cation-adjusted Mueller–Hinton broth to yield final test concentrations ranging from 19.5 to 2500 µg/mL. Each well received 50 µL of the essential oil dilution and 50 µL of bacterial suspension and

was incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration exhibiting complete inhibition of visible growth. Streptomycin served as the positive control, while DMSO (≤1%) served as a negative control. The major constituents, β-caryophyllene and germacrene D—were similarly evaluated under identical assay conditions.

2.6 Hierarchical Cluster Analysis (HCA)

Hierarchical cluster analysis was performed using XLSTAT version 2018.1.1 (Addinsoft, Paris, France). Major constituents of the Nigerian *S. officinalis* essential oil and previously published chemotypes (Addo et al., 2023; Barra, 2009; Jug-Dujaković et al., 2012) were included. Data were standardized, and the Euclidean distance matrix was generated. Clustering was conducted using Ward's minimum variance method. Dendrograms were examined to identify chemotypic relationships.

3 Results

3.1 Essential Oil Composition

GC–MS analysis of the *Salvia officinalis* leaf essential oil identified 73 constituents, representing 98% of the total composition. The oil was dominated by sesquiterpene hydrocarbons (45.0%), monoterpene hydrocarbons (21.0%), oxygenated monoterpenoids (5.0%), oxygenated sesquiterpenoids (5.0%), and other compounds (22.0%) (table 2). The major constituents were Caryophyllene oxide (3.9%), Dodecanoic acid (3.2%), β-caryophyllene (2.14%) and α-Bisabolol (2.0) (table 1).

Table 1 GC–MS Composition of *Salvia officinalis* Leaf Essential Oil

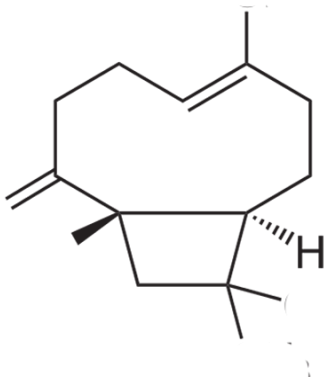
S/N	RT (mins)	RI (cal)	RI (db)	COMPOUND	% PEAK	CLASSIFICATION
1	6.9	801	801	Hexanal	0.1	Aldehyde
2	8.8	840	850	1,2,5,5-Tetramethyl-1,3 cyclopentadiene	0.1	Diene
3	12.1	932	925	α-Pinene	0.7	Monoterpene
4	12.5	964	932	Benzaldehyde	0.1	Aldehyde
5	14.6	973	969	1-Octen-3-one	tr	Ketone
6	14.7	978	972	β-Pinene	0.2	Monoterpene
7	15.0	988	978	2-Methyl-3-octanone	0.2	Ketone
8	15.4	989	984	Myrcene	0.1	Monoterpene
9	15.4	991	986	2-Pentylfuran	0.1	Monoterpene
10	15.6	1006	1005	Octanal	tr	Aldehyde
11	15.7	1017	1015	α-Terpinene	tr	Monoterpene
12	16.2	1025	1024	*p*-Cymene	0.1	Monoterpene
13	17.3	1030	1028	Limonene	0.2	Monoterpene
14	17.9	1031	1030	β-Phellandrene	0.1	Monoterpene
15	18.1	1032	1031	1,8-Cineole	0.8	Monoterpene
16	18.3	1034	1032	(*Z*)-β-Ocimene	0.1	Monoterpene
17	18.4	1045	1043	Phenylacetaldehyde	0.1	Aldehyde
18	18.5	1058	1056	γ-Terpinene	0.1	Monoterpene

19	19.2	1068	1069	Acetophenone	tr	Ketone
20	20.0	1086	1085	Terpinolene	tr	Monoterpene
21	21.8	1107	1105	Nonanal	0.1	Aldehyde
22	22.2	1180	1178	Terpinen-4-ol	0.1	Monoterpene
23	22.8	1198	1197	α-Terpineol	0.1	Monoterpene
24	23.2	1201	1201	Safranal	0.1	Aldehyde
25	26.5	1206	1205	Decanal	0.1	Aldehyde
26	27.0	1229	1227	Thymyl methyl ether	0.1	Sesquiterpenes
27	29.2	1272	1271	Nonanoic acid	0.3	Fatty acids
28	29.5	1293	1291	Thymol	0.1	monoterpenes
29	34.0	1300	1301	Tridecane	tr	Alkenes
30	37.8	1310	1312	4-Vinylguaiacol	0.1	Sesquiterpenes
31	41.0	1367	1371	Decanoic acid	0.9	Fatty acids
32	41.4	1367	1373	Cyclosativene	0.3	Sesquiterpenes
33	41.7	1375	1373	α-Copaene	0.6	Sesquiterpenes
34	44.2	1379	1378	(*E*)-β-Damascenone	0.2	Sesquiterpenes
35	44.3	1382	1381	β-Bourbonene	0.2	Sesquiterpenes
36	45.1	1387	1385	β-Cubebene	0.2	Sesquiterpenes
37	45.6	1390	1391	*trans*-β-Elemene	0.2	Sesquiterpenes
38	46.1	1396	1394	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.1	Alkenes
39	46.6	1396	1396	1,1,5-Trimethyl-1,2-dihydronaphthalene	0.1	Alkenes
40	47.8	1413	1412	*cis*-α-Bergamotene	0.2	Sesquiterpenes
41	48.0	1417	1418	α-Santalene	0.2	Sesquiterpenes
42	48.1	1418	1415	(*E*)-β-Caryophyllene	2.3	Sesquiterpenes
43	48.7	1425	1423	Florhydral	0.1	Sesquiterpenes
44	48.8	1430	1430	β-Copaene	0.1	Sesquiterpenes
45	49.1	1432	1432	*trans*-α-Bergamotene	3.2	Sesquiterpene
46	50.1	1447	1447	Geranylacetone	0.8	Sesquiterpenes
47	50.4	1452	1451	(*E*)-β-Farnesene	0.1	Sesquiterpenes
48	51.8	1454	1453	α-Humulene	0.8	Sesquiterpenes
49	52.9	1475	1473	γ-Muurolene	0.1	Sesquiterpenes
50	52.9	1485	1483	Dehydro-β-ionone	0.4	Ketone
51	53.2	1481	1481	(*E*)-β-Ionone	0.5	Ketone
52	53.6	1483	1483	Germacrene D	1.2	Sesquiterpenes
53	54.2	1483	1481	*trans*-β-Bergamotene	0.6	Sesquiterpenes
54	55.9	1489	1487	β-Selinene	0.1	Sesquiterpenes
56	56.1	1490	1490	γ-Amorphene	0.1	Sesquiterpenes
57	61.8	1497	1494	Bicyclogermacrene	0.4	Sesquiterpenes

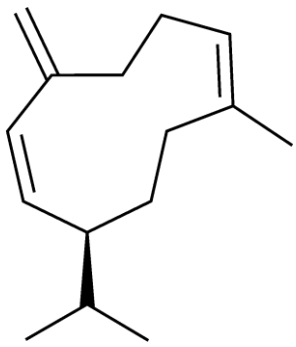
58	64.1	1500	1502	α-Muurolene	0.1	Sesquiterpenes
59	68.3	1501	1503	(*Z*)-α-Bisabolene	0.1	Sesquiterpenes
60	69.3	1508	1508	β-Bisabolene	0.1	Sesquiterpenes
61	69.9	1520	1522	7-*epi*-α-Selinene	0.1	Sesquiterpenes
62	70.7	1519	1520	*trans*-Calimenene	0.2	Sesquiterpenes
63	70.9	1533	1531	*trans*-Cadina-1,4-diene	0.2	Diene
64	71.1	1560	1561	Dodecanoic acid	3.2	Fatty acids
65	71.8	1562	1563	(*E*)-Nerolidol	0.7	Sesquiterpene
66	74.9	1563	1565	1-Tridecanol	0.3	Sesquiterpenoids
67	76.2	1587	1588	Caryophyllene oxide	3.9	Sesquiterpenoids
68	80.8	1611	1610	Humulene epoxide II	0.8	Sesquiterpenoids
69	82.3	1613	1615	Tetradecanal	0.1	Aldehyde
70	82.6	1676	1678	1-Tetradecanol	0.2	Fatty acids
71	83.6	1688	1690	α-Bisabolol	2.0	Sesquiterpenoid
72	85.3	1715	1720	Pentadecanal	0.3	Aldehyde
73	89.6	1723	1721	Methyl tetradecanoate	0.2	Sesquiterpenoids
				Total identified	98%	

Table 2 Classes of compound

CLASS OF COMPOUNDS	% COMPOSITION
Monoterpene hydrocarbons	21.0
Oxygenated monoterpenoids	5.0
Sesquiterpene hydrocarbons	45.0
Oxygenated sesquiterpenoids	5.0
Others	22.0



β-Caryophyllene



Germacrene D

Figure 1 major constituents of oils found in *S. Officinalis* leaf oil in Nigeria

3.2 Enantiomeric Distribution

Chiral GC–MS analysis identified five pairs of enantiomeric monoterpenoids and three enantiomerically pure sesquiterpenoids (β-caryophyllene, δ-cadinene, trans-β-ionone). Enantiomeric excess (ee%) ranged from 2.14% to 100%, with the highest purity observed for *β-caryophyllene*, *δ-cadinene*, and *trans-β-ionone* (table 3).

Table 3 Enantiomeric Composition and Enantiomeric Excess of Identified Chiral Compounds

Compound	Enantiomer Ratio (D: L)	Enantiomeric Excess (%)
α-Pinene	68.23 : 31.77	36.46
Sabinene	22.93 : 77.07	54.14
β-Pinene	20.84 : 79.16	58.32
Limonene	34.91 : 65.09	30.18
Linalool	51.07 : 48.93	2.14
α-Terpineol	32.35 : 67.65	35.00
β-Caryophyllene	0 : 100	100
δ-Cadinene	100 : 0	100
trans-β-Ionone	100 : 0	100
trans-Nerolidol	75.69 : 23.31	51.38

3.3 Antibacterial Activity

The essential oil exhibited antibacterial activity against all tested microorganisms, with MIC values ranging from 156.3 µg/mL to 2500 µg/mL. The strongest inhibition was recorded against *Streptococcus faecalis* (MIC = 156.3 µg/mL), while *Escherichia coli* and *Pseudomonas aeruginosa* showed the weakest susceptibility (MIC = 2500 µg/mL). In addition, the major constituents, germacrene D and β-caryophyllene, were screened and showed activity patterns comparable to those of the crude oil (Table 4).

Table 4 Minimum Inhibitory Concentration (MIC) of *S. officinalis* Essential Oil and Major Components

Bacterial Strain	Streptomycin (<19.5 µg/mL)	Germacrene D (µg/mL)	β-Caryophyllene (µg/mL)	Essential Oil (µg/mL)
S. aureus ATCC 25923	<19.5	220	312.5	625
Bacillus subtilis ATCC 6633	<19.5	300	312.5	312.5
Salmonella typhi ATCC 6539	<19.5	240	312.5	312.5
Proteus vulgaris ATCC 6380	<19.5	260	312.5	312.5
E. coli ATCC 25922	<19.5	300	312.5	2500
S. faecalis ATCC 9790	<19.5	280	312.5	156.3
P. aeruginosa ATCC 27853	<19.5	250	312.5	2500

3.4 Hierarchical Cluster Analysis (HCA)

Hierarchical cluster analysis was performed using the relative abundances of the major essential oil constituents of *Salvia officinalis* from this study together with published chemical profiles from different geographical regions. The dendrogram generated using Ward's method and Euclidean distance revealed six distinct chemotypic clusters among the compared *S. officinalis* samples. The first cluster comprised the 1,8-cineole–rich oils, which included samples from Tunisia, Albania, Croatia, Algeria, and the present Nigerian sample. The second cluster was characterized by high levels of α-humulene and linalool, also including the Nigerian sample. A third cluster grouped samples dominated by germacrene D, trans-nerolidol, α-pinene, sabinene, δ-cadinene, and β-thujone, representing oils from Brazil, Croatia, Albania, Algeria, and Nigeria. Another distinct group represented the β-caryophyllene–rich chemotype, which was previously reported and was also present in this study. Two additional clusters—camphor-rich and α-thujone–rich oils—were observed in samples from Tunisia, Albania, Brazil, Croatia, and Algeria; however, these chemotypes were absent in the Nigerian *S. officinalis* sample. Overall, the Nigerian essential oil grouped within multiple clusters dominated by sesquiterpenes such as β-caryophyllene, germacrene D, and δ-cadinene rather than the camphor or α-thujone chemotypes commonly reported in Mediterranean sage. This indicates a distinct sesquiterpene-rich chemotype for the Nigerian population.

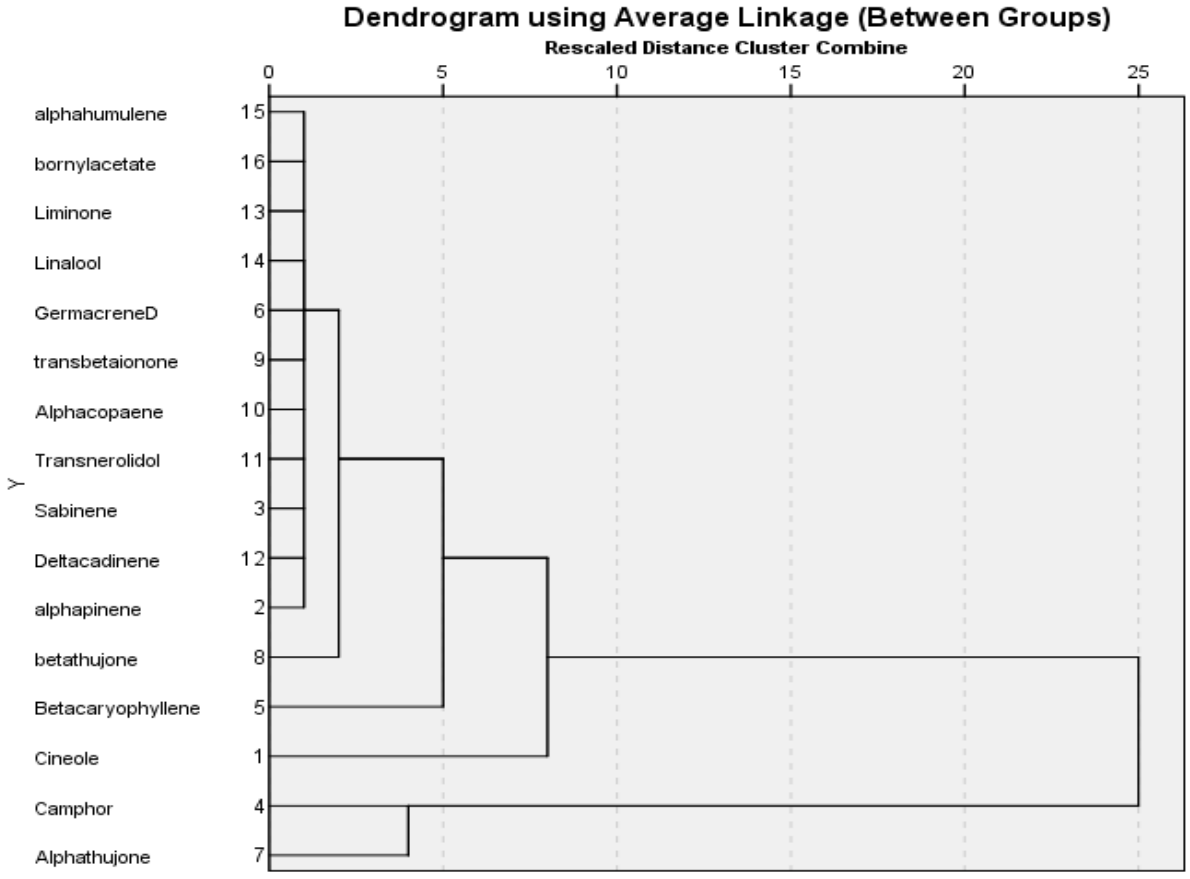


Figure 2 Hierarchical cluster analysis (HCA) of *Salvia officinalis* essential oils based on major chemical constituents using Ward's method and Euclidean distance. Six chemotypic clusters were identified: cineole-rich, humulene–linalool, sesquiterpene-dominant, caryophyllene-rich, camphor-rich, and thujone-rich clusters.

4 Discussion

The Nigerian *Salvia officinalis* essential oil's chemical makeup, enantiomeric distribution, and antibacterial activity demonstrated a chemotype different from those frequently documented in the Mediterranean and other parts of the world. Sesquiterpenes, specifically β-caryophyllene, germacrene D, trans-β-ionone, α-copaene, trans-nerolidol, and δ-

cadinene, predominated in the essential oil, suggesting a sesquiterpene-rich profile instead of the traditional camphor/α-thujone chemotype commonly linked to *S. officinalis* from Tunisia, Albania, Croatia, Algeria, and Brazil. Camphor, α-Thujone, and β-Thujone are major chemotypes found in Tunisia (Khedher et al., 2017), Albania (Hodaj-Çeliku et al., 2017), Algeria (Myrtaj et al., 2022), and Brazil (Porte et al., 2013), but they are not present in this study. In this study, as well as in Croatia (Jažo et al., 2023) and Brazil (Porte et al., 2013), α-pinene is a significant component. According to Barra (2009) and Jug-Dujaković et al. (2012), the absence of camphor/α-thujone chemotype in the Nigerian sample emphasises strong chemotypic variation that is probably caused by geographical, climatic, and ecological differences. Several studies show that the chemical makeup of S. officinalis volatile oils is strongly influenced by genetic, seasonal, and particularly climatic factors like temperature, precipitation, and soil conditions, which can lead to the emergence of distinct chemotypes (Karalija et al., 2022; Myrtaj et al., 2022).

The distinctiveness of this Nigerian chemotype is further supported by the significant amounts of α-pinene, sabinene, germacrene D, δ-cadinene, and nerolidol. Although α-pinene has been identified as a significant component of Brazilian and Croatian sage essential oils (Jažo et al., 2023; Porte et al., 2013), this study's elevated levels of sesquiterpenes, such as germacrene D and β-caryophyllene, are relatively higher than most previously reported samples. In particular, β-caryophyllene has been identified as a major constituent in certain sage species and has been acknowledged for its biological significance (Brunke & Hammerschmidt, 1985). Thus, the Nigerian S. officinalis adds to the species' known chemotypic diversity by representing a β-caryophyllene/germacrene-D chemotype.

High enantiomeric purity in β-caryophyllene, δ-cadinene, and trans-β-ionone (100% ee) was found in the enantiomeric analysis, indicating stereospecific biosynthetic pathways within the plant. The identification of enantiomeric pairs for α-pinene, β-pinene, limonene, sabinene, α-terpineol, and linalool is in line with the monoterpenoids' established chirality. Enantiomeric studies of other essential oils have shown similar findings (Valarezo et al., 2022). The present findings provide new stereochemical insights into *S. officinalis* from West Africa, where such data are almost non-existent. Enantiomeric distributions are crucial indicators of authenticity, chemotaxonomy, and biological activity. For African and tropical Salvia populations, this field is still in its infancy and has not received enough attention, underscoring the importance of reporting enantiomeric patterns in order to comprehend the chemotaxonomy and bioactivity of the plant.

The essential oil showed broad-spectrum antibacterial activity, showing low to negligible activity against *Pseudomonas aeruginosa* and *E. coli*, moderate activity against *Staphylococcus aureus*, and strong activity against *Streptococcus faecalis*, *Proteus vulgaris*, *Salmonella typhi*, and *Bacillus subtilis*. According to earlier studies (El Jery et al., 2020; Khedher et al., 2017; Lahlou et al., 2023; Najar et al., 2021), *S. officinalis* essential oils, especially those from Tunisia, Morocco, South Africa, and Saudi Arabia, have antimicrobial qualities. Due to the outer membrane barrier, these Gram-negative bacteria typically show greater intrinsic resistance to plant essential oils, which explains the comparatively weaker activity seen against E. coli and P. aeruginosa (Ogundajo et al., 2021). Germacrene D and β-caryophyllene exhibit activity levels similar to those of the entire oil, indicating that the dominant constituents are responsible for the specific antibacterial action. Previous studies against a variety of bacterial strains have demonstrated the antibacterial activity of germacrene D, whereas β-caryophyllene has been mechanistically demonstrated to damage cell membrane integrity, resulting in bacterial cell death, particularly in Gram-positive species like *Bacillus cereus*. β-caryophyllene is regarded as a major source of anti-staphylococcal and anti-bacillary activity and has been shown to be effective in a variety of plant-derived oils (El Jery et al., 2020; Moo et al., 2020; Pérez Zamora et al., 2018).

The Nigerian sample was grouped into clusters with cineole-rich, humulene-linalool, and sesquiterpene-rich chemotypes using hierarchical cluster analysis (HCA), which offered additional chemotaxonomic insight. The clustering pattern, which reflects the chemical deviations found in this study, clearly showed the lack of camphor- and thujone-dominant chemotypes frequently associated with Mediterranean sage. Mayer et al. (2009) and Jug-Dujaković et al. (2012) have reported similar HCA-based chemotype classifications for Salvia officinalis.

5 Conclusions

This study provides a comprehensive chemical, enantiomeric, and antibacterial characterization of the essential oil of *Salvia officinalis* cultivated in Nigeria. The oil demonstrated a distinctive sesquiterpene-rich chemotype dominated by β-caryophyllene, germacrene D, δ-cadinene, and trans-β-ionone, markedly different from the camphor- and thujone-dominated chemotypes widely reported in Mediterranean sage. The unique stereochemical features particularly the

high enantiomeric purity of β-caryophyllene, δ-cadinene, and trans-β-ionone highlight region-specific biosynthetic pathways and contribute important chemotaxonomic insights. The essential oil exhibited broad-spectrum antibacterial activity, with notable potency against *Streptococcus faecalis*, *Bacillus subtilis*, *Proteus vulgaris*, and *Salmonella typhi*. The activity of major constituents, especially β-caryophyllene and germacrene D, suggests that both major and minor components contribute synergistically to the antimicrobial efficacy. The weak activity against *E. coli* and *P. aeruginosa* aligns with the known resistance patterns of Gram-negative bacteria. Overall, the findings emphasize the pharmaceutical and nutraceutical potential of Nigerian *S. officinalis*, particularly as a natural antimicrobial agent in the face of rising antibiotic resistance. The chemical and stereochemical fingerprint generated in this study establishes a foundational reference for future research, quality control, and the development of sage-based therapeutic products in Nigeria and beyond.

Conflict of Interest

The authors declare that there is no conflict of interest.

Declaration of Use of Generative AI

Generative AI tools were used exclusively for layout formatting, paraphrasing, and grammar correction.

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