Received: 15-10-2025
Revised: 7-11-2025
Accepted: 18-12-2025
Published: 30-12-2025

Elicit Journal of Science Research
ISSN: 3093-0502

https://elicitpublisher.com

EMPIRICAL PAPER

Chemical and Enantiomeric Profiling, Chemotype Classification and Antibacterial Activity of a Phytone-Rich Essential Oil from *Lawsonia inermis* Leaves

Rayhana Olubukola Davies-Sani[1*], Alkali Hamza Sani[2], Moses Sunday Owolabi[1], Akintayo Lanre Ogundajo[1], William N. Setzer[3], Prabodh Satyal[3], Ambika Poudel[4], Lukman Olalekan Afolabi[5], Muhammad Sani Musbahu[6]

Affiliations

[1]Department of Chemistry, Lagos State University
[2]Department of Pathology, Me Cure Healthcare Ltd
[3]Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA
[4]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
[5]Indiana University School of Medicine, Indiana USA
[6]Department of Biochemical Technology,School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Thailand b

Orcid Identifiers

Davies-Sani Rayhana Olubukola
0009-0009-3191-5813
Sani Alkali Hamza
0009-0007-3978-6337
Owolabi Moses Sunday
0000-0002-8766-8649
Ogundajo Akintayo Lanre
0000-0001-8408-4894
Setzer William N.
0000-0002-3639-0528
Satyal Prabodh
0000-0002-2950-1074
Poudel Ambika
0009-0001-9353-4073
Afolabi Lukman Olalekan
0000-0001-7659-128X
Musbahu Muhammad Sani
0000-0001-8848-3403

Abstract

Purpose: This study examines the chemical composition, enantiomeric distribution, and antibacterial activity of Lawsonia inermis leaf essential oil to identify its chemotypic features and clarify links between composition and bioactivity.

Methodology: Essential oil was obtained from *L. inermis* leaves via hydro-distillation. Chemical characterization was performed using GC–MS, while chiral GC–MS was used to determine the enantiomeric distribution of selected terpenoids. Hierarchical cluster analysis (HCA) compared the oil with previously reported *L. inermis* chemotypes. Antibacterial activity of the crude oil and selected constituents (phytone, linalool, and trans-α-farnesene) was evaluated against seven ATCC bacterial strains using the microbroth dilution method.

Results: A total of 87 compounds were identified, representing 97.1% of the essential oil. The dominant constituents were phytone (15.4%), palmitic acid (14.6%), and tetradecanoic acid (5.5%). Enantiomeric profiling revealed several non-racemic terpenoids, including α-terpineol, sabinene, β-pinene, and trans-nerolidol, each exhibiting high enantiomeric excesses (>70%). HCA positioned the Nigerian oil within cineole- and linalool-related chemotypes, while also revealing a distinctive phytone-dominant profile. Antibacterial assays showed moderate activity of the crude oil (MIC 1250–5000 µg/mL), whereas isolated constituents particularly phytone and linalool demonstrated stronger antibacterial effects (MIC 78.1–625 µg/mL).

Novelty and Contribution: This study reports, for the first time, the enantiomeric distribution of *L. inermis* leaf essential oil and identifies a novel phytone-rich chemotype.

Practical and Social Implications: The findings support improved quality assessment and standardization of *L. inermis* essential oil and highlight its potential for developing plant-based antibacterial agents.

Keywords: Enantiomer, Henna, Gas chromatography, Hierarchical cluster, Chiral

*Corresponding author

E-mail address: bukeydavis@gmail.com

How to cite this article:

Davies-Sani, R. O., Sani, A. H., Owolabi, M. S., Ogundajo, A. L., Setzer, W. N., Satyal, P., Poudel, A., Afolabi, L. O., & Musbahu, S. M. (2025). Chemical and Enantiomeric Profiling, Chemotype Classification and Antibacterial Activity of a Phytone-Rich Essential Oil from Lawsonia inermis Leaves. *Elicit Journal of Science Research, 1* (1), 1-13.

1 Introduction

Medicinal plants have been integral to human society in the fight against diseases from the inception of civilisation. *Lawsonia inermis* (henna) belongs to the Lythraceae family, which comprises approximately 500 species, predominantly found in tropical climates, with relatively few species in temperate areas (Elaguel et al., 2019). The species Henna (*Lawsonia inermis*) L. is a tropical and subtropical shrub indigenous to North Africa, the Middle East, and the Indian subcontinent. Every portion of the plant has a longstanding history of ethnomedicinal application globally (Mosunmolar et al., 2025).

The plant can grow to a height of up to 6 meters and yields aromatic white or rose-red blossoms. *Lawsonia inermis L.*, commonly referred to as "Laali" in Nigeria, is utilised for dyeing fabrics and textiles, as well as in hair shampoos, conditioners, and dyes. The herb has been utilised for its astringent, anti-haemorrhagic, intestinal anti-neoplastic, cardio-inhibitory, hypotensive, jaundice, leprosy, and sedative qualities in traditional ethnomedicine (Ogunbinu et al., 2007). Contemporary pharmacological studies on henna leaves and their components have validated their anti-inflammatory, antipyretic, antibacterial, and analgesic properties, while also furnishing evidence of their anti-carcinogenic potential. Henna leaves are utilised as a decoction or ointment for the treatment of burns, skin inflammations, wounds, and ulcers. The leaves have antifungal and antibacterial properties (Mosunmolar et al., 2025).

Studies that have chemically profiled *L. inermis* essential oil report a varied composition based on plant origin, harvest period, and extraction method. Analyses utilising Gas chromatography–mass spectroscopy (GS-MS) have found dozens of compounds in leaf oils frequently reporting substantial levels of terpenes and oxygenated derivatives (Oyedeji et al., 2005). Similarly, Elaguel et al. (2019) demonstrated the existence of at least thirty of components of essential oil in L. inermis leaves. Monoterpene hydrocarbons were the predominant class of ingredients with 81.40% comprised the α-limonene (55.06%), β–limonene (24.06%) and β–myrcene (2.28%), followed by the linalool (2.41%). The generated essential oil was characterized by a higher concentration of monoterpene hydrocarbons. This compositional heterogeneity underscores the need for rigorous chemical characterisation when relating composition to biological action.

However, the enantiomeric distribution of chiral terpenoids is a more in-depth level of chemical complexity that is sometimes neglected in routine profiling (Das et al. 2024). Chiral essential oils, such as limonene, linalool, and α-pinene, can have significant effects on scent, pharmacology, and toxicity due to enantiomer differences. Modern analytical methods, such as enantioselective GC with chiral stationary phases or multidimensional GC, enable the resolution of these stereoisomers while also providing insight into biosynthetic routes and quality control criteria (De Sousa et al., 2023; Vallamkonda et al., 2024). Despite its significance, enantiomeric profiling of several therapeutic plant oils, including *L. inermis*, has received little attention.

The indiscriminate use of antibacterial drugs for therapeutic and preventative purposes in a range of industries, including animal husbandry and agriculture, has resulted in antibiotic resistance, which is now seen as one of the most serious global health issues (Galgano et al., 2022). Researchers have recently investigated plant sources of antibiotics. The antibacterial properties of Henna leaf have been attributed to its high phytochemical content, which includes flavonoids, tannins, and phenolic substances. Several studies have demonstrated that Henna extracts, when used at appropriate quantities, can prevent the growth of certain bacterial strains, giving it a promising biological alternative for treating bacterial infections (Habbal et al., 2011; Mosunmolar et al., 2025). Raja et al. (2013) used the disc diffusion method to test the antibacterial activity of *L. inermis* against Gram-positive bacteria (*B. subtilius, S. aureus, and Staphylococcus aureus*) and Gram-negative bacteria (*E. coli, S. flexneri, and Pseudomonas aeruginosa)*. This study found that methanolic leaf extracts of *L. inermis L* suppress the development of microorganisms in a dose-dependent manner. Phytochemical analysis of the extracts revealed glycosides, phytosterol, steroids, saponins, tannins, and flavonoids. While crude extracts and some oil fractions have demonstrated inhibitory effects against pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*, discrepancies in methodology and poor chemical characterisation make comparisons and mechanistic interpretation difficult. Comprehensive investigations combining rigorous chemical profiling (including enantiomeric analysis) with standardised antibacterial assays are required to determine whether ingredients or stereoisomeric forms drive activity and to assess the translational potential of *L. inermis* essential oil.

Taken together, these research identify two complementary needs in the study of *L. inermis* essential oil. First, high-resolution chemical analysis combining GC–MS identification with enantioselective techniques will provide a fuller

picture of the oil's composition and stereochemical profile. Second, pairing that chemical information with standardized antibacterial testing can help establish meaningful composition–activity relationships and reveal candidate compounds for further study. Addressing these gaps is important both for scientific understanding and for practical applications in pharmaceuticals, cosmeceuticals, and natural preservatives.

Therefore, this study aims to (i) perform detailed chemical profiling of *Lawsonia inermis* leaf essential oil using GC–MS and enantioselective GC, and (ii) evaluate its antibacterial activity against selected clinically relevant bacterial strains, with the objective of correlating compositional and enantiomeric features with observed antimicrobial effects

2 Materials and Methods

2.1 Plant Material Collection and Authentication

In May 2024, fresh leaves of *Lawsonia inermis 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 confirmed the plant material and put a voucher specimen (ABU6816) in the departmental herbarium for future reference.

2.2 Preparation of Plant Material

The leaves were air-dried under shade in a well-ventilated environment at ambient temperature for 5–7 days until a constant weight was achieved, in order to minimize loss of volatile constituents, and then they were ground up in an electric blender. The powdered substance was kept in airtight polyethylene containers until it was time to extract it.

2.3 Extraction of Essential Oil

Essential oil was obtained by hydro-distillation using an all-glass Clevenger-type apparatus in accordance with the British Pharmacopoeia method. Briefly, 500 g of the powdered leaf material was immersed in distilled water in a 5 L round-bottom flask and subjected to hydro-distillation for 4 h. The distillation time was adopted from standard pharmacopoeial procedures and was not further optimized. Distillation was carried out in replicate under identical conditions. The distillate was extracted with *n*-hexane, dried over anhydrous sodium sulfate, and the solvent removed under reduced pressure to afford a pale-yellow essential oil (Olubukola et al., 2024). The essential oil yields varied from 1.2 to 4.5% (v/w).

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

Chemical profiling of *L. inermis* essential oil was conducted using a Shimadzu GCMS-QP2010 Ultra system, operated under electron ionization (70 eV) with a scan range of 40–400 m/z. A ZB-5 fused-silica capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) coated with 5% phenyl-polymethylsiloxane was used as the stationary phase. Helium served as the carrier gas at a flow rate of 1.37 mL/min, and injection was carried out in split mode (30:1) at 250 °C.

The oven temperature program was as follows: initial temperature of 50 °C, increased at 2 °C·min^{-1} to 260 °C, with a final hold period. Essential oil samples were diluted to 5% (w/v) in dichloromethane, and 0.1 µL was injected. Each sample was analysed in replicate, and relative percentage compositions were calculated as mean values from replicate chromatograms.

Compound identification was based on comparison of mass spectra with NIST and FFNSC libraries, calculation of experimental retention indices (RI) relative to a homologous series of *n*-alkanes (C_8–C_{20}), and comparison with literature RI values. Where available, identifications were further confirmed by co-injection with authentic reference standards (Satyal et al., 2013; Mondello, 2016)

2.5 Chiral GC–MS Analysis

Enantiomeric profiling of monoterpenoids and sesquiterpenoids was conducted using a Shimadzu GCMS-QP2010S equipped with a chiral Restek B-Dex 325 capillary column (30 m × 0.25 mm ID × 0.25 µm film). The oven was

programmed at 50 °C, increased to 120 °C at 1.5 °C/min, then to 200 °C at 2 °C/min with a final hold of 5 min. Essential oil samples were diluted to 3% (w/v) in CH_2Cl_2, with 0.1 µL injected in split mode (1:45). Identification of enantiomers was based on retention indices compared with authenticated reference standards (Sigma-Aldrich) and literature data (Olubukola et al., 2024)

Enantiomeric excess (ee%) was calculated using peak area ratios following established methods (Mondello, 2016). Based on replicate injections, the estimated uncertainty in ee values was within ±2–3%.

2.6 Anti-bacterial Screening

The antibacterial activity of the L. inermis leaf essential oil was evaluated using the microbroth dilution method as previously described by (Olubukola et al., 2024). Seven standard bacterial strains were used in the assay: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Streptococcus faecalis* (ATCC 9790), *Salmonella typhi* (ATCC 6539), *Proteus vulgaris* (ATCC 6380), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853).

A stock solution of the essential oil (1% w/v) was prepared in dimethyl sulfoxide (DMSO). Serial two-fold dilutions were prepared in cation-adjusted Mueller–Hinton broth (CAMHB) to obtain final test concentrations ranging from 2500 to 19.5 $\mu g \cdot mL^{-1}$. The final DMSO concentration in assay wells did not exceed [≤1% v/v], a level that showed no inhibitory effect on bacterial growth, as confirmed by negative control wells.

Bacterial inocula were prepared from overnight cultures and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 $CFU \cdot mL^{-1}$). Plates were incubated at 37 °C for 24 h, and minimum inhibitory concentrations (MICs) were determined visually as the lowest concentration showing no visible growth. Streptomycin served as the positive control, while DMSO-containing wells without test compounds served as negative controls. All assays were conducted in at least two independent experiments, each performed in triplicate. The tested reference compounds were obtained from Sigma-Aldrich and had stated purities ≥95%. No solubility issues were observed at the concentrations tested.

2.7 Hierarchical Cluster Analysis

Hierarchical cluster analysis (HCA) was performed using XLSTAT version 2018.1.1.62926 to assess chemotypic relationships among L. inermis essential oils. The dataset comprised the 12 most abundant components (1,8-cineole, sabinene, α-pinene, (E)-β-farnesene, (E)-β-caryophyllene, α-terpinyl acetate, terpinen-4-ol, α-terpineol, bicyclogermacrene, caryophyllene oxide, limonene, and τ-cadinol), including the present study and published oils from different geographical origins (Barra, 2009; Brunke & Hammerschmidt, 1985). Euclidean distance was used as the dissimilarity metric, and Ward's method was applied for agglomeration. Prior to analysis, compound percentages were normalized to minimize bias from highly abundant constituents.

2.8 Data Processing and Statistical Analysis

GC–MS peak integration and quantification were performed using Shimadzu GCMS Solution software. Enantiomeric distribution and ee% calculations were performed manually using relative peak areas. All experiments were undertaken in triplicate, and results were expressed as mean values. Descriptive statistics and clustering were carried out using XLSTAT

3 Results

3.1 Chemical composition of the essential oil

The GC–MS analysis of the *L.inermis* leaf essential oil identified 87 constituents, which together accounted for 97.1% of the oil (Table 1). The oil was dominated by terpenoid and long-chain fatty constituents; hydrocarbon sesquiterpenes and hydrocarbon monoterpenes represented major fractions of the profile while oxygenated sesquiterpenoids, benzenoid aromatics and a mixture of other non-terpenoid components made up the remainder (classes: hydrocarbon sesquiterpenes 45.1%, hydrocarbon monoterpenes 18.0%, oxygenated sesquiterpenoids 5.0%,

benzenoid aromatics 1.0%, others 28.0%) (table 2). The "others" category represents identified non-terpenoid constituents, including fatty acids, long-chain aldehydes, esters, and related aliphatic compounds, rather than unidentified components.

The most abundant individual constituents (relative percentage) included phytone (15.4%), palmitic acid (14.6%), tetradecanoic acid (5.5%), caryophyllene oxide (3.9%), dodecanoic acid (3.2%), and trans-α-bergamotene (3.2%) (full composition presented in Table 1). numerous mono- and sesquiterpene hydrocarbons and oxygenated terpenoids were detected at lower abundances, creating a chemically complex profile typical of *L. inermis* leaf oils (Table 1).

Table 1 Chemical Composition of *Lawsonia inermis* Leaf Essential Oil (GC–MS Analysis)

S/N	RI (db)	RI (cal)	Compound	% Composition
1	801	801	Hexanal	0.1
2	850	840	1,2,5,5-Tetramethyl-1,3-cyclopentadiene	0.1
3	925	932	α-Pinene	0.7
4	932	964	Benzaldehyde	0.1
5	969	973	1-Octen-3-one	Tr
6	972	978	β-Pinene	0.2
7	978	988	2-Methyl-3-octanone	0.2
8	984	989	Myrcene	0.1
9	986	991	2-Pentylfuran	0.1
10	1005	1006	Octanal	Tr
11	1015	1017	α-Terpinene	Tr
12	1024	1025	p-Cymene	0.1
13	1028	1030	Limonene	0.2
14	1030	1031	β-Phellandrene	0.1
15	1031	1032	1,8-Cineole	0.8
16	1032	1034	(Z)-β-Ocimene	0.1
17	1043	1045	Phenylacetaldehyde	0.1
18	1056	1058	γ-Terpinene	0.1
19	1069	1068	Acetophenone	Tr
20	1085	1086	Terpinolene	Tr
21	1105	1107	Nonanal	0.1
22	1178	1180	Terpinen-4-ol	0.1
23	1197	1198	α-Terpineol	0.1
24	1201	1201	Safranal	0.1
25	1205	1206	Decanal	0.1
26	1227	1229	Thymyl methyl ether	0.1
27	1271	1272	Nonanoic acid	0.3
28	1291	1293	Thymol	0.1
29	1301	1300	Tridecane	Tr
30	1312	1310	4-Vinylguaiacol	0.1
31	1371	1367	Decanoic acid	0.9
32	1373	1367	Cyclosativene	0.3
33	1373	1375	α-Copaene	0.6
34	1378	1379	(E)-β-Damascenone	0.2
35	1381	1382	β-Bourbonene	0.2
36	1385	1387	β-Cubebene	0.2

37	1391	1390	trans-β-Elemene	0.2
38	1394	1396	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.1
39	1396	1396	1,1,5-Trimethyl-1,2-dihydronaphthalene	0.1
40	1412	1413	cis-α-Bergamotene	0.2
41	1418	1417	α-Santalene	0.2
42	1415	1418	(E)-β-Caryophyllene	2.3
43	1423	1425	Florhydral	0.1
44	1430	1430	β-Copaene	0.1
45	1432	1432	trans-α-Bergamotene	3.2
46	1447	1447	Geranylacetone	0.8
47	1451	1452	(E)-β-Farnesene	0.1
48	1453	1454	α-Humulene	0.8
49	1473	1475	γ-Muurolene	0.1
50	1483	1485	Dehydro-β-ionone	0.4
51	1481	1481	(E)-β-Ionone	0.5
52	1483	1483	Germacrene D	1.2
53	1481	1483	trans-β-Bergamotene	0.6
54	1487	1489	β-Selinene	0.1
55	1490	1490	γ-Amorphene	0.1
56	1494	1497	Bicyclogermacrene	0.4
57	1502	1500	α-Muurolene	0.1
58	1503	1501	(Z)-α-Bisabolene	0.1
59	1508	1508	β-Bisabolene	0.1
60	1522	1520	7-epi-α-Selinene	0.1
61	1520	1519	trans-Calimenene	0.2
62	1531	1533	trans-Cadina-1,4-diene	0.2
63	1561	1560	Dodecanoic acid	3.2
64	1563	1562	(E)-Nerolidol	0.7
65	1565	1563	1-Tridecanol	0.3
66	1588	1587	Caryophyllene oxide	3.9
67	1610	1611	Humulene epoxide II	0.8
68	1615	1613	Tetradecanal	0.1
69	1678	1676	1-Tetradecanol	0.2
70	1690	1688	α-Bisabolol	2.0
71	1720	1715	Pentadecanal	0.3
72	1721	1723	Methyl tetradecanoate	0.2
73	1725	1719	1-Phenylhepta-1,3,5-triyne	2.8
74	1753	1758	Tetradecanoic acid	5.5
75	1801	1800	Octadecane	0.5
76	1843	1841	**Phytone**	**15.4**
77	1863	1867	Pentadecanoic acid	0.6
78	1895	1893	Methyl (7Z,10Z,13Z)-hexadecatrienoate	1.0
79	1920	1915	(5E,9E)-Farnesylacetone	1.4
80	1925	1925	Methyl hexadecanoate	0.9
81	—	—	(7Z,10Z,13Z)-Hexadecatrienoic acid	2.2
82	1953	1958	**Palmitic acid**	**14.6**
83	2090	2093	Methyl linoleate	0.7

84	2093	2098	Methyl linolenate	1.3
85	2125	2128	Linoleic acid	0.9
86	2133	2134	Linolenic acid	2.5
87	2210	2212	Phytyl acetate	0.2

RIcalc = Retention index calculated with respect to a homologous series of n-alkanes on a ZB-5ms column RIdb = Reference retention index from the database, "Tr" = trace (<0.1%)

Table 2 Classes of compound

Class of Compounds	% Composition
Hydrocarbon monoterpenes	18.0
Hydrocarbon sesquiterpenes	45.1
Oxygenated sesquiterpenoids	5.0
Benzenoid aromatics	1.0
Others	28.0

3.2 Enantiomeric distribution

Enantioselective GC–MS analysis showed that several terpenoid constituents existed as non-racemic stereoisomeric mixtures (Table 3). A number of components displayed complete enantiomeric purity (100% enantiomeric excess), for example α-thujene, α-phellandrene, and β-caryophyllene (only a single enantiomer detected). Other constituents exhibited partial enantiomeric enrichment: α-pinene (+77.7 : −22.3; ee ≈ 55.3%), sabinene (+14.1 : −85.9; ee ≈ 71.8%), β-pinene (+14.3 : −85.7; ee ≈ 71.3%), limonene (+31.6 : −68.4; ee ≈ 36.8%), linalool (+63.7 : −36.3; ee ≈ 27.4%), α-terpineol (+5.1 : −94.9; ee ≈ 89.8%), and trans-nerolidol (+85.4 : −14.6; ee ≈ 70.8%) (Table 3). No racemic mixtures were observed among the major chiral terpenoids. Based on replicate injections, the estimated uncertainty in enantiomeric excess values was within ±2–3%.

Table 3 Enantiomeric Distribution in *L. inermis* Leaf Essential Oil

Compound	D (%)	L (%)	Enantiomeric Excess (%)
α-Thujene	0	100	100
α-Pinene	77.67	22.33	55.34
Sabinene	14.09	85.91	71.82
β-Pinene	14.33	85.67	71.34
α-Phellandrene	100	0	100
Limonene	31.61	68.39	36.78
β-Phellandrene	83.97	16.03	67.94
Linalool	63.71	36.29	27.42
α-Terpineol	5.09	94.91	89.82
β-Caryophyllene	0	100	100
trans-Nerolidol	85.39	14.61	70.78

3.3 Chemometric grouping (hierarchical cluster analysis)

Hierarchical cluster analysis (HCA) based on the relative abundances of major components grouped the present *L. inermis* leaf oil with published oils into four distinct chemotype clusters (Figure 1). The clusters are characterized as follows: (1) a 1,8-cineole / squalene / linalool / geranyl acetone cluster, which includes samples from Tunisia, Egypt, Nigeria and the current study; (2) an α-terpineol / β-ionone cluster that groups samples from Ethiopia, Nigeria and this study; (3) an α-limonene chemotype predominantly associated with Tunisian samples; and (4) a phytol / eugenol / β-limonene cluster comprising some Egyptian and Ethiopian samples but absent in the present material.

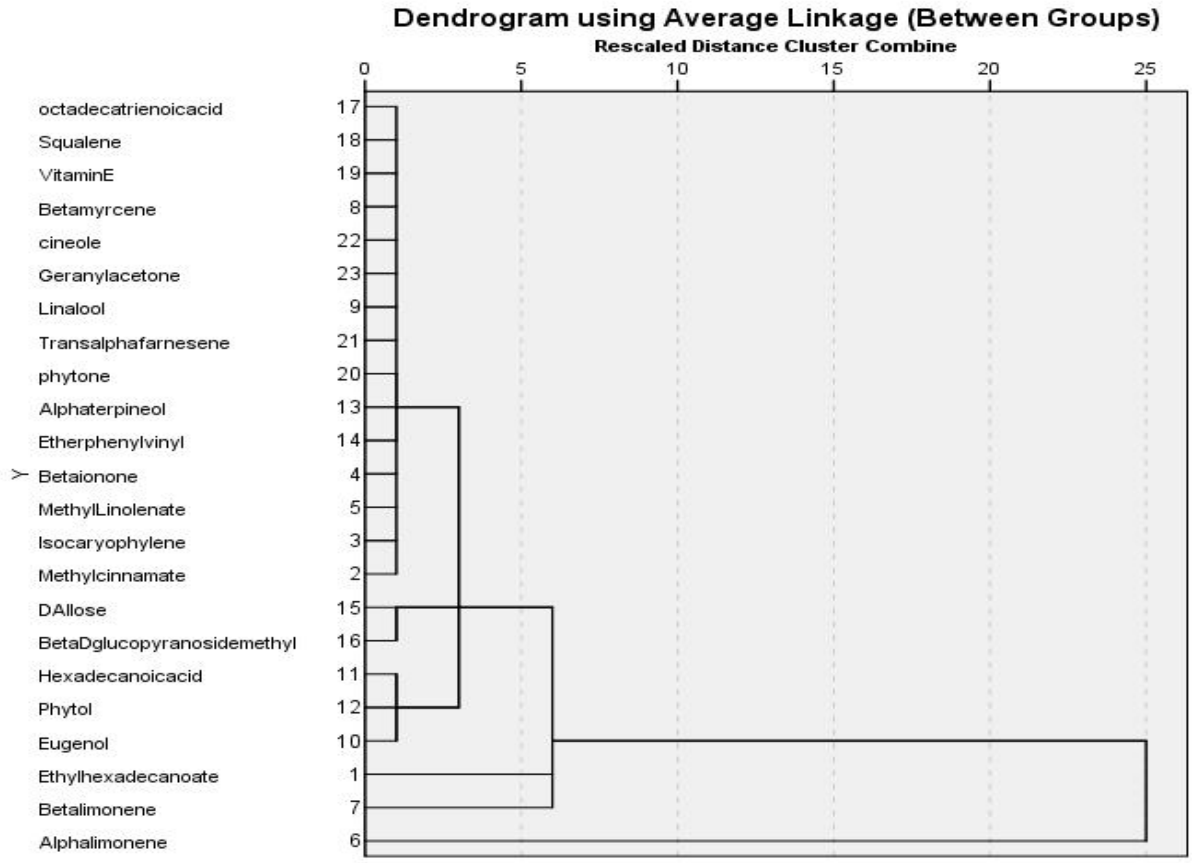

Figure 1 Dendrogram from hierarchical cluster analysis showing chemotype groupings of *L. inermis* essential oils (this study and literature samples).

3.4 Antibacterial activity

The antibacterial effects of the crude essential oil and three of its major constituents (trans-α-farnesene, linalool and phytone) were determined by microbroth dilution against seven ATCC bacterial strains; minimum inhibitory concentrations (MICs) are summarized in Table 4. The crude oil exhibited limited antibacterial potency, with MIC values ranging from 1250 to 5000 µg·mL^{-1}. The oil showed the greatest activity against *Escherichia coli* and *Proteus vulgaris* (MIC = 1250 µg·mL^{-1}) and reduced activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus faecalis* (MIC = 2500 µg·mL^{-1} for *S. aureus* and *P. aeruginosa*; *S. faecalis* MIC = 1250 µg·mL^{-1}). *Salmonella typhi* was the least susceptible species (MIC = 5000 µg·mL^{-1}). Such MIC values are generally considered indicative of low antibacterial potency for essential oils.

In contrast, several isolated major constituents displayed substantially stronger activity than the crude oil. Phytone was the most active constituent tested, with MICs as low as 78.1 µg·mL^{-1} (against S. aureus) and generally ranging between 78–625 µg·mL^{-1} across the panel. Linalool exhibited MIC values around 312.5 µg·mL^{-1} for most strains, whereas trans-α-farnesene showed variable activity (MICs 100–500 µg·mL^{-1}) (Table 4). Streptomycin, used as the positive control, inhibited all test organisms at concentrations < 19.5 µg·mL^{-1}. Dimethyl sulfoxide (negative control) produced no inhibitory effect, highlighting the substantially lower potency of the essential oil and its constituents relative to a standard antibiotic.

Overall, the data indicate that although the whole essential oil has modest antibacterial activity at high concentrations, specific constituents, particularly phytone and linalool, contribute disproportionately to the observed bioactivity and may represent leads for further antimicrobial evaluation.

Table 4 Antibacterial Activities (MIC Values, µg/mL) of *L. inermis* Essential Oil and Major Constituents

Bacterial Strain	Streptomycin	trans-α-Farnesene	Linalool	Phytone	*L. inermis* Essential Oil
S. aureus ATCC 25923	<19.5	250	312.5	**78.1**	2500
B. subtilis ATCC 6633	<19.5	312.5	312.5	312.5	1250
S. typhi ATCC 6539	<19.5	250	312.5	312.5	5000
P. vulgaris ATCC 6380	<19.5	500	312.5	312.5	1250
E. coli ATCC 25922	<19.5	312.5	312.5	312.5	1250
S. faecalis ATCC 9790	<19.5	100	312.5	625	1250
P. aeruginosa ATCC 27853	<19.5	>100	312.5	312.5	2500

4 Discussion

The GC-MS analysis of the essential oil from *Lawsonia inermis* leaves found 87 components, which made up 97.1% of the total oil composition. The significant chemotypic variation in L. inermis essential oils across different geographical regions is extensively documented in the literature. Previous investigations have documented pronounced geographical variation in *L. inermis* essential oils. For example, Ethiopian accessions were reported to be rich in α-limonene, while oils from Sudan, Nepal, Tunisia, and Egypt have been characterized by lawsone-, phytol-, limonene-, or phenolic glucoside-dominant profiles (Kidanemariam et al., 2013; EL-Kamali et al., 2018; Satyal et al., 2013; Benaissa, 2017). In contrast, the Nigerian oil analysed in the present study contained only trace amounts of limonene and was distinguished by a comparatively high phytone content. Rather than implying exact quantitative equivalence across studies, these differences should be interpreted qualitatively, reflecting broad chemotypic divergence likely driven by differences in genotype, climate, soil composition, harvest conditions, and post-harvest processing. Such variability is well documented in aromatic plants and highlights the importance of region-specific chemical characterization when linking composition to biological activity (Moutawalli et al., 2023).

The significant enantiomeric excess of α-terpineol (89.8% (−) enantiomer) corresponds with existing literature indicating that α-terpineol may possess various pharmacological activities, including antioxidant, anticancer, antifungal, and anticonvulsant effects. Studies indicate that α-terpineol functions as an NF-κB inhibitor and compromises fungal cell membrane integrity by reducing ergosterol levels (Ben Miri, 2025; Özek et al., 2010). Enantiomeric analysis of essential oils for chiral terpenoids such as linalool and α-terpineol underscores the substantial biological activity disparities among stereoisomers (Özek et al., 2010).

The hierarchical cluster analysis identified four unique chemotype clusters. The current *L. inermis* leaf oil was categorised with samples from Tunisia, Egypt, Nigeria, and additional sources into either a 1,8-cineole/squalene/linalool/geranyl acetone cluster or an α-terpineol/β-ionone cluster. Moutawalli et al. (2023) asserted that chemotypic variation in essential oils is a natural occurrence driven by genetic and environmental factors affecting terpenoid biosynthesis pathways, and that hierarchical clustering is an effective chemotaxonomic method for assessing intraspecific diversity. In the same way, Medina-Holguín et al. (2008) showed that where a medicinal plant comes from has a big effect on the composition of its essential oils. For example, populations that are only a short distance apart have different chemical profiles. In this study, the classification of Nigerian samples alongside certain Tunisian and Egyptian specimens indicates a common evolutionary history and/or analogous environmental conditions that promote specific metabolic pathways. The presence of phytone as the predominant single component (15.4%) in the current oil, in contrast to limonene-rich Tunisian oils or phytol-rich Egyptian oils, suggests that this Nigerian accession embodies a previously undercharacterized or newly emergent chemotype that merits further exploration.

Chemotypic variation has important medical applications. To guarantee constant bioactivity, standardised herbal remedies usually target particular chemotypes. HCA's ability to identify chemotype clusters offers a logical framework for pharmacological research, supply chain management, and quality assurance. Although direct comparisons necessitate identical assay methodologies, the current material's clustering with samples known to exhibit antibacterial activity (Elaguel et al., 2019; Satyal et al., 2013) provides preliminary evidence supporting its biological efficacy.

One of the study's main findings was the startling difference between the isolated major constituents, especially phytone and linalool, and the crude essential oil's weak antibacterial activity. Using the broth microdilution assay, the crude *L. inermis* essential oil showed moderate antibacterial activity, with minimum inhibitory concentrations (MIC) ranging from 1250 to 5000 $\mu g \cdot mL^{-1}$ for each of the seven ATCC bacterial strains examined. In comparison with previous studies on *L. inermis*, the essential oil evaluated in the present work appears to exhibit antibacterial activity within a similar or slightly higher range than that reported for crude plant extracts. Reported MIC values for *L. inermis* extracts and essential oils commonly fall within the $mg \cdot mL^{-1}$ range, with documented inhibitory effects against bacterial species such as *Staphylococcus aureus* and *Escherichia coli* (Youl et al., 2024). The observed activity may, in part, be associated with the presence of bioactive constituents known to influence antimicrobial potency. For instance, (−)-linalool is frequently reported as the predominant and more biologically active enantiomer in essential oils, which may contribute to antibacterial effects (Paudel et al., 2023). In addition, hexadecanoic (palmitic) acid has been reported to exert antibacterial and antifungal activity, potentially through mechanisms involving disruption of microbial membranes and induction of oxidative stress (Ghfil et al., 2025).

The superior activity of isolated phytone, linalool, and trans-α-farnesene may reflect their amphiphilic properties and ability to penetrate bacterial membranes more effectively than the whole oil preparation. The amphiphilic character possession of both hydrophobic and hydrophilic regions facilitates interaction with lipid bilayers and permits traversal of the bacterial cell envelope. Huang et al., (2022) demonstrated that terpenoids with amphiphilic character exploit their lipophilicity to disrupt the phospholipid bilayer of bacterial cells, penetrate the membrane, and diffuse inward, thereby exerting potent bactericidal effects.

5 Conclusions

This study provides the characterization of the chemical, stereochemical, and antibacterial properties of *Lawsonia inermis* leaf essential oil from Nigeria. GC–MS profiling revealed a complex mixture dominated by phytone, palmitic acid, fatty acids, and terpenoid derivatives, differing markedly from limonene-rich or phytol-rich chemotypes previously reported in other geographical regions. Enantioselective GC–MS further demonstrated that several monoterpenoids and sesquiterpenoids occur in highly enriched enantiomeric forms, offering valuable insights into the biosynthetic pathways and potential stereochemical drivers of biological activity. Hierarchical cluster analysis positioned the Nigerian oil within known cineole- and linalool-associated chemotype clusters, yet its distinctly phytone-dominant profile indicates the emergence of a unique chemotype not previously documented. This highlights the substantial influence of ecological, genetic, and environmental factors on *L. inermis* secondary-metabolite production. The antibacterial evaluation demonstrated that the crude essential oil possesses only moderate inhibitory effects; however, isolated constituents particularly phytone, linalool, and trans-α-farnesene exhibited significantly higher potency. These findings underscore the likelihood that the antibacterial activity of *L. inermis* is primarily attributable to individual bioactive molecules rather than the whole oil, supporting the direction of future research toward isolating and optimizing key compounds. Overall, the study advances the chemotaxonomic understanding of *L. inermis*, provides foundational enantiomeric data for future authentication and quality-control practices, and identifies promising antibacterial constituents with potential pharmaceutical and industrial relevance. Further work exploring mechanism of action, toxicity profiles, and synergistic interactions between constituents will be essential to fully unlock the therapeutic and commercial significance of this newly identified chemotype.

Practical Implication

The pronounced chemotypic and enantiomeric variability observed in *Lawsonia inermis* essential oil underscores the need for region-specific chemical characterisation prior to medicinal or commercial use. The identification of a phytone-dominant Nigerian chemotype demonstrates that essential oils derived from the same species may differ substantially in composition and, by extension, in biological activity. Consequently, phytopharmaceutical developers, herbal medicine practitioners, and regulatory bodies should prioritise chemotype-based standardisation and quality control, incorporating GC–MS and chiral analyses to ensure consistency, safety, and reproducible therapeutic efficacy.

From a pharmacological and formulation perspective, the weak antibacterial activity of the crude essential oil compared with the superior activity of isolated constituents such as phytone, linalool, and trans-α-farnesene suggests that targeted isolation or enrichment of bioactive components may be more effective than whole-oil preparations.

This finding has direct relevance for the development of plant-based antimicrobial agents, topical formulations, and natural preservatives, particularly in the context of rising antimicrobial resistance. The high enantiomeric excess of (−)-α-terpineol further highlights the importance of considering stereochemical composition when linking essential oil chemistry to biological function.

Finally, the successful application of hierarchical cluster analysis demonstrates its practical value as a chemotaxonomic and supply chain management tool, enabling the classification of accessions according to bioactive chemotypes. Such approaches can guide the selection of plant material for cultivation, conservation, and industrial sourcing, while supporting more rational pharmacological screening and product development strategies based on well-defined chemical profiles.

Limitations and Future Research Directions

This study is limited by the use of *Lawsonia inermis* leaves collected from a single geographical location, which restricts the broader generalisation of the chemotypic and antibacterial findings. Environmental factors such as seasonality, soil conditions, plant age, and post-harvest processing were not systematically assessed and may have influenced essential oil composition. In addition, antibacterial activity was evaluated only in vitro against a limited number of reference strains, and potential synergistic or antagonistic interactions among oil constituents were not examined.

Future studies should investigate *L. inermis* accessions from multiple regions and seasons to better characterise chemotypic diversity and its environmental drivers. Bioassay-guided fractionation, combination studies, and mechanistic investigations are recommended to clarify the contributions of individual and interacting compounds to antibacterial activity. Expanding testing to clinically relevant and resistant bacterial strains, alongside in vivo efficacy, toxicity, and formulation studies, will be essential for advancing *L. inermis* essential oil toward practical therapeutic applications.

Funding

The authors declare that no financial support was received for the research, authorship, and publication of this article.

Conflict of Interest

The author declare no conflicts of interest.

Declaration of Use of Generative AI

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

References

Barra, A. (2009). Factors Affecting Chemical Variability of Essential Oils: A Review of Recent Developments. *Natural Product Communications*, *4*(8), 934578X0900400827. https://doi.org/10.1177/1934578X0900400827

Ben Miri, Y. (2025). Essential Oils: Chemical Composition and Diverse Biological Activities : A Comprehensive Review. *Natural Product Communications*, *20*(1), 1934578X241311790. https://doi.org/10.1177/1934578X241311790

Benaissa, K. (2017). An optimization of the extraction and the physicochemical proprieties of the essential oil of Lawsonia inermis L. cultivated in Biskra (Department of Algeria). *Indian Journal of Pharmaceutical Education and Research*. https://doi.org/10.5530/IJPER.51.3S.31

Brunke, E.-J., & Hammerschmidt, F.-J. (1985). Constituents of the Essential Oil of Salvia Stenophylla—First Identification of (+)-Epi-α-Bisabolol in Nature. In A. B. Svendsen & J. J. C. Scheffer (Eds.), *Essential Oils and Aromatic Plants* (pp. 145–150). Springer Netherlands. https://doi.org/10.1007/978-94-009-5137-2_13

Das, D. D., Sharma, N., Chawla, V., & Chawla, P. A. (2024). Current Trends of Analytical Techniques for Bioactive Terpenoids: A Review. *Critical Reviews in Analytical Chemistry*, *54*(8), 2984–3000. https://doi.org/10.1080/10408347.2023.2219757

De Sousa, D. P., Damasceno, R. O. S., Amorati, R., Elshabrawy, H. A., De Castro, R. D., Bezerra, D. P., Nunes, V. R. V., Gomes, R. C., & Lima, T. C. (2023). Essential Oils: Chemistry and Pharmacological Activities. *Biomolecules*, *13*(7), 1144. https://doi.org/10.3390/biom13071144

Elaguel, A., Kallel, I., Gargouri, B., Ben Amor, I., Hadrich, B., Ben Messaoud, E., Gdoura, R., Lassoued, S., & Gargouri, A. (2019). Lawsonia inermis essential oil: Extraction optimization by RSM, antioxidant activity, lipid peroxydation and antiproliferative effects. *Lipids in Health and Disease*, *18*(1), 196. https://doi.org/10.1186/s12944-019-1141-1

EL-Kamali, H. H., Lateef, M. M. A. A. el, & Hassan, A. W. (2018). Chemical composition of Lawsonia inermis cultivated under Sudanese conditions-existence of chemotypes species. *International Journal of Advanced Biochemistry Research*, *2*(2), 01–03. https://doi.org/10.33545/26174693.2018.v2.i2a.13

Galgano, M., Capozza, P., Pellegrini, F., Cordisco, M., Sposato, A., Sblano, S., Camero, M., Lanave, G., Fracchiolla, G., Corrente, M., Cirone, F., Trotta, A., Tempesta, M., Buonavoglia, D., & Pratelli, A. (2022). Antimicrobial Activity of Essential Oils Evaluated In Vitro against Escherichia coli and Staphylococcus aureus. *Antibiotics*, *11*(7), 979. https://doi.org/10.3390/antibiotics11070979

Ghfil, Z. A. H. A., Aboalhur, F. J., Mshachal, M. A., & Alobaidi, A. A. A. (2025). Antioxdent, Antifungal Activities of n-Hexadecanoic Acid Extracted from Algae. *South Asian Research Journal of Pharmaceutical Sciences*, *7*(04), 125–127. https://doi.org/10.36346/sarjps.2025.v07i04.003

Habbal, O., Hasson, S., El-Hag, A., Al-Mahrooqi, Z., Al-Hashmi, N., Al-Bimani, Z., Al-Balushi, M., & Al-Jabri, A. (2011). Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Biomedicine*, *1*(3), 173–176. https://doi.org/10.1016/S2221-1691(11)60021-X

Huang, W., Wang, Y., Tian, W., Cui, X., Tu, P., Li, J., Shi, S., & Liu, X. (2022). Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants. *Antibiotics*, *11*(10), 1380. https://doi.org/10.3390/antibiotics11101380

Kidanemariam, T. K., Tesema, T. K., Asressu, K. H., & Boru, A. D. (2013). Chemical Investigation of Lawsonia inermis L. Leaves from Afar Region, Ethiopia. *Oriental Journal of Chemistry*, *29*(3), 1129–1134.

Medina-Holguín, A. L., Omar Holguín, F., Micheletto, S., Goehle, S., Simon, J. A., & O'Connell, M. A. (2008). Chemotypic variation of essential oils in the medicinal plant, *Anemopsis californica*. *Phytochemistry*, *69*(4), 919–927. https://doi.org/10.1016/j.phytochem.2007.11.006

Mosunmolar, B. W., Dahunsi, A. A., Akanbi, B. O., Ogbu, J. C., & Abubakar, S. (2025). *Phytochemical analysis and Antibacterial Activities of Henna Leaf (lawsonia inermis) against salmonella typhyi and Escherichia coli*. Research Square. https://doi.org/10.21203/rs.3.rs-6378359/v1

Mondello, L. (2016). FFNSC 3; Shimadzu Scientific Instruments: Columbia, Maryland, USA.

Moutawalli, A., Benkhouili, F. Z., Doukkali, A., Benzeid, H., & Zahidi, A. (2023). The biological and pharmacologic actions of *Lawsonia inermis* L. *Phytomedicine Plus*, *3*(3), 100468. https://doi.org/10.1016/j.phyplu.2023.100468

Ogunbinu, A. O., Ogunwande, I. A., Walker, T. M., & Setzer, W. N. (2007). Study on the Essential Oil of Lawsonia inermis (L) Lythraceae. *Journal of Essential Oil Bearing Plants*, *10*(3), 184–188. https://doi.org/10.1080/0972060X.2007.10643540

Olubukola, D.-S. R., Omololu, E. A., Owolabi, M. S., Ogundajo, A. L., Favour, N. A., Satyal, P., Poudel, A., & Setzer, W. N. (2024). Vitex agnus-castus L.: Chemical characterization, enantiomeric distribution, and antibacterial efficacy of the essential oil from north-central Nigeria. *Journal of Essential Oil and Plant Composition*, *2*(2), 115–123. https://doi.org/10.58985/jeopc.2024.v02i02.51

Oyedeji, A. O., Ekundayo, O., & Koenig, W. A. (2005). Essential Oil Composition of Lawsonia inermis L. Leaves from Nigeria. *Journal of Essential Oil Research*, *17*(4), 403–404. https://doi.org/10.1080/10412905.2005.9698943

Özek, T., Tabanca, N., Demirci, F., Wedge, D. E., & Başer, K. H. C. (2010). Enantiomeric Distribution of Some Linalool Containing Essential Oils and Their Biological Activities. *Rec. Nat. Prod.*

Paudel, P. N., Satyal, P., Setzer, W. N., Awale, S., Watanabe, S., Maneenet, J., Satyal, R., Acharya, A., Phuyal, M., & Gyawali, R. (2023). Chemical-Enantiomeric Characterization and In-Vitro Biological Evaluation of the Essential Oils from Elsholtzia strobilifera (Benth.) Benth. And E. blanda (Benth.) Benth. From Nepal. *Natural Product Communications*, *18*(7), 1934578X231189325. https://doi.org/10.1177/1934578X231189325

Raja, W., Ovais, M., & Dubey, A. (2013). *Phytochemical Screening and Antibacterial Activity of Lawsonia inermis Leaf Extract*.

Satyal, P., Paudel, P., Poudel, A., Dosoky, N. S., Pokharel, K. K., & Setzer, W. N. (2013). Bioactivities and Compositional Analyses of *Cinnamomum* Essential Oils from Nepal: *C. camphora, C. tamala* , and *C. glaucescens*. *Natural Product Communications*, *8*(12), 1934578X1300801232. https://doi.org/10.1177/1934578X1300801232

Vallamkonda, B., Sethi, S., Satti, P., Das, D. K., Yadav, S., & Vashistha, V. K. (2024). Enantiomeric Analysis of Chiral Drugs Using Mass Spectrometric Methods: A Comprehensive Review. *Chirality*, *36*(8), e23705. https://doi.org/10.1002/chir.23705

Youl, O., Sombié, E. N., Boly, R., Koala, M., Zoungrana, A., Savadogo, S., ... & Traoré, M. (2024). Phytochemical Analysis and Antimicrobial Activity of Lawsonia Inermis Leaf Extracts from Burkina Faso. *American Journal of Plant Sciences*, *15*(7), 552-576.https://doi.org/10.4236/ajps.2024.157038