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Chemical Composition and Antibacterial Properties of *Curcuma Longa* Volatile Oil, n-Hexane and Methanol Extracts against Selected Bacterial Isolates

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ABSTRACT

Curcuma longa is a bright yellow-orange rhizome used for traditional medicine as well as spices. This plant contains some bioactive compounds that inhibit bacterial growth. This study evaluated chemicals composition and antibacterial properties of *C. longa* volatile oil and extracts against selected bacteria. Clevenger apparatus was used to hydrodistillate fresh *C. longa*. Non-volatile compounds were extracted using methanol and n-hexane solvents. Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the chemical constituents of the oil and extracts. Antibacterial activity was assessed using agar-well diffusion method. Minimum Inhibitory Concentration (MIC) was determined using microdilution assay. The result showed that 'eighty-four' (84) compounds were detected in *C. longa* volatile oil, with ar-turmerone (19.01%) as the predominant constituent, followed by 1H-3a,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-[3R(3,alpha.,3a.beta.,7. beta.,8a.alpha.)] (10.13%) and benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (10.01%). Moderate amounts of turmerone (7.29%) and (+)-4-carene (4.74%) were also detected. Turmerone (13.39%) and 14-beta-H-pregna (9.05%) were present in *Curcuma longa* n-hexane extract contained 'seventy two' (72) compounds while for methanol extract, turmerone (12.46%) and 14-beta-H-pregna (8.72%) were found with a total of 109 compounds. The extracts and volatile oil inhibited bacteria that include *Staphylococcus aureus, Klebsiella ornithinolytica* and *Citrobacter gillenii.* The n-hexane extract significantly exhibited inhibition zones of 28±1.03, 26±1.63, and 25±0.33 mm against *C. gillenii, K. ornithinolytica,* and *S. aureus,* respectively. The MIC results indicated that volatile oil exhibited significant activity against *K. ornithinolytica* at a concentration of 6.25 mg/mL. This study revealed that volatile oils and extracts of *C. longa* possess potential as a natural agent against certain clinical bacterial species.

Keywords: Antibacterial Agents, Bacteria, *Curcuma Longa*, Chemical Composition, Gas Chromatography Analysis

INTRODUCTION

Bacterial infections remain a major cause of diseases in humans and negatively impacts public health worldwide (Murray *et al*., 2022; WHO, 2024). Antibiotics are chemically synthesized drugs commonly used against bacterial infections. However, rapid rise of antibacterial resistance has become a serious world health problem (Genova *et al*., 2023) and thus calls for solution. Recent reports indicated that antibacterial resistance has led to roughly 1.27 million deaths and also contributed to nearly 5 million deaths worldwide in 2019, highlighting its severe impact on public health (Murray *et al*., 2022). Data from the World Health Organization (WHO, 2025) revealed widespread resistance trends based on some factors behind bacterial infections, including bloodstream, urinary tract, and sexually transmitted infections. The increasing in bacteria resistance to antibiotics is largely attributed to their drug lethal dose and misuse (Mancuso *et al*., 2021). As a result, infections caused by resistant strains have become more difficult to manage, often leading to prolonged illness, treatment failure, and higher mortality rates (Haney and Hancock, 2022).

High resistance levels have been reported among clinically significant pathogens such as *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus* spp and *Clostridium* species (Koulenti *et al*., 2019). Among these, *S. aureus* is one of the major leading causes of infection associated with antibacterial resistance (Murray *et al*., 2022). Similarly, increasing resistance in bacteria has led to the frequent use of last-resort antibiotics which are also becoming less effective due to emerging resistance (Arato *et al*., 2021; Ardebili *et al*., 2023; Wang *et al*., 2024; Yang *et al*., 2023). There is an urgent need to explore alternative natural antibacterial agents that can destroy the growth of bacteria (Sahoo *et al*., 2021; Mishra *et al*., 2022).

Natural products, particularly those derived from plants have received considerable interest for their potential benefits (Arsene *et al*., 2021; Nourbakhsh *et al*., 2022; Kumar *et al.*, 2025). These natural products include medicinal plants known to contain bioactive compounds capable of inhibiting growth of pathogenic bacteria (Atta *et al*., 2023). Consequently, research has increasingly focused on identifying plant-based compounds that could serve as effective alternatives to synthetic antibiotics (Bishoyi *et al*., 2024). This supports Sustainable Development Goal 3, which focuses on improving people's health and quality of life by 2030 (UN, 2021).

Plant volatile oils and extracts exhibit biological activity due to their complex composition of bioactive compounds (Gupta *et al*., 2015). *Curcuma longa* is a perennial rhizomatous species belonging to the family *Zingiberaceae* and a native of India (El-Kenawy *et al*., 2019). *Curcuma longa* is widely cultivated across South and Southeast Asia (Nair *et al*., 2019) and is valued for both its culinary and medicinal uses. It is well known for its yellow colour and numerous biological activities (Fuloria *et al.*, 2022; Jyotirmaye *et al.*, 2022). It has antimicrobial properties health-promoting effects, such as anti-inflammation, protecting cells from damage, showing promise in fighting cancer, viruses and brain-related disorders (Tundis *et al*., 2023). The characteristic colour of *C. longa* is primarily due to the presence of curcuminoids such as curcumin, demethoxycurcumin and bis-demethoxycurcumin

(Kebede *et al*., 2021), which have demonstrated antibacterial activity against organisms like *Bacillus subtilis* and *Salmonella typhi* (Meng-Meng *et al*., 2020; Kebede *et al*., 2021).

In addition to curcuminoids, *C. longa* contains volatile compounds such as α-turmerone, ar-turmerone, and β-turmerone which are responsible for its distinctive aroma and taste (Dosoky *et al*., 2019). Other components of *C. longa* volatile oil include elemane, bisabolane, and germacrane, while components present in low amounts include cineole, α-phellandrene, β-caryophyllene and α-zingiberene (Dosoky *et al*., 2019; Jaiswal and Naik, 2021). These compounds have been reported to disrupt cell membranes of bacteria, thereby inhibiting their growth (Nourbakhsh *et al*., 2022). *Curcuma longa* also contains phytochemicals such as flavonoids, phenolics, terpenoids, fatty acids, and carotenoids, which contribute to its therapeutic potential. Due to these bioactive constituents, *C. longa* extracts and volatile oils are being explored as natural antibacterials agents. Hence, this study aimed at determining the chemical composition and antibacterial properties of *C. longa* volatile oil and extracts against selected bacterial isolates.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh *C. longa* rhizomes (1000 g) were collected from premises of Adekunle Ajasin University in Akungba-Akoko, Ondo State, Nigeria and verified at the Department of Plant Science and Biotechnology of the same school. The rhizomes were thoroughly washed with clean water to remove any attached soil particles, then air-dried at room temperature. After drying, they were ground into a fine powder using an electric grinder.

Extraction of *Curcuma Longa* Volatile Oil

Volatile oil was extracted from fresh *C. longa* rhizomes through hydrodistillation method using a Clevenger apparatus for 4 hours. The light yellow volatile oil obtained was dried over anhydrous sodium sulphate to remove any residual moisture. Volatile oil was subsequently stored at 4 °C in a refrigerator prior to further chemical and antibacterial analyses.

Extraction of *Curcuma Longa* Using N-Hexane

A Soxhlet apparatus was used to extract 200 g of powdered *C. longa* with n-hexane as the solvent for eight hours. After extraction, the solvent was removed under reduced pressure using a rotary evaporator, resulting in the n-hexane extract. The extract was collected and stored at 4 °C for further analysis.

Methanol Extraction of *Curcuma Longa* Residue

The residue remaining after n-hexane extraction was further processed using methanol. A 200 g of the residue was weighed and transferred into a polyethylene terephthalate (PET) container containing 500 mL of 95% methanol. The mixture was left to stand for 72 hours with occasional shaking to enhance the extraction process. It was then filtered using muslin cloth into a sterile conical flask to obtain the methanol extract.

GC-MS Analysis of Chemical Constituents

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the chemical constituents of *C. longa* volatile oil and solvent extracts using Agilent Technologies 7890B gas chromatograph with an HP-5MS silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) and a 5977A mass selective detector. A 1 μL aliquot of the samples were carefully dissolved in diethyl ether to ensure complete solubilization for further analysis (500 ppm) and was injected in split mode at a 20:1 ratio. The carrier gas was helium, which flowed at a steady rate of 1 cm^3/min. The temperature was first maintained at 50 °C for 2.25 minutes and then steadily increased to 290 °C at a controlled rate of 4 °C per minute. Transfer line, ion source, and quadrupole were kept at 300, 230, and 150 °C, respectively to ensure stable operation. Ionization was performed at 70 eV, and data were collected across a mass range of m/z 35–650.

Test Organisms

Six (6) clinical bacterial isolates were obtained from Health Centre of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The organisms included *Staphylococcus aureus*, *Streptococcus* sp, *Enterobacter agglomerans*, *Citrobacter gillenii*, *Klebsiella ornithinolytica* and *Escherichia coli*. Each isolate was grown in nutrient broth and incubated at 37 °C for 24 hours. The turbidity of the cultures was then standardized to match the 0.5 McFarland standard to ensure uniform inoculum density.

Media Preparation

Nutrient agar (NA) and Mueller-Hinton agar (MHA) were prepared according to manufacturer's instructions. Briefly, 28 g of NA and 38 g of MHA were each dissolved in separate 1000 mL of distilled water and then sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, the media were allowed to cool to around 45 °C before being carefully poured into sterile Petri dishes and left to solidify.

Antibacterial Sensitivity Test (AST)

The antibacterial activity of the *C. longa* volatile oil and extracts was evaluated using the agar well diffusion technique. Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions. Wells of 9 mm diameter were created in the agar using a sterile cork borer, with a spacing of about 20 mm between wells. Prepared solutions of the *Curcuma longa* volatile oil and extracts were introduced into the wells. Chloramphenicol was used as the positive control while 5% dimethyl sulfoxide was also used as negative control. The plates were incubated at 37 °C for 18 hours. Following incubation, the zones of inhibition around each well were measured in millimetres.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of *C. longa* volatile oil and extracts was evaluated using a cell viability test described by Adeoyo *et al*. (2019). First, a stock solution of 200 mg/mL was prepared, which was then diluted step by step to obtain lower concentrations of 100, 50, 25, 12.5 and 6.25 mg/mL. Each concentration was mixed with 2 mL of bacterial suspension that had been adjusted to the 0.5 McFarland standard, and the tubes were left to incubate at 37 °C for 18 hours to allow proper growth conditions. After incubation, to qualitatively detect the presence of live organisms, 0.4 mL of thiazolyl blue tetrazolium bromide (0.2 mg/mL) was added, and the mixture was left for another 30 minutes. A change in colour from yellow to purple indicated the presence of live organism.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 show the GC-MS analysis results of *C. longa* n-hexane extract, methanol extract, and volatile oil. The n-hexane extract contained different compounds such as cyclohexane, α-terpinene, α-lonone, turmerol, ar-turmerone and sinococuline (Table 1). The methanol extract also showed several compounds such as isobutanol, β-lonone, β-pinene,

turmerone, curcumen, β-turmerone, curcumene, α-curcumen, and selenophene compounds (Table 2). The volatile oil contained compounds like methylcyclohexane, turmerone, curcumen, curcumene, carvacrol, α-selinene, and atlantone (Table 3). Table 4 highlights the major compounds found in all the samples. Some of the major compounds include curcumene, 2-methylhept-2-en-4-one, atlantone, linoleic acid, curcumen, alpha-cadinol, allopregnanolone, palmitic acid, methylbenzene, and β-terpinene.

Seventy-two (72) compounds were present in n-hexane extract, 109 in the methanol extract, and 84 in the volatile oil, representing 98.05%, 99.51% and 99.76% of their total composition, respectively. Ten same compounds were found n-hexane extract, methanol extract, and volatile oil; turmerone (13.39, 12.4, and 7.29%), ar-turmerone (2.24, 1.46, and 19.01%), +-alpha atlantone (2.34, 5.59, and 0.30%), 9,12octadecadienoic acid (2.47, 1.67, and 0.35%), curlone (1.38, 1.84 and 1.63%) while others were present in *C. longa* n-hexane and volatile oil and they included 2-epi-alpha-funebrene (4.04 and 2.30%), toluene (1.48 and 0.69%), benzene 1(I,5 dimethyl-4-hexenyl)-4-methyl (2.49 and 10.010%), respectively. N-hexane and methanol extracts contained 14-beta-H-pregna (9.05 and 8.72%) and n-hexadecanoic acid (0.81 and 1.20%).

Table 1: Chemical Composition of *Curcuma Longa* N-Hexane Extract

S/N	Retention Time	Chemical Component	Area %
1	2.756	Cyclohexane, methyl-	0.14
2	2.839	Methylenecyclohexane-4,4-D2	0.30
3	3.516	Toluene	1.48
4	3.925	Cyclohexane, 1,2-dimethyl-, trans-	1.86
5	4.395	2- Chloropropionic acid, pentadecyl ester	0.75
6	4.571	n-Nonadecanol-1	0.15
7	4.961	Benzene, 1,3-dimethyl-	1.28
8	5.175	o-Xylene	1.44
9	5.365	p-Xylene	1.48
10	5.678	Nonane	0.63
11	5.888	1H-Indene, octahydro-, cis-	1.06
12	6.370	1,3,5-Trimethylbenzene	1.15
13	6.647	Benzene, 1,2,4-trimethyl-	1.02
14	6.841	Benzene, 1-ethyl-3-methyl-	1.24
15	7.226	Decane	1.36
16	7.385	Bicyclo [3.1.0] hex-2-ene, 2-methyl- 5-(1-methylethyl)-	0.63
17	7.625	Eucalyptol	0.87
18	7.721	Phenol, 2,6-dimethoxy-	0.54
19	7.808	Benzene, 1,2-diethyl-	1.00
20	7.919	p-Mentha-8-dehydeo-1,3-diene	1.31
21	8.333	4-Isopropylidene-1-cyclohexene	1.25
22	8.595	Undecane	0.72
23	8.703	trans-Decalin, 2-methyl-	0.80
24	8.915	Cyclodecene, 1-methyl-	0.87
25	9.215	Benzene, 2-ethyl-1,4-dimethyl-	0.31
26	9.322	Benzene, 1,2,3,4-tetramethyl-	0.21
27	9.434	Benzene, 1-ethyl-2,3-dimethyl-	0.36
28	9.586	2-(4'-methylphenyl)-propanal	0.10
29	9.696	Naphthalene	0.23
30	9.849	Dodecane	1.95
31	10.701	Bicyclo [2.2.1] heptan-2-ol, 3,3-dimethyl-	0.06
32	10.830	2-Piperidinone, N-[4-bromo-n-butyl]-	0.19
33	11.232	Tridecane	0.28
34	11.552	Naphthalene, 1-methyl-	0.16
35	11.807	(1R*,5R*)-1,5-Dimethylbicyclo[3.3.0]oct-3-en-2-one	0.10
36	12.037	1H-Inden-1-one, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	0.20
37	12.976	Caryophyllene	1.75
38	13.810	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	2.49
39	14.165	trans-.alpha.-Bergamotene	1.19
40	15.180	2-epi-.alpha.-Funebrene	4.40
41	15.437	Di-epi-.alpha.-cedrene-	3.67
42	17.300	Turmerone	13.39
43	17.769	aR-Turmerone	2.24
44	17.993	Curlone	1.38
45	18.260	(6R,7R)-Bisabolone	1.18
46	18.377	(+)-.alpha.-Atlantone	3.14
47	18.544	Bornyl tiglate	0.44
48	18.683	4-[dimethyl(phenyl)silyl]-2-oxanon	1.57
49	18.755	Spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester	1.29
50	18.896	Cyclohexane-1-methanol,3,3-dimethyl-2-(3-methyl-1,3-butadienyl)- 6-(2-Hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one 6-(3-Hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one	1.67

S/N	Retention Time	Chemical Component	Area %
51	19.092	n-Hexadecanoic acid	2.65
52	19.569	2-Pentene, 4,4-dimethyl-, (E)-	3.02
53	19.974	.beta-turmerone	0.81
54	20.112	cis-10-Heptadecenoicacid	1.03
55	20.202	4-Fluoropentacyclo [4.3.0.0(2,5).0(3,8).0(4,7)] nonane	1.56
56	20.438	3-(Dimethylphenylsilyl)-3-methylcyclopentanone	0.35
57	20.508	Methanone, dicyclohexyl-	0.59
58	20.662	9,12-Octadecadienoic acid (Z,Z)-	1.13
59	20.864	Linoleyl methyl ketone	0.29
60	21.147	14-.beta.-H-pregna	2.47
61	21.469	1-Docosene	1.58
62	21.704	9-Tricosene, (Z)-M	9.05
63	21.953	4-[(1Z)-(N-Hydroxyethanimidoyl)-2-methylpyridazin-3(2H)-one	0.21
64	22.163	1-Eicosene	0.54
65	22.250	1-Hexacosene	0.28
66	22.372	Hexacosane	0.30
67	23.035	Cyclohexane, 1,3,5-triphenyl-	1.43
68	23.163	Methoxymethyl(triethyl)stannane	0.64
69	23.460	1H-Indole, 5-methyl-2-phenyl-	0.76
70	24.205	B-Homomorphinan-7-one, 5-chloro-5, 6,8,14-tetradehydro-3-hydroxy-2,4,6-	1.03
71	24.335	trimethoxy-17-methyl-	0.84
72	26.329		0.41

Table 2: Chemical composition of *Curcuma longa* methanol extract

S/N	Retention Time	Chemical Component	Area %
1	3.224	1-propanol,2-methyl	0.80
2	3.455	Acetic acid	2.24
3	3.716	1-Butanol, 3-methyl-	6.34
4	3.874	Ethyl Acetate	0.07
5	4.173	(R, R)-Butane-2,3-diol	0.63
6	4.638	Dimethyl Sulfoxide	0.34
7	4.758	Methane, sulfinylbis-	0.20
8	4.820	1-Butanol, 3-methyl, acetate	0.20
9	5.077	Butane, 1-(1methylethoxy)-	0.14
10	5.254	3,4-O-Isopropylidene-d-galactose	0.08
11	5.437	1,3-Dihydroxyacetone dimer	0.38
12	5.655	Methane sulfinic acid methyl ester	0.07
13	5.736	2-Hydroxy-2-cyclopenten-1-one	0.15
14	6.003	Pentane, 1-(1-ethoxyethoxy)-	0.10
15	6.200	1,2-bis(2,4-dimethylphenyl) diazane	0.10
16	6.327	Acetaldehyde ethyl isoamyl acetal	0.11
17	6.484	1-Oxa-2-silacyclopentane,2,2 dimethyl-	0.10
18	6.604	Phenol	0.63
19	6.817	1,3-Cyclohexadiene, 2-methyl-5-(1- methylethyl)-	0.48
20	6.966	Butyric acid-2-D1	0.08
21	7.083	Glycerin	0.22
22	7.167	3-Cyclohexen-1-d-1-ol	0.23
23	7.578	Diglycerol	0.30
24	7.686	1,2,3-Propanetriol	0.26
25	7.844	Erythritol	0.32
26	7.961	Disulfide, methyl 1-(propylthio)ethyl	0.18
27	8.209	Phenol, 2-methoxy-	0.95
28	8.365	2,5-Disila-7-octene	0.16
29	8.537	2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-	0.14
30	9.072	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	0.08
31	9.384	[Ethyl 5-methylhexanoate	0.06
32	9.525	2-Phenyl-2-tert-butoxycyclopropane carboxylic acid, ethyl ester Benzo[b]thiophene, 2,3-dihydro-3-methyl	0.28
33	9.627	Octanoic acid, ethyl ester	0.08
34	9.723	3-isopropyl-6-methyl-7-oxabicyclo[4.1.0]hept-4-ene	0.27
35	9.893	4-Vinylphenol	0.05
36	10.150	Bicyclo[4.1.0]hepta-1,3,5-triene,7,7-difluoro-	1.34
37	10.373	4-(Methoxymethyl)m benzaldehyde	0.06
38	10.813	(5E)-2,6-dimethyl-4-octa-2,5,7-trienone	0.20
39	10.894	Bicyclo[2.2.1]hept-2-ene, 2,3-dimethyl-	0.11

S/N	Retention Time	Chemical Component	Area %
40	11.186	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	0.33
41	11.290	2-Cyclohexen-1-one,5,5-dimethyl-3-(2-methyl-1-propenyl)-	0.05
42	11.336	2-methoxy-4-vinyl-phenol	0.06
43	11.489	Vanillin	2.05
44	11.964	Eugenol	0.14
45	12.037	Ethyl 5-methylhexanoate	0.08
46	12.132	Butanoic acid, ethyl ester	0.13
47	12.237	Benzaldehyde, 4-hydroxy-	0.07
48	12.342	Decanoic acid, ethyl ester	0.42
49	12.460	Benzaldehyde, 3-hydroxy-4-methoxy-	0.53
50	12.636	Caryophyllene	0.97
51	12.910	Lauric acid, 2-methylbutyl ester	0.15
52	13.114	3-Methylplumbagin	0.05
53	13.207	Dimethyl phthalate	0.20
54	13.296	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2 yl)spiro[4.5]dec-7-ene	0.05
55	13.398	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	0.40
		1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	
56	13.642	.beta.-Sesquiphellandrene	0.93
57	13.790	1,3-Difluoroazulene	0.65
		2-Methyl-6-(p-tolyl)hept-2-en-4-ol	
58	14.155	Germacrene-b	0.74
59	14.637	Benzene, 1-(3 cyclopentylpropyl)-,4 dimethyl-	0.46
60	14.853	.beta. turmerone	1.05
61	14.968	aR-Turmerone	0.28
62	15.151	Curlone	2.67
63	15.321	Turmerone	0.68
64	15.437	.alpha.-turmerone	1.46
65	15.531	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl(1R)-	1.84
66	15.975	1-(4 Hydroxybenzylidene) acetone	12.46
67	16.168	3-Methyl-6-(6-methylhept-5-en-2-yl)cyclohex-2-enone (E)-Atlantone	5.59
68	16.552	2-Propenoic acid, 1,7,7-trimethyl bicycle [2.2.1] hept-2-yl ester,	0.18
69	16.692	3-Buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-	1.23
70	16.768	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	0.99
71	17.063	1,2,5-Oxadiazol-3-amine,4-[5-(1,methyl-5-pyrazolyl)-1,2,4-oxadiazol-3-	2.25
72	17.306	yl]-	0.89
		8-Acetoxy-3(dimethylphenylsilanyl)-3-methyl-1,2,3,4-tetrahydrobenzo	
73	17.538	[a]anthracene-7,12-dione	2.44
74	17.736	N-[(Benzoyl)(p-toluidino)-methyl] acetamide	0.77
		2-Norpinanone, 6,6-dimethyl-	
75	17.810	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)-cyclohex-2-enone	0.30
		Pentadecanoic acid, 14-methyl-, methyl ester	
76	18.063	6-(3-Hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one	0.39
		11H-Dibenzo[b,e][1,4]dioxepin-7-carboxylic acid, 2-chloro-3,8-dihydroxy-	
77	18.200	1,4,6,9 tetramethyl-11-oxo-, methyl ester	0.68
78	18.288	n-Hexadecanoic acid	0.12
79	18.412	(+)-alpha.-Atlantone	0.56
		Hexadecanoic acid, ethyl ester	
80	18.502	3-Nonen-5-one	1.17
81	18.651	1H-Cyclopentacyclododecene-1,7(4H) -dione, dodecahydro-	0.37
82	18.723	[1,2,4]Triazolo[1,5-a]pyrimidin-5-ol, 7-methyl-6-nitro-	0.33
		1-Heneicosanol	
		Cyclopropanol, 1-(3,7-dimethyl-1-octenyl)-	
83	18.915	9-Octadecenoic acid, methyl ester	1.22
84	19.041	Benzoic acid, 4-nitro-, 2-(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl)-1-	0.12
85	19.158	phenylhydrazide	0.40
86	19.296	Methyl stearate	1.11
87	19.546	9,12-Octadecadienoic acid (Z, Z)-	0.30
		Ethyl (9Z,12Z)-9,12-octadecadienoate	
88	19.705	14-beta.-H-pregna	0.25
89	19.846	Octadecanoic acid, ethyl ester	0.47
90	19.980	Z-14-Nonacosene	0.19
91	20.183	4-[(1Z)-(N-Hydroxyethanimidoyl)-2-methylpyridazin-3(2H)-one	1.25
92	20.280	Cyclotriacontane	0.17
		1-Hexacosene	
93	20.398	Baccharane	0.23
94	20.555	Z-14-Nonacosene	1.62

S/N	Retention Time	Chemical Component	Area %
95	20.742	cis-6-Ethyl-cis-4a,trans-8a-perhydro-trans-1-(2-methoxycarbonylethyl)-	0.57
96	20.941	trans-2,trans-6,8a-trimethylnaph thalene-2-carboxylic acid	8.92
97	20.998	Ergost-5-en-3.beta.-ol	0.15
98	21.958	Plucheasesquiterpenyl ester [4,8-Dimethyl dodeca-7Z-enyl-9'.alpha.,10'-	0.17
99	22.353	,alpha.-dihydroxyundecan-1'-oat Stigmasterol	0.19
100	23.134	3-Methylseleno-2-(1,3-dioxolan-2 yl) benzo[b]thiophene	3.81
101	23.487	(3S,8S,9S,10R,13R,14S,17R)-17-[(1R,3S)-3-ethyl-1,5-dimethyl-hexyl]-	0.95
102	24.085	0,13dimethyl2,3,4,7,8,9,11,12,14	1.99
103	24.280	,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	0.54
104	25.184		1.14
105	25.949		2.31
106	26.280		1.25
107	26.617		2.28
108	27.535		0.44
109	27.862		2.50

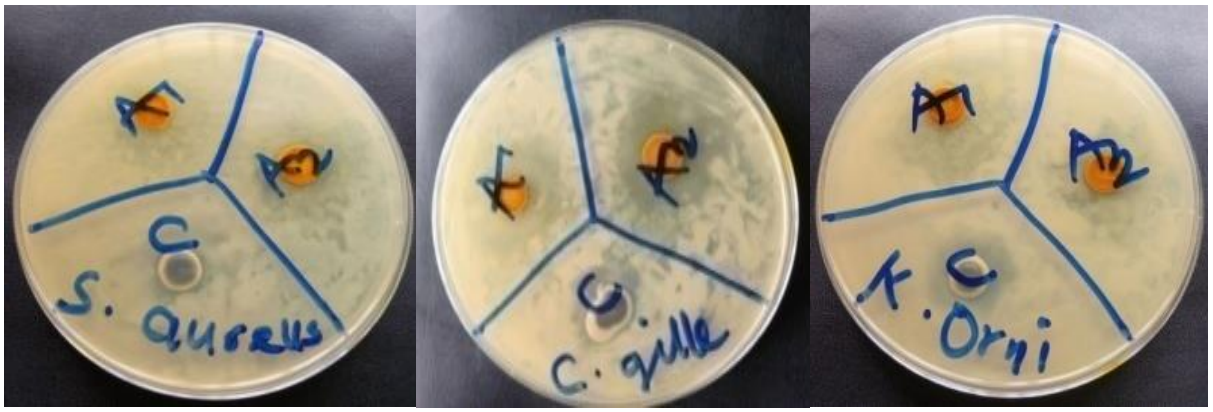
Table 3: Chemical Composition of *Curcuma Longa* Volatile Oil by GC-MS Analysis

S/N	Retention Time	Chemical Component	Area %
1	2.677	Cyclohexane, methyl-	0.03
2	3.212	Toluene	0.69
3	3.603	1,3,5-Cycloheptatriene	0.13
4	4.532	1-Docosene	0.01
5	4.645	Benzene,1,3-dimethyl-	0.02
6	5.087	Nonane	0.02
7	5.550	Bicyclo [3.1.0]hex-2-ene,2-methyl-5-(1 methylethyl)-	0.03
8	5.675	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	1.37
9	6.634	beta.-Myrcene	0.22
10	6.852	alpha.-phellandrene	2.65
11	7.586	Bicyclo[3.1.0] hex-2-ene,2-methyl-5-(1-methylethyl)-	3.51
12	7.800	o-Cymene	1.37
13	7.973	.gamma.-Terpinene	2.56
14	8.288	Cyclohexene,1-methyl-4-(1methylethylidene)-	1.11
15	8.459	(+)-4-Carene	4.78
16	9.583	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, cis-	0.38
17	9.688	Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	0.67
18	10.007	(3E)-2,7-dimethyl-2-octa-3,6-dieno	3.24
19	11.205	1-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene-	0.44
20	11.366	Cyclohexene, 4-methyl-1-(1-methylethenyl)-	0.25
21	11.468	1H-Inden-1-one, 3a,4,5,6,7,7a-hexahydro-3a-methyl-, cis-	0.93
22	11.950	Carvacrol	0.35
23	12.069	Tricyclo[3.3.1.13,7]decane, 2-methyl	0.33
24	12.372	p-Cymen-7-ol	0.44
25	12.440	Benzenemethanol, 4-(1-methylethyl)	0.19
26	13.810	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	10.01
27	14.470	1H-3a,7-Methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-Tetramethyl, 3R(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	9.41
28	15.685	2-epi-.alpha.-Funebrene	2.30
29	15.795	3-Cyclohexene-1-ethanol,.alpha.-ethenyl-.alpha.,3-dimethyl-6- (methylethylidene)-	0.54
30	15.862	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicycle[3.1.0] hex-2-ene aR-Turmerone	0.45
31	16.864	Turmerone	19.73
32	17.622	Curlone	7.29
33	18.397	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl) cyclohexa-1,4-diene	1.63
34	18.586	(Z)-.alpha.-Atlantone	1.84
35	18.784	Adamantane	2.54
36	18.936	(8-amino-1-naphthyl)-phenyl-amine	0.77
37	19.039		0.87

S/N	Retention Time	Chemical Component	Area %
		(3aR,8aR,9aR)-8a-methyl-3,5dimethylene	
38	19.112	3a,4,4a,6,7,8,9,9aoctahydrobenzo[f]benzofuran-2-one	1.33
		Tricyclo[5.2.1.0(2,6)]decan-3-one	
39	19.246	3-Nonen-5-one	0.82
40	19.357	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl) cyclohex-2-enone	0.81
41	19.417	2-Butanone,4-(4-hydroxyphenyl)-	0.53
		Dicyclohexyl sulfone	
42	19.477	3-Pentyl-4,5,6,7-tetrahydro-1H-indazole	0.48
43	19.568	Chloroacetic acid, undecyl ester	1.90
44	19.794	2-Bromopropionic acid, cyclohexyl ester	0.71
45	20.014	3-Methyl-2-butenoic acid, 2-methyloct-5-yn-4-yl ester	2.07
46	20.210	4,4'-Difluorobenzhydrol	0.36
47	20.308	3-Ethyl-5-methyl-1-heptyn-3-	0.444
48	20.390	2Nonadecanone	0.26
49	20.481	Aldrin-transdiol	0.33
50	20.555	9,12-Octadecadienoic acid (Z, Z)-	0.48
51	20.680	Coronarin A	0.35
52	20.807	3-Penten-2-one,1-[5-(3-furanyl)tetrahydro-2-methyl-2-furanyl]-4-methyl-	0.23
53	20.912	(+)-.alpha.-Atlantone	0.21
54	21.060	(E)-.gamma.-Atlantone	0.30
		(Z)-.gamma.-Atlantone	
55	21.165	(Z)-.alpha.-Atlantone	0.03
56	21.235	Trichloro(cyclohexylmethyl)silane	1.I6
57	21.378	Borinic acid, diethyl-, 1-cyclododecen-1-yl ester	0.04
58	21.553	1-Docosanol, methyl ether	2.54
59	21.622	Pulicanol	0.05
60	21.727	2-Butenoic acid, 2-methyl-, 2(acetyloxy)1,1a,2,3,4,6,7,10,11,11a-decahydro-	0.07
61	21.905	7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7a-epoxy-	0.18
62	22.057	5Hcyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester,	0.04
63	22.118	[1aR[1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*	0.05
		,8E,10R*,11R*(E),11aS*]]	
		Cyclohexanol, 2-methyl-3-(1-methylethenyl)	
		(1.alpha.,2.alpha.,3.alpha.)-	
		Cyclohexaneoctanoic acid, 2-butyl-, methyl ester	
		9-Octadecenamide,(Z)-	
64	22.201	(3-Methylbenzoyl) carbamic acid, (5-methyl- 2-nitroimidazol-1-yl) ethyl ester	0.03
65	22.448	2H-Isoindole-2-carboxylic acid,1.3-dihydro-1,3-dioxo-,ethyl ester	0.11
66	22.531	(E)-Ethyl-3-{4-methyl-2-(methylcarbamoyl) phenyl}acrylate	0.12
67	22.880	.alpha.-Longipinene	0.08
		1-Butyl-3-(1-methylethenyl)-1-methoxy-2methylcyclo propane	
68	23.029	3-Oxabicyclo [5.1.0] octane,5,5-dimethyl-4-(3-methyl-3-	0.10
		butenylidene)-2-methylene-, cis-(.+-.)-	
69	23.174	3-Bromo-5,5-dimethyl-cyclohex-2-enol	0.10
70	23.423	Silane, trichlorocyclohexyl-	0.12
71	23.549	2-(Phenylethynyl)benzonitrile	0.15
		4-(9-Acridinyl)-N, N-dimethylbenzen amine	
72	23.666	(3-Methylbenzoyl) carbamic acid,	0.17
		2-(5-methyl- 2 nitroimidazol-1-yl) ethyl ester	
73	23.795	(1Z, 8R*, 9R*)-8-Bromo-Chamigra-1,11(12)-dien-9-ol	0.29
74	23.990	Cyclopropa[1,2:1,3]dicyclopenten-3(3ah)-one,1,2,3b,6-tetrahydro-3a,6,6-	0.35
75	24.241	trimethyl-	0.30
76	24.368	Cyclohexanol,2-methyl-3-(1-methyl ethenyl).	0.09
77	24.650	(1.alpha.,2.alpha.,3.alpha.)-	0.10
		(1R,2S,5R)-(-)-Menthyl(S)-p-tolue Nesulfinate	
78	24.770	Cyclopropanol,1-(3,7-dimethyl-1-octenyl)-	0.16
79	24.956	(3-Methylbenzoyl)carbamic acid, 2-(5-methyl-2-nitroimidazol-1-yl)ethyl ester	0.11
80	25.026	2-Oxabicyclo[2.2.1]heptane-1-carboxylic acid, 4,7,7-trimethyl-3-oxo-,3-(5-	0.06
		ethoxy-3-methyl-5-oxo-1,3-pentadienyl)-2,4,4trimethyl-2-cyclohexen-1-	
81	25.187	yl,ester, [1S[1.alpha.[R*(1E,3E)],4.beta]]-	0.09
82	25.291		0.08
83	25.595		0.45
84	25.841		0.13

Table 4: The Predominant Compounds in *Curcuma Longa* Volatile Oil, Methanol, and N-Hexane Extracts

S/N	Name of Compounds	N-Hexane Extract	Methanol Extract	Volatile Oil
1	Turmerone	13.39	12.46	7.29
2	Ar-Turmerone	2.24	1.46	19.01
3	+-alpha altantone	3.14	5.59	0.30
4	9,12-octadecadienoic acid (Z,Z)	2.47	1.67	0.35
5	Curlone	1.38	1.84	1.63
6	2-epi-alpha-funebrene	4.04	------	2.30
7	14-beta-H-pregna	9.05	8.72	-----
8	n-hexadecanoic acid	0.81	1.20	-----
9	Toluene	1.48	------	0.69
10	Benzene-1(1,5dimethyl -4-hexenyl)-4-methyl	2.49	------	10.01



Staphylococcus aureus *Citrobacter gillenii* *Klebsiella ornithinolytica*

Figure 3: Antibacterial activity of *Curcuma longa* against *Staphylococcus aureus*, *Citrobacter gillenii*, and *Klebsiella ornithinolytica*

The AST results of *C. longa* volatile oil and extracts against selected bacterial species (Figure 3) revealed varying levels of activity. The n-hexane extract showed the strongest effect against *C. gillenii* with a zone of inhibition of 28±1.03 mm, followed by *Klebsiella ornithinolytica* (26±1.63 mm) and *Staphylococcus aureus* (25±0.33 mm). However, no inhibition zones were observed against *Streptococcus* sp, *E. coli*, and *E. agglomerans* (Table 5). For the methanol extract, the highest inhibition was recorded against *K. ornithinolytica* (22±2.01 mm), followed by *S. aureus* (20.5±0.65 mm), while the lowest activity was observed against *C. gillenii* (12.5±1.66 mm). Also, no inhibitory effect was detected against *Streptococcus* sp., *E. coli*, and *E. agglomerans* (Table 5). Similarly, the volatile oil exhibited its greatest activity against *C. gillenii* (23±0.00 mm), followed by *K. ornithinolytica* (20.5±0.11 mm) and *S. aureus* (17±0.50 mm). No zones of inhibition were observed against *Streptococcus* sp., *E. coli*, and *E. agglomerans* (Table 5).

Table 5: Antibacterial Sensitivity Test of Turmeric Volatile Oil and Extracts against Some Selected Bacterial Species

S/N	Organism	Turmeric n-Hexane Extract (mm)	Turmeric Methanol Extract (mm)	Turmeric Volatile Oil (mm)
1	*Streptococcus* sp.	0	0	0
2	*Staphylococcus aureus*	25±0.33	20.5±0.65	17±0.50
3	*Escherichia coli*	0	0	0
4	*Enterobacter agglomerans*	0	0	0
5	*Klebsiella ornithinolytica*	26±1.63	22±2.01	20.5±0.11
6	*Citrobacter gillenii*	28±1.03	12.5±1.66	23±0.00

Key: Zone diameter ≥ 20 mm = Sensitive; 15-19 mm = Intermediate, ≤ 14 mm = Resistance

Minimum Inhibitory Concentrations of *Curcuma Longa* Extracts and Volatile Oil against Bacterial Species

Tables 6, 7, and 8 show MIC of *C. longa* n-hexane, methanol extract, and volatile oil against *K. ornithinolytica*, *C. gillenii* and *S. aureus*. For *K. ornithinolytica*, the MIC values were 25 mg/mL for both the n-hexane and methanol extracts while the volatile oil showed a much lower MIC of 6.25 mg/mL. Also, *C. gillenii* n-hexane extract had a MIC value of 50 mg/mL whereas the volatile oil showed better activity (12.5 mg/mL). For *S. aureus*, both the n-hexane extract and the volatile oil recorded the same MIC value (25 mg/mL).

Table 6: Minimum Inhibitory Concentration *Curcuma Longa* against *Klebsiella Ornithinolytica*

Sample	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	6.25 (mg/mL)
N-hexane extract	+	+	+	-	-
Methanol extract	+	+	+	-	-
Volatile oil	+	+	+	+	+

Key: + = Sensitive, - = Resistance

Table 7: Minimum Inhibitory Concentration *Curcuma Longa* against *Citrobacter Gillenii*

Sample	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	6.25 (mg/mL)
N-hexane extract	+	+	-	-	-
Volatile oil	+	+	+	+	-

Key: + = Sensitive, - = Resistance

Table 8: Minimum Inhibitory Concentration *Curcuma Longa* against *Staphylococcus Aureus*

Sample	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	6.25 (mg/mL)
N-hexane extract	+	+	+	-	-
Methanol extract	+	+	+	-	-

Key: + = Sensitive, - = Resistance

Discussion

Curcuma longa rhizome is one of the oldest spices used as food ingredient and have medicinal benefits. it is widely recognized for its biological applications. Previous studies have also reported *C. longa* biological activities as antibacterial, antioxidant, anti-inflammatory, and anticancer effects (Adudu *et al*., 2018; Khatun *et al*., 2021; Tundis *et al*., 2023). These properties make *C. longa* an important plant for both traditional medicine and modern drug development. Also, a report on the antibacterial drugs and natural antibacterial compounds found in food plants supported *C. longa* as an antibacterial agent (Berteina-Raboin, 2025).

In this study, the GC-MS analysis of *C. longa* rhizomes revealed several bioactive compounds. The n-hexane extract was mainly dominated by turmerone, 14-beta-H-pregna, 2-epi-alpha-funebrene, and (+)-alpha-altatone (3.14%). The methanol extract showed turmerone as a predominant component, followed by 14-beta-H-pregna, 1-butanol-3-methyl-,alpha-turmerone, (+)-alpha-altatone). In the volatile oil, ar-turmerone was the most abundant compound, followed by 1H-3a,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-[3R-(3,alpha.,3a.beta.,7.beta.,8a.alpha.), benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl. Moderate amounts of turmerone and (+)-4-carene were also detected. Both turmerone and ar-turmerone are known to contribute to the characteristic aroma of *C. longa* (Li *et al.,* 2022). In addition, turmerone has been reported to have neuroprotective effects, including potential benefits against neurodegenerative diseases such as Alzheimer's disease (Goozee *et al.,* 2016). The biological activity of *C. longa* has been largely linked to its phytochemical composition (Saavedra *et al.,* 2026).

These compounds are believed to interact with bacterial cells by disrupting its membrane integrity and interfering with protein synthesis. Studies have attributed variations in antibacterial activities to environmental factors, plant characteristics, and differences in phytochemical content (Arulmozhi *et al.,* 2018; Asfaw *et al.,* 2023). Differences in chemical compositions and concentrations may be influenced by factors such as extraction method, plant age, chemotype, post-harvest and geographical location (Dosoky *et al*., 2019; Nourbakhsh *et al*., 2022). The antibacterial properties showed that *C. longa* volatile oil and extracts exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria. The strongest activity was observed against *S. aureus*, *K. ornithinolytica* and *C. gillenii*. In particular, *S. aureus* is a clinically important pathogen responsible for infections such as skin, urinary tract infections. These findings are consistent with earlier reports by Odo *et al*. (2023).

The MIC results showed that both n-hexane and methanol extracts inhibited *K. ornithinolytica* at 25 mg/mL while the volatile oil showed a stronger effect at 6.25 mg/mL, indicating higher potency. This stronger activity may be linked to higher levels of sesquiterpenoids such as turmerone and ar-turmerone, which are known to damage bacterial cell walls (Zhou *et al.,* 2022). MIC results indicated that volatile oil showed better activity against *C. gillenii*, this corroborate the findings of Oklo *et al*. (2023) who observed similar effect against some bacteria. Both n-hexane and methanol extracts showed antibacterial activity activities against *S. aureus*. This agrees with the result of Gupta *et al.* (2015) and Feghali *et al.* (2018) who reported that various fractions of *C. longa* rhizome extract showed antibacterial activity against *S. aureus*. The bacterium showed zone of inhibition that ranged between 9 mm and 21 mm. In this study, volatile oil demonstrated the strongest antibacterial activity among all the tested samples (20.5 to 26 mm), particularly against *K. ornithinolytica*.

CONCLUSION

This study showed that *C. longa* volatile oil and extracts have clear antibacterial effects. The strength of these effects depends on the post-harvest, chemotype, mode of extraction. Volatile oils are effective against Gram-positive bacteria while the extracts showed stronger effects against Gram-negative bacteria. These results agree with earlier studies, which linked the activity of *C. longa* to compounds called turmerones that help stop the growth of bacterial infection. The findings suggest that *C. longa* rhizome could be a useful as natural source for fighting both Gram-positive and Gram-negative bacteria (*S. aureus*, *K. ornithinolytica*, and *C. gillenii*).

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