Journal of Advances in Science, Engineering and Technology

Chemical metabolite profiling and *in vitro* antibacterial activities of selected medicinal plants of Kakamega tropical forest, Kenya

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ABSTRACT

Plants play a major role in the management of diseases, either exclusively or in combination with conventional medicines. This study investigates crude extracts of *Conyza floribunda*, *Zanthoxyllum gilletii*, *Olea capensis*, *Warburgia ugandensis*, and *Lantana trifolia* for antibacterial phytochemicals. Extraction of the phytochemicals was done by cold percolation in hexane, followed by 50% methanol in dichloromethane. The chemical composition of the non-polar hexane extracts was established using gas chromatography –mass spectrometry (GC-MS). The major chemical components were found to be; 1H-cycloprop[e]azulen-7-ol (8.41%, *C. floribunda*), 2,4-decadienamide (38.89%, *Z. gilletii*) isopimarol (29.08%, *O. capensis*), 2,2,5,7,8-pentamethyl-6-hydroxychroman (8.54%, *W. ugandensis* leaf), (1R,4aS,6R,8aS)-8a,9,9-trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol (10.17%, *W. ugandensis* bark) and tetrapentacontane (13.63%, *L. trifolia*). The *in vitro* antibacterial activities of the plant extracts were evaluated by agar well diffusion. Although each extract showed selective inhibition towards tested microbes, *W. ugandensis* stem-bark hexane extract showed the highest activities with zones of inhibition (ZI) of 16.0, 13.0, 11.0, 11.0, 10.0, and 9.0 mm against *S. aureus*, *E. coli*, methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa*, *P. aeruginosa* (7533), and *S. aureus* (29213), respectively. This activity could be attributed to the major constituent (1R,4aS,6R,8aS)-8a,9,9-trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol in addition to the synergistic effects of sesquiterpenes, which accounted for 80% of the total phytochemicals in the extract. This supports the ethno-medicinal use of *W. ugandensis* in the management of bacterial infections, further recommending its full utilization for the development of antimicrobial drugs.

ARTICLE INFO

Keywords:
Warburgia ugandensis,
Antibacterial activity,
GC-MS,
Kakamega.

Article History:
Received 9 October 2024
Received in revised form 2 May 2025
Accepted 21 May 2025
Available online 9 October 2025

1. Introduction

Bacterial infections pose a major global health concern, with the effects being higher in immunocompromised hosts. The infections are caused by different classes of either gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, or gram-positive bacteria, for example, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Chiang et al., 2017). In 2019, an estimated 13.7 million deaths related to bacterial infections were reported in the world. Among the pathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were leading causative agents and were responsible for 54·9% of the reported deaths (Ikuta et al., 2022). The management of microbial infections using

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Editor: Francis Orata, Masinde Muliro University of Science and Technology, Kenya.

Citation: Kamau R., Ochieno D., Baraza D., Nyongesa P., Sande E., & Shilaho C. (2025). Chemical metabolite profiling and in vitro antibacterial activities of selected medicinal plants of Kakamega tropical forest, Kenya. Journal of Advances in Science, Engineering and Technology 2(1), 1 – 8.

conventional medicines is complicated by their unavailability, microbial resistance, and their diverse side effects (Cloeckaert & Kuchler, 2020; Saga et al., 2009).

Traditional medicine has a rich history and plays a significant role in global healthcare, with reports indicating that up to 80% of the African population relies on it (Ikhoyameh et al., 2024). It includes indigenous practices and natural therapies used for disease diagnosis, treatment, and management. Countries like China and India have successfully integrated traditional medicine into their healthcare systems, enhancing its effectiveness alongside conventional treatments. Interestingly, around 40% of pharmaceuticals on the market originate from natural sources (Ikhoyameh et al., 2024).

For instance, the discovery of *penicillin G* from the fungus *Penicillium notatum* in the 1940s paved the way for better health for millions of people around the world (Lobanovska & Pilla, 2017). Later, the biosynthetic penicillin was found to be unstable in acidic media, which prompted the development of semi-synthetic methicillin, oxacillin, and ampicillin, which were more effective compared to the original *penicillin G* (Lobanovska & Pilla, 2017). The effectiveness of such naturally derived medicines highlights the need for ongoing research to find more accessible and dependable alternatives, aiming to address the existing challenges in bacterial infection treatment.

Different medicinal plant species are used in Kakamega County in the management of microbial infections, either solely or in combination with conventional treatments (Odongo et al., 2018a). Medicinal plants in the genus *Conyza* are used to treat throat infections, pimples, and tonsils. On the other hand, *Warbugia* species manage fever and stomach ache, while the genus *Lantana* is used in managing chest and eye infections. Additionally, *Olea* finds use in the treatment of diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, and asthma, whereas genus *Zanthoxyllum* is traditionally used to manage stomach infections, toothache, skin infections, and fever (Odongo et al., 2018b).

Although some phytochemical studies have been done on these medicinal plants, very few reports from the species from the Kakamega forest are available. This study, therefore, aimed to investigate and herein reports the GC-MS chemical profiles of hexane crude extracts from *Conyza floribunda*, *Zanthoxyllum gilletii*, *Olea capensis*, Warburgia ugandensis, and *Lantana trifolia*. Furthermore, the antibacterial activities of crude extracts from the medicinal plants were established and are herein reported.

2. Materials and Methods

2.1. Plant Materials

The plants selected for this study included: *Conyza floribunda, Zanthoxyllum gilletii, Olea capensis, Warburgia ugandensis*, and *Lantana trifolia*

2.2. Chemicals

The analytical grade hexane, dichloromethane, and methanol solvents were obtained from Scielab Chemiclas, Nairobi, Kenya.

2.3. Test microorganisms

Test microbes used in this study included two Gram-negative: *Escherichia coli*, *Pseudomonas aeruginosa*, and two gram positive: *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria, which were used as clinical isolates (the wild type). Standard sensitive isolates *P. aeruginosa* (7553) and *S. aureus* (29213) were also used for the study. These bacteria were a kind donation from the Department of Medical Microbiology, University of Nairobi, and all biosafety protocols were observed during the exchange of the microorganisms.

2.4. Collection, identification, and preparation of plant materials

The fresh parts of selected plant materials: *C. floribunda* (aerial parts), *Z. gilletii* (stem-bark), *O. capensis* (stem-bark), *W. ugandensis* (stem-bark and leaf), and *L. trifolia* (aerial part) were taxonomically identified and collected from the Kakamega forest catchment area. The plant voucher specimens were deposited in the laboratory of Botany at Masinde Muliro University of Science and Technology. The plant materials were separately air-dried, ground to fine powder using a RRH-1000A(K) multifunctional grinder, and then packaged in air-tight glass jars awaiting further investigations.

2.5. Extraction of phytochemicals from the selected medicinal plants

Extraction was done sequentially through cold percolation in non-polar and then mid-polar solvents as earlier described by Kamau et al. (2023). The ground plant parts were soaked in cold hexane for 24 hours, three times. Filtration was done, and the filtrates obtained were concentrated using a rotary evaporator (BUCHI Labortechnik AG, R-300 EL) to yield hexane extracts. The residues were then soaked in MeOH: CH_2Cl_2 (1:1) solvent mixture for 24 hours and filtered. This process was repeated twice more, and the resulting filtrates for each plant were combined and concentrated to obtain the MeOH: CH_2Cl_2 (1:1) extract.

2.6. Semi-purification of Z. gillettii stem-bark hexane extract

When the concentrated *Z. gillettii* stem-bark hexane filtrate was allowed to stand, a white precipitate was observed. Repeated recrystallization of the residue in cold hexane yielded 100 mg of white powder (R_f = 0.21, under 60% CH_2Cl_2/ $_n$-C_6H_{12}).

2.7. GC-MS analyses of hexane crude extracts and purified compounds

This was performed using a Shimadzu TQ8040 NXMass Spectrometer following the procedure as described by Abd El-Ghffar et al. (2017). An aliquot (1 mL) of diluted extract (1:10 hexane, v/v) was injected into the GC/MS apparatus, with helium used as the carrier gas, and was

adjusted to column velocity flow of 1.0 mL/min. Separations were done on a SH-RXi-5Sil column, 30 m in length, 0.25 mm in diameter, and 0.25 µm in thickness. The GC oven was set at an initial temperature of 50 ℃ and was held for 1 min at 260 ℃ and then for 10 min with a program rate of 4 ℃/min. The injector was in split mode, and detector temperatures were set at 250 and 230 ℃, respectively. The mass range was scanned from 50 to 550 amu with a scan speed of 1666 m/s. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The identification of components was based on their retention indices, and interpretation was done through comparison of their mass spectra with standard mass spectra in the National Institute of Standards and Technology (NIST) library.

2.8. Antibacterial activity of the crude extracts

2.8.1. *Maintenance of the bacterial cultures*

The cultures of the clinical isolates and the standard sensitive strains were maintained on agar slants. The nutrient agar slants were prepared according to the protocol outlined by Swami et al. (2024). The bacterial microorganisms were streaked on nutrient agar slants. The streaked agar slants were placed in an incubator at 37 ℃ and observed for growth every 24 hours. The microorganisms were subsequently sub-cultured every 48 hours to maintain their viability.

2.8.2. *Antimicrobial susceptibility testing*

The antibacterial activity of the plant extracts was evaluated by the agar well diffusion method (Valgas et al., 2007). Bacteria were grown in Muller-Hinton broth (MHB) (HiMedia Laboratories Ltd., India) to match the turbidity of 0.5 McFarland standards to be inoculated on MHB media. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Plant extracts (100 mg/mL) were prepared in dimethyl sulfoxide (DMSO). The wells were then filled with 100 µL of plant extracts and DMSO solvent to serve as a negative control. Commercially available gentamycin (10 µg) discs were used as a positive control in this study. Plates were incubated for 24 hours at 37 ˚C. The diameters of the zone of inhibition for different extracts against different bacteria were measured in millimeters. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate, and the average values were used for analysis. The strength of bioactivity of the crude extracts was compared to that of the standard drug, and the percentage inhibition was defined by Eq. 1

$$P(\%) = \frac{\text{Zone of Inhibition of extract}}{\text{Zone of Inhibition of Gentamicin}} \times 100 \quad (1)$$

Table 1: GC-MS profile of aerial parts of *C. floribunda* hexane extracts-

No	Retention Time (min)	Peak Area	Peak Area %	Name of the Compound	Class of Compound
1	7.78	77613886	3.01	gamma-Muurolene	Sesquiterpene
2	8.346	82096228	3.18	Bicyclo[3.1.1]heptane	Bicyclic hydrocarbon
3	8.49	132213363	5.12	(E)-beta-Famesene	Acyclic sesquiterpene
4	8.954	79158543	3.07	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	Sesquiterpene
5	9.131	188577730	7.3	alpha-Muurolene	Sesquiterpene
6	10.111	217051917	8.41	1H-Cycloprop[e]azulen-7-ol,	Sesquiterpene
7	12.53	136529136	5.29	Isopropyl myristate	Oxygenated hydrocarbon
8	12.749	332057377	12.86	Neophytadiene	Acyclic diterpene
9	14.247	98403067	3.81	Isopimarol	Diterpene
10	15.4	82853235	3.21	Phytol	Acyclic diterpene alcohol
12	17.267	177526034	6.88	Isopimarol	Diterpene
12	23.274	139744740	5.41	Hexatriacontane	Hydrocarbon
13	25.491	109536377	4.24	Tetrapentacontane	Hydrocarbon
14	26.021	184888276	7.16	Stigmasta-7,16-dien-3-ol	Steroid
15	26.706	144606068	5.6	beta-Amyrin	Triterpenoid
16	27.408	117564720	4.55	alpha-Amyrin	Triterpenoid

3. Results and Discussion

3.1. GC-MS chemical profiles of hexane crude extracts and purified compounds

3.1.1. GC-MS analysis of the aerial parts of *Conyza floribunda* hexane extracts

Hexane extracts from aerial parts of *C. floribunda* showed a total of sixteen compounds accounting for 89.10% of the total extracts in the range of 3.01-12.85% (Table 1). Terpenoids accounted for 75% of the total extract, whereby the acyclic diterpene, neophytadiene (12.86%) (Fig. 1) was the major terpenoid. This is the first report of the GC-MS profile of *Conyza floribunda* extracts. This report enriches the phytochemical knowledge of an earlier chromatographic separation of dichloromethane and methanol extracts of the whole plant, which afforded a total of four terpenoids with antimicrobial activities alongside six flavonoids (Opiyo et al., 2010).

3.1.2. GC-MS analysis of the stem-bark of *Zanthoxylum gilletii* hexane extracts

The hexane extract of *Z. gillettii* stem-bark yielded 2,4-decadienamide (38.89%) as the major compound among ten other phytochemicals identified in the extract (Table 2, Fig. 2). This is the first report on the chemical composition of hexane extracts of *Z. gilletii* stem-bark. Previously, the GC-MS analysis of the hexane extract of *Z. armatum* fruits yielded a mixture of classes of phytochemicals, including aromatics, phenolics, aliphatics, sesquiterpenes, and monoterpenes, with the major compound identified as 2-hydroxy cyclopentadecanone (27.37%) (Kayat et al., 2016)

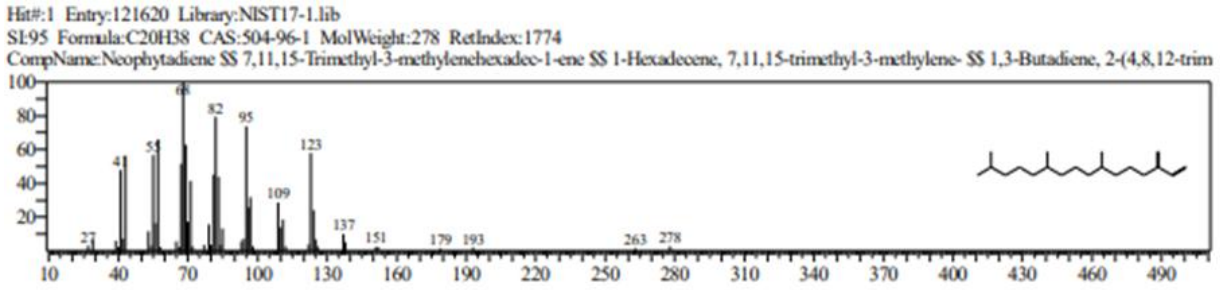

Fig. 1: GC-MS spectrum of the major terpenoid from the aerial parts of *C. floribunda* hexane extract

Table 2: GC-MS profile of the stem-bark of *Z. gilletii* hexane extracts

No	Retention Time (min)	Peak Area	Peak Area %	Name of the Compound	Class of Compound
1	9.044	282139901	7.51	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane	Hydrocarbon
2	10.033	153483503	4.09	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	Oxygenated hydrocarbon
3	11.256	140282145	3.74	(1R,3aS,5aS,8aR)-1,3a,5a-Trimethyl-4-methylenedecahydrocyclopenta[c]pentalene	Unsaturated hydrocarbon
4	11.631	241358644	6.43	(2E,4E)-N-Isobutylocta-2,4-dienamide	Acyclic amide
5	14.264	1.46E+09	38.89	2,4-Decadienamide	Acyclic amide
6	16.061	394364217	10.5	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	Unsaturated oxygenated hydrocarbon
7	17.639	291941414	7.78	3,4-Methylenedioxybenzylidene acetone	Phenolic
8	21.085	159688712	4.25	Supraene	Acyclic triterpene
9	24.123	147614616	3.93	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	Lignan
10	27.543	483637139	12.88	Lupeol	Triterpene

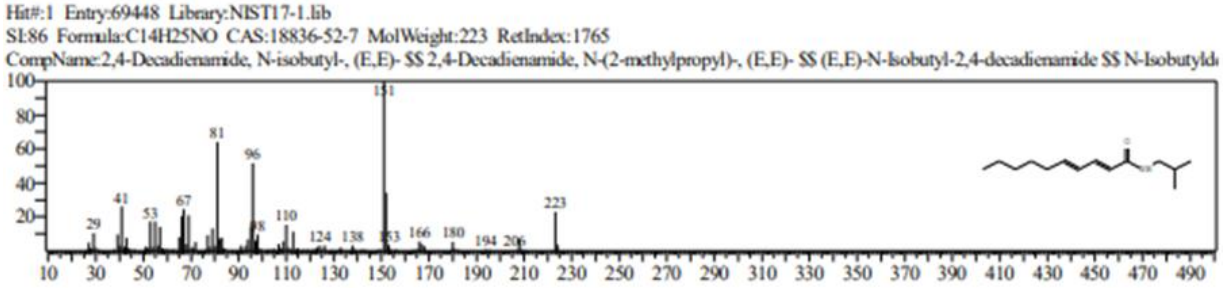

Fig. 2: GC-MS spectra of the major compound from the stem-bark of *Z. gilletii* hexane extracts

3.1.3. Lupeol from semi-purified Z. gilletii stem-bark hexane extracts

The UV-inactive white powder, whose thin-layer chromatography (TLC) spot turned blue on spraying with vanillin-H_2SO_4, was obtained from semi-purification of *Z. gilletii* hexane extract. The analysis of its solution gave a major compound with a retention time (RT) of 27.37 min and an area of 87.76%, which was identified as lupeol (Table 3, Fig. 2).

This confirms the presence of lupeol among the major compounds of the *Z. gilletii* hexane extracts (Table 4), at a retention time of 27.54 min. This compound had been reported earlier from the purification of MeOH:CH_2Cl_2 (1:1) extract obtained from the stem-bark of *Z. gilletii* (Nyaboke et al., 2018).

Table 3: GC-MS profile of lupeol

No	Retention Time (min)	Peak Area	Peak Area %	Name of the Compound
1	17.492	34739527	7.7	9-Octadecenamide
2	20.729	14255952	3.16	13-Docosenamide
3	27.366	395875160	87.76	Lupeol

3.1.4. GC-MS analysis of the stem-bark of Olea capensis hexane extracts

This study unveils for the first time the GC-MS profile of seven terpenoids (90.65% of the total extract) of O. capensis stem-bark hexane extract. The triterpene isopimarol (29.08%) formed the major constituents (Table 4, Fig. 3). Previous phytochemical investigations on *O. europaea* led to the isolation of biophenols, triterpenes, and benzoic acid derivatives, which have shown antimicrobial, among other activities (Hashmi et al., 2015).

3.1.5. GC-MS analysis of the stem-bark of *Warburgia ugandensis* hexane extracts

The analysis revealed ten sesquiterpenes in a mixture of twelve compounds of very close proportions (2.95-10.17%) (Table 5). Among the sesquiterpenes, (1R,4aS,6R,8aS)-8a,9,9-trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol, (10.17%) (Table 5, Fig. 4) was reported as the major compound. These findings add to the previous reports of various sesquiterpenoids, including cinnamolide-3β-acetate, muzigadial, and muzigadiolide, and unsaturated fatty

acids, from the plant (Drage et al., 2014). A different study reported drimane sesquiterpenes: polygodial, wurburganal, and muzigadial, with antimicrobial activities, from the stem-bark of *W. ugandensis* (Kitte et al., 2021).

Table 4: GC-MS profile of the stem-bark of *O. capensis* hexane extracts

No	Retention Time (min)	Peak Area	Peak Area %	Name of the Compound	Class of Compound
1	7.965	956271601	15.77	1,2,4-Metheno-1H-indene	Sesquiterpene
2	8.517	195725067	3.23	alpha-Guaiene	Sesquiterpene
3	9.517	977158105	16.12	alpha-Muurolene	Sesquiterpene
4	14.579	1.031E+09	17.01	Podocarpa-6,13-diene	Triterpene
5	16.107	252695699	4.17	1-Phenanthrenemethanol	Diterpene
6	17.712	1.763E+09	29.08	Isopimarol	Diterpene
7	18.226	319355666	5.27	Kaur-16-en-18-ol	Diterpene

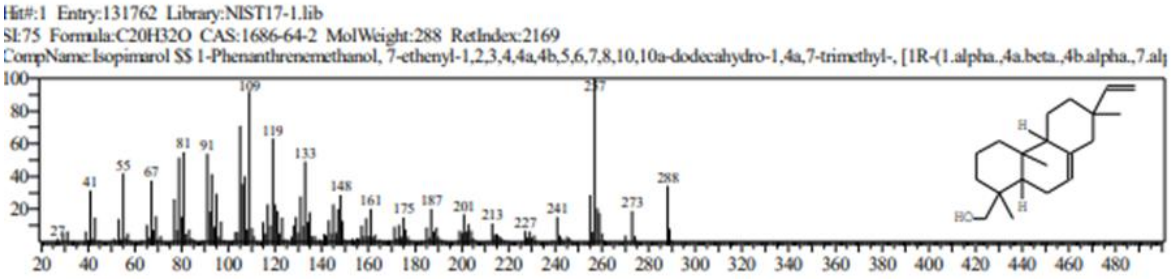

Fig. 3: GC-MS spectra of the major compound from the stem-bark of *O. capensis* hexane extracts

Table 5: GC-MS profile of the stem-bark of *W. ugandensis* hexane extracts

No	Retention Time (min)	Peak Area	Peak area %	Name of the compound	Class of compound
1	8.32	186078113	4.51	Caryophyllene	Bicyclic sesquiterpene
2	9.716	137432631	3.33	Nerolidyl acetate	Sesquiterpene
3	12.227	121804982	2.95	Drim-7-en-11-ol	Sesquiterpene
4	13.343	190957178	4.63	2-Butenal	Oxygenated hydrocarbon
5	13.723	238873690	5.79	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde	Sesquiterpene
6	14.132	145406129	3.53	(5a.alpha.,9a.beta.,9b.beta.)-5,5a,6,7,8,9,9a,9b-octahydro-6,6,9a-trimethylnaphtho[1,2-c]furan-1-(3H)-one (drimenin)	Sesquiterpene
7	14.847	419318347	10.17	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol	Sesquiterpene
8	15.035	167330277	4.06	Naphtho[2,1-b]furan	Sesquiterpene
9	15.523	141491248	3.43	Azuleno[4,5-b]furan-2(3H)-one	Sesquiterpene
10	15.779	261037606	6.33	2,4-Heptadiene	Unsaturated hydrocarbon
11	16.591	352027900	8.54	Sclaral (sclareolide lactol)	Sesquiterpene
12	18.699	173555050	4.21	4-Isopropyl-3,4-dimethylcyclohexa-2,5-dienone	Oxygenated hydrocarbon

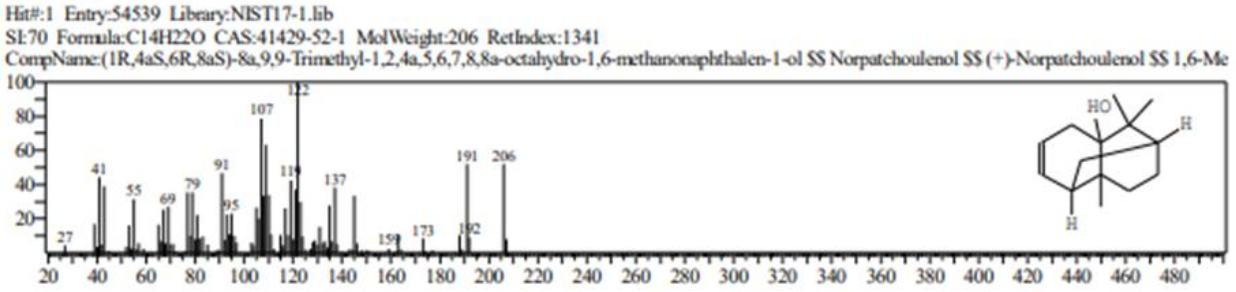

Fig. 4: GC-MS spectra of the major compound from the stem-bark of *W. ugandensis* hexane extracts

3.1.6. GC-MS analysis of the leaf of *Warburgia ugandensis* hexane extracts

The analysis showed a mixture of twelve compounds (Table 6). The compounds occurred in very close proportions, with the most abundant being a phenolic compound, 2,2,5,7,8-pentamethyl-6-hydroxychroman (Fig. 5), accounting for only 8.54% of the total area. Unlike stem-bark extract, which reported 80% sesquiterpenes, the leaf extract reported a lower (41.6%) percentage varying in the degree of oxidation, cyclization, unsaturation, and rearrangements.

Table 6: GC-MS profile of the leaf of *W. ugandensis* hexane extracts

No	Retention Time (min)	Peak Area	Peak Area %	Name of the Compound	Class of Compound
1	8.275	33723619	3.32	Caryophyllene	Bicyclic sesquiterpene
2	8.466	68387190	6.73	cis-.beta.-Farnesene	Acyclic sesquiterpene
3	13.182	42657987	4.19	2-Butenal	Oygemated acyclic hydrocarbon
4	13.524	52142565	5.13	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	Sesquiterpene
5	13.897	54594884	5.37	n-Hexadecanoic acid	Oxygenated hydrocarbon
6	14.183	41406313	4.07	1-Nonadecene	Unsaturated hydrocarbon
7	14.501	31421271	3.09	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol	Sesquiterpene
8	14.620	45311302	4.46	3H-3a,7-Methanoazulene	Sesquiterpene
9	14.949	86892776	8.54	2,2,5,7,8-Pentamethyl-6-hydroxychroman	Phenol
10	15.227	54026374	5.31	2,3-Pentadienoic acid	Oxygenated hydrocarbon
11	21.598	60401239	5.94	Hexatriacontane	Hydrocarbon
12	23.166	33763561	3.32	Tetrapentacontane	Hydrocarbon

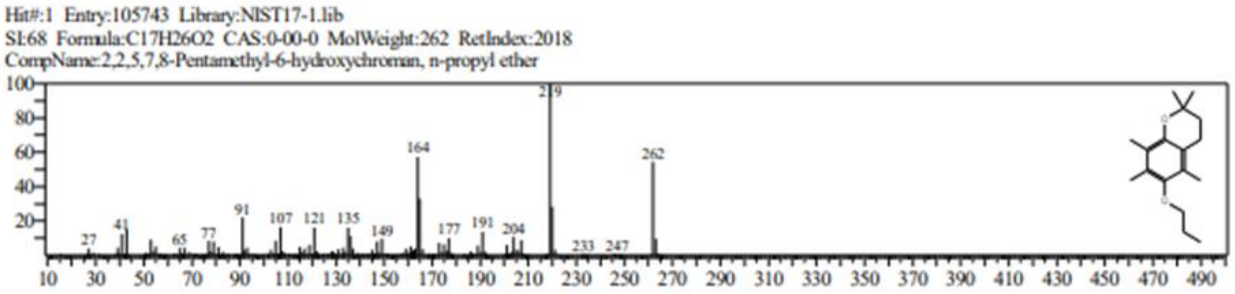

Fig. 5: GC-MS spectrum of 2,2,5,7,8-pentamethyl-6-hydroxychroman from the leaf of *W. ugandensis* hexane extracts

3.1.7. GC-MS analysis of the aerial parts of Lantana trifolia hexane extracts

The analysis revealed a straight chain alkane, tetrapentacontane (13.63%), as the major compound among twelve (86.97% of total extract) compounds belonging to terpenoid, hydrocarbon, and phenol classes. (Table 7, Fig. 6). There is a similarity in classes of compounds of the current hexane extracts with that reported for its methanol extracts (Madivoli et al., 2020), however, the identities were completely different, owing to the different polarities of solvents used for extraction, thus obtaining different compounds with different polarities.

Table 7: GC-MS profile of the aerial parts of *L. trifolia* hexane extracts

No	Retention Time (min)	Peak Area	Peak area %	Name of the Compound	Class of Compound
1	7.226	59514269	3.51	Cyclohexanol	Cyclic oxygenated hydrocarbon
2	12.492	117047363	6.91	Isopropyl myristate	Oxygenated hydrocarbon
3	14.178	199980077	11.81	n-Hexadecanoic acid	Oxygenated hydrocarbon
4	21.089	157914626	9.33	Supraene	Acyclic triterpene
5	21.629	64088936	3.78	Tetratriacontane	Hydrocarbon
6	23.262	117277428	6.93	Hexatriacontane	Hydrocarbon
7	23.609	72255163	4.27	.beta.-Tocopherol	Alkylated phenol
8	25.543	230836583	13.63	Tetrapentacontane	Hydrocarbon
9	26.033	151215855	8.93	.gamma.-Sitosterol	Steroid
10	26.736	57047007	3.37	.beta.-Amyrone	Triterpene
11	27.348	79235555	4.68	24-Norursa-3,12-diene	Steroids
12	28.470	92446212	5.46	Tetrapentacontane	Hydrocarbon

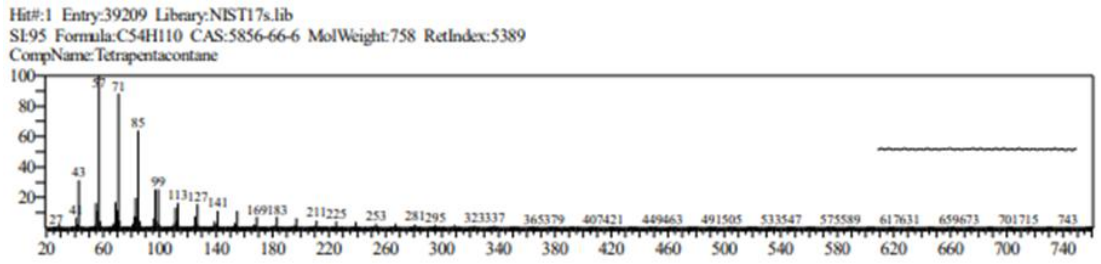

Fig. 6: GC-MS spectrum of the major compound of the aerial parts of *L. trifolia* hexane extracts

3.2. Bioactivity results of crude extracts from selected plants

All the hexane and 1:1 methanol/dichloromethane extracts from the five plants under investigation were screened for antibacterial activities against six microbial strains. *Wurbugia ugandensis* stem-bark hexane (WUBH) extract, *W. ugandensis* stem-bark 1:1 methanol/dichloromethane (WUBM), and *W. ugandensis* leaf hexane (WULH) extracts inhibited the growth of all of the studied microorganisms except for WUBM against *P. aeruginosa* (7553) (Table 8). The WUBH extract recorded the highest percentage inhibition (72.7%) against *S. aureus*, by exhibiting a ZI of 16.0 mm compared to 22.0 mm of gentamicin. Therefore, the high activity of the WUBH can be attributed to the higher percentage of sesquiterpenes (80%) (Table 5) compared to WULH, with a lower concentration (41%) (Table 6). On the other hand, the hexane extract (less polar) extracts showed higher bioactivity compared to the more polar 1:1 methanol/dichloromethane solvent mixture. Furthermore, the *Conyza floribunda* (CFH), *Olea capensis* (OCH), and *Lantana trifolia* (LTH) moderately inhibited *E. coli*, *S. aureus*, and *P. aeruginosa* with percentage inhibitions of 45, 36, and 30%, respectively. The absence of inhibition among the less inhibitive extracts may be that the concentrations of the compounds were lower than what would bring about the inhibition, there was antagonism among the compounds, or they did not possess the antimicrobial activity at all.

Table 8: Zones of inhibition of crude extracts against selected bacteria

Extract/ standard drug	Zone of Inhibition (mm)					
	S. aureus	*E. coli*	MRSA	*P. aeruginosa*	*P. aeruginosa* (7553)	*S. aureus* (29213)
WUBH	16.0 (72.7)	13.0 (65)	11.0 (47.8)	11.0 (47.8)	10.0 (40.0)	9.0 (39.1)
WUBM	10.0 (45.5)	10.0 (50.0)	11.0 (47.8)	10.0 (35.7)	-	9.0 (39.1)
WULH	10.0 (45.5)	8.0 (40.0)	9.0 (39.1)	10.0 (35.7)	9.0 (36.0)	9.0 (39.1)
CFH		9.0 (45.0)				
OCH	8.0 (36.4)	-	-	-	-	-
LTH	-	-	-	9.0 (32.1)	-	-
Gentamicin	22 (100)	20 (100)	23 (100)	28 (100)	25 (100)	23 (100)

Percentage (%) inhibition = $\frac{\text{ZI extract}}{\text{ZI gentamicin}} \times 100$ (given in brackets)

Zone of inhibition ≤ 6.0 mm.

WUB-*W. ugandensis* bark, WUL- *W. ugandensis* leaf, CF- *C. floribunda*, OC- *O. Capensis*, LT-*L. trifolia*, ZG- *Z. gillettii*

H- hexane extract, M-1:1 MeOH/CH_2Cl_2 extract

4. Conclusion

1H-cycloprop[e]azulen-7-ol, 2,4-decadienamide, isopimarol, (1R,4aS,6R,8aS)-8a,9,9-trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol, 2,2,5,7,8-pentamethyl-6-hydroxychroman and tetrapentacontane, were found as the major constituents of hexane extracts of, *C. floribunda* aerial, *Z. gilletii* bark, *O. capensis* bark, *W. ugandensis bark*, *W. ugandensis leaf* and *L. trifolia leaf*, respectively. The *W. ugandensis* extracts showed the highest activity against the tested bacteria (ZI 8.0-16.0 mm). The rest of the plants, only a few, indicated mild activity for some bacterial strains. *W. ugandensis* could be a potential antibacterial agent for controlling bacterial infections. Further work on *in vivo* and synergy activity, and possible formulations are recommended.

Acknowledgements

The authors acknowledge the Moto Mwaka Biodiversity Conservation team of the Kakamega Natural Forest Catchment Conservation Organization (KANFCCO) for ethno-medical information of the medicinal plants. This work was supported by the University Innovation Fund of MMUST under the theme 'Alternative Medicine for reducing vulnerability to Covid-19 infection' in the project 'Innovations for Integrated Control of COVID-19 among Vulnerable Populations with Underlying Predisposing Respiratory Diseases'.

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