Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Phytochemical Investigation of *Aristolochias ringens* (Vahl.) n-hexane root extract using GC-MS and FTIR

Seide M. Akoro*[1], Oyinlade C. Ogundare[1], Sunday O. Ajibade[2], and Deborah O. Awofeso [1]

[1]*Department of Chemical Sciences, College of Basic Sciences, Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria.*
[2]*Department of Chemical Sciences, Faculty of Natural Sciences, Redeemers University Ede, Osun State, Nigeria.*

ABSTRACT

Aristolochia ringens (Vahl.) has been featured in traditional medicinal practices to manage several diseases. This work aims to investigate the phytochemical contents of *Aristolochia ringens n*-hexane root extract and the functional groups present using Gas Chromatography-Mass Spectrometry (*GC-MS*) and Fourier Transform Infrared *(FTIR)* Spectroscopy methods of analysis and also to discuss the major and minor compounds detected with their biological activities. The plant material was extracted by macerating in *n*-hexane for 72 h. The extract was concentrated to dryness using a rotary evaporator and then screened for secondary metabolites. The phytochemical contents and *FTIR* were investigated using a GCMS-QP2010SE Shimadzu (Japan) fitted with an MS (Model EI) and an Agilent *FTIR* spectrophotometer (USA). Sixty-two compounds were detected by the GC-MS and identified using the NIST 11 library. The compounds consist of terpenes (28.97%), steroids and D-modified steroid (17.54%), cannabinoids (23.56%), esters (14.78%), epoxides (3.74%), alkanol, alkynol, and phenolic (3.00%), organosilicone (5.43%), alkanones and nitroalkanone (0.41%), hydrocarbons and chlorohydrocarbon (1.98%), pyrazine ester (0.11%), dioxocin (0.47%), and fatty acid (0.01%). Three cannabinoids are detected, and the most abundant of compounds and cannabinoid is cannabinol. *FTIR* detected OH-stretch (3327 cm^{-1}), C-H-stretch (2921 cm^{-1} and 2854 cm^{-1}), C=O stretch (1711 cm^{-1}), C=C (1640 cm^{-1} and 1462 cm^{-1}), C-O stretch for ether and epoxide (1380 cm^{-1}), and C-O stretch for alkanol (1171 cm^{-1} and 1074 cm^{-1}). This study revealed the rich phytochemical contents of the *n*-hexane *Aristolochia ringens* extract, with their diverse medicinal properties. These results further explained the ethnomedicinal uses of the plant.

Keywords: *Aristolochia ringens*, *n*-hexane extract, phytochemical contents, Gas Chromatography-Mass Spectrometry, Fourier Transform Infrared Spectroscopy.

Received 16 February 2025
Revised 21 February 2025
Accepted 22 March 2025
Published online 01 April 2025

Introduction

Plants have served as food and medicine to man throughout human history. Medicinal plants, in crude or modified forms, constitute about 25% of conventional medications used to manage ailments, especially in traditional practices, because of their phytochemical contents. [1, 2] The phytochemical contents of over 5000 plants, seeds, nuts, vegetables, and fruits have been determined and reported. [3] Despite these reports, the phytochemical contents of several other plants and medicinal plants are yet to be known. The knowledge of medicinal plants' chemical contents and bioactivity forms the basis for modern drug formulations and synthesis. [1,4] *Aristolochia ringens* is a plant known in the traditional medicine cycle for its numerous uses in the treatment and management of common and chronic ailments. [5, 6]

*Corresponding author. Email: akoro.sm@lasustech.edu.ng

Tel: +2348023216177

Citation: Akoro SM, Ogundare OC, Ajibade SO, Awofeso, DO. Phytochemical Investigation of Aristolochia ringens (Vahl.) n-hexane root extract using GC-MS and FTIR. Trop J Phytochem Pharm. Sci. 2025; 4(3) 111 – 122 http://www.doi.org/10.26538/tjpps/v4i3.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In the southwestern part of Nigeria, among the traditional medicinal practitioners, it is believed to have the ability to cure virtually all diseases; hence, it is known as "Akoogun" and also referred to as "Awo igba arun Oyo".[7] The plants are also cultivated in some areas for ornamental purposes because of their spectacular flowers. [8]

In ethnobotanical studies, the use of *A. ringens* for treating *Diabetes mellitus* and its complications was reported. [9, 10] The different parts of the plant have different medicinal properties, which are explored in traditional medicine practice: the root is used to treat diarrhoea and asthma in southwest Nigeria, and the aerial part is used for its antifungal, antibacterial, and cytotoxic properties. [11, 12, 13, 14] Some other researchers reported the traditional use of the root to manage piles. [15] The use as an analgesic and treatment for rheumatoid arthritis, insomnia, and asthma were reported. [10, 11, 12] The use of the root to treat ailments such as gastrointestinal disturbance, asthma, diabetes, and oedema in Nigeria was confirmed. [7, 8, 10, 16, 17] There are also pieces of evidence to confirm its use to manage conditions such as dyslipidaemia, inflammation, and snake bites. [10, 12, 16] Experiments on the anticancer properties of some of its extracts were carried out. [18, 19] The chloroform extract of the aerial part of *A. ringens* showed strong antimicrobial activity against *B. cereus*, antifungal activity against *C. albicans*, and cytotoxic activity against human breast carcinoma cells (HepG2), human breast carcinoma cells. [13] The trace element contents, phytochemicals, and alpha-amylase inhibitory and insecticidal activities of the root extracts were reported. [20, 21] Some other researchers determined and reported the volatile contents of the flowers, leaves, and stem. [22]

Gas Chromatography-Mass Spectrometry (GC-MS) is a common and versatile method of determining and quantifying the volatile phytochemical contents of plant extracts. [23, 24, 25, 26] In the electromagnetic spectrum, the infrared (IR) is in the low-energy region with a range from 12,800 to 10 cm^{-1}. [27, 28] The IR spectrum is made up of the far region, 400–10 cm^{-1} (25–1,000 µm wavelength); the mid-region, 400–4,000 cm^{-1} (25–2.5 µm wavelength); and the near region, 4,000–4,000 cm^{-1} (0.7–2.5 µm wavelength). [27, 28] The mid-region is appropriate for organic molecules, and the vibrational frequencies are suitable for functional group detection. [28] Previous works were carried out to determine the phytochemical contents of the flowers, leaves, and stem of *A. ringens* using GC-MS, but there were no reports on the root extract. [22] In this work, we are reporting the phytochemical contents of *A. ringens* n-hexane root extract using GC-MS, the functional group of the major components of the extract using *FTIR*, and also discussing the major and minor compounds detected and their biological activities.

Materials and Methods

Chemicals and Reagents

All chemicals used in this work are of analytical grade. The *n*-hexane (Fisher Scientific) was purchased from a local sales representative in Nigeria.

Collection of plant material

The dried sample of *A. ringens* stem was purchased at Mushin Market, Mushin Local Government, Lagos State, Nigeria. The plant material was authenticated at the University of Lagos Herbarium (LUH 5997).
Extraction of plant material. The plant material (100 g) was extracted by macerating in n-hexane (500 mL) for 72 h. The extract obtained was concentrated to dryness using a rotary evaporator. [26]

Phytochemical screening

The preliminary phytochemical screening of the extract was carried out to detect the presence of secondary metabolites: alkaloids, flavonoids, saponins, tannins, phlobatannins, cardiac glycosides, terpenoids, steroids, reducing sugars, and phenols using standard methods. [26, 29]

GC-MS analysis of the extract

GC-MS analysis of the plant extract volatile compound was carried out using a GC-MS QP2010SE Shimadzu (Japan) fitted with an MS (Model EI) directly connected with a capillary column. The detector is a secondary electron multiplier with a patented lens and conversion mode. The carrier gas was helium with a flow rate of 3.22 mL/min, and the pressure was maintained at 144.4 kPa. The injector and detector temperatures were 250°C. The column was set to an initial temperature of 60°C, which was maintained for 2 min. The temperature was increased to 260°C at 14°C/min and was maintained for 1.50 mins. This was then further increased to 300°C at 14°C/min and maintained for 3.30 mins. One microlitre (1 µL) of the sample was injected. Mass spectra were recorded at 70 eV ionisation energy armed with a metal quadrupole mass filter with a pre-rod. The total run time was 24.13 mins. The compounds were detected and identified using the NIST 11 Spectral Library. [26]

Infrared Spectroscopy

FTIR data for the extract was obtained using the Agilent Cary 360 ATR-FITR Infrared Spectrometer (Agilent Technologies, USA) equipped with Microlab PC software. The ATR sampling unit has a resolution of 8 cm^{-1}. The spectrum was obtained from 4000 to 650 cm^{-1}. [30]

Results and Discussion

Extraction of Plant Materials and Phytochemical Screening

The *n*-hexane extract (ArnH) was obtained as a brown sticky solid (2.80%). The preliminary phytochemical screening indicated the presence of saponins, steroids, flavonoids, cardiac glycosides, and phenols (Table 1). The medicinal properties of plants have been ascribed to the presence of secondary metabolites. [31] Several biological effects, such as antimicrobial, antifungal, antioxidant, antiviral, anticancer activities, and others, have been ascribed to the presence of secondary metabolites and their relative contents in plant materials and extracts. [26, 29, 32]

GC-MS Result

The GC-MS profile of the *A. ringens n*-hexane extract gave 70 horizontal peaks and indicated 62 volatile compounds (Figure 1, Table 2). The detected phytocompounds consist mainly of terpenes, steroids, cannabinoids, and some other minor compounds. The breakdown of indicated terpene (28.97%), steroids and D-modified steroid (17.54%), cannabinoids (23.56%), esters (14.78%), epoxide (3.74%), alkanol, alkynol, and phenolic (3.00%), organosilicon (5.43%), alkanones and nitroalkanone (0.41%), hydrocarbons and chlorohydrocarbon (1.98%), pyrazine ester (0.11%), dioxocin (0.47%), and fatty acid (0.01%)
Eighteen compounds out of the sixty-two have a percentage area composition between 2.0 and 13.0%, constituting 74.29% of the total compounds eluted, forming the significant contents of the *A. ringens n*-hexane extract. These compounds can be categorised into three groups. The first group is made up of eight compounds with an area composition between 2.0% and 3.0%, totalling 19.04%; the second group consists of three compounds with a percentage area composition between 3.0% and 4.0%, totalling 10.53%; and the third group consists of seven compounds with an area percentage between 4.0% and 13.0%, with a total composition of 44.72%.
The first group of eight compounds are 6. beta. -Hydroxy fluoxymesterone (RT: 12.295, 14.087 mins; total area 2.43%); 2H-Cyclopropa [a] naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a. alpha., 7. alpha., 7a. alpha., 7b. alpha.) - (RT: 13.626 mins, 2.58%); octadecanoic acid, 9,10-dibromo-, methyl ester (RT: 14.943 mins, 2.03%); (R)-(-)-14-methyl-8-hexadecyn-1-ol (RT: 16.242 mins, 2.24%); cannabidiol (RT: 18.229 mins, 2.3%); androst-5-en-17-one, 3-hydroxy-16-(1-methylethylidene)-, (3. beta.) - (RT: 18.42 mins; 2.82%), Stigmasta-7,22-dien-3-ol, acetate, (3.beta.,5.alpha.,22E)- (RT: 20.73 mins; 2.56%), and Pregn-5-en-20-one, 16-bromo-3,17-dihydroxy-, (3.beta.,16.beta.) - (RT: 20.935 mins, 2.08%).

Table 1: Contents of Aristolochia ringens n-hexane root extract from phytochemical screening

Alkaloids	Saponins	Tannins	Steroids	Flavonoid	Cardiac Glycoside	Reducing Sugar	Phenols
-	+	-	+	+	+	-	+

+: detected; - :not-detected

Table 2: Compounds detected from *A.ringens* n-hexane root extract by GC-MS

S/N	Peak	Retention Time	Name	Area %	Molecular formula	Molecular mass	Description
1	1	6.408	Cyclohexene, 1-methyl-4-(1-methylethenyl))-, (S)-	0.03	$C_{10}H_{16}$	136	monoterpene
2	2	7.401	trans-p-Mentha-2,8-dienol	0.3	$C_{10}H_{16}O$	152	monoterpene alkanol
3	3	7.544	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	0.14	$C_{10}H_{16}O$	152	monoterpene alkanol
4	4	7.637	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha., 5.alpha.)]-	0.02	$C_{10}H_{16}O$	152	monoterpene alkanol
5	5	7.678	5,10-Pentadecadienoic acid, (E,Z)-	0.01	$C_{15}H_{26}O_2$	238	Unsaturated fatty acid
6	6	8.135	p-Mentha-1(7),8-dien-2-ol	0.16	$C_{10}H_{16}O$	152	monoterpene alkanol
7	7	8.175	5,7-Octadien-2-ol, 2,6-dimethyl-	0.08	$C_{10}H_{18}O$	154	monoterpene alkanol
8	8	8.217	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	0.28	$C_{10}H_{18}O$	154	monoterpene alkanol
9	9	8.466	(2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide	0.08	$C_{10}H_{16}O_2$	168	monoterpene hydroperoxide
10	10	8.529	1-Nitro-bicyclo [6.1.0] nonan-2-one	0.07	$C_9H_{13}NO_3$	183	Nitroalkanone
11	11	8.909	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl) -	0.06	$C_{10}H_{14}O$	150	alkanone
12	12	9.379	Methyl 3-amino-2-pyrazinecarboxylate	0.11	$C_6H_7N_3O_2$	153	Pyrazine ester
13	13	9.533	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	0.27	$C_{10}H_{18}O_2$	170	Cyclic alkanol
14	14	9.657	4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	0.28	$C_{14}H_{24}O$	208	alkanone
15	15	9.921	Oxirane, 3-[5-(4-azido-2-nitrophenoxy)-3-methyl-3-pentenyl]-2,2-dimethyl-,	0.08	$C_{16}H_{20}N_4O_4$	332	Epoxide
16	16	10.403	Cyclohexanemethyl propanoate	0.01	$C_{10}H_{18}O_2$	170	ester
17	17	10.690	(-)-Aristolene	0.17	$C_{15}H_{24}$	204	Sequiterpene hydrocarbon
18	18	10.762	Aromadendrene, dehydro-	1.12	$C_{15}H_{22}$	202	Sequiterpene hydrocarbon
19	19	10.819	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	0.5	$C_{15}H_{24}$	204	Sequiterpene hydrocarbon
20	20	10.877	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylene bicyclo [4.1.0] heptane	0.47	$C_{15}H_{22}$	202	Sequiterpene hydrocarbon
21	21	10.961	Bicyclo [4.1.0]heptane, 7-chloro-	0.15	$C_7H_{11}Cl$	130	Bicyclic chlorohydrocarbon

22	22	11.056	Androstan-17-one, 3-ethyl-3-hydroxy-, (5. alpha.)-	0.24	$C_{21}H_{34}O_2$	318	steroid
23	23	11.336	Cubedol	0.37	$C_{15}H_{26}O$	222	Sesquiterpene alkanol
24	24	11.521	4-epi-cubedol	0.69	$C_{15}H_{26}O$	222	Sesquiterpene alkanol
25	25	11.683	alpha. -Calacorene	0.33	$C_{15}H_{20}$	200	Sesquiterpene alkanol
26	26, 28	11.786, 11.927	Calarene epoxide	0.84	$C_{15}H_{24}O$	220	Sesquiterpenes (Epoxide)
27	27	11.849	Glutaric acid, di(2-(2-methoxyethyl)heptyl)	0.32	$C_{25}H_{48}O_6$	444	Diester
28	29	12.037	(-)-Spathulenol	0.69	$C_{15}H_{24}O$	220	Sesquiterpene alkanol
29	30	12.097	Caryophyllene oxide|	1.1	$C_{15}H_{24}O$	220	Sesquiterpene
30	31. 43	12.295, 14.087	6. beta. -Hydroxy fluoxymesterone	2.43	$C_{20}H_{29}FO_4$	352	Steroid
31	32	12.353	Tricyclo[4.4.0.0.(2,7)]dec-8-ene-3-methanol,alpha.,alpha.,6,8-tetramethyl-,sterioisomer	0.44	$C_{15}H_{24}O$	220	Sesquiterpene alkanol
32	33	12.448	Alloaromadendrene oxide-(1)	3.12	$C_{15}H_{24}O$	220	Sesquiterpene (Epoxide)
33	34	12.526	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	1.34	$C_{15}H_{24}O$	220	Sesquiterpene hydrocarbon
34	35, 36	12.653, 12.732	Cadala-1(10),3,8-triene	4.09	$C_{15}H_{22}$	202	Sesquiterpene hydrocarbon
35	37, 42	12.846, 13.940	Murolan-3,9(11)-diene-10-peroxy	4.49	$C_{15}H_{24}O_2$	236	Sesquiterpene hydroperoxide
36	38	12.968	Isolongifolene, 9,10-dehydro-	1.05	$C_{15}H_{22}$	202	Sesquiterpene hydrocarbon
37	39	13.229	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	0.74	$C_{15}H_{24}$	204	Sesquiterpene
38	40	13.626	2H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a, 7b-octahydro-1,1,7,7a-tetramethyl-,(1a. alpha.,7. alpha.,7a.alpha., 7b.alpha.)-	2.58	$C_{15}H_{22}O$	218	Sesquiterpene alkanone
39	41	13.781	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	3.75	$C_{14}H_{22}O$	206	Sesquiterpene alkanol
40	44	14.713	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-,	3.66	$C_{15}H_{24}O_3$	252	epoxide
41	45	14.943	Octadecanoic acid, 9,10-dibromo-, methyl ester	2.03	$C_{19}H_{36}Br_2O_2$	454	ester
42	46	16.118	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	5.18	$C_{18}H_{30}O_6$	342	ester
43	47	16.242	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	2.24	$C_{17}H_{32}O$	252	alkynol
44	48	16.357	Adipic acid, butyl 2-cyclohexylethyl ester	4.27	$C_{18}H_{32}O_4$	312	ester

45	49	16.913	Tributyl acetylcitrate	1.98	$C_{20}H_{34}O_8$	402	ester
46	50	17.405	5.alpha. -Androstan-3.beta.-ol, 4,4-dimethyl-, acetate	1.19	$C_{23}H_{38}O_2$	346	steroid
47	51	18.229	Cannabidiol	2.3	$C_{21}H_{30}O_2$	314	Cannabinoid
48	52	18.420	Androst-5-en-17-one, 3-hydroxy-16-(1-methylethylidene)-, (3. beta.)-	2.82	$C_{22}H_{32}O_2$	328	Steroid
49	53, 54	18.911, 19.123	Dronabinol	9.17	$C_{21}H_{30}O_2$	314	cannabinoid
50	55, 56, 58	19.452, 19.630, 20.296	Cannabinol	12.09	$C_{21}H_{26}O_2$	310	cannabinoid
51	57	20.123	1,3-bis[(8E,10E)-Dodeca-8,10-dien-1-yloxy]-1,1,3,3-tetramethyldisiloxane	5.43	$C_{28}H_{54}O_3Si_2$	494	Organosilicone
52	59	20.554	D-Homoandrostane, (5. alpha.,13.alpha.)-	1.92	$C_{20}H_{34}$	274	D-modified Steroid
53	60	20.730	Stigmasta-7,22-dien-3-ol, acetate, (3. beta.,5. alpha.,22E)-	2.56	$C_{31}H_{50}O_2$	454	steroid
54	61	20.935	Pregn-5-en-20-one, 16-bromo-3,17-dihydroxy-, (3. beta.,16.beta.)-	2.08	$C_{21}H_{31}BrO_3$	410	Steroid
55	62	21.101	Allopregnane-7. alpha.,11. alpha.-diol-3,20-dione	1.08	$C_{21}H_{32}O_4$	348	Steroid
56	63	21.317	12-Hydroxy-3-keto-bisnor-4-cholenic acid	1.6	$C_{22}H_{32}O_4$	360	Steroid
57	64	21.441	Cholestane	0.91	$C_{27}H_{48}$	372	steroid
58	65	21.740	2-methylhexacosane	1.83	$C_{27}H_{56}$	380	alkane
59	66	21.963	Tetrahydrosmilagenin	0.71	$C_{27}H_{48}O_3$	420	steroid
60	67, 68	22.308, 22.520	Fumaric acid, 2-hexyl tetradecyl ester	0.99	$C_{24}H_{44}O_4$	396	ester
61	69	22.645	3H-3,10a-Methano-1,2-benzo dioxocin-3-ol, octahydro-7,7-dimethyl-, (3. alpha., 6a. beta., 10a.beta.)-	0.47	$C_{13}H_{22}O_3$	226.3	dioxocin
62	70	22.858	. gamma.-Tocopherol	0.49	$C_{28}H_{48}O_2$	416	phenolic

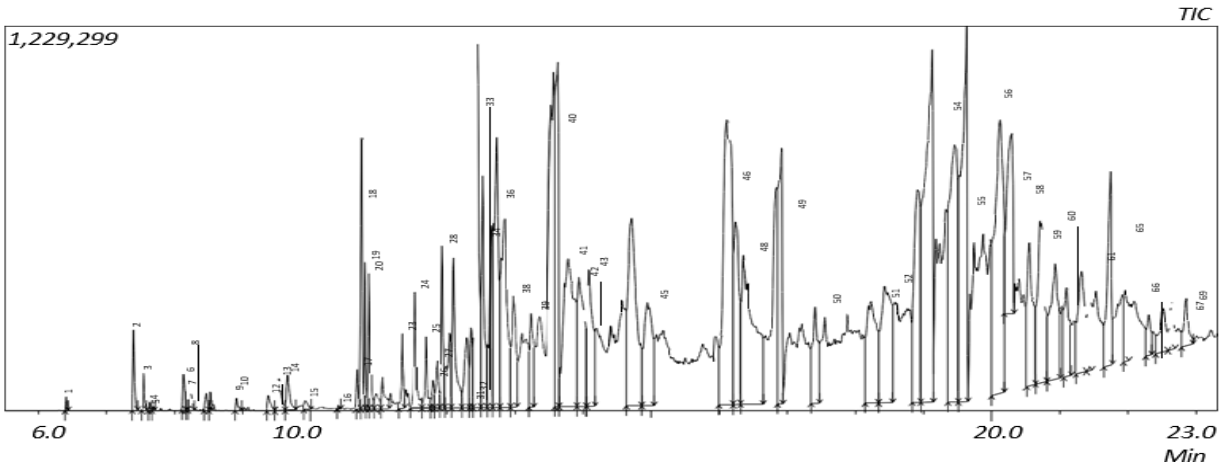

Figure 1: GC-MS Profile of *Aristolochia ringens* n-hexane root extract

The second group of compounds consists of alloaromadendrene oxide-(1) (RT: 12.448 mins, 3.12%). 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol (RT: 13.781 mins, 3.75%); and Spiro [tricycle [4.4.0.0(5,9)] decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy- (RT: 14.713 mins; 3.66%).

The third group of compounds are Cadala-1(10),3,8-triene (RT: 12.653, 12.732 mins; total area 4.09%); Murolan-3,9(11)-diene-10-peroxy (RT: 12.846, 13.94 mins; total area 4.49%); 1-Propene-1,2,3-tricarboxylic acid, tributyl ester (RT: 16.118 mins, 5.18%); Adipic acid, butyl 2-cyclohexylethyl ester (RT: 16.357 mins, 4.27%); Dronabinol (RT: 18.911, 19.123 mins; total area 9.17%); Cannabinol (RT: 19.452, 19.63, 20.296 mins; total area 12.09%); and 1,3-bis[(8E,10E)-Dodeca-8,10-dien-1-yloxy].-1,1,3,3-tetramethyl disiloxane (RT: 20.123 mins; 5.43%).

Some of the compounds showed multiple peaks, possibly due to column impairment, which tends to reduce efficiency, and this is related to high retention time. [33] Compounds with multiple peaks are Calarene epoxide (RT: 11.786, 11.927; Total: 0.21%); 6.beta.-Hydroxyfluoxymesterone (RT: 12.295, 14.087; Total: 2.43%); Cadala-1(10),3,8-triene (RT: 12.653, 12.732; Total: 4.09%); Murolan-3,9(11)-diene-10-peroxy (RT: 12.846, 13.94; Total: 4.49%); Dronabinol (RT: 18.911, 19.123; Total: 9.17%); Cannabinol (RT: 19.452, 19.63, 20.296; Total: 12.09%); and Fumaric acid, 2-hexyl tetradecyl ester (RT: 22.308, 22.52; 0.99); urolan-3,9 (11)-diene-10-peroxy (RT: 12.846, 13.94 mins; total area 4.49%); 1-Propene-1,2,3-tricarboxylic acid, tributyl ester (RT: 16.118 mins, 5.18%); Adipic acid, butyl 2-cyclohexylethyl ester (RT: 16.357 mins, 4.27%); Dronabinol (RT: 18.911, 19.123 mins; total area 9.17%); Cannabinol (RT: 19.452, 19.63, 20.296 mins; total area 12.09%); and 1,3-bis[(8E,10E)-Dodeca-8,10-dien-1-yloxy]. -1,1,3,3-tetramethyl disiloxane (RT: 20.123 mins; 5.43%).

In this extract, the terpene contents are in small area percentages ranging from 0.03% to 4.49% and in different classes, giving a significant total area of 28.97% (Table 2.0). They are the largest group of natural products, classified as monoterpenes, diterpenes, triterpenes, tetraterpenes, sesterpenes, sesquiterpenes, and polyterpenes, based on their isoprene units. [25] Terpenes have been reported for diverse medicinal properties, such as anti-cancer, antioxidant, anti-inflammatory, antiplasmodial, antiseptic, astringent, and diuretic activity. [34]

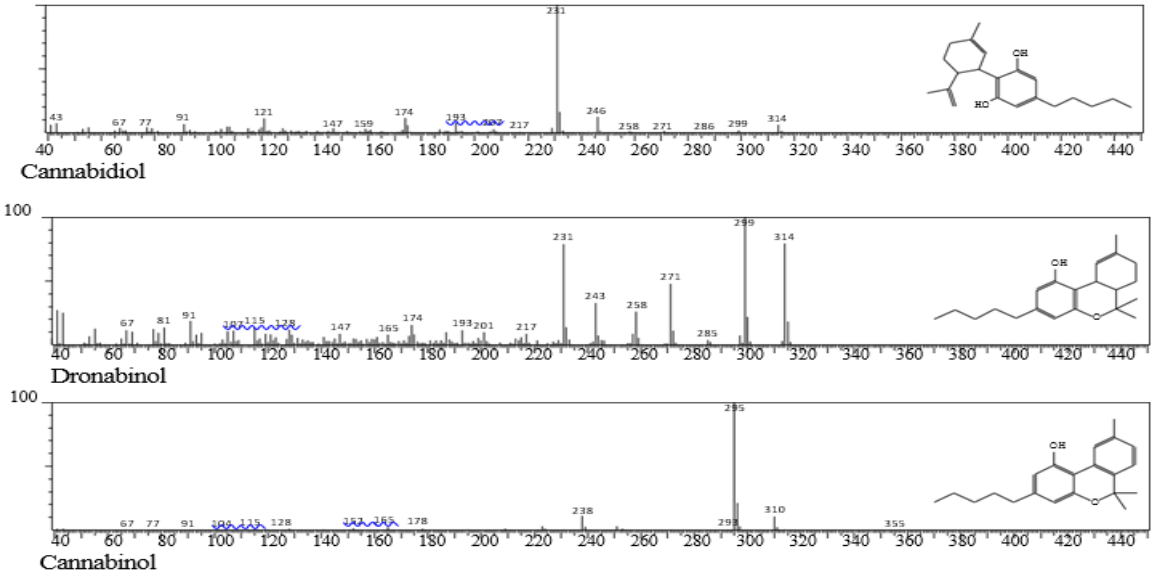

Figure 2: GC Mass spectra of Cannabidiol, Dronabinol and Cannabinol

Among the major compounds in the *A. ringens* n-hexane root extract are the cannabinoids (Figure 2), of which cannabinol is the most abundant as well as the most abundant compound in this extract. These compounds constitute 23.56% of the component compounds of the extract. The three cannabinoids are cannabidiol (2.3%), dronabinol (9.17%), and cannabinol (12.09%). The cannabinoids are compounds found originally in cannabis—a group of plants belonging to the class Cannabaceae. [35] Dronabinol constitutes about 74% of the content of the volatile compounds of cannabis. Comparing contents, dronabinol (delta-9-tetrahydrocannabinol) and cannabinol are 9.17% and 12.09% in *A. ringens* and 74.05% and 20.47% in cannabis. [36] Cannabidiol has been reported to possess antimicrobial, neuroprotective, analgesic, antiepileptic, anti-inflammatory, anti-anxiety, and anti-cancer properties. [37, 38, 39, 40, 41, 42] Dronabinol and cannabinol are psychoactive compounds with varying degrees of activity. [36] These two compounds, which have properties such as appetite stimulants and analgesics, were reported. [35, 43, 44] The activity of dronabinol as a sleep apnoea medication was reported. [45, 46] Cannabinol is a cannabinoid reported to possess antimicrobial, anti-inflammatory, immunosuppressive, neuroprotective, and analgesic effects. [47]

Several of the major compounds have peculiar medicinal properties. 6.beta.-hydroxy fluoxymesterone and pregn-5-en-20-one, 16-bromo-3,17-dihydroxy-, (3.beta.,16.beta.)- are halogenated steroids that exhibit anti-inflammatory, cytotoxic, anti-hormonal and antimicrobial activities. [48] The compound 6-beta-hydroxyfluoxymesterone (Figure 3) is a metabolite of fluoxymesterone, which is a drug used for the treatment of puberty delay and hypogonadism in young males and breast cancer in females. [49, 50] Alloaromadendrene oxide (1) (Figure 3) exhibits analgesic, antioxidant, anti-inflammatory, anti-tumour, and antibiotic activity. [51, 52] Cadala-1(10),3,8-triene (Figure 3) has antimicrobial properties, [53] stigmasta-7,22-dien-3-ol, acetate, (3.beta., 5.alpha.)- exhibits antiulcerogenic, alpha-amylase, antithrombotic and antibacterial activities, [54] and pregn-5-en-20-one, 16-bromo-3,17-dihydroxy-, (3.beta., 16.beta.) - have potential for the treatment of some reproductive disorders and some hormone-related cancers. [55]

Also significant is 1,3-bis[(8E,10E)-dodeca-8,10-dien-1-yloxy-1,1,3,3-tetramethyl disiloxane (RT: 20.12, Area % 5.30), the only organosilicon compound eluted from the *A. ringens* n-hexane extract (Figure 4). Siloxanes are organosilicon compounds that have the functional group (-R2Si-O-SiR2-), which forms the main structure of the silicones. [56, 57]

Some organosilicon compounds are from natural sources, such as plants, while others are synthesised. [58, 59] Some of these compounds show cytotoxic, anti-cancer, antitumour, anti-HIV, and antimicrobial activities. [58, 59]

The last sets of forty-four compounds eluted from *A. ringens* have an area percentage of 0.01%-2.0%, which is very low; hence, they have been considered minor constituents. Nine of these compounds have area percentages from 0.01% to 0.08%; twenty-five have area percentages from 0.1% to 0.99%, and the remaining ten have area percentages between 1.0% and 2.0%. The first nine of the forty-four compounds are cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)- (RT: 6.408, 0.03%); Bicyclo[3.1.1] heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha., 5.alpha.)]- (RT: 7.637, 0.02%); 5,10-Pentadecadienoic acid, (E, Z)- (RT: 7.678, 0.01%); 5,7-Octadien-2-ol, 2,6-dimethyl- (RT: 8.175, 0.08%); (2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide (RT: 8.466, 0.08%); 1-Nitro-bicyclo [6.1.0] nonan-2-one (RT: 8.529, 0.07%); 2-Cyclohexen-1-one, 3-methyl-6-(1-methyl ethenyl) - (RT: 8.909, 0.06%); Oxirane, 3-[5-(4-azido-2-nitrophenox)-3-methyl-3-pentenyl]-2,2-dimethyl-,(RT: 9.921, 0.08%); Cyclohexane methyl propanoate (RT: 10.403, 0.01%).

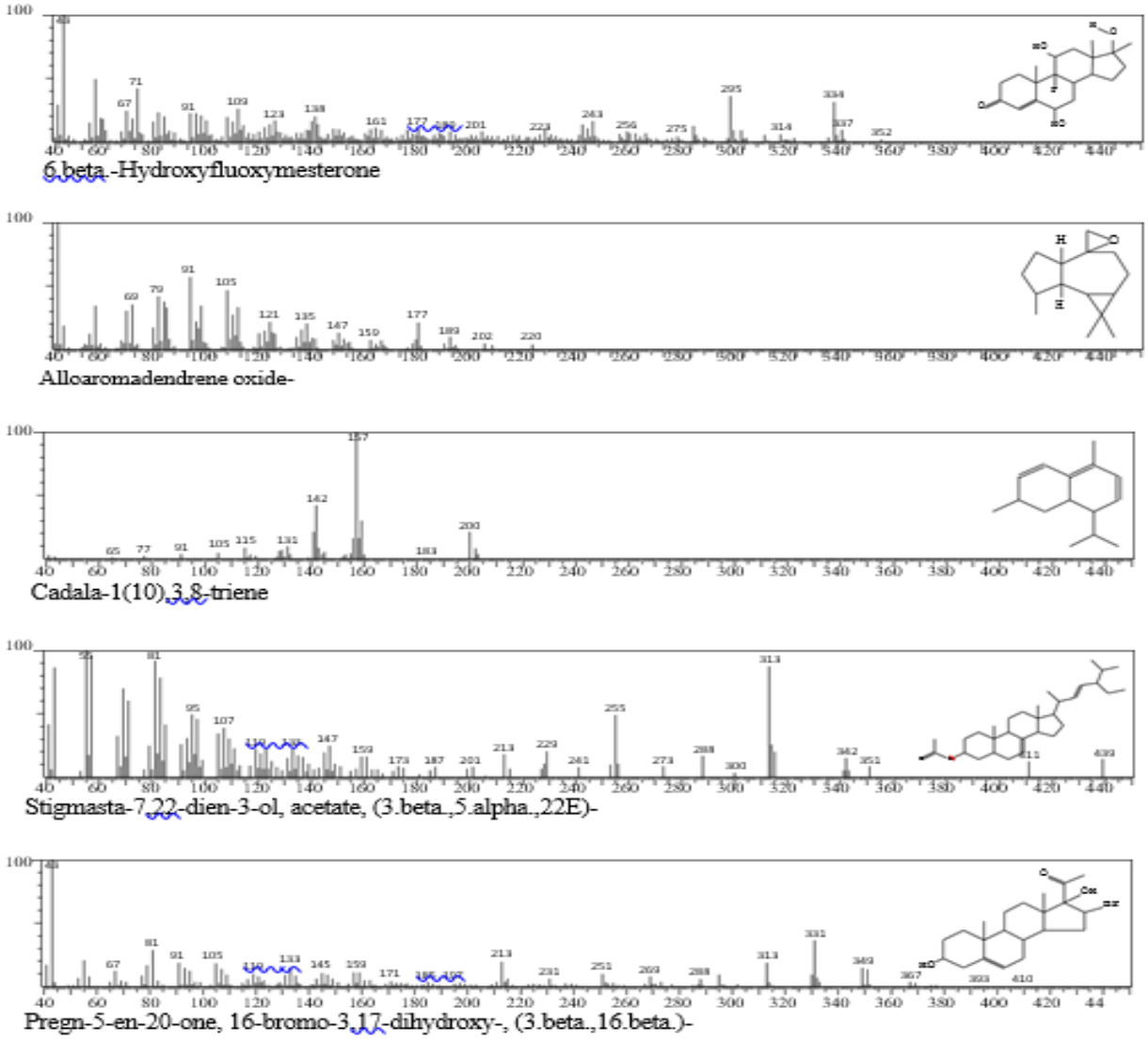

Figure 3: GC Mass spectra of 6.beta.-Hydroxyfluoxymesterone, Alloaromadendrene oxide-(1), Cadala-1(10),3,8-triene, Stigmasta-7,22-dien-3-ol, acetate, (3.beta.,5.alpha.,22E)- and Pregn-5-en-20-one.

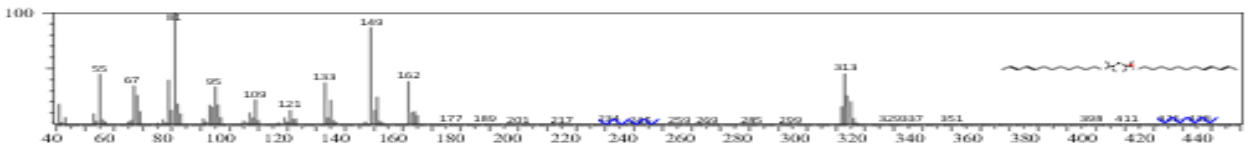

Figure 4: GC Mass spectrum of 1 ,3-bis[(8E,10E)-Dodeca-8,10-dien-1-yloxy]-1,1,3,3-tetramethyldisiloxane

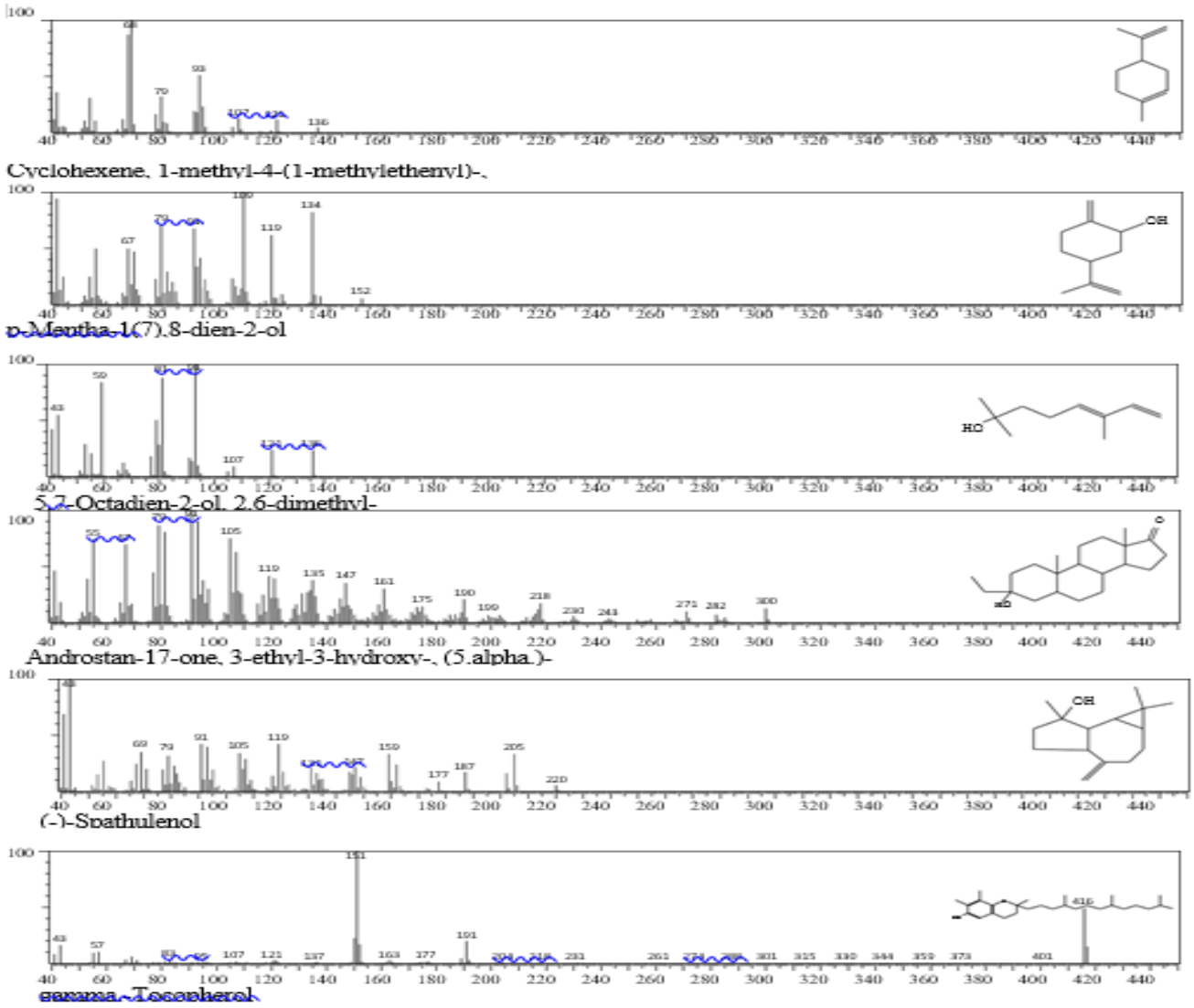

Figure 5: GC-Mass spectra of Cyclohexene, 1-methyl-4-(1-methylethenyl))-, (S)-, p-Mentha-1(7),8-dien-2-ol, 5,7-Octadien-2-ol, 2,6-dimethyl- ol, Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)- , (-)-Spathulenol, and .gamma.-Tocopherol

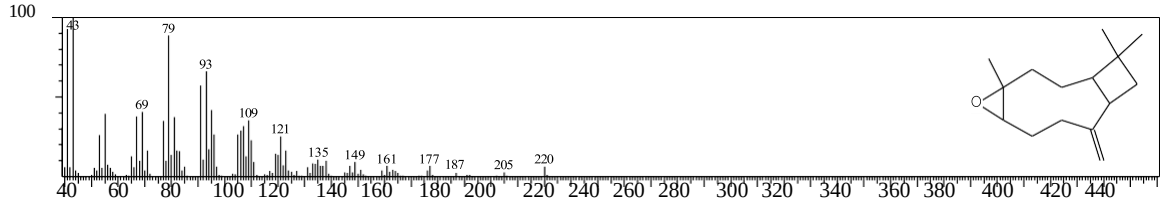

Figure 6: GC Mass spectrum of Caryophyllene oxide

Many of these nine minor compounds are monoterpenes, which are used as food additives for their flavouring and significant medicinal properties. [60] Significant for their medicinal activities among these compounds are limonene (cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-,) and trans-Pinocarveol (Bicyclo [3.1.1] heptan-3-ol, 6,6-dimethyl-2-methylene-). Limonene is used in the treatment of bronchitis. [61] The antioxidant, anticancer, anti-inflammatory, analgesic, and neuroprotective activities are recorded. [62, 63, 64.] Trans-Pinocarveol is a food flavour and also reported to exhibit anti-inflammatory, antibacterial, and insecticidal properties and tumour inhibitors. [65]

The next twenty-five compounds are trans-p-Mentha-2,8-dienol (RT: 7.401 mins, 0.3%); 2-cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans- (RT: 7.544 mins, 0.14%); p-Mentha-1(7),8-dien-2-ol (RT: 8.135 mins, 0.16%); Cyclohexanol, 2-methyl-5-(1-methyl ethenyl)- (RT: 8.217 mins, 0.28%); Methyl 3-amino-2-pyrazinecarboxylate (RT: 9.379 mins, 0.11%); 1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)- (RT: 9.533 mins, 0.27%); 4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one (RT: 9.657 mins, 0.28%); (-)-Aristolene (RT: 10.69 mins, 0.17%); 1H-Cyclopropa[a] naphthalene, 1a,2,3,5,6,7,7a,7b -octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alph a.,7.alpha., 7a.alp ha. ,7b.alpha.)]- (RT: 10.819 mins, 0.5%); 4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo [4.1.0] heptane (RT: 10.877 mins, 0.47%); Bicyclo[4.1.0]heptane, 7-chloro- (RT: 10.961 mins, 0.15%); Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)- (RT: 11.056 mins, 0.24%); cubedol (RT: 11.336 mins, 0.37%); 4-epi-cubedol (RT: 11.521 mins, 0.69%); alpha-Cacalorene (RT: 11.683 mins, 0.33%); Calarene epoxide (RT: 11.786, 11.927 mins; total area 0.84%); Glutaric acid, di(2-(2-methoxyethyl) heptyl) (RT: 11.849 mins, 0.32%);

(-)-Spathulenol (RT: 12.037 mins, 0.69%); Tricyclo [4.4.0.0 (2,7)] dec-8-ene-3-methanol, .alpha.,.alpha.,6,8-tetramethyl-, stereoisomer (RT:12.353 mins, 0.44%); Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methyl ethylidene)-, (4aR-trans)- (RT: 13.229 mins, 0.74%); Cholestane (RT: 21.441 mins, 0.91%); Tetrahydrosmilagenin (RT: 21.963 mins, 0.71%); Fumaric acid, 2-hexyl tetradecyl ester (RT: 22.308, 22.52 mins; total area 0.99%); 3H-3,10a-Methano-1,2-benzodi oxocin-3-ol, octahydro-7,7-dimethyl-, (3.alpha.,6a.beta., 10a.beta.)- (RT: 22.645 mins, 0.47%); and gamma.-Tocopherol (RT: 22.858 mins, 0.49%).

Significant among this set of twenty-five compounds for their bioactivity and medicinal uses are p-Mentha-2,8-dien-1-ol, p-Mentha-1(7),8-dien-2-ol, 5,7-Octadien-2-ol, 2,6-dimethyl-ol, Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-, (-)-Spathulenol, 3H-3,10a-Methano-1,2-benzodioxocin-3-ol, octahydro-7,7-dimethyl-, (3.alpha., 6a.beta.,10a.beta.)-, and gamma.-Tocopherol (Figure 5). Para-mentha-2,8-dien-1-ol exhibits antifungal and antibacterial activities, [65] p-Mentha-1(7),8-dien-2-ol and 5,7-Octadien-2-ol, 2,6-dimethyl-ol are fragrance ingredients that are used in decorative cosmetics, shampoos, fine fragrances, and other toiletries and detergents. [66, 67] Androstan-17-one, 3-ethyl-3-hydroxy-, (5. alpha.)- show antioxidant activities. [67] Spathulenol exhibits anticholinesterase, antioxidant, anti-oedematogenic, anti-proliferative, cytotoxic, and antimicrobial activities. [69, 70, 71, 72, 73] Some benzodioxocin derivatives are reported to show anticancer properties. [74] Gamma-tocopherol is a known form of vitamin E with anticancer and antioxidant activities. [26, 75]

The last set of ten compounds are Aromadendrene, dehydro- (RT: 10.762 mins, 1.12%); Caryophyllene oxide (RT: 12.097 mins, 1.1%); 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a. alpha., 4a. alpha., 7. beta., 7a. beta., 7b. alpha.)] - (RT: 12.526 mins, 1.34%); Isolongifolene, 9,10-dihydro- (RT: 12.968 mins, 1.05%); Tributyl acetylcitrate (RT: 16.913 mins, 1.98%); 5. alpha. -Androstan-3. beta. -ol, 4,4-dimethyl-, acetate (RT: 16.913 mins, 1.19%); D-Homoandrostane, (5. alpha.,13. alpha.) - (RT: 20.554 mins, 1.92%); Allopregnane-7. alpha., 11. alpha. -diol-3,20-dione (RT: 21.101 mins, 1.08%); 12-Hydroxy-3-keto-bisnor-4-cholenic acid (RT: 21.317 mins, 1.6%), and 2-methylhexacosane (RT: 21.74 mins; 1.83%). Significant among these compounds is caryophyllene oxide for its antifungal and cytotoxic activities (Figure 6). [76, 77]

Androstan-17-one, 3-ethyl-3-hydroxy-, (5. alpha.)- (RT: 11.056; 0.24%), 5. alpha. -Androstan-3. beta. -ol, 4, 4-dimethyl-, (RT: 17.405; 1.19%), and D-Homoandrostane, (5. alpha.,13. alpha.)- (RT: 20.554; 1.92%) are significant among the steroids from *A. ringens* n-hexane root extract as derivatives of the C19 steroidal hydrocarbon, Androstane. Androstane exists in two isomeric forms: 5alpha-androstane and 5beta-androstane, with the alpha isomer being the most potent. [78, 79] 5alpha-androstane functions as an androgen, serving as a foundation for several male hormones that are used in the management of male hormonal and sexual health challenges. [80] The detection of these compounds in small amounts within the n-hexane extract of *A. ringens* suggests the potential for sourcing these androgens from this medicinal plant.

Previous research work on *A. ringens* flowers, leaves, and stem indicated major monoterpenes like p-cymene (17.8%), limonene (20.0%), linalool (6.5%), and α-phellandrene (16.1%), as the contents of the stem, while the leaves consist majorly of sesquiterpenoids like trans 4(14), 5-muuroladiene (13.0%), β-caryophyllene (11.4%), spathulenol (8.0%), and methyl copalate (10.3%), a diterpenoid. [22] In this work on the n-hexane root extract, the results indicated the component compounds as terpenes, steroids, cannabinoids, esters, ethers, epoxides, alkanols, alkynes, phenolics, and organosilicon. Limonene and spathulenol are minor compounds in *A. ringens* n-hexane root extract.

FTIR Analysis

The FTIR spectrum (Figure 7) indicated the functional groups present in the component compounds in the extract. The technique is based on the principle that different molecules absorb frequencies of infrared light which correspond to the vibrational frequencies of the bonds in the molecule. [81] The absorption produces a characteristic spectrum from which the functional groups present in the molecule can be identified. [81,82] The FTIR analysis (Figure 7, Table 3) indicated O-H stretch for alkanols and phenols at 3327 cm^{-1}, [83] C-H stretch at 2921 cm^{-1} and 2854 cm^{-1}, [84] C=O stretch for alkanals, alkanones and esters at 1711 cm^{-1}, [84] C=C stretch for aromatics and others at 1640 cm^{-1} and 1462 cm^{-1}, [84] C-O stretch for ether and epoxide at 1380 cm^{-1}, and C-O stretch for alkanols at 1171 cm^{-1} and 1074 cm^{-1}, C-O. [83] This result is consistent with the functional groups in the compounds detected in the *A. ringens* n-hexane root extract.

Figure 7: FTIR spectrum of *A. ringens* n-hexane extract root extract

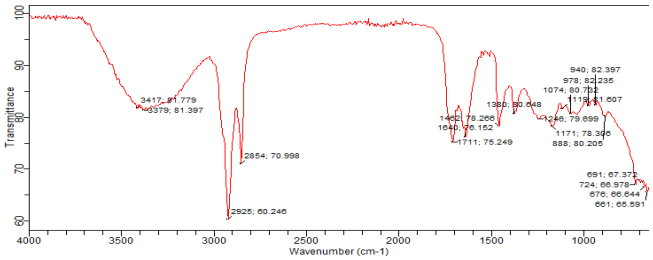

Table 3: FTIR analysis of *A. ringens* n-hexane root extract

S/N	Functional group	Vibrational Frequency (cm^{-1})	Description
1	O-H stretch	3327	Alkanol, phenols
2	C-H stretch	2921	Alkyl C-H stretch
3	C-H stretch	2854	Alkyl C-H stretch
4	C=O stretch	1711	Alkanal, Alkanone, Esters
5	C=C stretch	1640	Aromatic C=C
6	C=C stretch	1462	Aromatic C=C
7	C-O stretch	1380	ether and epoxide
8	C-O stretch	1171	Alkanol
9	C-O stretch	1074	Alkanol

Conclusion

This work has described the phytochemical composition of *A. ringens* n-hexane root extract using GC-MS. The result shows that the extract contains a wide array of volatile phytochemicals with known medicinal properties. The phytochemicals and their bioactivity are closely related to the ethnomedicinal uses of *A. ringens*. The detection of cannabinoids from this extract is, to the best of our knowledge, novel from the Aristolochia genus. The functional groups detected are related to the compounds eluted from this extract. This knowledge further authenticates the ethnomedicinal and other possible uses of this plant.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

References

1. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013; 10 (5):210-229. Doi: 10.4314/ajtcam.v10i5.2.

2. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE. Health benefits of herbs and spices: the past, the present, the future. Med J Aust. 2006; 185(S4): S1-S24.Doi: 10.5694/j.1326-5377.2006.tb00548.x.
3. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. 2013; 4(3): 384S-392S. Doi: 10.3945/an.112.003517.
4. Toroglu S. In-vitro antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. J Environ Biol. 2011; 32(1): 23-29.
5. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Niger. J. Biotechnol. 2007; 6:1690-1696.
6. Yu JQ, Liao ZX, Cai XQ, Lei JC, Zou GL. Composition, antimicrobial activity and cytotoxicity of essential oils from *Aristolochia mollissima*. Environ Toxicol Pharmacol. 2007; 23(2):162-167. Doi: 10.1016/j.etap.2006.08.004.
7. Minari JB, Idris MA. Forensic and Pharmacognostic Study of *Aristolochia ringens* Stem. J. Forensic Res. 2014; 6:1-4. Doi: 10.4172/2157-7145.1000257.
8. 8 Ruth AF, Olaide AO, Oluwatoyin SM. The aqueous root extract of *Aristolochia ringens* (Vahl.) Aristolochiaceae inhibits chemically induced inflammation in rodents. Pak J Pharm Sci. 2014; 27(6):1885-1889.
9. Olabanji S, Omobuwajo O, Ceccato D, Adebajo A, Buoso M, Moschini G. Accelerator-based analytical technique in the study of some anti-diabetic medicinal plants of Nigeria. Nucl. Instrum. Methods Phys. Res., B 2008; 266(10): 2387-2390. https://doi.org/10.1016/ j.nimb .2008 .03.016.
10. Sulyman AO, Akolade JO, Sabiu SA, Aladodo RA, Muritala HF. Antidiabetic potentials of ethanolic extract of *Aristolochia ringens* (Vahl.) roots. J Ethnopharmacol. 2016; 182:122-128. https://doi.org/10.1016/j.jep.2016.02.002.
11. Sonibare MA, Gbile ZO. Ethnobotanical survey of anti-asthmatic plants in South Western Nigeria. Afr J Tradit Complement Altern Med. 2008; 5(4):340-345. Doi:10.4314/ ajtcam. v5i4.31288.
12. Adeyemi OO, Aigbe FR, Badru OA. The antidiarrhoeal activity of the aqueous root extract of *Aristolochia ringens* (Vahl.) Aristolochiaceae. Nig Q J Hosp Med. 2012; 22(1):29-33.
13. Owolabi MS, Omowonuola A, Lawal OA, Labunmi L, Dosoky NS, Collins J, Ogungbe IV, Setzer WN. Phytochemical and bioactivity screening of six Nigerian medicinal plants. J Pharmacogn Phytochem, 2017; 6:1430-1437.
14. Mazadu EA, Misau MS, Gwallameji LB. Phytochemical screening and antimicrobial activity of some medicinal trees grown in Bauchi state, northeastern, Nigeria. J Pharmacogn Phytochem. 2018; 7:3503-3507.
15. Borokini TI, Ighere DA, Clement M, Ajiboye TO, Alowonle AA. Ethnobiological Survey of Traditional Medicine Practice for the Treatment of Piles and *Diabetes mellitus* in Oyo State. J. Med. Plants Stud. 2013; 1: 30-40.
16. Aigbe FR, Sofidiya OM, James AB, Sowemimo AA, Akindere OK, Aliu MO, Dosunmu AA, Chijioke MC, Adeyemi OO. Evaluation of the toxicity potential of acute and sub-acute exposure to the aqueous root extract of *Aristolochia ringens* Vahl. (Aristolochiaceae). J Ethnopharmacol. 2019; 244:112150. Doi: 10.1016/j.jep.2019.112150.
17. Odugbemi T. Textbook of Medicinal Plants from Nigeria. University of Lagos Press; 2008. 550 p.
18. Lerma-Herrera MA, Beiza-Granados L, Ochoa-Zarzosa A, López-Meza JE, Navarro-Santos P, Herrera-Bucio R, Aviña-Verduzco J, García-Gutiérrez HA. Biological Activities of Organic Extracts of the Genus *Aristolochia*: A Review from 2005 to 2021. Molecules. 2022; 27(12): 3937. Doi: 10.3390/molecules27123937.
19. Akindele AJ, Wani Z, Mahajan G, Sharma S, Aigbe FR, Satti N, Adeyemi OO, Mondhe DM. Anticancer activity of *Aristolochia ringens* Vahl. (Aristolochiaceae). J Tradit Complement Med. 2014;5(1):35-41. Doi: 10.1016/j.jtcme.2014.05.001.
20. Akoro SM, Ogundare OC, Omotayo MA, Durosimi D, Awofeso DO. Investigation of the phytochemical contents, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of *Aristolochia ringens* (vahl.) root. JRRS. 2022; 9: 33-39. Doi: 10.36108/jrrslasu /2202.90.0150.
21. Arannilewa ST. A simple laboratory prescreen for plants with grain protectant effects against the maize weevil; *Sitophilus zeamais* (Mots) (Coleoptera: Curculionidae). Agric J. 2007; 2:736-739. https://doi.org/aj.2007.736.739.
22. Stashenko EE, Andrés Ordóñez S, Marín NA, Martínez JR. Determination of the volatile and semi-volatile secondary metabolites and aristolochic acids in *Aristolochia ringens* Vahl. J Chromatogr Sci. 2009; 47(9):817-821. Doi: 10.1093/chromsci/47.9.817.
23. Grimm F, Fets L, Anastasiou D. Gas Chromatography Coupled to Mass Spectrometry (GC-MS) to Study Metabolism in Cultured Cells. Adv Exp Med Biol. 2016; 899:59-88. Doi: 10.1007/978-3-319-26666-4_5.
24. Ihegboro GO, Ononamadu CJ, Owolarafe TA, Onifade O, Udeh JJ, Saliu AO, Abolaji DD, Ibrahim YM. Title: In vitro Investigation and GC MS Analysis of the Chemical Constituents in the Fraction of Hexane Leaf Extract of *Tapinanthus bangwensis* (Engl. and K. Krause) Loranthaceae. Trop J Phytochem Pharm. Sci. 2024; 3(1):143-152. http://www.doi.org/10. 26538 /tjpps/v3i1.5.
25. Olaoye AB, Idowu KS, Awonegan AP. GC-MS Fingerprinting of Methanolic Extract of *Moringa oleifera* Stem, Leaf and Root. Trop J Phytochem Pharm. Sci. 2024; 3(4):254-260. https://www.tjpps.org/index.php/home/article/view/78.
26. Akoro S, Ogundare O, Oyedola A. Comparative GC-MS Analysis, Antioxidant and cytotoxic activities of *Garcinia kola* Heckel seed and stem-bark *n*-hexane extract. J. Med Herb. 2023; 14(2): 35-43. Doi: 10.30495/medherb.2023. 707916.
27. Skoog DA, Hanlan J, West DM. Principles of instrumental analysis. (7th ed.). Cengage; 2016. 303-566 p.
28. Madurapperumage A, Johnson N, Thavarajah P, Tang L, Thavarajah D. Fourier-transform infrared spectroscopy (FTIR) as a high-throughput phenotyping tool for quantifying protein quality in pulse crops. Plant Phenome j. 2022; 5(1): e20047. https://doi.org/10.1002/ppj2.20047.
29. Ijoma I, Ishmael V, Ajiwe V, Ndubuisi J. (2022). Evidence-based preferential in vitro antisickling mechanism of three native Nigerian plants used in the management of sickle cell disease. MJBMB. 2022; 3: 9-17.
30. Bolade OP, Akinsiku AA, Adeyemi AO, Williams AB, Benson NU. Dataset on phytochemical screening, FTIR and GC–MS characterisation of *Azadirachta indica* and *Cymbopogon citratus* as reducing and stabilising agents for nanoparticles synthesis. Data Brief. 2018; 20:917-926. https://doi.org/10.1016/j.dib.2018.08.133.
31. Teoh ES. Secondary Metabolites of Plants. In: Medicinal Orchids of Asia, Springer, Cham. 2016; 59–73.
32. Twaij BM, Hasan MN. Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses. Int. J. Plant Biol. 2022; 13(1):4-14. https://doi.org/10.3390/ijpb13010003.
33. Ogunlesi M, Okiei W, Osibote E. Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. Afr. J. Biotechnol. 2010; 9:1060-1067. Doi: 10.5897/AJBO9.941.

34. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and Medicinal Uses of Terpenes. Medicinal Plants. 2019; 333–359. Doi: 10.1007/978-3-030-31269-5_15.
35. Abyadeh M, Gupta V, Paulo JA, Gupta V, Chitranshi N, Godinez A, Saks D, Hasan M, Amirkhani A, McKay M, Salekdeh GH, Haynes PA, Graham SL, Mirzaei M. A Proteomic View of Cellular and Molecular Effects of Cannabis. Biomolecules. 2021; 11(10):1411. Doi: 10.3390/ biom11101411.
36. Nusantara GB, Rahmania T. Identification of Δ9-tetrahydrocannabinol compounds in *Cannabis sativa* using gas chromatography-mass spectrometry. Pharm Educ. 2024; 24:43-49. https://doi.org/10.46542/pe.2024.246.4349.
37. Blaskovich MAT, Kavanagh AM, Elliott AG, Zhang B, Ramu S, Amado M, Lowe GJ, Hinton AO, Pham DMT, Zuegg J, Beare N, Quach D, Sharp MD, Pogliano J, Rogers AP, Lyras D, Tan L, West NP, Crawford DW, Peterson ML, Callahan M, Thurn M. The antimicrobial potential of cannabidiol. Commun Biol. 2021; 4(1):7. Doi: 10.1038/s42003-020-01530-y.
38. Hussain SA, Zhou R, Jacobson C, Weng J, Cheng E, Lay J, Hung P, Lerner JT, Sankar R. Perceived efficacy of cannabidiol-enriched cannabis extracts for treatment of pediatric epilepsy: A potential role for infantile spasms and Lennox-Gastaut syndrome. Epilepsy behave. 2015; 47:138–141. Doi: 10.1016/j.yebeh.2015.04.009.
39. Kis B, Ifrim FC, Buda V, Avram S, Pavel IZ, Antal D, Paunescu V, Dehelean CA, Ardelean F, Diaconeasa Z, Soica C, Danciu C. Cannabidiol-from Plant to Human Body: A Promising Bioactive Molecule with Multi-Target Effects in Cancer. Int J Mol Sci. 2019; 20(23):5905. https://doi.org/10.3390/ijms20235905.
40. Wang X, Zhang H, Liu Y, Xu Y, Yang B, Li H, Chen L. An overview on synthetic and biological activities of cannabidiol (CBD) and its derivatives. Bioorg Chem. 2023; 140:106810. Doi: 10.1016/j.bioorg.2023.106810.
41. Śledziński P, Zeyland J, Słomski R, Nowak A. The current state and future perspectives of cannabinoids in cancer biology. Cancer Med. 2018;7(3):765-775. Doi: 10.1002/cam4.1312.
42. Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants (Basel). 2019; 9(1):21. Doi: 10.3390/antiox9010021.
43. Robson PJ. Therapeutic potential of cannabinoid medicines. Drug Test. Anal. 2014; 6: 24–30. https://doi.org/10.1002/dta.1529.
44. Shah SA, Gupta AS, Kumar P. Emerging role of cannabinoids and synthetic cannabinoid receptor 1/cannabinoid receptor 2 receptor agonists in cancer treatment and chemotherapy-associated cancer management. J Cancer Res Ther. 2021;17(1):1-9. Doi: 10.4103/jcrt.JCRT_488 _18.
45. De Vries M, van Rijckevorsel DC, Wilder-Smith OH, van Goor H. Dronabinol and chronic pain: importance of mechanistic considerations. Expert Opin Pharmacother. 2014;15(11):1525-1534. Doi: 10.1517/14656566.2014.918102.
46. Carley DW, Prasad B, Reid KJ, Malkani R, Attarian H, Abbott SM, Vern B, Xie H, Yuan C, Zee PC. Pharmacotherapy of Apnea by Cannabimimetic Enhancement, the PACE Clinical Trial: Effects of Dronabinol in Obstructive Sleep Apnea. Sleep, 2018; 41(1): zsx184. https://doi.org/ 10.1093/sleep/zsx184.
47. Sampson PB. Phytocannabinoid Pharmacology: Medicinal Properties of *Cannabis sativa* Constituents Aside from the "Big Two". J Nat Prod. 2021; 84 (1):142-160. Doi: 10.1021/acs.jnatprod.0c00965.
48. Kuzminac IZ, Bekić SS, Ćelić AS, Jakimov DS, Sakač MN. Antitumor potential of novel 5α,6β-dibromo steroidal D-homo lactone. Steroids, 2022; 188:109118. https://doi.org/10.1016/ j.steroids.2022.109118.
49. Manni A, Pearson OH, Marshall JS, Arafah BM. Sequential endocrine therapy and chemotherapy in metastatic breast cancer: effects on survival. Breast Cancer Res Treat. 1981;1(2):97-103. https://doi.org/10.1007/BF01805861.
50. Lenko HL, Leisti S, Perheentupa J. The efficacy of growth hormone in different types of growth failure. An analysis of 101 cases. Eur J Pediatr. 1982;138 (3):241-249. Doi: 10.1007/BF00441210.
51. Tebbaa M, Hakmaoui AE, Benharref A, Akssira M. Short and efficient hemisynthesis of α-eudesmol and cryptomeridiol Tetrahedron Lett., 2011; 52 (29): 3769-3771. https://doi.org/ 10.1016/ j. tetlet.2011.05.064.
52. Chen J, Ge S, Liu Z, Zhang D, Peng W. GC-MS explores health care components in the extract of Pterocarpus Macarocarpus Kurz. Saudi J Biol Sci. 2018; 25 (6):1196-1201. Doi: 10.1016/j.sjbs.2017.12.013.
53. Hamza YG, Danyaya AI, Lawal M. An In silico Analysis of Some Bioactive Compounds of *Psidium guajava* against Target Proteins of *Vibrio cholerae*. Asian J. Biochem. Gen. Mol. Biol. 2020;6 (4):14-20. Doi:10.9734/ajbgmb/2020/v6i430158
54. Madhubala M, Santhi G. Phytochemical and GC-MS analysis on leaves of selected medicinal plants in Boraginaceae family *Cordia dichotoma* L. Pramana Res. J. 2019; 9: 2249–2276.
55. Cabeza M, Bratoeff E, Flores E, Ramírez E, Calleros J, Montes D, Quiroz A, Heuze I. 5 Alpha-reductase inhibitory and antiandrogenic activities of novel steroids in hamster seminal vesicles. Chem Pharm Bull (Tokyo). 2002; 50 (11):1447-1452. Doi: 10.1248/cpb.50.1447.
56. Bains W, Tacke R. Silicon chemistry as a novel source of chemical diversity in drug design. Curr Opin Drug Discov Devel. 2003; 6 (4):526-43.
57. Röshe L, John P, Reitmeier R. "Organic Silicon Compounds" Ullmann's Encyclopedia of Industrial Chemistry. John Wiley and Sons: San Francisco, 2003
58. Mills JS, Showell GA. Exploitation of silicon medicinal chemistry in drug discovery. Expert Opin Investig Drugs. 2004;13(9):1149-1457. Doi: 10.1517/13543784.13.9.1149.
59. Gadhe CG, Cho SJ. Importance of Silicon Atom in the Drug Design Process. J. of the Chosun Natural Science. 2012; 5 (4): 229 – 232. Doi: 10.13160/ricns.2012.5.4.229.
60. Caputi L, Aprea E. Use of terpenoids as natural flavouring compounds in food industry. Recent Pat Food Nutr Agric. 2011; 3(1):9-16. Doi: 10.2174/2212798411103010009
61. Hirota R, Nakamura H, Bhatti SA, Ngatu NR, Muzembo BA, Dumavibhat N, Eitoku M, Sawamura M, Suganuma N. Limonene inhalation reduces allergic airway inflammation in Dermatophagoides farinae-treated mice. Inhal Toxicol. 2012; 24(6):373-381. Doi: 10.3109/08958378.2012.675528.
62. Eddin LB, Jha NK, Meeran MFN, Kesari KK, Beiram R, Ojha S. Neuroprotective Potential of Limonene and Limonene Containing Natural Products. Molecules. 2021; 26(15):4535. Doi: 10.3390/molecules26154535.
63. Chen X, Ding Y, Guan H, Zhou C, He X, Shao Y, Wang Y, Wang N, Li B, Lv G, Chen S. The Pharmacological Effects and Potential Applications of Limonene from Citrus Plants: A Review. Nat. Prod. Commun. 2024; 19. (5). https://doi.org/10.1177/1934578X241254229.
64. Scuteri D, Rombolà L, Crudo M, Watanabe C, Mizoguchi H, Sakurada S, Hamamura K, Sakurada T, Tonin P, Corasaniti MT, Bagetta G. Preclinical Characterization of Antinociceptive Effect of Bergamot Essential Oil and of Its Fractions for Rational Translation in Complementary Therapy. Pharmaceutics. 2022;14(2):312. Doi: 10.3390/pharmaceutics14020312.
65. Sánchez-Velandia JE, Valdivieso LM, Martínez O F, Mejía SM, Villa AL, Wärnå J, Murzin DY. Synthesis of trans-pinocarveol from oxidation of β-pinene using

multifunctional heterogeneous catalysts. Mol. Catal. 2023; 541:113104. https://doi.org/10.1016/j.mcat.2023. 113104.
66. Ambrosio CMS, Diaz-Arenas GL, Agudelo LPA, Stashenko E, Contreras-Castillo CJ, da Gloria EM. Chemical Composition and Antibacterial and Antioxidant Activity of a Citrus Essential Oil and Its Fractions. Molecules. 2021; 26(10):2888. Doi: 10.3390/ molecules 26102888.
67. Bhatia SP, McGinty D, Letizia CS, Api AM. Fragrance material review on p-mentha-1,8-dien-7-ol. Food Chem Toxicol. 2008;46 Suppl 11: S197-200. Doi: 10.1016/j.fct.2008.06.071.
68. Lapczynski A, Bhatia SP, Letizia CS, Api AM. Fragrance material review on ocimenol. Food Chem Toxicol. 2008;46 Suppl 11: S251-S252. https://doi.org/10.1016/j.fct.2008.06. 064
69. 69.Youssef AMM, Maaty DAM, Al-Saraireh YM. Phytochemical Analysis and Profiling of Antioxidants and Anticancer Compounds from *Tephrosia purpurea* (L.) subsp. *apollinea* Family Fabaceae. Molecules (Basel, Switzerland), 2023; 28(9): 3939. Doi: 10.3390/molecules28093939.
70. Dos Santos E, Radai JAS, do Nascimento KF, Formagio ASN, de Matos Balsalobre N, Ziff EB, Castelon Konkiewitz E, Kassuya CAL. Contribution of spathulenol to the anti-nociceptive effects of *Psidium guineense*. Nutr Neurosci. 2022;25(4):812-822. Doi: 10.1080/1028415X. 2020.1815330. 25(4):812-822.
71. Durán-Peña MJ, Botubol Ares JM, Hanson JR, Collado IG, Hernández-Galán R. Biological activity of natural sesquiterpenoids containing a gem-dimethylcyclopropane unit. Nat Prod Rep. 2015;32(8):1236-1248. Doi: 10.1039/c5np00024f.
72. Karakaya S, Yilmaz SV, Özdemir Ö, Koca M, Pınar NM, Demirci B, Yıldırım K, Sytar O, Turkez H, Baser KHC. A caryophyllene oxide and other potential anticholinesterase and anticancer agent in *Salvia verticillata* subsp. amasiaca (Freyn & Bornm.) Bornm. (Lamiaceae). J. Essent. Oil Res. 2020; 32(6): 512-525. Doi:10.1080/10412905.2020.1813212.
73. Martins A, Hajdú Z, Vasas A, Csupor-Löffler B, Molnár J, Hohmann J. Spathulenol inhibit the human ABCB1 efflux pump. Planta Med. 2010;76(12): P608. Doi:10.1055/s-0030-1264906.
74. Murugesan A, Kari S, Shrestha A, Assoah B, Saravanan KM, Murugesan M, Thiyagarajan R, Candeias NR, Kandhavelu M. Methanodibenzo[*b*,*f*][1,5]dioxocins as Novel Glutaminase Inhibitor with Anti-Glioblastoma Potential. Cancers (Basel). 2023;15(4):1010. Doi: 10.3390/cancers15041010.
75. Jiang Q, Christen S, Shigenaga MK, Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr. 2001;74(6):714-722. Doi: 10.1093/ajcn/74.6.714.
76. Gille L, Monzote L, Stamberg W, Staniek K. Toxicity of ascaridole from *Chenopodium ambrosioides* in mammalian mitochondria. BMC Pharmacol. 2010; 10: A10. Doi: 10.1186/1471-2210-10-S1-A10.
77. Yang D, Michel L, Chaumont JP, Millet-Clerc J. Use of caryophyllene oxide as an antifungal agent in an in vitro experimental model of onychomycosis. Mycopathologia. 1999;148(2):79-82. Doi: 10.1023/a:1007178924408.
78. Handa RJ, Sharma D, Uht RM. A Role for the Androgen Metabolite, 5alpha Androstane 3beta, 17beta Diol (3β-Diol) in the Regulation of the Hypothalamo-Pituitary–Adrenal Axis. Front. Endocrinol., 2011; 2:14098. https://doi.org/10.3389/fendo.2011.00065
79. Mariotti AJ. (2016). Steroid Hormones of Reproduction and Sexual Development. Pharmacology and Therapeutics for Dentistry (7th Ed.). 2016; 446-456. https://doi.org/10.1016/B978-0-323-39307-2.00032-1
80. Shelley J, Moir HJ, Petróczi A. The Use and Misuse of Testosterone in Sport: The Challenges and Opportunities in Doping Control. In: Bagchi D, Nair S, Sen C. (Eds.). Nutrition and Enhanced Sports Performance: Muscle building, endurance, and strength. (2nd ed.). Academic Press Ltd-Elsevier Science Ltd; 2019. 571-580 p. https://doi.org/10.1016/B978-0-12-813922-6.00048-5
81. Nandiyanto A. B. D., Oktiani R., Ragadhita R. (2019). How to read and interpret FT-IR spectroscopy of organic material. Indo. J. Sci. Technol. 4, 97–118. Doi: 10.17509/ijost. v4i1.15806.
82. Kassem A, Abbas L, Coutinho O, Opara S, Najaf H, Kasperek D, Pokhrel K, Li X, Tiquia-Arashiro S. Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Front Microbiol. 2023 Nov 21; 14:1304081. doi: 10.3389/fmicb.2023.1304081. Erratum in: Front Microbiol. 2023; 14:1342406. doi: 10.3389/fmicb.2023.1342406.
83. Smith BC. Alcohols—The Rest of the Story**.** Spectroscopy. 2017; 32 (4): 19-27.
84. Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacog J. 2017; 9 (6):952-956. Doi: 10.5530/pj.2017.6.149.