Sesquiterpenes: The Major Bioactive Constituent of the Nigerian *Chromolaena odorata* (Siam Weed) Leaf Extracts.

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Abstract- ***Employing phytochemicals from medicinal plants in drug synthesis is crucial since drug-resistant infections and diseases pose serious threat to life. This study investigated the chemical composition and antimicrobial activity of the Nigerian Chromolaena odorata based on its ethnomedical use. The crude extract was obtained by extracting the air-dried, powdered leaves of Chromolaena odorata using methanol. Using standard procedures, the extract was tested for phytochemicals. The fractions of the crude extract that were soluble in hexane, chloroform, and ethylacetate were separated. The activity of the crude extract and its fractions against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Candida albicans was assessed. By using GC-MS analysis, the chemical components of the chloroform-soluble fraction that exhibited the strongest inhibitory activity were identified. The phytochemical screening showed that saponins and glycosides were absent, but terpenoids, alkaloids, tannins, steroids/sterols, and volatile oils were present. The gram-negative bacterium E. coli was insensitive to the crude extract and fractions, while they showed inhibitory activity against the gram-positive bacteria S. aureus and B. subtilis. The only extracts that showed activity against Candida albicans were the ethylacetate and chloroform soluble fractions. Terpenes with known biological activity were the primary compounds identified by GC-MS analysis of the bioactive chloroform-soluble fraction extract. Terpenes may be responsible for the plant extracts' action. These results support the traditional use of Chromolaena odorata and show its potential as a natural source of antimicrobial compounds.***

Indexed Terms- ***Antimicrobial Activity, Chromolaena Odorata, Phytochemicals, Sesquiterpenes***

I. INTRODUCTION

Investigating medicinal plants' potential for the synthesis of novel drugs is essential and greatly advances the pharmaceutical industry. Alkaloids, flavonoids, terpenoids, and polyphenols are among the many bioactive substances found in these plants. These compounds have a variety of chemical structures and biological functions, making them promising for use in the development of new drugs [1]. They are ideal for treating a variety of illnesses, including cancer, infections, inflammation, and neurodegenerative diseases, due to their diverse pharmacological actions [2].

Utilising the resources of medicinal plants is essential given the growing threat of drug-resistant infections and illnesses. Plant-based compounds present promising approaches to address medication resistance and enhance the effectiveness of existing treatments [3].

Identification and extraction of bioactive chemicals from medicinal plants have been transformed by recent developments in biotechnology and screening techniques. The effectiveness of drug development procedures has significantly increased because of methods like high-throughput screening and genomic analysis [4].

The perennial herbaceous plant *Chromolaena*

odorata, also referred to as Siam weed or Chromolaena, is indigenous to the Americas but is found throughout tropical regions of the world. Different plant parts, including leaves, stems, and roots, are employed in traditional folk medicine due to their medicinal properties. Traditional uses include managing respiratory conditions, treating skin infections, and mending wounds [5].

The extract from *Chromolaena odorata* has been reported to be active against strains of *S. suis* [6]. Strong inhibitory activity was demonstrated by the leaf extracts in ethanol, methanol, and hexane solvents against the gram-negative bacterial strain *Proteus vulgaris* and the gram-positive bacterial strains *Propionibacterium acnes, Bacillus cereus, Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus aureus, and Streptococcus pyogenes*. While the hexane root extract demonstrated high inhibitory activity against *Enterococcus faecalis*, and *Klebsiella pneumoniae,* the hexane stem extract demonstrated greater inhibitory activity against *Pseudomonas aeruginosa*, *B. cereus*, and *Klebsiella pneumoniae*.[7].

The study intends to explore the chemical components and antimicrobrial potency of the Nigerian *Chromolaena odorata* leaf extracts used in traditional medicine.

II. MATERIALS AND METHODS

Plant material collection and identification

In March 2024, leaves of *Chromolaena odorata* were collected from Veritas University, Abuja. A specimen with the voucher number NIPRD/H/7407 was deposited after the leaves were verified at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu. After being weighed, the leaves were cleaned with running distilled water. For two weeks, they were kept at room temperature and out of the sun on a lab bench to dry. An electric blender was then used to grind the dried plant material into a powder.

Extraction of dried powdered plant

2000 mL of methanol was used to cold macerate the dried powdered leaf of *Chromolaena odarata* for 48 hours in a glass jar while being shaken occasionally. A glass funnel and filter paper were then used to filter the extract. The filtrate was dried by evaporating it in a rotary evaporator set to 40 °C. The result was a solid residue that was dark green in colour. The best component separation was seen using four spots on a thin-layer chromatography (TLC) plate, which is an aluminium sheet that has been pre-coated with silica gel. The crude extract was spotted on the plate using a 1:7 ethyl acetate/hexane mobile phase.

Fractionation

Hexane (2x150 cm^3) was added to a 250 ml beaker that held the crude methanolic extract, and the mixture was given a few minutes to stand. The hexane-soluble fraction was then extracted by decanting it. After hexane was removed from the residue, it was successively extracted using chloroform (2x150 cm^3), and ethyl acetate (2x150 cm^3) to give the chloroform-soluble, ethylacetate-soluble fractions (Figure 1). After evaporating each fraction to dry it out, the residue was weighed.

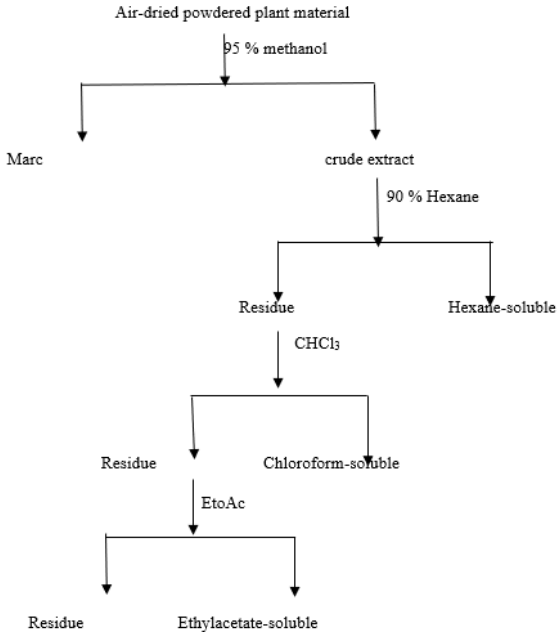


Fig. 1: Scheme for fractionating Chromolaena odorata, crude extract

Preliminary Phytochemical Screening

A preliminary qualitative screening of phytochemicals was conducted for the presence ofoils and fats,

phytosterols, alkaloids, tannins, glycosides, and saponins in the crude methanolic extract of *C. odorata* using the technique by Obi & Okwute [8].

Antimicrobial Screening

A standard protocol was used to conduct the antimicrobial screenings, which included sensitivity tests, minimum inhibitory concentration (MIC), and minimum bacteriocidal concentration (MBC) [8].

Sensitivity test for determining inhibitory activity

The agar well diffusion method was used to conduct the sensitivity test. A sterile swab stick was used to streak the standardised inocula of the bacterial and fungal isolates onto sterile Mueller Hinton and Potatoe dextrose agar plates, respectively. Each inoculated agar plate had four wells punched into it using a sterile cork borer. The extract concentrations (12.5, 25, 50, and 100, mg/ml, respectively) were used to appropriately label the wells. Each well received a 0.2 mL addition of the extract. To enable the extract to penetrate the agar, the infected plates containing the extract were left on the bench for approximately an hour. The incubated plates were then examined for indications of inhibition, which manifested as a distinct zone of inhibition surrounding the wells. A clear ruler that was calibrated in millimetres was us ed to measure these inhibitory zones' diameter.

Minimum inhibitory concentration (MIC) Determination

Mueller Hinton Broth was employed as a diluent in the tube dilution method to determine the extracts' minimal inhibitory concentration. In a test tube filled with Mueller Hinton broth, the extract was serially diluted to the lowest dose that inhibited each organism during the sensitivity test. Each tube containing the broth and extract was filled with the standardised organisms. For twenty-four hours, the infected tubes were incubated at 37 °C. Using turbidity as a criteria, the tubes were inspected for growth at the conclusion of the incubation time. The minimum inhibitory concentration (MIC) was determined by choosing the lowest concentration in the series that showed no growth or overt symptoms.

Minimum bacteriocidal concentration (MBC) Determination

The MIC test findings were used to calculate the extract's MBC.
Test tubes with no turbidity (clear) in the MIC test were filled with a sterile wire loop, and a loopful was streaked over sterile nutritional agar plates. For 18 to 24 hours, the plates were incubated at 37 °C. The plates were examined for growth after the incubation time. Determining whether the extract's antibacterial activity was bacteriostatic or bacteriocidal was the goal of this assessment.

GC-MS analysis of the chloroform-soluble fraction of Chromolaena odorata

A mass-selective detector was connected to an Agilent gas chromatographic column (30 m x 0.25 mm x 0.25 mm). Methanol and 1-pentanol were used as internal standards to dilute the chloroform-soluble extract sample. Three microlitres of the diluted chloroform-soluble sample were then added to the GC-MS for examination. The ion source's temperature was 230 ºC, and the injector's was 250 ºC. The oven's temperature ranges from 50 to 160 ºC. The MS had a scanning range of 30 to 400 amu.

III. RESULTS AND DISCUSSION

Extraction and fractionation

All the extracts were dark green gums. The cold maceration of 595.19 g of dried leaves produced 26.47 g of crude, a yield of about 4.45 % relative to the plant material. Fractionation of the crude methanolic extract yielded hexane-soluble, chloroform-soluble, and ethylacetate-soluble fractions. The chloroform-soluble fraction had the highest yield among the fractions (3.19 g, 12.05 %) (Table 1).

Table 1: Physical features and extractive yields of *Chromolaena odarata* leaves

Extractives	Yield in (g) (%) of crude and relative to crude	Colour and Consistency
Crude	(26.47) (4.45)	Dark green gum
Hexane-soluble	(0.55) (2.08)	Dark green gum
Chloroform-soluble	(3.19) (12.05)	Dark green gum
Ethylacetate-soluble	(0.13) (0.49)	Dark green shiny gum

Phytochemical Analysis

According to a phytochemical analysis of the crude leaf extract, volatile oils, terpenoids, alkaloids, tannins, and sterols were present while saponins and glycosides were absent (Table 2).

Table 2: Phytochemical screening of the crude methanolic extract of the leaf of *Chromolaena odorata*

Secondary Metabolites	Result
Tannins	+
Alkaloids	+
Sterols	+
Oils & fats	+
Saponins	-
Glycoside	-
Terpenoids	+

(+) represents present, (-) represents absent.

Antimicrobial screening

When screened for antimicrobial activity, the crude extract and fractions of *C. odorata* showed strong inhibitory activity against the gram-positive *S. aureus* and *B. subtillis* at MIC value of 6.25 mg/ml. The gram-negative bacterium, *E. coli* was insensitive to all the extracts (Table 3). The antifungal properties of the plant extract may be attributed to the chloroform and ethylacetate soluble fractions which were active against *C. albicans* at MIC value of 6.25 mg/ml. The crude extract had no activity against the fungus. Fractionation has therefore aided in the crude extract's distribution and increased activity against *C. albicans.*

Table 3: Inhibitory activity of the crude extract and fractions of *Chromolaena odorata*

TEST ORGANISM	Zone of Inhibition (mm) at different extract concentration (mg/ml)															
	HEX			$CHCl_3$			EtOAC			CRUDE			C
	25.0	12.5	6.25	25.0	12.5	6.25	25.0	12.5	6.25	25.0	12.5	6.25	
S. aureus	12	8	4	8	6	2	8	4	2	12	8	4	30	
B. subtillis	12	10	4	12	8	4	12	10	4	12	10	4	38	
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	33	
C. albicans	-	-	-	8	4	2	12	8	4	-	-	-	30	

KEY: (-) = No Activity

Hex = Hexane-soluble fraction

$CHCl_3$ = Chloroform-soluble fraction

EtOAc = Ethylacetate-soluble fraction

MeOH-$CHCl_3$ = Methanol-chloroform-soluble fraction

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) / Fungicidal Concentration (M.F.C) of the extracts

TEST ORGANISMS	MIC				MBC			
	HEX	$CHCl_3$	EtOAc	C	HEX	$CHCl_3$	EtOAc	C
S. aureus	6.25	6.25	6.25	30	25	12.5	12.5	30
B. subtillis	6.25	6.25	6.25	38	25	12.5	12.5	38
E. coli	ND	ND	ND	33	ND	ND	ND	33
C. albicans	ND	6.25	6.25	30	ND	ND	ND	30

KEY: ND/- = Not Determined for M.I.C/M.B.C

HEX = Hexane-soluble Fraction

$CHCl_3$ = Chloroform=soluble Fraction

EtOAc = Ethylaccetate-soluble Fraction

C = Control = Bacteria Fungi = Ciprofloxacin Nystatin

Analysis of the chloroform-soluble fraction of C. odorata using GC-MS

A 60% quality of characterisation and a 1 % area percentage indicated fifteen peaks in the gas chromatogram of the bioactive chloroform-soluble fraction of *C. odarata* (Figure 2).

The major compounds identified are cyclic terpenes (Fig. 3), especially sesquiterpenes (Table 5), which agrees with earlier phytochemical screening. Monoterpenes and sesquiterpenes display modest antioxidant, antibacterial and cytotoxic activities [9]. It has been observed that (E)-caryophyllene exhibits inhibitory effect against *Proteus mirabilis* and *Bacillus cereus*. (E)-caryophyllene did not affect the fungi *Candida albicans*, the bacteria *Micrococcus luteus, Klebsiella sp.,* or *Escherichia coli* [9]. Additionally, an essential oil containing caryophyllene, geijerene, and copaene showed excellent antibacterial action against *E. coli*, with a MIC value of 4.2 ± 0.5 mg/mL and an inhibitory zone of 16.4 ± 0.4 mm [10]. With minimum inhibitory concentrations (MIC) of 62.5 and 500.0 μg mL−1, respectively, (E)-caryophyllene, γ-muurolene, and viridiflorene were found to be the main oil constituents of a plant that had inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus* [11]. These compounds may therefore be responsible for the antimicrobial properties of *C. odorata* extracts.

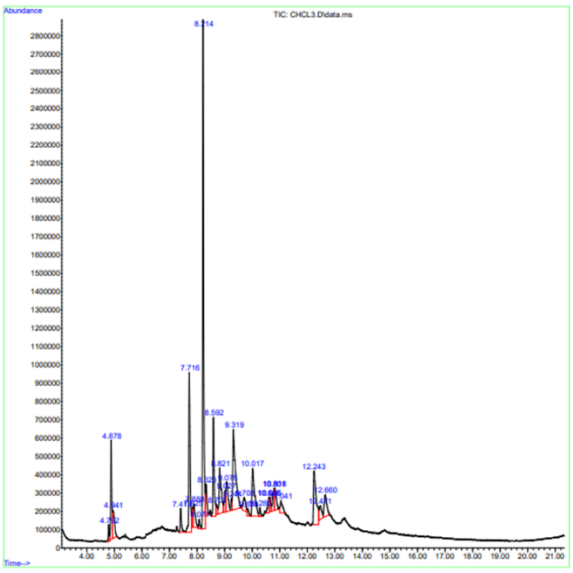
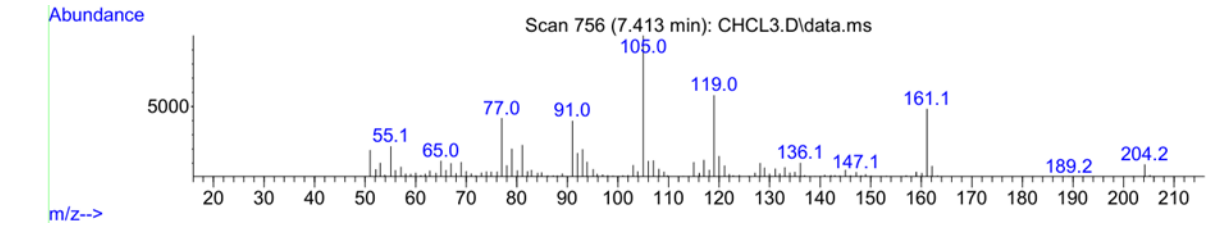


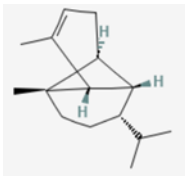
Fig. 2: Gas chromatogram for the bioactive chloroform-soluble fraction of *Chromolaena odorata*.

Table 5: The GC-MS analysis of the crude methanolic extract of *Chromolaena odorata* , showing its major compounds

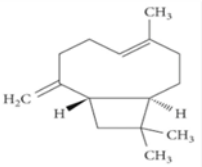
Name	RT(mins)	Area(%)	Quality	MW_t(g/mol)	Molecular Formula
Copaene	7.413	1.43	93	204.342	$C_{15}H_{24}$
gamma.-Muurolene	7.888	2.18	97	204.342	$C_{15}H_{24}$
Caryophyllene	8.214	24.95	99	204.342	$C_{15}H_{24}$
beta.-copaene	8.323	2.04	93	204.342	$C_{15}H_{24}$
Humulene	8.592	5.30	98	204.342	$C_{15}H_{24}$
1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-,[1S-(1.alpha, 2.alpha4-methyl-8-methylene-7-(1-methylethyl)-,[1S-(1α,3aβ,4α,7α,7aβ)]	9.078	2.65	94	204.342	$C_{15}H_{24}$
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	9.319	10.03	98	204.342	$C_{15}H_{24}$

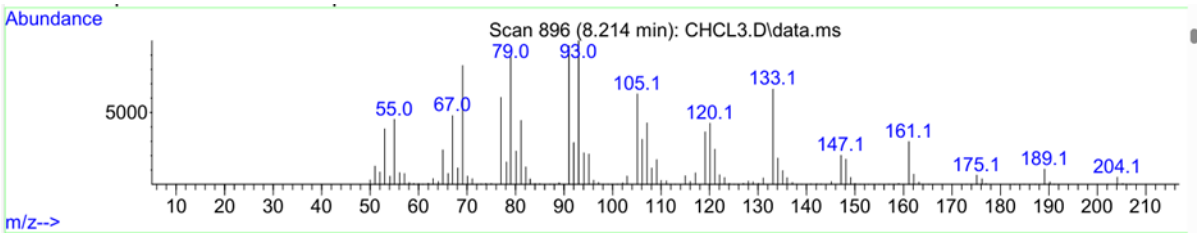
4-Isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene	9.708	1.37	38	200.319	$C_{15}H_{20}$
Caryophyllene oxide	10.017	5.78	93	204.342	$C_{15}H_{24}$
Alloaromadendrene	10.606	1.06	83	204.342	$C_{15}H_{24}$
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,7.alpha.,8a. alpha.)]-	10.801	1.62	95	204.342	$C_{15}H_{24}$
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	10.818	1.76	91	204.342	$C_{15}H_{24}$
Aromandendrene	11.041	1.27	64	204.342	$C_{15}H_{24}$
Neophytadiene	12.243	6.65	94	278.516	$C_{20}H_{38}$



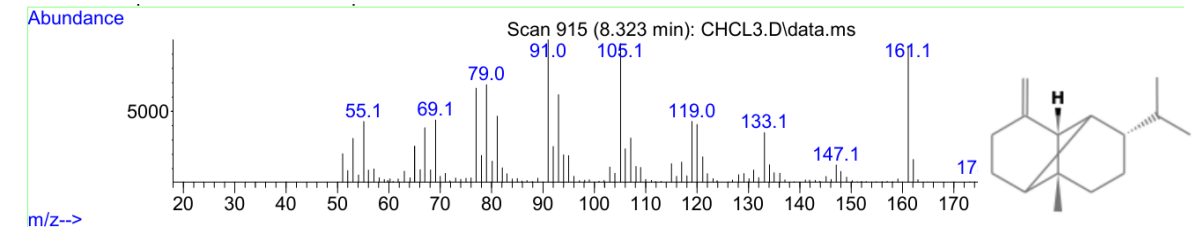


Copaene

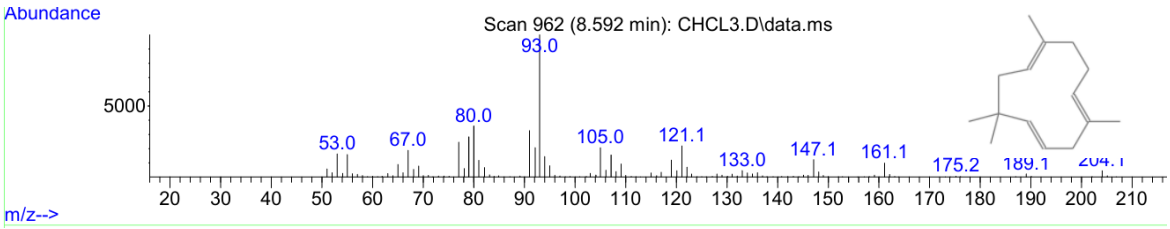




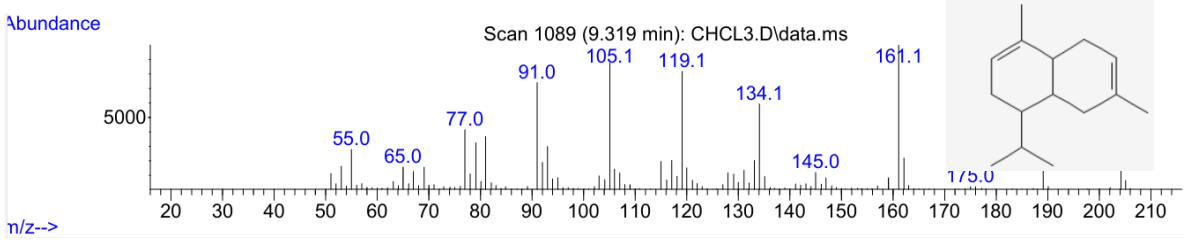
Caryophyllene



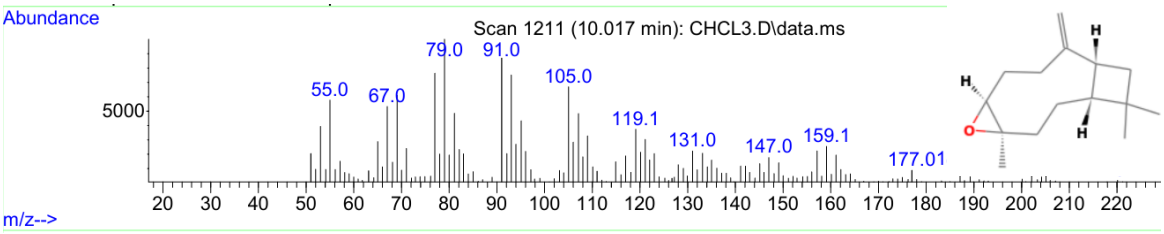
beta.-copaene



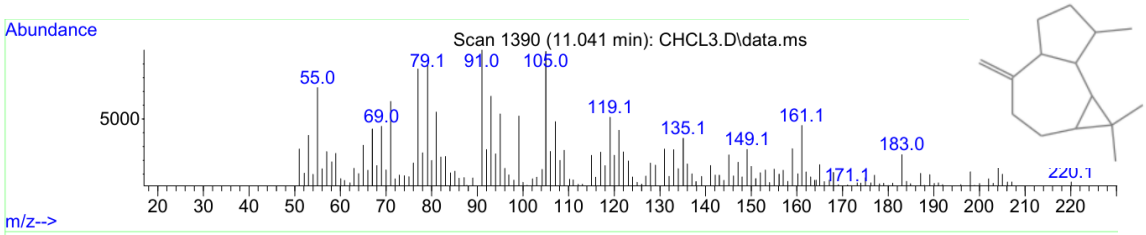
Humulene



1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene



Caryophyllene oxide



Aromandendrene

Fig. 3: MS spectra and structures of some components of the bioactive chloroform-soluble fraction of *Chromolaena odorata*

CONCLUSION

These findings provide scientific backing for the traditional use of *Chromolaena odorata* in herbal medicine and show the plant's potential as a natural source of antimicrobial compounds, particularly against Gram-positive bacteria. Terpenes and terpenoids found in the bioactive chloroform-soluble extract may be responsible for the plant extracts' biological activity.

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