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Original Article

Structural Elucidation of Phytoconstituents (Phenols, Long Chain Fatty Acids, Flavonoid, Terpenoids, Coumarin) Found in *Chromolaena odorata* through Gas Chromatography: Mass Spectroscopy (GC-MS) Technique

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ABSTRACT

Background: *Chromolaena odorata* (CO) is a well-known ethnopharmacological herb in Ayurveda and exhibits a comprehensive range of therapeutic potential. **Objectives:** The objective of this research was to study the phytochemical profile of ethanolic extract of CO by Gas Chromatography Mass Chromatography (GC-MS) analysis. **Materials and Methods:** Ethanolic extract of CO was prepared by soxhlet extraction and the extract was subjected to GC-MS analysis (Agilent 7890A GC System) for chemical characterization of the extract. The constituents were analysed by matching the mass spectra with MS libraries. **Results and Conclusion:** Total 24 compounds were identified in the extract and the constituents belonging to various chemical classes like phenols, coumarin, terpenoids, Long Chain Fatty Acids (LCFA) and flavonoid were identified. Compounds include phenol,4-ethenyl-acetate; 2-methoxy-4-vinyl phenol; 2H-1-Benzopyran-2-one,3,4-dihydro; 2-propenoic acid,3-(2-hydroxy phenyl); 1H-cyclopenta [1,3] cyclopropa [1,2] benzene, octahydro-7-methyl-3-methyene-4-(1-methylethyl)-(3As-(3aα,3bβ,7α,7aS*]; trans-z- -bisabolene epoxide; 1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl; 2-pentadecanone 6,10,14-trimethyl; hexadecanoic acid, methyl ester; hexadecanoic acid, ethyl ester; 9,12,15-octadecatrienoic acid; phytol; oleic acid; 2H-Pyran,2-(7-heptadecynyloxy) tetrahydro; squalene etc. The presence of these phytoconstituents could be of potential use of this plant and would help researchers to work with different *in vivo* and *in vitro* models.

Keywords: *Chromolaena odorata*, Phytoconstituents, Phenol, Flavonoid, Terpenoids, LCFA.

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INTRODUCTION

Phytochemicals are plant derived secondary metabolites which are important in investigation of various groups of chemical compounds which may further help in drug discovery and development of novel therapeutic agents.[1-3] GC-MS technique helps in the identification of new bioactive compounds, present if any, in the samples. GC-MS, a hyphenated system has become a technological platform for metabolite profiling in plant and the medicinal herbs having numerous bioactive compounds can be identified at less than 1 ng by using this technique.[4-8] *Chromolaena odorata* also known as *Eupatorium odoratum* is flowering shrub in the sunflower family, Asteraceae and is commonly known as "Hagonoy". Common names are siam weed, christmas bush, floss flower. In Ayurveda, this plant is used in the treatment of coughs, colds and skin diseases, wound healing etc. and have diverse pharmacological properties-anti-inflammatory, antimicrobial, antigonorrhoeal, antipyretic, antispasmodic, diuretic, analgesic, antiviral etc.[9-17] According to the World Health Organization (WHO), more than 80% of the world population relies on traditional medicine for their healthcare needs. Medicinal plants have been inexhaustible source of new drugs and chemical entities with reservoir of many novel phytoconstituents.

There are several classes of compounds that include alkaloids, flavonoids, coumarins, glycosides, gums, phenols, tannins, terpenes and terpenoids and these phytochemicals possess therapeutic potentials such as antioxidant, anti-diabetic, anticancer, Immunomodulatory activity. This study evaluates the chemical composition of ethanolic extract of *Chromolaena odorata* (whole plant) by GC-MS analysis. In the current research work different phytoconstituents belonging to chemical nature

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phenols, flavonoid, coumarin, terpenoids, long chain fatty acids etc. were identified. Phenolic acids and flavonoid compounds are the commonly appearing polyphenolic compounds in plants and various clinical studies showed the positive correlation between the intake of polyphenolic compounds or flavonoids and reduced cancer incidence. Long chain fatty acids play an important role in metabolic disorders and in chronic diseases where inflammation is involved.[18-20] Terpenoids are extensively studied for chemistry, biochemistry and biological origin and display a wide range of pharmacological properties-antibiotic, antitumor, antiviral, cytotoxic, immunosuppressive, antifungal etc.[21-29] The results may support researchers in developing formulations and to work with different animal and *in vitro* models. The research paper illustrates the mass spectra of each constituent identified in the analysis.

MATERIALS AND METHODS

Ethanolic extract of *Chromolaena odorata*

The whole plant was collected from Sanjay Gandhi National Park (SGNP), Mumbai. The plant was authenticated at Agharkar Research Institute, Pune, by Dr. R.K. Chaudhary. The plant was shade dried, crushed and finely powdered passing through sieve no. 80 to remove the twigs and associated materials. The powder was then extracted with ethanol (50 g in 500 mL) by using soxhlet extractor and dry form was obtained on rotary evaporator. The extract was stored at 8-15°C for further analysis.

GC-MS analysis

The extracts were subjected for GC-MS analysis at IIT Bombay. Mobile phase: Ethanol Electron Impact (EI)-MS spectrum was scanned at 70 eV with instrument details as follows:

GC Specification

Model	Agilent 7890A GC System
Detector Specification	Mass Spectrometer Model: The AccuTOFGCv/ JMS-T100GCv Make: JEOL
Column Specifications Name of column Length Dimension Column material	HP5 Column (30 m length*0.25 mm internal diameter*0.25 microfilm thickness) Column Material is Polysiloxane
Carrier Gas Used	Helium
Carrier Gas Flow Rate	1 mL/min
Oven Temperature	280°C
Injection Temperature	200°C
Injection Volume	1 µL
Sample flow rate	1 mL/min
Split Ratio	1:10

MS Specification

Model	Joel, AccuTOF GCV
Isonization source used	EI Positive
Mass range	35-800 amu
Split Ratio	1:10
Ion Source Temperature	220°C
Solvent Delay	4 min

The compounds were identified by comparing their mass spectra with NIST MS 2.0 structural library.

RESULTS

Constituents belonging to different important chemical classes were identified in this study. These constituents may have potential pharmacological role. The detailed information of various compounds elucidated by GC-MS analysis is shown in Table 1 and their chemical nature is described in Table 2. The mass spectrum peak of CO extract is shown in Figure 1. The mass spectrum of different components found in CO extract is shown from Figure 2a to 2c.

Total 24 peaks belonging to important phytoconstituents were identified in the ethanolic extract of *Chromolaena odorata*. The 1st and 2nd peak indicated was to be of phenol,4-ethenyl-acetate; and 2-methoxy-4-vinyl phenol (Figure 2a). The 3rd peak was found to be of 2H-1-Benzopyran-2-one,3,4-dihydro which is coumarin derivative. (Figure 2a). The 4th peak was determined to be 2-propenoic acid,3-(2-hydroxy phenyl) (Figure 2a).

The 5th and 6th peak were of sesquiterpenoids - 1H-Cyclopenta [1,3] cyclopropa [1,2] benzene, octahydro-7-methyl-3 methyene-4-(1-methylethyl)-(3As-(3aα,3bβ,7α,7aS*] and trans-z-α-bisabolene epoxide (Figure 2a). The 7th peak identified was 1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl (Figure 2b). The 8th and 9th peak identified were of sesquiterpene-cis-z-α-bisabolene epoxide and 2-pentadecanone 6,10,14-trimethyl (Figure 2b).

The 10th, 11th and 12th peaks were identified belonging to long chain fatty acids-hexadecanoic acid, methyl ester; hexadecanoic acid, ethyl ester; and 9,12,15-octadecatrienoic acid, methyl ester (Figure 2b). The phytoconstituent phytol (acyclic diterpenoids) was identified at 13th peak (Figure 2b). Long chain fatty acids-9,12-octadecadienoic acid; 9,12,15-octadecatrienoic acid; oleic acid; octadecanoic acid, ethyl ester; Eicosanoic acid, ethyl ester; and cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)

Table 1: Components found in ethanolic extract of *Chromolaena odorata* by GC-MS analysis.

Sl. No.	Components	Retention time	Molecular formula	Molecular weight	Figure Number
1	Phenol,4-ethenyl-acetate	8.76	$C_{10}H_{10}O_2$	162	2a
2	2-Methoxy-4-vinyl phenol	11.32	$C_9H_{10}O_2$	150	
3	2H-1-Benzopyran-2-one,3,4-dihydro	12.61	$C_9H_8O_2$	148	
4	2-Propenoic acid,3-(2-hydroxy phenyl)	13.54	$C_9H_8O_3$	164	
5	1H-Cyclopenta [1,3] cyclopropa[1,2] benzen e,octahydro-7-methyl-3-methyene-4-(1-methylethyl)-(3As-(3aα, 3bβ, 7α, 7aS*]	13.97	$C_{15}H_{24}$	204	
6	Trans-z-α-Bisabolene epoxide	15.60	$C_{15}H_{24}O$	220	
7	1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl	16.91	$C_{15}H_{20}O$	216	2b
8	Cis-z-α-bisabolene epoxide	18.24	$C_{15}H_{24}O$	220	
9	2-pentadecanone 6,10,14-trimethyl	19.05	$C_{18}H_{36}O$	268	
10	Hexadecanoic acid, methyl ester	20.25	$C_{17}H_{34}O_2$	270	
11	Hexadecanoic acid, ethyl ester	21.24	$C_{18}H_{36}O_2$	284	
12	9,12,15-octadecatrienoic acid, methyl ester (z,z,z)	22.72	$C_{19}H_{32}O_2$	292	
13	Phytol	22.97	$C_{20}H_{40}O$	296	
14	9,12-octadecadienoic acid	23.51	$C_{18}H_{32}O_2$	280	
15	9,12,15-octadecatrienoic acid	23.61	$C_{18}H_{30}O_2$	278	
16	Oleic acid	23.78	$C_{18}H_{34}O_2$	282	2c
17	Octadecanoic acid, ethyl ester	23.87	$C_{20}H_{40}O_2$	312	
18	Eicosanoic acid, methyl ester	25.42	$C_{21}H_{42}O_2$	326	
19	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-methyl ester	26.08	$C_{22}H_{38}O_2$	334	
20	2H-Pyran,2-(7-heptadecynyloxy) tetrahydro	26.62	$C_{22}H_{40}O_2$	336	
21	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	27.55	$C_{19}H_{38}O_4$	330	
22	Tetradecanoic acid,12-methyl-,methyl ester	28.54	$C_{16}H_{32}O_2$	256	
23	1,3-benzenedicarboxylic acid, bis (2-ethyl hexyl) ester	29.88	$C_{24}H_{38}O_4$	390	
24	Squalene	30.64	$C_{30}H_{50}$	410	

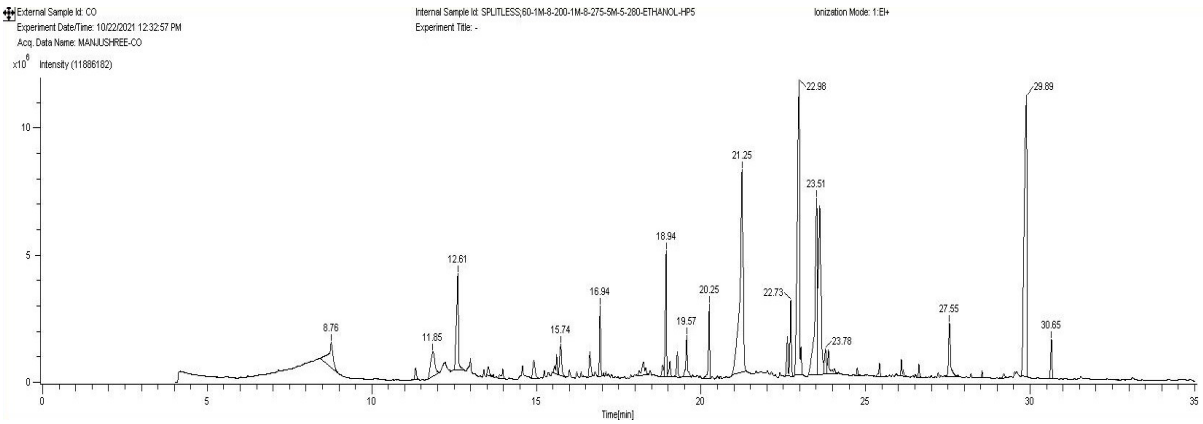


Figure 1: Mass spectrum of ethanolic extract of *Chromolaena odorata*.

methyl] cyclopropyl] methyl]-methyl ester was identified at 14th to 19th peaks (Figure 2b and 2c).

The flavonoid-2H-Pyran,2-(7-heptadecynyloxy) tetrahydro was identified at 20th peak (Figure 2c). Saturated long chain fatty acids– Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and Tetradecanoic acid, 12-methyl-,methyl ester was identified at peak no. 21 and 22 (Figure 2c). Phthalate ester-1,3-benzenedicarboxylic acid, bis (2-ethyl hexyl) ester was identified at peak no. 23 (Figure 2c). Triterpenoid squalene was identified at peak no. 24 (Figure 2c).

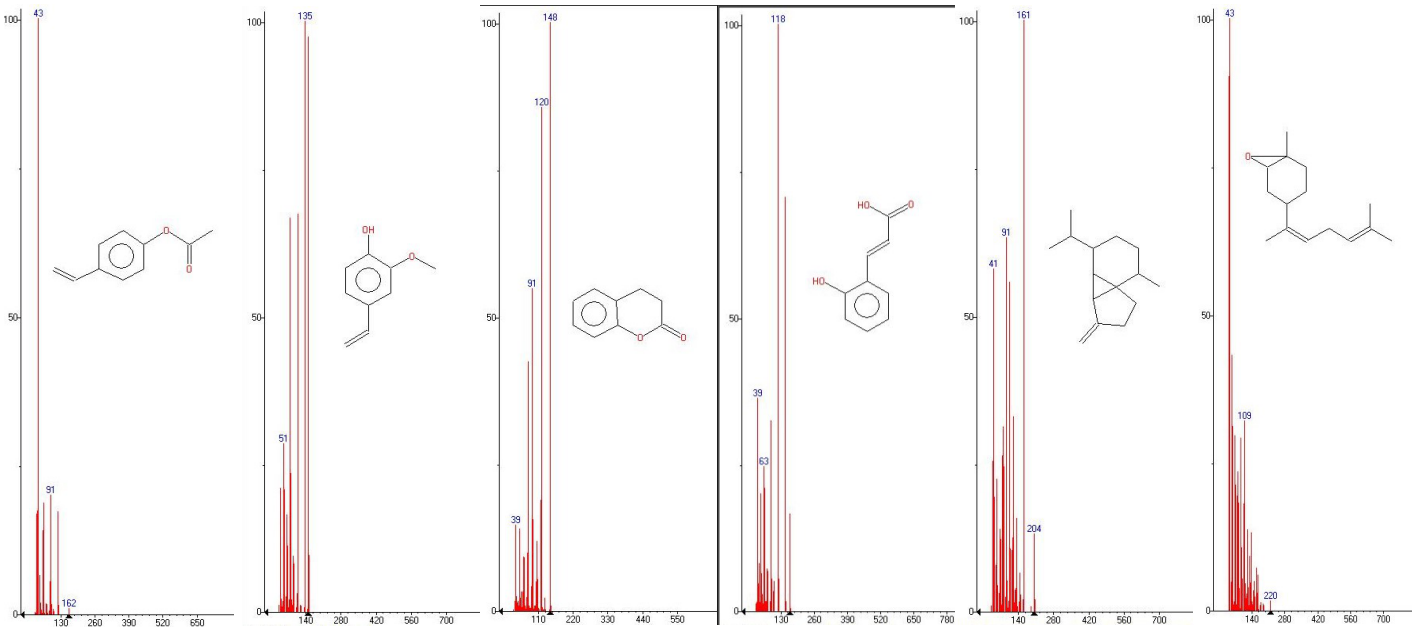
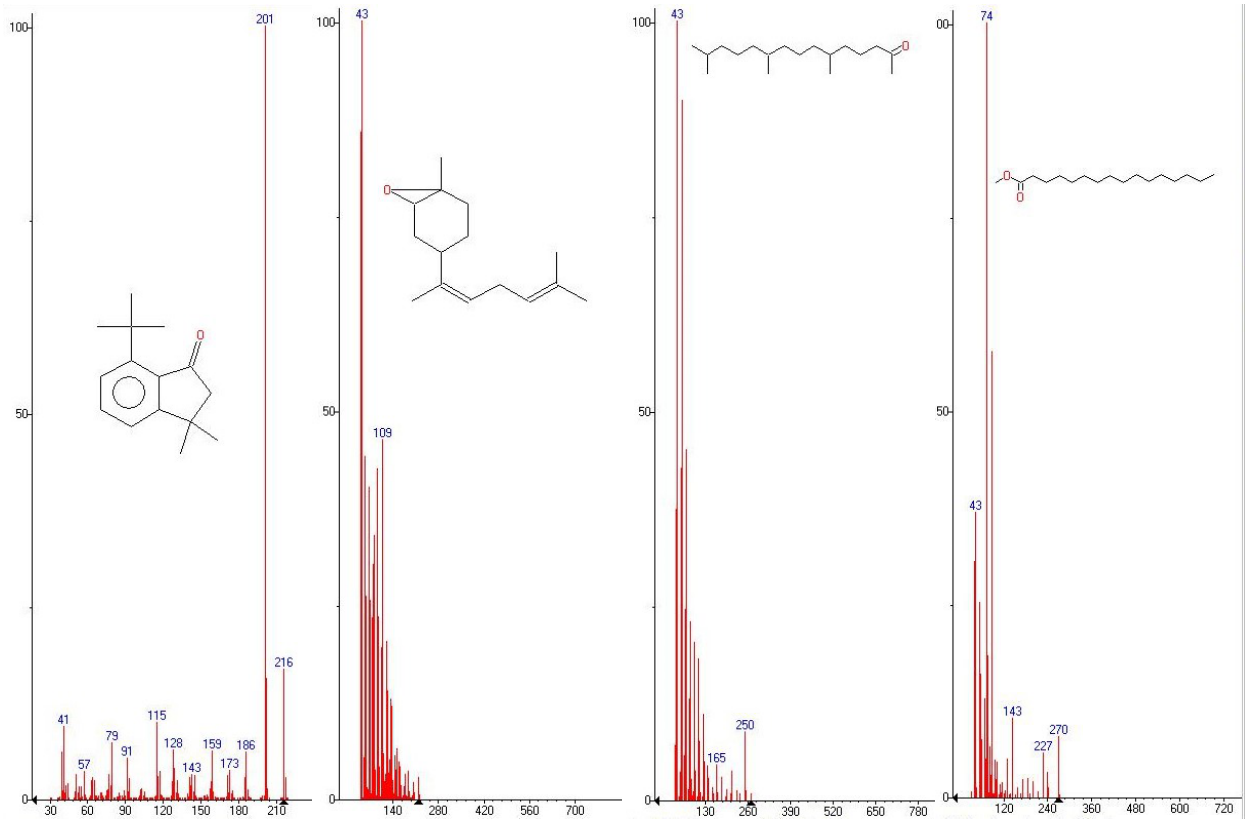


Figure 2a: Mass spectrum showing presence of Phenol,4-ethenyl-acetate; 2-Methoxy-4-vinyl phenol; 2H-1-Benzopyran-2-one,3,4-dihydro; 2-Propenoic acid,3-(2-hydroxy phenyl); 1H-Cyclopenta [1,3] cyclopropa[1,2] benzene,octahydro-7-methyl-3-methyene-4-(1-methylethyl)-(3As-(3aα, 3bβ, 7α, 7aS*]; Trans-z-α-Bisabolene epoxide in ethanolic extract of Chromolaena odorata.



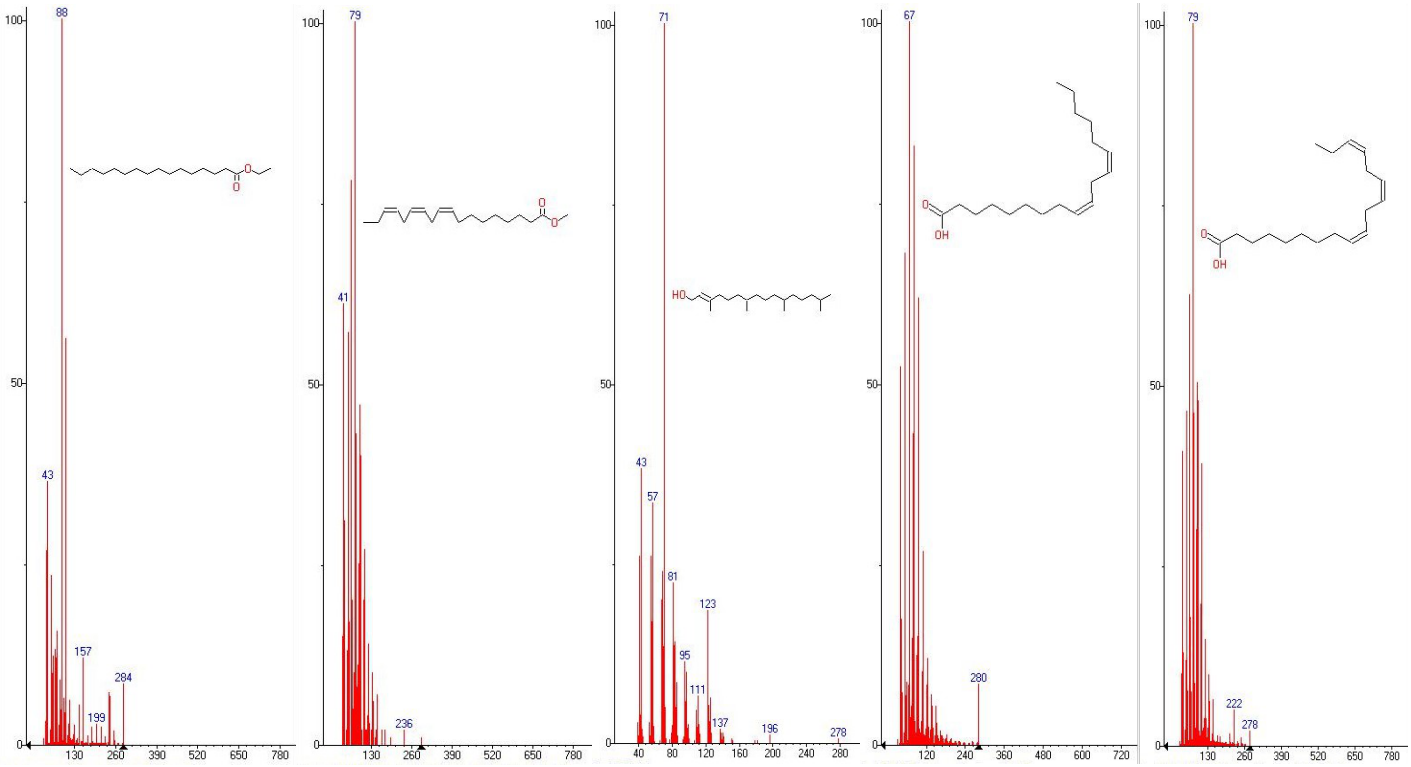
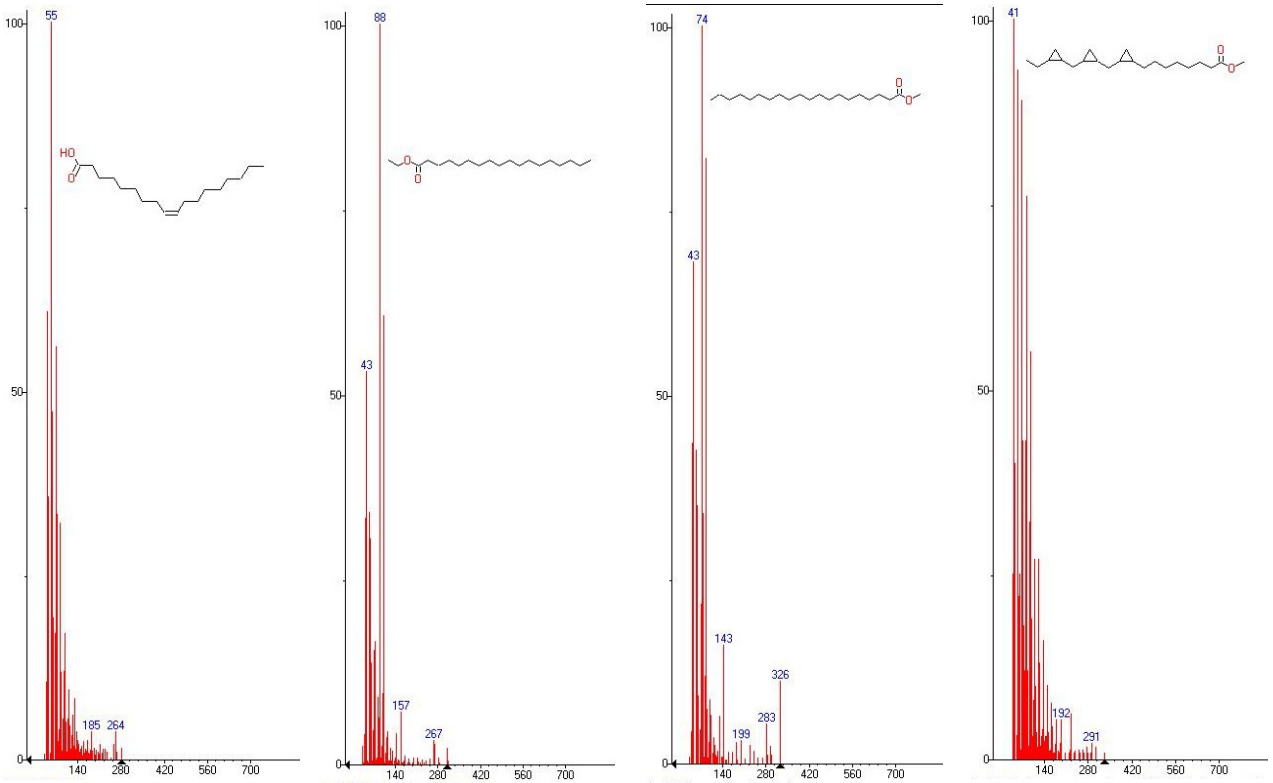


Figure 2b: Mass spectrum showing presence of 1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl; Cis-z-α-bisabolene epoxide; 2-pentadecanone 6,10,14-trimethyl; Hexadecanoic acid, methyl ester; Hexadecanoic acid, ethyl ester; 9,12,15-octadecatrienoic acid, methyl ester (z,z,z); Phytol; 9,12-octadecadienoic acid; 9,12,15-octadecatrienoic acid in ethanolic extract of Chromolaena odorata.



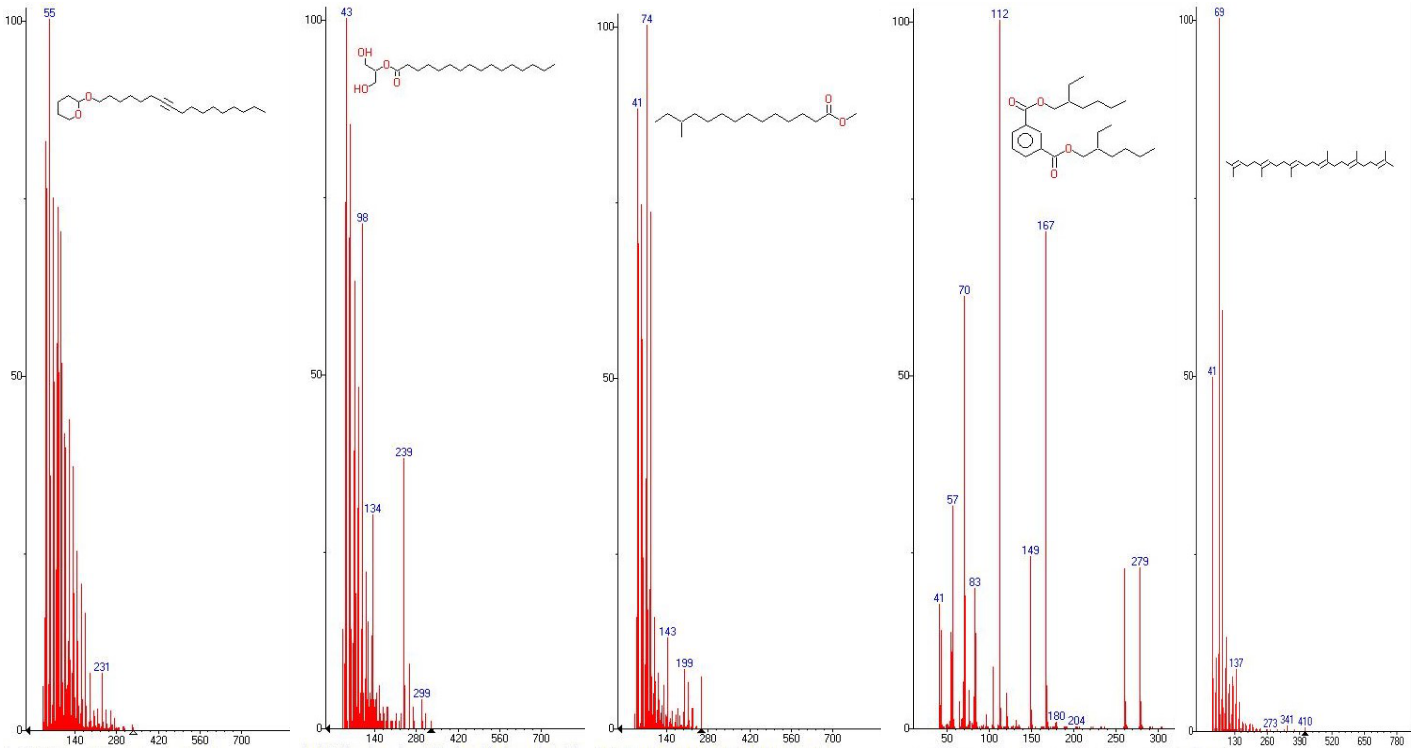


Figure 2c: Mass spectrum showing presence of Oleic acid; Octadecanoic acid, ethyl ester; Eicosanoic acid, methyl ester; Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-methyl ester; 2H-Pyran,2-(7-heptadecynyloxy) tetrahydro; Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester; Tetradecanoic acid,12-methyl-,methyl ester; 1,3-benzenedicarboxylic acid, bis (2-ethyl hexyl) ester; Squalene in ethanolic extract of *Chromolaena odorata*.

Table 2: Chemical profile of components found in ethanolic extract of *Chromolaena odorata*.

Sl. No.	Components	Chemical nature
1	Phenol,4-ethenyl-acetate	Phenols
2	2-Methoxy-4-vinyl phenol	Phenols
3	2H-1-Benzopyran-2-one,3,4-dihydro	Coumarin derivative
4	2-Propenoic acid,3-(2-hydroxy phenyl)	Hydroxycinnamic acid-polyphenolic compounds, aromatic acids, phenylpropanoids
5	1H-Cyclopenta [1,3] cyclopropa[1,2] benzene,octahydro-7-methyl-3-methyene-4-(1-methylethyl)-(3As-(3aα, 3bβ, 7α, 7aS*]	Sesquiterpenoids
6	Trans-z-α-Bisabolene epoxide	Sesquiterpene
7	1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl	Indanone
8	Cis-z-α-bisabolene epoxide	Sesquiterpene
9	2-pentadecanone 6,10,14-trimethyl	Sesquiterpene
10	Hexadecanoic acid, methyl ester	Saturated Long chain fatty acid methyl ester
11	Hexadecanoic acid, ethyl ester	Saturated Long chain fatty acid ethyl ester
12	9,12,15-octadecatrienoic acid, methyl ester (z,z,z)	Polyunsaturated Long chain fatty acid
13	Phytol	Acyclic diterpenoids
14	9,12-octadecadienoic acid	Polyunsaturated Long chain fatty acid
15	9,12,15-octadecatrienoic acid	Polyunsaturated Long chain fatty acid
16	Oleic acid	Long chain fatty acid-monounsaturated
17	Octadecanoic acid, ethyl ester	Saturated Long chain fatty acid
18	Eicosanoic acid, methyl ester	Saturated Long chain fatty acid

Sl. No.	Components	Chemical nature
19	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-methyl ester	Cyclopropane fatty acid
20	2H-Pyran,2-(7-heptadecynyloxy) tetrahydro	Flavonoid
21	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	Saturated Long chain fatty acid
22	Tetradecanoic acid,12-methyl-,methyl ester	Saturated long chain fatty acid
23	1,3-benzenedicarboxylic acid, bis (2-ethyl hexyl) ester	Phthalate ester
24	Squalene	Triterpenoid

DISCUSSION

GC-MS is the most precise method of analysis for the identification of various secondary metabolites present in plants. The chemical profile of ethanolic extract *Chromolaena odorata* was analysed by GC-MS analysis and total 24 peaks were identified belonging to various important class of phytoconstituents. These compounds play a major role in showing various pharmacological activities associated with the plant extracts. The compounds belonging to phenols, coumarin, long chain fatty acids, flavonoid and terpenoids were identified in this analysis. These phytoconstituents play important role in treatment of various diseases like cardiovascular disease, hypertension, diabetes, especially cancer. They can modulate host immune response to cancer, reducing inflammatory microenvironment and enhancing lymphocyte on Onco-surveillance. Hence, a growing interest is arising for phytoconstituents role in cancer prevention and treatment. Considering the richness of phytoconstituents found in this plant, to enhance the therapeutic efficacy of this plant, the ethanolic extract of *Chromolaena odorata* could be explored for different novel drug delivery systems like nano emulsion, liposomes and testing the formulation against various diseases.

CONCLUSION

Twenty-four compounds were identified in the ethanolic extract of *Chromolaena odorata* using GC-MS analysis. This justifies the benefit of whole plant for treating various diseases and the researchers could use this data for further analysis, preparation of formulation or to work with different animal and *in vitro* models and with different pharmacological activities. However, the isolation of these constituents from the plant and screening it for biological role by various *in vitro/in vivo* studies would prove the therapeutic efficacy of this plant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

GC-MS: Gas Chromatography Mass Spectrometry; **CO:** *Chromolaena Odorata*; **LCFA:** Long Chain Fatty Acid; **WHO:** World Health Organisation; **g:** Gram; **mL:** Milliliter; **µL:** Microliter.

SUMMARY

The objective of this research was to identify the compounds of pharmacological significance in ethanolic extract of *Chromolaena odorata* and to elucidate the structures of those compounds through GC-MS technique. The results revealed that the selected plant *Chromolaena odorata* is rich in phytoconstituents belonging to class - phenols, flavonoid, terpenoids, long chain fatty acids and coumarin. Considering its phytochemical profile and presence of various compounds of therapeutic efficacy, the extract of this plant could be further explored for different novel formulations or drug discovery. Also, the plant extract could be of great significance if screened for different *in vivo* and *in vitro* models.

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