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Exploring The Bioactive Components In *Ruellia Prostrata* Flower Extract Using Mass Spectrometry And Gas Chromatography Technique

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ABSTRACT

In recent years, Gas chromatography-Mass spectrometry (GC–MS) has commonly been employed for identification of various bioactive therapeutic compounds that are present in natural extracts. The present study was carried out to identify the possible bioactive components present in *Ruellia prostrata* flower extract. The GC-MS analysis revealed the presence of thirty chemicals bioactive compounds which include n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, Phytol, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid, Oleic Acid and Linoleic acid ethyl ester. The findings verified that the extract included a variety of phytochemicals. The identified compounds were likely to possess various biological activities such as antioxidant, hypocholesterolemic, antiinflammatory, antidiabetic, hepatoprotective, antieczemic, antiarthritic, antioxidant, and anticancer. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects.

Keywords: *Ruellia prostrata*, Phytochemicals, GC-MS analysis, Bioactive compounds. Biological activities

INTRODUCTION

Many therapeutic plants were found through the reciprocal link between humans and their natural surroundings. Eleven percent of the 255 important drugs, according to the World Health Organization (WHO), are derived from plants. Furthermore, a number of synthetic medications are created using natural precursors (Anichini *et al.,* 2020; Barman *et al.,* 2024). Chromatography refers to a method of separation when a stationary phase that is selectively absorbent comes into contact with a mobile phase that is transporting a mixture. Additionally, it is an essential analytical method for phytotherapeutical quality assurance and standardization (Abubakar and Lubabatu, 2025). Secondary metabolites found in medicinal plants have a wide range of biological actions, such as antibacterial, antiviral, antifungal, anti-inflammatory, and anticancer properties (Gololo *et al.,* 2021). Many phytochemical compounds have been utilized by thousands of physicians in their practices and are consumed under medical management by tens of millions of people (Yuan *et al.,* 2016).

Crude plant extracts and medicines manufactured on the values of natural compounds even by pharmaceuticals companies may lead to large-scale exposure of humans to natural products (Ghosh *et al.,* 2016). The major reason for continued use of herbal remedies is their usefulness, easy availability, low price, and moderately less or no toxic property (Alexandra *et al.,* 2018). It has the same potency as prescription medications and can initiate self-healing. It is relevant to look into the therapeutic effects of traditional Indian medicinal plants because several of them have been shown to contain phytochemicals with pharmacological activity (Kshetrimayum

et al., 2017). The most effective sensitive method for separating and identifying the numerous structurally complex components found in plant extracts is GC-MS. Considered bioactive molecules found in plants, phytochemicals have been shown to be safe, effective, reasonably priced, and lately suggested as a viable antibiotic alternative (Ahmed *et al.,* 2019). The precise method used to identify different bioactive pharmaceutical chemicals and detect functional groups in medicinal plants is gas chromatography-mass spectrometry (GC–MS) (Satapute *et al.,* 2019; Fan *et al.,* 2018). Since GC-MS analysis has shown itself to be a useful technique for the analysis of volatile essential oils, fatty acids, lipids, and alkaloids, as well as non-polar components, it has been used more and more in recent years for the investigation of medicinal plants (Sosa *et al.,* 2016). Therefore, the goal of the current work is to use GC-MS analysis to identify the bioactive chemicals found in the hydro-ethanolic extracts of the entire *Ruellia prostrata* flower.

MATERIALS AND METHOD

Materials

The flowers of *Ruellia prostrata* were collected from Kathattipatti (Palaiyapatti North), Thanjavur District, Tamil Nadu, India, during the month of March 2023. Identification of the plant species was confirmed with standard morphological characteristic features.

Preparation of extracts

The *Ruellia prostrata* flower was first washed well and during washing, the dust was removed from the flower. Then the flower was dried at room temperature and coarsely powdered. The powder was extracted with hydro-ethanolic (Ethanol and water (70:30)) for 24 hours. The extract was filtered using Whatman No.1 filter paper and filtrate used for further analysis.

GC –MS analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8 °C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al.,* 2013).

Identification of components

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST05) and WILEY 7 having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and WILEY library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

RESULTS AND DISCUSSION

Secondary metabolites are produced by plants in nearly infinite quantities and are found in various plant components, including leaves, fruits, buds, stems, flowers, bark, roots, and more. Tens of millions of individuals take numerous phytochemical compounds under medical supervision, and hundreds of doctors have used them in their professions. In recent years, Gas Chromatography-Mass Spectrometry (GC–MS) has commonly been employed for identification of various bioactive therapeutic compounds that are present in natural extracts (Fan et al., 2018). GC–MS is one of the best, fast and accurate techniques to detect various compounds, including

alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters and amino acids (Razack et al., 2015), and requires a small volume of extract. GC-MS was used to evaluate the various bioactive components found in the hydro-ethanolic extracts of *Ruellia prostrata* flowers. Tables 1 and 2 provided a summary of the extracts chromatograms, which were displayed in Figure 1. The existence of thirty distinct bioactive chemicals was revealed by peaks in the *Ruellia prostrata* flower extract GC-MS chromatogram. The results revealed that the percentage of major bioactive compounds viz., n-hexadecanoic acid, hexadecanoic acid ethyl ester, phytol, l-(+)-ascorbic acid 2,6-dihexadecanoate, 9,12-octadecadienoic acid, oleic acid and linoleic acid ethyl ester were found as the major compounds in the hydro-ethanolic extract of flowers of *Ruellia prostrata* (Table 2).

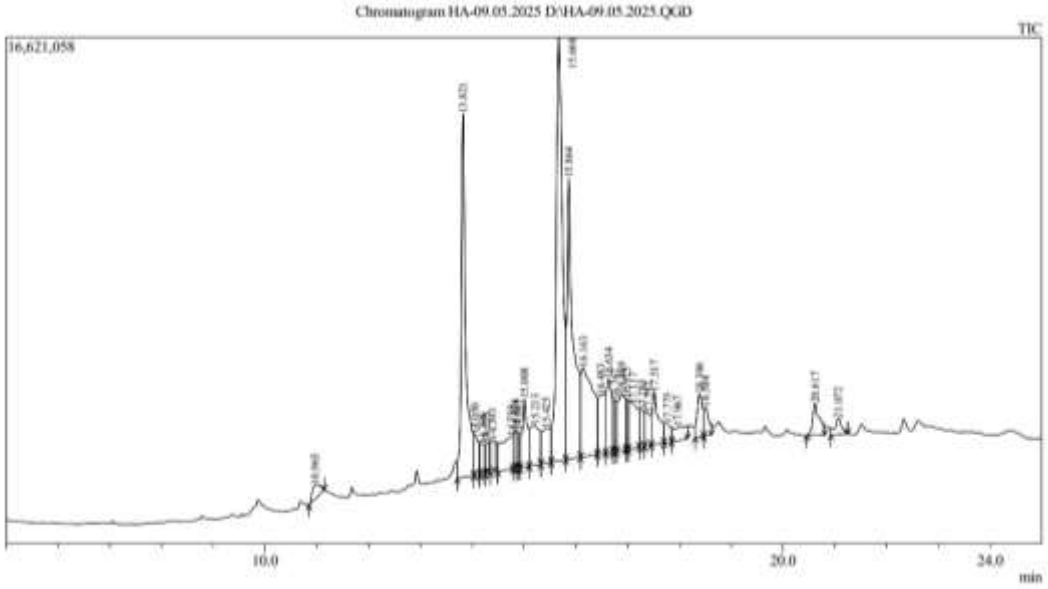

Figure 1: GC-MS chromatogram in hydro-ethanolic extract of *Ruellia prostrata* flower

Table 1: GCMS analysis of hydro-ethanolic extract of *Ruellia prostrata* flower

Peak #	R. Time	Area %	Height %	M. Weight (g/mol)	M. Formula	Phytochemicals
1	10.965	0.81	0.65	194	$C_7H_{14}O_6$	.beta.-D-Glucopyranoside, methyl
2	13.821	12.43	16.78	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
3	14.050	1.34	1.91	284	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
4	14.208	1.03	1.37	194	$C_7H_{14}O_6$	Methyl .beta.-d-galactopyranoside
5	14.275	0.76	1.40	296	$C_{20}H_{40}O$	Phytol
6	14.393	0.90	1.50	268	$C_{18}H_{36}O$	Oxirane, hexadecyl
7	14.758	3.00	1.51	196	$C_{12}H_{20}O_2$	4-Hydroxy-4-(2,6-dimethylcyclohex-3-enyl)butan-2-one
8	14.824	0.95	1.79	652	$C_{38}H_{68}O_8$	l-(+)-Ascorbic acid 2,6-dihexadecanoate
9	14.883	0.81	1.70	438	$C_{27}H_{50}O_4$	Fumaric acid, hexadecyl 4-heptyl ester
10	15.008	2.69	3.13	284	$C_{19}H_{40}O$	n-Nonadecanol-1
11	15.213	2.90	1.85	592	$C_{39}H_{76}O_3$	Oleic acid, 3-(octadecyloxy)propyl ester
12	15.425	2.35	1.61	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid

13	15.668	20.77	19.53	282	$C_{18}H_{34}O_2$	Oleic Acid
14	15.864	13.47	12.97	284	$C_{18}H_{36}O_2$	Octadecanoic acid
15	16.143	8.23	4.12	238	$C_{16}H_{30}O$	10,12-Hexadecadien-1-ol
16	16.483	3.18	2.72	262	$C_{19}H_{34}$	18-Norabietane
17	16.634	3.10	3.40	308	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester
18	16.767	1.18	2.37	243	$C_{12}H_{10}FN_5$	1H-Purin-6-amine
19	16.889	3.18	2.62	358	$C_{21}H_{42}O_4$	Octadecanoic acid, 2,3-dihydroxypropyl ester
20	16.975	1.01	2.36	278	$C_{18}H_{30}O_2$	2-(2-Methylpropenyl) cyclopropanecarboxylic acid, 2-isopropyl-5-methyl-cyclohexyl ester
21	17.117	3.11	2.05	681	$C_{47}H_{84}O_2$	Stigmast-5-en-3-ol, octadecanoate, (3beta)-
22	17.283	1.24	1.66	156	$C_{10}H_{20}O$	2-Decanone
23	17.392	1.47	1.51	412	$C_{29}H_{48}O$	22-Stigmasten-3-one
24	17.517	2.56	2.45	569	$C_{35}H_{68}O_5$	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester
25	17.775	0.90	0.85	326	$C_{21}H_{42}O_2$	2-Hexyldodecyl propionate
26	17.967	1.36	0.63	152	$C_{10}H_{16}O$	Trans-Decalone
27	18.386	1.76	1.99	266	$C_{18}H_{34}O$	5-Octadecenal
28	18.504	1.01	1.33	298	$C_{19}H_{38}O_2$	Nonadecanoic acid
29	20.617	1.69	1.50	884	$C_{57}H_{104}O_6$	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
30	21.072	0.80	0.75	624	$C_{39}H_{76}O_5$	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester

Table 2: Bioactive compounds of hydro-ethanolic extract of *Ruellia prostrata* flower

S. No	Compounds	Bioactivity
1	n-Hexadecanoic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic.
2	Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic.
3	Phytol	Antimicrobial, Anticancer, Cancer preventive, Diuretic, Antiinflammatory.
4	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant
5	9,12-Octadecadienoic acid	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic.
6	Oleic Acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge, Flavor.
7	Linoleic acid ethyl ester	Antiinflammatory, Hypocholesterolemic,

		Cancer preventive Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge.

The crude extract of the native plant is widely used for medical and other reasons by tribal and rural groups in India. Humans may be exposed to natural products on a wide scale as a result of crude extracts and medications made using natural components, even by pharmaceutical corporations. The biological and phytochemical screening of plant extracts from conventional preparations used in popular medicine is the first step in achieving this goal (Paz *et al.,* 1995; Rishikesh *et al.,* 2012). The phytocompounds have been shown to have therapeutic effects on a number of human ailments, including diuretics, skin conditions (Kirtikar and Basu, 1980), hypercholesterolemia (Sharma and Pant, 1992), and hyperglycemic disorders (Sharmila *et al.,* 2007; Rai *et al.,* 2008; Shiram *et al.,* 2024).

The GC-MS spectrum profile verified the existence of the primary components together with their retention duration. The peak heights indicate the relative amounts of each component found in the extracts. Using the NIST library and the constituent's mass spectrum, the phytoconstituents were discovered and described. The discovered phytocomponents' biological activity was determined using Dr. Duke's phytochemical and ethnobotanical database (available online) (Duke's, 2016; Kavitha and Nadu, 2021). The anti-inflammatory effects of hydrocortisone and bis (0-ethyloxime) are known. Gupta *et al.* (2023) state that the substance hexadecanoic acid, methyl ester, exhibits strong antioxidant, hypocholesterolemia, anti-androgenic, hemolytic, alpha reductase inhibitor, antibacterial, and antifungal effects. Methylene ester and octadecadienoic acid have antifungal and antibacterial effects (Wahyuni *et al.,* 2024). Utilizing the National Institute Standard and Technology (NIST) database, GC-MS was carried out. Thirty chemicals were examined from the Ruellia prostrata flower in total. Understanding the nature of active principles in medicinal plants and determining whether a plant species has a single chemical or a collection of compounds are two of the first steps in GC-MS analyses. All things considered, the *Ruellia prostrata* flower extract numerous bioactive components point to possible therapeutic use.

CONCLUSION

The present investigation was focused on identification of various bioactive compounds from the extract of *Ruellia prostrata* flower for the first time by GC–MS analysis. The biological activities of the identified phytocomponents have been found to have anti-inflammatory, anti-microbial, anti-diabetic, hepatoprotective, antioxidant, hypocholesterolemic, and anti-cancer properties, according to the findings of the current study. Overall, *Ruellia prostrata* flower extract contains rich source of phytochemicals which are possible to development of novel drugs for therapeutic effects.

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