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Screening of phytocomponents in ethanolic extract of Ruellia prostrata leaf using GC-MS technique

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Abstract

The present investigation was carried out to determine the possible bioactive components of *Ruellia prostrata* using GC-MS analysis. GCMS analysis of ethanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. 20 compounds were identified in the ethanolic extract of *Ruellia prostrata*. The prevailing compounds are Tridecane, 1-Tetradecanol, Pentadecane, 1,2-benzoldicarbonsaeure, Di-(Hex-1-EN-5-YL-ester), 2-propyldecan-1-OL 1-Hexadecyne, Di-lauryl thio-di-propionate, 1-Hexadecyne, Hexadecanoic acid, 2-Undecanone, Xycaine, 1-Octadecanol, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl and 1-Tridecyn-4-ol were found in Ruellia prostrataThese findings support the traditional use of *Ruellia prostrata.*

Keywords: gas chromatography and mass spectroscopy, *ruellia prostrata*, phytochemistry

Introduction

The study of plants involves the isolation and the structure elucidation of their compounds, for understanding and evaluating their therapeutic potential. But the plethora of different compounds within the different compound classes makes separation and isolation of unknown active compounds from plant material a difficult task. Activity-guided fractionation is the most frequently cited technique for separating plant compounds and isolating only those that exhibits the desired activity (Massiot *et al*., 1992; Adesina *et al*., 2000) [1, 2] and antimicrobial screening is the first step towards determining the presence of active compounds and to establish therapeutic potential of a plant extract (Palombo and Semple, 2001) [3]. A lot of analytical methods as spectrophotometry, high performance liquid chromatography, gas chromatography (GC) with flame ionization detection (FID), gas chromatography–mass spectrometry (GC–MS) are developed for plant active compounds study. However, the combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS as an ideal technique for qualitative and quantitative for volatile and semi volatile compounds (Iordache *et al*., 2009) [4]. In many cases, the GC-MS screening of plant samples has revealed compounds with unknown MS spectra, which has resulted in the isolation and spectroscopic identification of new natural bioactive molecules (Berkov *et al*., 2008) [5].

Negi and Dave, (2010) [6] reported antibacterial and antifungal compounds in the crude ethanolic extract of leaves of *Acacia catechu* (family-Fabaceae) with the help of GC-MS analysis. In another study, the crude hexane extract of *Spilanthes acmella* (Family-Asteraceae) flower heads were reported to have larvicidal activity against *Anopheles stephens*i larvae. The crude extract was separated into 85 fractions through silica gel column chromatography using hexane-ethyl acetate mobile phase and the compounds were detected by GC-MS analysis. In previous studies, various compounds have been isolated from medicinal plants belonging to family Asteraceae and a band. The isolation and scaling-up of the amount of most active antibacterial band is done by using preparative TLC.

Further, it is only once the compound has been characterized that it can be assessed in terms of its potential as a lead compound, and indeed if the compound is actually novel and worthy of further investigation. The aim of this study is to determine the organic compounds present in the *Ruellia prostrata* leaf extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

Material and Methods

Plant materials

The whole plant of *Ruellia prostrata* were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (JJVS 001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of extracts

The *Ruellia prostrata* were first washed well and dust was removed from the leaves. Then the leaves were dried at room temperature and coarsely powdered. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under

reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

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 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20min isothermal at 280 ºC. 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) [7].

Identification of components

The mass spectrum was interpreted with the aid of the database of National Institute Standard and Technology, Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) 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 library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013) [8].

Results and Discussion

Twenty compounds were identified in ethanolic extract of *Ruellia prostrata* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1. The prevailing compounds are Tridecane, 1-Tetradecanol, Pentadecane, 1,2-benzoldicarbonsaeure, Di-(Hex-1-EN-5-YL-ester), 2-propyldecan-1-OL 1-Hexadecyne, Di-lauryl thio-di-propionate, 1-Hexadecyne, Hexadecanoic acid, 2-Undecanone, Xycaine, 1-Octadecanol, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl and 1-Tridecyn-4-ol were found in *Ruellia prostrata*. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity (Table 2) will definitely give fruitful results.

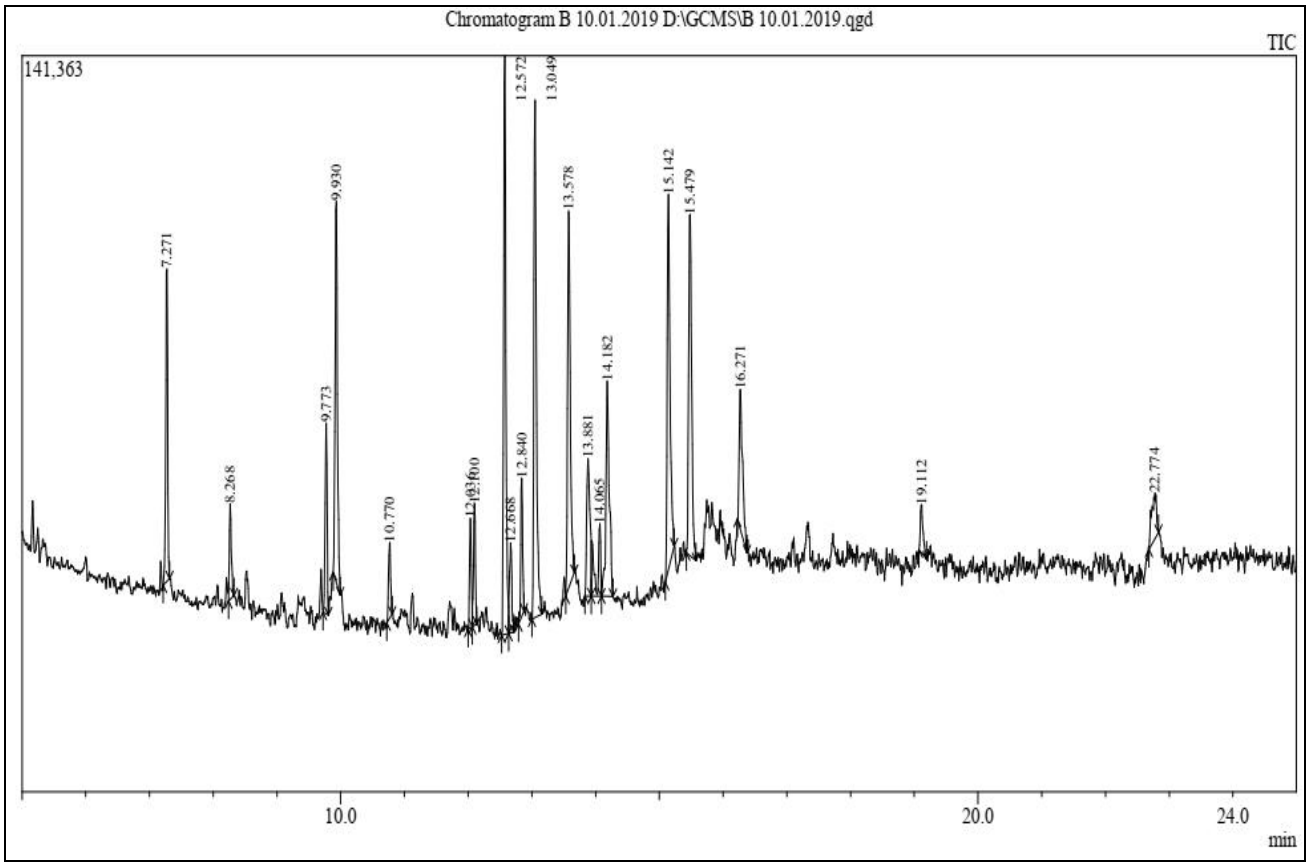

Fig 1: Chromatogram of *Ruellia prostrata* ethanolic extract

Table 1: GC-MS analysis revealed the presence of phytochemical component in leaves of *Ruellia prostrata*

peak #	R. Time	Area %	Height %	M. weight (g/mol)	Molecular formula	Name of the compounds	Chemical Name	Chemical Nature
1	7.271	12.840	7.16	184	$C_{13}H_{28}$	Tridecane	n- tridecane	Alkane
2	8.268	1.66	2.19	214	$C_{14}H_{30}O$	1-Tetradecanol	1-tetradecanol	Fatty alcohol
3	9.773	3.25	4.37	212	$C_{15}H_{32}$	Pentadecane	alkane hydrocarbon	Aliphatic hydrocarbon
4	9.930	8.02	8.73	330	$C_{20}H_{26}O_4$	1,2-benzoldicarbonsaeure, Di-(Hex-1-EN-5-YLester)	Carnosol	Phenolicditerpene
5	10.770	1.46	1.74	514	$C_{30}H_{58}O_4$	Di-lauryl thio-di-propionate	Dodecyl sulfanyl propanoate	DialkDiscussion:yl ester
6	12.036	1.78	2.51	130	$C_8H_{18}O$	1-Heptanol, 6-methyl-	1-octanol	Isooctyl alcohol
7	12.036	2.06	2.78	200	$C_{13}H_{28}O$	2-propyldecan-1-OL 1-Hexadecyne	Isotridecanol	Long chain primary fatty alcohol
8	12.036	11.82	13.27	222	$C_{16}H_{30}$	1-Hexadecyne	n-tetradecyla cetylene	Aliphatic hydrocarbon
9	12.668	1.85	2.06	170	$C_{11}H_{22}O$	2-Undecanone	Undecanal	Saturated fatty aldehyde
10	12.840	2.37	3.17	212	$C_{13}H_{24}O_2$	Citronellyl propionate	3,7Dimethyl 6-enyl propionate	Fatty esters
11	3.578	11.92	11.87	242	$C_{16}H_{34}O$	1-Hexadecanol	Cetyl alcohol	Long chain fatty alcohol
12	13.578	9.22	8.65	234	$C_{14}H_{22}N_2O$	Xycaine	Lidocaine	Monocarboxylic acid amide
13	13.881	4.16	3.14	256	$C_{16}H_{32}O_2$	Hexadecanoic acid	Palmitic acid	Saturated fatty acid
14	14.065	2.33	1.66	222	$C_{12}H_{14}O_4$	1,2-Benzenedicarboxylic acid, monobutyl este	Diethyl isopthalate	Di(n-butyl)phthalate
15	14.182	6.62	4.93	200	$C_{13}H_{28}O$	1-Tridecanol	11-methyl dodecan-ol	Long chain fatty alcohol
16	14.182	9.52	8.61	270	$C_{18}H_{38}O$	1-Octadecanol	Stearyl alcohol	Fatty alcohol
17	15.479	9.17	7.82	296	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	Phytol	Diterpenoid
18	16.271	4.12	3.19	254	$C_{12}H_{24}CL_2O$	1,1-dichloro-2-dodecanol	Dodecyl alcohol	Fatty alcohol
19	19.112	1.15	1.09	196	$C_{13}H_{24}O$	1-Tridecyn-4-ol	1-Tridecyn-4-ol	Cyclotridecanone
20	22.774	2.10	1.05	295	$C_{19}H_{21}NO_2$	Propan-1-one, 3-[methyl-(3-oxo-3	Alkaloid	Nuciferin

Table 2: Biological activity of compounds identified in *Ruellia prostrata* ethanolic extract using GCMS

S. No	R. Time	Name of the compounds	Biological activity**
1	7.271	Tridecane	Antimicrobial activity
2	8.268	1-Tetradecanol	Used cosmetics for its emollient property
3	9.773	Pentadecane	Antimicrobial and antioxidant activity
4	9.930	1,2-benzoldicarbonsaeure, Di-(Hex-1-EN-5-YL-ester)	Antimicrobial, **Anticancer** and Antifouling
5	12.036	2-propyldecan-1-OL 1-Hexadecyne	Gaucher disease, treatment Mucomembranous protector, Antiviral (Arbovirus), Skin diseases treatment
6	10.770	Di-lauryl thio-di-propionate	Antioxidant
7	12.036	1-Hexadecyne	Antibacterial, **Antioxidant**
8	13.881	Hexadecanoic acid	**Antioxidant**, hypocholesterolemic , Anti androgenic, hemolytic, Alpha reductase inhibitor.
9	12.668	2-Undecanone	Anti-mycobacterial drug
10	13.578	Xycaine	Local anesthetic and antiarrhythmic property
11	14.182	1-Octadecanol	Antimicrobial activity
12	19.112	1-Tridecyn-4-ol	Antimicrobial
13	15.479	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	**Cancer preventive,** anti-inflammatory anti-diuretic, **Antioxidant**

****Source:** Dr. Duke's phytochemical and ethno botanical database (online database)

Exploring plant biochemical diversity by GC-MS (Fiehn *in viz*., 2000; Kopka *et al*., 2004) [9, 10] has proved to be a fast and reliable approach, allowing identification of a large number of compounds. In some cases, the GC-MS screening of plant samples has revealed compounds with unknown MS spectra, which has resulted in the isolation and spectroscopic identification of new natural bioactive molecules (Berkov *et al*., 2008) [5]. This investigation has helped to identify the compounds present in the plant *Ruellia prostrata*. Thus, GC-MS analysis is the first step towards understanding the nature of active principles in these plants. However, further research on isolation of individual phyto chemical constituents their pharmacological potential and toxicological aspects were needed to develop safe and novel drug.

Conclusion

In the present investigation forty four bioactive compound have been identified from ethanolic extract of *Ruellia prostrata* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds in *Ruellia prostrata* proved that the pharmaceutical importance. Though, further studies will require finding out its bioactivity, toxicity profile.

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