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Research article

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF *HEDYOTIS LESCHENAULTIANA* DC (RUBIACEAE)

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ABSTRACT: The present investigation was carried out to determine the possible bioactive components of whole plant of *Hedyotis leschenaultiana* using GC-MS analysis. Fourteen components from the whole plant of the above said plant were identified. The prevailing compounds in the ethanol extract were 9-Octadecenoic acid (z)-, Methyl ester (22.57%), β-Sitosterol (17.65%), 3,7,11,15-Tetramethyl-2-hexadecenl-o1 (12.29%), Phytol (8.61%), Oxirane, (butoxymethyl)- (8.04%), Squalene (6.48%), 1,6 Anhydro- á – D- glucopyranose (levoglycosan) (5.03%), 9-12-Octadecadienoic acid (z,z)- (4.47%), Eicosane (3.35%), 1-Eicosanol (3.35%) and Diazoprogesterone (2.91%). This is the first report of identification of active constituents from the whole plant of *Hedyotis leschenaultiana.* This work will help to identify the compounds of therapeutic value.

Keywords: *Hedyotis leschenaultiana*, GC-MS, β-Sitosterol, Squalene.

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect it, but recent research demonstrates that, many phytochemicals can protect human against diseases. There are many phytochemicals in medicinal plant and each works differently. Plant and plant products are being used as a source of medicine since long (Kishorkumar *et al*., 2011). According to World Health Organisation (WHO) more than 80% of the world's population mostly in developing countries depend on traditional plant based medicines for their primary healthcare needs. India has about 45,000 plant species and among them many have been claimed to possess medicinal properties. The need for scientific validation of these useful medicinal plants is very essential. There is a growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Murugan *et al*., 2012; Kala *et al*., 2012). Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and Alzheimer's diseases (Sheeja and Kuttan, 2007; Mukherjee *et al*., 2007). The genus *Hedyotis* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Hedyotis* in the treatment of various ailments (Sasikumar *et al*., 2010). Taking into consideration of the medicinal importance of *Hedyotis*, the ethanol extract of whole plant of *Hedyotis leschenaultiana* DC were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value.

MATERIALS AND METHODS

PLANT MATERIAL

The whole plant of *Hedyotis leschenaultiana* DC was collected from Kothagiri, Nilagiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified with the help of local flora, voucher specimen were preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

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PLANT SAMPLE EXTRACTION

The whole plants were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC- MS ANALYSIS

GC–MS was analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (330mm x 0.25mm ID x 1µm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

IDENTIFICATION OF COMPONENTS

Interpretation of mass spectrum 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.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanol extract of *Hedyotis leschenaultiana.* These compounds were identified through mass spectrometry attached with GC. The compounds present in the ethanol extract of *H. leschenaultiana* identified by GC-MS analysis are shown in Fig.1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract were 9-Octadecenoic acid (z)-, methyl ester (22.57%), β-Sitosterol (17.65%), 3,7,11,15-Tetramethyl-2-hexadecenl-o1 (12.29%), Phytol (8.61%), Oxirane, (butoxymethyl)- (8.04%), Squalene (6.48%), 1,6 Anhydro- á – D- glucopyranose (levoglycosan) (5.03%), 9-12-Octadecadienoic acid (z,z)- (4.47%), Eicosane (3.35%), 1-Eicosanol (3.35%) and Diazoprogesterone (2.91%). Figure 2, 3, 4 and 5 shows the mass spectra and structures of 1- Eicosanol, Diazoprogesterone, Squalene and Phytol. Table 2 lists the major phytocomponents and their biological activities obtained through the GC-MS study of *H. leschenaultiana*.

Among the identified phytochemicals, Squalene has antioxidant activity. Recently it has been found that, squalene possesses chemopreventive activity against the colon carcinogenesis (Rao *et al*., 1998). Phytol, a bioactive principle, detected from *H. leschenaultiana* is also found to be effective at different stages of arthritis. It is found to give good as well as preventive and therapeutic results against arthritis. The results show that, reactive oxygen species-promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009). 9-12, Octadecadienoic acid (z,z) have the property of antiinflammatory and antiarthritic as reported by the earlier workers (Lalitharani *et al*., 2009; Kala *et al*., 2011). Squalene, Phytol and 9,12 Octadecadienoic acid (z,z)- found in the ethanol extract of whole plant of *H. leschenaultiana* which are being used for the pharmacological work.

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Table 1: Components detected in the whole plant ethanol extract of *Hedyotis leschenaultiana*

No.	RT	Name of the compound	Molecular formula	Peak area %
1.	7.70	Oxirane, (butoxymethyl)-	$C_7H_{14}O_2$	8.04
2.	8.16	1,6-Anhydro-á-D-glucopyranose (levoglucosan)	$C_6H_{10}O_5$	5.03
3.	11.39	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	12.29
4.	12.38	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	2.01
5.	14.44	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	4.47
6.	14.52	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	22.57
7.	14.66	Phytol	$C_{20}H_{40}O$	8.16
8.	19.96	Heptadecane, 2,6-dimethyl-	$C_{19}H_{40}$	1.45
9.	22.76	Eicosane	$C_{20}H_{42}$	3.35
10.	24.28	Squalene	$C_{30}H_{50}$	6.48
11.	25.53	1-Eicosanol	$C_{20}H_{42}O$	3.35
12.	28.54	Vitamin E	$C_{29}H_{50}O_2$	2.23
13.	30.03	Diazoprogesterone	$C_{21}H_{30}N_4$	2.91
14.	31.73	ß-Sitosterol	$C_{29}H_{50}O$	17.65

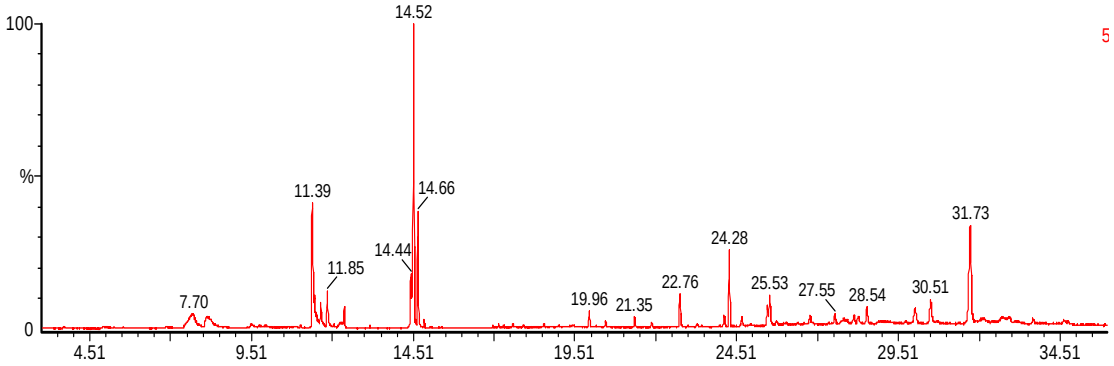

Fig. 1 GC-MS chromatogram of the ethanol extract of the whole plant of *Hedyotis leschenaultiana*

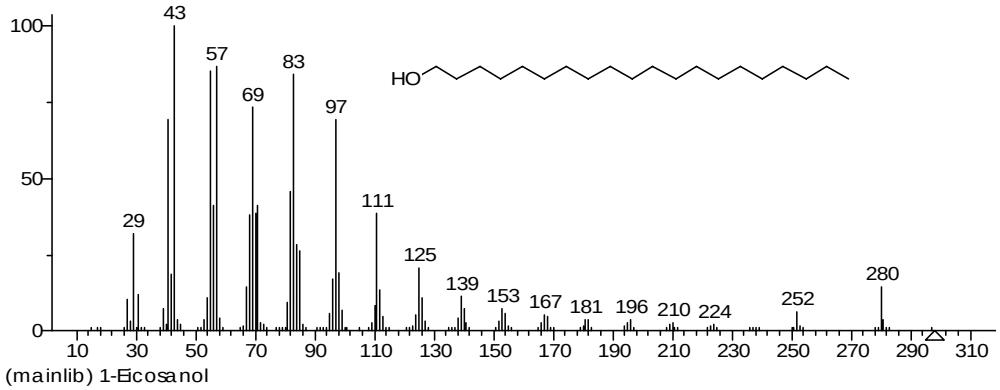

Fig. 2 Mass spectrum of 1-Eicosanol

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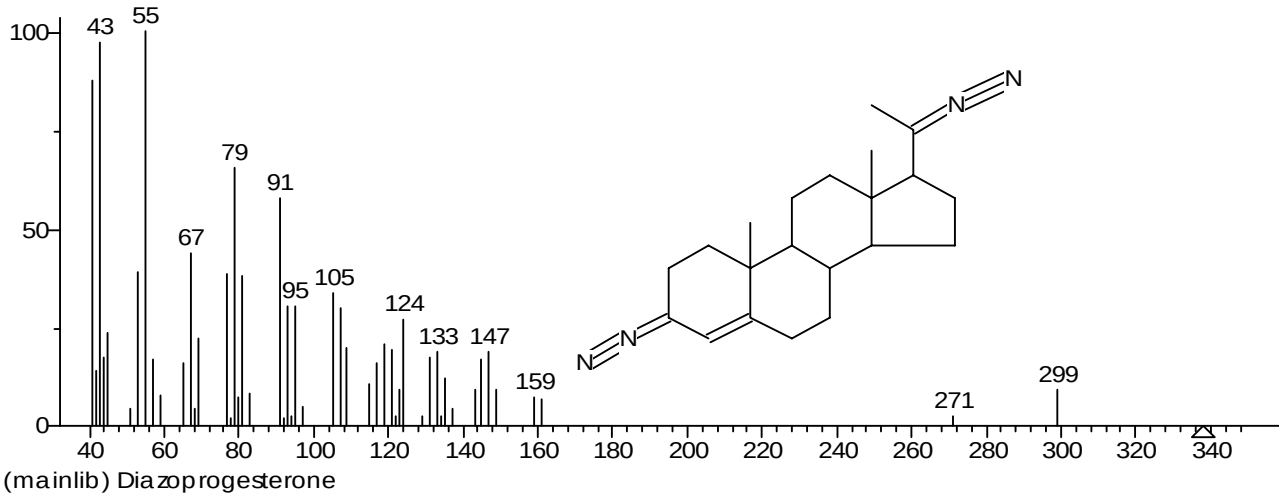

Fig. 3 Mass spectrum of Diazoprogesterone

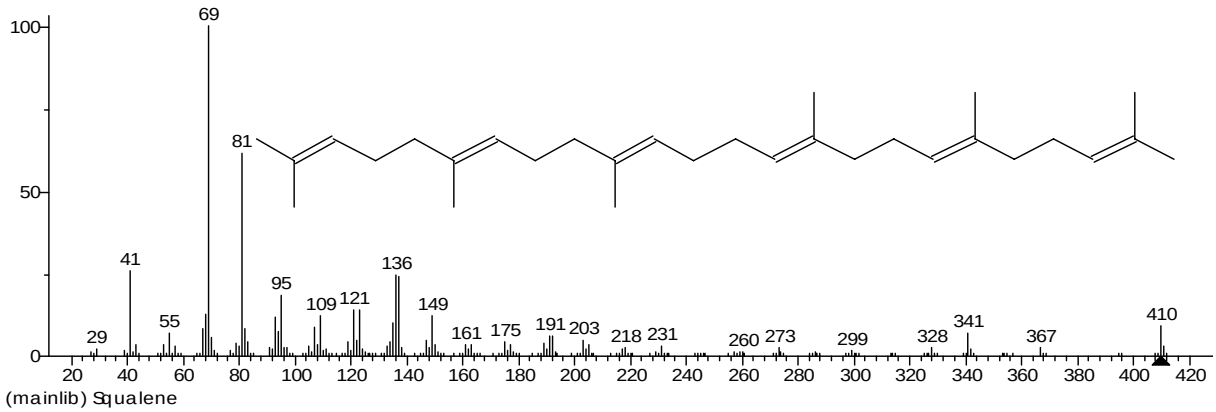

Fig. 4 Mass spectrum of Squalene

Fig. 5 Mass spectrum of Phytol

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Table 2: Activity of phytolcomponents identified in the ethanol extract of the whole plant of *Hedyotis leschenaultiana* by GC-MS.

RT	Name of the compound	Molecular formula	MW	Peak area %	Compound Nature	**Activity
8.16	1,6-Anhydro-á-D-glucopyranose (levoglucosan)	$C_6H_{10}O_5$	162	5.03	Sugar moiety	Preservative
11.39	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	12.29	Terpene Alcohol	Anti-inflammatory Antimicrobial
14.44	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.47	Linoleic acid	Antiinflammatory, Hypocholesterolemic, Cancer preventive Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, Alpha reductase inhibitor. Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
14.52	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	22.57	Oleic acid ester	Antiinflammatory, Antiandrogenic Cancer preventive ,Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
14.66	Phytol	$C_{20}H_{40}O$	296	8.16	Diterpene	Antimicrobial Anticancer Anti-inflammatory Diuretic
24.28	Squalene	$C_{30}H_{50}$	410	6.48	Triterpene compound	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
25.53	1-Eicosanol	$C_{20}H_{42}O$	298	3.35	Alcoholic compound	Antimicrobial
28.54	Vitamin E	$C_{29}H_{50}O_2$	430	2.23	Vitamin compound	Antiageing, Analgesic, Antidiabatic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic, Anticoronary
30.03	Diazoprogesterone	$C_{21}H_{30}N_4$	338	2.91	Nitrogen compound	Antimicrobial
31.73	ß-Sitosterol	$C_{29}H_{50}O$	414	17.65	Steroid	Antimicrobial Anti-inflammatory Diuretic Anti asthma Antiarthritic

Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

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In the present study, 14 compounds have been identified from the whole plant of *H. leschenaultiana* by Gas Chromatography- Mass Spectrometry (GC-MS analysis). The gas chromatogaram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of it's to analyze the chemical constituents of *H. leschenaultiana* using GC-MS. Thus, GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

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