Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4); 802-809

ISSN: 0975-4873

Research Article

Determination of Bioactive Components of *Psychotria nilgiriensis* Deb & Gang (Rubiaceae) by GC-MS Analysis

Lalitha S[1], Parthipan B[2*], Mohan V.R[3]

[1]*Microbiologist, Vivek Laboratories, K.P. Road, Nagercoil-629 003, Tamil Nadu, India.*
[2]*P.G. & Research Department of Botany , S.T. Hindu College, Nagercoil-629 002, Kanyakumari District, India*
[3]*Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin- 628 008, Tamil Nadu, India*

Available online:21st July, 2015

ABSTRACT
The investigation was carried out to determine the phytocompounds of ethanol extract of *Psychotria nilgiriensis* Deb & Gang leaf. GC-MS analysis of ethanol extract was performed using a Perkin-Elmer GC clarus 500system and Gas Chromatograph interfaced to a Mass Spectometer (GC-MS) equipped with a Elite-1, fused silica capillary column(30mm×0.25mm 10x1µmdf, composed of 100% Di methyl poly siloxene). Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute standard and Technology (NIST). The GC-MS analysis revealed the presence of 22 compounds from the leaf of *P. nilgiriensis*. The prevailing compounds were n-Hexadecanoic acid (25.08%), 9,12-Octadecadienoic acid, methyl ester (19.97%), 1-Hexadecyne (9.02%), β-Sitosterol (7.52%), Resorcinol (5.12%), 1-Octadecyne (3.93%), 1,14-Tetradecanediol (3.56%), 4-Hexen-3-one, 4,5-dimethyl- (3.46%), 2-Dodecylcyclobutanone (3.24%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.01%), Benzene, 1-methyl-3-[(2-methylpropyl)thio]- (2.27%), Stigmasterol (1.87%) and Vitamin E (0.87%) major compound in the leaf extract. The ethanol extract of leaf of *P. nilgiriensis* possesses antioxidant, antiinflammatory and antiarthritic effects so that it can be recommended as a plant of phytopharmaceutical importance.

Key words: *Psychotria nilgiriensis*- n-Hexadecanoic acid, 9,12-Octadecadienoic acid methy ester , Vitamine E.

INTRODUC TION

Plants produce a remarkable diverse array of over 5, 00,000 low and high molecular mass natural products which are known as secondary metabolites[1]. Distinguished example of these compounds includes flavonoids, phenols, saponins and cyanogenic glycosides[2,3]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially helpful properties for further chemical and pharmacological investigation[4]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of healthcare, the screening of medicinal plants for active compounds have become very Significantly[5].

GC-MS is a technique used for screening /identification/ quantification of many susceptible compounds in plant extracts. Gas chromatography (GC) is used to separate drugs that might be present in the sample. The retention time (RT) is an identifying characteristic of a drug. The combination of speed, sensitivity and a high resolving power in gas chromatography provides a very adequate technique for the separation of complex samples. Moreover, the coupling to spectrometric methods such as mass spectrometry (MS) direct identification of unknown compounds is easy to establish[6]. In recent years GC-MS studies have been increasing applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar compounds and essential oil, fatty acid, lipids and alkaloid[7].

The genus *Psychotria* belongs to the Rubiaceae family contains medicinally valuable indole alkaloids namely psychotridine and brachycerine. These alkaloids are widely used to cure problems in central nerves system of human. Ethnobotanical and chemotaxonomical studies on species of *Psychotria* resulted in the discovery of a set of novel bioactive monoterpinoid indole alkaloids (MIAs), some of them with clear pharmaceutical potential. Few reports have been published on antioxidant activities of the crude extracts or compounds isolated from *Psychotria.* Tender fruit of *P. nilgiriensis* (commonly called as odai kaapi patchilai in Tamil) is consumed along with honey for its action against rheumatism. It is being used by Kanikkar tribes of Kalakad, Mundanthurai at Tirunelveli district, and Irula tribes of Thottabeta at Nilgiris district, Tamilnadu, India[8]. Even though the plant has been reported for the medicinal property and uses, very limited reports are available regarding the phytochemical and pharmacological aspects. To our knowledge, no chemical analysis has been previously

**Author for Correspondence*

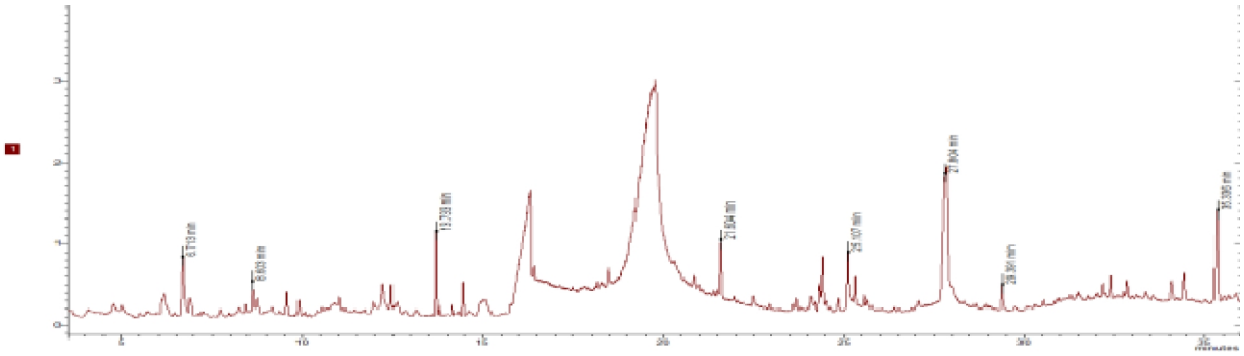
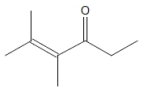
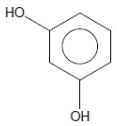
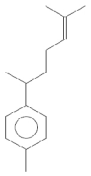
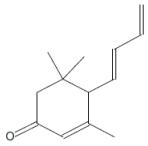
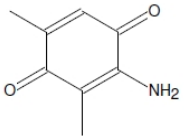
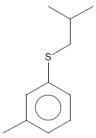
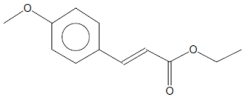
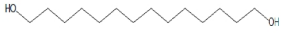
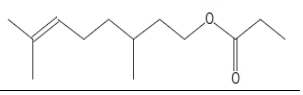
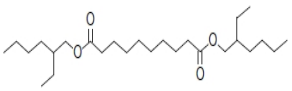
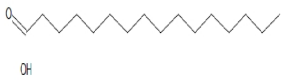
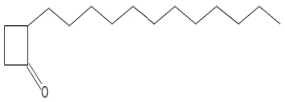
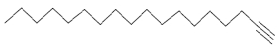
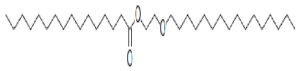
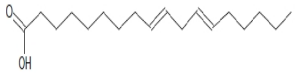
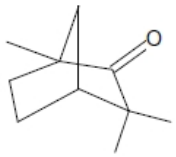
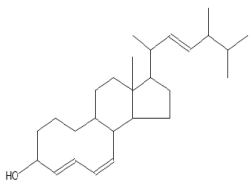
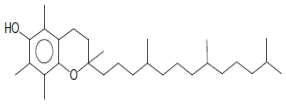
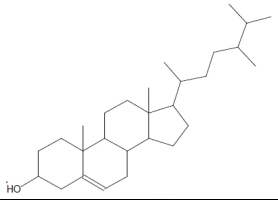


Figure 1: GC-MS chromatogram of ethanolic extract of leaves of *Psychotria nilgiriensis*

Tabe 1: Phytocomponents identified in the ethanol extract of *P. niliriensis* leaf by GC-MS

No.	RT	Name	Mol. Formulae	MW	Peak Area %	Structure
1.	6.17	4-Hexen-3-one, 4,5-dimethyl-	C8H14O	126	3.46	
2.	6.71	Resorcinol	C6H6O2	110	5.12	
3.	8.60	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202	1.46	
4.	9.63	Megastigmatrienone	C13H18O	190	1.11	
5.	9.91	2,6-Dimethyl-3-aminobenzoquinone	C8H9NO2	151	0.68	
6.	12.23	Benzene, 1-methyl-3-[(2-methylpropyl)thio]-	C11H16S	180	2.27	
7.	12.47	Ethyl p-methoxycinnamate	C12H14O3	206	0.96	
8.	13.73	1,14-Tetradecanediol	C14H30O2	230	3.56	
9.	14.45	6-Octen-1-ol, 3,7-dimethyl-, propanoate	C13H24O2	212	1.51	

Tabe 1: Phytocomponents identified in the ethanol extract of *P. niliriensis* leaf by GC-MS

No.	RT	Name	Mol. Formulae	MW	Peak Area %	Structure
10.	15.09	Sebacic acid, ethyl tridecyl ester	C25H48O4	412	1.46	
11.	16.31	n-Hexadecanoic acid	C16H32O2	256	25.08	
12.	19.77	1-Hexadecyne	C16H30	222	9.02	
13.	21.60	2-Dodecylcyclobutanone	C16H30O	238	3.24	
14.	24.45	1-Octadecyne	C18H34	250	3.93	
15.	25.15	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	3.01	
16.	27.80	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294	19.97	
17.	29.39	L-Fenchone	C10H16O	152	1.39	
18.	32.36	Ergosta-4,6,22-trien-3α-ol	C28H44O	396	0.99	
19.	32.83	Vitamin E	C29H50O2	430	0.87	
20.	34.04	Campesterol	C28H48O	400	1.51	

Tabe 1: Phytocomponents identified in the ethanol extract of *P. niliriensis* leaf by GC-MS

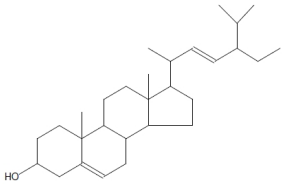
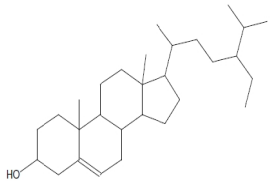
No.	RT	Name	Mol. Formulae	MW	Peak Area %	Structure
21.	34.44	Stigmasterol	C29H48O	412	1.87	
22.	35.33	β-Sitosterol	C29H50O	414	7.52	

Table 2: Activity of phytocomponents identified in the ethanol extracts of leaf of *Psychotria nilgiriensis*

No	RT	Name of the compound	Molecular formula	MW	Peak area %	Compound Nature	**Activity
1	6.17	4-Hexen-3-one, 4,5-dimethyl-	C8H14O	126	3.46	Ketone compound	No activity reported
2	6.71	Resorcinol	C6H6O2	110	5.12	Poly Phenolic compound	Pesticide Fungicide Antiacne Aldose reductase inhibitor Antibacterial Antioxidant Antiseptic
3	8.60	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202	1.46	Aromatic compound	No activity reported
4	9.63	Megastigmatrienone	C13H18O	190	1.11	Ketone compound	No activity reported
5	9.91	2,6-Dimethyl-3-aminobenzoquinone	C8H9NO2	151	0.68	Amino compound	Antimicrobial
6	12.23	Benzene, 1-methyl-3-[(2-methylpropyl)thio]-	C11H16S	180	2.27	Sulfur compound	Antimicrobial
7	12.47	Ethyl p-methoxycinnamate	C12H14O3	206	0.96	Cinnamic acid compound	Antimicrobial Anti-inflammatory
8	13.73	1,14-Tetradecanediol	C14H30O2	230	3.56	Alcoholic compound	Antimicrobial
9	14.45	6-Octen-1-ol, 3,7-dimethyl-, propanoate	C13H24O2	212	1.51	Flavoring ingredient	Flavoring uses
10	15.09	Sebacic acid, ethyl tridecyl ester	C25H48O4	412	1.46	Ester compound	Used in Candle manufacture
11	16.31	n-Hexadecanoic acid	C16H32O2	256	25.08	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
12	19.77	1-Hexadecyne	C16H30	222	9.02	Alkene compound	No activity reported
13	21.60	2-Dodecylcyclobutanone	C16H30O	238	3.24	Ketone	No activity reported

Table 2: Activity of phytocomponents identified in the ethanol extracts of leaf of *Psychotria nilgiriensis*

No	RT	Name of the compound	Molecular formula	MW	Peak area %	Compound Nature	**Activity
14	24.45	1-Octadecyne	C18H34	250	3.93	compound Alkene compound	No activity reported
15	25.15	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	3.01	Palmitic acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
16	27.80	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294	19.97	Linoleic acid ester	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
17	29.39	L-Fenchone	C10H16O	152	1.39	Monoterpene oxide	Anti-tumor, Analgesic, Antibacterial Antiinflammatory, Sedative, Fungicide, Hypocholesterolemic, Insecticide, Insectifuge Chemo preventive, Pesticide, Antiacne,
18	32.36	Ergosta-4,6,22-trien-3α-ol	C28H44O	396	0.99	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic
19	32.83	Vitamin E	C29H50O2	430	0.87	Vitamin E compound	Antiageing, Analgesic, Antidiabatic Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic,

Table 2: Activity of phytocomponents identified in the ethanol extracts of leaf of *Psychotria nilgiriensis*

No	RT	Name of the compound	Molecular formula	MW	Peak area %	Compound Nature	**Activity
							Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
20	34.04	Campesterol	C28H48O	400	1.51	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic
21	34.44	Stigmasterol	C29H48O	412	1.87	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic
22	35.33	β-Sitosterol	C29H50O	412	7.52	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiarthritic Antiasthma Diuretic

**Activity Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

reported by GC-MS on this plant except their fruit[9]. Therefore, this study was designed to identify the possible phytoconstituents present in the ethanol extract of *P. nilgiriensis* leaves using GC-MS study.

MATERIALS AND METHODS

Collection of plant sample

The fresh leaves of *Psychotria nilgiriensis* were collected from Thottabetta, Nilgiris, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical survey of India, Southern circle, Coimbatore, Tamil Nadu. The voucher specimens preserved in the P.G and Research Department of Botany, S. T. Hindu College, Nagercoil, Tamilnadu.

Preparation of plant extract

Leaves of *P. nilgiriensis* 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 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC- MS analysis.

GC - MS Analysis

GC - MS analysis of stem extracts were performed using a Perkin - Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectometer (GC - MS) equipped with a Elite - 1, fused silica capillary column (30 mm x 0.25 mm 10 x 1 μmdf, composed of 100% Di methyl poly siloxene). For GC - MS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2μ 1 was employed (split ratio of 10:1); injector temperature 250ºC; ion-source temperature 280º C. The oven temperature programmed from 110º C (isothermal for 2 min) with an increase of 100º C/min to 2000 C, then 50ºC/min to 280º C, ending with a 9 min isothermal at 280º C, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36 minutes. The relative % 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. Interpretation on mass spectrum GC - MS was conducted using the database of National Institute of 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

The results pertaining to the GC-MS chromatogram are presented in Figure- 1. On comparison of the mass spectra of the constituents with the NIST library the 22 phytoconstituents were characterized and identified, which are listed with their retention time (RT), molecular

formula, molecular weight and mass spectrum in table 1. The prevailing compounds were n-Hexadecanoic acid (25.08%), 9,12-Octadecadienoic acid, methyl ester (19.97%), 1-Hexadecyne (9.02), β-Sitosterol (7.52), Resorcinol (5.12), 1-Octadecyne (3.93), 1,14-Tetradecanediol (3.56), 4-Hexen-3-one, 4,5-dimethyl- (3.46), 2-Dodecylcyclobutanone (3.24), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.01), Benzene, 1-methyl-3-[(2-methylpropyl)thio]- (2.27), Stigmasterol (1.87), 6-Octen-1-ol, 3,7-dimethyl-, propanoate (1.51), Campesterol (1.51), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (1.46), Sebacic acid, ethyl tridecyl ester (1.46), L-Fenchone (1.39), Megastigmatrienone (1.11), Ergosta-4,6,22-trien-3α-ol (0.99), Ethyl p-methoxycinnamate (0.96), Vitamin E (0.87), 2,6-Dimethyl-3-aminobenzoquinone (0.68). Table 2 listed the major phtocompounds and its biological activities obtained through the GC-MS study of the leaf of *P. nilgiriensis.*

DISCUSSION

In the present study, 22 compounds have been identified from ethanol extract of the leaf of *P nilgiriensis* by Gas Chromatography- Mass spectroscopy (GC-MS) analysis. Among the identified phytochemical n- Hexadecanoic acid have the property of antioxidant activity and 9,12-Octadecadienoic acid , methyl ester have the property of antiinflammatory and antiarthritic as reported by the earlier worker[10]. Vitamin E is thought to be important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution and immunocompetence, membrane and DNA repair and decreasing oxidative DNA damage[11]. *In vitro* studies showed that vitamin E can prevent oxidation of DNA by inhibiting activated neutrophils. Vitamin E can protect the conjugated double bond of β-carotene from oxidation[12] Similar compounds and their biological activites are found in *Nothapodytes nimmoniana* were reported by[13]. Stigmasterol is used as a precursor of semisynthetic progesterone[14], a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogens effect, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. It is also used as the precursor of Vitamin D3[15,16].

CONCLUSION

In the present study, 22 components from leaf of *P. niligiriensis* were identified by GC-MS analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. So that it might be utilized for the development of traditional medicines and further investigation is need to elute novel active compounds from the medicinal plants which may create a new way to treat many uncurable diseases.

ACKNOWLEDGEMENT

The authors are thankful to the Management and Principal of S.T. Hindu College, Nagercoil and V.O.Chidambaram College, Tuticorin for their support and their encouragement.

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