

**PHYTOCHEMICAL AND GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC
ANALYSIS OF ETHANOLIC EXTRACT OF *RAUVOLFIA SERPENTINA* ROOT****¹Thulasi Sivaraman*, ²N. S. Sreedevi and ³S. Meenatchisundaram**¹Research Scholar, Department of Microbiology, Nehru Arts and Science College, Coimbatore, Tamil Nadu, India.²Research Scholar, Department of Microbiology, Nehru Arts and Science College, Coimbatore, Tamilnadu, India.³Director, Research and Projects, Associate Professor, Department of Microbiology, Nehru Arts and Science College, Coimbatore, Tamil Nadu, India.**Corresponding Author: Thulasi Sivaraman**

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Article Received on 14/12/2016

Article Revised on 03/01/2017

Article Accepted on 25/01/2017

ABSTRACT

Rauvolfia serpentina which is also known as Indian snakeroot or sarpagandha is a member of the family Apocynaceae is an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. Qualitative analysis of root extracts of *Rauvolfia serpentina* indicates the presence of alkaloids, flavanoids, protein, coumarin and tannins. Ethanolic extract of *Rauvolfia serpentina* root extract was analyzed through GC-MS analysis. The compounds were identified by comparison of GC-MS spectrum with library searches which led to identification of twenty three compounds. The major constituents were found to be Phenol,2,4-bis (1,1-dimethylethyl) (30.97%) Benzoic acid, 4-ethoxy-, ethyl ester (1.25%), Eicosane (2.32%), 3-Heptadecanol (3.25%), Isopropyl myristate(1.96%), 3-Undecanol, 3-ethyl(1.46%), Heneicosane (1.86%), Hexadecanoic acid (3.12%), Hexadecanoic acid, Ethylester (1.67%), Cyclohexanol, 1-butyl(1.47%), Tetrapentacontane (1.71%), D-Ribose, 2-Deoxt-bis(Thioheptyl) (2.08%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (33.30%), Octadecane (1.17%), Diethylphthalate (2.07%), Octadecanoic acid, 2,3-dihydroxypropyl ester (5.94%), Beta-Yohimbine(1.47%). Among the constituents Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester covers 33.30% peak area with retention time of 22.14.

KEYWORDS: *Rauvolfia serpentina*, Phytochemicals, Gas chromatography-mass spectrometry.**INTRODUCTION**

Historically plants have played an important role in medicine. Through observations and experimentations, human beings have learn that plants promote health and welfare. The use of these herbal remedies is not only gainful but also safe and almost free from serious side effects.^[1] A wide range of medicinal plant part is used for extract as raw drug and they acquire varied medicinal properties.^[2] It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituent that produce definite physiological effects on human body. These natural compounds formed the foundation of modern drugs as we use at present.^[3] Herbal medicines are becoming popular in modern world as people resort to natural therapies. Natural products isolated from higher plants and microorganisms have been providing novel clinically active drugs. The medicinal plant could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them.^[4, 5]

Rauvolfia serpentina L. Benth. Ex Kurz. is an evergreen, woody, glabrous and perennial shrub with maximum height upto 60 cm.^[6] The plant belongs to the family Apocynaceae and occurs in habitats of tropical and subtropical regions. The family includes 50 species, distributed worldwide in the region of the Himalayas, Indian peninsula, Burma, Indonesia and Sri Lanka and is native to India, Bangladesh and other regions of Asia.^[7] The plant is commonly known as Sarpagandha, Chandrabagha, Snake root plant, Chotachand, Chandrika and Harkaya etc.^[8] The roots, leaves and juice are of medicinal importance and have attracted the attention of practitioners of indigenous system of medicine, as it contain a large number of secondary metabolites (N-containing indole alkaloids) localized mainly in the roots and rhizomes.^[9] It is used as an antidote against snake bite and bites of other poisonous insects.^[10] The roots of *Rauvolfia serpentina* is used in Ayurvedic medicines as a treatment for curing hypertension, sleeplessness, mental agitation, gastrointestinal disorders, anticipation epilepsy, trauma, anxiety, stimulation, schizophrenia, sedative insomnia and psychosis.^[11]

The major alkaloid present in root, stem and leaves of the plant is Reserpine varies from 1.7 to 3.0%.^[12] While Ajmalinine, Ajmalicine, Chandrine, renoxidine, reserpinine, Sarpagine, Tetraphyllicine, Yohimbine, 3-epi-ayohimbine are the other alkaloids present in *Rauvolfia serpentina*.^[13] The plant is known to cure various circulatory disorders due to the presence of alkaloids.^[14] Therefore, the present study was carried out to determine the phytochemical constituents of root of *Rauvolfia serpentina* root by using gas chromatography mass spectrometry (GC-MS).

MATERIALS AND METHOD

Collection and authentication of plant material:

Plant sample was collected from Anakkal region, Malampuzha, Palakkad district, Kerala after questionnaire with tribal people and from vaidyars in and around Palakkad district. It was authenticated by Dr.M.Palinisamy, Scientist 'D' in Charge, Botanical Survey of India Southern Regional Centre, Coimbatore.

Preparation of Extracts:

20 g of powdered sample of the herb was extracted by soaking in 180 ml of distilled water in a beaker, stirred for about 6 min and left over night. Thereafter, the solution was filtered using filter paper (What man No. 1) and the extracts were evaporated to dryness under reduced pressure below 40°C. The plant extracts were expressed in terms of dry weight.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Test for Alkaloid^[15]

Solvent free extract, 50 mg of the plant sample was stirred with one ml of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids.

Mayer's Test: To the filtrate, a drop of Mayer's reagent was added along the sides of the test tube. A white precipitate indicates the test as positive.

Test for Flavanoids

Alkaline reagent test: Two ml of aqueous solution of the extract was treated with 1 ml of 10 % ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Test for Saponins

50 mg of the plant sample was ground with 3 ml of distilled water and diluted with the same, made-up to 20 ml. The suspension was shaken in a graduated cylinder. After 15 min, a two cm layer of foam indicates the presence of saponins.

Test for Phenols

Ferric chloride test: 50 mg of the sample was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

Test for Protein

To 1 ml of the extract few drops of Barfoed's reagent was added to give blue color products.

Test for Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

Test for Reducing Sugar^[16]

To 0.5 ml of the aqueous extract of the plant sample, 1 ml of water and 5 -8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Tannins

One ml of water and 1-2 drops of ferric chloride solution was separated and 1 ml of aqueous extract of the plant sample. Blue color was observed for gallic tannins and green black for catecholic tannins.

GC-MS ANALYSIS

GC MS analysis was carried out at Kerala Forest Research Institute, Peechi, Thrissur, Kerala. It is one of the key techniques generally used for screening/identification of many group of plant phytochemicals. Different constituents of ethanolic root extract of *Rauvolfia serpentina* was determined on a Shimadzu GCMS-QP 2010S system. Chromatography was performed on a 30 m x 0.25 mm ID x 0.25 µm thickness DB-5MS column. Injector temperature was set at 260°C, Interface temperature was set at 280°C and the ion-source temperature was at 200°C. Helium was used as a carrier gas with a flow rate of 1ml/m and the sample injected was 1µl. The oven temperature programmed from 80°C for 2 minutes to 260°C to 10 minutes. Total GC running time was 40 m. Split ratio set as 50 and the injector used was splitless mode. The chromatogram obtained from the GC was then analysed in the mass spectroscopy (MS) to get the mass of all the fractions. Interpretation of mass spectrum of GC -MS was done using the database of NIST 11 and WILEY 8. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analyses

The results of phytochemical screening of root extracts of *Rauvolfia serpentina* is presented in **Table 1**. Qualitative tests for alkaloids, flavonoids, saponins, phenols and other phytochemicals were carried out in order to know the presence of primary and secondary metabolites in these crude extracts of the plant.

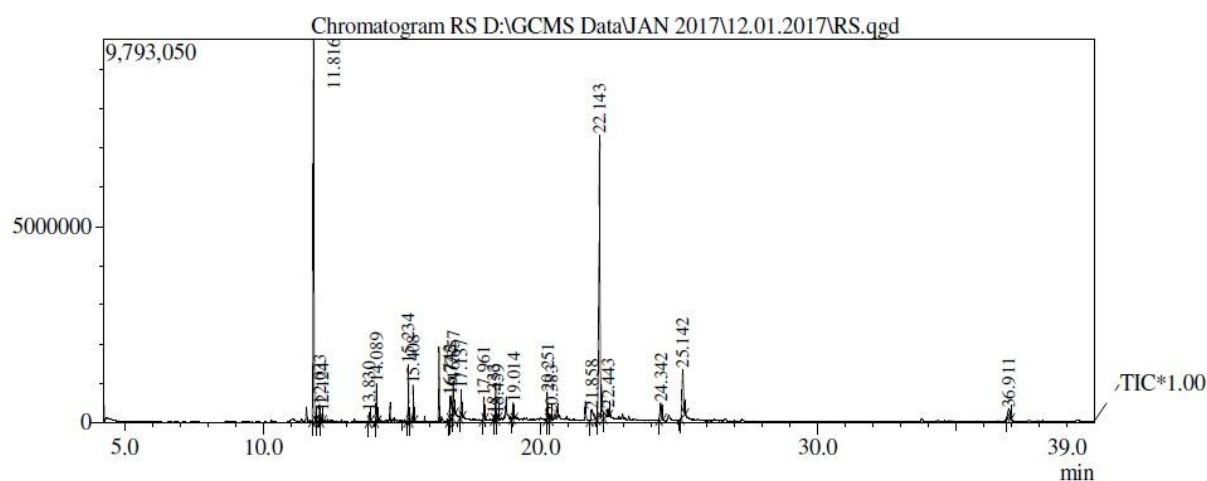
Table 1 Qualitative Phytochemical Analysis

Phytochemical constituents	Qualitative analysis of <i>Rauvolfia serpentina</i>
Alkaloids	+
Flavonoids	+
Saponins	-
Phenol	-
Protein	+
Coumarin	+
Reducing sugar	-
Tannins	+

GC-MS Analysis

GC-MS chromatogram of the ethanolic extract of root of *Rauvolfia serpentina* (Figure 1) showed twenty three peaks indicating the presence of twenty different

compounds, besides a number of peaks with very narrow retention time. The chemical compounds identified in the ethanolic extract of root of the *Rauvolfia serpentina* are presented in Table 2.

(Figure 1) GC-MS chromatogram of the ethanolic extract of root of *Rauvolfia serpentina*Table 2 Phytochemical components identified in the ethanolic extract of root of *Rauvolfia serpentina* by GC-MS

Peak	RTime	Area	Area %	Molecular formula	Molecular weight g/mol	Name of the components	Base m/z
1	11.816	2203346	30.97	C ₁₇ H ₃₀ OSi	278.511	Phenol,2,4-bis(1,1-dimethylethyl)	191.15
2	12.033	887017	1.25	C ₁₁ H ₁₄ O ₃	194.23	Benzoic acid, 4-ethoxy-, ethyl ester	121.05
3	12.124	479191	0.67	C ₁₉ H ₄₀	268.529	Nonadecane	57.05
4	13.830	458933	0.65	C ₁₆ H ₃₃ Cl	260.886	1-Chlorohexadecane	57.05
5	14.089	1648143	2.32	C ₂₀ H ₄₂	282.556	Eicosane	57.05
6	15.234	2310603	3.25	C ₁₇ H ₃₆ O	256.474	3-Heptadecanol	59.05
7	15.408	1394477	1.96	C ₁₇ H ₃₇ O ₂	270.457	Isopropyl myristate	60.00
8	16.742	1039277	1.46	C ₁₃ H ₂₈ O	200.3608	3-Undecanol, 3-ethyl-	87.10
9	16.775	1321972	1.86	C ₂₁ H ₄₄	296.583	Heneicosane	57.05
10	16.857	2223169	3.12	C ₁₆ H ₃₂ O ₂	256.4241	Hexadecanoic acid	73.00
11	17.137	1188287	1.67	C ₁₈ H ₃₆ O ₂	284.4772	Hexadecanoic acid,Ethylester	88.05
12	17.961	1046206	1.47	C ₁₀ H ₂₀ O	156.2652	Cyclohexanol, 1-butyl-	81.10
13	18.333	157095	0.22	C ₁₀ H ₁₈ O	154.249	(E)1-Allyl-2-methylcyclohexanol	95.10
14	18.459	128865	0.18	C ₁₄ H ₃₀	198.39	Tetradecane	57.05
15	19.014	658955	0.93	C ₂₀ H ₄₀ O ₂	312.5304	Octadecanoic acid,Ethylester	88.05
16	20.251	1216048	1.71	C ₅₄ H ₁₁₀	759.474	Tetrapentacontane	57.05
17	20.251	201429	0.28	C ₂₈ H ₄₂ O ₂	410.63	O O'-Biphenol, 4,4',6,6'-Tetra-T-butyl,	57.05
18	21.383	1482771	2.08	C ₁₉ H ₄₀ O ₃ S ₂	380.646	D-Ribose, 2-Deoxt-bis(Thioheptyl),	117.05
19	22.143	23688858	33.30	C ₁₉ H ₃₈ O ₄	330.503	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	98.10

20	22.443	831192	1.17	C ₁₈ H ₃₈	254.502	Octadecane	57.05
21	24.342	1474927	2.07	C ₁₂ H ₁₄ O ₄	222.24	Diethyl phthalate	130.10
22	25.142	4228567	5.94	C ₂₁ H ₄₂ O ₄	358.5558	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.10
23	36.911	1048348	1.47	C ₂₁ H ₂₆ N ₂ O ₃	354.45	Beta-Yohimbine	353.20

The major constituents were found to be Phenol,2,4-bis(1,1-dimethylethyl) (30.97%) Benzoic acid, 4-ethoxy-, ethyl ester (1.25%), Eicosane (2.32%), 3-Heptadecanol(3.25%), Isopropyl myristate(1.96%), 3-Undecanol, 3-ethyl(1-46%), Heneicosane (1.86%), Hexadecanoic acid (3.12%), Hexadecanoic acid,Ethylester (1.67%), Cyclohexanol, 1-butyl(1.47%), Tetrapentacontane (1.71%), D-Ribose, 2-Deoxt-bis(Thioheptyl) (2.08%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (33.30%), Octadecane(1.17%), Diethyl phthalate (2.07%), Octadecanoic acid, 2,3-dihydroxypropyl ester(5.94%), Beta-Yohimbine(1.47%). Among the constituents Hexadecanoic acid.2-hydroxy-1-(hydroxymethyl)ethyl ester covers 33.30% peak area with retention time of 22.14.

In accordance with the previous findings, most of the identified compounds from this study have also been reported elsewhere in other species. According to^[17] Hexadecanoic acid, ethylester and Octadecanoic acid, ethylester were present in leaf extracts of *Neolamarckia cadamba* and it possess Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic activity. Corresponding to the present study^[18] observed, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester were present in Petroleum ether leaf extract of *Clerodendrum phlomidis*. Similarly,^[19] tetradecane and heneicosane were present in the GCMS analysis of Petroleum ether of *Pulicaria crispa* and^[20] Hexadecanoic acid were present in methanolic extract of *Leucas aspera* and it possess anti-inflammatory PLA2 inhibition activity. In the current investigation^[21] nonadecane and hexadecanoic acid were present in essential oil of *Jasminum officinale* L var *Grandiflorum* Flower and^[22] detected nonadecane is an antioxidant. In another report^[23] eicosane were present in Leaves of *Vernonanthura patens*. Similarly^[24] Diethyl phthalate were present in GC-MS Analysis of n-Hexane Extract of *Rauvolfia serpentina* root. So it is recommended as a plant of phyto pharmaceutical importance, however, further studies will need to be undertaken to ascertain fully its pharmacological activity.

CONCLUSION

Phytochemical investigation of root extracts *Rauvolfia serpentina* indicates the presence of alkaloids, flavonoids, protein, coumarin and tannins. GC-MS chromatogram of the ethanolic extract of root of *Rauvolfia serpentina* showed different peaks indicating the presence of different compounds. The compounds were identified by comparison of GC-MS spectrum with library searches which led to identification of twenty compounds. Further studies are required to confirm the compound detected by GC-MS analysis.

ACKNOWLEDGEMENT

Authors Thank to the Kerala Forest Research Institute(KFRI), Peechi, Thrissur, Kerala India. For GC-MS analysis.

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