Vol 6 | Issue 1| 2016 | 11-15.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

POTENTIAL BIOACTIVE COMPONENTS OF *MALAXIS RHEEDEI* SW. (ORCHIDACEAE)

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ABSTRACT

Malaxis rheedei Sw. belongs to the family Orchidaceae, is commonly Known as *Jeevakam*. The present investigation was carried out to determine the possible bioactive components whole plant extract of *Malaxis rheedei* by using GC-MS analysis. The GC-MS analysis provides different peaks determining the presence of 42 compounds were identified from the whole plant extract of *Malaxis rheedei.* The main compounds in the Methanol extract of whole plant extract of *Malaxis rheedei* were identified as 2,6,10,14,18,22-Tetracosahexaene, 2,6, (17.07%), n-Nonadecanol-1 (11.47%), 4,7,10,13,16,19-Docosahexaenoic acid(8.83%), Oxirane, hexadecyl-(7.19%), Phytol (6.97%) and 2,6,10-Trimethyl, 14-Ethylene-1(6.26%). The aim of the present work was to understand the chemical constituents of plant and also highlight the actual significance of the traditional medicinal practices of this potential medicinal plant.

Keywords: *Malaxis rheedei*, Orchidaceae, Methanol extracts, GC-MS Analysis.

INTRODUCTION

The first record of Indian orchid *Malaxis rhedei* used in ayurvedic medicine is discussed in 'Charaka Samhita', a classic ancient Indian medicinal treatise written by Charaka in Sanskrit, a few thousand years ago [1]. *Malaxis* genus is distributed throughout the world. It is found in India, Bangladesh, Eastern Himalayas, Bhutan, Andaman Islands, Myanmar, Thailand, Malaysia, Combodia, China, Vietnam, Java, Sumatra, Philippines and Australia [2].

Malaxis rheedei Sw. (Orchidaceae) commonly named as '*Jeevakam*' is a rare, terrestrial, endangered and medicinal orchid [3]. In recent times GC-MS studies widely used for the analysis of medicinal plants because this technique has proved to be an effective method for the valid analysis of biological compounds [4]. The whole plant extract of *Malaxis rheedei* is used by *Kattunayaka* tribes in Nilambur area, Malappuram district at Kerala. *Kattunayakans* used *Malaxis rheedei* Sw. (Orchidaceae) is one of the best medicines for against snake poisons, fever, joint pain and burns. This potential plant has been selected for the present study.

PLANT DESCRIPTION

Botnaical Name: ***Malaxis rheedei*** Sw. (Orchidaceae)
Synonyms: *Seidenfia rheedei* (Sw.) Szlach.
Microstylis rheedei (Sw.) Lindl.

Botanical description

Stem swollen towards base, to 15 cm long. Leaves broadly ovate or elliptic, to 12 x 6cm, with purple shades. Scape to 18 cm long. Bracts subulate, deflexed. Flowers orange yellow, 0.5 cm across. Sepals and petals linear, 3 mm long. Lip reniform, margin pectinate (Fig.1).
Distribution: India, China, Thailnad and Sri Lanka
Local Names: Jeevakam
Habit: Erect herb
Habitat: Semi-evergreen and moist deciduous forests

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MATERIALS AND METHODS

Solvent Extraction

The fresh whole plant parts of *Malaxis rheedei* Sw. (Orchidaceae) were washed with tap water and shade dried for two month and powdered coarsely. Then they were finely powdered mechanically using Pulverizer and passed through 40 mesh sieve and stored in airtight containers. About 250g of powdered aerial and root were extracted in soxhlet apparatus with methanol (6.1 and 10.3). The process of extraction continues till the solvent in siphon tube of an extractor become colourless. The extract was taken in a beaker and kept it for air dry till the solvent got evaporated. The last traces of the solvent were removed under vacuum drier and the solid mass obtained was stored at 4°C until further use.

GC-MS Analysis

Gas Chromatography (GC) analysis was carried out using Varian 5975 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fit with VF 5 MS capillary column (30 m × 0.25 mm). The injector temperature was set at 240ºC, and the oven temperature was initially be at 70ºC then programmed to 300ºC at the rate of 10ºC / minute and finally held at 300ºC for 10 min. Helium was used as carrier gas with the flow rate of 1.51ml/min. The percentage of composition of extract was calculated by GC peak areas. The compounds were identified based on comparison of their retention indices (RI), retention time (RT) and mass spectra.

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name of the components of the test materials was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

This is done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and traditional use as a herbal medicine, in addition to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their biological or therapeutic relevance.

RESULTS

The GC-MS analysis of *Malaxis rheedei* revealed that the presence of 42 compounds (phytochemical constituents) in whole plant part of methanolic extracts that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area (%) and retention time (RT) The first compound identified with less retention time (5.835and 7.029 min) was Ethinamate, Cyclopentane, 1,3-BIS (METHYL) and Propanamide, 3,3,3-trifluoro-2-(trifluoro) respectively. Whereas Tetrapentacontane and 2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6 was the last compound which took longest retention time (42.541 and 43.193 min) to identify. The highest peak area for 2,6,10,14,18,22-Tetracosahexaene, 2,6, (17.07%), n-Nonadecanol-1(11.47%), 4,7,10,13,16,19-Docosahexaenoic acid (8.83%), Oxirane, hexadecyl-(7.19%), Phytol (6.97%) and 2,6,10-Trimethyl,14-Ethylene-1(6.26%). The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed (Table-1 & Fig. 2).

Figure 1. Image of *Malaxis rheedei* Sw. (Orchidaceae)

Figure 2. GC-MS Chromatogram of *Malaxis rheedei* Sw. (Orchidaceae)

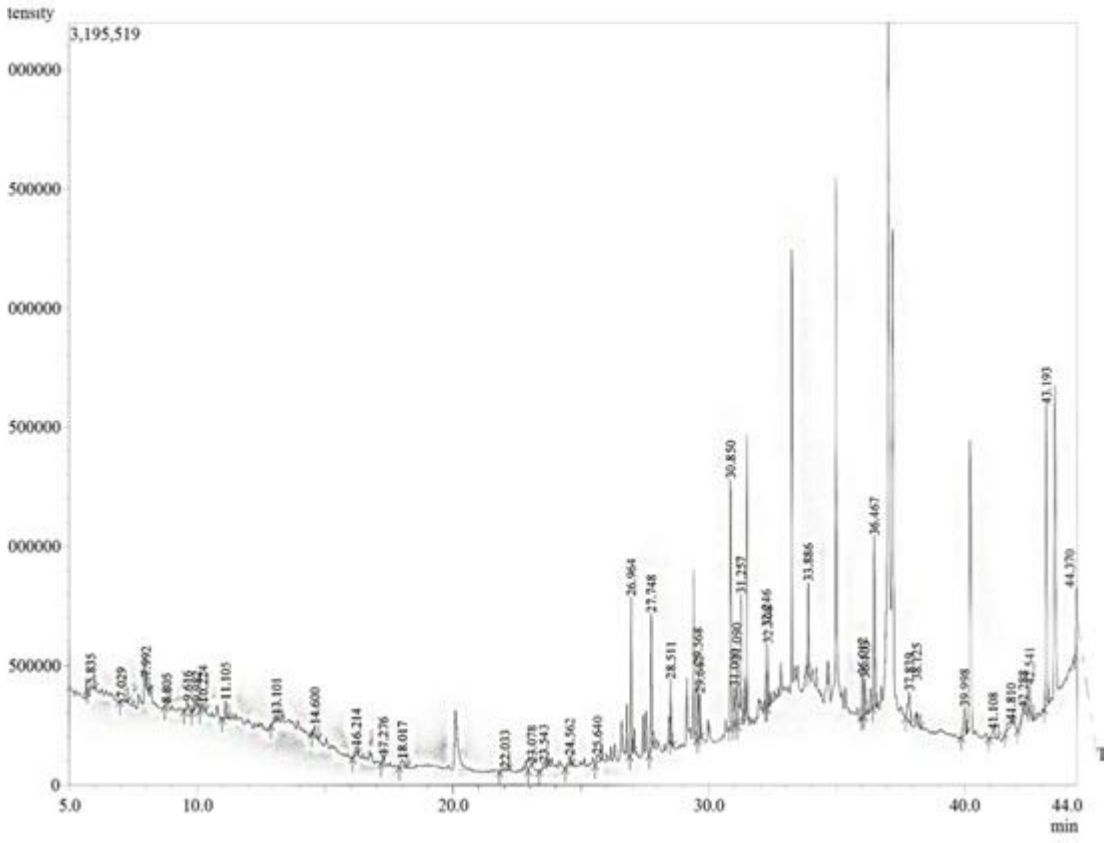

Table 1. Phytochemical compounds identified in the methanolic extract (Whole parts) of *Malaxis rheedei* by GC-MS analysis

S.No	R/T	Peak Area	Name of the compounds	Molecular formula	Molecular weight
1	5.835	-0.37	Ethinamate	$C^9H^{13}NO^2$	167
2	7.029	0.37	Cyclopentane, 1,3-bis(methyl	C^7H^{10}	94
3	7.992	-1.19	Propanamide, 3,3,3-trifluoro-2-(trifluoro	$C^4H^3F^6NO$	195
4	8.805	0.01	Benzenamine, 2-methyl	C_7H_9N	107
5	9.616	0.58	2-Nonanol, 5-Ethyl	$C_{11}H_{24}O$	172
6	9.982	0.41	2-Butanol, 3-(1-methylbutoxy)-	$C_9H_{20}O_2$	160
7	10.224	0.57	4-Nitro-N-(2,6-xylyl)benzenesulfonami	$C_{14}H_{14}N_2O_4S$	306
8	11.105	0.67	Alpha.-D-Galactopyranosid	$C_{13}H_{25}NO_6$	291
9	13.101	0.51	1-Heptanol, 2,4-diethyl-	$C_{11}H_{24}O$	172
10	14.600	0.59	Hexane, 2,4,4-trimethyl-	C_9H_{20}	128
11	16.214	0.83	Trichloroacetic acid, tridec-2-ynyl ester	$C_{15}H_{23}C_{l3}O_2$	340
12	17.276	0.09	Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22O	206
13	18.017	0.92	Oxalic acid, 6-ethyloct-3-yl heptyl ester	$C_{19}H_{36}O_4$	328
14	22.033	0.36	Benzonitrile, 2-benzylthio-4-nitro	$C_{14}H_{10}N_2O_2S$	270
15	23.078	0.18	1-Undecanol	$C_{11}H_{24}O$	172
16	23.543	0.27	3-Fluorobenzoic acid, 2-tridecyl ester	$C_{20}H_{31}FO_2$	322
17	24.562	0.74	Dodecane, 1-chloro-	$C_{12}H_{25}Cl$	204
18	25.640	0.74	2(4H)-benzofuranone, 5,6,7,7A-T	$C_{11}H_{16}O_3$	196
19	26.964	6.26	2,6,10-trimethyl,14-ethylene-1	$C_{20}H_{38}$	278
20	27.748	7.19	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268
21	28.511	2.26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270

22	29.568	3.34	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
23	29.647	1.70	Heneicosane	$C_{21}H_{44}$	296
24	30.850	11.47	n-Nonadecanol-1	$C_{19}H_{40}O$	284
25	31.000	2.04	Ethyl (9Z,12Z)-9,12-octadecadi	$C_{20}H_{36}O_2$	308
26	31.090	3.44	9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292
27	31.257	6.97	Phytol	$C_{20}H_{40}O$	296
28	32.246	3.08	Octadecanoic acid, Ethyl ES	$C_{20}H_{40}O_2$	312
29	32.308	2.02	Dotriacontane	$C_{32}H_{66}$	450
30	33.886	3.51	Icosapent	$C_{20}H_{30}O_2$	302
31	36.012	2.28	1-Heptacosanol	$C_{27}H_{56}O$	396
32	36.103	1.59	Dotriacontane	$C_{32}H_{66}$	450
33	36.467	8.83	4,7,10,13,16,19-Docosahexaenoic acid	$C_{23}H_{34}O_2$	342
34	37.839	2.69	Bicyclo[2.2.1]Heptane-1-Metha	$C_{10}H_{16}O_3$	184
35	38.125	-0.32	Dotriacontane	$C_{32}H_{66}$	450
36	39.998	2.41	Dotriacontane	C_32H_{66}	450
37	41.108	0.58	Ethanol, 2-(eicosyloxy)-	$C_{22}H_{46}O_2$	342
38	41.810	1.14	Stigmasterol	$C_{29}H_{48}O$	412
39	42.288	-0.40	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337
40	42.541	1.01	Tetrapentacontane	$C_{54}H_{110}$	758
41	43.193	17.07	2,6,10,14,18,22-Tetracosahexaene, 2,6,	$C_{30}H_{50}$	410
42	44.370	3.55	Tetrapentacontane	$C_{54}H_{110}$	758

DISCUSSION

The gas chromatogram shows that the relative concentrations of various compounds are getting eluted as a function of retention time. The height of the peaks indicates the relative concentrations of the compounds present in the plant. The mass spectrometer analyzes of the compound present in the plant. The mass spectrometer analyses of the compound eluted at different times to identify the nature and structure of the compound. The large compounds fragments into small compounds give rice to the appearance of peaks at different ratios. These mass spectra are fingerprint of that compound which can be identified from the data library [5].

In the present study, the GC-MS analysis of *Malaxis rheedei* revealed the presence of 42 compounds (phytochemical constituents) in whole plant part of methanolic extract that could contribute the medicinal quality of the plant (Table-1). The identification of the phytochemical compounds was confirmed based on the peak area (%) and retention time (RT). In recent years Gas chromatography – Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc [6,7]. The GCMS analysis of the methanolic extract resulted many compounds which have diverse use. Compounds having anti-diabetic, antibacterial, antifungal, antioxidant and anticancer properties have been identified. In addition to these, the plant is extensively used against snake poison by tribal people of the area.

CONCLUSION

GC-MS method is a direct and fast analytical approach for identification of potential bioactive from plant extracts. The results obtained through such studies are supporting the medico-potentiality some valuable plants. In this present study, there are about 42 compounds present in methanol extract of whole plant part of *Malaxis rheedei* by GC-MS method. Such results also highlight the potentiality of these species in anticarcinogenic, antidiabetic, antimicrobial and antioxidant properties.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

1. Sahaya S, Chitra D, Sarmad M, and Servin W. Evaluation of Bioactive Potential of *Coelogyne nervosa* A. Rich. - An endemic medicinal orchid of Western Ghats, India. *Asian Journal of Pharmaceutical and Clinical Research,* 6(1), 2013, 114 - 118.
2. Arenmonla T, Chitt RD. Germination of immature embroys and multiplication of *Malaxis accuminata* D. Don, *Indian Journal of Biotechnology*, 11, 2012, 464 - 469.
3. Sharma P, Mahajan N, Garg P, Singh G, Dadhwal S, Sharma S. *Malaxis acuminata* A Review. *International Journal of Research in Applied Pharmacology,* 2(2), 2011, 422 – 425.

4. Srinivasa Reddy CH, Ammani K, Rose M. GC-MS Studies of *Maba baxifolia* (Rottb.) Juss. Stem. *Journal of Global Biosciences,* 4(1), 2015, 1193-1197.
5. Antony SM, Paul JP, Yesu Raj. Phytochemical Analysis of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS. *Research Journal of Chemical Sciences,* 3(11), 2013, 14-23.
6. Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja, PS. Plant-based anticancer molecules: A chemical and biological profile of some important leads. *Bioorganic and Medicinal Chemistry,* 13, 2005, 5892-5908.
7. Konovalova A, Lotte SA, Lee K. Regulated proteolysis in bacterial development *FEMS. Microbiology Reviews***,** 38(3), 2013, 493-522.