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Original Article

GC-MS Analysis of *Mussaenda roxburghii* Hk.f..: A Folk Food Plant Used Among Tribes Of Arunachal Pradesh, India

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ABSTRACT

Introduction: *Mussaenda roxhburgii* Hk.f. shoot is widely used as folk food among the tribal people of Arunachal Pradesh. This study was carried out to establish the phytoconstituents of *Mussaenda roxburghii* shoot. **Methods:** Fresh shoot were collected from Mone forest of East Siang district of Arunachal Pradesh and identified by the corresponding author himself. The samples were shade dried and pulverized to powder using a mechanical grinder. 500 g powder of the sample was soaked in ethanol for 72 hours with intermittent shaking then filtered through Whatmann No. 41 filter paper and concentrated under reduced pressure at 40°C by using rotary evaporator to obtain a viscous semi solid extract. Gas-Chromatography Mass Specrometry (GC-MS) analysis of the ethanol extracts was carried out in Shimadzu GCMS-QP-2010 plus system. The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, US) and WILEY 8. **Results:** The GCMS chromatogram of *Mussaenda roxburghii* shoot ethanolic extract gave forty nine phytochemical compounds. The most prevailing compounds were hydrocoumarin with 13.6%, hexadeconoic acid, 2-hexadecen-1-ol, 3, 7, 11, 15 tetramethyl [R-[R-) with 17.52%, ethyl ester with 6.24% and Vitamin E with 2.8%. The study gave some compounds with biological interest like gamma tocopherol, Vitamin E, Neophytadiene, Squalene etc. which have been reported to be Antitumur, Antiinflammatory, Antiaging, antidiabetic, anti-inflammatory, Antipyretic, perfume, pesticide and sunscreen.

Key words: *Mussaenda species*, Folk food, Tribal people, Nutraceuticals, GC-MS, Hydrocoumarin.

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INTRODUCTION

Food without dal, wheat, potato, oil and spice is the main characteristic features of the indigenous food system ofArunachal Pradesh and it is an interesting researchable domain.[1] They use locally available wild and semi domesticated herbs in their diet for food and medicines since antiquity. Let food be your medicine, once said Hippocrates (c. 460–c. 370 BC) over 2500 years ago.[2,3] In the word of Etkin and Ross[4] and Guarrera and Savo;[2] wild plants retained in local food cultures are inseparable from traditional therapeutic systems hence, there is no clear dividing line between food and medicinal plants especially in indigenous and local traditions. Therefore, Food and medicine represents a continuum domain.

Nature has endowedArunachal Pradesh with full of bioresources. *Mussaenda roxburgii* Hk.f.[5] is one among hundreds of bioresources used among tribal people of Arunachal Pradesh as food. This wild herb is consumed as a vegetable almost every day. Indigenous people of this far remote Indian state prefer to cook this folk food by boiling with rice powder and dry bamboo shoot.

Plant kingdom basically produced two types of compounds; plants nutrients to function directly for primary metabolic processes to regulate growth development and reproduction and allele-chemicals or plant secondary compounds as plant chemical defences.[6] Plants provide a great challenge in metabolomics due to the rich chemical diversity of metabolites that they possess across a huge range of concentrations; estimates of 100000-200000 metabolites have been made for the plant kingdom.[7]

And Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample[8] likewise, Gas chromatography-mass spectrometry (GC-MS) is the most commonly used technique for the analysis of liposoluble constituents, especially volatile/semi-volatile compounds, and their metabolites in biological fluids due to its high resolution, selectivity and sensitivity.[9] GC-MS have been used to study phytocompounds in numbers of research works.[10-12] Hence, the present study has been taken up to study phytocompounds present in the folk food plant used in Arunachal Pradesh by using GCMS.

MATERIALS AND METHODS

Plant material

Mussaenda roxburghii Hk.f. an erect shrub; stem light brown, leaves elliptic, entire, acuminate, membranous, pale beneath, lateral nerve 8-10 on either half, petiole stought, stipule lenciolate, flower orange, in dense corym cyme, calyx teeth long, persistant, berry oblong crowded in head like mass.[5] This herb is commonly found in all the 19 districts of Arunachal Pradesh. The materials for this study was collected from Mone forest of East Siang district, Arunachal Pradesh and identified by the corresponding author himself.

Preparation of extract

The shoot of Mussaenda *roxburghii* Hk.f. which is consumed as food was collected wild from Mone forest of East Siang District, Arunachal Pradesh. The sample was shade dried and pulverized to powder using a mechanical grinder. 500 g of plant powder of the sample was soaked in ethanol for 72 hrs with intermittent shaking then filtered through Whatmann No. 41 filter paper and concentrated under reduced pressure at 40°C using rotary evaporator to obtain a viscous semi solid mass/extract.

GC-MS Analysis

Gas-Chromatography Mass Specrometry (GC-MS) analysis of the ethanol extracts of *Mussaenda macrophylla* was carried out in Shimadzu GCMS-

Table 1: Phytocompoundstable from *M.roxburghii* shoot extract.

Peak	R. time	Area	Area %	Name
1	7.260	561382	0.71	2-propen
2	10.464	322920	0.41	Ethyl dacylate
3	10.629	10274629	13.06	Hydrocoumarin
4	11.365	3897097	4.95	Chromen-2-one
5	12.149	872385	1.11	Methyl 3-(2-hydroxyphenyl) propionate
6	12.295	87121	0.11	1,8(2H,5H)-Napthalenedione,hexahydro-8a-methyl-,cis
7	12.515	120842	0.15	2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a trimethyl
8	12.929	562624	0.71	Chromanone
9	13.682	299526	0.38	Ethyl.alpa.-d-glucopyranoside
10	14.970	98754	0.13	Tetradecanoic acid
11	15.222	219123	0.28	Heptadecanoic acid, ethy ester
12	15.335	144231	0.18	Calendin
13	15.717	484169	0.62	Neophytadiene
14	15.962	298672	1.03	Perhydrofarnesyl acetone
15	15.975	288318	0.37	3,7,11,15-tetramethyl-2-hexadecen-1-ol
16	16.171	201836	0.26	(E)-phytol
17	16.614	3059444	3.89	Hexadecanoic acid, methyl ester
18	17.093	2698429	3.43	Pentadecoinic acid
19	17.217	13.0596	0.17	Methyl hexadec-9-enoate
20	19.295	4909706	6.24	Hexadeconoic acid, ethyl ester
21	18.288	1583959	2.01	Linoleic acid methyl ester
22	18.362	2187072	2.78	Methyl linolenate
23	18.515	13789501	17.52	2-hexadecen-1-ol,3,7,11,15 tetramethyl [R-[R-)
24	18.729	182842	0.23	9,12,octadecadinoic acid(Z,Z-)
25	18.807	463478	0.59	Cis,cis,cis-7,3,13 hexadecatrienal
26	18.908	2119825	2.69	Ethyl linoleate
27	18.982	2814981	3.58	Ethyl (9Z,12Z)-9,12-octadecadenoate
28	19.160	1825939	2.32	Octadeconoic acid, ethyl ester
29	19.404	97342	0.12	Phytol, acetate
30	20.141	142152	0.18	Heptadeconoic acid, ethyl ester
31	20.447	202509	0.13	Octanamide, N-(2-hydroxyethyl)-
32	20.523	268592	0.34	Methyl 18-methylnonadecanoate
33	20.621	214189	0.27	Linolenic acid, ethyl ester
34	20.968	260320	0.33	4,8,12,16-tetramethylheptadecan-4-olide
35	21.313	627508	0.80	Octadecanoicacid,ethyl ester
36	28.295	248909	0.32	(Z,Z)-6,9-cis-3,4-epoxynonadecaciene
37	27.836	2498599	3.18	Squalene
38	28.295	485280	0.62	Heptadecafluorononanoic acid, udecyl ester
39	28.620	547656	0.70	Triacontylpentafluoropropionate
40	29.392	663359	0.84	Neryl linalool isomer
41	29.535	169258	0.22	Geranyl linalool isomer
42	31.652	496101	0.63	.gamma-tocopherol
43	33.391	2200287	2.80	Vitamin E
44	35.851	797850	1.01	Erost-5-EN-3-ol, (3,beta)
45	36.721	555011	0.71	Stigmasterol
46	38.691	11362048	14.44	Beta sitosterin
47	39.398	502811	0.64	2,6,10-trimethyl,14-ethylene-14,pentadecne
48	41.709	392174	0.50	Stigmasta-3,5-dien-7-one
49	43.086	1049574	1.33	Almefrol
		78692677	100	

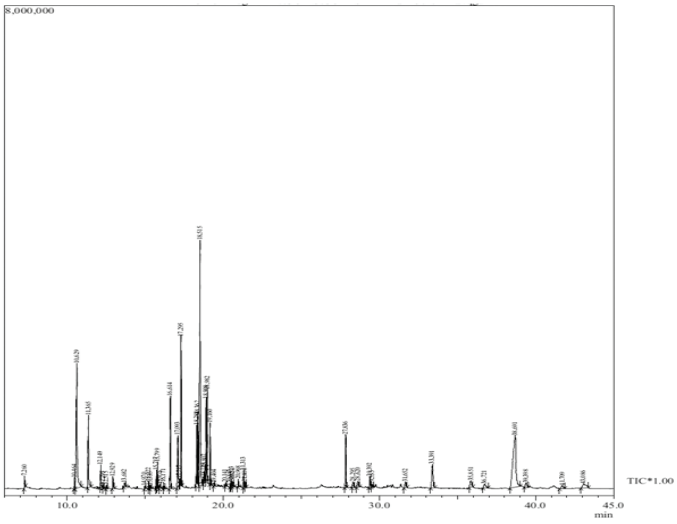

Figure 1: GC-MS chromatogram of *M. roxburghii* ethanolic extracts.

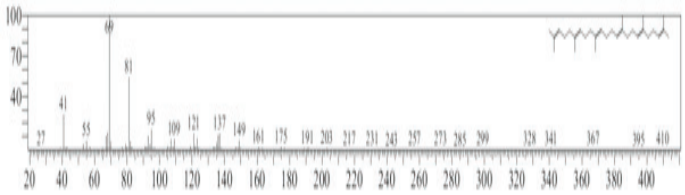

Figure 2: Squalene.

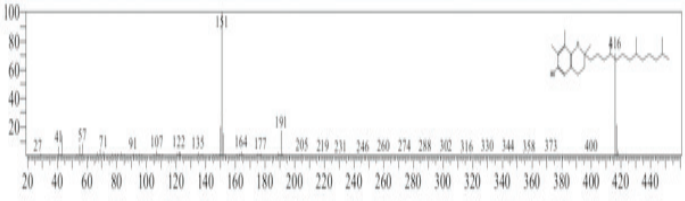

Figure 3: Gamma tocopherol.

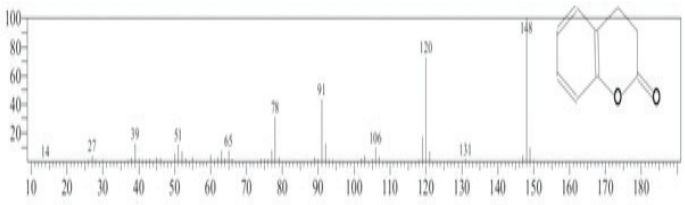

Figure 4: Hydrocaumarin.

QP-2010 plus system. RTx-5 Sil MS column (30 m×0.25 mm id×0.25 film thickness) was used for the analysis. The operating conditions of the column were as follows:

Oven temperature program from 80°C to 210°C at 4°C/min withhold time of 2 min and from 210°C to 300°C at 15°C/min withhold time of 5 min, and the final temperature was kept for 20 min. The injector temperature was maintained at 270°C, the volume of injected sample was 0.3 µl; pressure 85.4kPa, total flow 76.8 mL/min, column flow 1.21 mL/min, linear velocity 40.5 cm/sec, purge flow 3.0 mL/min, split ratio: 60.0; ion source temperature 230°C; scan mass range of m/z 40-600 and interface line temperature 280°C. The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, US) and WILEY 8.

Identification of phytocomposition

The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, US) and WILEY 8.

RESULTS AND DISCUSSION

The GCMS chromatogram of *Mussaenda roxburghii* Hk.f. ethanolic extract (Figure 1) shows 49 peaks indicating the presence of at least forty nine phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST 11 library and Willet 8 library, the forty nine phytocompounds were characterized and identified (Table 1). Some fragmentation patterns of compounds have been given (Figure 2, 3, 4). Of the forty nine phytoconstituents, the most prevailing compounds were hydrocoumarin with 13.6%, hexadeconoic acid, 2-hexadecen-1-ol,3,7,11,15 tetramethyl [R-[R-) with 17.52%, ethyl ester with 6.24% and Vitamin E with 2.8%.

The compounds, gamma tocopherolhave been reported to be Anticancer; Antiinflammatory; Antioxidant;[13] Vitamin E has been reported to be Antiaging, antioxidant, antitumour, antialzheimeran, antidiabetic, anti-inflammatory, antispasmodic, antistroke; hepatoprotective, immunostimulant, Insulin-Sparing;[13] Neophytadieneis reported to be Antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant; while Squaleneis reported as Antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive, lipoxygenaseinhibitor, pesticide, diuretic, perfumer, pesticide, sunscreen.[13]

From this work, it could be concluded that *Mussaenda roxburghii* contains various phytocompounds useful for numbers of health problems like cancer, inflammatory, diabetic and antioxidant, antbacterial etc. in addition to the usual macro and micronutrients. This work may lead this folk plant as a source of nutraceutical in future.

CONCLUSON

The GC-MS study of the ethanolic exract of the shoot of *Mussaenda roxburghii* shoot shows the presence of numbers of useful phytocompounds that are useful for numbers of health problems like cancer, inflammatory, diabetic, antioxidant and antibacterial etc. There should be further scientific studies on this food plant in future.

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CONFLICT OF INTEREST

None declared.

Source of support: Nil.

ABBREVIATION USED

GC-MS: Gas Chromatography-Mass Spectrometry; **C:** *circa*; **BC:** Before Christ.

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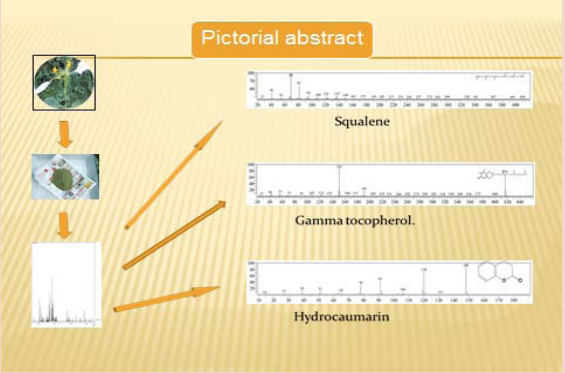
PICTORIAL ABSTRACT



SUMMARY

- Hydrocoumarin, hexadeconoic acid, 2-hexadecen-1-ol, 3, 7, 11, 15 tetramethyl [R-[R-), ethyl ester and Vitamin E are the main constituents of *Mussaenda roxhburgii* shoot.

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