



## GC-MS Analysis of *n*-Hexane Extracts of *Hibiscus micranthus* Linn.

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*Hibiscus micranthus* Linn., is an undershurb which grows wild in India, Ceylon, tropical Africa and Saudi Arabia. The plant is used as a febrifuge in the traditional medicine in Saudi Arabia and in Ceylon. The current study is aimed at to characterize the non-polar chemical components from *n*-hexane extracts of *Hibiscus micranthus* Linn., with the help of GC-MS technique. The *n*-hexane extraction of the powder of dried leaves, stem and root parts of *Hibiscus micranthus* Linn., by Soxhlet extraction yielded thick green residue (leaves and stems) and red thick residue (roots), respectively, which were analyzed by the GC-MS. A total of 23 compounds were identified from the *n*-hexane extract of leaf of *H. micranthus* Linn. The major components of the leaf extract are  $\beta$ -ionone, cyclopentane carboxylic acid, decyl ester, oleic acid, hexadecane 2,6,10,14-tetramethyl, 1-octanol-2-butyl, cyclopentadecanol, octadecane, phosphonofluoric acid (1-methylethyl)-cyclohexyl ester. However cyclopentadecanol was the most abundant followed by oleic acid. From the *n*-hexane extract of the stem, 14 compounds were identified. Pentadecanoic acid-14-methyl, methyl ester, 9,12-octadecadienoic acid methyl ester, cyclopropanepentanoic acid-2-undecyl-methyl ester and oleic acid were the major components of the stem extract and the oleic acid was the most abundant one. Thirty nine compounds were identified from the GC-MS analysis of the *n*-hexane root extract of *H. micranthus* Linn. 2-(Methyl)-cycloheptanone, dihydrocarveol, 1,2,4-trioxolane-2-octanoic acid 5-octyl-methyl ester, *n*-heptanoic acid methyl ester, 4-germaspiro(3,4)octane, cinerin-II, 4-decen-6-yne dioic acid dimethyl ester, 1- $\alpha$ -18O-1,25-dihydroxycholecalciferol, naphtho(3,2,1-c,d)isoindol-4(5H)-one-2-hydroxy-1,5-dimethoxy-, 1,3,2-dioxarsenane-4-methyl-2-phenyl-, methyl-2,3,4-tri-O-acetyl-6-O-methyl- $\alpha$ -D-galactopyranoside, 1,2-dicarbododecaborane 1-hexyl- and 1,2,3,6,9-pentaazaspiro(4,4)non-2-ene, 1,4,4,6,9-pentamethyl-, were the major components of the root extract and cinerin-II was the most abundant one followed by 4-germaspiro(3,4)octane. The GC-MS analysis revealed that the *n*-hexane extract of leaves are mainly composed of hydrocarbons (25 %) followed by aldehydes and ketones (together 13.8 %), sulphur compounds (8.3 %), fatty acids, esters and alcohols were least (5.5 %), while stem extracts composed fatty acid esters (33.3 %) followed by fatty acids (14.8 %) and least in hydrocarbons (3.7 %). The non-polar root extract composed fatty acid esters (19.1 %) followed by aldehydes and ketones (13.2 %), hydrocarbons (10.2 %), fatty alcohols (8.8 %) and least were fatty acids. It is concluded that the *n*-hexane extracts of leaf, stem and root of *Hibiscus micranthus* Linn., contains various potent bioactive compounds of phytopharmaceutical importance.

**Key Words:** *Hibiscus micranthus*, *n*-Hexane extracts, GC-MS Analysis.

### INTRODUCTION

*Hibiscus micranthus* Linn., is an undershurb which grows wild in India, Ceylon, tropical Africa and Saudi Arabia. The plant is used as a febrifuge in the traditional medicine in Saudi Arabia and in Ceylon<sup>1</sup>. In certain parts of Gujarat the fruits and flowers of this plant are used as hypoglycemic agent<sup>2</sup>. Antipyretic, antiinflammatory, hematological effects<sup>3</sup>, antimicrobial, antiviral, antitumor<sup>4</sup>, female antifertility, viralizing<sup>5</sup> and anabolizing<sup>6</sup> activities of this plant has been scientifically validated. Certain phytoconstituents (whose concentration is rich) like phenolic acids, flavonoids,  $\beta$ -sitosterol, alkanes, fatty alcohols and acids have been identified by conventional column

chromatographic analysis<sup>4</sup>. However, some of the non-polar chemical compounds (trace phytoconstituents) yet to be identified using newer and sensitive analytical techniques.

In the current study an attempt was made to characterize the non-polar chemical components from *n*-hexane extracts of *Hibiscus micranthus* Linn., with the help of GC-MS technique.

### EXPERIMENTAL

The study materials were collected from the wildy grown plants in Bharat Institute of Technology Campus, Mangalpally, Ibrahimpatnam. The plant was authenticated by a renowned Taxonomist Jayaraman at the National Institute of Herbal

science, Chennai, India. In order to ensure the sample used was from the same source throughout the experiment, the sample was collected in sufficient quantities at a time.

The plant *Hibiscus micranthus* Linn., was washed thoroughly with running tap water, followed by rinsing with distilled water and then leaves, stem and roots were separated and cut into small pieces. The leaves and stems were shade dried at room temperature, while roots were dried in an oven at 45 °C for 2 weeks. The dried parts of the plant were powdered to a mesh size of 150 and stored in an airtight container till further use.

All the chemicals used in the experiment were of analytical grade (SD fine chemicals) and were procured from the central store of the institution.

**Extraction:** The powdered leaves, stems and roots were exhaustively extracted individually with *n*-hexane for 1 week in a Soxhlet extractor. The resultant extracts were filtered and evaporated under vacuum, yielded thick green residue (leaves and stems) and red thick residue (roots), respectively. The residues were dried and analyzed by GC-MS.

**Sample preparation:** 200 mg of the respective extracts were dissolved in 1 mL of *n*-hexane. The mixture was sonicated for 15 min to ensure homogenous solution. Inject 3 µL of the test solution directly into the GC-MS system.

**Chromatographic analysis:** The samples were analyzed using Shimadzu GC-MS-QP 2010 Plus instrument equipped with quadrupole detector and split injection system. The GC was fitted with a ZP-624 capillary column (30 mm × 1.4 mm, film thickness 0.25 µm). The temperature programmed was as follows: injector temperature 220 °C, initial oven temperature at 120 °C for 2 min, then rise to 250 °C at the rate of 10 °C per minute maintained at 250 °C for 25 min, transfer line temperature 220 °C. Helium was used as carrier gas at 35.6 Kpa pressure with flow 2.5 mL/min and electronic pressure control on. The EM voltage was 952.9 V with lower and upper mass limits set at 30 and 350 m/z. Samples were solved in *n*-hexane and injected automatically. MS spectra of separated compounds were compared with one from Wiley 7 Nist 05 mass spectral database. The identity of the spectra above 95 % was needed for the identification of compounds.

## RESULTS AND DISCUSSION

The *n*-hexane extracts of leaves, stems and roots of *Hibiscus micranthus* linn., were obtained by Soxhlet extraction. The GC-MS analysis of the individual extracts indicated a complex mixture of constituents of different chemical classes. Previously reported non-polar soluble constituents of these extracts by conventional column chromatography analysis were also detected along with many other constituents which are reported for the ever first time in this plant. Twenty three compounds (above 95 %) were identified from the *n*-hexane extract of leaf of *H. micranthus* Linn. (Table-1).

Hydrocarbons were in maximum number 9 (25 %) followed by aldehydes and ketones together 5 (13.8 %), sulphur compounds 3 (8.3 %), fatty acids, esters and alcohols were in equal number 2 (5.5 %). β-Ionone, cyclopentanecarboxylic acid, decyl ester, oleic acid, hexadecane 2,6,10,14-tetramethyl, 1-octanol-2-butyl, cyclopentadecanol, octadecane, phospho-

TABLE-1  
PHYTOCONSTITUENTS FROM *n*-HEXANE EXTRACT OF LEAF OF *H. micranthus* Linn., DETECTED BY GC-MS

Comp. No.	Retention time (RT)	Compound	Matching with Wiley library (%)
1	8.12	2,4-Heptadienal	99
2	8.61	Hexanoic acid	76
3	9.12	3-Hexenoic acid	93
4	9.42	Nonanal	96
5	9.98	Di- <i>tert</i> -butyl disulfide	99
6	10.20	<i>n</i> -Propyl sec-butyl disulfide	97
7	10.96	Disulfide, <i>bis</i> (1-methylpropyl)	99
8	11.47	1-Hexanol, 2-ethyl	97
9	11.81	2,4-Decadienal	93
10	12.37	1-Hexanol, 2-ethyl	76
11	12.98	Tetradecane	98
12	13.09	1,4-Hexadiene, 3-ethyl	99
13	13.45	2,4-Decadienal	96
14	13.89	Octadecane, 1-chloro	98
15	14.25	Phenol, 2-methoxy-4-(2-propenyl)	98
16	14.38	Hexadecane	99
17	14.50	Propanoic acid, 2,2-dimethyl	99
18	14.76	Naphthalene, 2,3-dimethyl	97
19	17.89	Neryl acetone	98
20	15.35	Hexadecane, 3-methyl	93
21	15.70	Pentadecane	99
22	15.77	β-Ionone	99
23	16.11	Phenol, 3,5- <i>bis</i> (1,1-dimethylethyl)-	94
24	16.33	Hexanoic acid, 1,1-dimethylethyl ester	88
25	16.49	Cyclopentanecarboxylic acid, decyl ester	98
26	16.74	9-octadecenoic acid	99
27	16.94	Octadecane	99
28	17.00	Hexadecane, 2,6,10,14-tetramethyl	97
29	17.41	1-octanol, 3,7-dimethyl-	95
30	17.52	1-Octanol, 2-butyl-	98
31	18.09	Cyclopentadecanol	86
32	18.26	Octadecane	93
33	18.39	Hexadecane, 2,6,10,14-tetramethyl	98
34	18.65	Phosphonofluoridic acid, (1-methylethyl)-cyclohexyl ester	98
35	18.85	Nonadecane, 9-methyl-	96
36	18.85	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	54

nofluoridic acid (1-methylethyl)-cyclohexyl ester were the major components of the leaf extract and cyclopentadecanol was the most abundant followed by oleic acid.

From the *n*-hexane extract of the stem, 14 compounds (above 95 %) were identified and presented in Table-2.

Fatty acid esters 9 (33.3 %) were more in number followed by fatty acids 4 (14.8 %) and hydrocarbon 1 (3.7 %). Pentadecanoic acid-14-methyl, methyl ester, 9,12-octadecadienoic acid methyl ester, cyclopropanepentanoic acid-2-

TABLE-2  
PHYTOCONSTITUENTS FROM *n*-HEXANE EXTRACT OF STEM OF *H. micranthus* Linn., DETECTED BY GC-MS

Comp. No.	Retention time (RT)	Compound	Matching with Wiley library (%)
1	20.18	Dodecanoic acid methyl ester	99
2	20.82	Dodecanoic acid	97
3	22.47	Cyclohexanecarboxylic acid, ethenyl ester	97
4	23.28	Tetradecanoic acid, methyl ester	98
5	23.81	Decanoic acid	96
6	24.89	Z-4-Nonadecen-1-ol acetate	99
7	24.97	2-Undecanone	94
8	25.22	1-Dodecyne	91
9	25.49	1-Tridecyne	96
10	26.11	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	73
11	26.11	Pentadecanoic acid, 14-methyl, methyl ester	99
12	26.67	<i>n</i> -Hexadecanoic acid	99
13	27.08	9-Octadecenoic acid, methyl ester	94
14	27.39	Eicosanoic acid methyl ester	97
15	27.54	7-Nonynoic acid, methyl ester	90
16	28.29	9,12-Octadecadienoic acid, methyl ester	99
17	28.42	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester	99
18	28.69	Octadecanoic acid methyl ester	99
19	29.16	Oleic acid	99
20	29.34	<i>n</i> -Hexadecanoic acid	95
21	29.57	5,8-Octadecadienoic acid, methyl ester	93
22	31.46	8,11,14-Eicosatrienoic acid methyl ester	69
23	31.46	9-Octadecenoic acid, methyl ester	94
24	31.95	Heptacosanoic acid, methyl ester	95
25	33.52	Dodecane	93
26	36.29	13-Docosenoic acid, methyl ester	93
27	37.08	Heptacosanoic acid, methyl ester	97

undecyl-methyl ester and oleic acid were the major components of the stem extract. However concentration of oleic acid was most abundant.

Thirty nine compounds were identified by the GC-MS analysis of the *n*-hexane root extract of *H. micranthus* Linn. Fatty acid esters 13 (19.1 %) were predominant in number followed by aldehydes and ketones together 9 (13.2 %), hydrocarbons 7 (10.2 %), fatty alcohols 6 (8.8 %) and least in number were fatty acids and are presented in Table-3.

2-(Methyl)-cycloheptanone, dihydrocarveol, 1,2,4-trioxolane-2-octanoic acid 5-octyl-methyl ester, *n*-heptanoic acid methyl ester, 4-germaspiro(3,4)octane, cinerin-II, 4-decen-6-ynedioic acid dimethyl ester, 1- $\alpha$ -18O-1,25-dihydroxycholecalciferol, napho(3,2,1-c,d)isoindol-4(5*H*)-one-2-hydroxy-1,5-dimethoxy-, 1,3,2-dioxarsenane-4-methyl-2-phenyl-, methyl-2,3,4-tri-O-acetyl-6-O-methyl- $\alpha$ -D-galactopyranoside, 1,2-dicarbododecaborane-1-hexyl- and 1,2,3,6,9-pentaazaspiro(4,4)non-2-ene, 1,4,4,6,9-pentamethyl, were the major components of the root extract and cinerin-II was the most abundant one followed by 4-germaspiro-(3,4)-octane.

On comparing the results of GC-MS analysis of *n*-hexane extracts of leaf, stem and root of *H. micranthus* linn., it was revealed that, few constituents were restricted to particular plant part. The sulphur compounds like di-*tert*-butyl-disulfide, *n*-propyl *sec*-butyl disulphide and *bis*(1-methylpropyl) disulphide were found in leaf extract, while nitrogen, bromine containing compounds 2-(2-(4-nitrophenoxy)ethoxy)benzaldehyde, 1-nitro-naphthalene-2-carboxylic acid, 1,2,3,6,9-pentaazaspiro(4,4)non-2-ene, 1,4,4,6,9-pentamethyl, 3,1,2-azaazoniaboratin, 2,2-(1,5-cyclooctandiyl)-4,6-diethyl,

phenol, 4-(aminomethyl)-2-methoxy-, 5-chlorovaleric acid, dodec-9-ynyl ester, 2-methyl-2-bromosuccinic acid 1-ethyl 4-methyl ester were detected in root extracts. From leaf extract phosphorous and fluorine containing compound phosphofluoric acid (1-methyl ethyl)-cyclohexyl ester was detected. Fat soluble vitamins 1 $\alpha$ -18O-1,25-dihydroxycholecalciferol, vitamin A aldehyde and acetylated sugar methyl-2,3,4-tri-O-acetyl-6-O-methyl- $\alpha$ -D-galactopyranoside were also detected in root extracts. The oxygen containing compounds were maximum in stem extract (88.88 %) followed by root extract (79.41 %) and least in leaf extract (58.33 %). The aromatic compounds were very few and exclusively found in root 7.35 % and leaf 4.41 %.

Plants are considered as biochemical factories, which synthesize array of metabolites. Numerous uses are being identified since last few decades as therapeutic, nutraceutical, cosmesuetical and also as source of lead molecules.

*n*-Hexane extracts of plants are reported to be rich in hydrocarbons are found at the outer surface. These compounds are directly derived from fatty acids and utilized as biofuels, chemical feedstock<sup>7</sup>, serve as a barrier to water penetration and evaporation, affect the absorption of chemicals, act as sex attractants (or antiaphrodisiacs)<sup>8-10</sup>.

Polyacetylenic compounds such as 1-tridecyne and 5-chlorovaleric acid dodec-9-ynyl ester, cyclohexanecarboxylic acid dodec-9-ynyl ester, 4-decen-6-ynedioic acid dimethyl ester were detected in the *n*-hexane extracts from stem and root parts of the plant, respectively. Indeed polyacetylenic compounds are reported to be cytotoxic to tumour cells. Based on the above it may anticipated that, *Hibiscus micranthus* stem and root extracts may be exploited for their antitumour activity<sup>11</sup>.

TABLE-3  
PHYTOCONSTITUENTS FROM *n*-HEXANE EXTRACT OF ROOT OF *H. micranthus* Linn., DETECTED BY GC-MS

Comp. No.	Retention time (RT)	Compound	Matching with Wiley library (%)
1	4.84	1,2-Epoxy-nonane	84
2	5.16	Heptanoic acid	99
3	6.13	9-Heptadecanone	79
4	6.50	Heptanoic acid, ethyl ester	73
5	7.13	8-Hexadecenal, 14-methyl	97
6	7.31	Benzoic acid	89
7	7.94	2-(Methyl)-cycloheptanone	98
8	8.58	Carveol, dihydro	99
9	8.78	Cyclopropane, 1-(1'-propenyl)-2-hydroxymethyl	98
10	9.19	Thiirane, octyl	99
11	9.80	1,3,3-Trideuterio-endo-6-hydroxy-9-oxabicyclo(3,3,1)nonan-2-ene	98
12	9.89	9,12,15-Octadecatrienoic acid, methyl ester	100
13	10.10	7-Nonynoic acid	89
14	10.58	Hexadecanoic acid, 2,3-dihydroxypropyl ester	97
15	11.11	2-(2-(4-Nitro-phenoxy)-ethoxy)-benzaldehyde	97
16	11.57	Cyclodecanone	94
17	11.67	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-methyl ester	91
18	12.02	1,6-Methano(10)annulene	21
19	12.37	9-Heptadecanone	96
20	12.76	Methyl-15-oxohexadecanoate	99
21	12.92	Vitamin A aldehyde	99
22	13.02	$\alpha$ -D-Glucofuranose, 1,2-O-(1-methylethylidene)	94
23	13.15	Octanedioic acid	98
24	13.36	Cyclohexanemethanol, 2-(2-propenyl)-	97
25	13.54	Eicosanoic acid	96
26	13.94	1R-Acetamido-4C-acetoxy-5,6C-epoxy-2C,3T-dimethoxy-cyclo	97
27	14.24	Chloroacetic acid dodecyl ester	99
28	14.37	2-Pentadecanone,6,10,14-trimethyl	98
29	14.63	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-methyl ester	99
30	14.74	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-methyl ester	98
31	14.89	Z-7-Pentadecenol	99
32	15.01	<i>n</i> -Heptanoic acid, methyl(tetramethylene)silyl ester	95
33	15.23	1,2-Benzenedicarboxylic acid, mono(phenylmethyl)ester	97
34	15.38	3-Cyclohexene-1-propanol	98
35	15.50	Naptho(2,3-b)oxirene, decahydro	97
36	15.61	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-methyl ester	95
37	15.74	10-Nonadecanone	98
38	16.19	4-Germaspiro(3,4)octane	96
39	17.46	Cinerin-II	98
40	18.18	4-Decen-6-ynedioic acid, dimethyl ester	98
41	18.51	<i>n</i> -Octanoic acid, methyl(tetramethylene)silyl ester	99
42	18.82	1 $\alpha$ -18O-1,25-Dihydroxycholecalciferol	99
43	19.42	Naptho(3,2,1-c,d)isoidol-4(5H)-one, 2-hydroxy-1,5-dimethoxy-	98
44	19.77	Tetracyclo(16,1,0,0E2,9,0E10,17)nonadeca-2(9),10(17)-dien,19,19-D	99
45	19.85	1-Nitro-naphthalene-2-carboxylic acid	87
46	20.18	1,3,2-Dioxarsenane, 4-methyl-2-phenyl	98
47	20.56	<i>n</i> -Butyl laurate	96
48	20.78	Phenol,4-(aminomethyl)-2-methoxy-	97
49	21.09	1,18O-1,25-Dihydroxycholecalciferol	96
50	21.32	Benzo(3,4)cyclobuta(1,2)cyclooctene, 4b,5,6,7,8,9,10,10a-octahydro-	94
51	21.59	2-Methyl-2-bromosuccinic acid, 1-ethyl, 4-methyl ester	98
52	21.83	Methyl-3-(2,3-dimethoxyphenyl)-propanoate	98
53	22.29	Pentadecane,2,6,10,14-tetramethyl-	98
54	22.68	3,1,2-Azaazoniabornatin,2,2-(1,5-cyclooctandiyl)-4,6-diethyl	99
55	23.11	Methyl-2,3,4-tri-O-acetyl-6-O-methyl- $\alpha$ -D-galactopyranoside	98
56	23.79	Furan-3-carboxylic acid, 5-(benzo(1,3)dioxol-5-yl)-2-methyl-	97
57	24.14	1,2-Dicarbododecaborane(12),1,hexyl	96
58	25.34	Pentadecane,2,6,10,14-tetramethyl-	97
59	26.14	2(5H)-Furanone,4,5,5-trimethyl-3-(3-methyl-2-methylenebutyl)	98
60	26.94	5,5-Dimethyl-1-oxa-5-silacyclononanone-9	97
61	27.42	1-Methyl-8-propyl-3,6-diazahomoadamantan-9-ol	98
62	29.23	1,2,3,6,9-Pentaazaspiro(4,4)non-2-ene, 1,4,4,6,9-pentamethyl	99
63	30.95	Z-7-Pentadecenol	97
64	31.77	1,2-Benzenedicarboxylic acid, dioctyl ester	99
65	32.32	5-Chlorovaleric acid, dodec-9-ynyl ester	98
66	32.83	Decanoic acid, 2,3-dihydroxypropyl ester	98
67	34.18	1,2,3,6,9-Pentaazaspiro(4,4)non-2-ene, 1,4,4,6,9-pentamethyl	99
68	35.96	Cyclohexanecarboxylic acid, dodec-9-ynyl ester	98

The presence of aldehydes such as 2,4-heptadienal and *n*-nonanal have been identified in the leaf extract of *Hibiscus micranthus*. These types of aldehydes have been used as perfuming agent, flavouring agent, antiseptic and insect repellent<sup>12-14</sup>. The *n*-hexane extract of leaf contained some sulphur compounds including di-*tert*-butyl-disulfide, *n*-propyl *sec*-butyl disulphide, bis(1-methylpropyl)disulphide. Sulphur compounds reported to attract some insects<sup>15</sup> and are used to improve the fragrance and taste of some foods<sup>16</sup>.

The root and stem extract exhibited the presence of two important polyunsaturated fatty acids (9,12,15-octadecatrienoic acid, 9,12-octadecadienoic acid)<sup>17</sup>. These types of fatty acids are important for growth, neural functions, as cardio-protectives, antiarthritis, antiinflammatory and as anticancer<sup>18,19</sup>. Plants found to contain high content of organogermanium compounds have beneficial effect in the treatment of malignant tumors. Spiro germanium is reported to be effective in killing cancers cell both *in vivo* and *in vitro*. Germaspiro (3,4)octane was most abundant compound found in *n*-hexane root extract of the title plant<sup>20-22</sup>.

Cinerin-II an scientifically validated insect repellent compound was also detected and was second most abundant compound present in the root extract<sup>23</sup>.

Aromatic compounds were also detected, phenolics like eugenol were identified usually possess antioxidant, antimicrobial, antifungal and several other therapeutic uses<sup>24</sup>.

Based on the above studies, it is concluded that, the *n*-hexane extracts of leaf, stem and root of *Hibiscus micranthus* Linn., contains various potent bioactive compounds of phytopharmaceutical importance. These primary investigations inspire to isolate the compounds in their pure form and validate for their reported biological activity for the welfare of mankind.

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