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# GC-MS analysis on the methanolic extract of *Trichosanthes anguina* L. root and leaves

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**Abstract**
Phyto Chemical Screening of *Trichosanthes anguina* L. (snake gourd) root and leaves were done by GC-MS analysis for the identification of secondary metabolites. In this paper, the results of the analysis of methanolic extract analysed by GC-MS technique (Thermo GC-Trace Ultra Version 5.0, Fisher, USA) are presented. The 1.0 ml/min volume of methanolic extract sample was injected into the instrument and the compounds are identified and analyzed by splitless injection technique. The compounds were separated using Helium (1.0 ml/min) as the carrier gas. The GC-MS analysis for *Trichosanthes anguina* L. provides eight major peaks determining the presence of twelve major compounds namely ester (15.13%); alcohol (2.12 %); hetero cyclic compound (19.67%); amine/amide (0.51/0.41%); thioether (0.51%); diene (9.88%); aldehyde (9.88 %); nitro compound (1.35 %); ketone (0.56%); alkene (0.56 %); amino compound (1.17 %) and amino acid (0.61%).The presence of various main bioactive compounds namely furan, pyrrole-1-oxide, 4-cyanomethyl-3- pyrrolepropione, nitrilemethyl esters, hetero cyclic compounds which confirm the medicinal importance of the plant.

**Keywords:** Phytocomponents, *Trichosanthes anguina* L, methanolic extract, medicinal plants

## Introduction
Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants which grow in different parts of the country. Herbal medicine plays an important role in health care programs in the developing countries. *Trichosanthes anguina. L.* is mainly constituted with a series of secondary metabolites as flavonoids, carotenoids, phenolics acids, etc. The nutritional values make the plant pharmacologically and therapeutically active. It has a prominent place in alternative system of Ayurvedic and Siddha medicine due to the various Paramacological activities like anti-diabetic, hepto-protective, cytotexins, anti-inflammatory and carvicidal effects (Longman, 2002) [1]. The species possess antiviral compounds like *Trichosanthin and Trichoanguin.* The *Trichosanthin* is reported to have anti-HIV properties (Ferrari *et al*, 1991). These compounds have been used in the treatment of skin diseases, cough, ulcer etc. The plant is known as an appetizer, digestive, germicide, laxative and aphrodisiac (Sivarajan and Balachandran, 1994; Chatterjee and Prakashi, 1997) [2, 3].
Plants remain a major source of Pharamaceuticals and fine chemicals. Plants produce an array of natural products which are known as" *Secondary Metabolites*". They are not essential for plant growth and are normally produced in small amount (Kim *et al*., 2002) [4]. The utilization of plant cells, tissues or organs for the production of natural and recombinant compounds of commercial interest has gained increasing attention over the past decade (Center *et al* 2005; Murthy *et al*, 2008) [5, 6].
Screening of active compounds extracted from the plants has led to the invention of new medical drugs which have efficient protection and treatment roles against various diseases including cancer. (Sheeja and Kuttan, 2007) [7] and alzheimers disease (Mukherjee *et al*, 2007) [8]. Flavonoid 'quercetin' was isolated and identified from the leaf and not of utrullus colocynthis (Meena and Patni, 2008) [9]. The dried samples were separately extracted in 80% methanol.
A variety of instruments and devices are available for the estimation of secondary metabolites. Of which a gas chromatography – mass spectrometry (GC-MS) analysis is a versatile technique used for the identification and analysis of secondary metabolites present in the plants. In this work it is decided to identify the phyto components from *Trichosanthes anguina.* L. leaf extract by GC-MS analysis.

## Materials and Methods

### Collection and Preparation of Plant Materials

The fresh leaves (fig.1) of *Trichosanthes anguina*. L. were collected from the experiment garden. The samples were washed thoroughly in running tap water to remove fungal contaminations and adhered debris and finally washed with sterile distilled water. The leaves were shade dried and ground into fine powder, sieved, lebelled and kept in amber coloured containers and stored in refrigerator for GC-MS.

### Plant sample extraction

1 gm of the sample was transferred to 2 ml effendroff flask and to that contents 2ml of HPLC grade methanol was added and the tube was tightly sealed. The sealed tube kept in a sonicator for 20-30 minutes for sonication. After that the dissector was kept under reduced pressure for 4-7 days for the evaporation, to remove the excess amount of methanol present in it. To the residue obtained, methanol was added to dissolve it and was centrifuged for 8 minutes at 800 rpm. The supernatant liquid was decanted by simple filtration and sterilized. The sterilized filtrate was used for the GC-MS analysis.

## Results

The GC-MS analysis of the phytochemicals present in the methanolic extract of *Trichosanthes anquina* L. leaves are Tabulated (Table No.1). The presence of eight major peaks are shown in Fig.1,1(a) &1(b), Where all the noted phyto constituents are characterised and identified. The retention times (RT) of compounds are given in minutes.

The eleven major compounds are identified according to the active functional groups with the RT. Heterocyclics compounds (7.14; 8.32; 8.79; 16.16; 20.48; 27.39; 31.48 and 36.95 min. ester/ hydroxy ester at the retention time of 5.40; 18.18; 19.16 and 36.48 min; Lactose/ketone at the retention time of 5.42; 7.81; 10.6; 14.41; 6.3 and 30.77 min; fatty acids (stearic and suberic acid) at RT of 15.45; 20.07; 21.99 and 25.74 min.; heterocyclic alkaloid compound at the retention time of 29.39 and 33.60 min.; amino acid derivative and amino acid at the RT of 6.67 and 9.07 min.; alcohol at RT of 13.8 in.; cyclo alkyl derivative at RT of 17.24 min.; phosphonyl salt at RT of 36.95 min. and steroid at the RT 36.95 min. and steroid at the RT of 24.58 min are identified and compared with the standard values.

The above mentioned compounds are named according to their functional groups (with serial numbers1-32) present in the constituents. Heterocyclic compounds alkaloids and bifuran-4,6,14, 19,22,26,28 and 32; ester/ hydroxyl ester (I,16, 17,22-39); Lactone/ ketone (2,5,10, 12, 23 and 25); Fatty acids (stearic and suberic acid, 13,18, 20 and 22); amino acid derivative/ amino acid (3 and 8); steroid (21); alcohol(11); cyclo alkyl derivative (1) and phosphonyl salt (31). Among the compounds the high percentage occurred are heterocyclic compounds (methyloxazone, methyeno oxolane, furanone, isoquinoline, imidazone, alkaloid) esters amino acids and ketones.

**Fig 1:** *Trichosanthes anguina* L. CV.UKB 200561 (snake gourd)

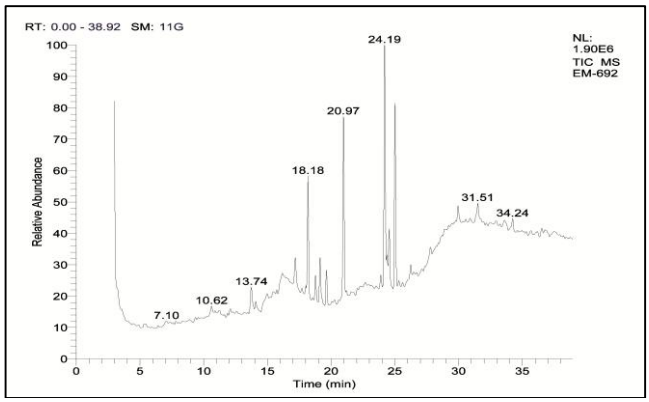


**Fig 1(a):** Chromatogram (GC-MS) of methanolic extract root of *Trichosanthes anguina* L.

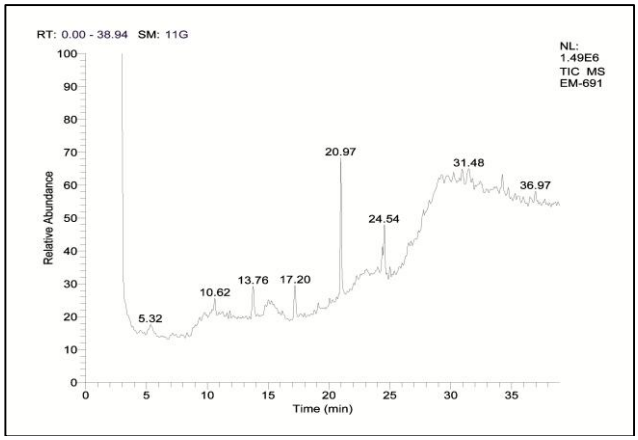


**Fig 1(b):** Chromatogram (GC-MS) of methanolic extract leaf of *Trichosanthes anguina* L.

**Table 1:** GC-MS data of the bioactive compounds present of the methanolic extract roots of *Trichosanthes anguina* L.

| S. No. | RT | Name of the compound | M.F. | M.W | Area (%) | (Common name) / Functional group |
|---|---|---|---|---|---|---|
| 1 | 3.22 | 4-Cyano butanamide | $C_5H_8N_2O$ | 112 | 1.12 | Amide |
| 2 | 3.22 | Cyanovaleramide | $C_6H_{10}N_2O$ | 126 | 1.12 | Amide |
| 3 | 10.60 | N-Phenylacetyl-N-methylglycine | $C_7H_{15}oNO_3$ | 207 | 2.38 | Amino acid |
| 4 | 12.07 | 4-Butylpyridazine | $C_8H_{12}N_2$ | 136 | 1.87 | Heterocyclic |
| 5 | 13.76 | 2,3-dimethyl-1H-Indole | $C_{10}H_{11}N$ | 145 | 3.25 | Heterocyclic |
| 6 | 14.08 | Butocarboxim | $C_{27}H_{14}N_2O_2S$ | 190 | 1.32 | Oxime |
| 7 | 14.68 | 5-(Dimethylamino)-4,4-dimethyl-2-(trifluoromethyl)-4H-imidazol | $C_8H_{12}F_3N_3$ | 207 | 6.58 | Heterocyclic |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 15.43 | (S)-1-Phenyl-4-(triisopropylsilyloxy)-1-butanol | $C_{19}H_{34}O_2Si$ | 322 | 1.03 | Alcohol |
| 9 | 15.80 | Sydnone,3-(1,1-dimethylethyl | $C_6H_{10}N_2O_2$ | 142 | 18.37 | Ketone |
| 10 | 18.18 | Dodecanal | $C_{12}H_{24}O$ | 184 | 9.35 | Aldehyde |
| 11 | 18.77 | Citronellyl isobutyrate | $C_{14}H_{26}O_2$ | 226 | 1.90 | |
| 12 | 19.12 | (E)-4-nonen-1-ol | $C_9H_{18}O$ | 142 | 4.08 | Alcohol |
| 13 | 19.63 | 11,1-Dideuterio-2-methyl-1-propene | $C_4H_6D_2$ | 56 | 1.76 | Alkene |
| 14 | 20.97 | Pentadecanoic acid, methyl ester | $C_{16}H_{32}O_2$ | 256 | 11.36 | Ester |
| 15 | 21.75 | Bicyclo[5,1,0]octan-2-one | $C_8H_{12}O$ | 124 | 1.19 | Ketone |
| 16 | 21.75 | 3-Amino-2-methylheptane | $C_8H_{19}N$ | 129 | 1.19 | Amino compound |
| 17 | 22.21 | Dehydrovomifoliol | $C_{13}H_{18}O_3$ | 222 | 0.74 | Alcohol |
| 18 | 23.88 | Nonacosane | $C_{29}H_{60}$ | 408 | 0.98 | Alkane |
| 19 | 23.88 | 2-Ethyl-6-methyltetrahydro-4H-pyran-4-one | $C_8H_{14}O_2$ | 142 | 0.98 | Ketone |
| 20 | 24.19 | 3-Chloromethylfuran | $C_5H_5ClO$ | 116 | 8.58 | Heterocyclic |
| 21 | 25.01 | Myrtenol | $C_{10}H_{16}O$ | 152 | 2.76 | Alcohol |
| 22 | 26.23 | 2,2-Dimethyl-4-hydroxy-3-decanone | $C_{12}H_{24}O_2$ | 200 | 0.81 | Ketone |
| 23 | 26.93 | Ethyl3-[(P-tolylsulfuonyl)amino]-2-(4'-methoxyphenyl)propanoate | $C_{19}H_{23}NO_5S$ | 377 | 0.81 | Acid derivative |
| 24 | 27.78 | Methyl-4-dimethylamino-3-(fluoromethyl)butyrate | $C_8H_{16}FNO_2$ | 177 | 1.67 | Amino compound |
| 25 | 27.78 | 4-Amino-5-ethyl-3-methyl5H-1,2-oxathiole 2,2-dioxide | $C_6H_{11}NO_3S$ | 177 | 1.67 | Heterocyclic |
| 26 | 29.33 | 1,2:4,5-Bis(diisopropylidenedioxy)-3-[methoxymethoxy]dodec-6-ene | $C_{20}H_{36}O_6$ | 372 | 2.62 | Alkene derivative |
| 27 | 29.96 | 3-Ethyl-3-methylbicyclo[3.1.0]hexane-2,4dione | $C_9H_{12}O_2$ | 152 | 0.90 | Diketone |
| 28 | 29.96 | (2E)-i-Propyl 5-Chloropent-2-enoate | $C_8H_{13}ClO2$ | 176 | 0.90 | Phenol/derivative |
| 29 | 30.91 | 2-(2-Methylbenzyl)hexanal | $C_{14}H_{20}O$ | 204 | 0.63 | Aldehyde |
| 30 | 30.91 | 1-Cyclopropyl-2-methyl-4-phenylbutan-1-one | $C_{14}H_{18}O$ | 202 | 0.63 | Ketone |
| 31 | 31.36 | 3-Ethyltetrahydro-2H-pyran-2-ol | $C_7H_{12}O_2$ | 128 | 1.09 | Alcohol |
| 32 | 33.60 | 1,Bis(bromoacetyl)cyclohepta-1,3,5-triene | $C_{10}H_{18}OSi$ | 182 | 1.27 | Alkene |

**Table 2:** GC-MS data of the bioactive compounds present of the methanolic extract leaves of *Trichosanthes anguina* L.

| S. No. | RT | Name of the compound | M.F. | M.W | Area (%) | (Common name) / Functional group |
|---|---|---|---|---|---|---|
| 1 | 5.42 | Ethyl 4-oxotrideconate | $C_{14}H_{26}O_3$ | 242 | 2.84 | Ester |
| 2 | 5.42 | (2R-)-2-Acetyl-2-hydroxo-3-methyl-c-butyrolactone | $C_7H_{10}O_4$ | 158 | 2.84 | Lactone |
| 3 | 6.67 | N-Benzyl-(2-deuterio)alanine | $C_{11}H_{12}DNO_3$ | 207 | 1.03 | Amino acid (Derivative) |
| 4 | 7.14 | 5-methyloxazole | $C_4H_5NO$ | 83 | 1.53 | Heterocyclic compound |
| 5 | 7.81 | 1-Cyclopentyl-3,3-dimethyl-2-butanone | $C_{11}H_{20}O$ | 168 | 0.88 | Ketone |
| 6 | 8.32 | 2-Cyclopentyl-4-methyleneoxolane | $C_{10}H_{16}O$ | 152 | 1.02 | Heterocyclic compound |
| 7 | 8.79 | 2,5-Dimethyl-2,2'-bistetrahydrofuran | $C_{10}H_{18}O_2$ | 170 | 5.96 | Bifuran (Heterocyclic) |
| 8 | 9.07 | N-L-analyl analine | $C_6H_{12}N_2O_3$ | 160 | 2.77 | Amino acid |
| 9 | 9.50 | (-)-(R)-5-Ethyl-2(5H)-furanone | $C_6H_{18}O_2$ | 112 | 1.94 | Heterocyclic compound |
| 10 | 10.6 | %-Methyoxypyrrolidine-2-one | $C_5H_9NO_2$ | 115 | 8.10 | Heterocyclic/ Ketone |
| 11 | 13.8 | 1-Octadecanol | $C_{18}H_{38}O$ | 270 | 2.19 | Alcohol |
| 12 | 14.41 | (S) - () – Pipecolic acid | $C_6H_{11}NO_2$ | 129 | 5.80 | Ketone |
| 13 | 15.45 | 4-Ethoxy benzoic acid | $C_{11}H_{14}O_3$ | 194 | 0.82 | Acid |
| 14 | 16.16 | 1-(2-bromopent-1-en-5oyl)indole-3-carbaldyhde | $C_{14}H_{12}BrNO_2$ | 305 | 2.17 | Heterocyclic aldehyde compound |
| 15 | 17.24 | 2-(N,N-Dimethylamino)-thia-3-3-oxacyclopentane | $C_5H_{10}NOS$ | 132 | 1.58 | Cycloalkane derivative |
| 16 | 18.18 | Neopentyl hydroxyacetate | $C_7H_{14}O_3$ | 146 | 1.02 | Hydroxy ester |
| 17 | 19.16 | Verrucosin-3 | $C_{25}H_{40}O_5$ | 420 | 3.54 | Hydroxy ester |
| 18 | 20.07 | (anti,syn)-2-hydroxy-5-methylcyclopentane carboxylic acid | $C_8H_{14}O_3$ | 158 | 1.29 | Acid |
| 19 | 20.48 | 2-[2-(Triphenylphosphoranylidene) aminoethyl]indole | $C_{28}H_{25}N_2P$ | 420 | 1.33 | Heterocyclic compound |
| 20 | 21.99 | Octadecanoic acid | $C_{18}H_{36}O_8$ | 284 | 33.22 | Stearic acid |
| 21 | 24.58 | Methyl lithocholate | $C_{25}H_{42}O_3$ | 390 | 1.15 | Cholesterol derivative |
| 22 | 25.74 | Octanedioic acid | $C_8H_{14}O_4$ | 174 | 7.04 | Suberic Acid |
| 23 | 26.23 | Bicycle[10,1,0]tridec-1(12)-en-13-one | $C_{13}H_{20}O$ | 192 | 1.90 | Cyclo ketone |
| 24 | 27.39 | N-[2,3-dihydro-4-oxo-4H-pyran-2-oyl]-(S)-proline-t-butyl ester | $C_{15}H_{21}NO_5$ | 295 | 1.23 | Heterocyclic compound (Alkaloid) |
| 25 | 30.77 | Campherenone | $C_{15}H_{24}O$ | 220 | 1.66 | Ketone |
| 26 | 31.48 | N-(2',4'-Dimethoxybenzyl)6,7-ethoxy-1,2,3,4 tetrahydroisoquinoline | $C_{20}H_{25}NO_4$ | 343 | 1.20 | Heterocyclic compound |
| 27 | 33.60 | 2-Acetoxy-5-benzyl-6-(diacetyl amino)-3-methylpyrazine | $C_{18}H_{19}N_3O_4$ | 341 | 0.94 | Heterocyclic compound (Alkaloid) |
| 28 | 34.26 | 1,2-Benzene dicarboxylic acid/dicyclohexyl ester | $C_{20}H_{26}O_4$ | 330 | 1.79 | Acid / ester |
| 29 | 36.48 | 1,2-dihydroacenaphthylen-1-yl-cyclohepta-2,4,6-trienecarboxylate | $C_{20}H_{16}O_2$ | 288 | 1.03 | Ester |
| 30 | 36.48 | Methyl-3-[(t-butoxy carbonyl]-2-benzylbutyrate | $C_{17}H_{25}NO_4$ | 307 | 1.03 | Ester |
| 31 | 36.95 | (Triphenylphosphonylaminoaceta nitrile salt | $C_{28}H_{24}N_3P$ | 433 | 1.32 | Phosphonyl salt |
| 32 | 36.95 | 1-Methyl-2-(N,N-di-t-carbamoyl)4,5-bis(hydroxymethyl)imidazole | $C_{20}H_{33}N_5O_8$ | 471 | 1.32 | Heterocyclic compound |

## Discussion

More than 80% of the World's Population relies on traditional medicine for their primary health care needs (Pierangeli *et al*., 2009) [11]. With growing interest of use of plant extracts in the food and pharmaceutical industries, screening of plant extracts for their properties and uses has become significance importance. (Zollo *et al*., 1998) [12]. In this work the presence of various chemical constituents of *Trichosanthes anguina* L. leaves have been screened for their therapeutic potential. The GC-MS chromatogram showed the relative concentrations of

various compounds getting eluted as a function of retention time. The height of the peaks indicates the relative concentrations of the components present in the extract. Most of the compounds fragmented in to small compounds giving rise to appearance of peaks at different m/z ratios.

**Root:** The most abundant phytochemical constituents found in the root extract are ester derivatives of the compounds namely hexyl propanotes, 2-enoate, methyl ester of deconoic acid and nanonoic acid, phytol acetate, iavandulyl acetates, methyl ester of octadecanoic acid and methyl ester of henecosanoic acid, hept- entyl-2-acetate and neopentyl hydroxyl acetate. In addition, sporadic appearance of important medicinal compound such as amino compounds and amino acids are noted in the methanolic extract.

**Leaves: T**he chemical composition of the extract obtained from the leaves mostly revealed the presence of heterocyclic compounds. The compounds present include 5-methyloxazole, methylenexolane, 1-indole-3-carbohydrate, 2-aminoethyl indole, hetecyclic alkaloid, tetrahydroisoquinoline, acetoxy-bezyl-methyl pyrazine alkaloid and methyl-hydroxy methyl imidazone. Apart from the secondary metabolites, fatty acids are also present which include steroids and lactones/ ketones are also screened. In our study the presence of n-hexadecanoic acid and oleic acid was in accordance with the presence of the same in the whole plant extracts of Solanum surattence (Abirami and Rajendran, 2011) [17].

## Conclusion

The GC-MS results of the two samples, root and leaf extract revealed that both contains phytochemical compounds of medicinal importance. The adundancy of secondary metabolites which include heterocyclic compounds, alkaloids, steroids and fatty acids (steric acid and suberic acid) are more characteristic in leaf extract whereas, the root extract showed less abundance of heterocyclic compounds and their derivatives but predominantly, esters, aldehydes/ketones and alcoholic derivatives are common. The amino compounds and amino acid derivatives are more predominant in roots than in leaf sample. Almost 90% of the medicine are made up of heterocyclic compounds. Most of the heterocyclic compounds are useful as anti-inflammatory, anti-cancerous anti-tumourous drugs. Our samples showed derivatives of heterocyclic compounds such as oxazole, oxalane, furanone. Pyrrolidine, isoquinoline (in leaf extract) and pyrrol, furan in root extract. Alkaloid compounds like pyrazine are found in leaf extract. They are basic in nature. Phenols have antibiotic and antioxidant properties. Fatty substances namely cholate found less abundancy in leaf extract. Among the two, methanolic leaf extract exhibits more bio-active components. It is therefore suggested that detailed phytochemical screening of *Trichosanthes anguina* L. has to be carried out to isolate the therapeutically important compounds. From the literature survey it is observed that this report is the first of this kind to analyze the chemical constituents of *Trichosanthes anguina* L. using GC-MS technique.

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