Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2458-2463
Received: 11-11-2017
Accepted: 12-12-2017

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GC-MS analysis of phytocomponents in the methanolic extract of *Gynocardia odorata* R.Br.- A poisonous plant from Arunachal Himalayan Region

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Abstract
The present research was designed to investigate the bioactive phytochemicals present in the metabolic leaf extract of the species. The identification of the phytochemical compounds was confirmed based on the peak area, RT and molecular formula. In total 50 compounds of different molecular weight were detected from the methanolic extracts of *Gynocardia odorata* R.Br. leaf and the identification and the characterization of each known compound was done with the help of the Wiley and NIST library. Stigmast-5-En-3-Ol, (3.Beta.) had the highest abundance with (7.72% of peak area) with 414 D molecular mass. So, there is a need of proper investigation of the plants with the help of sophisticated instruments like HPLC, GC-MS, LCMS, NMR for the isolation and characterization of the active bioactive molecules.

Keywords: achariaceae, *Gynocardia odorata*, GC-MS profile, poisonous, phytochemicals

1. Introduction
Plants have been used as food, medicine and poisonous agents throughout the course of human civilization. Poisonous plants have been used by hunter-gatherers worldwide and are still widely used in countries like South America, Africa and Asia. Arrow poisoning and water poisoning have been the prevalent methods to kill civilians as well as combatants in many areas of the world since ancient period. Poisoned arrows have featured in mythology, notably the Greek story of Heracles slaying the centaur Nessus. Traditional usage of poisonous plants is highlighted in the classical text like the *Charak Samhita*, *Mahabharata* and the *Ramayana*. However, in the present state of global affairs where nuclear and chemical weapons are gaining popularity, there is need to preserve such ancient knowledge on poisonous plants which was earlier used in traditional war tactics for self defense, territorial and national security, kalita *et al.,* (2017) [9].

In Arunachal Pradesh, tribal communities use poisonous plants mainly for fishing and animal hunting kalita *et al.,* (2017) [9] our literature survey has revealed that no specific research has been done on poisonous plants found in Arunachal Pradesh till date. Even in India, only few works on poisonous plants have been described viz. Ballantyne *et al.,* (1995) [2]; Singh *et al.,* (1999) [7]; Jain (1991, 1999) [6, 8]; and Caius (2003) [4].

Gynocardia odorata R.Br. is an evergreen tree belonging to the Achariaceae family. The trees grow up to 30-35 m tall. The leaves are glossy, entire, and alternate. The flowers are yellow and Sweet-scented, Staminate flowers 3-4 cm in diam., fragrant; calyx lobes ca. 7 mm, obtuse to rounded, outside glabrous or with short, sparse, appressed hairs; petals yellowish green, oblong or slightly obovate, 1-2.5 cm, glabrous, apex obtuse; epipetalous scale oblong or ovate, ca. 5 × 3mm, densely ciliate, apex obtuse; stamens ca. 1.5 cm, filaments villous, anthers ca. 5 mm. Pistillate flowers larger than staminate flowers; petals ca. 2 cm; staminodes 8-16, villous; styles short, slender; stigmas peltate or cordate. Berry yellowish brown, globose, (5-) 8-12 cm in diam.; pericarp grayish, ca. 3.5 mm thick (http://www.efloras.org/florataxon.aspx? flora_ id=2&taxon_ id=220005929).

The fruit is round, ash-coloured, and when developed, its average weight is about 9 to 15 pounds. The seeds are grayish, irregularly ovoid, compressed, and angular and smooth, a little over an inch long, and have an oily taste and a peculiar nauseous odor, *Gynocardia odorata* R.Br. is a poisonous plant growing wildly throughout India and tropical countries of the world. The species is found in moist forests of mountain valleys in South Asia - India, South-east Xizang and Yunnan in China, Bangladesh, Nepal and Myanmar. In the present study the *Gynocardia odorata* R.Br. plant reported from Arunachal Pradesh is commonly used by the

Adi, Idu Mishimi, Digaro & *Wangsu* tribes as a fish poison. Therefore, the candidate plant was undertaken for the GC-MS profiling of the secondary metabolites present in the leaf. The present study was aimed to identify the phytochemicals present in *Gynocardia odorata* R.Br. by using GC-MS analysis.

2. Materials and Methods

2.1 Collection of plant material

Gynocardia odorata R.Br. leaves were collected from Pasighat, East Siang District of Arunachal Pradesh, India. Herbarium specimen for the collected species has been prepared following Jain and Rao (1977) [6] and deposited in the Departmental Herbaria, Department of Botany, Rajiv Gandhi University for future reference. Authentication of the specimen was carried out with the help of Botanical Survey of India, Arunachal Pradesh (BSI ARUN). Further detail taxonomic characters and distribution pattern was verified consulting standard flora viz., Flora of British India (Hooker, 1872) and Materials for the Flora of Arunachal Pradesh (Chowdhury, 1996). The accepted name and synonyms were verified through website (www.theplantlist.org) (The plant List, version 1.1., 2013).

2.2 Preparation of Plant Extract

The fresh leaves of *Gynocardia odorata* R.Br. Were washed thoroughly under running tap water and shade dried. The dried leaves were then powdered and were stored in air tight polythene bags until further use. For GC-MS analysis about 100 gms. of the powdered leaves were taken and was soaked in 95% methanol for 12 hrs followed by filtering through Whatman filter No. 41 along with sodium sulphate to remove the sediments and traces of water in the filtrate. The filtrate was then concentrated by rotary evaporator and stored in air tight container in 4°C refrigerator.

2.3 Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS analysis was carried out on a GC Clarus 500 Perlin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrophotometer (GC - MS) instrument employing the following conditions: Sample volumes of 1 µl were injected with a splitless mode in to a GC-MS-QP2010 Ultra (Shimadzu) system which consists of TSQ Quantum XLS GC-MS/MS (Thermo Scientific Co.). The GC column used for the analysis was TG-5MS with an inner diameter of 0.25 mm, 30 m length and 0.25 µm film thicknesses. Helium gas was used as carrier gas at a flow rate of 1 mL/min. The extracted sample using M/C technique was analyzed under the following oven temperature program: injection at 250^0C followed by 2 ÀC/min oven temperature ramp to 70^0C and then by 5 ÀC/min to 250 ÀC and finally with 24 min isothermal at 280^0C. For the plant samples, the temperature program was set according to Jiang H *et al*., 2005. Mass spectra were acquired using full scan monitoring mode with a mass scan range of 40-650 m/z. The chromatogram and mass spectra were evaluated using the Xcalibur™ software embedded in the GC-MS/MS system.

2.4 Identification of components

Identification was based on the molecular structure, molecular mass and calculated fragments. The name, molecular weight and structure of the components of the test materials were ascertained by Wiley and NIST library. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. This was done in order to determine whether these plant species contained any individual compound or group of compounds, which may substantiate its potential to be used as a poison in different areas of use besides aiding in determining the most appropriate methods of extracting these compounds that would provide a new insight in determining the potential of these poisonous plants to formulate the effective dosage of its use for therapeutic relevance.

3. Results

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, ester etc. The GC-MS analysis of *Gynocardia odorata* R.Br., leaves revealed the presence of 50 compounds (phytochemical constituents) that could contribute to the poisonous quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula (Table-1). The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 1. The first compound identified with less retention time (16.659 min) was Stigmast-4-En-3-One, was the last compound which took longest retention time (62.214min) to identify.GC-MS analysis revealed the presence of 50 compounds among which Stigmast-4-En-3-One and Phytol compound was present to be maximum (Peak area 17.45 and10.52) while Ledene oxide-(II) and 1,1,4,7-Tetramethyl-1a,2,3,4,5,6,7,7b-Octahydro compound was found to be the lowest (Peak area 0.14 and 0.17) present in the roots of *Gynocardia odorata* R.Br. which accounts for their biological activities. The biological activities of the phytoconstituents screened out from literature studies are listed in Table 1.

4. Discussion

The GC-MS analysis of *Gynocardia odorata* R.Br. leaves revealed the presence of Fifty compound, Phytochemical constituents of medicinal or poisonous plants have different roles for the protection of the plants and also the different poisonous properties. Different secondary metabolites of a particular plant can be determined by using sophisticated chromatographic techniques like HPLC, GC-MS, LCMS etc. The GC-MS analysis of a plant parts gives an imaginary picture of the chemical constituents which are present in the candidate plants. The above table provides adequate information about the safety and credibility of the *Gynocardia odorata* leaf extracts. The identified compounds possess many biological properties. Most of the compounds such as 1H-Cyclopropa[A]Naphthalene, Decahydro-1,1,3a-Trimethyl-7-Methylene, 1h-Cycloprop[E]Azulene, 1a,2,3,5,6,7,7a,7b-Octahydro, Kw3 AusEpiglobulol, Beta.-Guaiene,9-Isopropyl-1-Methyl-2-Methylene-5-Oxa-Tricyclo, Tetradecanal, 1-Dodecanol, 3,7,11-Trimethyl-,2-Hexadecene,3,7,11,15-Tetramethyl-,[R-[R*,R*-(E)]-,2,6,10-Trimethyl,14-Ethylene-14-Pentadecne,5,9,13 Pntadecatrien - 2-One, 6,10,14-Trimethyl-,,2,6,10-Trimethyl,14-Ethylene-14-Pentadecne, 2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*, 2-Pentadecanone, 6,10,14-Trimethyl-,5,9,13-Pentadecatrien-2-One, 6,10,14-Trimethyl-,Hexadecanoic Acid, Ethyl Ester, Oxybenzone, Heptadecanoic Acid, Ethyl Ester Phytol and Pentadecanoic Acid are mainly responsible for their psychopharmacological activities, which validates the efficacy of the leaf of this plant as an active psychopharmacological agent. These reports are in

accordance with the results of this study. Due to psychopharmacological activity of the compounds already reported, local tribal communities use the raw paste of the leaf and put into the slow moving stream or ponds which cause temporary hallucination to fishes. Fishes on unconscious state float over the water surface and from which the fisherman easily collect. At higher dose, the above mentioned chemicals present in *Gynocardia odorata* R.Br. leaf extract could cause toxicity in the central nervous system and may cause cardiac and hepatic impairment. Toxicological study using acute and chronic models in animal is required to validate the toxicity effects of these phytocompounds on animal physiology.

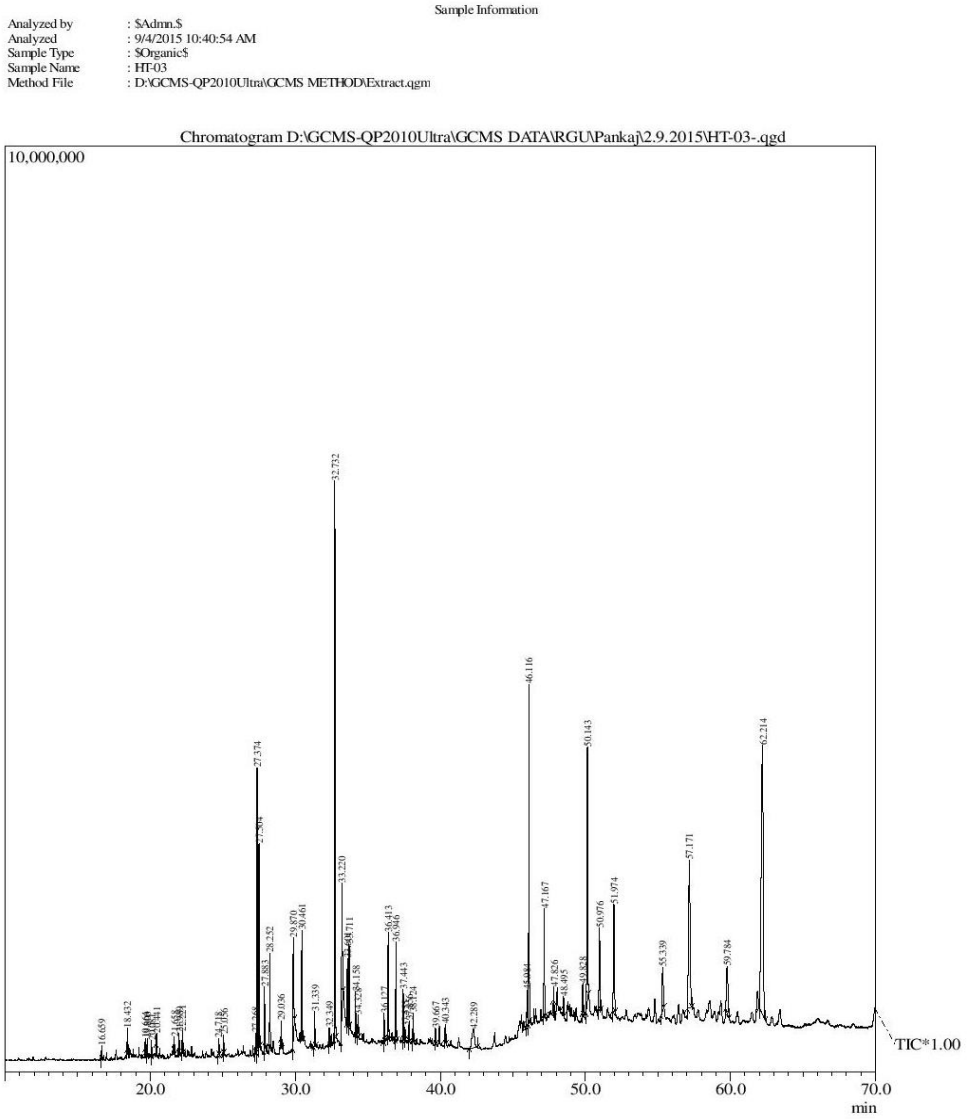

Fig 1: GC-MS peak of *Gynocardia odorata* R.Br

Table 1: The list of 50 compounds identified from leaf extract of *Gynocardia odorata* using GC-MS are listed below along with their Molecular formulae, Molecular Weight, Retention Time (min), Peak Area (%) and Biological Activities

Compound Identified	Molecular formula	Molecular weight(mw)	Retention time(min)	Peak area (%)	Biological Activity
1,1,4,7-Tetramethyl-1a,2,3,4,5,6,7,7b-Octahydro	$C_{15}H_{24}$	204	16.659	0.17	Antioxidant Activity
1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-Octahydro	$C_{15}H_{24}$	204	18.432	0.37	Essential Oils
1H-Cyclopropa[A]Naphthalene, Decahydro-1,1,3a-Trimethyl-7-Methylene	$C1_5H_{24}$	204	19.664	0.18	Anti-Candida, Anti-Inflammatory
1h-Cycloprop[E]Azulene, 1a,2,3,5,6,7,7a,7b-Octahydro	$C_{15}H_{24}$	204	19.800	0.27	Anti cancer Activity
Kw3 AusEpiglobulol	$C_{15}H_{24}$	204	20.081	0.27	Anti-Cancer
Beta.-Guaiene	$C_{15}H_{24}$	204	20.441	0.22	Essential Oil From
Ledene oxide-(II)	$C_{15}H_{24}$	204	21.658	0.14	No Activity Reported
9-Isopropyl-1-Methyl-2-Methylene-5-Oxa-Tricyclo	$C_{15}H_{24}O$	220	21.658	0.26	Anti-Candida, Anti-Inflammatory, Anti diabetic
3-Methyl-5-(2,6,6-Trimethyl-1-Cyclohexen-1-Yl)	$C_{15}H_{24}O$	220	21.989	0.40	No Activity Reported

Tetradecanal	$C_{15}H_{24}O$	220	22.221	0.27	Anti microbial Activity
1-Dodecanol, 3,7,11-Trimethyl-	$C_{14}H_{28}O$	212	24.718	0.29	Anti microbial And Cytotoxic Activity
2-Hexadecene, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-	$C_{15}H_{32}O$	228	25.056	0.32	Anti-Inflammatory And Analgesic *Activities*
2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{40}$	280	27.268	5.01	Antibacterial Activity And Toxicological
2-Pentadecanone, 6,10,14-Trimethyl-	$C_{20}H_{40}O$	296	27.374	3.65	Anti microbial And Anti-oxidant Activity
2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{18}H_{36}O$	268	27.504	1.09	Anti proliferative
2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,	$C_{20}H_{40}O$	296	27.883	1.76	Anti malarial And Antifungal Activity
5,9,13-Pentadecatrien-2-One, 6,10,14-Trimethyl-,	$C_{20}H_{40}O$	296	28.252	0.36	Antioxidant
Pentadecanoic Acid	$C_{18}H_{30}O$	262	29.036	2.21	Ant ibacterial And Anti fungal Activities
Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	284	30.461	1.74	Anti-oxidant, Hypo-cholesterolemic, Nematicide, Pesticide, Anti-androgenic Flavor, Hemolytic, Alpha reductase Inhibitor 4
Oxybenzone	$C_{14}H_{12}O_3$	228	31.339	0.70	No Activity Reported
Heptadecanoic Acid, Ethyl Ester	$C_{19}H_{38}O_2$	298	32.349	0.28	Anti-microbial
Phytol	$C_{20}H_{40}O$	296	32.732	10.52	Anti-microbial, Anti-cancer, Anti-Inflammatory, Diuretic
Octadec-9-Enoic Acid	$C_{18}H_{34}O_2$	282	33.220	3.68	Anti-Fungal Activity
Ethyl (9z,12z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	308	33.601	1.13	Anti-cancer Activity
Cis-9-Hexadecenal	$C_{16}H_{30}O$	238	33.711	2.05	Anti-Inflammatory Properties
Octadecanoic Acid, Ethyl Ester	$C_{20}H_{40}O_2$	312	34.158	0.72	No Activity Reported
Benzoic Acid, Tetradecyl Ester	$C_{20}H_{32}O_2$	318	34.328	0.41	Anti-Mycotic Activity
Benzoic Acid, Tetradecyl Ester	$C_{20}H_{32}O_2$	304	36.127	0.47	Anti-Mycotic Activity
2-Propenoic Acid, 3-(4-Methoxyphenyl)-, 2-Ethylhexyl Ester	$C_{18}H_{26}O_3$	290	36.413	2.08	Cancer-Preventive *Activity*
4,8,12,16-Tetramethylheptadecan-4-Olide	$C_{21}H_{40}O_2$	324	36.946	1.74	Anti-oxidant *Activities*
(2e,6e,10e)-3,7,11,15-Tetramethyl-2,6,10,14-Hexadecatetraenyl	$C_{22}H_{36}O_2$	332	37.443	0.92	Anti-microbial And Cytotoxic Activity
Heptadecanoic Acid, Ethyl Ester	$C_{19}H_{38}O_2$	298	37.555	0.16	Anti-oxidant, Anti-microbial
Benzoic Acid, Tetradecyl Ester	$C_{21}H_{34}O_2$	318	37.856	0.33	Anti-Mycotic Activity
Octadecanal	$C_{18}H_{36}O$	268	38.124	0.50	Anti-oxidant And Anti-bacterial Properties
Benzoic Acid, Tetradecyl Ester	$C_{21}H_{34}O_2$	318	39.667	0.25	Anti-oxidant Properties, Anti-bacterial, Anti-fungal Properties
1,2-Benzene dicarboxylic Acid	$C_{24}H_{38}O_4$	390	40.343	0.31	Anti-Mycotic
Gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	42.289	1.63	Inflammatory *Activity*
(2e,6e,10e)-3,7,11,15-Tetramethyl-2,6,10,14-Hexadecatetraenyl	$C_{22}H_{36}O_2$	332	45.984	0.74	Anti-oxidant And Cytotoxic *Activities*
Squalene	$C_{30}H_{50}$	410	46.116	6.22	Anti-bacterial, Anti-oxidant, Anti-tumor, Cancer Preventive, Immuno-stimulant, Chemo Preventive, Pesticide
Tetracontane	$C_{40}H_{82}$	562	47.167	2.34	No Activity Reported
(6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-	$C_{30}H_{50}O$	426	47.826	0.33	Toxicity Fertilizer
Ergosta-5,7,9(11),22-Tetraen-3-Ol, (3.Beta.,22e,24s)-	$C_{28}H_{42}O$	394	48.495	0.22	Cytotoxic *Activities*
Beta.-Tocopherol	$C_{28}H_{48}O_2$	416	49.828	0.82	No Activity Reported
Gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	50.143	7.34	Anti-tumor, Anti-oxidant, Antimicrobial
Tetracontane	$C_{40}H_{82}$	562	50.976	2.30	No Activity Reported
Vitamin E	$C_{29}H_{50}O_2$	430	51.974	3.58	Analgesic, Anti-DiabaticAnti-inflammatory, Anti-oxidant, Anti-dermatitic, Anti-leukemic, Ant-itumor, Anti-cancer, Hepato-protective, Anti-spasmodic
Stigmasterol	$C_{29}H_{48}O$	412	55.339	1.78	Anti-hepatotoxic, Anti-viral, Anti-oxidant, Cancer preventive, Hypo-cholesterolemic
Stigmast-5-En-3-Ol, (3.Beta.)-	$C_{29}H_{50}O$	414	57.171	7.72	Hepato-protective
4,22-Stigmastadiene-3-One	$C_{29}H_{46}O$	410	59.784	2.31	Anti-microbial Activity
Stigmast-4-En-3-One	$C_{29}H_{48}O$	412	62.214	17.45	Hepato-protective

Phytochemical constituents identified from the methanol extract (leaf) of *Gynocardia odorata* R.Br. using GC-MS analysis: List of major compounds identified from *Gynocardia odorata* leaf extract

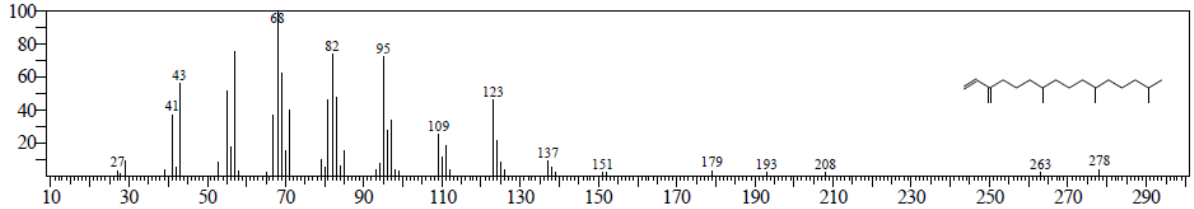

Fig 2.A: Neophytadiene

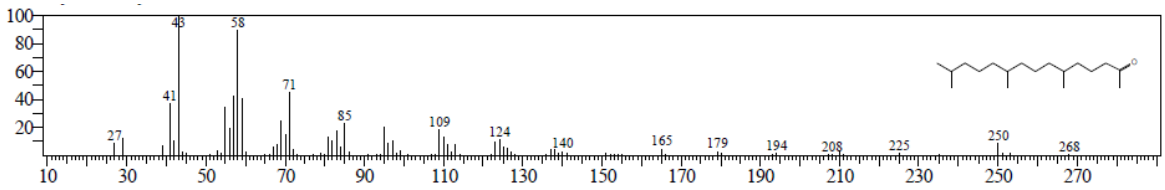

Fig 2.B: Perhydrofarnesyl acetone

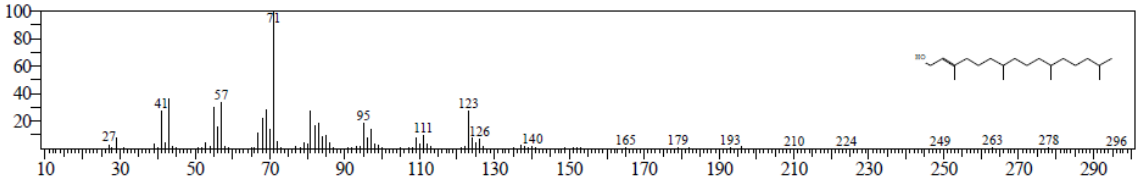

Fig 2.C: Phytol

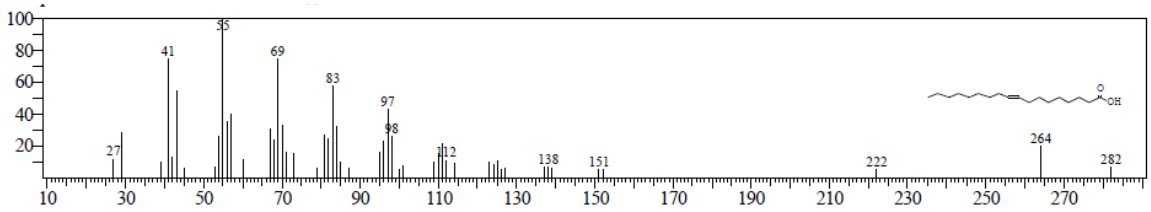

Fig 2.D: Octadec -9-Enoic Acid

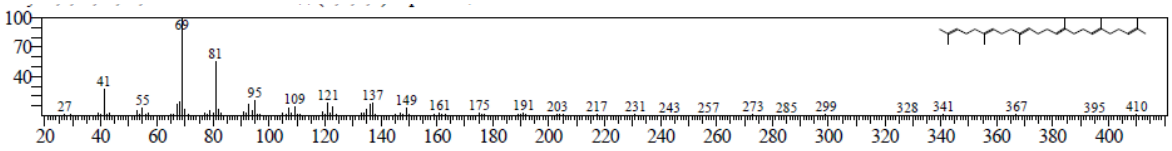

Fig 2.E: Squalene

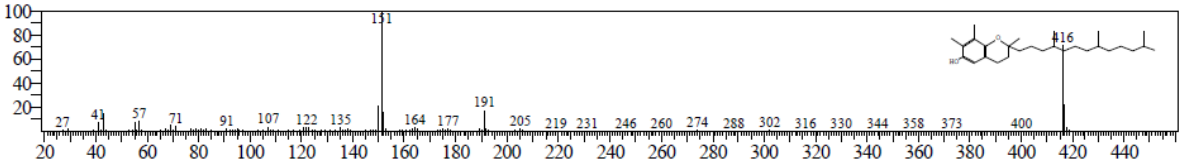

Fig 2.F: Squalene

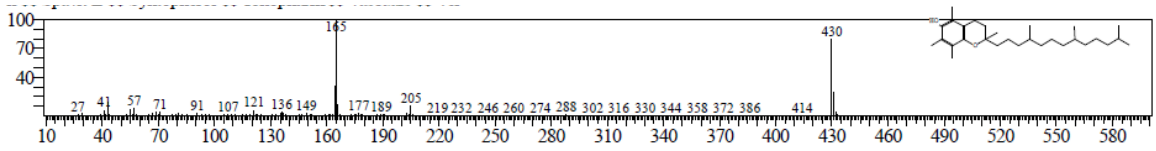

Fig 2.G: Vitamin E

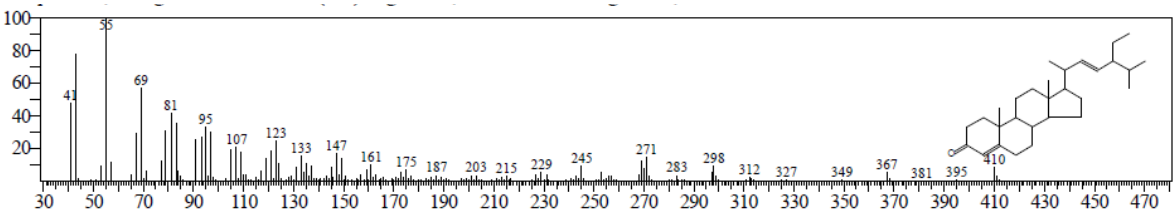

Fig 2.H: Stigmast-5-En-3-Ol, (3.Beta.)

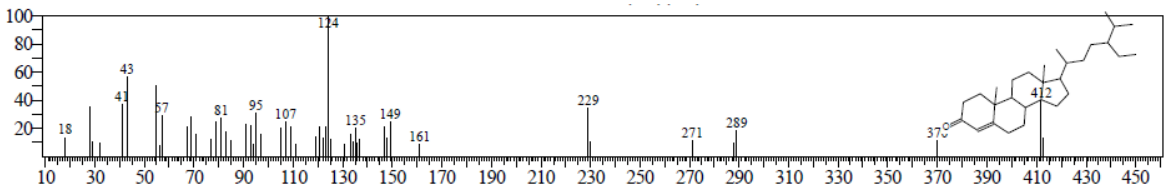

Fig 2.I: R-4-Stigmasten-3-One

5. Conclusion

In the present study the *Gynocardia odorata* R.Br., a poisonous plant reported from Arunachal Pradesh is commonly used by the *Adi*, *Idu Mishimi*, *Digaro* & *Wangsu* tribes as a fish poison. Therefore the candidate plant was undertaken for the GC-MS profiling of the secondary metabolites present in the leaf. Phytochemical constituents of medicinal or poisonous plants have different roles for the protection of the plants and also the different poisonous properties. Different secondary metabolites of a particular plant can be determine by using sophisticated chromatographic techniques like HPLC, GC-MS, LCMS etc. The GC-MS analysis of a plant parts gives an imaginary picture of the chemical constituents which are present in the candidate plants.

6. Conflict of interest statement

Authors declare that they have no conflict of interest.

7. Acknowledgements

The first and last author is also thankful to Director of DRL Tezpur, Assam for facilities and encouragement for partial funding support during field work. The extract was then analyzed in GC-MS at Advanced Instrumentation Facility, Jawaharlal Nehru University (JNU), and New Delhi. The contributions of the traditional knowledgeholders and local herbalists during field work in different districts of Arunachal Pradesh are duly acknowledged and Authors are thankful to Department of Biotechnology, New Delhi, Government of India for providing financial support, They also acknowledge the support of the Director of IASST, Gauhati, Assam.

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