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GC-MS Analysis of the Ethanol Extract of *Tragia plukenetii* R. Smith

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Abstract
A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. Gas Chromatography (GC) and Mass Spectroscopy (MS) can be used to study Traditional Medicines and characterize the compound of interest. *Tragia plukenetii* R.Smith is herb distributed in hill slopes of southern peninsular India. The root is diaphoretic and alterative and is given for fevers to cause perspiration. Sterols, triterpenes, polar and other constituents in whole plant of *Tragia plukenetii* were analyzed by gas chromatography-mass spectrometry. Over 38 compounds were identified. Sitosterol and stigmasterol were the most abundant of sterols identified in the sterol fraction.

Keywords: Gas Chromatography (GC), Mass Spectroscopy (MS), *Tragia plukenetii*.

Introduction
Biological screening is necessary to provide a scientific basis for validating the traditional utilization of medicinal plants. A great number of screening programs are going on worldwide for new plant based bioactive molecules. Gas Chromatography (GC) and Mass Spectroscopy (MS) can be used to study Traditional Medicines and characterize the compound of interest. *Euphorbiaceae* is a complex heterogeneous family consisting of about 322 genera and 8900 species in the world (Hutchinson, 1959). In India, this family is represented by 73 genera and 410 species (Punt, 1987). The family is essentially tropical and occurs in diverse habitats from arid regions to humid tropics. Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of *Euphorbiaceae* particularly from the rural areas are unexplored which deserve attention and research. *Tragia,* a genus of perennial, usually climbing or twining herbs, with stinging hairs, found in the tropical and sub-tropical parts of the world. *Tragia plukenetii* is such plant which has not been explored extensively by the scientific world so far. *Tragia plukenetii* R. Smith (Tamil name: Karunkanchori) the root is diaphoretic and alterative and is given for fevers to cause perspiration (Ranjani, 2010).

Materials and Methods
Plant material
Mature and healthy plants of *Tragia plukenetii* were collected from Southern Western Ghats in the district of Tirunelveli, South India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the *'Flora of the Presidency of Madras'* (Gamble. 1915 - 1936). The taxonomic features collected from the species have been confirmed with the '*Flora of Tamilnadu Carnatic'* (Mathew. 1988) [8]. Voucher specimens are documented in the herbarium of St. Xavier's College (XCH), Palayamkottai, Tamil nadu, India.

Soxhlet extraction
About 60 g dried sample was refluxed with 250 ml of the ethanol for 5 hour on a steam bath. The extract was collected and concentrated.

Procedure
The GC - MS analyses were carried out in a Shimadzu GC – MS - QP 2010 gas chromatograph fitted with a DB1 (methylphenylsiloxane, 30 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.7 mL/min; column oven temperature 70 °C, 5 min in 180 °C, 180-260 °C at 3 °C/min, 5 min in 260 °C, 260-280 °C at 0.2 °C/min, and finally 5 min in 280 °C; injector temperature, 280 °C detector temperature, 290 °C, Volume injected, 1 µL of TMS ether derivatives in *n*-hexane (2%); Split ratio, 3:0. The MS operating parameters were as follows: ionization potential 70 eV; ion source temperature 200 °C; quadrupole 100 °C, Solvent delay 6.0 min , scan speed 2000 amu/s and scan range 30-600 amu, eV voltage 3000 volts.

The concentrated extract is injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with an HP-1 glass capillary column). The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moves through the column, various components are separated due to their affinity for the stationary phase of the column and can be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample has a distinct retention time measured in minutes, shown in a peak on a graph which measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass selective detector breaks up each chromatographic component into fragment ions, which are shown by their abundance, with each ion represented as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum is unique to that chemical. This mass spectrum forms a ''fingerprint'' that can identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12·9 min retention time value if they were present two modes of GC/MS were possible with this instrumental method. First, there is a ''Scan'' mode which looks at all the constituents of a sample, listing whatever chemical components are present.

Compound Identification

Components of the ethnolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST '98 MS computer library (Wiley). GC/MS analysis was carried out with the assistance of Sargam laboratory Pvt. Ltd, 2 Ramavaram road, Manapakkam, Chennai.

Results and Discussion

Tragia plukenetii R. Smith. (Synonym: *T. cannabina* L.f) is a hispid climbing shrub with sting hairs, the leaves tripartite, with long narrow lobes. In the GC-MS analysis, 38 bioactive phytochemical compounds were identified in the ethanolic extracts of *T. plukenetii*. The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the ethanol extracts of leaf of *B. sensitivum* are presented in Table 1, and the total running time was 36 min. The spectra of the compounds were matched with Wiley 9.0 and National Institute of Standards and Technology libraries. The chromatogram of the constituents indicated three major peaks (Fig: 1).

Table 1: GC-MS analysis of *Tragia plukenetii*

Peak	Retention Time	Area	Area%	Name
1	5.517	123831	0.24	Melamine
2	6.587	161181	0.31	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One
3	9.605	138615	0.27	Eugenol
4	12.205	496142	0.95	Lauric acid
5	13.025	960317	1.84	beta-Methylglucoside
6	13.340	641373	1.23	2,5-Monoformal-I-rhamnitol
7	14.386	302315	0.58	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
8	14.476	621157	1.19	Myristic acid
9	14.806	123162	0.24	(-)Loliolide
10	15.226	1115110	2.13	Neophytadiene
11	15.481	173199	0.33	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	15.532	125886	0.24	Stearic acid
13	15.674	218857	0.42	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	16.589	11125043	21.28	Palmitic acid
15	16.828	1718458	3.29	Ethyl Palmitate
16	17.951	1224229	2.34	Phytol
17	18.230	2775460	5.31	Linoliec acid
18	18.288	6966736	13.32	(7Z,10Z,13Z)-7,10,13-Hexadecatrienal # $$
19	18.407	1512347	2.89	Methyl linoleate
20	18.465	2562852	4.90	Dichloroacetic acid, tridec-2-ynyl ester
21	18.685	356444	0.68	Ethyl stearate
22	21.549	386986	0.74	Bis(2-Ethylhexyl) Phthalate
23	23.424	304574	0.58	Pentacontanoic acid, ethyl ester
24	23.568	950606	1.82	Spinacene
25	24.127	265796	0.51	Hexatriacontane $$ n-Hexatriacontane $$
26	24.557	268353	0.51	Piperin
27	25.271	326165	0.62	gamma-Tokoferol
28	25.478	240989	0.46	Octacosane$$ n-Octacosane $$
29	25.843	1741309	3.33	Vitamin E
30	26.825	368999	0.71	Ergost-5-en-3-ol, (3.beta.)-
31	27.054	451828	0.86	Stigmasterol
32	27.680	6210537	11.88	gamma.-Sitosterol
33	28.232	785347	1.50	Methyl Comate A
34	28.343	471177	0.90	9,19-Cyclolanost-23-ene-3,25-diol,(3.beta.,23E)-
35	28.432	746583	1.43	Lupenone
36	28.608	1024848	1.96	Cycloartenol
37	28.793	3627519	6.75	Lupenol
38	29.193	776481	1.48	Cholest-4-en-3-one

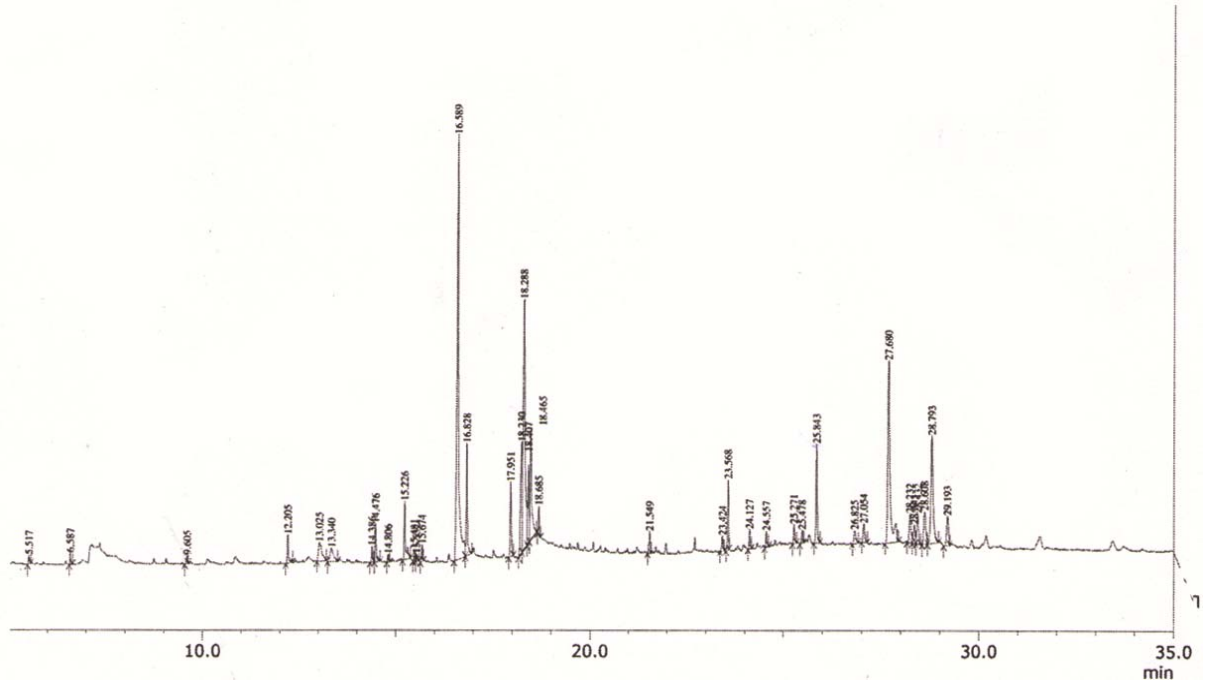

Fig 1: Chromatogram of *Tragia plukenetii* ethanol extract

The most prevailing major compounds in *T. plukenetii* were Cholest-4-en-3-one with *RT* 29.193has peak area 1.48 %, Lupenol with RT (28.793) and cycloartenol with RT 28.608 ranks next having peak area 6.75% and 1.96 % respectively. Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common fatty acid (saturated) found in animals, plants and microorganisms. Structural and kinetics studies that the fatty acid, n-hexadecanoic acid, is an inhibitor of phospholipase A (2), hence, an anti-inflammatory compound. The inferences from the present study validate the rigorous use of medicated oils rich in n-hexadecanoic acid for the treatment of rheumatic symptoms in the traditional medical system of India, Ayurveda. Linoleic acid, (9,12,15-Octadecatrienoic acid (Z,Z,Z) (IUPAC Name)) an *n*−3 fatty acid, is a member of the group of essential fatty acids (EFAs), so called because they cannot be produced within the body and must be acquired through diet. Most seeds and seed oils are much richer in an *n*−6 fatty acid, linoleic acid. In present study reported the presence of linoleic acid (Rt: 18.230, area: 5.31%). Linoleic acid is essential for maintenance of growth and α-linoleic acid for neural functions. Preliminary research has found evidence that α-linoleic acid is related to a lower risk of cardiovascular disease (Simon *et al*., 2009) [12].

Tocopheryl acetate, also known as vitamin E acetate, is a common vitamin supplement with the molecular formula $C_{31}H_{52}O_3$ (for 'α' form). It is the ester of acetic acid and tocopherol (vitamin E). It is often used in dermatological products such as skin creams. Tocopheryl acetate is not oxidized and can penetrate through the skin to the living cells, where about 5% is converted to free tocopherol and provides beneficial antioxidant effects (Beijersbergen *et al*., 1995) [2]. Vitamin E Acetate, is the stable form of Vitamin E most often used in cosmetic formulations for its skin care benefits. Vitamin E protects cell membranes from damage by oxygen free radicals. It can prevent premature aging of the skin induced by UV irradiation and lipid peroxidation. Therefore, antioxidant constituents from *Tragia plukenetii* ethanol extract could hold promise for future application in therapy.

Different types of sterols were present in considerable amounts in the chosen species. Gamma-sitosterol and stigmasterol were found in this fraction. Sterols are important constituents of all eukaryotes and play vital role in plant cell membranes. Plant sterols possess valuable physiological activities; they are biogenetic precursors of many hormones and oviposition stimulants of some insects (Harborne, 1928). Stigmasterol was found to markedly inhibit tumor promotion in two-stage carcinogenesis in mice (Yasukawa *et al.*, 1991; Kasahara *et al.*, 1994) [7, 14] and to exhibit significant inhibitory effect on HIV reverse transcriptase (Akihisa *et al.*, 2001) [1]. A mixture of stigmasterol and sitosterol was shown to possess anti-inflammatory activity after topical application (Gomez *et al.*, 1999) [4]. Therefore, the presences of these sterols in chosen species are of practical importance. Sitosterol possesses antihyperlipoproteinaemic, antibacterial and antimycotic activity and has been shown to act as inhibitor of tumor promotion *in vivo* (Yasukawa *et al.*, 1991) [14] and to inhibit carcinogenesis (Raicht *et al.*, 1980) [11].

Traditional use of the *Tragia plukenetii* for pain relief is well supported by the presence of stigmasterol and lupenol. Lupeol and b-amyrin both have a hepatoprotective effect (Sunitha *et al.*, 2001; Oliveira *et al*., 2005) [13, 10] and lupeol also has a nephroprotective effect (Nagaraj, 2000) [9]. Some of main constituents identified in study are reported to have antibacterial property. Therefore, antibacterial constituents from *Tragia plukenetii* ethanol extract could hold promise for future application in therapy. Further experiments, are planned to establish the influence of the components of these mixtures on the pharmacological activity.

References

1. Akihisa T, Ogihara J, Kato J, Yasukawa K, Ukiya M, Yamanouchi S *et al*. Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase. Lipids 2001; 36:507-512.
2. Beijersbergen van Henegouwen G, Junginger H, de Vries H. "Hydrolysis of RRR-alpha-tocopheryl acetate (vitamin E acetate) in the skin and its UV protecting activity (*an in vivo* study with the rat)". Journal Photochem. Photobiol. 1995; 29(1):45-48.
3. Gamble JS. *Euphorbiaceae* In: Flora of the Presidency of Madras, Bishen Singh Mahandra Pal Singh Dehra Dun, 1915-1936.
4. Gomez MA, Saenz MT, Garcia MD, Fernandez MA. Study of the topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation models. Z Naturforsch 1999; 54(11):937-941.
5. Herbone JB. Phytochemical methods. Chapman and Hall, London, New York, 2nd edition, 1928.
6. Hutchinson, J. 1959. The families of flowering plants. I. Dicotyledons. Oxford University Press, London. p: 510.
7. Jayaweera DMA. Medicinal Plants (Indigenous and Exotic) used in Ceylon. Volumes I-V. National Science Council of Sri Lanka. Colombo, Sri Lanka, 1980-1982.
8. Kasahara Y, Kumaki K, Katagiri S, Yasukawa K, Yamanouchi S, Takido M *et al*. Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. Phytotherapy Res. 1994; 68:327-331.
9. Matthew KM. The Flora of Tamil Nadu Carnatic, the Rapinat Herbarium, Tiruchirappali, 1983-1988, II.
10. Nagaraj M, Sunitha S, Varalakshmi P. Effect of lupeol, a pentacyclic triterpene, on the lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure. Journ Appl. Toxicol 2000; 20(5):413-417.
11. Oliveira FA, Chaves MH, Almeida FR, Lima RC, Silva RM, Maia JL *et al*. Protective effect of alpha- and betaamyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. Trunk wood resin, against acetaminophen-induced liver injury in mice. Journ Ethnopharmacol. 2005; 98(1-2):103-108.
12. Rajani P. Pharmacognostic and antihyperglycemic studies of *Tragia plukenetii* M.Pharmacy Thesis Submitted to Osmania University, Hyderabad, India; 2010.
13. Raicht R, Cohen B, Fazzini E. Protective effect of plant sterols against chemically induced colon tumours in rats. Cancer Res 1980; 40:403-405.
14. Punt W. A survey of pollen morphology I n Euphorbiaceae with special reference to *Phyllanthus.* Botanical Journal of the Linnean Society, 2008; 94(12):127 - 142.
15. Simon JA, Chen YH, Bent S. The relation of alpha-linolenic acid to the risk of prostate cancer. American Journal of Clinical Nutrition 2009; 89(5):1558S-1564S.
16. Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. Fitoterapia 2001; 72(5):516-523.
17. Yasukawa K, Takido M, Matsumoto T, Takeuchi M, Nakagawa S. Sterol and triterpene derivatives from plants inhibit the effects of tumour promoter and sitosterol and betulinic acid inhibits tumour formation in mouse skin two-stage carcinogenesis. Journ Oncology. 1991; 41:72-76.