

Research in Pharmacy 4(4): 01-07, 2014

ISSN: 2231-539X

www.researchinpharmacy.com

Regular Article

GC-MS Analysis of Phytocomponents in *Spermacoce articularis* L. f. leaf

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Spermacoce is a genus of the plant family Rubiaceae. Approximately 280 species distributed in subtropical and tropical regions in Asia and Africa etc. It is commonly known as Nathaisuri in Tamil. Leaf extract of the plant is used in against hemorrhoids, galls tones, jaundice and conjunctivitis. Roots are used to mouthwash to relieve toothache, decoction of the herb used to relieve headache, while seeds are demulcent in diarrhea, dysentery and antimicrobial activity. *In vitro* and *In vivo* leaf also contains alkaloids, glycosides, steroids, flavonoids and tannins. Liver diseases are a major public health. A 50% methanolic extract of the leaf were subjected to phytochemical studies and further investigated by GC-MS Analysis.

Keywords : GC-MS analysis, *Spermacoce articularis*, Methanol extract, *In vitro* leaf, *In vivo* leaf

Introduction

Primitive man tried to cure diseases from plants growing abundantly around him. His experience through trial and taught him a lot about the medicinal properties of different plants. The active secondary metabolites possess various medicinal applications as drugs or as model compounds for drug synthesis (De-Fatima *et al.*, 2006). Phytochemical analysis of plants, used in folklore has yielded a number of compounds with various pharmacological activities. Hence medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act a disease curing agents (Haznagy Radnal *et al.*, 2007). Review of literature revealed no work as been done form the angle(separate *In vitro* and *In vivo* leaf) identification of phytocomponents by Gas Chromatography-Mass Spectrometry (GC-MS) analysis on this plant. Hence it was decided to do so.

Plants are now occupying important position in allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Medicinal plants are the sources of many important drugs of the modern world. Many of these indigenous medicinal plants are used as spices and food plants; they are also sometimes added to foods meant for pregnant mothers for medicinal purposes (Chaman Lal and Verma, 2006).

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc (Betz *et al.*, 1997). In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Milne & Beamish, 1999).

Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents (Andrew Marston, 2007). Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients (Thanga Krishnakumari *et al.*, 2012).

Spermacoce articularis L.f. is an important medicinal plant used widely in Indian folk medicine. Leaf extract of the plant is in against hemorrhoids, galls tones, jaundice and conjunctivitis. Roots are used to mouthwash to relieve toothache, decoction of the herb used to relieve headache, while seeds are demulcent in diarrhea, dysentery and antimicrobial activity. It also contains alkaloids, glycosides, steroids, flavonoids and tannins (Soosairaj *et al.*, 2013). Liver diseases are a major public health. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in inherent indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. Hippocrates (460-377 BC) discussed the illness and treatment of several diseases in a chronological manner and earned his reputation as the 'Father of Medicine'.

Materials and Methods

Sample Collection and Extraction

Whole plant of *Spermacoce articularis* L.f. were collected from Karur district, Tamil Nadu, (India) during the month of January, 2012. The voucher specimen was identified, authenticated and submitted at Botanical Survey of India (BSI/ SRC/ 5/23/2013-14/Tech, 1643) Coimbatore. *In vivo* and *In vitro* Leaves and roots of the collected plants were washed thoroughly with distilled water and shade dried for ten days. A 1000 g dried leaves were ground to a fine powder using mixer grinder and subjected to extraction thrice in 50% methanol using cold maceration technique. The extract was concentrated in rotary vacuum evaporator and stored at 40C until further use (yield = 8.24%).

GC-MS Analysis

GC-MS analysis of these extracts performed with GC clarus 500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite - 1 fused silica capillary column (30 mm x 0.25 mm 1D x 1 um df, composed of 100% Dimethyl poly siloxane). For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml / min and an injection volume of 2 ul was employed (Split ratio of 10:1); Injector temperature 2500C; ion-source temperature 2800C. The oven temperature was programmed from 1100 C (isothermal for 2 min) with an increase of 100C/min, to 2000C, then 50C/min to 2800C, ending with a 9 min isothermal at 2800C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbo mass.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass

spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

The plant sample on subjecting to GC-MS provides result of different peaks determining the presence of different compounds. The molecular weight of these compounds is also known. By interpreting these compounds, it is found that this plant possess various therapeutically uses.

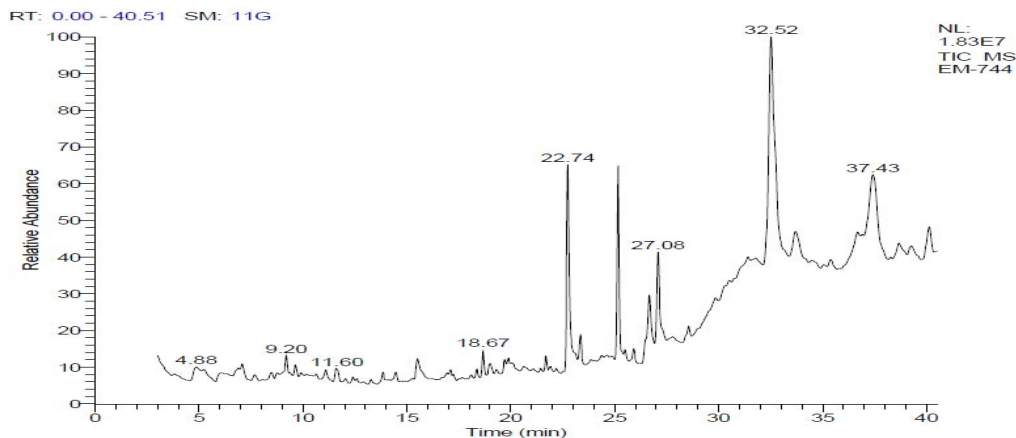


Figure 1: GC-MS Chromatogram of *Spermacoce articularis* L.f. *in vivo* leaf

In vivo leaf

The Chromatogram (Figure 1) shows 6 prominent peaks in the retention time range 4.88 - 40.12. The sample of *In vivo* leaf methanolic extract was run for 37.51 minutes. GC-MS analysis was carried out on the *In vivo* leaf of *Spermacoce articularis* L.f. and 30 compounds were identified.

The GC-MS analysis of major phytochemicals active principles with their retention time (RT), Name of the Compound, Molecular Formula, Molecular weight (MW), Peak area%, major phytochemicals chemical nature and its biological activities of *Spermacoce articularis* L.f. were presented in table 1.

The Major prevailing phytochemicals in methanol extract of *In vivo* leaf were 3-[4'-(2"-Chlorophenyl)-2'-thiazolyl]-2, 4-dioxo-1,2,3,4-tetrahydroquinazoline, 2-N-Propyl-1-d1-Aziridine, 6-Imino-2,3,4,6-tetrahydro1, 3thiazini[3,2b] isoquinoline-11 carbonitrile, 7,8 Bis (trimethylsilyl) benzo (5,6-g)-1H, 3H-quinazoline-2,4-dione, Cycloheptasiloxane, tetradecamethyl, 3-ethylcyclohexanone, Cytidine (CAS), Dihydroactinidiolide, Neophytadiene, 6-Methyl-3-methylmercapto-5-thioxo-1,2,4-triazine, Phytol, Octadecanoic acid, 9,10-dichloro- methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, isochiapin b , Silicone oil, 5 α -cholestane-3 α ,7 α ,12 α ,25-tetrol tms ether, 3',4'-Dihydro-Stephasubine, 1,6-Dibromo-3,4 bis[(Triisopropylsilyl) ethynyl] hex-3-en-1,5-diyne, 5,10,15-tribenzylidene-truxene, Mixtures of chlorine. The above compounds were represented by less than 2% area.

Table 1. List of Phytochemicals identified in the methanolic extract of *in vivo* leaf in *Spermacoce articularis* L.f. by GC-MS

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
1	19.89	2-Pentadecanone, 6,10,14-trimethyl- (CAS)	C ₁₈ H ₃₆ O	268	2.28	Ketone Compound	No Activity reported
2	22.72	Hexadecanoic acid (CAS)	C ₁₆ H ₃₂ O ₂	256	9.29	Palmitic acid	Antioxidant Antimicrobial
3	25.17	Phytol	C ₂₀ H ₄₀ O	296	9.38	Diterpene	Antimicrobial Anti-inflammatory Antioxidant Diuretic
4	26.67	9,12-Octadecadienoic acid (Z,Z)- (CAS)	C ₁₈ H ₃₂ O ₂	280	5.34	Magnesium Stearate	Anti-adherent Vegetable
5	27.08	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(CAS)	C ₁₉ H ₃₂ O ₂	292	4.50	Fatty acid	Anticancer
6	31.41	5-Nonanone, 2,8-dimethyl	C ₁₁ H ₂₂ O	170	4.53	Amide	Antioxidant Anti-inflammatory Antimicrobial
7	32.52	Cyclohexane, 1,3,5-trimethyl-2-octadecyl- (CAS)	C ₂₇ H ₅₄	378	24.30	Alcohol	Anticancer
8	33.68	Tungsten,	C ₂₁ H ₂₆ BO ₅	612	3.14	Carbide	Antimicrobial Anticancer
9	37.43	Dioxa-2-stanna-[d,h]dibenzocyclo nonene	C ₂₁ H ₂₆ N ₂ O ₄ Sn	490	9.66	Polycyclic Aromatic Hydrocarbon	Anticancer
10	40.12	Colchicine	C ₃₀ H ₃₀ N ₄ O ₈	574	2.91	Colchicine	Anti-inflammatory Antioxidant

The highest coverage peak area of *In vivo* leaf *Spermacoce articularis* L.f. were 2-(4-Chlorophenyl)-6-phenyl-4-trifluoromethylpyridine (78.48), Strychnine (7.58), (5R,11R)-2,8-Dimethoxy-5,11-dipropyl-5,6,11,12-tetrahy drochrysenone (6.87) (Table 1).

In vitro leaf

The Chromatogram (Figure 2) shows 6 prominent peaks in the retention time range 4.77– 38.23. The sample of *in vivo* leaf methanolic extract was run for 37. 49 minutes. GC-MS analysis was carried out on the *Spermacoce articularis* L.f and 29 compounds were identified.

The major phytochemicals active principles with their retention time (RT), Name of the Compound, Molecular Formula, Molecular weight (MW), Peak area%, major phytochemicals chemical nature and its biological activities obtained through GC-MS study of *Spermacoce articularis* L.f.

Table 2: List of Phyto-Compounds identified in the methanolic extract of *in vitro* leaf in *Spermacoce articularis* L.f.

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
1	4.77	12-Octahydroindolo (2,3-A)Quinolizine	C ₂₁ H ₂₈ N ₂ O ₃	356	2.19	Alkaloid	Anesthesia Diabetes mellitus Anticancer Anti-HIV Agent Anti-inflammatory Antimicrobial
2	7.06	Cyclohexasiloxane, dodecamethyl-	C ₁₂ H ₃₆ O ₆ S _{i6}	444	5.93	Cosmetics	Antimicrobial
3	9.17	Cycloheptasiloxan, tetradecamethyl-	C ₁₄ H ₄₂ O ₇ S _{i7}	518	4.04	Cosmetics	Antimicrobial
4	11.54	Cyclooctasiloxane, hexadecamethyl-	C ₁₆ H ₄₈ O ₈ S _{i8}	592	2.29	Cosmetics	Antimicrobial
5	15.63	Guanosine (CAS)	C ₁₀ H ₁₃ N ₅ O ₅	283	7.74	Nucleic acid	Antimicrobial
6	19.02	Hexaborane	B ₆ H ₁₂	78	5.97	Borane Compound	No Activity
7	25.15	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-RRR-(E)-(T)- Phytol	C ₂₀ H ₄₀ O	296	4.46	Phytol	Antimicrobial
8	26.69	8-Deacetyl-7,8-dehydro-7,17-secoyunaconitine-3,13,14-triol	C ₂₅ H ₄₁ NO ₇	467	4.71	Diterpene	Antimicrobial Anti-inflammatory Antioxidant Diuretic
9	30.00	Spiro[Adamantylidene-1,1'-2',2'-diphenyloxirane	C ₂₃ H ₂₄ O	316	13.09	Heteroxylic Compound	Biological & Physiological Studies
10	31.17	Decanoic acid,	C ₄₀ H ₆₄ O ₈	672	6.98	Fatty acid	Antimicrobial
11	36.16	Ethyl3-amino-4-{4'-[(5"-chloro2"-methoxybenzoylamino]phenyl}-6-(p-fluorophenyl)thieno[2,3-b]pyridine	C ₃₀ H ₂₃ ClF ₃ N ₃ O ₄ S	575	13.75	Thieno pyridine	Antiplatelet Activity Anti-inflammatory
12	38.23	Cholesta-7,14-dien-3-ol, 4,4-dimethyl-, (3á,5à)	C ₂₉ H ₄₈ O	412	6.12	Methanol	Anticancer

The Major prevailing phytochemicals in methanol extract of *In vivo* leaf were 6-Bromo-3-(4-Phenyl-Piperazine-1-Carbonyl)-Chromen-2-one, Estragole, 2-Pyrrolidinone, 5-(Hydroxymethyl), 5-Thiazoleethanol, 4-methyl- (CAS), 1,1,3,3,5,5-Hexamethyltrisilazane, 1,3-Bis(4-Chlorobenzyl)-5,6-Dihydrobenzo[f]quinazoline, Silicone oil, 1-(3-Chloro-4-fluorophenyl)-3-(1,2,3,4-Tetrahydro-naphthalen-1-yl)-thiourea, Eicosamethyl cyclodecasiloxane, Quercetin 7,3',4'-trimethoxy, Phenformin, Pentadecanoic acid, methyl ester (CAS), Octadecamethyl cyclonona siloxane, 1-(+)-Ascorbic acid 2,6-Dihexadecanoate, 3,4,5,6-

Tetrabromo-2Methoxyimino-1-benquinone, Hexadecanoic acid, 2,3-Dihydroxypropyl ester (CAS), 4-(4-Chlorobenzoyl)-1-Cyclohexyl-5-tosylamino-1H-1,2,3-triazole.

The highest coverage peak area of *In vitro* leaf *Spermacoce articularis* L.f. were Ethyl 3-amino-4-[4'-(5"-chloro-2"-methoxybenzoyl) amino]phenyl]-6-(p-fluorophenyl) thieno [2,3-b] pyridine (13.75), spiro [Adamantylidene-1,1'-2',2'-diphenyloxirane (13.09), Guanosine (CAS) (7.74), Decanoic acid, (6.98), Cholesta-7,14-dien-3-ol, 4,4-dimethyl-, (3á,5à) (6.12), Hexaborane- (5.97), Cyclohexasiloxane, dodecamethyl- (5.93), 8-Deacetyl-7,8-dehydro-7,17-secoyunaconitine-3,13,14-triol (4.71), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-,[r-[r*,r*-(e)] - (t-phytol) (4.46), Cyclooctasiloxane, Hexadecamethyl (2.29) and 12á Octahydroindolo[2,3-a]quinolizine (2.19).

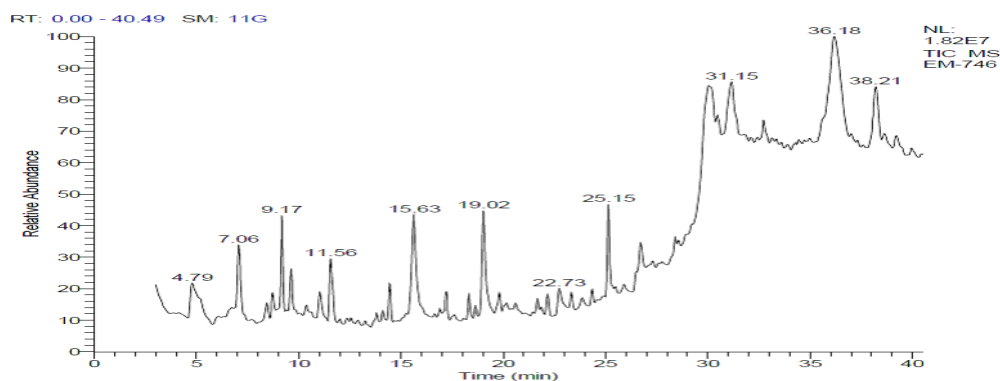


Figure 2: GC-MS Chromatogram *Spermacoce articularis* L.f. *in vitro* leaf

The plant sample on subjecting to GC-MS provides the result of different peaks determining the presence of seven different compounds. The molecular weight of these compounds is also known. By interpreting these compounds, it is found that this plant possess various therapeutically uses. This typical gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The numbers at various peaks are the retention time in minutes. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios (Kalimuthu et al., 2013).

Conclusion

In the present study the presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Spermacoce articularis* L.f. contains various bioactive compounds. So it is recommended as a plant of phyto-pharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

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