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GC-MS Analysis of methanolic extract of *In vitro* and *In vivo* explants of *Spermacoce articularis* L. *f*.

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ABSTRACT

Spermacoce articularis L.F. (Rubiaceae) is an important medicinal plant used widely in Indian folk medicine. Its commonly undertaken to standardize a protocol for *in vitro* known as Nathaisuri in Tamil. 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 diarrrhea, dysentery and antimicrobial activity. It also contains alkaloids, glycosides, steroids, flavonoids and tannins. Liver diseases are a major public health. A 50% methanolic extract of explants (Root, Leaf) were subjected to phytochemical studies and further investigated for GC-MS Analysis the chemical components present in it. Each explants contain 29-30 chemical compounds were identified.

1. INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli et al., 2009). Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemo therapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik et al., 2002; Chaman Lal and Verma, 2006). Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fatima et al., 2006). A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information

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may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne et al., 1993). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (Jie et al., 1988) and alkaloids (Betz et al., 1997).

Spermacoce articularis L.F. (Rubiaceae) is an important medicinal plant used widely in Indian folk medicine. It's commonly undertaken to standardize a protocol for *in vitro* known as Nathaisuri in Tamil (Soosairaj et al., 2013). 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 diarrrhea, dysentery and antimicrobial activity. It also contains alkaloids, glycosides, steroids, flavonoids and tannins. Liver diseases are a major public health.

2. MATERIALS AND METHODS

Collection of plant material

The whole Plant of *Spermacoce articularis* L.F. was collected from Karur District., Tamilnadu (India), was collected in the month of October. The plant was collected by Botanical Survey of India (BSI/ SRC/ 5/23/2013-14/ tech, 1643) Coimbatore.

Plant Sample Extraction

The fresh aerial plant were carefully washed with tap water to remove soil particles and adhered debris, rinsed with The fresh aerial plant were carefully washed with tap water to remove soil particles and adhered debris, rinsed with (root, stem and leaf) and dried in room temperature for weeks. Then they were ground in to powder with the help of Hand Mill (mechanical Grinder) and stored in room temperature for air-tight container. The defatted powder material (marc) thus obtained was Further extracted with ethanol (95% v/v) with same method and fresh powder used for aqueous extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

GC-MS Analysis

Gas chromatography and Mass spectroscopy studies were performed to identify the bioactive compounds. Methanolic extract of above four samples has been subjected to GC-MS analysis. Each sample consists of 30 chemical constituents were identified. 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 0.25um (film thickness)df, composed of 100% Dimethyl poly siloxane).Coated with DB5 MS Capillary Standard Non-Polar Column. For GC-MS detection, and electron ionization system with ionizing energy of 70 eC 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 2600C. The oven temperature was programmed from 1100 C (isothermal for 2 min) with an increase of 100C/min, to 2000C, then 60C/min to 2800C, ending with a 9 min isothermal at 2800C. Mass spectra were taken at 70 eC, a scan interval of 0.5 seconds and fragments from 45 - 450 Da. Total GC running time was 37 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.

3. RESULTS AND DISCUSSION

GC-MS chromatogram of the methanolic extract of *Spermacoce articularis L.f.,* showed 30 peaks indicating the presence of thirty phytochemical constituents for both in vitro leaf, root and in vivo leaf, root. On comparison of the mass spectra of the constituents with the NIST library the thirty phyto constituents were characterized and identified. The various phytochemicals contributes to the medicinal activity of the plant were shown in Table 1. The plant sample *In vivo* Leaf, *In vivo* Root, *In vitro* Leaf and *In vitro* Root the large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. The GC-MS analysis of 25 compounds and its biological activity were presented in table 1. Same results are presented in GC-MS analysis of *in vitro* leaf samples of *B.monnieri*

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indicated the presence of various alkaloids, flavonoids and steroidal compounds. Especially in the leaf samples there are 6 compounds recorded by their peak values. They are alkaloids, terpenoids and saturated aliphatic compounds.

4. 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.

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Table 1

Highest Activity of phyto-components identified in methanolic extract of different explants in *Spermacoce articularis* L.f. by GC-MS

	In vivo leaf			In vivo root			In vitro leaf			In vitro root		
S.NO	Compound Name	Probability	Peak Area %	Compound Name	Probability	Peak Area %	Compound Name	Probability	Peak Area %	Compound Name	Probability	Peak Area %
1	Cyclohexane, 1,3,5-trimethyl-2-octadecyl- (CAS)	8.03	24.30	3-Chloro-[3-(4diphenylmethyl)-2-propenal	94.49	78.48	*Ethyl 3-amino-4-* pyridine	8.23	13.75	Spiroketal	42.85	20.49
2	8-Bromo-7,10-dimethoxy-5-(methoxymethyl)-phenanthridin-6-one	19.59	9.66	3,14-Didehydroapotacamine	59.88	7.58	2-phenyl-4-trifluoromethylquinoline	18.83	13.09	à-D-Glucopyranoside, á-D-fructofuranosyl (CAS)	24.81	12.92
3	Hexadecen-1-ol, 3,7,11,15-tetramethyl-,	67.64	9.38	DIHETHOXY-DIHYDROSANGUINARINE	68.17	6.87	à-D-Glucopyranoside, á-D-fructofuranosyl (CAS)	19.67	7.74	Benzyl-á-D-ribofuranosyl-5-pentyl-pyridazine	53.38	10.83
4	Hexadecanoic acid (CAS)	77.47	9.29	Methyl 2,3-dideoxy-4,6-O	51.78	1.57	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,	6.03	6.98	1,7 Dipyrrolidinyl perylene-	28.67	8.08
5	Oxacycloheptadec-8-en-2-one, (8Z)	11.01	5.34	à-D-Glucopyranoside, á-D-fructofuranosyl (CAS)	51.70	1.31	TRIMETHYL-3-CYCLOHEXENYL)-3 -HYDROXY-BUTAN-1-ONE	7.67	6.12	1,3-Diphenyl-6-(p-chlorophenyl)--(1H,6H)-dione-	69.81	7.90

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