IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume **8** | Issue 1 | January, 2013 | 116-119

RESEARCH ARTICLE

GC–MS analysis of biologically active compounds in *Indigofera viscosa* Lam.

■ **E. RAJABUDEEN, A. SARAVANA GANTHI, M. PADMA SORNA SUBRAMANIAN AND K. NATARAJAN**

SUMMARY

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. *Indigofera viscosa* Lam. is herb distributed in hill slopes of southern peninsular India. The macerate of the crushed whole plant is used as rectal application, twice a week for one week, to stop diarrhea. Sterols, triterpenes, polar and other constituents in whole plant of *Indigofera viscosa* Lam. were analyzed by gas chromatography-mass spectrometry. Over 23 compounds were identified. Sitosterol and stigmasterol were the most abundant of sterols identified in the sterol fraction.

Key Words : Gas chromatography (GC), Mass spectroscopy (MS), *Indigofera viscosa*

How to cite this article : Rajabudeen, E., Ganthi, A. Saravana, Subramanian, M. Padma and Natarajan, K. (2013). GC–MS analysis of biologically active compounds in *Indigofera viscosa* Lam.. *Internat. J. Plant Sci.,* **8** (1) : 116-119.

Article chronicle : Received : 06.08.2012; **Revised :** 24.09.2012; **Accepted :** 14.11.2012

A large proportion of the world's population depends on traditional medicines to meet its needs. Recently, WHO introduced guidelines on research evaluation of traditional medicine and practice. These guidelines have a major objective of developing traditional drugs and aim to ensure quality and safety of botanicals before being evaluated for its efficacy. On this background phytochemistry is playing a paramount role in the evolution of novel medicines, taking lead from natural products. 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. The Fabaceae family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill *et al.*, 1981; Judd *et al.*, 1999). Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of Fabaceae are unexplored which deserve attention and research. *Indigofera viscosa* Lam. is such plant which has not been explored extensively by the scientific world so far. The genus *Indigofera* comprises around 700 species that are distributed geographically in tropical regions (Bakasso, 2008). *Indigofera viscosa* Lam. is herb distributed in hill slopes of southern peninsular India. The macerate of the crushed whole plant is used as rectal application, twice a week for one week, to stop diarrhea (Kusamba Chifundera, 2001). The objective of the present work was to identify the biologically active compounds in *Indigofera viscosa* Lam.

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
A. SARAVANA GANTHI, Department of Botany, Rani Anna Govt. College for Women, TIRUNELVELI (T.N.) INDIA
saran_gan@rediffmail.com

Address of the Co-authors:
E. RAJABUDEEN, Dr. Zahir Husain College, ILAYANKUDI (T.N.) INDIA

M. PADMA SORNA SUBRAMANIAN, Siddha Medicinal Plants Garden, C.C.R.S., METTUR DAM (T.N.) INDIA

K. NATARAJAN, Annai Arts and Science College for women, KARUR (T.N.) INDIA

MATERIALS AND METHODS

Plant material :

The medicinal plant *Indigofera viscosa* Lam. was

collected from Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with voucher specimen No: 5087 available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai.

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 x 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.7 mL/min; column oven temperature 70^0 C, 5 min in 180^0 C, 180-260^0 C at 3^0 C/min, 5 min in 260^0 C, 260-280^0 C at 0.2^0 C/min, and finally 5 min in 280^0 C; injector temperature, 280^0 C detector temperature, 290^0 C, Volume injected, 1µL 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 200^0 C; quadrupole 100^0 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.

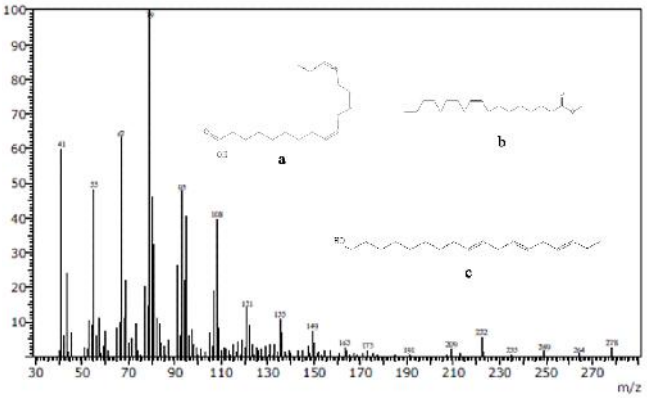
Compound identification :

Components of the methnolic 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).

RESULTS AND DISCUSSION

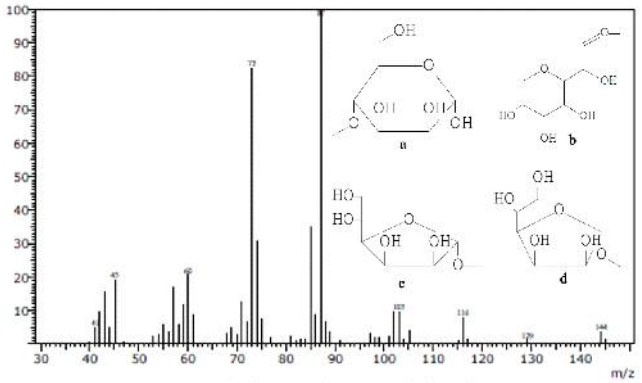
A total of 23 compounds were identified from the whole plant of *I. viscosa*. The details are presented in Table 1. Mome Lnositol, n-Hexadecanoic acid, 2E-3, 7, 11, 15-Tetramethyl-2-Hexadecan-1-ol and alpha.-Linolenic acid were the major components and n-Hexadecanoic acid was the most abundant one (18.02%) (Fig. 1, 2, 3). Four major peaks were observed in the chromatogram (Fig. 4). Their retention times were 14.060, 17.000, 18.465 and 18.711 min., respectively.

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).



a. 9,12,15-Octadecatrienoic acid, (Z,Z,Z)
b. 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)
c. 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)

Fig. 1: Mass spectrum for *Indigofera viscosa*



a. 4-O-Methylmannose, b. 3-O-Methyl-d-glucose
c. alpha.-d-Mannofuranoside, methyl
d. alpha.-Methyl mannofuranoside

Fig. 2: Mass spectrum for *Indigofera viscosa*

Stigmasterol was found to markedly inhibit tumor promotion in two-stage carcinogenesis in mice (Yasukawa *et al.*, 1991; Kasahara *et al.*, 1994) and to exhibit significant inhibitory effect on HIV reverse transcriptase (Akihisa *et al.*, 2001). A mixture of stigmasterol and sitosterol was shown to possess anti-inflammatory activity after topical application (Gomez *et al.*, 1999). 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) and to inhibit carcinogenesis (Raicht *et al.*, 1980).

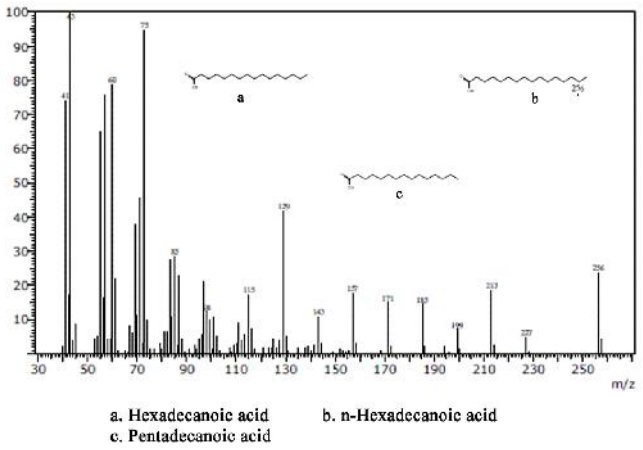


Fig. 3: Mass spectrum for *Indigofera viscosa*

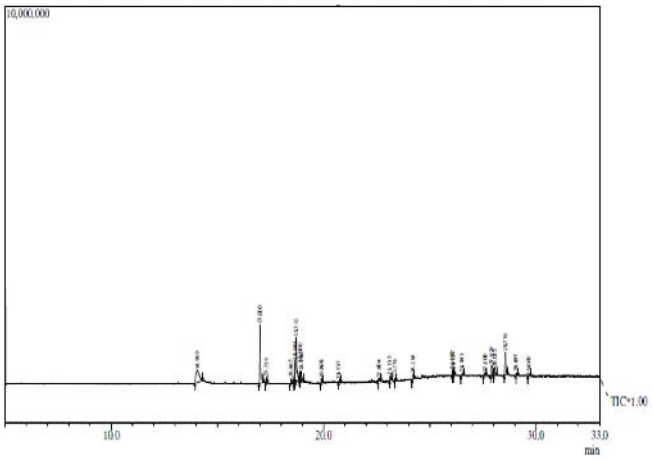


Fig. 4: Chromatogram for *Indigofera viscosa*

Table 1: Composition of the methanolic extract of the whole plant of *Indigofera viscosa* (Peak Report TIC)

Peak #	R.time	Area	Area %	Name
1.	14.060	3510809	17.79	Mome Inositol
2.	17.000	3556362	18.02	n-Hexadecanoic acid
3.	17.310	223401	1.13	Ethyl palmitate
4.	18.465	294936	1.49	(2E)-3,7,11,15-Tetramethyl-2-Hexadecen-1-OL
5.	18.646	1340014	6.79	Linolic acid
6.	18.711	3493443	17.70	.alpha.-Linolenic acid
7.	18.900	523092	2.65	(6z)-6-Pentadecen-1-ol
8.	18.960	448280	2.27	Ethyl linolenate
9.	19.898	224315	1.14	Methyl linolenate
10.	20.737	143896	0.73	(+)-Noe's reagent
11.	22.604	321807	1.63	(-)-5-Oxatricyclo[8.2.0.0(4,6)] Dodecane,, 12-Trimethyl-9-Methylene, [1R-(1R*, 4R*, 6R*, 10S*)]
12.	23.135	308368	1.56	1,1,4A, 7-Tetramethyl -2, 3,4,4A, 5,6,7,8-Octahydro-1H-Benzo[A]Cyclohepten-7-OL
13.	23.376	128217	0.65	2-Nonadecanol
14.	24.214	441469	2.24	Allethrin-2
15.	26.082	262587	1.33	Hexatriacontane
16.	26.137	194492	0.99	Methyl erucate
17.	26.543	205876	1.04	dl-.alpha.-Tocopherol
18.	27.600	281790	1.43	Ergost-5-EN-3-OL
19.	27.929	803161	4.07	Stigmasterol
20.	28.055	744498	3.77	1-Heptacosanol
21.	28.576	1940478	9.83	Gamma.-Sitosterol
22.	29.097	240447	1.22	Olean-12-EN-3-One
23.	29.669	104904	0.53	3-Hydroxy-7-Isopropenyl-1,4A-Dimethyl-2,3,4,4A,5,6,7,8-Octahydro-2-Naphthalenyl Acetate
		19736642	100.00	

The fatty acids are well known active metabolites. They serve as an important energetic substrate for the cells. Linolenic acid is essential for maintenance of growth and α-linolenic acid for neural functions. Both acids were shown to be potent cycloxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis inhibitors (Ringbom *et al.*, 2001). Pain-relieving activity of a plant may be due to the anti-inflammatory effect of stigmasterol (Garcia *et al.,* 1999; Gomez *et al.*, 1999). Some of main constituents identified in study were reported to have antibacterial property. Therefore, antibacterial constituents from *I. viscosa* methanol 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

Akihisa, T., Ogihara, J. Kato, J. Yasukawa, K. Ukiya, M. Yamanouchi, S. and Oishi, K. (2001). Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase. *Lipids,* **36**: 507-512.

Bakasso, S.A., Lamien-Meda, C.E., Lamien, M., Kiendrebeogo, J., Millogo, A.G., Ouedraogo, and Nacoulma, O.G. (2008). Polyphenol contents and antioxidant activities of five *Indigofera* species (*Fabaceae*) from Burkina Faso.

Garcia, M.D., Saenz, M.T., Gomez, M.A. and Fernandez, M.A. (1999). Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. *Phytother. Res.,* **13** (1): 78- 80.

Gomez, M.A., Saenz, M.T., Garcia, M.D. and Fernandez, M.A. (1999). Study of the topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation models. *Z Naturforsch,* **54** (11): 937-941.

Herbone, J.B. (1928). *Phytochemical methods*. Chapman and Hall, LONDON, NEW YORK, 2nd Ed.

Judd, W.S., Campbell, C.S., Kellogg, E.A. and Stevens, P.F**.** (1999). *Plant systematics: a phylogenetic approach*. Sinauer Associates, Sunderland 464pp.

Kasahara, Y., Kumaki, K., Katagiri, S., Yasukawa, K., Yamanouchi, S., Takido, M., Akihisa, T. and Tamuta, T. (1994). Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytotherapy Res.*, **68**: 327-331.

Kusamba, Chifundera (2001).Contribution to the inventory of medicinal plants from the Bushi area South Kivu Province Democratic Republic of Congo. *Fitoterapia,* **72**: 351-368.

Polhill, R.M., Raven, P.H. and Stirton, C.H. (1981). Evolution and systematics of the Leguminosae, pp. 1-26. *In*: R. M. Polhill & P. H. Raven (eds.), *Advances in legume systematics* Part 1, Royal Botanic Gardens, Kew 425.

Raicht, R., Cohen, B. and Fazzini, E. (1980). Protective effect of plant sterols against chemically induced colon tumours in rats. *Cancer Res.*, **40**: 403-405.

Ringbom, T., Huss, U., Stenholm, A., Flock, S., Skatteboel Perera, P. and Bohlin, L**.** (2001). COX-2-inhibitory effects on naturally occurring and modified fatty acids. *J. Nat. Prod*., **64**: 745-749.

Yasukawa, K. Takido, M. Matsumoto, T. Takeuchi, M. and Nakagawa, S. (1991). 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. *J. Oncol.,* **41**: 72-76.

****
*****