Journal of Advanced Chemical Sciences 1 (2015) 6–8

ISSN: 2394-5311

Contents List available at JACS Directory

Journal of Advanced Chemical Sciences

journal homepage: www.jacsdirectory.com

GC-MS Analysis of the Methanol Extract of *Indigofera aspalathoides* Vahl ex DC

E. Rajabudeen[1], A. Saravana Ganthi[2,*], M. Padma Sorna Subramanian[3], K. Natarajan[4]

[1] *Dr. Zahir Husain College, Ilayankudi, TN, India.*
[2] *Rani Anna Govt. College for Women, Tirunelveli, TN, India.*
[3] *Siddha Medicinal Plants Garden, CCRS, Mettur Dam, TN, India.*
[4] *Annai Arts and Science College for Women, Karur, TN, India.*

ARTICLE DETAILS

Article history:
Received 5 December 2014
Accepted 8 December 2014
Available online 10 December 2014

Keywords:
Gas Chromatography (GC)
Mass Spectroscopy (MS)
Indigofera aspalathoides

ABSTRACT

Medicinal plants are the plants rich in secondary metabolites like alkaloids, glycosides, coumarins, flavonoids, steroids etc. are used as potential sources of drugs. Standardization of herbal drug has done through the five main steps. They are organoleptic study, botanical study, physical analysis, chemical analysis and biological study. Chemical studies include qualitative, quantitative analysis, chromatographic study and spectroscopy studies. Gas Chromatography (GC) and Mass Spectroscopy (MS) have become important technique for secondary metabolite profiling in plants. *Indigofera aspalathoides* Vahl ex DC widely used in traditional medicines has tremendous medicinal potential owing to its biological functions. The methanol extract possesses hepatoprotective activity. Whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases. The GC-MS analysis has shown the presence of different phytochemical compounds in the methanolic extract of *Indigofera aspalathoides*. Over 23 compounds were identified. Sitosterol and stigmasterol were the most abundant of sterols identified in the sterol fraction.

1. 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. The *Fabaceae* family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families [1, 2]. Many plants of this family have been used in traditional systems of medicine. Still, several potent pants of *Fabaceae* are unexplored which deserve attention and research. *Indigofera aspalathoides* Vahl ex DC. 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 [3]. The plant *Indigofera aspalathoides* Vahl ex DC. is commonly known as 'Shivanarvembu' in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoots are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous affections [4]. The leaves are also applied to abscesses. The whole plant is used in edematous tumors and the ashes are used to treat dandruff [5]. The methanol extract of *Indigofera aspalathoides* also possesses hepatoprotective activity [6]. The stem is traditionally used for various skin disorders and cancers [7]. Whole plant powder or potion is used for joint pains [8]. Powdered barks mixed with coconut oil are applied on the affected parts cautiously for six months to cure leprosy [9]. The ash of the whole plant is added with coconut oil and applied topically to treat psoriasis [10]. The whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases [11]. Shivanarvembu Kuli Tailam is prescribed for chronic weeping eczema in children, frequently occurring scalp on lower limbs, insect bites, leprosy, chronic ulcers and boils [12, 13]. The objective of the present work was to identify the biologically active compounds in *Indigofera aspalathoides* Vahl ex DC.

2. Materials and Methods

2.1 Plant material

The medicinal plant *Indigofera aspalathoides* Vahl ex DC. was collected from Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen No: 7010 available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai.

2.2 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.

2.3 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

*Corresponding Author. *Ph.:* +91 9442908041
Email Address : saran_gan@rediffmail.com (A. Saravana Ganthi)

Cite this Article as: E. Rajabudeen, A. Saravana Ganthi, M. Padma Sorna Subramanian, K. Natarajan, GC-MS analysis of the methanol extract of *Indigofera aspalathoides* Vahl ex Dc, J. Adv. Chem. Sci. 1 (1) (2015) 6–8.

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.

2.4 Compound Identification

Components of the methanolic 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).

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

Peak #	R. Time (min)	Area	Area %	Name
1.	14.184	7865384	7.47	Mome Inositol
2.	17.002	3597098	3.42	Hexadecanoic acid
3.	17.308	475591	0.45	Hexadecanoic acid, Ethyl ester
4.	17.365	172872	0.16	n-Heneicosane
5.	18.696	1923656	1.83	Oleic Acid
6.	18.804	465075	0.44	Cis-(-)-2, 4a,5,6,9a-Hexahydro-3, 5,5,9-tetramethyl(1H)benzocycloheptene
7.	18.902	452170	0.43	Octadecanoic acid
8.	19.325	216688	0.21	Valerenol
9.	19.660	9031244	8.58	Labda-8(20), 13(16), 14-triene
10.	19.704	1723797	1.64	Kauran-16-OL
11.	19.842	1080715	1.03	Kaur-16-ENE
12.	20.187	447493	0.43	Isopimaradien-3-one
13.	20.346	1435377	1.36	Labda-8(20), 12,14-triene
14.	20.444	310976	0.30	14-Isopropyl-3,7,11-Trimethyl-1,3,6,10-Cyclotetradecatetraene
15.	20.860	1323649	1.26	Kaur-16-EN-19-OL
16.	21.081	14192035	13.49	Kaur-16-EN-18-OIC Acid
17.	21.458	1068364	1.02	2-Methoxy-[4-(2-pyridin-4-yl-ethylimino)-methyl]-phenol
18.	21.517	1430814	1.36	7-Cyano-4a, 7-dimethyl-8-(2-oxoethyl) tetradecahydro-2-phenanthrenyl acetate
19.	21.567	5578437	5.30	Kaur-16-EN-18-OIC ACID
20.	22.124	1820567	1.73	5-(4-Hydroxy-3-Methoxy-Benzyl)-1-(4-Methoxy-Phenyl)-Pyrimidine-2,4,6-Trione
21.	22.477	631515	0.60	9-Methoxy-6A,11A-Dihydro-6H-[1] Benzofuro [3,2-C] Chromen-3-OL
22.	22.713	1501472	1.43	Acridin-9-yl-(4-methoxy-phenyl)-amine
23.	22.820	433031	0.41	Dehydroisoandrosterone acetate
24.	22.882	1164697	1.11	Kauren-19-YL-Acetate
25.	23.105	926032	0.88	3-(2,4-Dimethoxyphenyl)-7-chromanol
26.	23.233	866618	0.82	Nonadecane
27.	23.376	2459340	2.34	4-[2-(3,4-Dimethoxy-Phenyl)-Ethylamino]1-OXA-Spiro[4,5]DEC-3-EN-2-ONE
28.	23.568	610417	0.58	5,7-Dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxy-2,3-dihydro-4H-chromen-4-one
29.	23.634	765930	0.73	2-(3,4-Dimethoxyphenyl)-3,7-dihydroxy-4H-chromen-4-one
30.	23.707	1157169	1.10	5,7-Dihydroxy-2-(3,4,5-trimethoxyphenyl)-4-chromanone
31.	24.204	4381436	4.16	3-(2-Hydroxy-3,4-Dimethoxyphenyl)-7-Chromanol
32.	24.258	2402381	2.28	1-Benzyl-7-methoxy-1,2,3,4-tetrahydro-6-isoquinolinol
33.	24.480	27352537	25.99	5,7-Dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxy-2,3-dihydro-4H-chromen-4-one
34.	24.557	1354625	1.29	2-(4-Methoxy-2,6-dimethylphenyl)-3-methyl-2H-benzo[g] indazole
35.	24.647	766119	0.73	Hexatriacontane
36.	24.820	405235	0.39	5,7-Dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxy-2,3-dihydro-4H-chromen-4-one
37.	27.930	1872680	1.78	Stigmasterol
38.	29.102	1564763	1.49	Norolean-12-ENE

3. Results and Discussion

Thirty eight constituents were identified in *Indigofera aspalathoide*s. The chemical profiles of the extract, the amount (%) of the individual components, and gas chromatographic and mass spectral data are summarized in Table 1. GC/MS chromatogram of *I. aspalathoides* methanol extract gives two prominent peaks (Fig. 1) with retention time 13.49 and 25.99 indicating the presence of two compounds such as Kaur-16-en-18-oic acid and 5,7-Dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxy-2,3-dihydro-4H-4-chromen-4-one. The mass spectrum of two compounds Kaur-16-en-18-oic acid and 5, 7-Dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxy-2, 3-dihydro-4H-4-chromen-4-one is presented in the Fig. 2 and Fig. 3. These confirmed with complied data from known compounds. Earlier report on this plant also revealed the presence of many compounds that are biologically active such as salicylic acid, -sitosterol-D-glucopyroside and erythroxydiols 'X' and 'Y' [14].

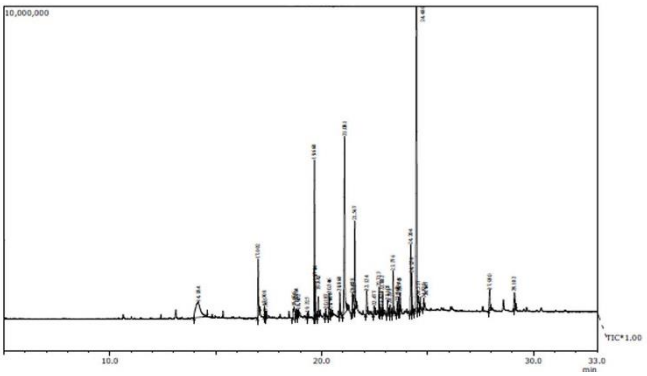
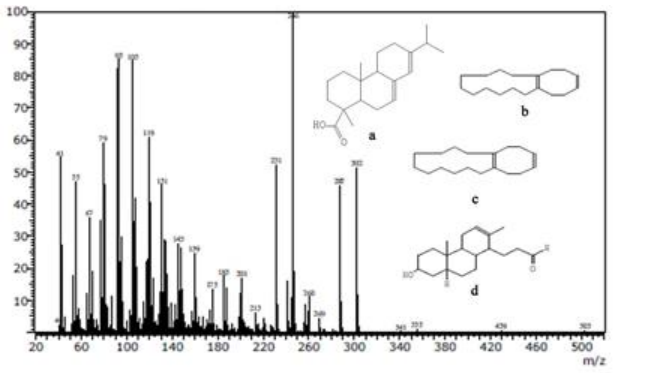
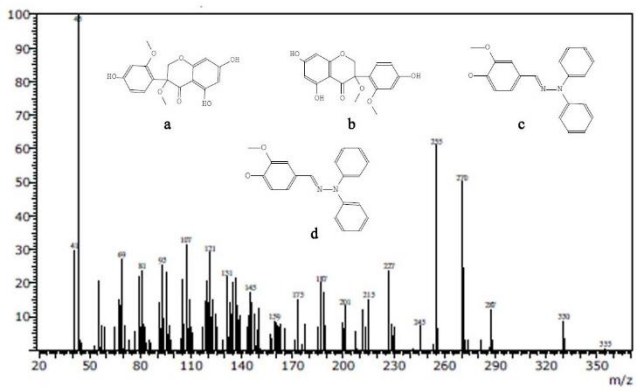


Fig. 1 Chromatogram for *Indigofera aspalathoides*



a. Abietic acid, b. Bicyclo[10.6.0]octadeca-1(12),15-diene
c. 1,2,5,6,7,8,9,10,11,12,13,14,15,16-Tetradecahydrocyloocta[A]cyclododecene
d. 3-(7-Hydroxy-2,4b-dimethyl-1,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1-yl)-propionaldehyde

Fig. 2 Mass spectrum for *Indigofera aspalathoides*



a. 4h-1-Benzopyran-4-One, 2,3-Dihydro-5,7-Dihydroxy-3-(4-Hydroxy-2-Methoxyphenyl)
b. 4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-3-(4-hydroxy-2-methoxyphenyl)-3-methoxyphenyl
c. 3,4-Dimethoxybenzaldehyde Diphenylhydrazone
d. Benzaldehyde, 3,4-Dimethoxy-, Diphenylhydrazone

Fig. 3 Mass spectrum for *Indigofera aspalathoides*

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 [15]. Stigmasterol was found to markedly inhibit tumor promotion in two-stage carcinogenesis in mice [16, 17] and to exhibit significant inhibitory effect on HIV reverse transcriptase [18]. A mixture of stigmasterol and sitosterol was shown to possess anti-inflammatory activity after topical application [19]. 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* [16] and to inhibit carcinogenesis [20].

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 [21]. Pain-relieving activity of a plant may be due to the anti-inflammatory effect of stigmasterol [22, 19]. Some of main constituents identified in study are reported to have antibacterial property. Therefore, antibacterial constituents from *Indigofera aspalathoide*s 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.

4. Conclusion

The presence of various bio-active compounds detected after GC-MS analysis using the methanolic extract of *I. aspalathoide*s justifies the use of whole plant for various elements by traditional practitioner. This type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

Acknowledgement

The authors are thankful to the Principal, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu for providing the required facilities for completion of the work.

References

[1] R.M. Polhill, P.H. Raven, C.H. Stirton, Evolution and systematics of the Leguminosae in: R.M. Polhill, P.H. Raven (Eds.), Advances in legume systematics Part 1, Royal Botanic Gardens, Kew, 1981, p. 425.
[2] W.S. Judd, C.S. Campbell, E.A. Kellogg, P.F. Stevens, Plant Systematics: a phylogenetic approach. Sinauer Associates, Sunderland, 1999, p. 464.
[3] S.A. Bakasso, C.E. Lamien-Meda, M. Lamien, J. Kiendrebeogo, A.G. Millogo, O.G. Ouedraogo Nacoulma. Polyphenol contents and antioxidant activities of five *Indigofera* species (*Fabaceae*) from Burkina Faso, Pak. J Biol. Sci. 11 (2008) 1429–1435.
[4] K.R. Kirtikar, B.D. Basu, Glossary of Indian Medicinal plants, Vol. I, M/s Periodical Experts, New Delhi, 1975, p. 338.
[5] The Wealth of India. A Dictionary of Indian Raw Materials and industrial Products, Vol. V, H-K Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi, India, 1959, p. 176.
[6] M. Gupta, U.K. Mazumder, P.K. Haldar, C.K. Kandar, L. Manikandan, G.P. Senthil, Hepatoprotective activity of *Indigofera aspalathoides* against carbon tetrachloride induced liver damage in rats, OPEM (2004) 100–103.
[7] B. Rajkapoor, B. Jeyakar, N. Murukash, Antitumor activity of *Indigofera aspalathoides* on *Ehrilich ascites* carcinoma in mice, Indian J Pharmacology 38(1) (2004) 38–40.
[8] C.S. Murugasa Mudaliyar, Gunapadam Mooligai Vaguppu, IVth Ed., Sivakami Vilas Publications, Chennai, 1988.
[9] G.M. Jeeva, S. Jeeva, C. Kingston, Traditional treatment of skin diseases in South Trivancore, south peninsular India, Indian J Traditional Knowledge 6(3) (2007) 498–501.
[10] P. Revathi, T. Parimelazhagan, Traditional Knowledge on Medicinal Plants used by the Irula Tribe of Hasanur Hills, Erode District, Tamil Nadu, India, Ethnobotanical Leaflets 14 (2010) 136–160.
[11] S.N. Yoganarasimhan, *Indigofera aspalathoides*: Medicinal plants of India, Interline Publishing Private Ltd., Bangalore India, 2000, p. 284.
[12] Anonymous, Govt. Siddha Formulary Part – I, published by Ministry of Health and Family Welfare, Govt. of New Delhi, 1992, pp. 189–192.
[13] Anonymous, Bharathathin Siddha Maruntukal Ceymuraikkurippu Nool Part. I, published by Ministry of Health and Family Welfare, Govt. of New Delhi, 1984, p. 221.
[14] A. Brindha, A. Saraswathy, R. Vijayalakshmi. Pharmacognostic studies on 'Sivanarvembu' – A Siddha drug, B.M.E.B.R. XXV (1-4) (1997) 70–85.
[15] J.B. Harborne, Phytochemical methods: A guide to modern technique of plant analysis, Champman and Hall, London, 1998.
[16] K. Yasukawa, M. Takido, T. Matsumoto, M. Takeuchi, S. Nakagawa. 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 Oncology 41 (1991) 72–76.
[17] Y. Kasahara, K. Kumaki, S. Katagiri, K. Yasukawa, S. Yamanouchi, M. Takido, T. Akihisa, C. Tamuta, Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis, Phytotherapy Res. 68 (1994) 327–331.
[18] T. Akihisa, J. Ogihara, J. Kato, K. Yasukawa, M. Ukiya, S. Yamanouchi, K. Oishi, Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase, Lipids 36 (2001) 507–512.
[19] M.A. Gomez, M.T. Saenz, M.D. Garcia, MA. Fernandez, Study of the topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation models, Z Naturforsch. 54(11) (1999) 937–941.
[20] R. Raicht, B. Cohen, E. Fazzini, Protective effect of plant sterols against chemically induced colon tumours in rats, Cancer Res. 40 (1980) 403–405.
[21] T. Ringbom, U. Huss, A. Stenholm, S. Flock, P. Skatteboel Perera, L. Bohlin, COX-2-inhibitory effects on naturally occurring and modified fatty acids, J Nat. Prod. 64 (2001) 745–749.
[22] M.D. Garcia, M.T. Saenz, M.A. Gomez, M.A. Fernandez, Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models, Phytother. Res. 13(1) (1999) 78–80.