OPEN ACCESS

Botanical report

ISSN 2319 - 8230 (Print)

Article ID : br151213101

2013: Volume No. 2(2): 1-6.

HPLC analysis of Phenolic compounds of *Indigofera aspalathoids* D.C

***G.Raju, N. Subash and M. Maridass**

Received: 01 November 2013 / Accepted: 30 November 2013 / Published Online: 15 December 2013

Abstract

Aim of this paper was study on HPLC fingerprints of flavonoids identification of medicinal herbs of leaf and stem of *Indigofera aspalathoids* DC. The HPLC profiles of chemical constituents of *I. aspalathoids* were 40^{o}C temperature on a C18 column using eluted with acetonitrile- water (25:1) and methanol as mobile phases in a linear gradient elution with the flow rate of 1mL/min^{-1}. The elution of the flavonoids compounds were identified by comparison of retention time and authentic samples. The results of HPLC finger print analysis of flavonoids contains gallic acid, caffeic acid, rutin, quercetin and ferulic acid were performed in leaf and stem of *Indigofera aspalathoids* DC. The total phenolic compound of stem was higher than leaves of *I. aspalathoids* statistically significant.

Keywords: *Indigofera aspalathoides* DC.,stem, leaves, HPLC fingerprinting, phenolic compounds, flavonoids

Citation

Raju,G.,Subash,N. and Maridass,M.2013. HPLC analysis of Phenolic compounds of *Indigofera aspalathoids* D.C. *Botanical Report*,2(2):1-6.

Present Address
G. Raju, N. Subash and M. Maridass
Department of Zoology,
Pioneer Kumaraswamy College
Nagercoil-629003,South India

To whom Corresponding Author

E-mail to: rajumaran@yahoo.co.in

Manuscript Type : **Research Article**
Received Manuscript : **Via Email**
Approved Letter : **Received**
Funding Source: UGC, New Delhi
Conflict of Interest : **Nil**
Manuscript Full Responses: **Authors**

Submission manuscripts info:
botanicalreport@yahoo.com

Article ID : br151213101 2013: Volume No. 2(2): 1-6.

1. Introduction

There is an increase in the research areas of newly development and prevention of diseases especially the role of flavonoids and phenolic acids as antioxidants moreover flavonoids and phenolic acids components play important roles in the control of different human diseases (Saxena *et al*.,2012). Several plant and plants containing phenolic compounds have been used treatment for certain diseases such as cancer, daiabetic and wound healing. However, pre-clinical studies on several plants for assessing the pharmacognostical, phytochemical, toxic and biological properties of any herbal drug are very essential before its clinical administration. In particular, the qualitative and quantitative analysis of the phenolic compounds found in many plant parts following systematic scientific methodology and its comparison with standard phenolic compounds is very important for establishing its efficacy (Mradu *et al*,2012).

Indigofera aspalathoides DC*.,* is a small herb, which is belongs to the family Leguminosae. They were growing in throughout the region of South India. Vernacular name of *I. aspalathoides* DC*.,* well known as *Shivanar vembu in South India*. The whole plants of *I. aspalathoides* DC has been used for cooling, demulcent and odematous tumors, leprosy, cancer and various skin disorders (Kirtikar and Basu,1975; Wealth of India,2001). Earlier report on *n*-butyl ester of nanodecanoic acid, 1-octadecanol, 4-heneicosanone, *α*-amyrin, *n*-octacosanol, *β*-sitosterol, salicylic acid, erthroxydiol X, erythroxydiol Y and *β*-sitosterol-3,*β*-D-glucopyranoside were isolated from different plants parts of *I. aspalathoides* (Rosy *et al*., 2010; Saraswathy *et al*., 2013). Hepatoprotective activity of stem and roots were reported in this plant (Gupta *et al*., 2004; Claime *et al*., 2012). The present study is established in HPLC fingerprint identification of flavonoid constituents in leaves and stem of *I. aspalathoides.*

2. Materials and Methods

2.1 Plant materials

The plant materials of *Indigofera aspalathoides* DC were collected from Thenkalam, Tirunelveli District, Tamil Nadu and authentication plants were identified by third author.

2.2 Extraction of plant materials

Air dried of plant materials of *Indigofera aspalathoides* DC were powdered and extracted with 95% ethanol, and then concentrated to dryness under reduced pressure. Yield of extract was qualitative and quantitative analyzed by HPLC method.

2.3 Total phenolic compounds

The total phenolic compounds was determined by according to Sadasivam *et al*.,(1996) method.

2.4 HPLC analysis

The chromatographic analyses were performed by system of HPLC Shimadzu CLASS-VP V6.14 SP2 model. The separation flavonoids constituents were developed on C18 column by gradient elution with Acetic Acid-Water (25:1) and methanol as mobile phase at a flow rate of 0.1 mL/min, the detection wavelength at 280nm and column temperature at 40^{o}C. The HPLC fingerprint chromatogram of *I.aspalathoides* DC was produced 5 peaks identified in flavonoid compounds. These compounds were identified by comparing retention time and reference compounds.

2.5 Data Analysis

Results of all data were regression analyzed by SPSS-11.5 Version.

3. Results and Discussion

The results of total phenolic compound are present in the ethanolic extract of *I. aspalathoides* were shown in Table-1.

Table-1: Total phenol content of leaf and stem of *I. aspalathoides*

Plant parts	Result (mg / 100gm)
leaf	845
stem	139

Peaks of the flavonoids constituents 1-5 were assigned by HPLC chromatograms and compared by individual peak and retention times with those of authentic reference compounds (Fig.1 and 2). Leaves and stem of *I. aspalathoides* were HPLC fingerprinting compounds of

Article ID : br151213101 2013: Volume No. 2(2): 1-6.

flavonoids shown in table-2 and 3. Fig.3 showed on HPLC fingerprint analysis of flavonoid compounds was comparative analysis of leaf and stem of *I. aspalathoides* statistically significant level at<0.5%. Major flavonoid compound of gallic acid was to be found in stem and ferulic acid was present in leaves of *I. aspalathoides* (Fig.3). The regression analysis of phenolic compounds of stem was higher than leaves of *I. aspalathoides (*Table-4).

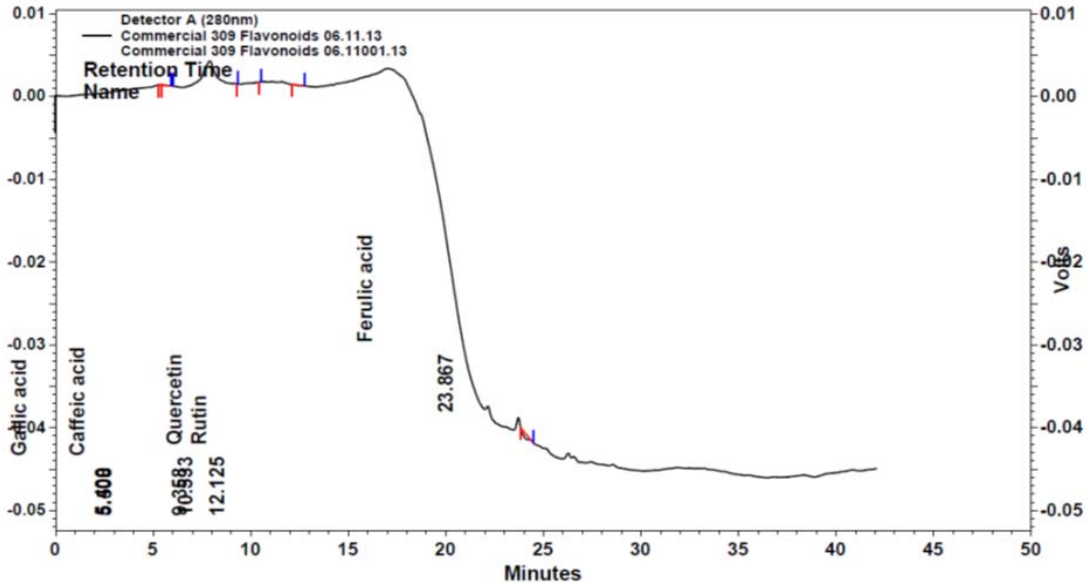

Fig.1: HPLC Chromatogram of leaf of *Indigofera aspalathoides*

Table-2: HPLC Chromatogram of *I. aspalathoides* leaf

Detector A (280nm)				
Retention Time	Area	Height	Concentration (mg/g)	Name*
5.508	96	61	Below Detection Limit	Gallic acid
9.358	115	44	Below Detection Limit	Caffeic acid
10.533	327	89	Below Detection Limit	Rutin
12.125	1786	0	0.001	Quercetin
23.867	12657	0	0.008	Ferulic acid

Earlier studies on flavonoid constituents were exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties (Balasundram *et al*.,2006; Wang *et al*.,2012; Gill *et al*.,2011). These activities of flavonoids compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol and catechin (Balasundram *et al*.,2006; Wang *et al*.,2012; Gill *et al*.,2011).

Article ID : br151213101 2013: Volume No. 2(2): 1-6.

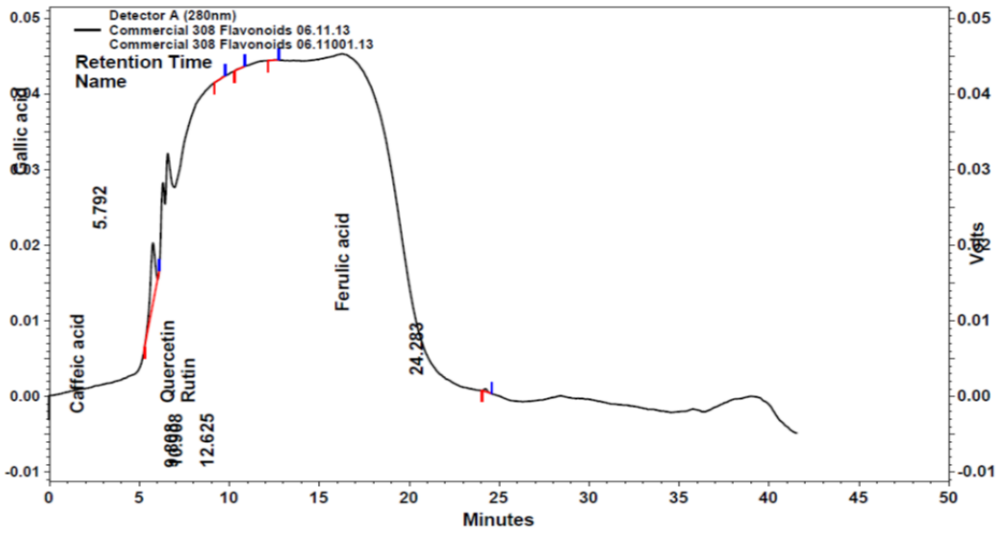

Fig.2: HPLC Chromatogram of stem of *I. aspalathoides*

Table-3: HPLC Chromatogram of *I. aspalathoides* stem

Detector A (280nm)				
Retention Time	Area	Height	Concentration (mg/g)	Name*
5.808	110231	5838	0.019	Gallic acid
9.867	7883	14	0.005	Caffeic acid
10.742	261	7	Below Detection Limit	Rutin
12.700	578	41	Below Detection Limit	Quercetin
23.808	1661	0	0.001	Ferulic acid

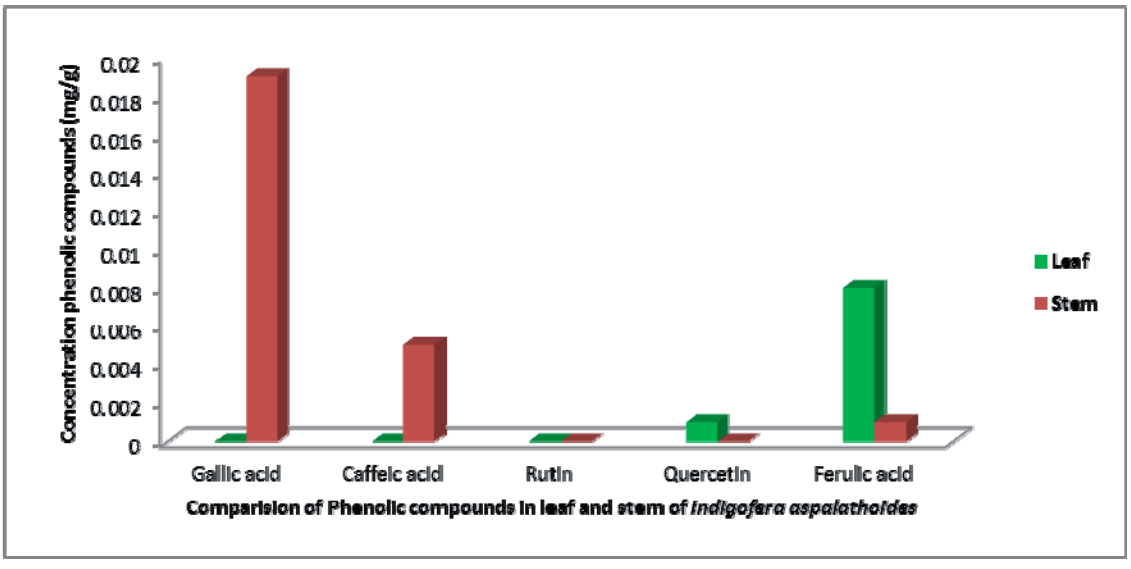

Fig.3: Comparative analysis phenolic compounds in leaf and stem of *Indigofera aspalathoides*

Article ID : br151213101 2013: Volume No. 2(2): 1-6.

Table-4: Regression analysis of phenolic compounds in leaves and stem of *Indigofera aspalathoides*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.327(a)	.107	-.191	.00381

a Predictors: (Constant), stem

ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.000	1	.000	.360	.591(a)
	Residual	.000	3	.000		
	Total	.000	4			

a Predictors: (Constant), STEM; b Dependent Variable: LEAF

Coefficients (a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.003	.002		1.210	.313
	stem	-.141	.235	-.327	-.600	.591

a Dependent Variable: LEAF

In the present study was HPLC fingerprint of flavonoids constituents was successfully identified in gallic acid in stem and ferulic acid in leaves represent the characteristic chemo markers of this plant. The development of HPLC fingerprint method is preferred for qualitative and quantitative estimation of therapeutically active constituents of medicinal herbs of *I. aspalathoides*.

4. Acknowledgements

The authors are grateful to the University Grants Commission, New Delhi for financing assistance of this work.

5. References

Saxena,M., Jyoti Saxena, and Alka Pradhan,2012. Flavonoids and phenolic acids as antioxidants in plants and human health. *Int. J. Pharm. Sci. Rev. Res*., 16 (2):130-134.

Mradu, G., Saumyakanti,S., Sohini,M. and Arup,M.2012. HPLC Profiles of standard phenolic compounds present in medicinal plants. *J. Pharmacog. Phytoche. Res*., 4(3): 162-167.

Kirtikar, K.R. and Basu, B.D.1975. Glossary of Indian Medicinal plants; New Delhi: Periodical Experts,Vol. 1:338.

Article ID : br151213101 2013: Volume No. 2(2): 1-6.

The Wealth of India, 2001. A Dictionary of Indian Raw Materials and industrial Products, Raw Materials; Council of Scientific and Industrial Research, New Delhi, Vol. 5: 176.

Rosy, B.A., Henry Joseph and Rosalie,2010. Phytochemical, pharmacognostical, antimicrobial activity of *Indigofera aspalathoids* vahl. (Fabaceae). *Int. J. Biol. Tech*., 1(1):12-15.

Saraswathy, A., Mathuram, V. and Allirani, T. 2013. Chemical constituents of *Indigofera aspalathoides* Vahl. Ex.DC*. J. Pharmacog. and Phytochem*., 2 (2):74-80.

Gupta, M., Mazumder, U.K., Haldar, P.K., Manikandan, L., Senthilkumar, G.P. and Kander, C.C.2004. Hepatoprotective activity of *Indigofera aspalathoides* against carbon tetrachloride induced liver damage in rats. *Orient. Pharm. Exp. Med*.,4:100-3.

Claimer, C.S., Mahesh, A., Sinilal, B., Rao, D.M. and Thangadurai, D. 2012. Protective Effect of *Indigofera aspalathoides* roots on N-Nitrosodiethylamine-induced Hepatocarcinogenesis in Mice. *Ind. J. Pharmaceutical Sciences*,74(2): 157-160.

Sadasivam, S. and Manickam A.1996 Biochemical methods, 2nd edition. New Age International Publishers,pp.116-117.

Wang, M., Li, K., Nie, Y., Wei, Y. and Li, X.2012. Antirheumatoid arthritis Activities and chemical compositions of phenolic compounds-rich fraction from *Urtica atrichocaulis*, an endemic plant to China. *Evidence-Based Complementary and Alternative Medicine,* doi:10.1155/2012/818230.

Balasundram, N., Sundram, K. and Samman, S.2006. Analytical, nutritional and clinical phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry,* 99(1): 191-203.

Gill, N.S., Arora, R. and Kumar, S.R.2011. Evaluation of antioxidant, anti-inflammatory and analgesic potential of the *Luffa acutangula* Roxb. Var. amara. *Res. J. Phytochem*., 5: 201-208.