J. Indian bot. Soc. Vol. 90 (1&2) 2011 : 97-102

PHYTOCHEMICAL INVESTIGATIONS ON *INDIGOFERA CORDIFOLIA* HYENE EX ROTH.

S. P. GUDADHE*, V. S. DHORAN, V. N. NATHAR

Department of Botany
Sant Gadge Baba Amravati University, Amravati.
*Author for corresspondance**
E-mail: smitagudadhe@rediffmail.com

Indigofera cordifolia Hyene ex Roth.a dicotyledonous plant. It is a member of fabaceae family of herbs, shrubs and trees with a great variety of habitat, distributed in temperate and tropical areas. *Indigofera* is a large genus consisting of over 800 species all over the world (Hutchinson, 1964). In India about 60 species and 10 varieties of *Indigofera* are found (Hajra *et al*., 1995).*Indigofera* has important medicinal uses. It is used as forage for animals due to high crude protein and calcium content.

The present paper deals with the phytochemical screening of therapeutic importance from *Indigofera cordifolia* Hyene ex Roth. This study involves the preliminary screening, quantitative analysis, qualitative thin layer chromatographic separation of amino acids from leaves and seeds of *Indigofera cordifolia* and IR analysis for the study of functional groups.

Key Words: *Indigofera cordifolia*, IR analysis, Phytochemical screening.

During the last century, most agronomic research was aimed to increase the yield of food and fiber (Abelson 1994) plants However, during the last decades more attention is being focused on the production of new and alternative crops and their by-products for industrial and pharmaceutical use Medicinal plants are of great importance to the health of individuals and communities (Hill 1988). The demand of medicinal plants is steadily increasing in both developing and developed countries due to the growing recognition of drugs based on natural products, food supplements and flavours. Being non-narcotic, having no side effects, these products sometimes are the only source of health care available to the poor Medicinal plants have traditionally occupied an important position in the social, cultural, spiritual and medicinal area of rural and tribal lives in India (Ramawat 2004). India owns a rich biodiversity of such natural products and foods, although few studies have been conducted to document the nutritive value of less familiar foods (Rajalakshmi and Geervani 1990, Mohan and Janardhan 1995) but information available is quite meager. Hence, in an attempt to explore some such untapped sources of good nutrition, the present investigation has been undertaken in *Indigofera cordifolia* Hyene ex Roth. with a potential to contribute towards food and nutrition security of the masses.

Indigofera cordifolia Hyene ex Roth. is a creeping, prostrate annual herb of the family Fabaceae distributed throughout the tropical and subtropical regions of the world (Dalwitz, 1980). Generally, *Indigofera cordifolia* is used as forage for animals due to the high crude protein content and good palatability (Nath *et al*. 1971). Due to these potentialities this plant is very valuable cover crop spread in semi-arid and arid areas (Hassen *et al*. 2006). The seeds are nutritionally important.

In this study the proximate compositions, phytochemical analysis, IR analysis for the functional chemical groups are carried out.

Received on April 19, 2010 Accepted on February 4, 2011

HABITAT

FLOWER

FRUITS

MATERIAL AND METHODS

Indigofera cordifolia was collected from the campus of Sant Gadge Baba Amravati University, Amravati in the month of September and is deposited in the form of specimen for herbarium.

Different parts of *Indigofera cordifolia* were evaluated for their proximal composition. Lowry's method was used for estimation of crude protein content. Total carbohydrate content was estimated by Anthrone method. Nitrogen estimation was done by the Micro-Kjeldahl method of Oser (1976). Dry matter and moisture content was determined by the method described by Sadashivam and Manikam (2005). Total oil content was estimated by Soxhlet method. Ash content was determined by the method described in AOAC (1984). TLC was done to detect the amino acids present (Thimmaiah 1999).

For phytochemical analysis the plant material was shade dried, powdered and extracted with different solvents such as Petroleum ether, Benzene, Chloroform, Acetone, Ethanol. The different plant parts such as leaves, stems and seeds were used for the extraction. Phytochemical screening of extracts in various solvents was carried out as per standard methods (Raman 2006, Trease and Evans 1987 and Harborne 1973, Yadav and Yadav 2001). Response to varios tests was denoted by +, ++, +++, indicating weak, moderate and strong reactions respectively. IR

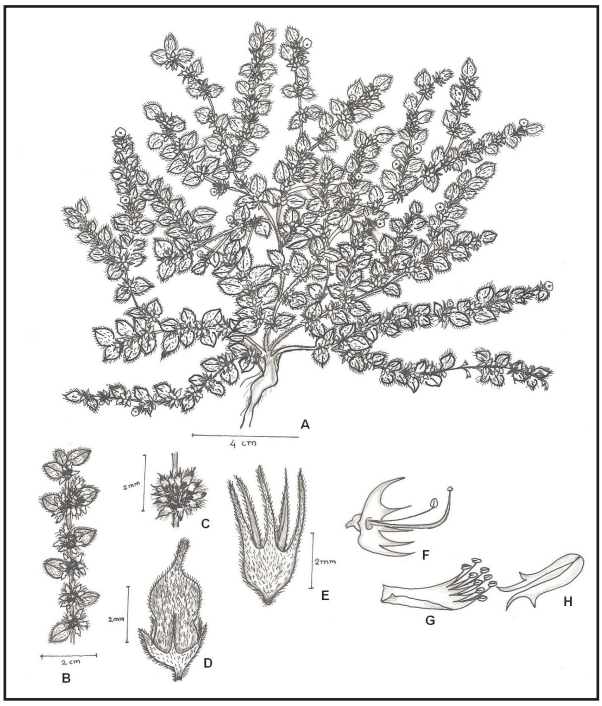


Figure.1:- A-H: *Indigofera cordifolia* ; A. Habit, B. Inflorescence, C. Arrangement of fruits on the disk, D. Single fruit, E. Calyx, F-H. Floral parts

Table-1: Amino Acids in *Indigofera cordifolia* plant parts.

Sr. No.	Rf Values			
	Leaves	Colour	Seeds	Colour
1.	0.59	Pink	0.48	Pink
2.	0.43	Yellow	0.41	Yellow
3.	0.39	Violet	0.33	Pink
4.	0 28	Pink	0.25	Purple
5.	-	-	0.20	Dark Pink
6.	-	-	0.16	Violet

spectra analysis of leaf extract was performed to identify the active chemical compounds present. It is carried out in KBr pellets on Perkin Elmer spectrophotometer in the ranges of 4000-5000 cm^{-1} for characterization of different chemical groups.

RESULT AND DISCUSSION

The data of qualitative separation of amino acids in leaves and seeds of *Indigofera cordifolia* by thin layer chromatograph four types of amino acids in leaves and six in seeds as given in Table-1.These are mostly Serine, Alanine, Proline, Valine and Lysine, Arginine, Aspartic acid, Threonine, Proline, Tyrosine, respectively when compared with standards.

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. In Table-2 the proximate analysis shows that the seeds contain 32.6% of protein and 36.7% carbohydrate and leaves contain 22.4% of protein and 24.8% of carbohydrate which revealed that the seeds and leaves of *Indigofera cordifolia* are good source of protein and carbohydrate. Due to this, consumers are more benefited when they use this plant as a forage crops for animals and the seeds when mixed

Contents	Plant Parts		
	Stem	Leaves	Seeds
Proteins	8.7 %	22.4 %	32.6 %
Carbohydrates	10.11 %	24.8 %	36.7 %
Oil content	0.9 %	1.2 %	2.9 %
Dry matter content	26.9 %	30.3 %	23.1 %
Moisture content	11.4 %	12.3 %	9.2 %
Ash content	3.2 %	3.4 %	2.4 %
Nitrogen content	1.98 %	2.62 %	3.85 %

Table- 2: Proximal compositions of various plant parts of *Indigofera cordifolia.*

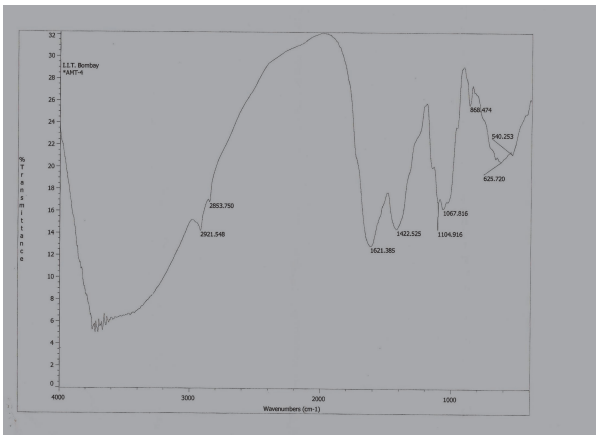
with Jawar and Bajra seeds for various food preparations. Church (1901) reported that the seeds of *I. cordifolia Heyne ex Roth.* when eaten unmixed, edema and death may occur. The minute seed is noted to be highly nitrogenous, but somewhat unpleasant in taste. It grows with grass at the commencement of the monsoon and is ripe in the month of November. When the moisture content of the plant material is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% (African Pharmacopoeia, 1986). The various parts of *Indigofera cordifolia* does not show great variation in moisture content. Therefore no microbes can attack this plant easily. The high ash content i.e. 3.4% and 3.2% indicates that the *Indigofera cordifolia* leaves and stem are good source of inorganic minerals. The percentage of dry matter content is more in leaves i.e. 30.33%. This study confirms that the leaves are not good source of oil. Therefore, it will not enhance the use of many important structural, genetic and metabolic compounds in plant cells play major role in making possible many of the biochemical reactions in plants. The seeds of *Indigofera cordifolia* are good source of nitrogen and that is approximately 3.85% of nitrogen, which is the higher concentration.

Table-3: Qualitative Analysis of Secondary metabolites of various extracts from various parts of *Indigofera cordifolia.*

S. No	Phytochemical Tests	Reagents	Petroleum Ether			Benzene			Chloroform			Ethanol			Acetone		
			S1	L	S2	S1	L	S2	S1	L	S2	S1	L	S2	S1	L	S2
1)	Alkaloids	Mayer's	-	+++	++	+	++		-	++	+	+++	-	-	-	+	+
		Wagner's	-	+++	+	-	+	+	-	+	-	+	+++	+	-	-	+
		Hager's	+	++	-	++	++	+	+	+	-	-	+	+	+	++	+
		Dragendorff's	+	+++	+	-	+++	+	+	++	-	-	+++	-	+	+++	-
2)	Saponins	Foam test	+	+++	-	-	+++	+	++	-	+	+	++	++	-	+++	++
3)	Tannins & Flavonoids	Ferric Chloride Gelatin	++	+++	+	++	+++		-	+++	-	++	+++	+	++	++	+
			++	+++	+	+	++		-	++	+	+	+++	+	+	+++	-
		Lead Acetate	-	++	+	+	-		+	-	+	-	++	-	-	+	-
		Alkaline	+	+	++	-	+	++	+	++	+	-	-	-	-	++	-
		Magnesium and Hydrochloric acid tests	++	++	-	-	++	+	-	+	-	+	+++	+	-	-	+

[Where as **S1** = Stem, **L** = Leaves, **S2**=Seeds, **(-)** absent, (+) weak, (++) moderate, (+++) strong]

The result of phytochemical screening is presented in Table-3. This reveals the weak, moderate, and strong concentration of alkaloids, flavonoids, saponins and tannins, which could make the plant useful for treating different ailments and having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound(s). Comparatively Petroleum Ether, Benzene and Ethanol extract of the leaves showed higher concentration of alkaloids when tested by the reagents. Saponins posses



IR spectrum *Indigofera cordifolia* leaves

hypocholesterolemic and antidiabetic properties (Rupasinghe *et al.* 2003) and also responsible for many other important activities anthelmentic, antiulcerogentic, antioxidant, anticancer, immunomodulatory, antimalerial and antibacterial and hepatoprotective (Shi *et al.* 2004). The foam test of saponins gave the positive and strong result mainly in leaves of *Indigofera cordifolia*. Tannins have been reported to have several pharmacological activities such as spasmolytic activity in smooth muscle cells (Tona *et al.* 1999). The analysis of tannins and flavonoids done by ferric chloride, gelatin test showed strong presence in leaves and moderately by alkaline reagent and magnesium and hydrochloric acid reduction test. The Petroleum Ether extract, Benzene extract comparatively gave strong positive test for alkaloids, saponins, tannins and flavonoids. In comparison leaves are best source of phenolic compounds. Infrared (IR) absorption spectroscopy is the measurement of wavelength and intensity of the absorption of mid infrared light by a sample. Mid infrared light (2.50μm 4000-2000cm^{-1}) is energetic

Table-4: Observations for IR-Spectrum:AMT -4

Sr. No.	Absorption observed (cm^{-1})	Assignment	Absorption expected (cm^{-1})
1.	2921.54	Carboxylic acid	3400-2400
2.	2853.75	-OH-stretching	3400-2400
3.	1621.38	Aromatic ketonic stretching	1670-1600
4.	1422.52	-CH3 Bending	1450-1375
5.	1104.91	C= N bonding	1350-1000
6.	1067.81	C-N-C grouping	1200-1000
7.	868.47	Aromatic Stretching (out of plane)	900-690
8.	625.72	C-Cl stretching	785-540
9.	540.25	C-Br stretching	600-500

Whereas AMT-4 is *Indigofera cordifolia* leaves

enough to excite molecular vibrations to higher energy levels. Plant powder clearly shows 2921.54 cm^{-1} stretching of COOH group, 2853.75cm^{-1}stretching of OH group, 1621.38cm^{-1} stretching due to the presence of aromatic ketonic >C=C< group. Stretching at 1422.52 cm-1 due to bending of CH_3 group which enhances the potency of drug, -C=N amino group stretching at 1104.91cm^{-1}.The spectrum also showed 1067.81 cm^{-1} peaks due to C-N-C grouping in the molecule, peak at 868.47 cm^{-1} shows aromatic streleting is due to presence of C-Cl stretching . The spectrum also shows C-Br stretching at 540.25 cm^{-1} .The wave lengths of IR-bands are characteristics of specific types of chemical bonds. Phenolic group is generally present in analgesic drugs and possesses antidiabetic property as our spectrum shows the phenolic group. Thus the plant powder of *Indigofera cordifolia* Hyene ex Roth. contain analgesic and antidiabetic property. CH_3 group is electron donating group which is responsible to increase the potency in drug action.

Hence, for the first time the data is generated on the plant *Indigofera cordifolia* which have provided a basis for its wide use as therapeutic agent for treating various ailments. As it contains various secondary metabolites in different parts of plant, this has helped to go for quantitative analysis of secondary metabolits and protein in seeds on *Indigofera cordifolia* by Gel Electrophoresis.

The authors are indebted to IIT, Pawai, Mumbai for providing the IR facilities and Department of Botany, Sant Gadge Baba Amravati University Amravati for laboratory facilities.

REFERENCES

Abelson PH 1994 Continuing evolution of U. S. *Agron Sci* 264:1383

African Pharmacopoeia 1986 General methods for analysis 1st Ed. Vol II (OAU/STRC) Lagos 123.

AOAC 1984 Official Methods of Analysis 14th Ed. Association of Official Analytical Chemistry, Washington DC, USA.

Church AH 1901 Food grains of India with supplement: Chapman and Hall. London, England.

Dallwitz MJ 1980 A general system for coding taxonomic description. *Taxon* **29** 41-164.

Hajra PK, Sashtry ARK & Sanjappa M 1995 Fascicles of Flora of India Fascicle **21** *Botanical Survey of India.*

Harborne JB 1973 Phytochemical methods A Guide to modern technique of plant analysis, Chapman and Hall, London, New York.

Hassen A Rethman NFG & Apostolides 2006 Morphological and agronomic characterization of *Indigofera* species using Multivariate analysis Tropical **40** 45-59.

Hill AF 1988 A Text Book of Useful plants and plant Products, TATA McGraw-Hill Publishing Company Ltd, New Delhi.

Hutchinson J 1964 The genera of flowering plants (Angiospermae), Oxford University Press, Amen House, London, 1:400

Mohan VR & Janardhanan K 1995 Chemical determination of nutritional and antinutritional properties in tribal pulses. *J. food. Sci. Technol*. **32** 465-469.

Nath K, Malik NS & Singh ON 1971 Chemical composition and nutritive value of *Indigofera enneaphylla* and *I. cordifolia* as sheep feeds, *Australian Journal of Experimental Agriculture and Animal Husbandry*, **11 (49)**178-185.

Oser BL 1976 Hawks Physiological Chemistry. 14th Edn. Tata Mc Graw Hill Publishing Co. Ltd. New York.

Rajlakshmi P & Geeravani P 1990 Studies on tribal foods of South India: Effects of processing methods on the vitamins and *in vitro* protein digestibility of cereals/millets and legumes. *J. Food Sci. Technol.* **27** 260-263.

Raman N 2006 Phytochemical Techniques, New India Publishing Agency, New Delhi.

Ramawat KG 2004 Biotechnology of Medicinal Plants Vitalizer and Therapactic New Delhi.

Rupasinghe, H P, Jackson C J, Poysa V, Di Berado, Bewley J D & Jenkinson J 2003. Soyasapogenol A and B distribution in Soybean (Glycine Max L.Merr)in relation to seed physiology, genetic variability and growing location. *J. Agric. Food Chem.,* **51** 5888- 5894

Sadasivam S & Manickam A 2005 Biochemical Methods, IInd Ed. New Age International Limited Publishers, New Delhi.

Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G & Jiang Y. Guelph 2004 Saponins from edible legumes: chemistry, processing, and health benefits. *J Med Food.* **7 (1)** 67-78.

Thimmaiah SR 1999 Standard Methods of Biochemical Analysis, Kalyani Publishers, New Delhi.

Tona L, Kambu K, Mesia K, Cimanga K, Apers S, Bruyne T, Pieters L, Totte J. & Vlietinck AJ 1999 Biological screening of traditional preparations from some medinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*, **6** 59-66.

Trease GE & Evans WC 1987 Pharmacognosy 13th Ed. Brialliar Tiridel Can. Macmillian Publishers.

Yadav VK & Yadav N 2001 Biochemistry and Biotechnology. A Laboratory Manual. Pointer Publishers, India.