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Phytochemical Screening of Bioactive Constituents in *Indigofera cassioides* Rottle. DC.

KEYWORDS: Phytochemical screening, Indigofera cassioides Rottle.DC. TLC and IR analysis.

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ABSTRACT *Indigofera L. is a dicotyledonous plant, a member of Fabaceae family and has high medicinal uses. As there are large number of species each found its use in different manner depending upon its secondary constituents. The species, Indigofera cassioides Rottlt. DC. is a shrub with many important uses because of its secondary constituents and used as a vegetable by trible people and forage for animals.*

The present work deals with the phytochemical screening of medicinally important bioactive constituents of Indigofera cassioides qualitatively and quantitatively from the different plant parts along with the thin layer chromatography and the IR studies. The study will provide the referential information for use of this plant as a crude drug.

Introduction
Medicinal plants are the rich source of therapeutic agents, traditional medicines and raw drugs for the prevention of various diseases and ailments. Due to the presence of various phytochemicals, bioactive constituents, secondary metabolites in different parts of the plant give the medicinal as well as neutraceutical properties to the plant. Plants have primary and secondary metabolites as well as mineral nutrient that play a very vital role in growth and developmental process of plants they benefit the human life too. The diversity of structure and uneven distribution of phenolic products between and within different plant families makes it almost certain that a single physiological role can not be ascribed to these compounds. To study such type of bioactive constituents in plants various biochemical analysis techniques refer to a set of methods, assays and procedures that enable scientist to analyze the substances found in living organisms and the chemical reactions underlying life processes.

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. The legume family (Fabaceae) is the third largest family of flowering plants, with approximately 650 genera and nearly 20,000 species (Doyle, 1994). The economic importance of Indigofera species dates back since neoplithic times (Burkill, 1995). Other new products would include new food sources but the majority would provide industrial products and pharmaceutical products such as, fiber, pulp, vegetable and dyes from Indigofera L.

Indigofera cassioides Rottle D. C. is annual shrub which grows to a height of 3.5 m with wide distribution, mostly found in India ranges from East Asia to Himalayas. Branches are erect, angular, glabrous. The flower of this genus is largest, in axillary, pink or pinkish purple, dense racemes. The pods are spreading or descending, linear, subcylindric. The seeds are ovoid, dark brown and smooth. The species shows great variations in size of plant parts. The branches are used for fences and fuel. The flowers are occasionally eaten as a vegetable (Kunkel, 1984). A decoction of the roots is used in the treatment of coughs. The root is dried, ground in to powder and applied externally in the treatment of pains in the chest (Chopra et al, 1996).

Hence, in an attempt to explore some such untrapped sources of good nutritional and medicinal property, the present work deals with the phytochemical screening of bioactive constituents in various plant parts of Indigofera cassioides Rottle. DC. TLC to study the amino acids important for the proximal analysis and IR analysis for the study of functional groups.

Materials and Methods
Collection of Plant Material
I. cassioides Rottle DC. was collected from the slopes of hill at Chikhaldara, Amravati District. The plants were collected during November to December and were brought to the laboratory and Herbarium specimens were also prepared.

Proximal analysis of Plant parts
Different parts of Indigofera cassioides 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).

Thin Layer chromatography
TLC was done to detect the amino acids present in the leaves extract and seed extract of the plant (Thimmaiah, 1999).

Phytochemical Screening:
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). Response to various tests was denoted by positive signs (**+, ++, +++**) indicating weak, moderate and strong reactions respectively.

IR analysis:
IR 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.

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Observations


Fig-1: Habit


Fig-2: Inflorescence


Fig-3: Fruit


Fig-4: Seed

Table- 1: Amino Acids in Indigofera cassioides plant parts

Sr. No.	Rf Values			
	Leaves	Colour	Seeds	Colour
1.	0.39	Violet	0.21	Purple pink
2.	0.41	Yellow	0.29	Violet
3.	0.44	Pink	0.37	Purple
4.	0.64	Pink	0.46	Yellow
5.	-	-	0.54	Pink

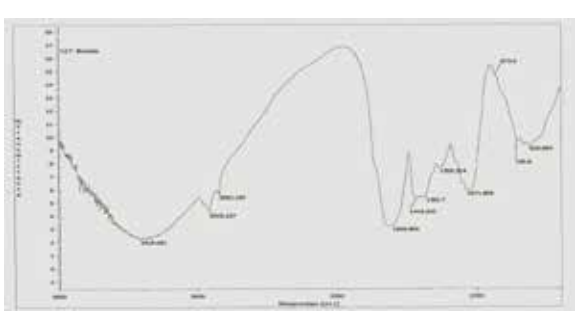
Table- 2: Proximal compositions of plant parts of Indigofera cassioides

Contents (%)	Plant Parts Stem	Leaves	Seeds
Proteins	7.9	12.3	27.9
Carbohydrates	8.8	23.2	39.2
Oil content	0.5	1.1	3.3
Dry matter content	42.9	43.2	37.9
Moisture content	52.9	57.5	27.2
Ash content	3.4	3.8	3.0
Nitrogen content	2.3	3.6	3.8

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

S. N0	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 and Flavonoids	Ferric Chloride	-	++		++	+++	+		+++	++	+++	++	+	-	++	-
		Gelatin	-	+++	-	-	-	-	++	+++	++	-	+++	++	-	+++	-
		Lead Acetate	-	+++	++	+++	+++	-	-	++	+	+++	+++	++	-	++	++
		Alkaline	++	-	-	+++	+++	-	-	++	+	-	-	-	++	+++	-
		Magnesium and Hydrochloric acid tests	+++	+++	-	-	+++	+	+++	+++	++	++	+++	++	-	+++	+

[Whereas S1-Stem, L-Leaves, S2-Seeds, (-) absent, (+) weak, (++) moderate, (+++) strong]



IR spectrum of Indigofera cassioides leaves

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Table -4: Observations for IR- Spectrum

Sr. No.	Absorption observed (cm^{-1})	Assignment	Absorption expected (cm^{-1})
1.	3416.08	- OH Phenol (stretching)	3500-3300
2.	2919.10	Carboxylic acid	3400-2400
3.	2850.19	-OH-stretching	3400-2400
4.	1608.90	Aromatic ketonic stretching	1670-1600
5.	1443.24	-CH3 Bending	1450-1375
6.	1382.7	-CH3 Bending	1450-1375
7.	1265.31	Sulphoxide group	1350-1140
8.	1071.80	C-O stretching	1300-1000
9.	873.6	Aromatic Stretching (out of plane)	900-690
10.	720.8	Tri/Disubstituted ring stretching	785-540
11.	620.69	C-Cl stretching	785-540

IR spectroscopic analysis of Indigofera cassioides Rottle DC.

Results and Discussion

The results associated with the various parameters selected for the phyochemical screening of bioactive constituents are tabulated in various tables.

Thin Layer Chromatography

The data of TLC summereised in Table-1. The chromatogram when compared with the standards showed four spot of Alanine (Violet), Proline (Yellow), Tyrosine (Pink), Cystine (Pink) at Rf 0.39, 0.41, 0.44 and 0.64 respectively in leaves and in seeds five spot of Arginine (Purple pink), Glutamic acid (Violet), Alanine (Purple), Proline (Yellow) and Valine (Pink) at Rf 0.21, 0.29, 0.37, 0.46 and 0.54 respectively which indicates that the plant have adequate level of essential amino acids which can provide information about their suitability as field crops and to examine the possible deleterious constituents.

Proximal Composition Analysis

The essential life nutrients are proteins, fat and carbohydrates. They contribute to caloric content of the dietary, minerals including trace elements, vitamins and water. In the proximal analysis of Indigofera cassioides it is found that the seeds are rich in proteins (27.9%), carbohydrates (39.2%) and oil (3.3%) than other parts. The quality and quantity of protein in the seed are basic factors important in the selection of plants for nutritive value, systematic classification and plant improvement programs (Siddique, 1998). The seeds and leaves of Indigofera cassioides have high percentage of nitrogen i.e. 3.8% and 3.6% respectively. The dry matter and moisture content is higher in leaves and stems. The high percentage of ash content in the leaves (3.8%) and stems (3.4%) confirms that are good source of inorganic minerals.

Phytochemical Screening

The phytochemical screening of all the parts of Indigofera cassioides is shown in Table-3 which reveals the weak, moderate and strong concentration of alkaloids, flavonoids, saponins and tannins. The number of positive signs indicates the intensity of reactions that reflects the quantity present. Comparatively Petroleum ether, Chloroform and Acetone extract gave maximum positive test when tested with different reagents for the presence of these phytoconstituents. Leaves extract of Indigofera cassioides showed the maximum intensity for the presence of alkaloids, saponins, tannins and flavonoids.

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Hence the leaves extract of Indigofera cassioides can be a good source and helpful in maintaining health and combating human diseases.

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just et al., 1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al., 2000 and Okwu, 2004). Saponin was found to be present in Indigofera cassioides extracts and can be supported the usefulness of this plant in managing inflammation and other foaming products preparations.

Plants that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). Tannins are secondary metabolites responsible for antimicrobial properties in various plants (Chung, 1998). Hence the presence of tannins in Indigofera cassioides can support the traditional medicinal use of this plant in the treatment of different ailments and also exhibited antimicrobial properties.

Flavonoids, another constituent of plant extracts a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997). The presence of these phenolic compounds in this plant contributed to their antioxidative properties and thus the usefulness of these plants in herbal medicament.

Infrared (IR) absorption spectroscopy

Infrared (IR) absorption spectroscopy is the measurement of wavelength and intensity of the absorption of mid infrared light by a sample. Mid infrared light (2.50um, 4000-2000cm^{-1}) is energetic enough to excite molecular vibrations to higher energy levels. Plant powder clearly shows –OH phenolic stretching at 3416.08 cm^{-1}, 2919.10 cm^{-1} peak due to stretching of –COOH group, 2850.19 cm^{-1} stretching due to the presence of –OH phenolic group, 1608.90 cm^{-1} stretching due to presence aromatic ketonic >C=C< group, stretching at 1443.24 cm-1 and 1382.7 cm^{-1} due to bending of $–CH_3$ group which enhances the potency of drug. The spectrum also showed the peak 1265.31 cm^{-1} stretching shows sulpoxide group, the peak at 1071.80 cm^{-1} due to C-O stretching, peak at 873.6 cm^{-1} shows aromatic stretching and peak at 720.8 cm^{-1} is due to the presence of di/trisubstituted cyclic ring. The spectrum also showed C-Cl stretching at 620.69 cm^{-1} (Table-4).

Thus the preliminary phytochemical tests of Indigofera cassioides are helpful in finding such phytoconstituents in the plant material that may helpful in traditional medicinal practices which form an integral part of complementary or alternative medicine. As rich source of primary, secondary bioactive constituents, phenolic groups, the plant can be a potential source of useful drugs.

Conclusion

The results obtained by the present analysis of Indigofera cassioides confirmed the presence of the bioactive phytochemicals which contribute medicinal as well as physiological properties to the plants. Therefore, extracts from these plants could be a good source for useful drugs and used as a famine food.

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