Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

PHARMACOGNOSTIC STUDIES OF *INDIGOFERA HIRSUTA* L.

A. SUVARNALATHA, N. YASODAMMA, C. ALEKHYA, D. CHAITHRA.

Dept. of Botany, Sri Venkateswara University, Tiurpati.-517501

Email: yasodanpalli@gmail.com

Received: 20 Dec 2013 Revised and Accepted: 02 Apr 2014

ABSTRACT

Objective: *I.hirsuta* leaf juice has been given as a drug to improve infant immunity by the local herbalists paved a way for the pharmacognostical screening of the crude drug in a systematic way to prove its therapeutic activity and also to Quantify and Qualify the drug for better uses in the formulations and to check for its adulterants.

Methods: All physico chemical studies were carried out by the standard methods of Anonymous, Khandelwal, Trease, Kokate and Johanson, and the results were given tables and figures.

Results: *I.hirsuta* an under shrub; plant parts covered with hirsute hairs with bright brick red flowers; Main phytoconstituents are lignins, mucilages, starches, alkaloids, tannins, saponins, suberins, fats / oils and sugars are localized in xylary elements. Total ash, acid insoluble ash and water soluble ash were high in all parts. Extractive values are high in aqueous, alcohol, methanol and chloroform solvents in all parts.

Conclusion: *I. hirsuta* pharmacognostic studies proved its high quantities of phytoconstituents in all parts in relation to its herbal uses than other *Indigofera* species.

Keywords: Infant immunity, Pharmacognostical screening, Adulterants, Phytoconstituents, *Indigofera* species.

INTRODUCTION

Indigofera hirsuta (Fabaceae) is commonly known as hairy indigo has several medicinal uses. In Africa and in Kenya, it is used as chest medicine and in Tanganyika whole plant is prepared as an external application for back ache [1]. Whole plant extract is used in case of injury to the eye ball and inflammation of eye lids; root decoction is used in most parts of Nigeria to counteract various poisons. [2]. Leaf is used against infant immunity [3]; urinary complaints [4] decoction of leaf is used in case of stomach problems. [5], used against diarrhea [6]. *I. hirsuta* leaf methanol and ethanol extracts showed effective activity at 100 µg/ml on *E.coli* and *B.subtilis* than *P.aeruginosa* and *S.aureus*, which also supports the presence of flavonoids like rutin, quercetin, kaempferol, luteolin, apigenin, orientin and phenols like protocatecheuic acid, chlorogenic acid, trans-p-coumaric acid, caffeic acid, cis-p-coumaric acid, P-hydroxy benzoic acid, coumarin and cinnamic acid [7].

Fruits of *I. hirsuta* consist of nearly 14 Phyto constituents mainly in aqueous, methanol, alcohol, ethyl acetate and chloroform extracts. Fixed oils are totally absent. Antibacterial activity against selected four bacterial strains was very effective than the control drug *Ampicillin* and the MIC values ranges from 0.019 mg to 0.312 mg. Hence *I. hirsuta* proved its herbal usage against diarrohea, chest and body pains, infant immunity and skin diseases. [8].

Due to the presence of various bioactive constituents like alkaloids, tannins, lignins, phenols, flavonoids, terpenoids and glycosides *I.hirsuta* leaf, fruit alcohol and methanol extracts showed effective anthelmintic activity than the control drug *Albendazole.* [9]. Due to high medicinal significance the crude drug *I.hirsuta* has to be evaluated pharmacognostically for future design of drugs.

MATERIALS AND METHODS

Plant Material Collection and Identification

Plant material *Indigofera hirsuta was* collected from S. V. Agricultural College during the months of August- January, 2010, and was authenticated by Prof. N.Yasodamma the voucher specimen SVUTYIH1293 was preserved in the herbarium Department of Botany, S.V.University, Tirupati as per the standard method [10].

Macroscopic - Morphological studies

The characters like colour, odour, taste, size, texture and shape of leaf, stem, root and fruit were noted.

Microscopic – Anatomical studies

Cross sections and photography was taken and observed the distinguished characters of the tissue systems of the leaf, stem, root and fruit using digital microscope attached with computer system (Olympus Mic –D). Epidermal membranous layers (in fragments) were cleaned with chloral hydrate, mounted and observed under microscope.

Histochemistry: [11]

Hand sections of fresh leaf, stem, root and fruit were stained with a series of histochemical reagents. a) Safranine (1% safranine in 50% alcohol); lignins b) Iodine solution; cellulose c) Ruthenium red; mucilage d) Iodine; starch e) Wagner's reagent; alkaloids e) Dilute Fecl3 solution; tannins f) Millon,s reagent; proteins g) conc. H2SO4; saponins h) Sudan-III; fat/oil globules i) 20% aq.NaOH; sugars j) conc. Hcl; calcium oxalate crystals k) heating ; strong KOH; sulphuric acid.

Physico-Chemical Analysis

For the determination of ash values, leaf, stem, root and fruit powders were sifted through sieve no. 20 and the following tests were performed as per the standard methods [12, 13].

Total ash

About 3 g of each powder was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less

filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated ash

A silica crucible was heated to red for 10 min. and was allowed to cool in desiccators and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800^{o}C ± 25^{o}C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid were added and heated ignited as before and was allowed to cool and weighed.

Swelling index

It is defined as the volume in milliliters occupied by 1g of drug. The drug is treated with 1.0 ml ethanol (96%) and 25 ml water in a graduated cylinder, shaken every 10 minutes for 1 h and allowed to settle. The drugs have mucilage (swell after absorbing plenty of water) as a Phyto-constituent may have different swelling index and therefore, provide the useful information.

Moisture content

Air dried material of 10g was dried in an oven at 105°C. The loss of weight was calculated and values were tabulated.

Foreign matter

100g of the powdered drug is taken and spread out in a thin layer on a slide and observed free from foreign matters like soil, insect parts or animal excreta. They are separated and weighed and the percentage was calculated.

Extractive value determination

Coarsely powdered air-dried material 20 g was placed in a glass stopper conical flask with 200 ml of solvents shaking frequently, and then allowing it to stand for 18 hours.

Filter it rapidly through Whatmann No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to flat- bottom dish and evaporate it on a water bath. Dry at 105^{o}C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in% of air-dried material by the standard method. **[14]**

Fluorescence Analysis

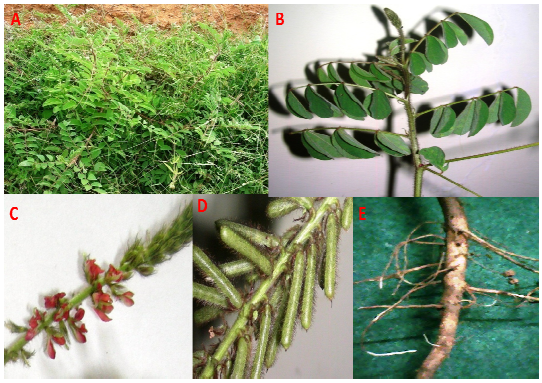
A small quantity of dried and finely powdered leaf, stem, root and fruit were placed on a grease free clean microscopic slide and added 1-2 drops of Conc. Sulphuric acid, 50% Sulphuric acid, Conc. Hydrochloric acid, 50% Hydrochloric acid, Conc. Nitric acid, 50% Nitric acid, 10% Sodium hydroxide, 5% Ferric chloride, 5% Potassium hydroxide, Water and Acetic acid separately and gently tilting the slide waited for 1-2 min.

Then the slide was placed inside the Ultra Violet viewer chamber and viewed in Day light, Short (245nm) and long (360nm) Ultra Violet radiation.The colors observed by application of different reagents in different radiations were recorded. [14]

RESULTS

Macroscopic – Morphological studies: (Plate: 1)

Sub shrub reaches 1m in height, branchlets white – pilose. Leaves: odd pinnate. 15 x 6cm, leaflets 5 pairs (Sub) opposite, elliptic – oblanceolate, 2-3.5x1-1.15cm, chartaceous, densely pilose, base sub acute, margin entire, apex obtuse, apiculate, petiole 3cm; stipules subulate – linear, setose 1cm. Racemes axillary, dense, 2-20cm, peduncle 2 - 1.5cm. Flowers : 5 mm across; calyx tube 0.5mm, hirsute, pubescent pink to brick red, Corolla : standard broadly obovate, 5.5mm, narrow at base, wings 5mm, keels 6mm. Androecium : staminal sheath 4mm. Gynoecium : ovary sessile, 2mm, style 1.5mm. Fruit: straight, tetragonous, 1cm, deflexed, tomentose, mucronate. Seeds: cuboid, 5 angular, 2mm, pitted Flowering: September – February. Fruiting: November onwards.



A: Natural Habitat; B: Leaf; C: Flower; D: Fruit; E: Root

Plate: 1: Morphology

Microscopic Anatomical studies

Leaf Lamina

The lamina is distinctly bifacial. It consists of square or rectangular, wide, thin walled epidermal cells with 15-20 µm wide. The mesophyll is differentiated in to adaxial zone of palisade cells, median level of circular cells and adaxial zone of spongy parenchyma cells. The palisade cells are in two rows; they are narrow and cylindrical and compactly arranged. The median circular cells in one - two rows. The spongy parenchyma cells are three layered; they are lobed and loosely arranged. Midrib-Transverse sections showed a unistratified epidermis with stomata on both sides and usually with adpressed trichomes simple, nonglandular, uniseriate, but having a higher density of stomata on the lower face. Epidermal cells were more or less isodiametrical, polyhedral and randomly arranged. Beneath epidermis on lower side 2-3 layers of collenchyma is present, followed by 2-3 layers of thin walled parenchyma; vascular bundle single collateral and crescent shaped and situated in median part. The vascular bundle consists of xylem elements and small group of phloem elements.

Stem

The thickness of the stem consists of intact epidermis with abundant trichomes (T-shaped), wide heterogeneous cortex, secondary phloem, secondary xylem and wide pith. Epidermis consists of small spindle shaped thick walled cells. The inner epidermis consist of one or two layers of chlorenchyma cells followed by a few layers of parenchyma cells next to the parenchyma zone occurs a discontinuous cylinder of gelatinous fibers of 3 or 4 cells wide. The vascular bundle comprises outer narrow, continuous zone of secondary phloem unsheathing thick secondary xylem cylinder. Secondary phloem elements are arranged in short thin compact rows, secondary xylem includes major portion of thick walled lignified xylem fibers. Vessels occur in thin walled uniseriate radial rows with wide gaps in between the rows.

Root

It consists of fairly wide, superficial, Periderm with wide shallow fissures. Periderm is followed by parenchymatous, thick continuous sclerenchyma cells. Secondary phloem is wide and continuous. Secondary xylem is dense and compact, compressing wide, circular thick walled diffuse mass of vessels and sclerenchymatous ground tissue. The root measures 2-5 mm thick. It has wider and deeply fissured, periderm, discontinuous; radial segments of fibers in cortical region. The xylem cylinder is 1.5 mm thick and consists about nine, fan- shaped radial bands of vessels, and fibers. The radial xylem further cleaved into smaller units by dilated rays; xylem with circular thick walled chain of vessels. The fibers ensheating the vessels are wider and lignified. Lateral part of the xylem bands are small, with gelatinous fibers; 20-60 µm wide.

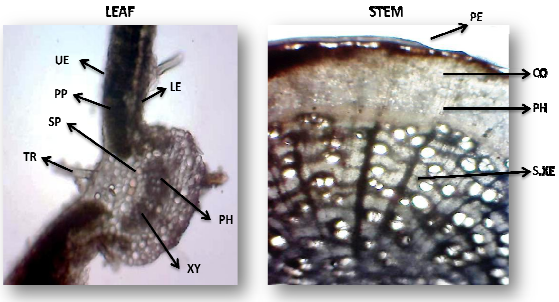
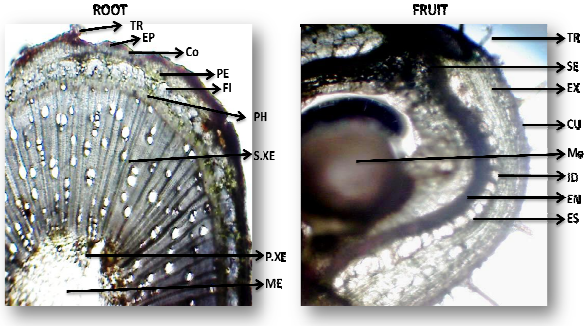


Plate: 2: Plant Anatomy

Fruit

The outer surface of the fruit with reticulate flakes of wax depositions. Simple unicellular biramious trichomes, with lignified walls, spread over the surface. And also simple secretory, trichomes are present on the surface. The exocarp with a thin cuticle, uniseriate, cuboidal cells. The mesocarp was divided into four regions. Outer most hypodermis, 1-2 rows containing large idioblasts and parenchyma cells; collateral vascular bundles not delimitated by fibers, but accompanied by parenchyma cells; fiber caps, ran along the dorsal and ventral sutures of the fruit; four rows of mesocarp mesophyll also with idioblasts scattered throughout. The endocarp consists of lignified fibers outside and several layers of parenchyma cells on inside. Fibers oblique 3-4 rows. Prismatic crystals were observed in the outer fibers. The septum dividing the fruit internally consists of thick walled parenchyma cells forms the inner region of the endocarp. Vascular bundle consists on dorsal and ventral sutures of fruits.



UE: Upper Epidermis; LE: Lower Epidermis; PP: Palisade Parenchyma; SP: Spongy Parenchyma; TR: Trichomes; XE: Xylem; P. XE: Primary Xylem; S.XE: Secondary Xylem; PH: Phloem; PE: Periderm; CO: Cortex; Co: Collenchyma; PE: Parenchyma; ME: Medulla; EX: Exocarp; CU: Cuticle; Me: Mesocarp; ID: Idioblast; EN: Endocarp; ES: Endospermic Seed.

Histochemistry: (Table: 1), (Plate: 3, 4, 5, 6)

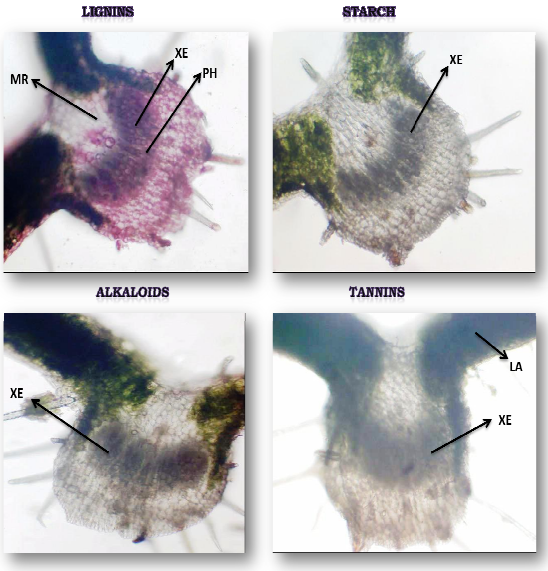
Lignins, alkaloids, tannins and fats/oils are most common phytoconstituents in all parts of the plant. Xylary elements of leaf, epidermis, cortex and vascular elements of stem and root, fruit carpellary layers consists majority of the secondary metabolites.

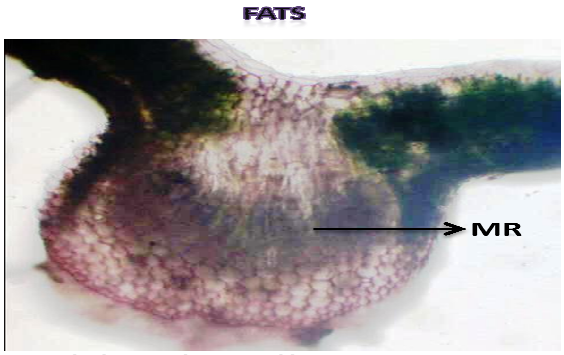
Table 1: Histochemistry

Test for	Reagent used	Nature of change	Leaf	Stem	Fruit	Root
Lignins	Safranine (1%)	Red	Midrib, xylem, phloem	Epidermis, cortex, vascular bundle	Epicarp, mesocarp, endocarp	Rhizodermis, vascular cylinder
Mucilage	Ruthenium Red	Pink	-	Cortex, phloem, medulla	Epicarp, endosperm	Rhizodermis, vascular cylinder
Starch	Iodine	Blue	Xylem	-	-	-
Alkaloids	Wagner's Reagent	Thick Pink	Xylem	Epidermis, cortex, vascular bundles	Epicarp, mesocarp	Cortex, vascular cylinder
Tannins	Dil.$FeCl_3$ solution	Bluish Black	Xylem, lamina	-	Endocarp	Rhizodermis, xylem
Saponins	Conc. H_2SO_4	Light Yellow	-	Cortex, phloem	-	Rhizodermis, phloem
Suberins	KOH+ H_2SO_4	Brown	-	Cortex, phloem	Endosperm	-
Fats/oils	Sudan III	Pink	Midrib	Cortex, vascular bundle	Epicarp	-
Sugars	20% Aq. NaOH	Yellow	-	Xylem, medulla	Epicarp, endosperm	cortex

Table 2: Physico Chemical Analysis: (% w/w)

S. No.	Parameters	Leaf	Stem	Fruit	Root
1	Total ash	40.0	25.3	50.0	41.6
2	Acid insoluble ash	15.0	8.6	22.2	8.0
3	Water soluble ash	15.0	10.0	12.7	19.0
4	Sulphated ash	2.0	1.0	1.5	3.0
5	Moisture Content	40.0	20.0	33.0	36.8
6.	Swelling index	18	8.0	16.0	7.0
7.	Foreign matter	10.0	5.0	15.0	16.0





MR: Mid Rib; **XE:** Xylem; **PH:** Phloem; **LA:** Lamin

Plate: 3: Leaf

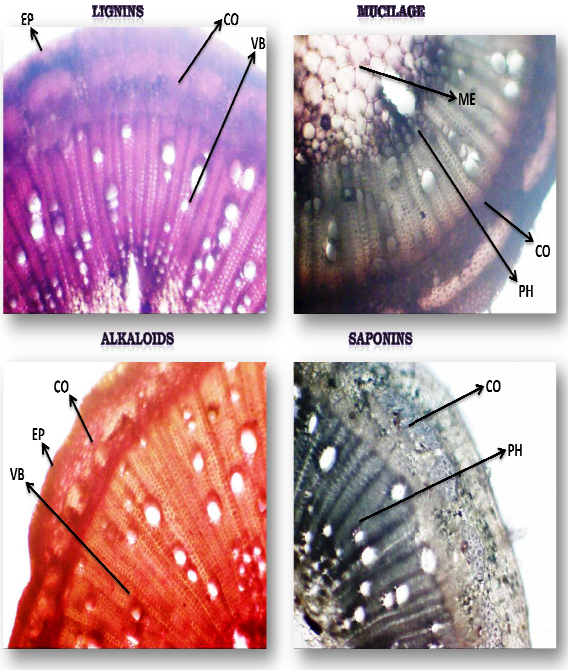
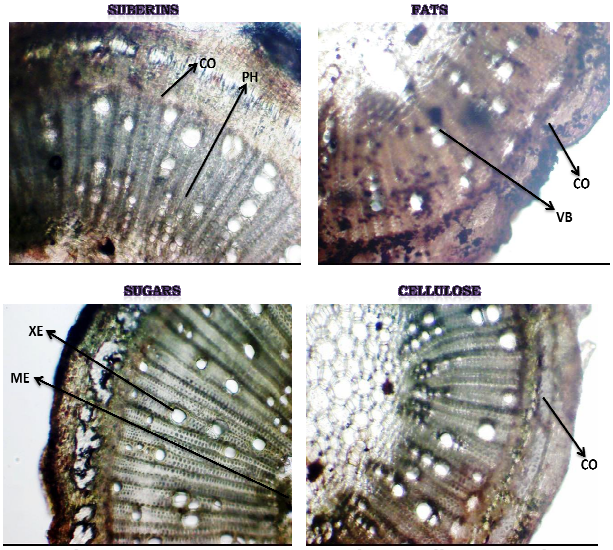
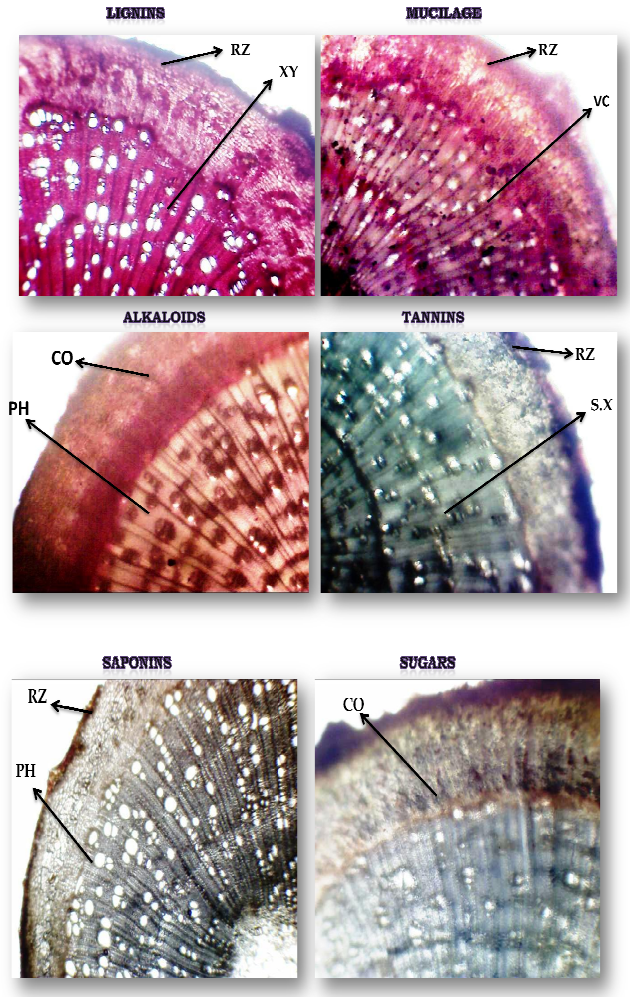


Plate: 4: Stem



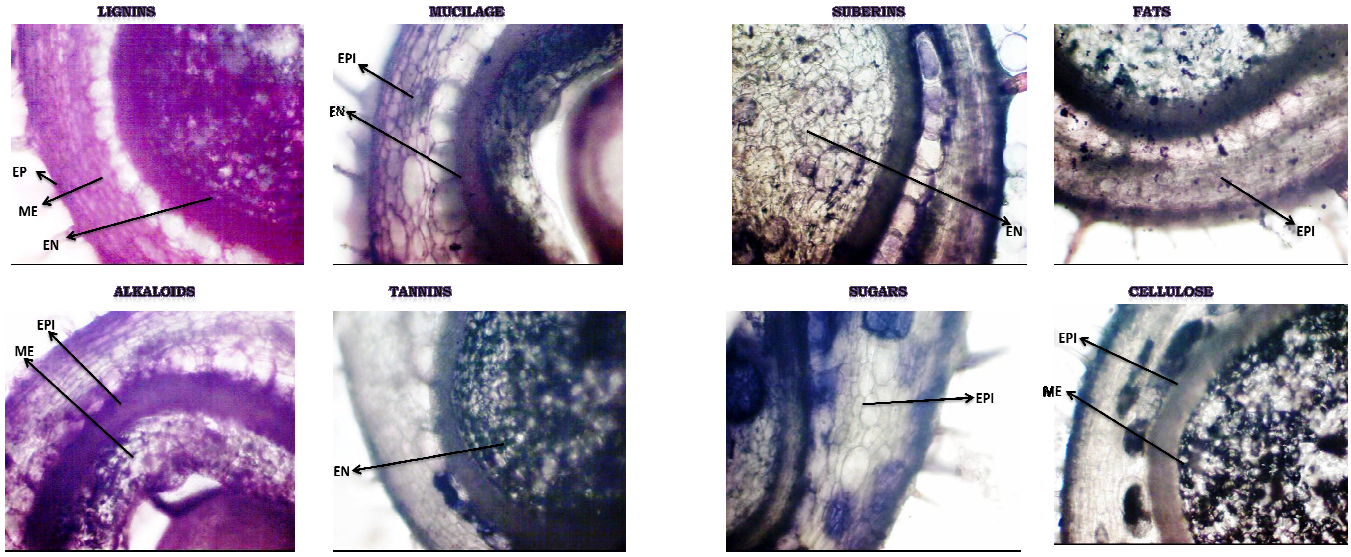
EP: Epidermis; **CO:** Cortex; **VB:** Vascular Bundles; **XE:** Xylem; **PH:** Phloem; **ME:** Medulla

Plate: 5: Root



RZ: Rhizodermis; **CO:** Cortex; **XY:** Xylem; **SX:** Secondary Xylem; **PH:** Phloem

Plate: 6: Fruit



EP: Epidermis; **EPI:** Epicarp; **ME:** Mesocarp; **EN:** Endocarp

Physico Chemical Analysis: (Table: 2)

Total ash content and acid insoluble ash is highest in fruit 50 and 22.2%w/w followed by root, leaf and stem. Water soluble ash and sulphated ash are highest in root 19.0 and 3.0%w/w followed by leaf, fruit and stem. Moisture content is high in leaf followed by root, fruit and stem.

Extractive Values: (Table: 3)

Crude drug extractability was highest in fruit with all solvents followed by leaf, root and stem.

Alcohol extract yielded highest amount followed by aqueous, methanol and chloroform as in fruit 43.7, 40.0, 33.8 and 30.5 mg respectively.

Fluorescence Analysis: (Table: 4)

Powders of all plant parts reflects Bluish green to fluorescent brown under daylight, UV light and fluorescence light when treated with H_2So_4; thick brown to orange red with HCl and HNo_3; Bluish red to fluorescent blue with NaoH, $Fecl_3$, and water treatments.

Table 3: Extractive Values: (mg)

S.No	Parameters	Leaf	Stem	Fruit	Root
1	Aqueous	24.6	13.3	30.0	32.1
2	Methanol	30.0	7.6	33.8	21.5
3	Alcohol	35.6	13.0	43.7	20.6
4	Ethyl acetate	5.0	6.4	2.0	4.0
5	Chloroform	25.0	15.8	30.5	21.2
6	Benzene	3.0	1.2	0.5	0.6
7	Petroleum ether	3.8	2.3	8.0	4.6
8.	Hexane	6.0	3.2	5.0	2.2

Table 4: Fluorescence Analysis

Chemical analysis	Day light				250-270nm				360-390nm			
	L	S	R	F	L	S	R	F	L	S	R	F
Powder as such	L.G	G.W	L.Br	Br	F.G	F.G	F.Br	F.Br	Br	F.W	F.Br	Br
Conc. H_2SO_4	Bl.G	B	Bl.Br	Bl.Br	P	Bu	V	V	R.B	R	F.R	F.Bu
50% H_2SO_4	G	Br.	Br	Bl.Br	T.G	Br	Br.G	V	F.R	O	F.Br	Br
Conc. Hcl	T.G	T.Br	Br	T.Br	Bl	P	V	Bl	Br	F.R	O	R
50% HCl	G	Br	Bl.Br	T.Br	T.G	L.G	F.G	T.G	V	V	F.Bu	R
Conc. HNO_3	O	O	O	T.O	L.G	Y	Y	Y	R	R	R	O
50% HNO_3	B. R	O	O	T.O	L.G	F.G	Y.G	Y	O	R	O	R
10% NaOH	Bl. R	T.Br	T.Br	Bl. R	Bu	Bu	Br.Y	V	F.Bu	F.Bu	F.Bu	Br
5% $FeCl_3$	T.G	G.Y	Y.Gr	B	Bu.G	Y	Bu	Bl	V	F.Bl	R	Bl
5% NaOH	R.G	Br	Br	B.R	Bu	Bu. Y	Y	Bl	F.Bu	Br	O	F.Bu
With Water	L.G	L.Br	L.Br	T.Br	L.G	Bu	Bu	G	F.Br	F.R	O	O
Acetic Acid	T.G	Br	Br	T.Br	T.G	G	Bu	Br	O	O	R	B.R

L: Leaf; **S:** Stem**; R:** Root **F:** Fruit. **LG**: Light Green; **Br:** Brown; **D.G:** Dark Green; **G:** Green; **B. R**: Brick Red; **L. Y:** Light Yellow; **D. Br:** Dark Brown; **Br. G:** Brownish Green**; B. G:** Blackish Green; **T.G:** Thick Green; **O**:Orange; **Bl. R**: Blackish Red; **R.G:** Reddish Green; **G.W**: Greenish White; **B:** Black; **L.Br:** Light Brown; **Bl.Br:** Blackish Brown; **Y.G:**Yellowish Green;**T.O:**Thick orange; **F.G:** Fluorescent Green; **P:**Purple; **Bu.G:** Bluish Green; **Bu:** Blue; **Bu.Y:** Bluish Yellow; **F.Br**: Fluorescent Brown; **V**:Violet; **Br.Y:** Brownish Yellow: **F.R:** Fluorescent Red; **F.Bu**: Fluorescent Blue; **F.W:**Flourescent White

DISCUSSION AND CONCLUSION

Comparative account on Indigofera Species: [15-19]

Plant Name	Parts	*I. aspalathoides*	*I. caerulea*	*I. tinctoria*	*I.hirsuta*
Medicinal uses		Leaves, flowers and tender shoots as cooling and demulcent, leprosy, cancerous affections and abscesses.	Dye yielding plant	Expectorant, anthelmintic, splenomegaly, cephalgia, cardiopathy, hepatoprotective, anticancer, epilepsy, skin diseases	Stomachache, diarrhea, chest and body pains, infant immunity, skin diseases, injury to the eyeball and inflammation of the eyelids. Root decoction antidote to poisons
Macroscopic		Ht-75cm; Leaves-digitately 3or5 foliolate. 4 x 0.5 mm; Petiole absent, corolla-red; Fruit-cylindrical	-	1.2-1.8m ht;7-13 leaflets; 1-2.5cm long leaflet, mucronate tip, flowers red or pinkish color	1m ht; 5 pairs of leaflets; 1-1.5 cm long ; thickly hirsute, flowers pink (or) brick red; fruit- tetragonous
Microscopic	Leaf Stem Root	Folded adaxially, wide circular canals in the midrib and leaf margin, adaxial epidermis consists of papillae cells, calcium oxalate crystals present	-	calcium oxalate crystals rarely present in mesophyll cells.	Calcium oxalate crystals absent; palisade cells in two rows; vascular bundle with secondary tissue periderm, sclerenchymatous ground tissue; Biramous trichomes, idioblasts in hypodermis, endocarp with lignified fibers, prismatic crystals.

Plant Name	Parts	Extractive Values							
		Aq	**H**	**Al**	**M**	**C**	**EA**	**PE**	**B**
I. caerulea	Leaf	-	2.7	-	17.5	3.9	-	-	-
I. tinctoria	Leaf	23.20	6.0	-	28.0	-	-	7.85	-
I.hirsuta	Leaf	24.6	6.0	35.6	30.0	25.0	5.0	3.8	3.0
	Stem	13.3	3.2	35.6	7.6	15.8	6.4	2.3	1.2
	Root	32.1	2.2	20.6	21.5	21.2	4.0	4.6	0.6
	Fruit	40.0	5.0	43.7	33.8	30.5	2.0	8.0	0.5

Aq: Aqueous; **H:** Hexane; **Al:** Alcohol; **M:** Methanol; **C:** Chloroform; **EA:** Ethyl acetate; **PE:** Pet Ether; **B:** Benzene.

Plant Name	Parts	Physico Chemical Analysis				
		Total ash	**Acid insoluble ash**	**Sulphated ash**	**Water soluble ash**	**Moisture Content**
I. aspalathoides	Leaf	18.06	1.13	17.89	1.24	-
I. caerulea	Leaf	5.2	3.0	-	-	4.7
I. tinctoria	Leaf	39.20	0.39	-	23.20	11.80
I.hirsuta	Leaf	40.0	15.0	2.0	15.0	-
	Stem	25.3	10.0	1.0	10.0	-
	Root	41/6	8.0	3.0	19.0	-
	Fruit	50.0	22.2	1.5	12.7	-

I. hirsuta is having many herbal uses than other species. Morphologically similar pink flower with *I. tinctoria*. It is distinct in *hirsuta* hairs covered plant parts. Microscopically calcium oxalate crystals are absent in *I.hirsuta* present in *I. aspalathoides* and in *I. tinctoria*. Pharmacognostic studies of *I. hirsuta* all parts extractive values are high in aqueous and methanol extracts to that of *I. tinctoria*, and also in chloroform and alcohol extracts. Similarly water soluble ash and in total ash contents are very high to that of *I. tinctoria* along with acid insoluble ash in all parts.Hence *I. hirsuta* has been proved its herbal uses having more extractive values, water soluble and total ash content in all parts when compared to other *Indigofera* herbal drugs. This species can be recommended for the isolation of bioactive compounds for their therapeutic activities in curing diarrhoea, skin diseases, eye ball inflammation and to improve infant immunity.

REFERENCES

1. Burkill, JH. A dictionary of Economic Products of Malaysia Peninsular, Ministry of Agriculture, Kuala Lumpur, Malaysia. 1935.
2. Hutchinson J, Dalziel M I, Flora of west tropical Africa. Crown agents for overseas Government and Administration, Mill bank, London. 1958; 533-543.
3. Suvarnalatha, A, Phytochemical studies of three medicinal plants. 2004, M. Phil. Dissertation, Department of Botany, Sri Venkateswara University, Tirupati, India.
4. Gaur. R D, Jyotsana Sharma and Painuli R M, Plants used in traditional healthcare of livestock by Gujjar community of Sub-Himalayan tracts, Uttarakhand, India Indian Journal of Natural Products and Resources ; 2010; 1(2), 243-248
5. Faridah Hanum and Vander Maesen, L.J.G, Plant Resources of South-East Asia 11- Auxiliary plants, LIPI Press, Jakarta, Indonesia 2007.
6. Siva, R. Status of natural dyes and dye-yielding plants in India. Curr. Sci., 2007 92: 916-925.
7. Yasodamma. N Suvarna latha.A Santosh Kumar Mehar and Ch. Paramageetham Phytochemical and antibacterial studies of *Indigofera hirsuta* Linn. J. Trop. Med. Plants, 2009;10. (1), 27 - 29.
8. Suvarnalatha. A, Yasodamma. N, Chaithra. D. Phytochemical and antibacterial studies of *Indigofera hirsuta* L. fruit extracts.

Indo American Journal of Pharmaceutical Research, 2013; 3(2); 4026-4032.

9. Suvarnalatha A, Yasodamma. N, Alekhya. C. Anthelmintic activity of *Indigofera hirsuta* L. leaf and Fruit extracts. Indo American Journal of Pharmaceutical Research, 2013; 3(5).
10. Jain, S.K., Rao, R.R., A Handbook of Field and Herbarium Methods. Today and tomorrow's Printers and Publishers, New Delhi, 1977.
11. Johansen DA. Plant micro technique, New York, USA: McGraw-Hill Book Co. Ltd, 1940.
12. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, Edition 16th, Nirali Prakashan, Pune, 2006.
13. Evans and Trease. Pharmacognosy. Edition-12, Eastbourne, U.K, 1983.
14. Kokate, C.K. Practical Pharmacognosy, 4th edition, VallabhPrakan, New Delhi, 1994.
15. Bojaxa A Rosy, Henry Joseph and Rosalie. Phytochemical, Pharmacognostical, Antimicrobial activity of *Indigofera aspalathoids* Vahl. (Fabaceae). International Journal of Biological Technology, 2010; 1(1); 12-15.
16. Puratchikody, A and Swarnalatha, S. Pharmacognostical, Pharmacological, Investigation on *Indigofera aspalathoides* Vahl: A Review. Journal of Pharmaceutical and Biomedical Sciences. 2011; 5(23); 1-5.
17. Tamilselvi, N. Dhamotharan, R., Krishnamoorthy, P. Sivakumar, S. Anatomical studies of *Indigofera aspalathoids* Vahl. (Fabaceae). Journal of Chemical and Pharmaceutical Research, 2011; 3(2); 738-746.
18. Devarajan Natarajan, Andimuthu Ramachandran, Kesavan Srinivasan, Chokkalingam Mohanasundari. Screening for antibacterial, phytochemical and pharmacognostical properties of *Indigofera caerulea* Roxb. Journal of Medicinal Plants Research. 2010; 4(15), pp. 1561-1565.
19. Saraswathi Motamarri. N, Karthikeyan M, Rajasekar. S, Gopal. V. *Indigofera tinctoria* Linn. A Phytopharmacolgical Review. International Journal of Research in Pharmaceutical and Biomedical Sciences.2011; 3(1); 164-169.