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Full Length Research Paper

Microscopical and physicochemical studies of *Indigofera barberi* (Fabaceae) stem

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The present study mainly focuses on the establishment of pharmacognostical standards of *Indigofera barberi* stem. *I. barberi* is a small diaceous shrub which belongs to the family Fabaceae and is distributed in India. The whole plant is medicinally reported or claimed to cure several diseases in traditional system of medicine folklore in particularl. This study aimed for the pharmacognostic study of the stem has been carried to establish the pharmacognostical standards. The parameters selected were microscopical studies, proximate analysis, fluorescence analysis, behavior of powder drug with different chemical reagents and preliminary phytochemical screening. In physico-chemical evaluation, the ash values and extractive values were studied. Fluorescence analysis performed showed a wide range of fluorescence colours for the crude powder as well as the extracts. Behavior of powder drug with different chemical reagents showed the different colors. The powder of *I. barberi* was successively extracted with petroleum ether, benzene, chloroform, ethylacetate, ethanol and water. In the ethanol was the identification of the best solvent because preliminary phytochemical screening carried out for ethanol extract gave maximum chemical constituents and percentage yield. Phytochemical tests performed identified different chemical constitutions like flavonoids, steroids, cardiac glycosides, phenols and tannins.

Key words: *Indigofera barberi*, proximate analysis, microscopical, fluorescence, ethanolic extract, preliminary phyto chemical screening.

INTRODUCTION

Indian sub-continent is a rich source of plant and animal wealth which is due to its varied geographical and agro-climatic regions. Besides its varied biodiversity, it has a diverse cultural heritage too. Medicinal plants have played an essential role in the development of human culture, for example in religions and different ceremonies. Medicinal plants are resources of new drugs. It is estimated that there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity (Madhavan et al., 2011).

Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world. Many food crops have medicinal effects, though at present Indian health care delivery consists of both traditional and modem systems of medicines, both organized traditional systems of medicine like Ayurveda, Siddha and Unani and unorganized systems folk medicine have been flourishing well. Most of the crude are obtained from plant sources, and parameters like phytochemical analysis,

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pharmacognostic evalution and qualitative analysis are done on them for standardization (Kannan, 2007).

MATERIALS AND METHODS

Collection of plant material and authentication

Plant was collected in the forest regions of Thalakona (Nelakona regions) of Chittoor district, Andhra Pradesh, India in the month of November, 2011. The plant material was taxonomically identified by the taxonomist from Nalgonda. A voucher specimen was certified under Voucher No: NCOP NLG/ph'cog/2010-11/041 which has been preserved in our laboratory for future reference (Madhava Chetty et al., 2008).

Chemicals

All the chemicals and reagents like chloral hydrate, phloroglucinol, glycerin, iodine, petroleum ether, benzene, chloroform, ethyl acetate, ethanol, distilled water and sodium hydroxide used were of laboratory grade and obtained from various other commercial sources.

Equipment

Soxhlet apparatus, rotary vacuum evaporator (Indosati, India), heating mantel (Bio-technics,India), ultra violet (UV) chamber (Secor,India), muffle furnace, silica crucible, stoppered conical flask, microscope were used for this study.

Pharmacognostic study

Transverse section of stem

Dried stems of *Indigofera barberi* were sectioned to obtain a thin transverse section and the micro anatomy was studied.

Preparation of sample for sectioning

This included three simple steps; boiling of the sample, section cutting, mounting and microscopy. Free hand sectioning was done because transverse section (TS) has different parts. A section of the stem was boiled slightly to soften the tissue taken in between the potato. Phloroglucinol, hydrochloric acid and glycerin were used as a stain and mounted on a glass slide and focused under a microscope (Randhawa et al., 2004). The sections were stained with phluroglucinol and hydrochloric acid in the ratio 1:1. Photo micrographs of different magnifications were taken to study the anatomical features (Khandelwal, 2005).

Microscopic Study

Dried stem of *I. barberi* section to obtain a thin transverse section and the micro anatomy was studied (Khandelwal, 2005; Ansari, 2005; Kokate, 2008).

Powder microscopy: Shade dried plant was powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of the plant was subjected to powder microscopy as per standard procedures mentioned (Khandelwal, 2005; Ansari, 2005; Kokate et al., 2009; Iyengar, 1998).

Determination of physico chemical parameters

Total ash, acid insoluble ash, water soluble ash, sulphated ash, crude fiber content, moisture content, foreign organic matter, alcohol soluble extractive value, and water soluble extractive value of stem of *I. barberi* were determined as per standard procedures (Khandelwal, 2005; The Ayurvedic Pharmacopoeia, 2004; Iyengar, 1998; Trease and Evens, 2009; Indian pharmacopoeia, 2007).

Measurement of cell structure and content

The length and width of phloem fibres, calcium oxalate crystals, trichomes and starch grains were measured using stage micrometer and the eye piece micrometer by standard methods (Khandelwal, 2005; Kokate et al., 2009).

UV fluorescence analysis

Powdered whole plant parts of *I. barberi* were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account, that is observation under long UV (365 nm), short UV (256nm) and normal day light (Madhavan et al., 2009). Similarly, extracts were also subjected to UV chamber and fluorescence was observed, and consistency was noted as an additional character for identification (Kalaskar et al., 2010; Sama venkatesh et al., 2008).

Behaviour of the powdered drug with different chemical reagents

Small quantity of the powdered drug sample was taken in a watch glass and mixed with different chemical reagents. The change in the color was observed under short UV, long UV and day light (Madhavan et al., 2009; Kalaskar et al., 2010; Sama venkatesh et al., 2008; Kirtikar et al., 2001).

Preliminary phytochemical screening

The extracts obtained from crude drug include petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water. These extracts were subjected to qualitative test for the identification of various plant constituents. The test which was performed gave a broad idea of the organic chemical constituents (Khandelwal, 2005; Pulokk mukherji, 2000). Initially, 25 gm of crude whole plant powder were taken and packed in a packing paper. This packing was placed in a soxhlet extractor for 24 h (approximately) with different solvents (that is petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water) and temperature was adjusted as per the solvent been used in the extraction. After six successive extractions, the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery (The Ayurvedic Pharmacopoeia., 2004; Suurabh jain et al., 2010; Wen et al., 2010; Bojaxa et al., 2010; Puratchikody et al., 2011).

RESULTS

Transverse section of stem

The transverase section is represented in Figure 1. A thin transverse section of young dicot stem when examined under the microscope showed the following regions from outside to inside.

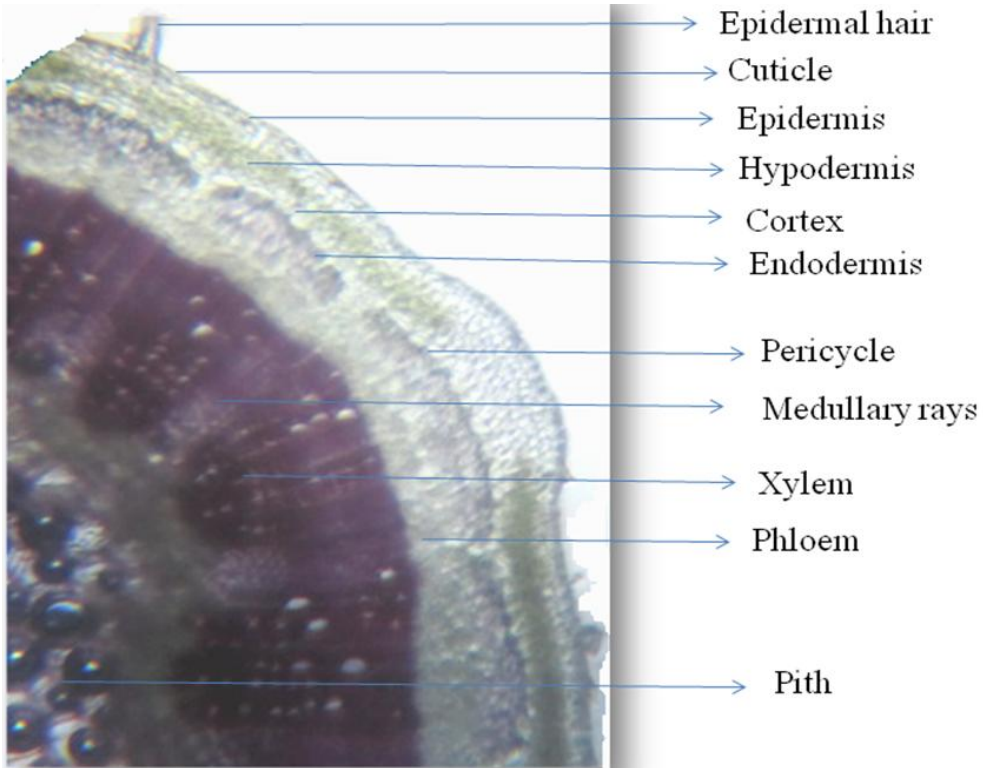

Figure 1. Section stained with phluroglucinol-HCl.

Epidermis: It was the outermost region of the stem and was formed of a single layer of rectangular cells. The outer surface of the epidermis is covered by a layer of cuticle.

Hypodermis: The hypodermis lies just below the epidermis and consist of a few layers of collenchymatous cells. In young stem, the collenchyma contains chloroplast.

Cortex: It is the region next to the hypodermis and is formed of thin walled parenchymatous cells arranged in single layer.

Endodermis: It is a wavy layer of barrel shaped cells end is the innermost layer of the cortex. The cells of endodermis are thickened at their radial walls.

Pericycle: It lies inside the endodermis and is formed of several layers of cells. The pericycle is distinguished in to alternately occurring sclerenchymatous and parenchy-matous region, the former situated outside the vascular bundles and the latter in between them. The sclerenchymatous regions of the pericycle provide mechanical support to the vascular region.

Vascular bundles: The vascular bundles in stem are wedge shaped in TS, they are arranged in a ring just inside the pericycle. Each bundle consists of phloem on the outside and xylem on the inner side, both lying on the same radius. Such vascular bundles are called conjoint and collateral. It consists of 4 to 5 layers of cells.

The protoxylem: This is the xylem formed earlier and lies towards the centre, while the metaxylem, that is the later formed xylem, is towards the periphery. This condition is called endarch. Pholem consists of phloem parenchyma and phloem fibres. All elements are lignified. Endarch proto xylem and exarch, together with meta xylem was observered.

Medullary rays: The region between the vascular bundles is called medullary rays. They are formed of radially arranged thin walled parenchymatous cells. The medullary rays are concerned with radial conduction of food and water.

Pith: It constitutes the central region of the stem and is composed of loosely arranged thin walled parenchymatous cells. Pith stores food in its cells. Abundant starch grains were observed.

Table 1. Measurements of different stem cellular components.

Parameter	Length (µm)	Width (µm)
Phloem fibers	125-250-500	12.5-25-37.5
Covering trichomes	50-125-175	12.5-25-37.5
Knee–shaped trichomes	25-50-75	12.5-25-37.5
Calcium oxalate crystals	18.75-37.5-62.5	12.5-25-37.5
Xylem vessels	12.5-37.5-62.5	12.5-25-37.5
Stone cells	12.5-18.75-25	12.5-18.75-25
Starch grains	12.5-18.75-25	12.5-25-37.5

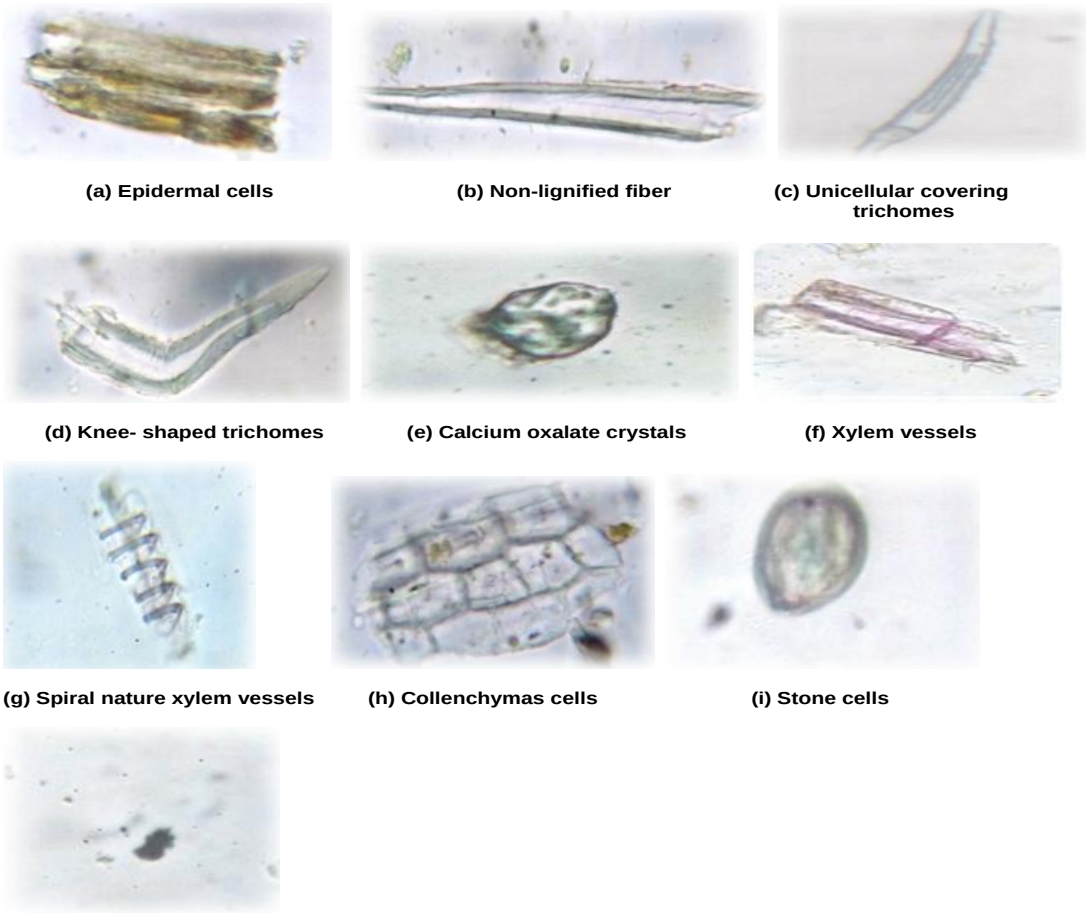

Figure 2. Parts of the stem.

Powder microscopy of stem

Powder microscopy (Table 1) was done according to the standard procedures mentioned. Powder microscopy revealed the presence of the following (Figure 2a to j):

1. Epidermal cells: These cells were present in outer region; the walls may be straight and brownish in colour.
2. Non-lignified fiber: They were present in mid rib region (sclerenchyma region). They are thin walled narrow lumen, and pointed ends.
3. Trichomes: Two types of trichomes were present. (A) Uni cellular covering trichomes: they were found to be long, slender and bent at the base and pointed apex. (B) Knee shaped trichomes: they were present at lamina region, these types of trichome are also present in vasaka leafs.
4. Calcium oxalate crystals: They were found in meophyll

Table 2. Measurements of different proximate values of stem.

S/No	Parameters	Yield (% w/w)
1	Total ash	3
2	Acid insoluble ash	0.1
3	Water soluble ash	1.2
4	Sulphated ash	2.2
5	Loss on drying	0.75
6	Crude fiber content	24
7	Foreign Organic Matter	1.5
8	Water soluble extractive value	8.92
9	Ethanol soluble extractive value	4

Table 3. Fluorescence analysis of stem.

Reagent	Long (365 nm)	Short (256 nm)	Day
50% H_2SO_4	Light yellow	Green	Light yellow
50% HNO_3	Black	Green	Light red
5% NaOH	Black	Green	Dark brown
1 N Me NaOH	Yellow	Light green	Light yellow
1 N KOH	Black	Green	Pale brown
5% KOH	Black	Green	Yellowish black
5% $FeCl_3$	Black	Green	Yellow
Methanol	Light brown	Yellow	Yellow
Conc HCl	Black	Light green	Greenish yellow
Conc H_2SO_4	Black	Light green	Reddish black
Ammonia	Black	Green	Yellow
Conc HNO_3	Black	Green	Wine red

region, they are prismatic and circular in shape.
5. Xylem vessels: The walls were thickened and lignified. They give mechanical support.
6. Spiral vessels: Spiral annular vessels are typical of proto xylem.
7. Collenchyma: Simple, polygonal collenchymas cells were present.
8. Stone cells: Stone cells were strained with green when treated with sulphuric acid. They are single circular in shape. They are heavily lignified with varying lumen (the middle space left over after the lignification). It also varies in shape and size. These are mainly present in stem part and provide valuable diagnostic characters.
9. Starch grains: They were abundant starch grains in hypodermis region which contained chlorophyll containing tissue. They were small and circular; it strained with iodine, showing blue to violet colour.

Proximate analysis of stem

Proximate analysis of *I. barberi stem* were determined by standard method and the results shown in Table 2.

Fluorescence analysis of stem with different chemical reagents

Powdered stem was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. The findings are shown in Table 3.

Behavior of stem powder with different chemical reagents

Powdered stem was subjected to behavioral analysis with different reagents. The findings are shown in Table 4.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water extracts (Khandelwal, 2005; Tadigoppula et al., 2006; Puratchikody et al., 2011) was performed. Powdered drug was subjected to successive solvent extraction with different solvents. The obtained extracts were subjected to preliminary phytochemical screening according to the standard procedures mentioned. Findings are shown in Table 5.

DISCUSSION

The microscopy of the plant specimens showed valuable information regarding the microanatomy of the *I. barberi*. Section of stem appeared with regions like epidermis, hypoderis, cortex, endodermis, pericycle, vascular bundles, medullary rays, pith which were observed. It consisted of epidermis in the outermost layers covered by cuticle on the surface and unicellular trichomes were also present. Hypodermis consisted of few layers of collenchymatous cells. In the cortex, thin walled parenchymatous cells were arranged in single layer. Endodermis is a wavy layer which is barrel shaped. Pericycle provides mechanical support to vascular region. Vascular bundles were arranged in a ring inside the pericycle. Endarch proto xylem and exarch meta xylem was observed. The medullary rays supply the food and water. Pith is inner part which stored food in its cells and starch grains which were also observed. Powder microscopy of the stem powder showed the presence of epidermal cells, non-lignified fiber, unicellular covering trichomes, knee-shaped trichomes, calcium oxalate crystals, xylem vessels, collenchymas cells, stone cells and starch grains which were present and were considered as tissues of diagnostic importance.

Extractive values play a vital role for the evaluation of the crude alcohol, and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. Ash values were used to detect the presence of any siliceous contamination and

Table 4. Behaviour of stem powder with different chemical reagents.

Reagent	Observation	Inference
Powder + iodine	Black colour observed	Presence of starch
Powder + $HgCl_2$	No Bule colour observed (black colour)	Absence of Alkaloids
Powder + Ammonia	Light pink colour observed	Presence of cardiac glycosides
Powder + $AgNO_3$	No ppt formed	Absence of protiens
Powder + Picric Acid	No colour change (brown)	Absence of alkaloids
Powder + Water (shaking)	Foam not appeared	Absence of saponins
Powder + Conc H_2SO_4	Black	Presence of starch
Powder + $FeCl_3$	Bluish black	Presence of tannins
Powder + Conc HNO_3	Orange yellow	Presence of tannins

Table 5. Preliminary phytochemical analysis.

Phyto constituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Water
Carbohydrates	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-
Proteins	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Phenols and Tannins	+	+	+	+	+	+
Steroids	-	-	-	+	+	+
Volatile oils	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+
Saponins	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	-

presence of any water soluble salts and incorrect preparation. Sulphated ash was used to detect the sulphates and phosphates (how much percent solubility in the ash). Crude fibre content is a useful technique for differentiation of the similar drugs and for the detection of adulteration. Moisture content is an inevitable content in the crude drug, it should be eliminated as much as possible.

While processing, drying of plant material plays crucial role. It helps to fix the constituents, and also aid in preservation. The values obtained aids to establish the suitable monograph of the plant. Fluorescence analysis of the powdered drugs were performed and tabulated, which helps to detect the adulteration, because phytoconstituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug. Prior to the phytochemical screening, a rough estimation of phytoconstituents was done by the behavior of powder drug with different chemical reagents in which powdered drug showed different colours when it got mixed the particular reagents, which reflects the presence of phytochemicals in accordance with the colours obtained.

Phytochemical evaluations like preliminary phytochemical screening were performed according to the standard procedures. The investigation revealed the presence of various active phytoconstituents like flavonoids, steroids, glycosides, phenols and tannins. Based on literature review, successive solvent percentage yield and preliminary phytochemical screening ethanol was taken majorly as a solvent. The preliminary chemical tests confirmed that ethanol was a suitable solvent for extraction of the active principles from the stem of *I. barberi.* The detailed phytochemical investigation strengthens the resourcefulness of the extracts for the further pharmacological evaluations. All these results put together will help in filing a suitable monograph for the stem of *I. barberi*.

Conclusion

The phytochemical screening revealed the presence of flavonoids, cardiac glycosides, steroids, phenols and tannins. All these phytochemicals have potential therapeutic or physiological actions on human system, in that the stem can stand as a potential source of some vital drugs. It may be concluded that *Indigofera barberi*

contains rich amount of phytochemical constituents which may have a variety of pharmacologically activities.

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REFERENCES

Anonymous, Indian pharmacopeia (2007). Government of India. Ministry of Health, Controller of Publications, New Delhi, India. Vol. I, 78:134.

Ansari SH (2005). Essentials of Pharmacognosy, published by Birla publications Private Limited, Ed. IV, 589-593.

Bojaxa AR, Henry JR (2010). Phytochemical, Pharmacognostical, Antimicrobial activity of *Indigofera aspalathoids* vahl. (Fabaceae) in Int. J. Biol. Technol. 1(1):12-15.

Iyengar MA (2004). Pharmacognosy of powdered crude drugs. Ed. IX, 21-31.

Kailasnath ST, Rama KKN, Sai SRKV, Gourinath A, Satanarayana RP, Editor BB (2003). Intermediate first year botany, Telugu academy, Hyderabad, Andhra Pradesh, Ed. I: 197–242.

Kalaskar MG, Shah DR, Raja NM, Surana SJ, Gond NY (2010). Pharmacognostic and phytochemical investigation of *ficus carica* linn. Ethnobot. leaflets 14:599-609.

Kannan (2007). Thesis entitled Pharmacognostical studies on some selected medicinal plants submitted to Tamil university, Thanjavur, Tamilnadu, India.

Khandelwal KR (2005). Practical Pharmacognosy. Nirali Prakashan, Pune India, Ed. I: 27-35.

Kirtikar KR, Basu BD (2001). Indian medicinal plants, Published by International book distributor's book sellers and publisher, volume - I, 709.

Kokate CK (2008). Practical Pharmacognosy. Vallabh Prakashan, Delhi, Ed. III, 107-121.

Kokate CK, Purohit AP, Gokhale SB (2009). Pharmacognosy. Published by Nirali Prakashan, Ed. XXXXIII, 1.3 - 9.19.

Madhava CK, Sivaji K, Tulasi R (2009). Book of Flowering plants of Chittoor district, Andhra Pradesh, India, Ed. II, 90–95.

Madhavan V, Hema B, Guru DMR, Yoganarsimhan SN (2009). Pharmacognostical evaluation of *Drosera burmannii* Vahl (Droseraceae). Indian J. Trad. Knowledge 8(3):326-333.

Pulokk MK (2008). Quality Control of Herbal Drugs, galaxy printers, Ed. I, 187-190: 357.

Puratchikody A, Swarnalatha (2011). Pharmacognostical, Pharmacological investigation on *Indigofera aspalathoides Vahl.* Rev. J. Pharmaceut. Biol. Med. Sci. 5(23).

Sama V, Reddy YSR, Ramesh M, Swamy MM, Mahadevan NBS (2008). Pharmacognostical studies on *Dodonaea viscose* leaves. Afri. J. Pharm. Pharmacol. 2(4):83-88.

Tadigoppula N, Tanvir K, Anju P, Ramesh C (2006). Reported Antidyslipidemic activity of furano-flavonoids isolated from *Indigofera tinctoria.* Bioorganic. Med. Chem. Letters 16:3411-3414.

The Ayurvedic Pharmacopoeia of India (2004). Published by the controller of publications civil lines, Delhi, Part-1, Volume - IV, Ed. I, 156-160.

Trease EWC, Evans D (2009). Pharmacognosy, International edition, A catalogue record of book available in British library cataloguing in publication data, Library of congress cataloguing in publication data, Ed. XVI, 9.3, 49 :436.

Wen E, Liang H, Zhongguo ZYZZ (2010). Chemical constituents of *Indigofera pseudotinctoria.* School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China, 35(20): 2708-2711.