Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(1): 216-220
Received: 26-11-2015
Accepted: 29-12-2015

Geetha DH
Assistant Professor,
PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode-638012, Tamil Nadu, India.

Indhiramuthu Jayashree
Assistant Professor,
PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode- 638012, Tamil Nadu, India.

Rajeswari M
Associate Professor & Head,
PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode- 638012, Tamil Nadu, India.

Correspondence
Geetha DH
Assistant Professor,
PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode-638012, Tamil Nadu, India.

Micro-morphological and phytochemical studies of aerial parts of *Indigofera enneaphylla* Linn.

Geetha DH, Indhiramuthu Jayashree, Rajeswari M

Abstract
Indigofera enneaphylla Linn. is an important medicinal plant of the family Fabaceae (Common name: Birdsville Indigo; Tamil name: Sheppunerunji). It is a small trailing, much-branched annual or biennial herb distributed throughout India. The aim of the present review is carried out the micro-morphological and the phytochemical constituents in the aerial parts of aqueous and ethanolic extracts of above said plant.

Keywords: Micro-morphological studies, phytochemical, *Indigofera enneaphylla*, aqueous and ethanolic extracts.

1. Introduction
Nature has created inimitable medicinal plants in the world for every ailment and there is a cure for every disease and man has to find it. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Many herbal drugs are shifts from fringe to main stream as they enjoy the advantages of comparatively less toxic than synthetic drugs, more harmony with the biological system and affordable to all classes of people [1]. Medicinal plant research has now got a momentum among the scientists of the world. Plants are considered as state-of-art chemical laboratories capable of biosynthesizing number of biomolecules of different chemical classes. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [2]. There is a need for documentation of research work carried out on traditional medicines [3]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [4]. The old trial and error is modernized into "high throughput screening" of hundreds of molecules, and the time for 'bingo' here is in days rather than years. But the principle is quite the same.
Indigofera enneaphylla Linn. is an important medicinal plant of the family Fabaceae. *Indigofera* in Greek means indigo dye which is famous for the natural blue colors obtained from the leaflets, branches and has been used as folk medicine. In traditional system of medicine this plant is claimed to be useful in treating ulcer, solid tumours, insect stings and snake bites, epilepsy [5-6], anti-nociceptive, anti-inflammatory [7], antimicrobial [8] and antidyslipidemic [9] activities. *I. enneaphylla*, which has not been explored extensively by the scientific world so far. Hence, in an attempt to explore some such untapped sources of good nutritional and medicinal property, the present work is aimed to study the micro-morphological and the phytochemical constituents in the aerial parts of this plant. This investigation will be a useful marker for identification of the crude drugs obtained from the investigated taxa.

2. Materials and Methods
The plant material was collected from Nochipalayam, Erode district, Tamil Nadu, India. The plant was identified and authenticated by the taxonomists at the PG and Research Department of Botany, Vellalar College for Women, Erode and the herbarium specimen has been deposited at the college for further reference. The shade dried aerial plant parts were ground into a coarse powder with the help of a blender. The powder was stored in an airtight container and kept in a cool, dark and dry place until further analysis.

Morphological studies were performed by using simple microscope [10]. The fresh aerial plant parts to determine the color, odour, taste, shape, size, texture, etc. For micro characterization, free hand sections of about 10-20µm thickness of stem, rachis and leaflet cleared with chloral hydrate solution and stained with aqueous safranin (0.5%) solution. After washing, the stained sections were mounted on clean micro slides and examined. The preparation was further observed in an image analyzer (Nikon S700) and the anatomical peculiarities were photo documented [11]. The dried material was subjected to powder microscopy studies, which includes the identification of organoleptic characters, behaviour of the powder with different chemical reagents/solvents according to methods described in Indian pharmacopoeia [12]. Phytochemical reporting of the plant, 10gm of the dried powder was subjected to cold extraction with ethanol and aqueous extracts for 7 days; the extract concentrated and then carried out preliminary phytochemical following the method of [13-15].

3. Results and Discussion

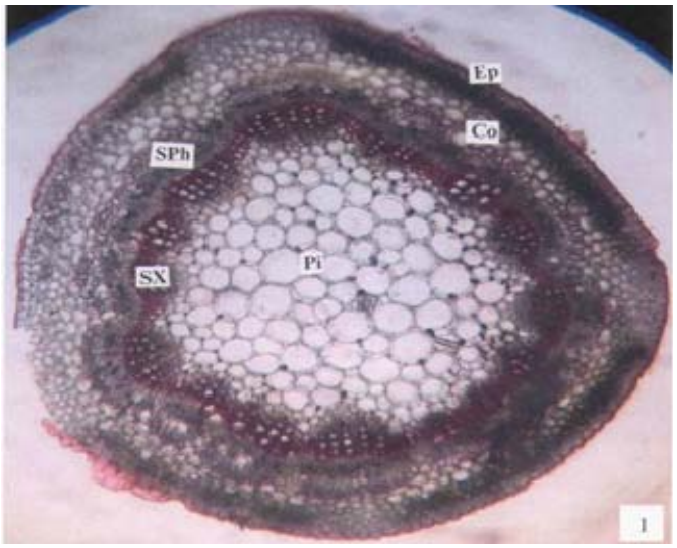
3.1. Macroscopic Analysis: The macroscopic characters are useful in the quick identification of plant material and also serve as an important criterion for standardization of drugs. *Indigofera enneaphylla* is a prostrate, much branched herb. Stem is trailing, velvet-hairy, with white hairs pressed against the stem. Leaves are compound, with 7-9 alternately arranged leaflets, 7-12mm long, 2-5mm broad, not glandular, stalk-less, with a rounded or notched tip, velvety on both sides (Figure 1). Inflorescence is an axillary raceme, sepal cup shaped 3-4mm long, velvety towards the outer side, teeth longer than the cup. Pea-shaped flowers are bright red, with the standard petal. Fruit long cylindrical pods, velvety, pale greenish grey when young and dark brown on ripening with 1-3-seeds.

Fig 1

3.2. Microscopic Analysis: Microscopic evaluation allows more detailed examination of a plant and is considered to be a source of fascination for correct identification of plant taxa. The anatomical studies were made from very thin sections of stem, rachis and leaflet showed the following features:

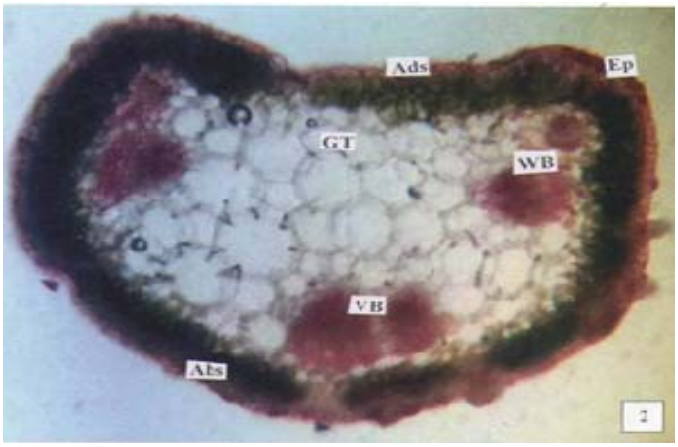
Stem: The stem is circular in sectional view with smooth and even surfaces. It has distinct and continuous epidermal layer of small, rectangular, thick walled cells and trichomes (T-shaped). The cortical zone is heterogeneous comprising of outer cortex made up of discrete small compact chlorenchyma cells; inner cortex has fairly wide, compact parenchyma cells of varying dimensions. Phloem occurs in continuous sheath encircling the xylem. It is a narrow zone of radial files of small cells; rays appear prominently in the phloem zone. Xylem cylinder is hollow, thick, wide and dense. It consists of xylem fibres are thick walled with reduced lumen; the walls are lignified. The vessels occur in thin uniseriate radial lines, widely separated from each other. The vessel lines extend from the inner to outer boundaries of the cylinder. The vessels are narrow, circular and thick walled. The pith is parenchymatous, thin walled and delicate and the cells tend to disintegrate while processing the stem for sectioning. Calcium oxalate crystals are scattered in distribution and fairly abundant in the inner cortex of the stem. The crystals are mostly prismatic type (Figure 2.1). Similarly, Suvarnalatha *et al.* [16] and Tamilselvi *et al*. [17] reported the presence of unicellular T-shaped trichomes and calcium oxalate in the leaflet and stem of *Indigofera hirsuta* and *Indigofera aspalathoides*, respectively. In addition to this, the epidermal micro-morphological features help in the authentication of foliar drugs in pharmacognosy and thus serve as biomarkers [18]. Generally, frequency distribution and size of crystals are used as diagnostic characters in microscopical and powder studies of herbal drugs. Extensive survey of crystals in plants is mostly related to the taxonomic studies of plants [19-21].

Rachis: The adaxial side of the rachis is flat, with two lateral short wings and abaxial side of the rachis is boat shaped in sectional view (Figure 2.2). It consists of thick darkly stained epidermal cells. The ground tissue is homogenous and parenchymatous. The cells are angular, thick walled and compact. Calcium oxalate crystals are scattered in some of the cells. The two smaller vascular bundles are located at the abaxial side near to the epidermis, oval in outline and conjoint, collateral and closed. Vascular bundles are made up of mass of xylem elements, and abaxial cluster of phloem elements. The xylem elements are narrow, angular, thick walled and compact. The two wing bundles placed within the wing are circular, small and collateral, comprising of a cluster of xylem and phloem cells. This was on par with the observations of Rajabudeen *et al.* [22] in *Indigofera aspalathoides.*



Ep- Epidermis, Co- Cortex, SPh – Secondary Phloem, SX – Secondary Xylem, Pi- Pith

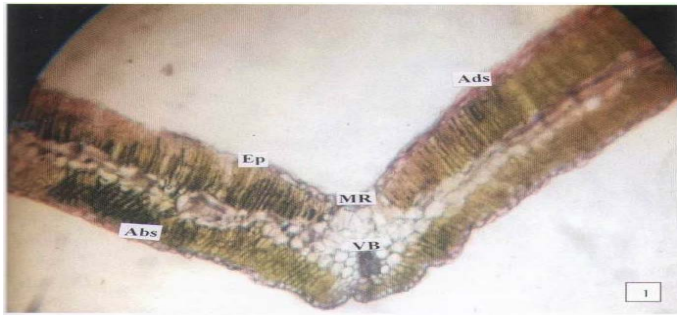
Fig 2a: T.S. of Stem



Ads-Adaxial side, Abs-Abaxial side, Ep- Epidermis, GT-Ground Tissue, VB-Vascular Bundle, WB- Wing Bundle

Fig 2b: T.S. of Rachis

Leaflet: The leaflet is either flat or folded adaxially forming V shaped outline (Figure 3.1). In the folded leaflet, the midrib has wide adaxial groove and semicircular abaxial side. The epidermis along the adaxial groove is smooth and has rectangular cells. Cells along the abaxial part are circular and papillate and wide. The vascular bundle of the midrib is collateral with adaxial triangular mass of xylem elements and abaxial cluster of phloem elements. The xylem elements are narrow, angular, thick walled and compact. The leaflet margin is slightly dilated and it is semicircular in sectional view. The epidermal layer is thick and the cells are papillate. The mesophyll tissue is differentiated into palisade and spongy parenchyma similar to the middle part of the leaflet. The adaxial and abaxial part has broad palisade zone, in between irregularly shaped and loosely arranged spongy parenchyma cells are present (Figure 3.2). This was in agreement with the records of Rajabudeen *et al.* [22] in *Indigofera aspalathoides.*



Ads-Adaxial side, Abs-Abaxial side, Ep- Epidermis, MR-Midrib, VB-Vascular Bundle

Fig 3a: T.S. of leaflet (Midrib)

Ads-Adaxial side, Abs-Abaxial side, Ep- Epidermis, PM-Palisade Mesophyll, SM-Spongy Mesophyll

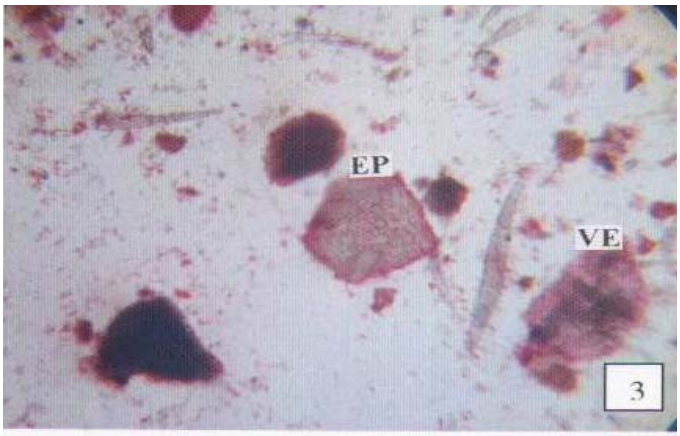
Fig 3b: T.S. of leaflet (Lamina)

3.3. Powdered-drug Analysis

The leaf powder consists of thin and small fragments of epidermal peelings. Epidermal trichomes are T-shaped, unicellular, unbranched, wide and straight with pointed tip and non-granular inclusions. The trichomes have thick warty walls with broad lumen, medifixed hairs which are constricted in the middle (both arms almost spindle shaped) and more warty. The middle portion of hair was the broadest (Figure 4.1). Minute prismatic crystals are sparsely seen on the epidermal cells. The stem powder showed epidermal peeling consisting of polygonal cells with thick anticlinal walls, extremely thick walled wide fibres with wide lumen, T-shaped trichomes and vessel elements (Figure 4.2, 4.3). Similar to the present study, Rajeswar *et al.* [23] stated the presence of trichomes and calcium oxalate crystals in the stem of *Indigofera barberi*.

Leaf Powder

Stem Powder



Cr: Crystal, Tr: Trichome, EP: Epidermal Peeling, VE: Vessel Elements

Fig 4: Powder Microscopy

3.4. Organoleptic evaluation

The results of organoleptic study offer a scientific basis for the traditional use of *I. enneaphylla* which possessed characters like green colour, characteristic odour and taste Table 1. The powder aerial plant parts when treated with various chemicals exhibited green, yellow colour shades showed in Table 2. Related studies were done by Rajeswar *et al.* [23].

Table 1: Organoleptic evaluation of aerial plant parts

S. No.	Features	Observation
1	Nature	Coarse powder
2	Color	Green
3	Odour	Characteristic
4	Taste	Acid

Table 2: Behavior of plant powder with different chemical reagents

S. No.	Treatment	Observation
1.	Powder Untreated	Green colour
2.	Powder + Conc. HCl	Green colour
3.	Powder + Conc.HNO_3	Yellow green
4.	Powder + H_2SO_4	Dirty green
5.	Powder + Ferric chloride	Grass green
6.	Powder + Acetic acid	Yellowish green
7.	Powder + Ammonia solution	Grass green
8.	Powder +KOH solution	Yellowish green
9.	Powder + NaOH	Greenish white
10.	Powder + Distilled water	Green colour

3.5. Preliminary phytochemical screening

Extractive values play an imperative role in the evaluation of the crude drugs. In the present study, the plant materials were extracted with ethanol and water using cold percolation. Obtained extracts were dried, weighed and the percent yield was calculated as depicted in Table 3. Quantitative chemical tests are also of the prime importance in drugs evaluation [24] for the detection of inferior or exhausted materials or substitution by of a worthless article. Phytochemical analysis consists of identifying in a plant, chemical compounds showing pharmacological interest. In the present investigation, the qualitative screening by using prepared ethanol and aqueous extracts revealed the presence of a wide range of phytoconstituents. Principal chemical groups of *I. enneaphylla* identified are consigned in the Table 4. The result showed the presence of carbohydrates proteins and amino acids, alkaloids, flavonoids, tannins, phenolic compounds, triterpenoids, steroids, coumarins, saponins, quinines and anthraquinones and absence of terpenoids, glycosides and fixed oils. The detailed phytochemical investigation strengthens the resourcefulness of the extracts for the further pharmacological evaluations.

Table 3: Percentage yield

S. No.	Extract	Colour	Consistency	Percentage (%)
1.	Ethanol	Dark green	Sticky	32.6%
2.	Distilled water	Light green	Solid	22.0%

Table 4: Phytochemical screening of powder of aerial plant parts of *Indigofera enneaphylla*

S. No.	Phytochemical constituents	Reagents used	Solvents	
			Ethanol	Water
1.	Carbohydrates	Fehling's Reagent	+	+
		Molisch's Reagent	-	-
2.	Proteins and Amino acids	Biuret Reagent	+	-
		Ninhydrin	+	-
3.	Alkaloids	Mayer's Reagent	+	-
		Wagner's reagent	-	+
4.	Flavonoids	Extract + $FeCl_3$	+	+
		Extract + NaoH	+	+
5.	Tannins	Extract + $FeCl_3$	+	-
6.	Phenols	Extract + $FeCl_3$	+	+
		Extract + Lead acetate	-	+
7.	Terpenoids	Extract + Chloroform + Conc. H_2SO_4	-	-
8.	Triterpenoids	Libermann - Burchard's test	+	-
9.	Steriods	Salkowski Test	+	+
10.	Coumarin	Extract + 10% NaOH	-	+
11.	Saponins	Foam Test	+	+
12.	Quinine	Extract + Conc. H_2SO_4	+	+
13.	Anthraquinine	Borntrager's Reagent	-	+
14.	Glycosides	Anthrone + H_2SO_4	-	-
15.	Fixed oil	Spot test	-	-

"+" Present "–" Absent

4. Conclusion

The plant studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and the claims about the therapeutic values of this plant as curative agent and we therefore, suggest further the isolation, identification, purification, characterization and elucidation of the structure of the bioactive compounds of the *I. enneaphylla* that would be obtained with a view to obtain useful chemotherapeutic agent.

5. References

1. Muthulingam M, Mohandoss P, Indra N, Sethupathy S. Antihepatotoxic efficacy of Indigofera tinctoria (Linn.) on paracetamol induced liver damage in rats. IJPBR. 2010; 1(1):13-18.
2. Trease GE, Evans WE. Phamacognosy, 15thEdition, W.B. Saunders Company Limited, London, 2002; 585.
3. Dahanukar SA, Kulkarni RA, Rege NN. Ind. J Pharmacol. 2000; 32:81-118.
4. Ozarkar KR. Studies on anti-inflammatory effects of two herbs Cissus quadrangularis Linn. and Valeriana wallichi DC using mouse model. Ph.D., Thesis, University of Mumbai. 2005.
5. Kritikar KB, Basu BD. Indian Medicinal Plants, Oriental enterprises, Dehra Dun, 2001; 997.

6. Mail RG, Hundiwale JC, Gavit RS, Patil DA, Patil KS. Herbal abortifacients used in North Maharashtra, Nat Prod 2006; 5:315-318.
7. Raju SK, Rajkapoor B, Perumal P. Anti-inflammatory and anti-nociceptive activities of methanolic leaf extract of Indigofera cassioides Rottl. Ex. DC. Journal of Acute Disease. 2013; 2(4):322-326.
8. Esimone CO, Adikwu MV, Muko KN. Antimicrobial properties of Indigofera dendroides leaves, Fitoterapia, 1999; 70(5):517-520.
9. Narender T, Khalia T, Anju P, Ramesh C. Antidyslipidemic activity of furano-flavonoids isolated from Indigofera tinctoria. Bioorganic and Medicinal Chemistry Letters, 2006; 16(13):3411-3414.
10. Evans WC. Trease and Evans, Pharmacognosy. (15thed.) Saunders, Elsevier, 2009; 519-521.
11. Anonymous. Indian Pharmacopia. Ministry of Health and Family Welfare, Govt. of India, Controller of Publication, New Delhi, 1985; 310.
12. Wallis TC. Text book of Pharmacognosy. 5th Ed, EBS Publications, New Delhi, India, 1985; 111-117.
13. Harbone JB. Phytochemical methods. A guide to modern Techniques of plant analysis. Chapman and Hall, London. 1973; 267-270.
14. Trease GE, Evans WE. Phamacognosy, 15thEdition, W.B. Saunders Company Limited, London, 2002; 585.
15. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. 27th edition, Nirali Prakashan, Pune, India, 2004.
16. Suvarnalatha A, Yasodamma N, Alekhya C, Chaithra D. Pharmacognostic studies of Indigofera hirsuta L. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(4):111-117.
17. Tamilselvi N, Dhamotharan R, Krishnamoorthy P, Shivakumar. Anatomical studies of Indigofera aspalathoides Vahl (Fabaceae). J. Chem. Pharm. Res. 2011; 3(2):738-746.
18. Gohil Dipa, Daniel M. Micromorphological characters as biomarkers for some of the medicinal plants of Gujarat. Herbal Technology: Recent Trends and Progress. Scientific Publishers (India), 2007; 77-84.
19. Solereder H. Systematic Anatomy of the Dicotyledons Boodle, L.A. and Fritich, F.E. (Transl.), Clarendon Press, Oxford, 1908.
20. Chattaway MM. Crystals in woody tissues. Part II, Tropical Woods, 1956; 104(9):100-124.
21. Arnott HJ, Pautard FGE. Development of raphide idioblasts in Lemna. Am. J. Bot. 1965; 52:618.
22. Rajabudeen E, Saravana Ganthi A, Padma Sorna Subramanian M. Pharmacognostical studies on Indigofera aspalathoides Vahl ex DC (Fabaceae). Journal of Pharmacognosy and Phytochemistry. 2014; 3(3):86-91.
23. Rajeshwar T, Yadagiri Rao T, Rao KNV, Sandhya S. Microscopical and physicochemical studies of Indigofera barberi (Fabaceae) stem. Journal of Pharmacognocy and Phytotherapy. 2013; 5(8):153-159.
24. Rangari A. Textbook pharmacognosy and phytochemistry. 1sted. Allahabad: Carrier publication, 2004; 98-102.