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## Pharmacognostical Studies on *Indigofera aspalathoides* Vahl ex DC (Fabaceae)

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### Abstract

Nature has created plants in the world for every ailment and there is a cure for every diseases and man has to find it. *Indigofera aspalathoides* Vahl ex DC is herb distributed in hill slopes of southern peninsular India. The methanol extract possesses hepatoprotective activity. whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases. Systematic and detailed Pharmacognostical studies were performed on *Indigofera aspalathoides*. The studies include anatomical characters of leaf, stem and roots, fluorescence analysis of the leaf, stem and root powders and their extracts in petroleum ether (40 - 60°), benzene, chloroform and methanol in the selected species. Quantitative determination such as moisture content, total ash, water-soluble ash, acid insoluble ash, water extractive values and sulphated ash have also been made. Preliminary phytochemical analysis of the extracts was done and the results showed that tannins, protein and steroid were predominantly present in all the form extracts of leaf, stem and root.

**Keywords:** *Indigofera aspalathoides*, fluorescence analysis, Quantitative determination, Pharmacognosy

### 1. Introduction

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety and lesser costs. Proper uses of plants depend upon the correct identification of these plants and appropriate methods of extraction or processing of the plant products. In the field of Indian medicine certain synonyms are used for more than one or two plant drugs. To remove controversies, confusion and selection of genuine drugs we need physicians and pharmaceutical experts. Pharmacognosy deals with all these aspects.

The present investigation aims at the screening of *Indigofera aspalathoides* (Fabaceae) for pharmacognostic characteristics. The *Fabaceae* family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill *et al.*, 1981; Judd *et al.*, 1999) [21, 14]. Many plants of this family have been used in traditional systems of medicine. Still, several potent parts of *Fabaceae* are unexplored which deserve attention and research. *Indigofera aspalathoides* Vahl ex DC. Is such plant which has not been explored extensively by the scientific world so far. The genus *Indigofera* comprises around 700 species that are distributed geographically in tropical regions (Bakasso, 2008) [5]. The plant *Indigofera aspalathoides* Vahl ex DC. is commonly known as 'Shivanarvembu' in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoots are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous affections (Kirtikar and Basu, 1975) [16]. The leaves are also applied to abscesses. The whole plant is used in edematous tumors and the ashes are used to treat dandruff (The Wealth of India, 1959) [25]. The methanol extract of *Indigofera aspalathoides* also possesses hepatoprotective activity (Gupta *et al.*, 2004) [11]. The stem is traditionally used for various skin disorders and cancers (Raj Kapoor *et al.*, 2004) [22]. Whole plant powder or potion is used for joint pains (Murugasa Mudaliyar, 1988) [20]. Powdered barks mixed with coconut oil are applied on the affected parts cautiously for six months to cure leprosy (Jeeva *et al.*, 2007) [12]. The ash of the whole plant is added with coconut oil and applied topically to treat psoriasis (Revathi and Parimelazhagan, 2010) [23]. (Yoganarasimhan, 2000) [27]. reported that the whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases. Shivanarvembu Kuli Tailam is prescribed for chronic weeping eczema in children, frequently occurring scalp on

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lower limbs, insect bites, leprosy, chronic ulcers and boils (Anonymous, 1984, 1992) [2,3]. The objective of the present work was to identify the biologically active compounds in *Indigofera aspalathoides* Vahl ex DC. In view of its medicinal importance and the fact that no Pharmacognostical work is available on the species, the present investigations were undertaken. This will help in evaluating or assuring the quality of raw drug.

## 2. Materials and Methods

The identified plant of *Indigofera aspalathoides* was collected from Sivanthipatti hills near Palayamkottai. It was confirmed with voucher specimen (No: 4303) deposited at the Survey of Medicinal Plants Unit, Govt. Siddha Medical College, Palayamkottai. The taxonomic features of the plant confirmed with the Flora of Presidency of Madras (Gamble, 1915 – 1921) and The Flora Tamil Nadu Carnatic (Mathew 1983 – 1988). The plant parts were soaked in 70% alcohol, free hand sections of the leaf, stem and root were taken for detailed microscopic observations and figures were drawn by following Johansen (1946). Dry powder of the leaf, stem and root was used for chemical analysis. Physico chemical analysis was carried out as per standard procedure (Anonymous 1966) [1]. The fluorescence analysis of the powder drug under Ultra Violet was done according to the methods described by (Chase and Pratt 1949) [7]. The preliminary phytochemical analysis was done by the methods described by (Brindha *et al.* 1981) [6]. Biochemical estimation for protein (Lowry *et al.* 1951) [17], Phenol (Farkes and Kiraly 1962), Starch (Sadasivam and Manickam 1992) [24], Amino acid (Jeyaraman, 1981) and Tannin (Aparna Buzarbarua, 2000) [4] were carried out.

## 3. Results and Discussion

### Macroscopic studies

The plant is a **sub shrub** that grows up to 75 cm height. The branches have silvery pubescent. The **leaves** are digitately three foliolate; the leaflets are linear, membranous, pubescent, margins entire, base cuneate, margin entire, apex obtuse, apiculate; petiole 0; stipules linear. **Flowers** axillary, solitary; pedicel to 3 mm. **Calyx** - tube has five sepals 0.5 mm; lobes 1 mm, pilose, **Corolla** five petals, polypetalous, papilionaceous; purple to brick red.; standard orbicular, 4 mm, puberulous without, shortly clawed; wings 3 mm; keels 4 mm. **Staminal** sheath 4 mm. **Ovary** sessile, 4 mm, pubescent; style 1 mm, glabrous. The **pods** straight and cylindrical terete. **Seeds** cuboidal and smooth.

Regional names: Tamil: Shivanar vembu Sanskrit: Sivanimba Malayalam: Manali Telugu: Mil

### Microscopical characters of *Tephrosia villosa*

#### Leaflet

The leaflet is either flat or folded adaxially forming V-Shaped outline (Plate: 1). The leaflets are characterized by the presence of wide, circular cavities in the midrib and at the margin. The cavities may be present only in the midrib, only at the margin or both in the midrib and two margins; in some of the leaflets, the cavities are absent. In the folded leaflet, the midrib has wide adaxial groove and semicircular abaxial side. The midrib part is 170  $\mu\text{m}$  thick vertically and 200  $\mu\text{m}$  horizontally. The epidermis along the adaxial

groove is smooth and has rectangular cells. Cells along the abaxial part are circular and papillate and wide. The cells are 15  $\mu\text{m}$  in radial plane and 10  $\mu\text{m}$  in horizontal plane.

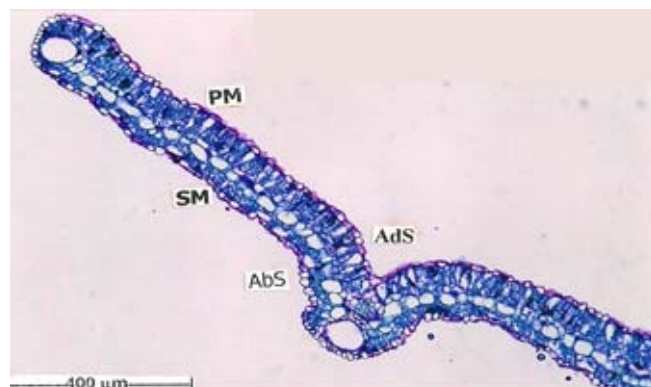


Plate 1: T.S. of leaf

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 secretory canal is located beneath the phloem unit and it occupies the major portion of the midrib.

The leaf margin is slightly dilated and it is semicircular in sectional view. It is 130  $\mu\text{m}$  thick. The epidermal layer of the leaf margin is thick and the cells are papillate measuring 10  $\mu\text{m}$  in height. The mesophyll tissue is differentiated into palisade and spongy parenchyma similar to the middle part of the leaflet. The adaxial part has broad palisade zone, the abaxial part has spongy parenchyma and in the median part a row of circular cells is present.

### Venation pattern of the leaflet

The lateral veins and veinlets are uniform in thickness. They form dense network. Along the lateral margin of the leaflet, the lateral branches of the veins are inter-connected by sub marginal vein. In the middle part of the lamina, some of the vein-islets are distinct, wide and polygonal in outline. These are distinct vein terminations which are long, slender and curved. At the extreme end of the vein termination, there are short, spherical clusters of short, cylindrical tracheids. These terminal tracheids have spiral or annular thickenings. The number of tracheids in the terminus varies from two to five.

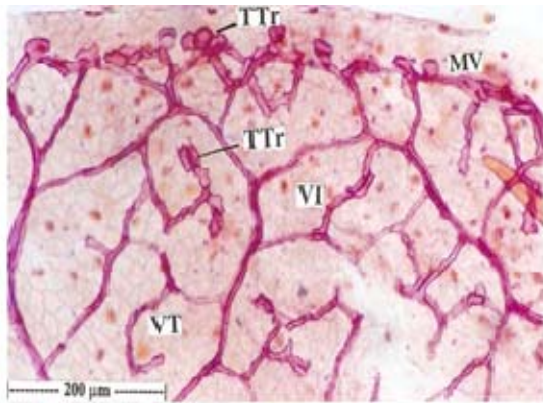
### Stem

The stem is circular in sectional view with smooth and even surfaces. It is 900  $\mu\text{m}$  thick. The stem has distinct and continuous epidermal layer of small, rectangular, thick walled cells. The epidermal cells are 5  $\mu\text{m}$  thick. The cortical zone is 100  $\mu\text{m}$  wide. It is heterogeneous comprising of outer part of four or five layers of small compact cells, in addition to wide isolated group of two or three cells; the middle part has prominent, discrete, circular masses of gelatinous or mucilage fibres, arranged in a ring, all around the stem (Plate: 2). The inner cortex has four or five layers of fairly wide, compact parenchyma cells of varying dimensions.

Phloem occurs in continuous sheath encircling the xylem. It is a narrow zone of five or six layers of radial files of small

cells. Thin phloem rays appear prominently in the phloem zone.

Xylem cylinder is hollow, thick and dense. The entire cylinder is 750  $\mu\text{m}$  wide; the xylem segment is 200  $\mu\text{m}$  thick. Xylem cylinder consists of xylem fibres as the ground tissue; the 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. They are up to 20  $\mu\text{m}$  wide. The pith is 350  $\mu\text{m}$  wide. It is paranchymatous; the cells are thin walled and delicate and the cells tend to disintegrate while processing the stem for sectioning.



**Plate 2:** Paradermal section showing vein

#### Crystals in the stem

Calcium oxalate crystals are fairly abundant in the inner cortex and xylem rays of the stem. The crystals are mostly prismatic type. They are scattered in distribution.

#### Anatomy of the root

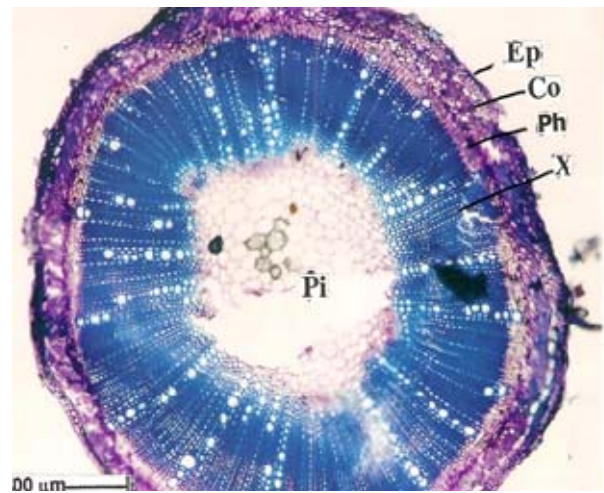
Root of different thicknesses was studied. The thickness of the roots ranges from 850  $\mu\text{m}$  to 3 mm. The thin as well as thick roots have shallow fissured periderm and uneven cross-sectional outline.

The epidermis is crushed into a thin dark line; the periderm in a thin root consists of four or five layers of tubular, suberised phellem cells (Plate: 3). This is followed by a narrow cortex of small, rectangular cells. Secondary phloem consists of outer dark cylinder of crushed phloem and sclerenchyma elements; inner phloem has non collapsed, radial files of elements without sclerenchyma cells. Secondary xylem is circular, solid and dense cylinder. It consists of randomly distributed vessels and dense xylem fibres. The vessels are solitary and are narrow in the centre, become much wider towards the periphery. The wide vessel in the outer zone is 50  $\mu\text{m}$  in diameter. The xylem fibres are libriform type and lignified. Along the periphery of the xylem cylinder, the fibres are gelatinous type; their inner walls are non-lignified and stain purple with toluidine blue stain.

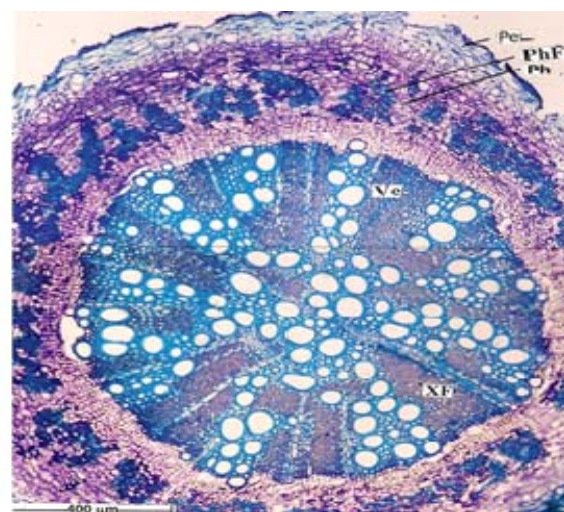
In the older root, the periderm is wider comprising of outer suberised phellem and inner radially oblong phellderm cells. The cortex is narrow and less distinct. Secondary phloem is differentiated into outer collapsed phloem and inner non collapsed phloem. The collapsed phloem is much wider and consists of funnel shaped dilated rays alternating

with triangular cones of fibres and crushed phloem elements. The dilated rays and cones of crushed phloem are clearly visible in old root.

The non-collapsed phloem is narrow, comprising of small cells arranged in radial files. The rays are narrow and sclerenchyma elements are absent in the non-collapsed phloem. Secondary xylem cylinder is circular with even circumference. It consists of several wedge – shaped, radial vessel bands separated laterally by wide radial sheets of xylem fibres. Fairly prominent xylem rays run radially segregating the vessel bands and fibre sheets. The fibres are gelatinous type; they have inner gelatinous unlignified walls and outer lignified walls. However, the fibres that occur within the vessel bands are libriform type with thick lignified walls. Both wide and narrow vessels occur, mixed in the vessels bands. The narrow vessels are 20  $\mu\text{m}$  in diameter and widest vessels are 70  $\mu\text{m}$  in diameter. The vessels are mostly solitary, thick walled, circular or elliptical.



**Plate 3:** T.S. of stem



**Plate 4:** T.S. of root

AdS: Adaxial side, AdH: Adaxial Hood, PM – Palisade Mesophyll SM- Spongy Mesophyll, VI – Vein islet VT- Vein termination, TTr – Terminal tracheid MV – Marginal vein, Ep – Epidermis, Co – Cortex, Ph – Phloem, X: Xylem, Pi: Pith, PE: Periderm, Ph F – Phloem Fibre, XF – Xylem fibre, Ve – Vessel.

### Crystals

Calcium oxalate crystals are abundant in collapsed phloem and in the xylem rays. The crystals are prismatic type. In the collapsed phloem the crystals are scattered; in the xylem rays they are in radial chains. Starch grains are also abundant in the cortex, phloem and xylem parenchyma.

### Fluorescence analysis

The leaf, stem and root powder of *Indigofera aspalathoides* and the extracts of the powder on various solvents were examined under ordinary light and UV light. These powders were also treated with different reagents and the change in colour was recorded. These results were presented in Table – 1

**Table 1:** Comparative Fluorescence analysis of leaf, stem and root of *Indigofera aspalathoides*

S. No.	Treatment	Plant parts					
		Leaf		Stem		Root	
		Visible light	UV light	Visible light	UV light	Visible light	UV light
1.	Powder + acetone	light green	green	pale yellow	yellowish green	brownish yellow	yellowish green
2.	Powder + ethyl alcohol	yellowish green	green	brownish yellow	yellowish green	pale brown	greenish yellow
3.	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	yellowish green	green	yellow	green	yellow	dark green
4.	Powder + 1 N HCl	pale yellow	yellowish green	pale brown	yellowish green	pale brown	greenish yellow
5.	Powder + 1N NaOH	yellowish brown	green	brown	green	brown	yellowish green
6.	Powder + 50% HNO <sub>3</sub>	yellow	green	Dark green	brown	brown	dark green
7.	Pet – ether extract	dark green	green	yellow	greenish yellow	light brown	brownish yellow
8.	Benzene extract	pale yellow	light green	pale yellow	light green	pale	light yellow
9.	Chloroform extract	pale yellowish green	yellowish green	pale yellow	brown	pale yellow	yellow
10.	Methanol extract	yellow brown	brown	reddish brown	greenish yellow	dark brown	brownish red
11.	Powder + H <sub>2</sub> O	pale green	yellowish green	light yellow	Brown	pale white	yellow

*Indigofera aspalathoides* stem powder shows yellowish green under UV light when treated with acetone, ethyl alcohol, and 1 N HCl. Methanol extract exhibits light yellow colour under visible light and brown colour in UV light

### Quantitative Physico – chemical Analysis

The percentage of total ash, water soluble ash, acid

insoluble ash, water soluble extractive value, sulphated ash and moisture content value were presented in Table - 2. It is helpful for determining the quality and purity of crude drugs especially in the powdered form. The amount of water soluble ash is 10.3% in leaf. The value of sulphated ash is much higher in leaf samples than in stem and root samples.

**Table 2:** Comparative analyses of physicochemical characters of leaf, stem and root of *Indigofera aspalathoides*

S. No	Test	Leaf %	Stem %	Root %
1.	Total ash	13.0	5.1	6.5
2.	Water soluble ash	10.3	2.9	4.5
3.	Acid insoluble ash	9.8	1.5	5.3
4.	Sulphated ash	5.5	1.5	2.6
5.	Moisture content	53.80	64.12	49.4
6.	Water soluble extractive	7.86	0.57	5.14
7.	Alcohol soluble extractive	8.75	2.25	2.75
Extractive value				
8.	Petroleum ether extract	6.2	6.2	4.2
9.	Benzene extract	8.2	6.4	6.2
10.	Chloroform extract	6.2	4.1	2.2
11.	Methanol extract	10.6	7.2	7.3

### Phytochemical screening

The preliminary phytochemical screening of *I. aspalathoides* shows that the plant contains different types of chemical constituents and the results are presented in Table 4.7. Tannin, alkaloid, sugar and protein are

predominantly present in leaf, stem and root samples. Saponins are reported in the water extracts of stem, leaf and root, and absent in methanol extract. Amino acids are reported in the benzene and water extract. Catechin is absent in leaf extract, but present in root and of stem

extracts. The presence of alkaloid and flavones in *Indigofera aspalathoides* has also been reported by other

researchers and this plant is widely used in herbal medicine (The Wealth of India, 1959) [25].

**Table 3:** Phytochemical screening of leaf, stem and root of *Indigofera aspalathoides*

S. No	Extract	Samples	Saponin	Tannin	Alkaloid	Flavones	Amino acids	Protein	Phenol	Steroid	Triterpenoid	Catachin	Anthroquinone	Sugar
1.	Pet. ether	Leaf	-	+	+	-	-	+	+	-	-	-	-	-
		Stem	-	+	-	-	-	+	+	-	-	-	+	-
		Root	+	+	+	+	-	+	+	-	-	+	+	-
2.	Benzene	Leaf	+	+	+	-	+	-	+	+	-	-	-	+
		Stem	-	+	+	-	+	+	-	+	+	-	+	+
		Root	-	+	+	-	-	+	-	+	+	-	+	+
3.	Chloroform	Leaf	+	+	-	-	-	+	-	+	-	-	-	+
		Stem	+	+	+	+	-	+	-	-	+	+	+	+
		Root	-	+	+	+	-	+	+	-	+	-	+	+
4.	Methanol	Leaf	-	+	+	+	-	+	+	+	+	-	+	+
		Stem	-	+	+	+	-	+	+	+	+	+	+	+
		Root	-	+	+	+	-	+	+	+	+	+	+	+
5.	Water	Leaf	+	+	+	+	+	+	-	-	-	-	+	+
		Stem	+	+	-	-	-	+	+	+	-	-	-	-
		Root	+	+	-	-	-	+	+	+	+	-	-	-

**NOTE:** - + denote PRESENT - denote ABSENT

#### Quantitative Phytochemicals Analysis

Table: 4 shows the results of quantitative estimation Starch, sugar, amino acids, lipids, protein, tannin and phenolic compounds in the dry powder of the leaf, stem and root. The analyses yielded significant results. Phenols are stimulating, antiseptic, anti-infectious and detoxifying

activities (Kenner and Requena, 1996). Tannins have important roles such as stable and potent antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003; Lutete *et al.*, 1994) [8, 18].

**Table 4:** Phytochemicals analysis of stem, leaf and root of *Indigofera aspalathoides*

Estimation	Part	Quantity
Starch (mg/g/dry wt.)	Stem	136.45
	Leaf	154.85
	root	131.53
Sugar (mg/g/dry wt.)	Stem	53.00
	Leaf	72.00
	root	78.00
Amino acids (mg/g/dry wt.)	Stem	35.80
	Leaf	53.20
	root	16.75
Protein (mg/g/dry wt.)	Stem	68.67
	Leaf	75.73
	root	39.83
Tannin ( $\mu$ g/g/dry wt.)	Stem	560.00
	Leaf	280.00
	root	155.00
Phenol (mg/g/dry wt.)	Stem	1.92
	Leaf	3.25
	root	1.45
Lipid (mg/g/dry wt.)	Stem	24.92
	Leaf	45.08
	root	23.86

#### 4. Conclusion

The macroscopic and microscopic characters, fluorescence analysis, physico-chemical determination and preliminary phytochemical screening can be used as a diagnostic tool in the correct identification of plants. The adulterants if any in the plant material can also easily identified by these studies.

#### 5. Acknowledgement

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