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PHARMACOGNOSTICAL INVESTIGATION OF *Indigofera barberi Gamble* (FABACEAE) – A THREATENED MEDICINAL HERB

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ABSTRACT

Plan: *A preliminary Pharmacognostical study on the leaves and stems of Indigofera barberi Gamble*

Methodology: *The Indigofera barberi Gamble leaves and stems were collected, in the forest regions of Thalakona (Nelakona regions) of Chittoor district, Andhra Pradesh, India in the month of November. The collected drug were dried and studied to determine various Pharmacognostical parameters such as macroscopy, microscopical characters of leaf and stems including its powder microscopical characters. The shade dried powder and various solvent extracts (viz., petroleum ether, chloroform, dichloromethane ethanol and water) have been analysed for their phytochemicals, behaviour of powder with different chemical reagents and fluorescence characters.*

Outcome: *The data generated for the Pharmacognostical evaluation on Indigofera barberi Gamble leaves and stems. The results may be useful as a reference material in the preparation of standard monograph.*

1. INTRODUCTION

Medicinal plants play an important role in the lives of rural people particularly in remote parts of developing counties with few health facilities. It is estimated that around 70,000 plant species from lichens to towering tree has been used for medicinal purpose[1]. The discovery of new biologically active compounds derived from natural products seems to be the main objective of many scientific researchers and pharmaceutical companies. Screening of natural products for this objective with the greatest possibility of success is always needed. Using plants in this area, especially, has a huge advantage owing to their long-term use in health care.

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The traditional medicine is now a day's revealed by an extensive activity of research on different plant species and their therapeutic principles, as plants produce a lot of anti-oxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with liver protective activity.

Gradually accumulate practice and systemic medical knowledge in India is called ayurveda. This system of medicine provides an approach to prevention and treatment of different disease by a large number of medical procedures and pharmaceuticals. One of the clinical specialities of this ayurveda is rasayana. Rasayana is not only a drug therapy but is a specialized procedure practice in the form of rejuvenating recipes dietary regimen promoting good habit. Indigofera, commonly known as indigo, is a member of the legume family Fabaceae (Leguminosae) Indigofera consists of approximately 700 species worldwide and occurs on all major land masses, but is most abundant in Africa and Asia.

In the traditional system of medicine, medical plants play the major role in cure of various diseases. *Indigofera barberi Gamble* is threatened medicinal herb belongs to the family *Fabaceae*. The plant selected for the present study is used traditionally to treat various skin diseases, renal disease and liver disease[2, 3]. Further, it has not yet been studied pharmacognostically. Hence, the present study is aimed to know the Pharmacognostical and microscopical investigation of leaf and stem part of the plant *Indigofera barberi Gamble*.

2. MATERIALS AND METHODS

2.1. 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. The plant material was taxonomically identified by Prof.P.Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu and India. The voucher herbarium specimens (PARC/2012/1246) have been preserved in our laboratory for further reference.

2.2. Chemical and equipment requirement

All the chemicals and reagents like petroleum ether, chloroform, ethyl acetate, ethanol, distilled water and sodium hydroxide, sulphuric acid nitric acid, and methanol used were of analytical grade and obtained from S.D.Fine chemicals, Mumbai. Soxhlet apparatus, Rotary vacuum evaporator (Indosati, India), heating mantel (Biotechnics, India), UV chamber (Secor, India), silica crucible, stoppered conical flask, microscope, stage micrometer, and eye-piecemicrometer (Edison).

2.3. Drying of plant material

The aerial part of the plant material of *Indigofera barberi Gamble* was subjected to shade drying for about 10 weeks. The shade dried plant material was further crushed to powder and the powder was passed through the mesh 22 and stored in airtight container for further analysis.

2.4. Extraction of powdered plant material

The plant material collected from their natural habitat was cleaned, shade dried at room temperature, coarsely powdered and stored in an air tight glass container. 100gm of coarse powder was successively extracted with increasing the polarity of the solvent in Soxhlet extractor for 18 hours. Successive Solvent Extraction are done using petroleum ether, chloroform, dichloromethane, ethanol and water, by successive solvent extraction method based on the increasing order of polarity of solvent. The extraction temperature was adjusted as per the solvent been used in the extraction. The percentage yield obtained was calculated and reported, the values are presented in table no: 7.4. The extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulphite below 60°C. Freshly prepared extract was subjected to phytochemical evaluation for the detection of various constituents using conventional protocol. The presence of various phytoconstituents, i.e. alkaloids (Dragendorff 's test), steroids and terpenoids (Liebermann Burchard test), tannin and phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), amino acids (ninhydrin test), etc., was detected by the usual methods prescribed in standard texts.[4,5]The phytochemical screenings of various fractions are showed the presence of glycosides, steroids, flavonoids, tannins and carbohydrates.

2.5. Macroscopy observation

The fresh plant of *Indigofera barberi Gamble* was used for macroscopical study. The size shape, colour, taste, and odour were observed. The powder of the plant were sieved and investigated in different organoleptic features by repeated observation. Morphological studies, such as shape, size, apex, surface, base, margin, venation, taste and odour of leave was performed according to the prescribed procedure[6, 7].

2.6. Microscopic analysis

The microscopy of the plant studied according to the prescribed procedure. Transverse sections of Leaf and Stem were prepared and stained with Safranin and Fast green as per the procedure[6]. Powder microscopy is performed according to the prescribed procedure[8, 9]. Photographs of different magnifications were taken with a Nikon Labphot2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against a dark background.[10]

2.7. Measurements of different leaf components

Determination of leaf Constant, Stomatal Index, Vein-Islet Number, Vein termination Number, palisade ratio.[4, 8, 11]

2.8. Determination of behaviour of plant powder

Behaviour of powdered plant material with different chemical reagents was determined under natural light.

2.9. Fluorescence analysis

Powdered leaves were subjected to analysis under violet light after treatment with various chemical and organic regents. Three parameters were taken into account i.e observation under long UV light (365 nm), Short UV light (256 nm) and normal day light. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification.[12, 13, 14] .

2.10. Phytochemical study

Freshly prepared extracts were subjected to phytochemical screening for the detection of various constituents using conventional protocol.[11]Total ash, Acid insoluble ash, Water soluble ash, Moisture content, Alcohol soluble extractive value and Water soluble extractive value of *Indigofera barberi Gamble* were determined as per standard procedures.[1, 15]

3. RESULTS

3.1. Macroscopy of plant

Habit: Under shrub, Habitat: Endemic or occasional in deciduous Forests, Ecological Status: Common, Description: flowers: Flowers red aggregate in 3 cm long axillary racemes. Plant Type: Diecious, Stem Type: Erect stems or greyish, pubescent, Leaf Arrangement: Leaves simple. Leaves were tri-foliolate, entire, mucronate, obtuse and pubescent below, gland dotted. Leaf Shape: Ovate – cordate. Leaflets obovate: 1.5-3.5 x 0.5-1.5 cm. Leaf Base: Rounded, Leaf Surface: Tomentose beneath, Leaf Length: 3 cm long, Standard petal: 2-7 mm across, Inflorescence: Axillary racemes, Flower Type: Red, in axillary racemes, Stamen: Diadelphous (9+1). Flowering & fruits Month: September – December, Fruit Shape: Ovoid, Ribbed. Fruiting Month: September–October, Pods: subterete, angular torulose, white pubescent. Pods shape: cylindric deflexed, appressedly white – villous, torulose. Pods length: 1 x 0.2 cm. Seeds: 4 – 6 seeded. Seeds shape: Oblong – ellipsoid.

A Transverse section of *Indigofera barberi Gamble* shows dorsiventral nature. Following are the important tissues in the lamina and the midrib region. In cross sectional view then leaf let exhibit distinct dorsiventral symmetry and fairly thick midrib (Fig 1.1). The midrib is plant convex with flat adaxial side and semi-circular abaxial side. The midrib is 370 µm thick and 400 µm broad. On the adaxial side of the midrib there is a shallow concavity will wide and thick layer of epidermal cells and a short band called sub epidermal layer (Fig 1.2). The abaxial part of the midrib has slightly thin layer of epidermal cells which are thick walked and squarish in shape. There is a single collateral, top shaped vascular strand located in the median part of the midrib and surrounded by thick walked, angular parenchymatous ground tissue (Fig 1.2). The vascular strand has broad, several parallel compact lines of narrow, thick walled xylem elements and a thin of arc of phloem elements.

Transverse section of leaf and stem

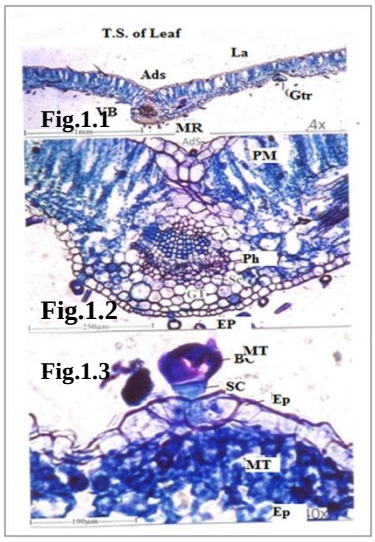

Fig. 1.1, 1.2, 1.3 AdS-Adaxial side, **BC**-Body cell, **EP**-Epidermis, **GT**- Ground Tissue, **LA**- Lamina, **MR**-Midrib, **MT**-Mesophyll Tissue, **PH**- Phloem, **PM**-Palisade Mesophyll Tissue, **PH**-Phloem, **PM**- Palisade Mesophyll, **SC**-Stalk cell, **VB**- Vascular Bundle, **X**-Xylem, **SC**- Sclerenchyma, **GTr**-Glandular Trichorne

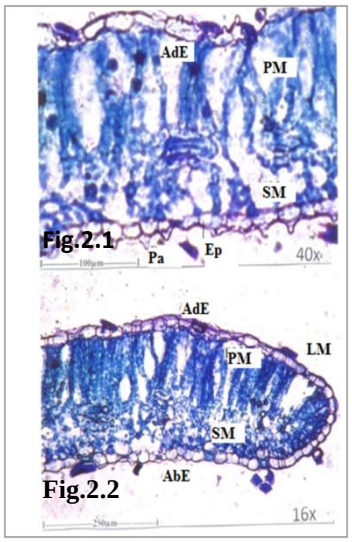

Fig.2.1, 2.2, AbE – Abaxial Epidermis, **AdE** – Adaxial Epidermis, **EP** –Epidermis, **LM**-Leaf Margin, **PA**- Parenchyma, **PM**-Palisade Mesophyll, **SM**-Spongy Mesophyll.

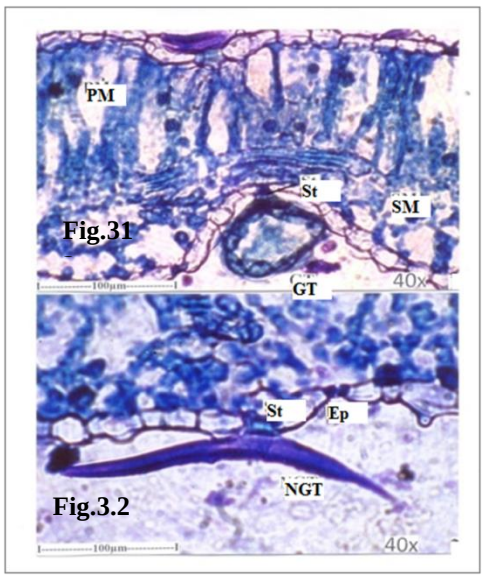

Fig.3.1, 3.2, NGT-Abaxial epidermis of the lamina showing T- shaped non- glandular Trichome, **EP**-Epidermis, **GT**- Glandular Trichome, **PM**- Palisade Mesophyll, **SM**-Spongy Mesophyll, **ST**- Stomata

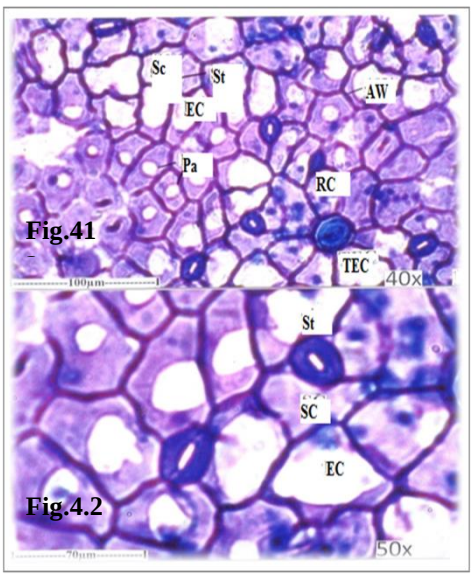

Fig.4.1, 4.2, EC- Epidermal Cell, **RC** – Rosette cell, **ST**- Stomata, **TEC**- Trichome bearing epidermal cell, **SC** - subsidiary cell, **PA** – Parenchyma, **AW** - Anticlinal wall.

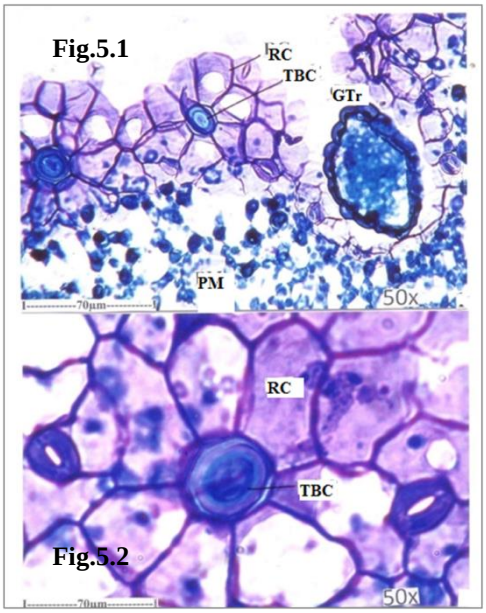

Fig.5.1, 5.2, GTr – Glandular Trichome, **PM** - Palisade Mesophyll, **RC** - Rosette cell, **TBC** – Trichome Bearing cells

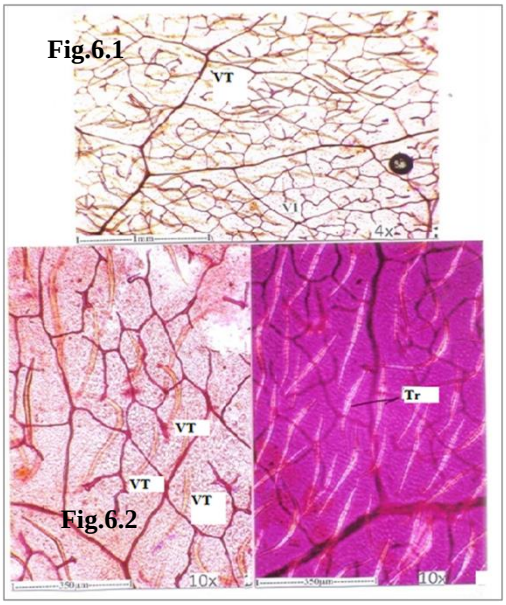

Fig.6.1, 6.2, **Tr**-Trichome, **VI**-vein Islet, **VT**-vein termination

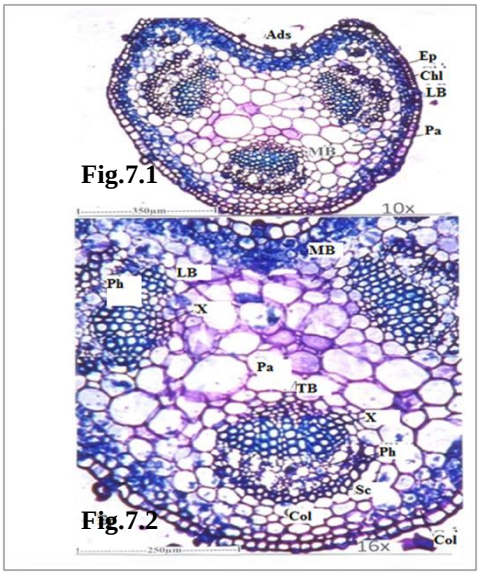

Fig.7.1, 7.2, AdS - Adaxial side, **COL** - Collenchyma, **SC** - Sclerenchyma, **CHL** - Collenchyma, **EP** - Epidermis, **LB** - Lateral Bundle, **MB**- Mediax Bundle, **PA** - Parenchyma, **PH** - Phloem, **X** - Xylem.

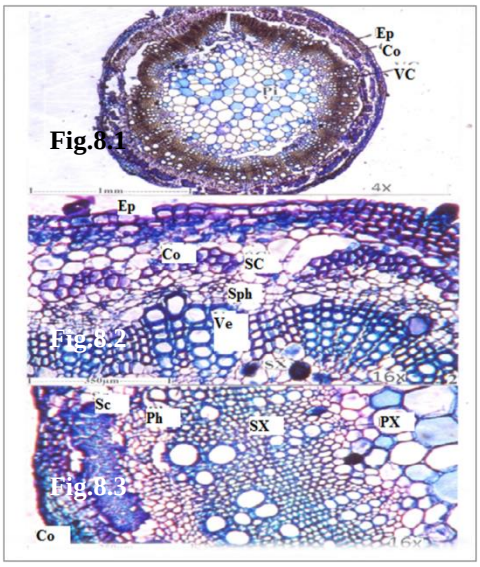

Fig.8.1, 8.2, 8.3, CO- Cortex, **EP**- Epidermis, **SC**- Sclerenchyma, **Sph**- Secondary Phloem, **Sx**- Secondary xylem, **Vc**- Vascular cylinder, **Ve**– Vessel, **Px**- Primary xylem.

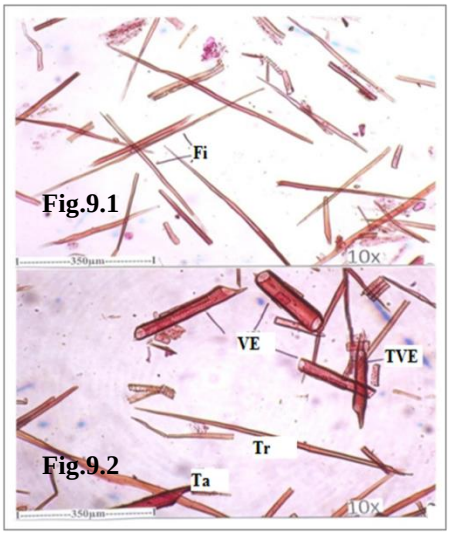

Fig.9.1, 9.2 Fi- Fibre, **Ta-** Tail, **Tr-** Trichome, **VE-** Vessel Element, **TVE-** Tailed Vessel Element

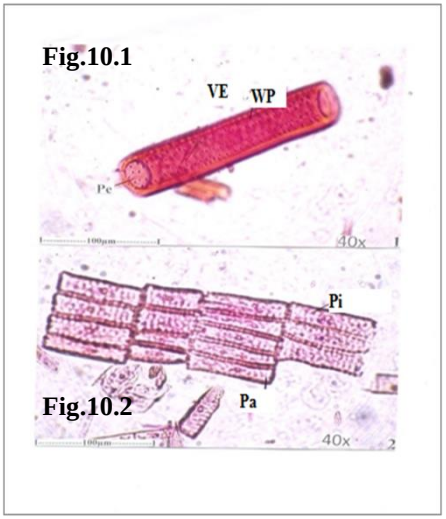

Fig.10.1,10.2 Fi- Fibre, **Ta-** Tail, **Tr-** Trichome, **VE-** Vessel Element, **TVE-** Teiled Vessel Element, **LPW-** Lateral Wall Pits, **Pe** – Perforation, **Pi-** Pits, **Pa-** Parenchyma, **VE-** Vessel Element.

Two or three layers of sclerenchyma cell form an abaxial sheath abutting phloem are. There are glandular and non-glandulartrichomes are on the abaxial side of the lamina. The glandular trichomes are short stalked and peltate type. (Fig 1.1) or capitate type (Fig 1.3). The capitates trichome has a single rectangular stalk cell and spherical, multicellular darkly stained body cells. The trichome is 50 µm in height and 60 µm thick (Fig 1.3). The trichomes are usually situated within shallow, wide epidermal concavity (Fig 3.1). The trichome has short, thin stalk and horizontally wide cup shaped body. These trichomes are 70 µm in height and 70 µm wide. The non-glandular trichomes are more abundant and are dense on the abaxial side of the leaf let (Fig6.3). The non-glandular trichome is unique in shape, it is T-shaped. It consists of a short rectangular stalk cell which bears horizontally oriented 2-armed thick walked trichome (Fig3.2). The trichomes are 340 µm long and 20 µm thick. The walls are lignified.

3.1.1. Lamina

The lamina is smooth on the adaxial side and the epidermal cells are rectangular and thin walked. The abaxial epidermis consists of thick-walled oblong cells with prominent, papillate outgrowths on the tangential walls. The lamina is 170 µm thick. The palisade tissue is differentiated into adaxial zone of thin cylindrical palisade cell layer with wide air spaces. The spongy parenchyma of the abaxial part consists of small, lobed cells which form reticulate structure with wide air spaces (Fig.2.1)

3.1.2. Leaf margin

It is straight and possesses small, epidermal cells with thick cuticle. The palisade tissue is similar to middle part of the lamina. The marginal part is 170 µm thick (Fig 2.2).

3.1.3. Epidermal cell and stomata

The lamina is amphistomatic -stomata occur both on the adaxial and abaxial sides. The stomata are broadly elliptical with wide stomatal opening. The stomata are mostly anisocytic with three unequal subsidiary cells surrounding the guard cells. The epidermal cells are polygonal in outline with thick, straight anticlinal walls. In the centre of the epidermal cells are seen circular white areas. These areas represent papillate outgrowth of the tangential walls of the epidermis (Fig 4.1). The epidermal cells surrounding the epidermal cell which bears the non-glandular trichome form a radiating circle of rosette cells. (Fig 5.1). Trichome bearing cell is circular and highly thick walled (Fig 5.2).

3.1.4. Venation

The venation is densely reticulated formed by thin, straight veins. The veins islets are distinct with defined bordering of thin, straight vein boundaries (Fig 6.2). The vein terminations are short and unbranched or long, branched once or twice. (Fig 6.2).

3.1.5. Rachis

The rachis is semi-circular with wide shallow adaxial concavity. It is 750 µm thick and 800 µm wide. The surface of the rachis is smooth and even. The epidermal cells are prominent squarish and thick walled. Inner to the epidermis are two or three layers of thick walled collenchyma cells. Along the adaxial part of the rachis there are three or four layers of chlorenchymatous cells. The ground tissue is parenchymatous with thin, compact polygonal parenchyma cells. The vascular system consists of three prominent vascular strands, one of them being abaxial median and the other two are adaxial lateral in position (Fig 7.1). The vascular bundles have long. Compact parallel lines of thick walled narrow xylem elements and wide band of phloem elements and there are of sclerenchymatous bundle cap (Fig 7.2).

3.1.6. Stem

The stem is circular and even in outline the young stem is 1.8 µm thick. It consists of single layer of epidermis, chlorenchymatous outer cortex and parenchymatous inner cortex; the vascular cylinder is complete, wide and hollow with central wide pith (Fig 8.1) the epidermis is intact; in case of young stem the epidermal cells are squarish and thick walled. The chlorenchyma tissue includes two or three layers of circular cells with chloroplast. The parenchymatous tissue includes wide compact, angular cells. The inner boundary of the cortex is marked by a discontinuous circle of discrete segments of sclerenchyma cells. The vascular cylinder includes outer secondary phloem in which some of the phloem cylinder includes several, wedge shaped segments of wide, thick walled elements are collapsed and inner phloem elements are intact. The secondary xylem vessel elements arranged in radial files.

These vessel segments are interconnected by thick radial files of inter fascicular secondary xylem elements (Fig 8.2). In the case of thick stem, the phloem zone is wider with radial files of elements.

The xylem cylinder consists of inner zone of primary xylem vessel elements and outer zone of secondary xylem which includes xylem fibres and wide circular solitary vessel elements. The vessel elements are 40 µm in diameter (Fig 8.3).

3.1.7. Powder microscopy

Shade dried plant was powdered with the help of an electric grinder till a fine powder was obtained. The stem powder includes xylem fibers, vessel, elements and parenchyma cells.

3.1.8. Xylem fibres

The fibres are long narrow with tapering ends. The walls are thick lignified and the cells are narrow. The fibres are 350-750µm long and 10µm thick.

3.1.9. Vessel elements

The vessel elements are long narrow and cylindrical some of the have short or fairly long tails at both or one end. The vessels elements are single, wide circular perforation at the walls (Fig 10.1). The perforation may be horizontal or slightly oblique. The lateral walls are circular multi serrate and dense (Fig 9.2). The vessel elements are 220-250µm long.

3.1.10. Parenchyma cells

Fairly wide elongate parenchyma cells are seen attached with each other in their pad (Fig 10.2). The tangential walls of the cells are thick walled and there are prominent, circular pits on the tangential walls (Fig 10.2). The parenchyma cells are 10µm in size.

4. DISCUSSION

Traditional medicaments play an important role in our day to day life but only a few poly herbal formulations are accepted in modern medicine due to lack of accurate method for their standardization and evaluation. The main aim of pharmacognostical study is to assess the true identity of the raw material, which would reduce drastically many errors in wrong identification and handling of the final product for the required standard. Thus diagnostic features have been evolved to identify and to discriminate the *Indigoferabarberi Gamble* from other crude drugs and their adulterants. Microscopic evaluation is an important tool for the identification of medicinal herbs and is one of the essential parameters in preparation of modern monograph. The transverse section of the leaf shows dorsiventral nature.

The midrib portion of the leaf shows thick walled epidermal cell, they are squarish in shape; Inner vascular tissues are surrounded by angular parenchymatous ground tissue. The vascular strand has broad, several parallel compact lines of narrow, thick walled xylem elements and a thin of arc of phloem elements also shows the presence of two to three layers of sclerenchyma cells. Epidermal portion of the leaf are shows the presence of both glandular and non-glandular types. Spongy parenchyma is presents in the leaf lamina portion.

The marginal part of the leaf is having small epidermal cells with thick cuticle having anisocytic type of stomata. The stem is circular and young stem is 1.8 µm thick. The stem consists of single layer of epidermis, chlorenchymatous outer cortex and parenchymatous inner cortex. The stem powder shows the presence of xylem fibres, vessel, elements and parenchyma cells.

The phytochemical screenings of various fractions are showed the presence of glycosides, steroids, flavonoids, tannins and carbohydrates results were shown in table 1.

Among this five different factions of plant extracts, dichloromethane and ethanolic factions are shows this major classes of phytoconstituents Behaviour of powder of *Indigofera barberi Gamble* with different chemical reagents is detected results were shown in table 2. The colour changes, when observed under day light and UV-light by method and results are presented in the table 3.The percentage yield of successive solvent extraction, colour of the extract, extracts consistency and their physiochemical parameters are shown in the table 4 and 5.

5. CONCLUSION

Medicinal plant research that look for a finding a pharmacologically and economically valuable lead molecules. Since India having great potential to make use of these resources and skills acquired through experience base in traditional medicines as population as well as for economical benefit. There is an immediate attention necessary for the documentation of our traditional knowledge. In this contest, the present investigation throws light to various standardized parameters such as macroscopy, microscopy, phytochemical screening etc which could be helpful in authentication of *Indigofera barberi Gamble*. The results of present study may also serve as a reference in preparation of standard monograph.

Table 1: Phytochemical analysis of aerial plant extract of *Indigofera barberi Gamble*

Phytocompounds	*Pet. ether extracts*	*Chloroform extracts*	*Dichloromethane extracts*	*Ethanol extracts*	*Water extracts*
Alkaloids	–	–	–	–	–
Glycosides	+	+	+	+	–
Steroids	–	–	+	+	+
Flavonoids	+	+	+	+	+
Tannins and phenolic compounds	+	+	+	+	+
Proteins	–	–	–	–	–
Carbohydrates	+	+	+	+	–
amino acids	–	–	–	–	–
Volatile oils	–	–	–	–	–
Saponins	–	–	–	–	–

(+): presence (-): absence

Table 2: Behavior analysis of aerial plant powder of *Indigofera barberi Gamble* with different chemical reagents

Reagent	*Observation*	*Inference*
Powder + Iodine	Black colour observed	Presence of starch
Powder + $Hgcl_2$	No blue colour observed (Black colour)	Absence of Alkaloids
Powder + Ammonia	Light Pink colour observed	Presence of glycosides
Powder + $AgNo_3$	No precipitate formed	Absence of proteins
Powder + Picric acid	No colour changed	Absence of Alkaloids
Powder + Water shaking	Foam not appeared	Absence of Saponins
Powder + Con. H_2So_4	Black	Presence of Starch
Powder +$FeCl_2$	Bluish black	Presence of tannins
Powder + Con. HNO_3	Orange brown	Presence of tannins

Table 3: Fluorescence analysis of aerial plant powder of *Indigofera barberi Gamble*

Reagent	*Long (365nm)*	*Short (254nm)*	*Visible/Day light*
1 N HCL	Black	Light Green	Olive Green
50%HCL	Black	Light Green	Olive Green
50%H_2SO_4	Black	Light Green	Greenish Yellow
50%HNO_3	Black	Light Green	Light Red
1N NaOH	Black	Green	Dark brown
Alcoholic NaOH	Brick Red	Dark Green	Light Yellow
Water	Black	Green	Light Yellow
Methanol	Reddish black	Green	Green

Table 4: Successive solvent extraction consistency, color, and percentage yield of Indigofera barberi Gamble

Parameters	*Extracts*				
	Petroleum ether	*Chloroform*	*Dichloromethane*	*Ethanol*	*Water*
Consistency	Waxy	Oily	Viscous	viscous	Waxy
Colour (Visible/ Day light)	Greenish black	Green	Brownish green	Reddish black	Cream (Light green)
% Yield	1.24%	3.14%	2.96%	3.28%	2.24%

Table 5: Physicochemical parameters of *Indigofera barberi Gamble*

S. No	*Parameters*	*Result*
1	Total ash	19.5±0.3155
2	Acid insoluble ash	8.25±0.2341
3	Water soluble ash	16.4±0.3041
4	Loss on drying	0.4±0.0115
5	Water soluble extractive value	10.28±0.2768
6	Alcohol soluble extractive value	4±0.1985

Mean (w/w) ± SEM (n = 3)

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