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Pharmacognostical Standardization of *Tephrosia purpurea* Pers Root

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ABSTRACT

Wild Indigo or Purple Tephrosia or fish poison occurs throughout the Indian subcontinent. It is widely used in the treatment of inflammation, diabetes, rheumatism, asthma, diarrhoea and many other ailments. But so far the pharmacognostic standardization has not been reported for its proper identification. Hence the present study is a pharmacognosy work carried out for the root part. This may help in the identification of the plant species. A thin transverse section, powder microscopy, measurement of the dimensions of cell structures, fluorescence analysis and physico chemical parameters were conducted for the root. From the T.S, the secondary xylem fibres and vessels were found to be the tissues of diagnostic importance. The xylem vessels were of two types: narrow and long; broad and short. The important characters in the powdered microscopy were vessel elements, fibres and xylem parenchyma cells. The different fluorescent light shades were obtained under short and long UV light for both powder as well as the extracts of the root. The proximate analysis values were also obtained in a satisfactory way. Combining all these data a suitable root profile for plant can be constructed which may help in the identification of quality of the plant part.

Key words: *Tephrosia purpurea, rotenone, transverse section, fluorescence analysis, vessel elements*

Introduction:

Tephrosia purpurea Pers. is a pan tropical coastal shrub that grows up to 1 m in height[1]. It is also known as ahuhu ʻauhola, or hola[2]. It is a species of flowering plant in the pea family, *Fabaceae*. The plant is an erect herb with leaf lets obovate, pubescent and ovate. It has pinkish blue or purple colored flowers. The fruits are pods and seeds are with strophiole in the middle. The roots of the plant are used in the treatment of various ailments like dyspepsia, diabetes, rheumatism, asthma, diarrhea, urinary complaints and cough. The whole plant is used to treat ulcers, fever and liver disorders. The pods are used in vomiting and inflammation[3]. Previous phytochemical investigations on this plant have shown the presence of coumarins, flavonoids, rotenoids, flavanones, isoflavanones and quercetin[4-10]. Recently invented constituents are an isoflavone, 4-dihydroxy-3, 5 dimethoxyisoflavone, chalcone, tephropurpurin, purpurin, pongamol, lanceolatin B, maackiain, 3-hydroxy-4-methoxy-8, 9-methylene-dioxypterocarpan and medicarpin. Three novel flavonoids, (+)-tephrorins A and B and (+)-tephrosone, were isolated from *Tephrosia purpurea*[11,12]. The toxic properties of *T. purpurea* are due to the presence of flavonoids, rotenone and several of its isomers named deguelins. One of the deguelins, tephrosin, is poisonous to fish, but not to mammals. The leaves contain up to 2.5% rutin[13].

Methods:

Plant materials:

The plant specimen for the proposed study was collected from Talakona region, Thirupathy in Andhra Pradesh, South India. It was identified and authenticated by Dr. Madhava Chetty, Taxonomist, S.V University, Thirupathy. A herbarium specimen (NCOP-NLG/ph'cog/2009-10/011) was prepared and deposited in Department of Pharmacognosy, Nalanda College of Pharmacy, for further reference.

Transverse section of root

The roots were taken cleaned and fixed in formalin, acetic acid and ethanol. After twenty four hours of fixing the specimens were dehydrated with graded series of tertiary butyl alcohol[14]. Infiltration of the specimen was carried out by gradual addition of paraffin wax until the solution attained super saturation. The specimens were cast into paraffin blocks and sectioned with the help of rotary microtome. Dewaxing was done by customary procedure[15]. The sections were stained with toluidine blue, safranin, fast green and iodine[16]. Photo micrographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells polarized light were employed. Magnifications of the figures were indicated by the scale bars. Temporary preparations of the slide were made by mounting on glycerin. The terms described in the anatomical features are as per standard books[17-19].

Powder microscopy

Shade dried root of the plant was powdered with the help of an electric grinder till a fine powder was obtained. Powdered materials of roots were cleared with sodium hydroxide and stained with phloroglucinol: concentrated hydrochloric acid (1:1) and mounted in glycerin medium[20].

Measurement of dimensions of cell characters

Different cell structures were studied and measured microscopically as per procedures mentioned by Kokate[20].

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Fluorescence analysis

The root powder was individually mixed with different solvents like dilute hydrochloric acid, dilute sulphuric acid, 50% nitric acid, 5% potassium hydroxide 40% sodium hydroxide and acetone. They were observed under the short UV light (254nm) and long UV light(365nm) to detect the emission of fluorescence[21,22]. The 25g of root powder was exhaustively extracted in a soxhlet apparatus with different solvents like petroleum ether, chloroform, ethyl acetate and methanol. The crude extract obtained was subjected to fluorescence analysis under short and long UV light.

Determination of physico chemical parameters

The various physicochemical parameters like ash values and extractive values were determined. They were performed according to the official methods prescribed in standard books[23,24].

Results

Transverse section of root

The root measuring 110mm in diameter was studied to identify the anatomical structures and arrangement of the different type of cells present (Fig.1-3). The root had a distinct rhizodermis or epidermis which was broken at certain places. When it was intact the rhizodermal cells were wide and cylindrical. The cortex had 2-3 layers of highly dilated compact thin walled cells. No periderm was evident. Phloem cells were found in thin layer around the xylem. The phloem elements were not preserved in the material except at certain regions.

Secondary xylem was a solid cylinder. It consisted of a wide central core of pith where the cells were wide thick walled and lignified. Outer to the central portion were xylem elements which were in regular radial rows, they were rectangular in outline thick walled and lignified. The vessels were scanty, they could be recognized by wide lumen and angular outline. Major component of secondary xylem were fibres.

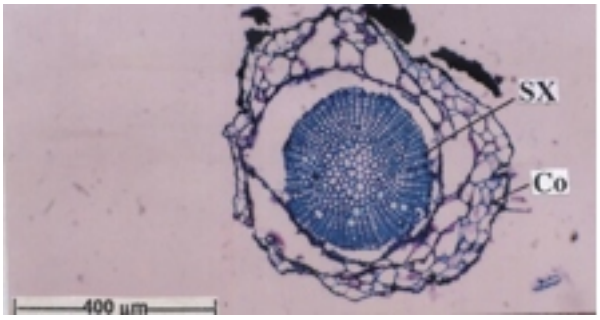

Figure 1. T.S of root-entire view

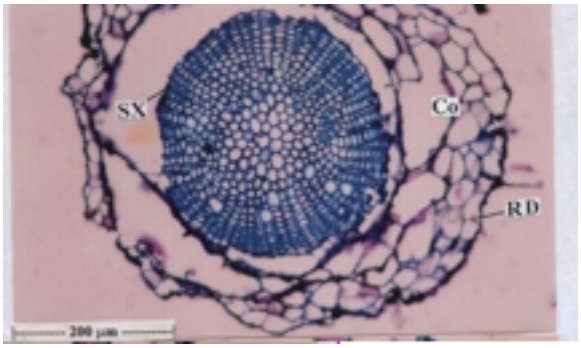

Figure 2. T.S of root-entire view (under high magnification)

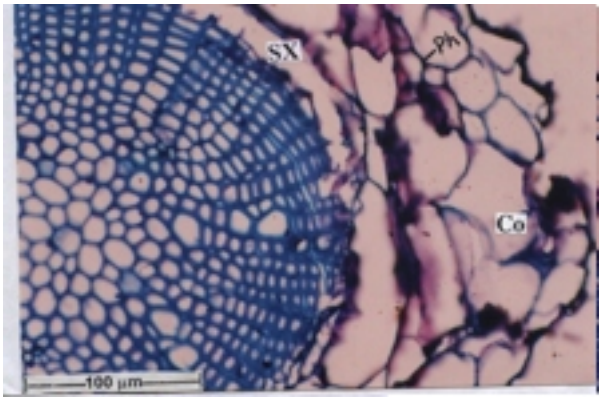

Figure3. T.S of root-A sector enlarged

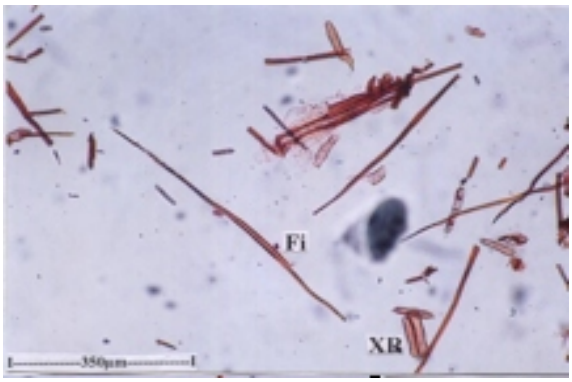

Figure 4. Fibres and parenchyma cells.

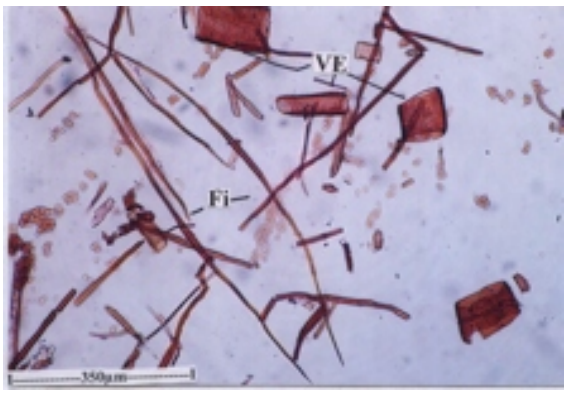

Figure 5. Fibres and vessel elements.

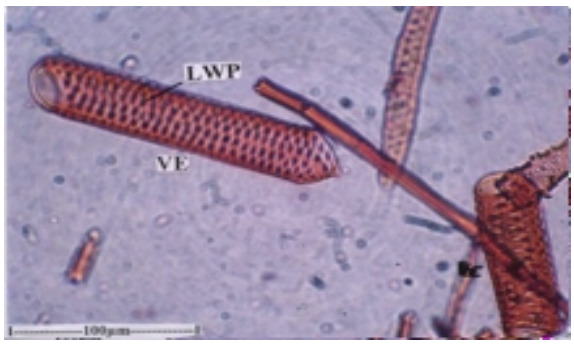

Figure 6. Structure of vessel elements-long narrow vessel element.

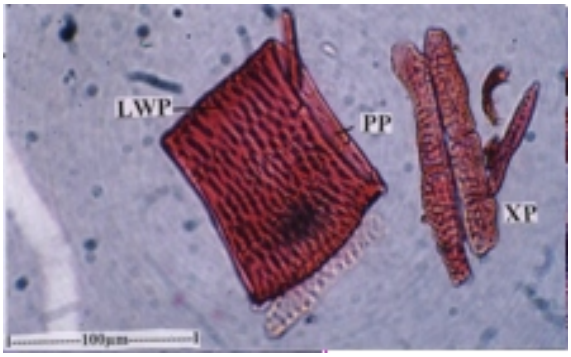

Figure 7. Structure of vessel elements-short, broad vessel element.

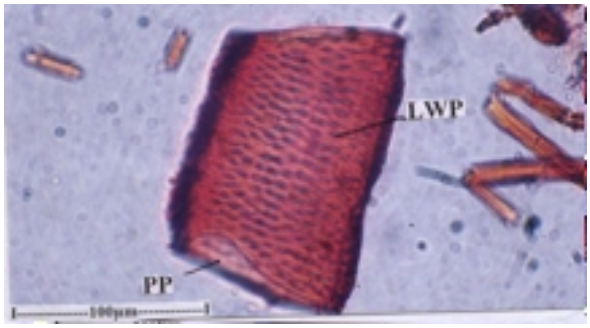

Figure 8. Short, broad vessel element.

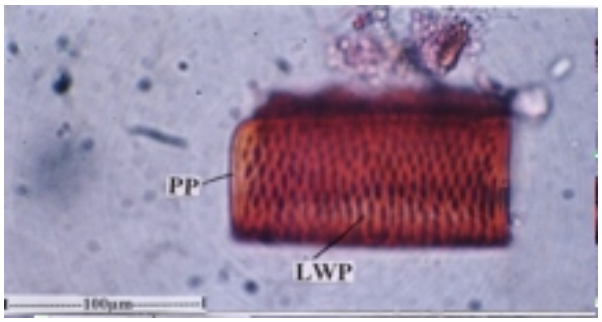

Figure 9. A vessel element showing lateral wall pits with perforation plate.

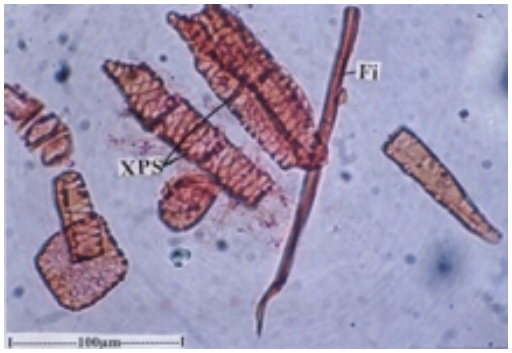

Figure 10. Fibre and xylem parenchyma strand.

Legends for the pictures in the transverse section

Co-cortex; Ph-Phloem, RD-Rhizodermis, SX-secondary xylem.

Powder microscopy

Vessel elements, fibres and xylem parenchyma cells were the common inclusions in the root powder. Xylem fibres were long wider in the middle and narrow and tapering at the ends. The walls were thick and lignified. Pit was not evident (Fig.4,5).

Vessel elements were equally abundant as the fibres. Some of the vessel elements were long, narrow and cylindrical (Fig.6). Others were short wide and drum shaped (Fig.7,8).

Some of the vessel elements were wide, long and cylindrical. The perforation plate was wide simple and slightly oblique (Fig.9). The lateral wall pits were either circular or horizontally elongated and elliptical. The pits were multiseriate, alternate and densely crowded.

Xylem parenchyma:

Cells found with in the xylem cylinder such as parenchyma cells were frequently met with in the powder. These cells were square shaped or rectangular and thick walled. The walls were unlignified. Abundant simple pits were seen in the cell walls (Fig.10).

Legends for the figures of powder microscopy

Fi-Fibres, VE-Vessel element, XP-Xylem parenchyma, LWP-Lateral wall pits, PP-Perforation Plate, XPS-Xylem parenchyma strands.

Measurement of dimensions of cell characters

The dimensions obtained are tabulated in Table1. The secondary xylem was found to possess long fibres and wide vessels. The dimension obtained for the xylem parenchyma shows that it is polygonal in shape.

Table 1. Dimensions of cell structures

Cell Structure	Measurements
Secondary xylem	290µm in diameter
Xylem fibres	10 µm wide 500 µm -800 µm long
Xylem vessels	20 µm wide 80 µm -130 µm long
Xylem parenchyma	40 µm ×40 µm × 20 µm × 70 µm

Table 2. Fluorescence analysis of root powder

Reagent used	Long UV light (365 nm)	Short UV light (254 nm)	Day light
Powder +HCl	Cream colour	Light green	Yellowish brown
Powder +40%NaOH	Straw colour	Light green	Light brown
Powder + 5% KOH	Dark brown	Green	Yellowish brown
Powder+50%HNO_3	Black	Green	Deep orange
Powder +H_2SO_4	Off white colour	Pale green	Brown
Powder +acetone	Emerald green	Pale green	Brownish yellow

Table 3. Fluorescence analysis of root extract

Extract	Long UV light (365 nm)	Short UV light (254 nm)	Day light
Petroleum ether extract	Black	Greenish black	Dark brown
Ethyl acetate extract	Black	Brownish black	Brown
Chloroform extract	Greenish brown	Black	Dark brown
Methanol extract	Parrot green	Olive green	Reddish brown

Table 4. Physico chemical values

Parameters	Values
Total ash	7%w/w
Acid insoluble ash	4%w/w
Water soluble ash	6%w/w
Alcohol soluble extractive	8%w/w
Water soluble extractive Crude fibre content Loss on drying	11.2%w/w 29.5%w/w 14%

Fluorescence analysis

The fluorescence analysis performed for the powdered drug as well as the extracts are tabulated in Table 2& 3. For root powder the long UV light produced a colour ranging from cream to black whereas in short UV different shades of green colour was seen, in day light shades of brown and orange were noted. For the root extract the petroleum ether extract, chloroform and ethyl acetate extract produced colours ranging with in black and green, whereas the methanolic extract exhibited quite different colours like parrot green, olive green and reddish brown colour.

Physico chemical parameters

The physico chemical parameters like ash values and extractive values are tabulated in Table 4. Total ash, loss on drying and crude fibre content was found to be 7%w/w, 29.5%w/w and 14% which can be assumed as a standard for the drug.

Discussion

Microscopical evaluation is indispensable in the initial identification of herbs as well as in detection of adulterants and identifying the plant by characteristic tissue features. The important distinct feature observed in the T.S was the distinct rhizodermis and the major portion of the xylem fibres. The tissue of diagnostic importance can be concluded as the xylem fibres which have taken up the majority of the section. In the powdered microscopy abundant fibres and xylem parenchyma were seen which is an identifying tool for the root of the plant. The values obtained for the linear measurements will help in the identification of adulterants or in distinguishing the various species of *Tephrosia.* Fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colors and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration. The root powder has exhibited different colour shades in the long and short UV light which can be utilized in detecting impurities. In the case of the root extracts the petroleum

ether, chloroform and ethyl acetate extracts produced similar kind of fluorescent colour but the methanol extract produced entirely different fluorescence which can be an effective tool while setting the standards. The significance of performing the ash values is to find out the amount of inorganic impurities, resistant materials like sand, soil and stone in crude drugs. The ash obtained after incinerating the powdered root is of significance as this usually consists mainly the carbonates, phosphates, silicates and silica. The values obtained in the work reflect the presence of these substances as the sand, soil and stones often contain these minerals and hence it assumes that the values obtained can be set as a standard reference.

Standardization is the prime need of time. These help in the establishment of quality and identity profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. *Tephrosia purpuea* is known as 'Sharpunkha' in Sanskrit and is given the name of 'Sarwa wran vishapaha' in Ayurvedic literature which means it has the power to cure all kinds of wounds. Hence it is very essential to establish a Pharmacognostical standardization. The results obtained in the present investigation are encouraging and can be used as an effective reference data for the standardization of *Tephrosia purpurea root.*

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