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Pharmacognostical Evaluation Study on *Crotalaria juncea* Linn

Sathis Kumar Dinakaran, David Banji, Prashanthi Godala and A. Harani

Nalanda College of Pharmacy, Charlapally, Nalgonda-508001 Andhra Pradesh, India

Abstract: This paper deals with the detailed pharmacognostical evaluation of the *Crotalaria juncea* L. (Fabaceae). Morphology of the entire plant have been studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO recommended physio-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establishes the authenticity of *Crotalaria juncea* L.and can possibly help to differentiate the drug from its other species /varieties.

Key words: *Crotalaria juncea* L. · Microscopy · Nutritional value · Microscopy · Preliminary chemical tests

INTRODUCTION

Crotalaria juncea L. belongs to Fabaceae family, widely distributed in the tropical and subtropical regions of India, Nepal, Sri Lanka and Southern Africa. It is commonly known as Sun hemp or Indian hemp [1]. It has great potential as an annually renewable, multi-purpose fiber crop. It is used as a medicine, edible, culinary purpose by many tribal communities [2]. In the folk [3] and Ayurvedic medicines [4], it is used as blood purifier, abortificient, astringent, demulcent, emetic, purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis[5]. It is an annual, erect, stiff branched, half-woody herb, usually about 1 meter high, with all the parts finely hairy. Leaves are simple, linear-oblong to oblong, 4 to 10 cm in length. Leaf is simple, 2.5-10.5 cm long, 6-20 mm broad, linear or oblong, obtuse or subacute, apiculate, pubescent on both sides, hairs appressed, silky; petiole 1.2-2.5 mm long; stipules almost absent. Flowers are large; in erect terminal & lateral 12-20 flowered racemes often reaching 30cm.long; Inflorescence an erect terminal and lateral raceme, up to 30 cm long, 12-20-flowered, pedicles are 3-6 mm long pubescent; bracts minute, linear subulate; bracteoles 2, beneath the calyx, minute, linear-subulate [6]. Calyx is 2 cm long, clothed with fulvous hairs; teeth linear lanceolate, very deep. Corolla is bright yellow slightly exserted; standard ovate oblong subacute. Pods are 2.5-3 cm long, sessile, clothed with short fulvous silky hair. Seeds are numerous, small, flattened, dark-gray to black, loose in the pod at maturity, 33,000 seeds per kg [7]. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals. Hence, in the present work the pharmacognostical standardization has been performed for the leaf of the plant.

MATERIALS AND METHODS

Collection and Authentication: Plant material of *Crotalaria juncea* L. (CJ) was collected from local areas of Nalgonda andhra Pradesh and plant was authentified by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda andhra Pradesh. Plant was dried in the shade and ground into uniform powder using milling machine [8]. For the microscopic studies, transverse sections were prepared and stained [9]. The leaves were boiled separately with saturated chloral hydrate solution for surface studies and quantitative microscopical observation of leaf [10].The selected whole plant of CJ was subjected to organoleptic (colour, odour and taste were recorded) [11], microscopic (essential for powdered crude drugs consist of the fragments of cells in the form of recognizable tissues and the study of surface constants like fibres, lignified vessel, epidermal cells, calcium oxalate crystals, starch grains, etc.) [12] and physico-chemical values such as the percentage of total

Corresponding Author: Dr. Sathis Kumar, Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh, India.
Tel: +91 8682 247910, Mob: +91-9966796051.

ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractives, crude fiber content and foreign matter were calculated as per the Indian Pharmacopoeia [13] and fluorescence analysis of crude powder were estimated using various chemical and organic reagents [14].

Determination of Stomatal Index: Leaf fragments were observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, vein islet number and veinlet termination number. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.

Preparation of Extracts: The extracts of leaves of CJ were prepared by successive soxhlation with various solvents. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (60-80°C), chloroform and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 hours to obtain the aqueous extract. Each extract was concentrated by evaporating the solvent on the water-bath and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were tabulated.

Phyto Chemical Screening: All the extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents viz. triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, etc. were detected by usual prescribed methods.

RESULTS AND DISCUSSION

Macroscopic Studies: The macroscopical studies revealed that Leaves are simple 3-10.5 cm long, 1-2 cm broad, linear or oblong, obtuse or subacute, apiculate, reticulate venation with entire margin, lanceolate lamina. Stems are erect, 2 cm in diameter, cylindrical and ribbed. Much branched and lobed nodules, up to 2.5 cm in length. Roots are appeared in brown colour, 10.5-12.5 cm long, 0.3-1 cm broad, strong taproot and having well developed lateral roots. Organoleptic properties are green color, characteristic taste and odour.

Transverse Section of Leaf: The transverse section of the leaf showed dorsiventral nature. It was divided into three sections epidermis, mesophyll & vascular bundles. Epidermis was found on either side of the leaf i.e. Upper epidermis and Lower epidermis. Upper epidermis was single layered rectangular epidermal cells with distinct cuticle and it consists of covering trichomes. Mesophyll was differentiated into palisade and parenchyma. Palisade cells were elongated arranged compactly in single layer and were discontinued over midrib. Parenchyma consists of loosely arranged 4-5 layers of parenchymatous cells. Lower epidermis was single layered with rectangular cells. Beneath the lower epidermis was two layered with collenchymatous cells. Vascular bundles were found to be open type; these were enclosed by a bundle sheath. Lower portion consists of ground tissue (Fig. 1).

Transverse Section of Stem: It consists of different regions from outside to inside. Epidermis was the outer most layers with closely arranged cells and they are elongated. These cells a re covered with cuticle.It may produce a few unicellular stem hairs. Hypodermis consists of closely arranged chlorenchymatous cells with thin walled. Cortex consists of thin walled parenchymatous cells. Pericycle was below the endodermis and it consists of sclerenchymatous cells. Vascular bundles were arranged in a ring just inside the pericycle. Each bundle consists of phloem on the outside, xylem on the inside. These are called collateral vascular bundles. Medullary rays were the regions between the vascular bundles. Pith constitutes the central region of the stem with loosely arranged parenchymatous cells (Fig. 2).

Transverse Section of Root: The transverse section of the root was found to be different regions. Epiblema was found to be the outer most layers. The layer was covered with the cuticle. It consists of thin walled rectangular parenchymatous cells. These cells were arranged compactly without intercellular spaces. Cortex was found to be just beneath the epiblema. It consists of loosely arranged thin walled parenchymatous cells. Endodermis was constituted by the inner cortical layers. Pericycle was found to be lying inner to the endodermis. Vascular bundles were radially arranged. Medullary rays were found in between vascular bundles (Fig. 3).

Powder Microscopy: In the powdered preparation, Square shaped Calcium oxalate crystals were present. Xylem vessels were found to be lignified, pitted walls and some of these having spiral arrangements. Cork cells were found to be thin walled & polygonally arranged. Xylem and phloem parenchymatic cells, spongy parenchyma, unicellular trichomes and anisocytic stomata were

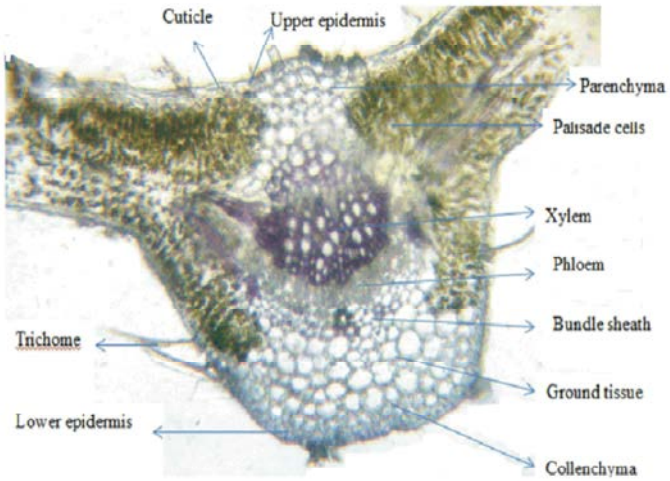

Fig. 1: Leaf T.S of *Crotalaria juncea* L.

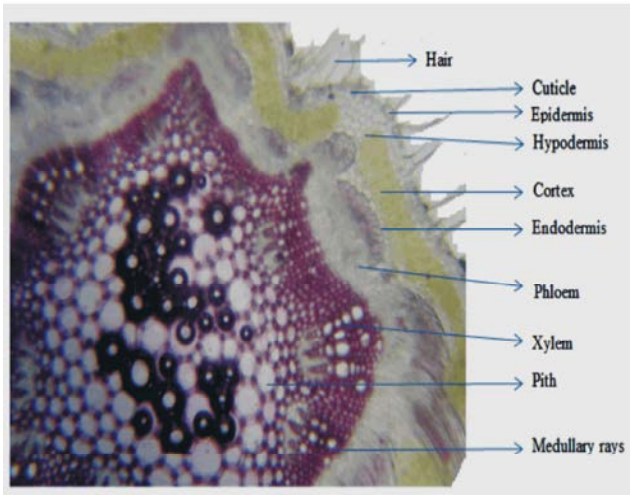

Fig. 2: Stem T.S of *Crotalaria juncea* L.

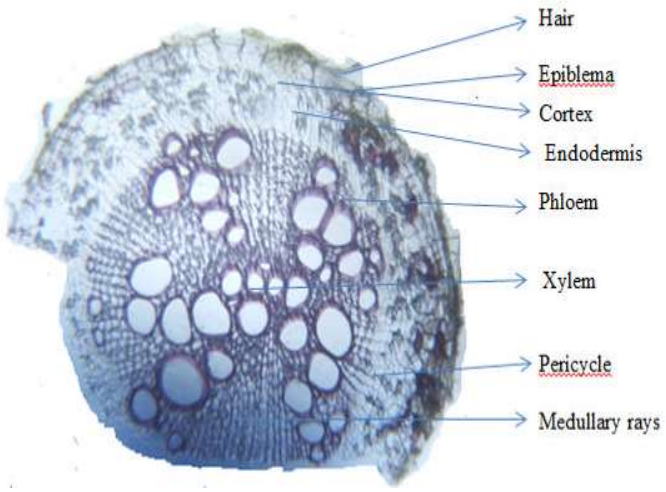

Fig. 3: Root T.S of *Crotalaria juncea* L.

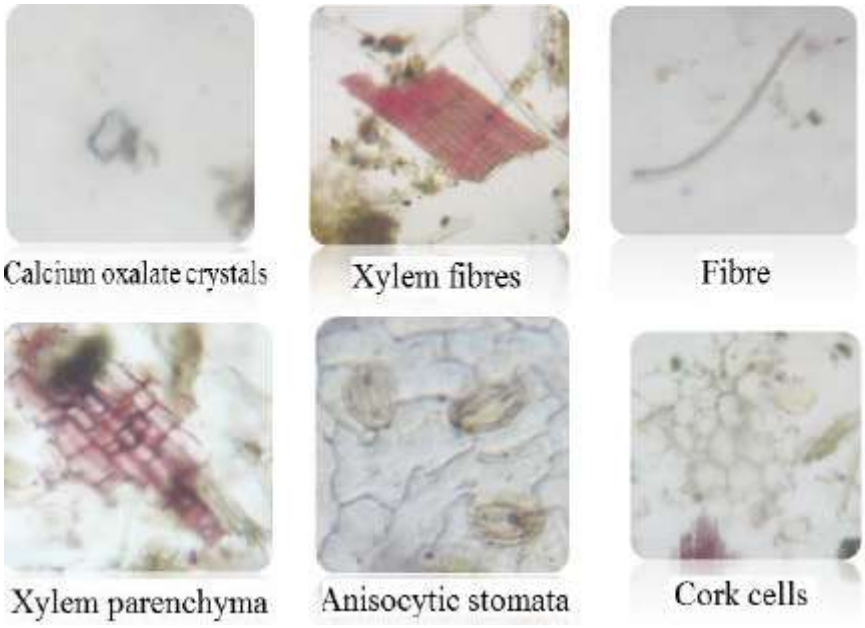

Fig. 4a: Powder microscopy studies

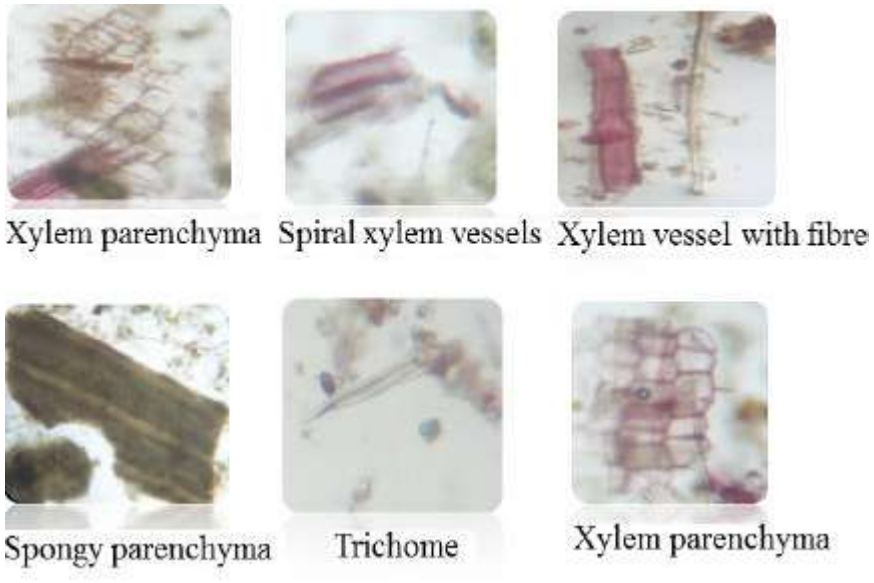

Fig. 4b: Powder microscopy studies

Fig. 4c: Powder microscopy studies

Table 1: Quantitative microscopy of leaf / leaf powder of *Crotalaria juncea* L.

Parameter	Value
Phloem fibers (width)	2-6µ
Phloem fibers (length)	32-46 µ
Starch grains (diameter)	2-3µ
Calcium oxalate crystals (length)	2-6/mm^2
Calcium oxalate crystals(width)	2-4/mm^2
Stomatal number (lower epidermis)	19
Stomatal index (lower epidermis)	25.6
Stomatal number (upper epidermis)	13
Stomatal index (upper epidermis)	20.9
Vein islet number	9/ mm^2
Veinlet termination number	6 / mm^2
Palisade ratio	1:4
Length of Unicellular Trichomes	14-20 µ
Width of Unicellular Trichomes	4-6 µ
Length of xylem vessels	38-82µ
Width of xylem vessels	8-16 µ

Table 2: Physico chemical properties of *Crotalaria juncea* L.

Quantitative Parameter	Values obtained %w/w
Total ash value	5.9
Acid insoluble ash	2.7
Water soluble ash	3.9
Sulphated ash	5.1
Moisture content	11
Foreign matter	0.04
Alcohol soluble extract value	5.84
Water soluble extract value	20.4
Crude fiber content	52.6

Table 3: Fluorescence analysis of *Crotalaria juncea* L.

Solvents used	Daylight	UV light (254nm)	UV light (366nm)
Conc.H_2SO_4	Brown	Greenish brown	Brownish black
50%H_2SO_4	Pale brown	Green	Brown
Conc.HCl	Green	Yellowish green	Black
50%HCl	Pale brown	Pale green	Brownish black
Ammonia	Pale yellow	Green	Black
HNO_3	Brownish yellow	Greenish yellow	Brownish black
50%HNO_3	Pale brown	Brownish green	Black
5% $FeCl_3$	Greenish yellow	Green	Brown
5% KOH	Light yellow	Pale yellow	Black
5% NaOH	Yellow	Green	Greenish black
1N KOH	Pale yellow	Light yellow	Brownish black
1N NaOH	Brownish yellow	Blackish green	Brownish black
Methanol	Green	Greenish yellow	Greenish brown
1NMethanolic KOH	Greenish yellow	Light yellow	Greenish black
1NMethanolic NaOH	Brownish yellow	Green	Blackish brown

Table 4: Qualitative Preliminary phytochemical studies of *Crotalaria juncea* L.

Extracts	Pet. ether	Chloroform	Alcohol	Water
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	-
Alkaloids	-	+	-	-
Proteins	-	-	-	-
Aminoacids	-	-	+	+
Phenolic compounds	-	-	-	-
Steroids	-	-	-	-
Tannins	-	-	-	-
Saponins	-	-	-	+
Volatile oils	+	+	+	+
Fats & Oils	-	-	-	-
Glycosides	-	-	-	-

+ present, - absent

observed. Starch grains present were circular to oval in shape. all The results of quantitative microscopy of leaf / leaf powder of CJ were present in Table 1.

Physico Chemical Properties: The physico-chemical characters of powdered drug of whole plant of CJ such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol soluble extractives, moisture content and foreign matter were presented in Table 2. The fluorescence analysis of the powdered drug of CJ in various solvents and chemical reagents was performed under normal and Ultra Violet (UV) light (Table 3).

Preliminary Phytochemical Screening: After extraction with different solvents, the residues were dried and measured. The residue obtained was 0.8%, 1.6%, 7.88 % and 13.52%w/w for petroleum ether, chloroform, ethanol and water extract of CJ, respectively. The yellowish green, greenish black, brownish black and brownish black residues were for petroleum ether, chloroform, ethanol and water extract of CJ, respectively. The petroleum ether and chloroform extracts were sticky in nature. The ethanol and water extracts were gummy and powdery in nature respectively. The preliminary phytochemical investigation of the petroleum ether, chloroform, ethanol and water extracts of CJ showed the presence of carbohydrates, aminoacids, volatile oils, alkaloids, flavonoids, saponins (Table 4).

DISCUSSION

As a part of standardization study, the macroscopically examination of CJ was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the CJ can serve as diagnostic parameters. The extractive value, ash value, moisture content and fluorescent analysis of whole plant extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug from the CJ in various solvents was performed under normal and UV light. All the whole plant extracts are examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent constituents.

CONCLUSION

The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

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