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Research Article

PRELIMINARY PHYTOCHEMICAL AND PHARMACOGNOSTIC STUDIES ON *TEPHROSIA COLLINA* VAR *LANUGINOCARPA* V.S.SHARMA– AN ENDANGERED SPECIES OF WESTERN INDIA

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ABSTRACT

Introduction: *Tephrosia collina* var *lanuginocarpa* V.S.Sharma is an extremely rare, endangered plant species of western India. It is distributed in Gujarat and Rajasthan.

Objective: The present study gives first insight of anatomical, pharmacognostical, histochemical, physicochemical and preliminary phytochemical analysis of the root, stem and leaf of *T. collina* var *lanuginocarpa.*

Method: All the analysis was done according to WHO norms.

Results: The anatomical features seen in the root, stem and leaf of this plant species is presences of rhomboidal calcium oxalate crystals in medullary rays and pericycle. The micro-morphological parameters such as the stomatal index, palisade ratio and vein-islet number were distinctly quantified. The powder study showed the presence of warty unicellular trichomes and anisocyctic stomata in leaf and stem, along with presence of the rhomboidal calcium oxalate crystals in the leaf, stem and root powder. The histochemical test performed showed the presence of starch grain, lignin and calcium oxalate crystal. The physicochemical parameters such as ash values, extractive values and loss on drying of all the three plant parts have been studied. The phytochemical screening shows the presence of carbohydrates, lignans, flavonoids, Quinones and anthocyanidins in all the three plant parts. The results of these studies provide referential data for identification of some diagnostic indices for identification, authentication and preparation of monograph of this plant.

Keywords: *Tephrosia collina* var *lanuginocarpa* V.S.Sharma, Anatomy, Micromorphology, Phytochemical, Calcium oxalate crystals.

INTRODUCTION

The *Tephrosia collina* var *lanuginocarpa* is one of the extremely rare and endangered plant species of genus *Tephrosia* pers. [1] This species of *Tephrosia* have restricted distribution in semi arid region of India's states like Gujarat and Rajasthan. [2, 3, 4] In both geographical locations, this species is found within small area confined to hills, among montane grasses. This species was first discovered by V.S.Sharma from Ajmer district Rajasthan.[5] Later this species was reported rare from the Rajpipla, southern Gujarat[6] and Motividi Jamjodhpur Taluka from western Gujarat.[1] Till today, only morphological description of this rare endangered species (*T. collina* var *lanuginocarpa*) has been scientifically documented. Thus the present research is fresh look on the anatomical, pharmacognostical, histochemical, physicochemical and preliminary phytochemcial analysis of root stem and leaf of this rare endangered species.

MATERIALS AND METHODS

Collection

T. collina var lanuginocarpa was collected in October 2012 from Motividi, Jamjodhpur Taluka, Jamnagar, and Gujarat, and were grown at arboretum of the M. S. University of Baroda, Vadodara Gujarat, India. It was identified, authenticated and deposited at Botanical Survey of India (BSI) Arid Zone Jodhpur, Rajasthan. (The voucher specimen No.BSI/AZC/112012/Tech/2012-12 (pl.id)/550).

Physicochemical parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive values and alcohol soluble extractive, swelling index , loss on drying and foaming index of the all three parts of the plants species has been calculated as per WHO guidelines.[7,8]

Anatomy

The free hand sections of all the three plant parts were done. Sections of 10-15µm thickness were selected. These selected sections were stained with safranin (0.5%) in water and the mounted in 50% glycerine. The slides were the observed in the tissues were measured using an ocular micrometer. The least count of the micrometer was calculated for this purpose. The sections were photographed under Lecia DM 2000 Microscope connected to digital Canon Camera.

Micromorphology

Fresh plant materials were washed and small fragments of leaves were taken from the middle region of the lamina of the mature leaves. The epidermal layer was peeled off with the help of pointed needle or blade and was washed in water, stained with safranin (0.5%) and then mounted of slide and viewed under the microscope. Stomatal index, palisade ratio, vein termination number and vein islet number were then calculated using standard procedures. [9,10]

Powder Studies

Completely dried plant material was finely powdered and sieved through BSS mesh No.44. The fine powder obtained was stained using safranin in water. The stained powder was mounted on a slide and observed under a microscope to locate and identify the distinguishing characters. The Characters observed were photographed under Lecia DM 200 microscope connected to digital Canon Camera.

Histochemcial Tests

Specific reagents for identification of important classes of compounds were prepared according to procedure prescribed in the WHO guidelines. Sections and powder of roots, stem and leaves were treated with reagents and mounted on slides for observations under microscope. [8]

Preliminary Phytochemical Screening

The coarsely powdered leaves, stem and roots were extracted with different solvents by soxhlet's apparatus and analyses using simple chemical tests for preliminary screening of various groups of phyto-constituents such as carbohydrates, proteins, saponins, tirterpenoids, iridiodes, alkaloids, flavonoids and so on as WHO guidelines. [11]

RESULT AND DISCUSSION

Physicochemical parameters

Various physicochemical parameters of powdered drug are shown in Table 1. Ash values used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. The values of total ash, water soluble ash, acid insoluble ash and water extractive of leaves in *T. collina var lanuinocarpa* are higher in comparison to root and stem. Moreover the alcohol extractive of Stem was higher then root and leaves.

Table 1: Physicochemical parameters of *T.collina* var *lanuginocarpa*

Parameters	Values		
	Root	Stem	Leaves
Loss on drying	7.6%	7.6%	7.2%
Total ash	2.16±0.18	2.31±0.11	9.40±0.25
Water soluble ash	1.09±0.08	1.05±0.02	2.58±0.07
Acid insoluble ash	0.23±0.03	0.14±0.08	1.02±0.09
Water extractive	0.675%	7.1%	12.75%
Alcohol extractive	3.75%	5.43%	5.25%
Foaming index	<1	<1	<1
Swelling index	ABSENT	ABSENT	ABSENT

Anatomy

The transverse section of *T. collina* var *lanuginocarpa* root (Figure no.1-A and B) is 1-10mm thick and circular in outline. The epidermis one layer is replaced by many layer phellem. Phellem arises from outer layer of cortex. The cork is 198-365 µm composed of small squares cell of 31-36 X 67-79 µm. The ground tissues of cortex four-five layer composed of parenchyma (81.3-114 X 53-59 µm). The phloem (36-94 X 46-127 µm) is composed of sieve tube and phloem fiber. The 2/3 of the area of root is occupied by secondary xylem composed of xylem fiber, trachieds and xylem vessel of 38-120 X 27-141µm size. The pith was not observed.

The transverse section of the stem (Figure no.2-A, B and C) is slightly angular in outline with 1-10mm thickness. The epidermis is single layered (15-29 X 25-59 µm)), covered by thick cuticle and uniseriate warty trichomes (241-373 X 12-60µm) with narrow lumen. The hypodermis consist of collenchymas (29-70 X 15-50 µm) patches alternating with chlorenchyma (117 X 80 µm) patches. The endodermis is single layered, encircling the sclerenchymatous pericycle 3-4 layered (216.4 X 111.1 µm). Pericycle shows presence of prim shape calcium oxalate crystals (28X 49 µm). Secondary phloem (21-44 X 23-45µm) encircles secondary xylem composed of sieve tube and phloem parenchyma. The secondary xylem consists of medullary ray, xylem vessel (81.3X 83.36µm) and trachied. Pith is parenchymatous show presence of starch grain and center portion is voids.

The leaf is dorsiventrally flat, transverse section (Figure no. 3) shows presences of upper and lower epidermis (21-39 X 18-35 µm) covered by thick cuticle (13-28µm) and along with trichomes and stomata. Stomata (47 X 77 µm) are anisocyctic type and trichomes are unicellular warty (241-372 X 12-60µm). The mesophyll tissue is differentiated into palisade and spongy. Palisade composed of elongated, linearly arranged rows of 2-3 cells. Spongy tissue is made up of three layers of rounded loosely arranged cells with little air space. The midrib portion of leaf contains vascular bundles with sclerenchymatous pericycle (97-123µm) encircling on either side of it. Pericycle show the presence of prim shape calcium oxalate crystal (73 X 32 µm). Phloem composed of few thin walled cells. Xylem composed of 4-5 smaller size vessels (50-98 X 46-87µm) and few trachieds with protoxylem pointed upwards. The ground tissue is parenchymatous (36-121 X 46-107µm) and toward either side epidermis there is double layered of collenchymas (36-50 X 39-54µm) hypodermis.

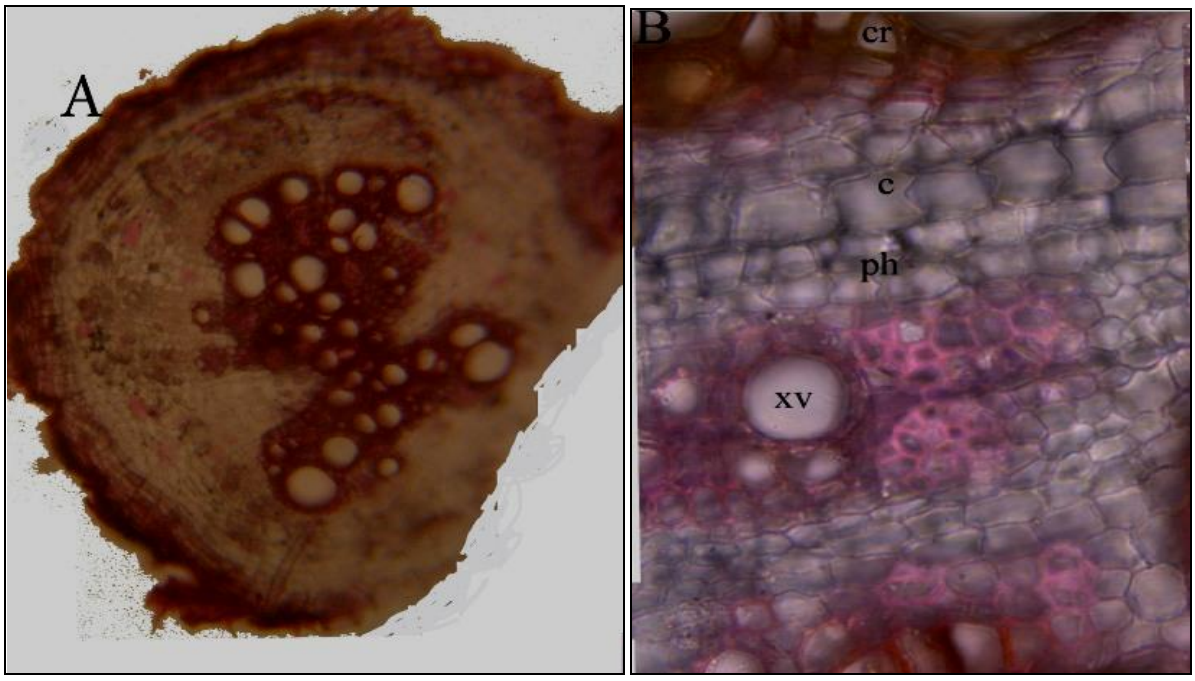

Fig. 1: (A) T. S of root of *T. collina* var *lanuginocarpa,*(B) Magnigied position of root cortex; cr-cork, c-cortex, ph-phloem, xv- xylem vessel.

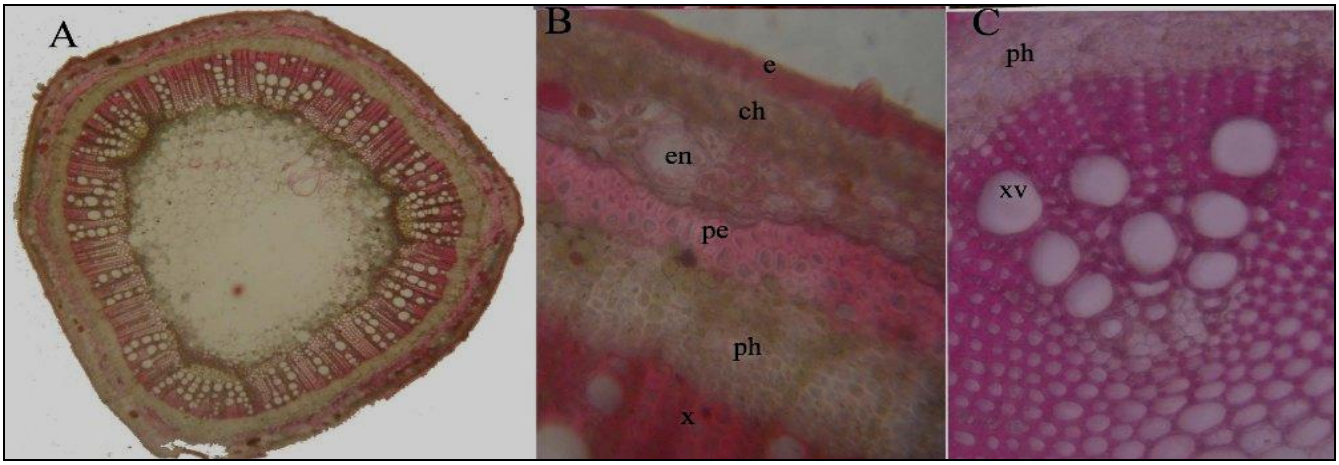

Fig. 2: (A) T. S. of stem, (B) Magnified Portion of cortex of stem; e-epidermis, ch-chlorenchyma, en-endoermis, pe pericycle,ph-phloem and x-xylem. (C) xv-xylem vessel.

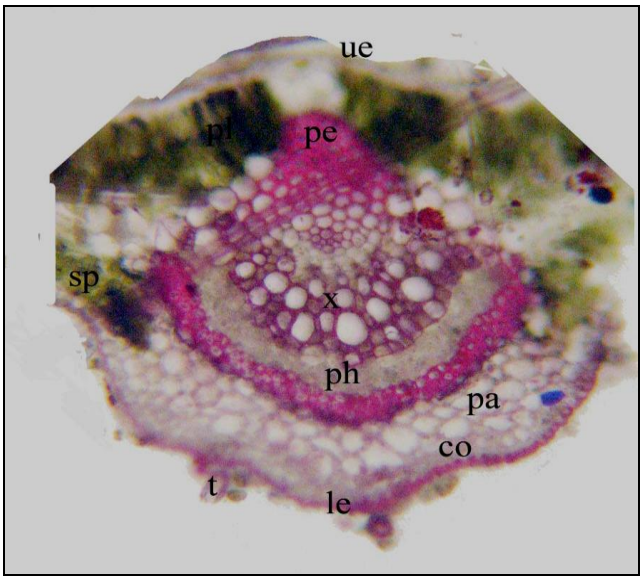

Fig. 3: T.S of Leaf; ue-upper epidermis, pl-palisade, pe-pericycle, sp-spongy, ph-phloem, pa-parenchyma, co-collenchyma, t-trichome, le-lower epidermis

Micromorphology

The leaves and stem of the *T. collina* var *lanuginocarpa* show the presence of the anisocytic stomata and warty unicellular trichomes (Figure no.4- A and B). The values of the stomatal index, vein termination no, vein islet no and palisade ratio are given in Table 2.

Powder study

The powder of leaves is dark green, stem light green and root is brownish in colour. The powder of all the three plants parts are coarse in texture, bitter in taste and have a characteristic odour. The microscopic observations of all three plants showed following characteristic. The warty unicellular elongated trichome, anisocytic stomata, palisade, spongy, parenchyma and collenchymas in powder of leaves and stem, chlorenchyma in stem and cork cell in root powder, xylem vessel showing large rhomboidal calcium oxalate crystals xylem vessel with annular and pitted thickening. (Figure no. 5,A-F)

Histochemical tests

The results of histochemical analysis are given in Table 3.

Preliminary phytochemical screening

Preliminary Phytochemical screening revealed the presence of Carbohydrates, protein, lignans, anthocyanidin flavonoids and quinones given in Table 4.

Table 2: Results of *T. collina* var *lanuginocarpa* micromorphology of leaves

Parameters	Values
Stomatal index	14 ±0. 02
Palisade ratio	3.57 ±0.83
Vein islet No	2.95
Vein termination No.	1or 2

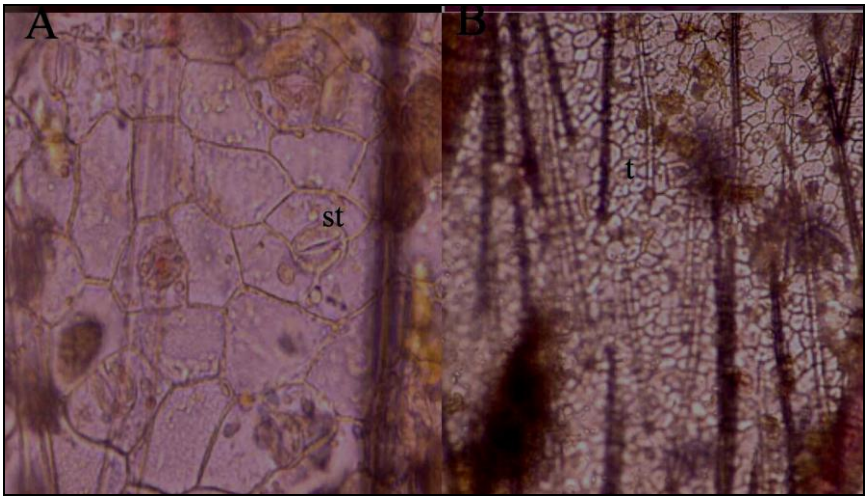

Fig. 4: (A) Anisocyctic stomata-st, (B) Trichomes –t

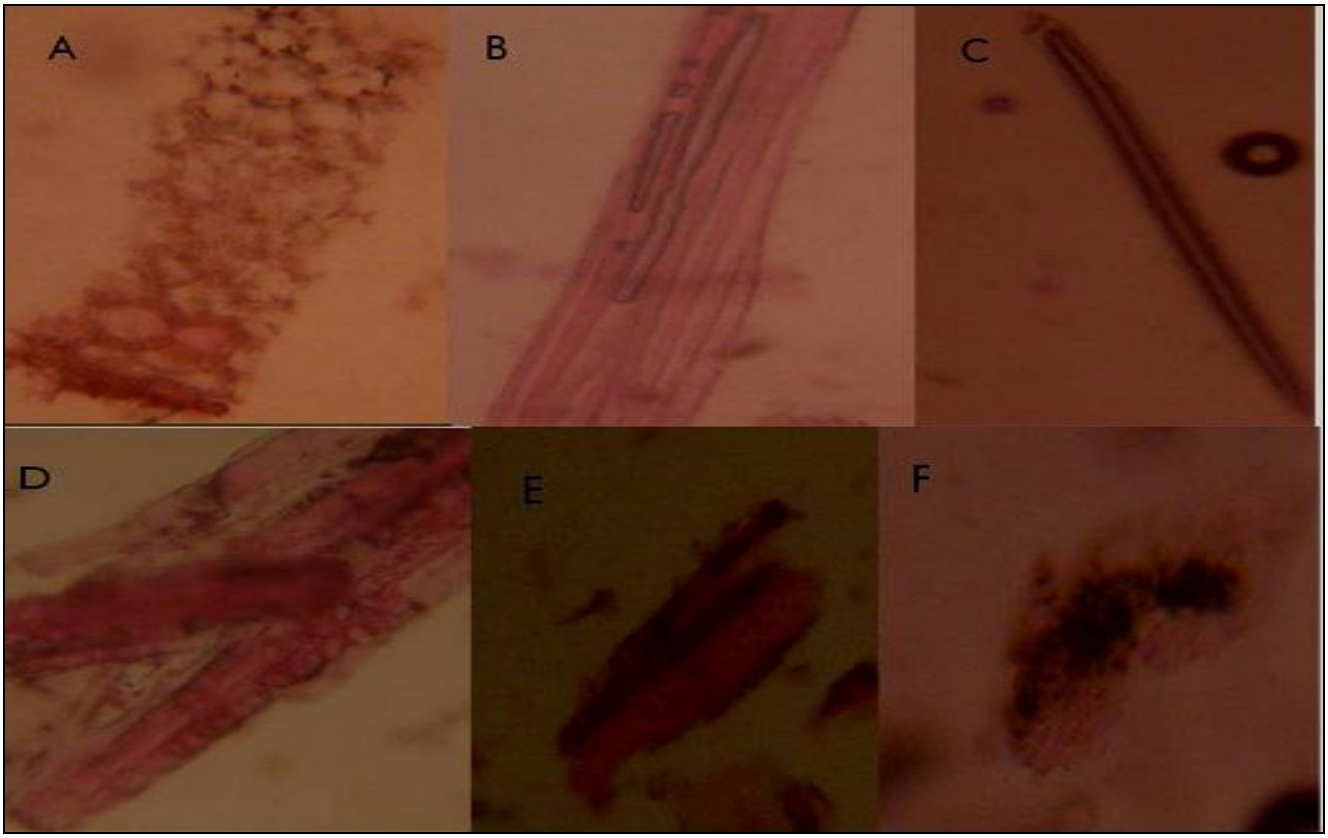

Fig. 5: (A) Parenchymatous and collenchymatous tissue, (B) Xylem fibers, (C)Unicellular warty trichome, (D) Xylem fibers with calcium oxalate crystals, (E) pitted xylem vessel, (F) Mesophyll tissue

Table 3: Histochemical test on the section of *T.collina* var *lanuginocarpa*

Cell content	Reagent used	Root T.S.	Stem T.S.	Leaves T.S.
Lignified cell wall	Phloroglucinol+ HCl	+	+	+
Calcium oxalate	HCl	+	+	+
Starch grain	Iodine	-	+	-
Tannin	$FeCl_3$	ND	ND	ND
Cellulose	Iodine + H_2SO_4	+	+	+
Alkaloids	Dragendorff	ND	ND	ND
Aleurone grains	Iodine	-	+	-

+= Present, - =absent, ND= not define

Table 4: Result of preliminary phytochemical screening on powder of *T.collina* var *lanuginocarpa*

Phytoconstituent Group	Roots	Leaves	Stem
Carbohydrates	+	+	+
Protein	+	+	-
Alkaloids	-	+	-
Tannin	-	-	-
Triterpenoids	ND	-	-
Steroids	+	ND	-
Saponins	-	-	-
Lignans	+	+	+
Anthocyanidins	+	+	+
Iridiodes	-	-	-
Flavonoids	+	+	+
Quinones	+	+	+

+= Present, - =absent, ND= not define

DISCUSSION

The above observations and analysis are the first report providing information regarding the physicochemical, pharmacognostic and preliminary phytochemical studies of the endangered plant species *T. collina* var *lanuginocarpa* Sharma. The values of total ash, water soluble ash, acid insoluble ash and water extractive of leaves of are higher then root and stem while the alcohol extractive of Stem was higher then root and leaves. In general the microscopy characters like presence of warty unicellular trichomes, rhomboidal calcium oxalate crystals and anisocytic stomata are the chief characteristic feature for identifying the quality of a crude drug. However, the similar microscopic feature was observed another endemic species *Tephrosia jamnagarensis* Sant. of same region.[12] The preliminary phytochemical screening of the all the plant parts showed significant results, carbohydrates, lignans, anthocyanidins, flavonoids, and Quinones is present in all extracts of root, stem and leaf while protein was found present in root and leaves and alkaloids only in leaves. Iridiodes, saponins and tannin are found to be absent in extracts of all three plant parts. Thus this researches helps us developed some basic profile of the *T. collina* var *lanuginocarpa* Sharma which would be further helpful in developing the detail monograph for identification.

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