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PHARMACOGNOSTICAL EVALUATION OF AERIAL PARTS OF *PUERARIA TUBEROSA*

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ABSTRACT

This research was done to assess the pharmacognostical factors for *Pueraria tuberosa's* aerial parts (leaves and stem) (Fabaceae). The flower is traditionally calming and aphrodisiac. The tuber clears the voice, cures leprosy, biliousness, disorders of the blood, "vata", burning sensation, and urinary discharges. It is indigestible and has cooling, aphrodisiac, tonic, galactagogues, diuretics, and alterative properties. generates "kapha" (Ayurveda). Refrigerator root tuber, hyperglycemia, and bronchial asthma. To fully harness this folk herb's therapeutic potential, an effort has been made to correctly identify it. According to this perspective, the morphoanatomy of the leaves and stem, along with quantitative microscopy, microscopic linear measurements, WHO-recommended physico-chemical determinations, and genuine phytochemical procedures, are the key diagnostic characters that have been carried out to help the full pharmacognostical evaluation of the plant. The parameters discussed in this research could be suggested as the benchmarks for determining *Pueraria tuberosa's* veracity. This research aids in separating this medication from its other species.

INTRODUCTION

Early man investigated his local natural environment, experimented with a wide range of plants, animals, and minerals, and created a wide range of medicinal substances. Man has developed a variety of health care methods and techniques in an effort to achieve eternal health and longevity as well as to find relief from suffering. A increasing corpus of medical literature supports the clinical usefulness of herbal remedies, and plants are valued in pharmaceutical research as a significant source for new medicines[2]. The medicinal plants play a significant part in practically all traditional medicines and are the foundation of traditional medicine. Due to their varied Content, which might take the form of whole plants, plant parts, or extracts made from them[3,4], standardising natural goods is a challenging undertaking. The starting material must be properly controlled if herbal products are to be of a consistently high quality. Authentication is the initial step in confirming the calibre of the beginning material. Despite current methods, pharmacognostical investigations are more reliable for identifying plant-based medications. The macroscopic and microscopic description of a medicinal plant is the first step towards confirming the identity and level of purity of such materials, according to the global health organisation (WHO, 1998), and should be carried out before any tests are carried out[5,6].

The Fabaceae family includes *Pueraria tuberosa*[7-9] (Syn: Hedysarum tuberosum Roxb). Locally known as Akasagaruda and Nagadonda. In the western Himalaya, it is dispersed to Sikkim, Kumaon, Punjab, Mt. Abu, Bengal, southern India, and Andhra Pradesh. Rare in Andhra Pradesh. in dry deciduous woodlands. Japali Theertham in Talakona is next to a temple region. The flower has cooling and arousing properties. The tuber clears the voice, cures leprosy, biliousness, disorders of the blood, "vata", burning sensation, and urinary discharges. It is indigestible and has cooling, aphrodisiac, tonic, galactagogues, diuretics, and alterative properties. generates "kapha" (Ayurveda). Despite the plant's many uses, there is no scientific evidence to distinguish the authentic sample. In order to standardise the medicine, the current inquiry was undertaken to determine the identity of aerial portions morphologically, microscopically, and physicochemically. Habitat and Habit woody, large twiner vines. Large tuberculous roots are found in a chain-like structure. stem rough. 3-foliolate, ovate, thin, coriaceous, white silky below, sparse thin hairs above, whole, acutemucronate leaves with herbaceous stipules. Flowers In axillary panicles, pale violet. racemes with clawed petals, 15–30 cm in length, Bracteoles are 1.5 mm long, oblong, and silky. Pedicels are 2-3 mm long, silky-pubescent, and fascicled along a more or less pubescent rhachis. Calyxes are 6 to 8 mm long, thickly silky, and have teeth that are oblong, obtuse, and ciliate. Corolla has a spurred standard length of 1.3 cm and a bluish colour. Membranous, flat, constricted pods that are 5 to 7.5 cm long and covered in long, silky, bristly brown hairs between the seeds. 3-6 sub-orbicular, oblong-hilar seeds. Fruits & Flowers February through August.

EXPERIMENTAL MATERIAL AND METHODS

Collection and authentication of plant material: The study's chosen herb, *Pueraria tuberosa*, was gathered from its native habitat at Tirumala Hills in Chittoor District, Andhra Pradesh, India, namely from Talakona Hills and Nagapatla Reserve Forest. Prof. P. Jayaraman, a taxonomist and the director of the Plant Anatomy Research Centre (PARC), in Chennai, Tamil Nadu, recognised it. The college of pharmaceutical sciences, AU, Visakhapatnam has received the *Pueraria tuberosa* voucher specimens (PARC/2008/298). For the investigation of macroscopical and microscopical features as well as quantitative microscopy, the specimens (leaf and stem) were employed. The extracted values, ash values, qualitative chemical analysis, and phytochemical components present in the chosen plants were all determined using the dried powdered material.

Instruments and chemicals

The main equipment and tools utilised for the investigation were a rotary microtome, a compound microscope, watch glass, glass slides, cover slips, and other glassware. A Nikon Labphoto[2] Microscope was used to take the microphotographs. Petroleum ether, chloroform, and ethanol (95%) are examples of solvents, and toluidine blue, phloroglucinol, glycerin, HCl, chloral hydrate, and sodium hydroxide are examples of reagents. The analytical-grade reagents were provided by Ranbaxy Fine Chemical Ltd. in Mumbai, India, or Sigma Chemicals Co. in St. Louis, USA..

Macroscopic and microscopic analysis: The approach of Brain and Turner[10] was used to examine the leaves' macroscopy and microscopy. Cross sections were produced and stained according to Johansen's[11] method for microscopical examinations.

Physico-chemical analysis: According to the official procedures outlined in the Indian Pharmacopoeia[12] and WHO standards on quality control methods for medicinal plant materials, physical and chemical analyses, including percentage of ash values and extractive values, were carried out. WHO/QCMMPM recommendations[13].

Preliminary phytochemical screening

Initial phytochemical screening was done using the guidelines outlined by Kokate[14] and Harborne[15].

RESULTS AND DISCUSSION

Macroscopical characters: It is a woody twiner with tuberous roots, terete branchlets, and nodes with protruding wings. The leaves are subcoriaceous, orbicular or elliptic-

obovate, whole, undulate, and longitudinally folded. Flowers in axillary peduncled cymes are yellow. Oval seeds with a long, white, silky coma at the tip.

Microscopic characters of *Pueraria tuberosa*

Microscopy of the *P. tuberosa* leaf (Fig. 1)

The leaflet is dorsiventral with prominently projecting midrib and thin bilaterally differentiated lamina (Fig. 1.1).

Midrib: The midrib has thick, blunt, errect adaxial hump and quite wider, semicircular abaxial part (Fig. 1.2 & 1.2). The midrib is 1.1 mm thick along the vertical axis; the adaxial hump is 250 µm is height and 250 µm in thickness. The abaxial part is 900 µm wide. The midrib has thin, continuous epidermal layer comprising of small, squarish thick walled cells which have papillate outer tangential walls. The ground tissue within the adaxial hump consists of small, thick walled compact cells (Fig. 1.2). The abaxial part of the midrib has large, angular thin walled parenchyma cells. The vascular system of the midrib is multistranded and consists of five, prominent and discrete vascular bundles. Of the five bundles, the abaxial bundle is the largest; of the remaining four bundles, one is adaxial and other three are lateral in position. All the bundles are collateral and have wide, thick walled angular, clusters. Phloem occurs in wide band beneath the xylem. Embedded in the phloem zone are wide, circular secretory canals placed in an arc (Fig. 1.2 & 2.1). The cavities are up to 60 µm in diameter (Fig.2.1). The vascular bundles have thick and prominent sclerenchyma sheath along the phloem zone. The sclerenchyma cells have thin walls and wide lumen.

Lamina (Fig. 2.2 & 2.3): It has even and smooth adaxial side and slightly undulate abaxial side; wherever the lateral veins are located the abaxial side is slightly raised. The lamina is 90 µm thick in between the lateral veins and 110 µm thick in the region of the vein. The adaxial epidermis is wide and the cells are widely tabular or rectangular with thin walls. The abaxial epidermis is thin and the cells are narrowly cylindrical and stometiferous (Fig. 2.2 & 2.3). The adaxial epidermis is 20 µm thick while abaxial epidermis is 10-12 µm thick. The mesophyll tissue is differentiated into a narrow band of unistratose, short cylindrical palisade cells and three or four layers of lobed spongy parenchyma cells. The lateral veins have narrow vertical row of xylem phloem elements with a layer of wide, hyaline bundle sheath cells which extend both adaxially and abaxially (fig. 2.3).

Adaxial epidermis (Fig.3.1): The adaxial epidermis is apostomatic (lacking stomata). The epidermal cells are random in orientation. The anticlinal walls of the epidermis are thin, highly wavy and smooth. Due to the wavyness of the walls, the cells appear lobed and amoeboid in surface outline (fig. 3.1). There are epidermal trichomes sparsely distributed especially along the veins. They arise from circular, thick walled epidermal cells (fig.3.1).

Abaxial epidermis (Fig. 3.2): The veins and vein-islets are more prominently seen on the abaxial side of the lamina (fig. 3.2). The abaxial epidermal cells are much lobed with thin and smooth walls. They appear amoeboid in surface view (fig. 3.2). The abaxial epidermis is stomatiferous (having stomata).

Stomata (Fig.3.2)**:** The stomata are anomocytic type, the guard cells are elliptic and the stomatal pore in district. They 10 x 15 µm is size.

Venation (Fig.4): When the lamina is cleared and made transparent it shows the venation pattern and trichome distribution (Fig.4.1). The secondary veins are thick and straight. The tertiary veins branch profusely forming dense reticulate venation. The vein-islets are distinct and well defined. They are rectangular, square shaped or polyhedral. No vein terminations are evident and the aereoles are mostly empty (Fig. 4.2).

Trichomes: These are abundant on the lower surface of the leaf. They are dense and random in distribution (Fig. 5.1). The trichomes are lopsided and uniform in orientation (Fig.5.2). They are unicellular, unbranched, thick walled and wide lumened. They are 240-450 µm long and 10 µm thick. The tip is pointed (Fig. 5.2). The trichome arises from a circular, thick walled epidermal cell; the epidermal cells around the basal cell

of the trichome are radially elongated and rosette in shape (Fig.5.2).

Crystals: calcium oxalate crystals are sparsely seen in the midrib and bundle sheath cells of the lamina. The crystals are prismatic type and rhomboidal or cuboidal in shape. They occur in the subepidermal cells of the midrib (Fig.6.1) or in the parenchyma cells that form the bundle sheath of veins (fig. 6.2). The crystals are up to 12 μm in size.

Table No.1: Quantitative microscopy (leaf constants) of *P. tuberosa*

S.No	Parameter →	Stomatal Number and Stomatal Index per sq. mm			
	Epidermis →	Lower (40X)			
	Trial No. →	I	II	III	IV
	No. of Stomata per sq. mm (S)	7	7	8	8
	No. of epidermal cells / sq. mm (E)	21	23	24	22
	Stomatal Index S I= (S/E+S)x100	25.00	23.33	25.00	26.66
	Average Stomatal No.	7.5 per sq. mm			
	Average Stomatal Index	24.99 per sq. mm			
	Parameter →				
	Trial No. →	I	II	III	IV
	No. of epidermal cells (E)	4	4	4	4
	No. of Palisade cells/sq.mm (P)	21	25	16	21
	Palisade ratio	5.25	6.25	4.00	5.25
	Average Palisade Ratio	5.18			
	Parameter →				
	No. of Vein-Islet per 4 sq.mm	72	68	68	72
	No. of Vein-Islet per 1 sq.mm	18	17	17	18
	Average Vein-Islet No.	15.75			
	No. of Veinlet-Terminations per 4 sq. mm	16	12	12	12
	No. of Veinlet-Terminations per 1 sq. mm	4	3	3	3
	Average Veinlet-Termination No.	3.25			

Table No.2: Quantitative determinations (ash and extractive values) of *P. tuberosa*

Parameter →	Ash values (% w/w)
Parts used →	Aerial parts
Total ash	9.00
Water soluble ash	4.50
Acid insoluble ash	3.50
Sulphated ash	5.00
Parameter →	Extractive values (% w/w)
Ether soluble	1.77
Alcoholic soluble	5.35
Water soluble	5.18

Table No.3: Physical characteristics of extracts of *P. tuberosa*

Physical characteristics of aerial parts extracts				
S. No		**Nature**	**Colour**	**% yield (w/w) g**
1	Petroleum ether	Waxy	Dark brown	1.77
2	Chloroform	Waxy	Brownish green	1.79
3	Alcoholic	Viscous	Brownish green	5.35
4	Aqueous	Sticky	Brown	5.18

Table No.4: Qualitative chemical tests for phytoconstituents of *P. tuberosa*

Part used →	Aerial parts				Part used →	Aerial parts			
Plant constituents and Chemical tests↓	Pet. Ext	Chl. Ext	Alc. Ext	Aq. Ext		Pet. Ext	Chl. Ext	Alc. Ext	Aq. Ext
Tests for Steroids (a) Salkowski test	-	-	-	-	(c) Wagner's test	-	-	-	-
(b) Liberman Burchards test	-	-	-	-	(d) Hager's test	-	-	-	-
Triterpenes (a) Salkowski test	+	+	+	+	**Tests for Carbohydrates.** (a) Molisch's test	-	-	+	+
(b) Liberman Burchard test	+	+	+	+	(b) Fehling's test	-	-	+	+
(c) Tschugajeu test	+	+	+	+	(c) Benedict's test	-	-	+	+
(d) Briekorn and Brinars test	+	+	+	+	(d) Barfoed's test	-	-	+	+
Tests for Saponins. (a) Foam test	-	-	-	-	**Tests for Flavanoids.** (a) Shinoda test	-	-	-	-
(b) Haemolysis test	-	-	-	-	(b) Ferric chloride test	-	-	-	-
Tests for Steroidal saponins. a) Salkowski test	-	-	-	-	(c) Lead acetate test	-	-	-	-
					(d) ZnCl/HCl reduction test	-	-	-	-
(b) Haemolysis test	-	-	-	-	**Tests for Tannins** (a) Ferric chloride test	-	-	-	-
Tests for Triterpenoidal saponins (a) Salkowski test	-	-	-	-	(b) Gelatin test	-	-	-	-
(b) Liberman Burchard test	-	-	-	-	**Tests for Glycosides** (a) Baljet's test	+	+	+	+
(c) Tschugajeu test	-	-	-	-	(b) Legal's test	+	+	+	+
(d) Briekorn and Brinars test	-	-	-	-					
Tests for alkaloids. (a) Mayer's test	-	-	-	-	(c) Keller-Killiani test	+	+	+	+
					Tests for Bitters (a) Vanillin sulphuric acid	-	-	-	-
(b) Dragendorff's test	-	-	-	-	(b) Serial dilutions	-	-	-	-

Note: "+": Present, "-": Absent, Pet. Ext: Petroleum ether extract, Chl. Ext: Chloroform extract, Alc Ext: Alcoholic extract and Aq Ext: Aqueous extract, MB: Moderately bitter in taste.

Fig. 1: Anotomy of the leaf P. *tuberosa*

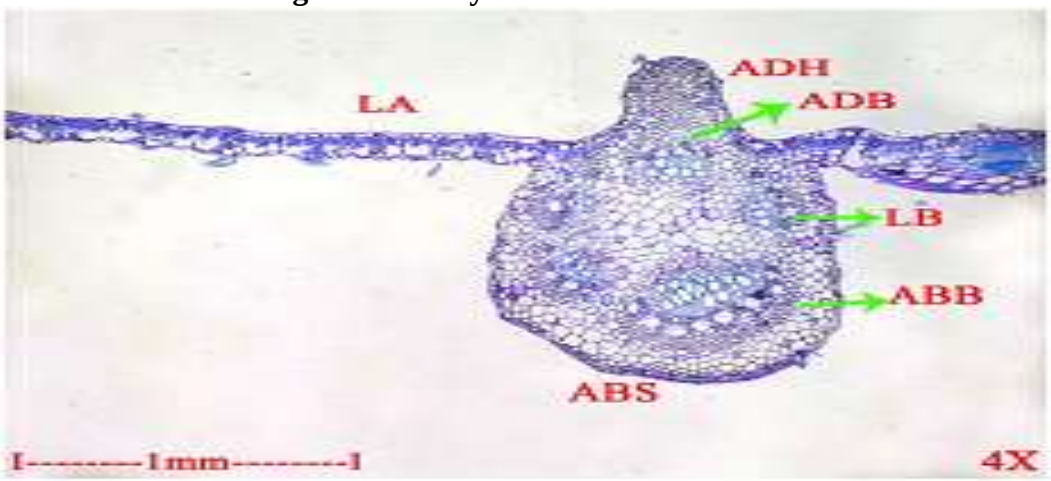

Fig. 1.1: T.S of leaf through midrib with lamina

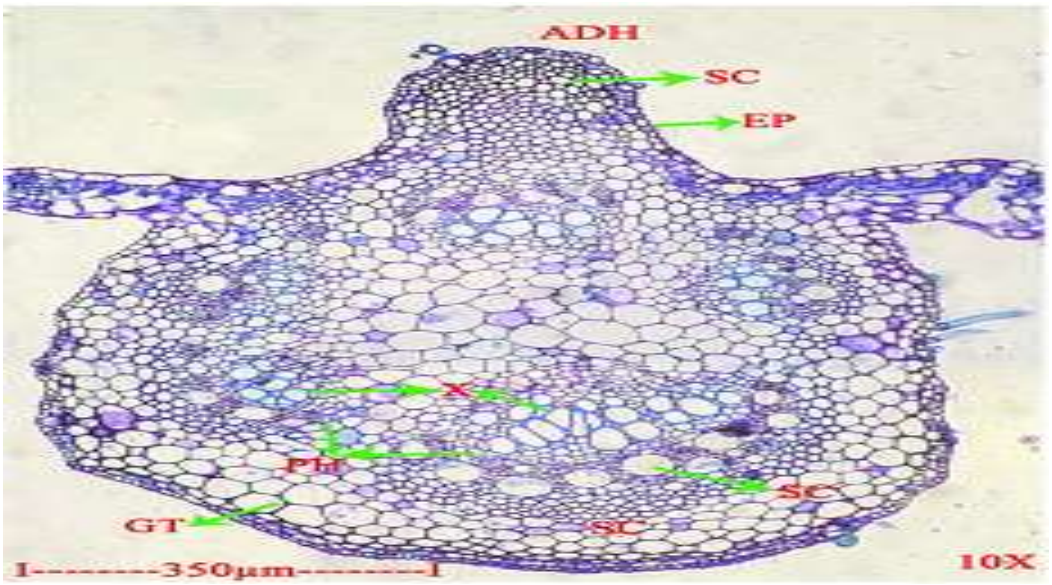

Fig. 1.2: T.S leaf of midrib with lamina enlarged

ABB-Abaxial bundle; ABS- Abaxial side; ADB- Adaxial bundle; ADH- Adaxial hump; ADS- Adaxial side; EP- Epidermis; GT- Ground tissue; LA- Lamina; LB- Lateral bundle; PH-Phloem; SC- Sclerenchyma; SC- Secretory cavity; X-Xylem

Fig. 2: Anatomy of the lamina and vascular bundle of the midrib *P. tuberosa*

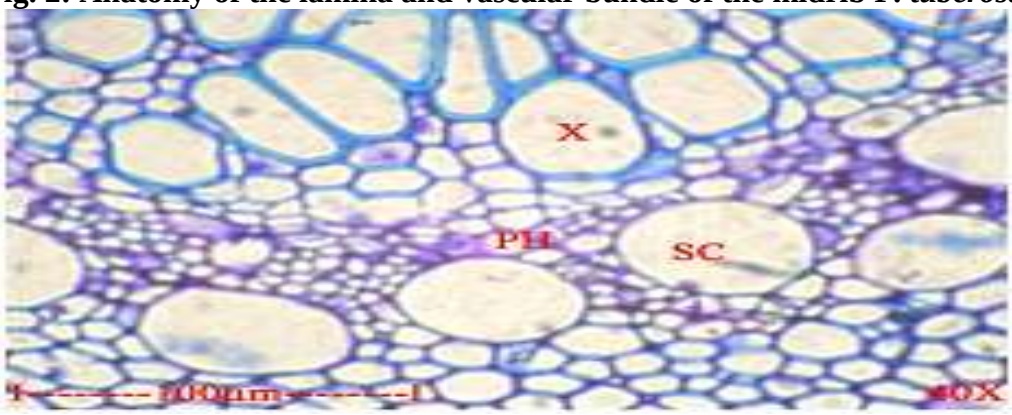

Fig. 2.1: T.S of midrib vascular bundle showing secretory cavities in the phloem tissue

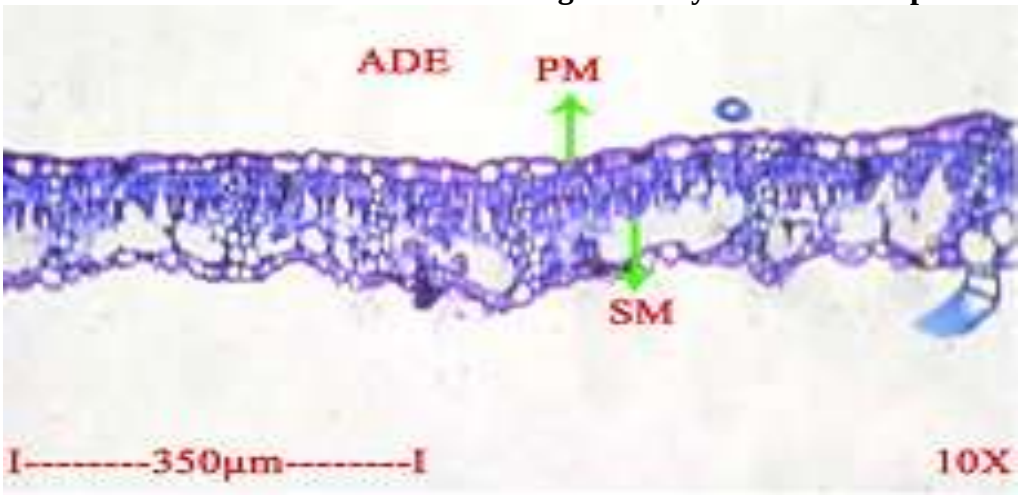

Fig. 2.2: T.S of lamina under low magnification

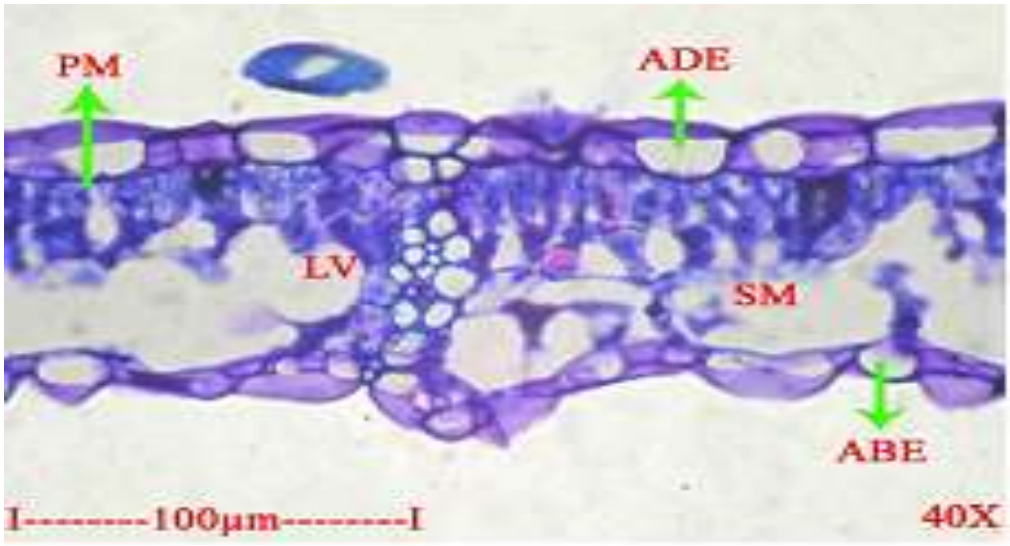

Fig. 2.3: T.S of lamina through lateral vein

ABE-Abaxial epidermis; ADE-Adaxial epidermis; LV- Lateral vein; PH- Phloem; PM-Palisade mesophyll; SM-Spongy mesophyll; SC- Secretory cavity; X-Xylem

Fig. 3: Epidermal morphology of *P. tuberosa*

Fig. 3.1: Adaxial epidermis

Fig. 3.2: Abaxial epidermis with stomata and trichome

ABE-Abaxial epidermis; ADE-Adaxial epidermis; AW- Anticlinical wall; BC- Basal cell of trichome; ETR- Epidermal trichome; GC- Guard cells; ST- Stomata

Fig. 4: Venation pattern of *P. tuberosa*

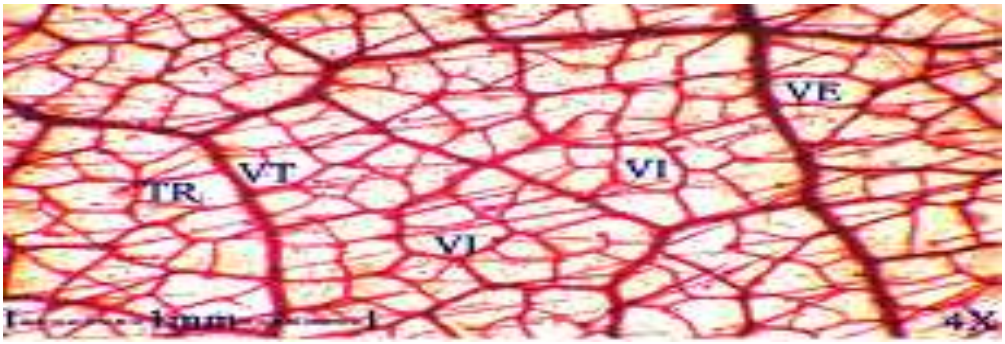

Fig. 4.1: Cleared leaf showing vein-islets and vein-termination

Fig. 4.2: Enlarged cleared leaf sowing vein-islets and vein-termination
TR-Trichome; VE- Vein; VI-Vein-islets; VT- Vein-termination
Fig. 5: Trichome morphology of *P. tuberosa*

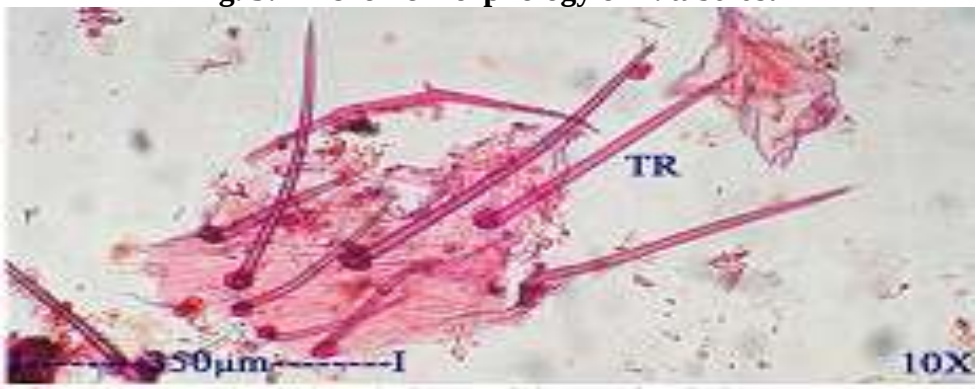

Fig. 5.1: Non-glandular epidermal trichomes

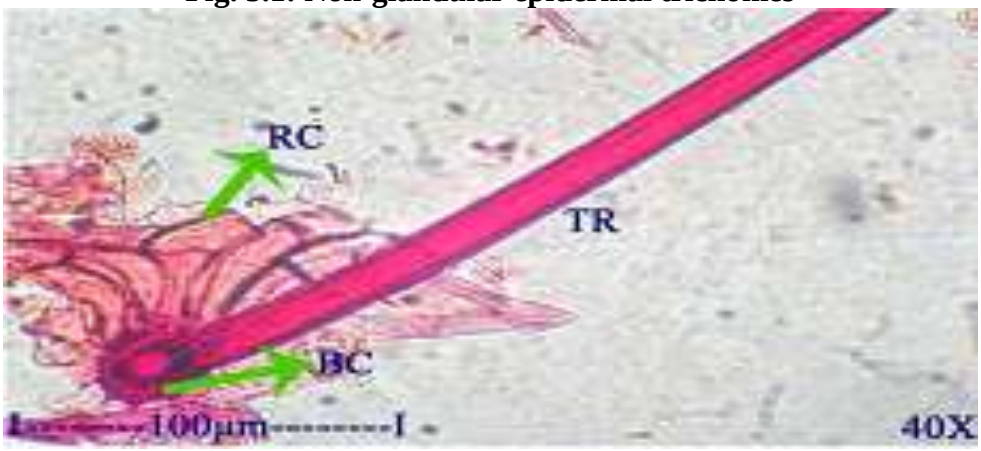

Fig. 5.2: A trichome with basal cell of trichome
BC- Basal cell of trichome; RC-Rosette cell; TR- Trichome
Fig. 6: Crystal distribution in the leaf (Under polarized light microscope)

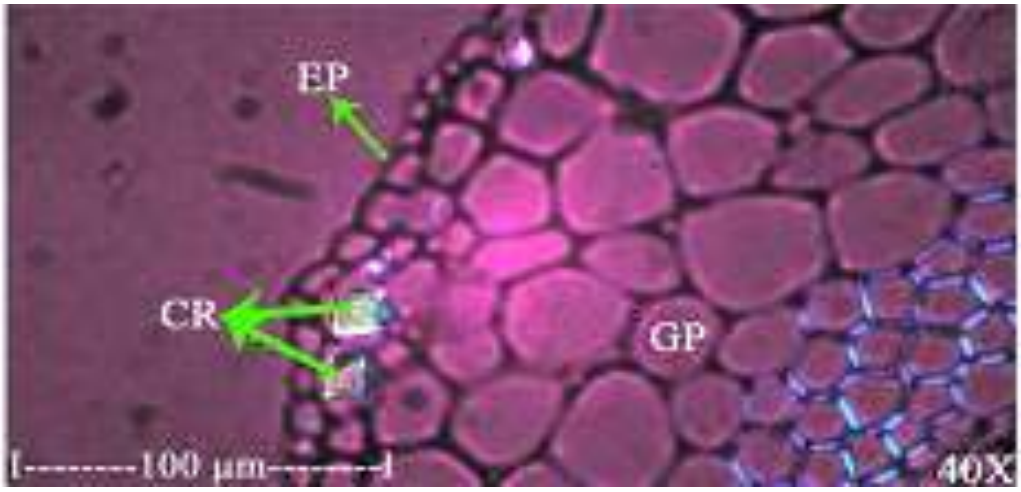

Fig. 6.1: T.S of midrib showing crystals along epidermis

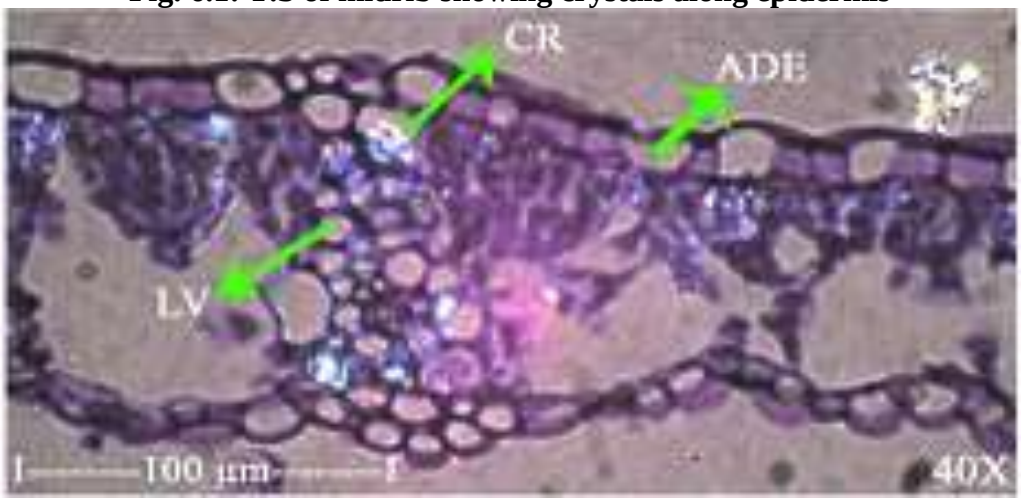

Fig. 6.2: T.S of lamina showing crystals abutting the leaf lateral vein
ADE- Adaxial epidermis; CR- Crystal; EP-epidermis; GP- Ground parenchyma; LV- Lateral vein

CONCLUSION

As a result, the current investigation into the pharmacognostical evaluation of Pueraria tuberosa will be able to distinguish it from other closely related species and offer helpful information regarding its correct identity. The additional measurements made could help identify the plant in the future.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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