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PHARMACOGNOSTICAL EVALUATION OF AERIAL PARTS OF *HOLOSTEMMAADA-KODIEN*

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ABSTRACT

With this study, the various pharmacognostic and phytochemical standards for the aerial portions (leaves and stem) of the plant *Holostemmaada-kodien* were to be established (Asclepiadaceae). The plant *Holostemmaada-kodien* is traditionally used as an alterative and astringent to the bowels; it also has medicinal properties for ulcers, biliousness, "kapha," blood disorders, worms, itching, leucoderma, and vesicular calculi (Ayurveda). Diabetes, stomachic, gonorrhoea, cough, tonic. 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 the veracity of *Holostemmaada-kodien*. This research aids in separating this medication from its other species.

KEYWORDS

Holostemmaada-kodien (Asclepiadaceae), Pharmacognostical and Leaf and stem.

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INTRODUCTON

The medicinal plants play a significant part and form the foundation of both the herbal medicines industry as well as local populations' traditional medicine. The majority of the basic medications used in practically all traditional treatments come from wild plants. When obtained from markets, the raw materials are frequently contaminated[1]. The issues of adulteration and substitution at the raw material level are generally a problem for local

communities and herbal industries around the world[2]. 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 in this situation 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].

Holostemma ada-kodien[7-9] is a member of the Asclepiadaceae family. Locally known as Akasagaruda and Nagadonda. China, Ceylon, and the Western Peninsula all receive it. Rare in open hill areas and on fences in the tropical Himalaya, Burma, and Andhra Pradesh. Tirupati, Tirumala, and Talakona campuses of S.V.U. Traditionally The plant is alterative and astringent to the bowels; it treats ulcers, biliousness, "kapha," blood disorders, worms, itching, and leucoderma; it is also helpful for vesicular calculi and gonorrhoea (Ayurveda). Diabetes, gastrointestinal, cough, gonorrhoea, toni. Branching, glabrous and shiny stem. 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.

EXPERIMENTAL

MATERIAL AND METHODS

Collection and authentication of plant material

The study's chosen herb, *Holostemmaada-kodien*, was gathered from its natural 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 voucher specimens for *Holostemmaada-kodien* (PARC/2007/182). 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. Using a Nikon Labphoto 2 Microscopic equipment, microphotos were taken. 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 used 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

Preliminary phytochemical screening was carriedout by using standard procedures described byKokate[14] and Harborne[15].

RESULTS AND DISCUSSION

Macroscopical characters

It is a big, glabrous perennial climber or twining shrub. Fusiform tubers with linear-oblong, cylindric

roots that taper to a blunt end are present. branching, glabrous, and shiny stem. Thick, ovate-oblong, acuminate, glabrous above, more or less pubescent (especially on the nerves), and reticulately veined be beath leaves that measure 7.5-12.5 by 5-7.5cm. The base of the leaves is deeply cordate, with rounded lobes, and there are frequently a few small glands at the base of the midrib above. Petioles are robust, 2.5-5cm long, and glabrous. Flowers in sublateral few-flowered cymes are fragrant, tasty, reddish scarlet on the interior, and frosted white or pale pink on the outside. Long peduncles, measuring 2.5 to 5cm, that emerge near to the petiole's base outside the leaf axil. Longer pedicels than peduncles. Calyx dove almost to the ground. The corona of the corolla, which arises from the base of the staminal column and is made up of a fleshy, 2.5mm-high truncate ring, is subrotate, divided approximately 2/3 of the way down, and has lobes that are 1.3 by 1cm, ovate-oblong, obtuse, and overlapping to the right. Big anthers, enormous stiff wings, and inflexed membranous tips over the column; Long, waxy, compressed, linear-clavate, slightly curved pollen masses that are attached to linear pollen-carriers by 1.25mm long, black caudicles. Follicles are linear-oblong, cylindrical, and taper slightly to a blunt end, measuring 10-12.5 by 0.6cm. The coma is 2-2.5cm long, and the seeds are 6-mm long, oblong, extremely thin, greatly flattened, somewhat truncate, and not crenate at the base. Fruits and flowers from July through January.

Microscopic characters of *Holostemmaada-kodien*

Microscopy of the *Holostemmaada-kodien* leaf

The leaf has prominent midrib and uniformly thin and dorsiventral lamina (Figure No.1). The midrib is planoconvex with flat adaxial side and semicircular abaxial side (Figure No.2).

Midrib

Is 550μm vertical plane and 650μm in horizontal plane. It has a thin adaxial epidermal layer of small rectangular cells; the abaxial epidermis is thinner and has small circular thick walled cells. The ground tissue of the midrib is parenchymatous. The cells are fairly large, compact, thin walled and angular in outline.

The vascular strand is single large and arc-shaped. It is 350μm wide and 150μm thick. The vascular strand in collateral and possesses upper, dense radial rows of small, thick walled xylem elements. A thin layer of phloem occurs beneath the xylem arc.

The lateral vein is also prominent and planoconvex in sectional view (Figure No.3). The abaxial part is semicircular and the adaxial side is flat. The lateral vein is 350 μm thick and abaxial part is 250μm wide. The vein has thin epidermal layer, parenchymatous ground tissue extending up to the adaxial epidermis. The vascular strand top shaped with conical xylem and narrow arc of phloem (Figure No.3).

The veinlet is flat both on the adaxial and abaxial sides. The vascular strand of the vein is prominent having four or five short rows of xylem elements and two or three phloem nests (Figure No.4). The vascular strand has parenchymatous, dilated cells on the abaxial and adaxial sides forming extensions. The lateral veinlet is 160μm thick.

Leaf-margin

Is thick, blunt and semicircular and measures 130μm thick. It has wide circular or elliptical adaxial epidermis with papillate cuticle. The lower epidermis has smooth and even cuticle. The mesophyll consists of palisade and spongy parenchyma similar to the middle part of the lamina (Figure No.6).

Lamina

The lamina is bilaterally symmetrical with adaxial-abaxial differentiation. The adaxial epidermis is wide and consists of large barrel shaped cells; the outer tangential walls have thick cuticle with short, thick papillae (Figure No.5). The cells are 30μm thick in vertical plane. The abaxial epidermis is narrow and stomatiferous. The cells are rectangular or cylindrical; they are 10μm thick.

The mesophyll is differentiated into adaxial palisade tissue and abaxial spongy parenchyma. The palisade cells are one or two layered, narrowly cylindrical and loosely arranged. The palisade tissue is 60 μm

in the height. The spongy parenchyma consists of five or six layers of lobed, loosely interconnected parenchyma cells (Figure No.5).

Abaxial epidermis (Figure No.7)

The abaxial epidermis is stomatiferous. The stomata are paracytic type. The stoma has two, equal subsidiary cells, one on either side. The epidermal cells are small, polyhedral in outline and have thick, straight anticlinal walls. The epidermal cells lying around the basal cell of the trichome radiate into oblong cells forming rosette-cells (Figure No.7).

Adaxial epidermis (Figure No.8)

The adaxial epidermal cells are apostomatic.They are slightly wider than the abaxial cells. The cells are polyhedral and random is orientation. Their anticlinal walls are thick and straight. Cuticular markings are not evident. Trichomes are also wanting on the adaxial epidermis (Figure No.8).

Venation (Figure No.9)

The lamina was made transparent by clearing technique. In surface view, the leaf shows distinct venation. The primary lateral veins are fairly thick. The secondary and tertiary veins are also prominent forming distinct vein-islets. The shape of the islets is squarish, rectangular, triangular or polyhedral (Figure No.9). Most of the islets have well developed vein-terminations. The terminations are also variable: they are short and thick, long, slender and wavy; sometimes they are forked once or twice.

Microscopy of the *Holostemmaada-kodien stem* (Figure No.10 and No.11)

Both young and old stem were studied. The young stem is 1.3mm thick. It has narrow epidermis with circular, papillate cells. The cortex is parenchymatous and the cells are circular thick wally and compactly arranged. The cortical zone is 150 μm wide.

Along the inner boundary of the cortex, these are wide, circular isolated masses of sclerenchyma cells. These sclerenchyma masses occur all along the boundary of the xylem.

The vascular cylinder, that is the stele of the young stem, consists of thin cylinder of xylem which has numerous short compact rows of five or six xylem elements. Outer to the xylem is a narrow zone of phloem which encircles the xylem cylinder (Figure No.10). The pith is parenchymatous and homogeneous. The cells are circular and less compact.

Along the outer peripheral part of the pith occur small nests of inner phloem or medullary phloem. These are also narrow thick walled, darker cells in the pith which are thin laticiferous canals.

Young stem has unilacunar node with one leaf-trace. The stele breaks to form a small gap without the vascular tissues. From this gap a single leaf trace pinches off and enters the cortex (Figure No.10). From the cortex it later passes into the petiole.

Microscopy of the *Holostemmaada –kodien* old stem (Figure No. 12-15)

The old stem has a thin continuous epidermal layer of narrow, rectangular thick walled cells. The outer cortical cells are circular, and compact. The middle zone of the cortex has distorted masses of sclerenchyma cells. These cells are circular, wide and thin walled. Large spaces are seen in between these cells (Figure No.12).

The inner cortex has radially oblong, compact wider cells. The cells of the inner cortex have accumulation of starch grains.

The vascular cylinder is thin and wide. It has outer narrow zone of xylem and inner zone of primary xylem (Figure No.13). The secondary xylem has regular radial rows of fibres and similar type of wider cells or the vessels. The vessels are angular, polygonal and thick walled (Figure No.15). The primaryxylem has two wide metaxylem elements and narrow two or three protoxylem elements.

Phloem occurs both on the outer portion and inner portions of the xylem. Both the outer (normal) phloem and inner (medullary) phloem are seen in discrete islands of phloem elements (Figure No.14 and Figure No.15). The xylem elements are wide, angular and thick walled.

The pith is wide and parenchymatous. The pith cells are polyhedral, thin walled and compactly arranged. There are small cells with thick walls in the pith region. These cells are the laticifers.

Crystals (Figure No. 16-18)

Calcium oxalate crystals of druses or sphaerocrystals located in the midrib and stem

cortex. The druses are wide and have echinate surface. The druses in the cortex of the stem are often circular and platelike measuring 22μm in diametr. The druses in the midrib are larger measuring 40μm wide.

Table No.1: Quantitative microscopy (leaf constants) of *Holostemmaada -kodien*

S.No	Parameter →	Stomatal Number and Stomatal Index per sq. mm			
1	Epidermis →	Lower (40X)			
2	Trial No. →	I	II	III	IV
3	No. of Stomata per sq. mm (S)	4	3	4	3
4	No. of epidermal cells / sq. mm (E)	11	10	10	12
5	Stomatal Index S I= (S/E+S)x100	26.66	23.07	28.57	20
6	Average Stomatal No.	3.5 per sq. mm			
7	Average Stomatal Index	24.57 per sq. mm			
8	Parameter →				
9	Trial No. →	I	II	III	IV
10	No. of epidermal cells (E)	4	4	4	4
11	No. of Palisade cells/sq.mm (P)	37	35	45	35
12	Palisade ratio	9.25	8.75	11.25	8.75
13	Average Palisade Ratio	9.5			
14	Parameter →				
15	No. of Vein-Islet per 4 sq.mm	64	60	64	64
16	No. of Vein-Islet per 1 sq.mm	16	15	16	16
17	Average Vein-Islet No.	15.75			
18	No. of Veinlet-Terminations per 4 sq. mm	36	40	32	32
19	No. of Veinlet-Terminations per 1 sq. mm	10	8	7	7
20	Average Veinlet-Termination No.	8			

Table No.2: Quantitative determinations (ash and extractive values) of *Holostemmaada -kodien*

S.No	Parameter →	Ash values (% w/w)
1	Parts used →	Aerial parts
2	Total ash	8.25
3	Water soluble ash	6.00
4	Acid insoluble ash	3.00
5	Sulphated ash	3.50
6	**Parameter →**	**Extractive values (% w/w)**
7	Ether soluble	1.60
8	Alcoholic soluble	10.72
9	Water soluble	9.40

Table No.3: Physical characteristics of extracts of *Holostemmaada-kodien*

S.No	Physical characteristics of aerial parts extracts			
		Nature	**Colour**	**% yield (w/w) g**
1	Petroleum ether	Greasy	Dark green	1.60
2	Chloroform	Greasy	Green	2.15
3	Alcoholic	Paste	Brownish green	10.72
4	Aqueous	Sticky	Brown	9.40

Table No.4: Qualitative chemical tests for phytoconstituents of *Holostemmaada-kodien*

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) LibermanBurchard 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 Triterpenoidalsaponins (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.

Anotomy of the leaf *Holostemmaada –kodien*

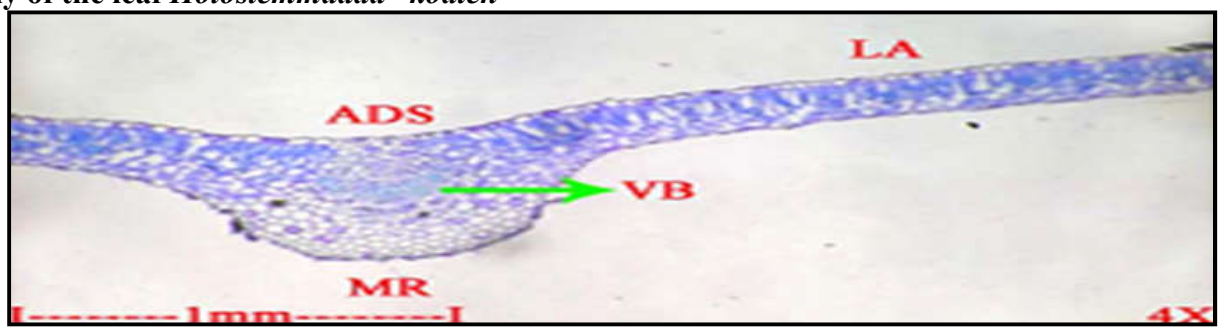

Figure No.1: T.S of leaf through midrib with lamina

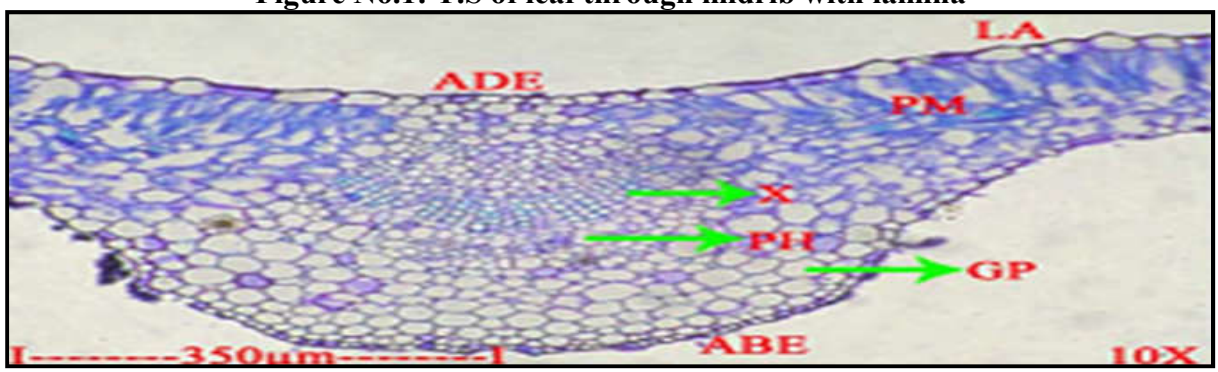

Figure No.2: T.S of leaf midrib with lamina enlarged

Anatomy of the lateral vein *Holostemmaada-kodien*

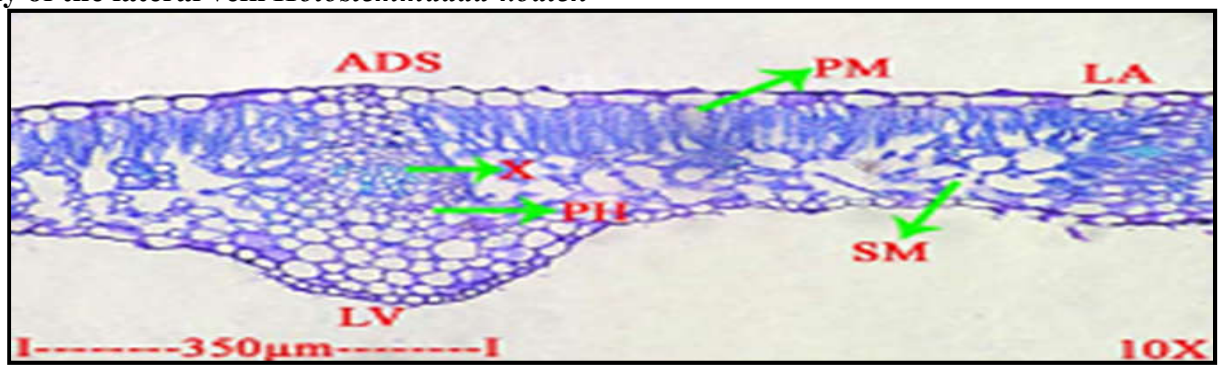

Figure No.3: T.S of lamina through lateral vein

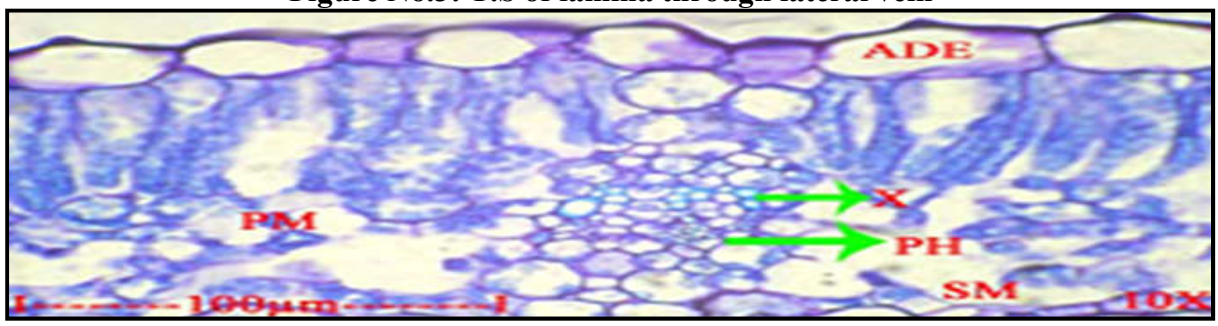

Figure No.4: T.S of lateral vein-enlarged

ABE-Abaxialepidermis; ADE- Adaxial epidermis; ADS- Adaxial side; GP-Ground parenchyma; LA-Lamina; LV-Lateral vein; MR-Midrib; PH-Phloem; PM- Palisade mesophyll; SM-Spongy mesophyll; VB-Vascular bundle; X-Xylem

Anatomy of the lamina *Holostemmaada-kodien*

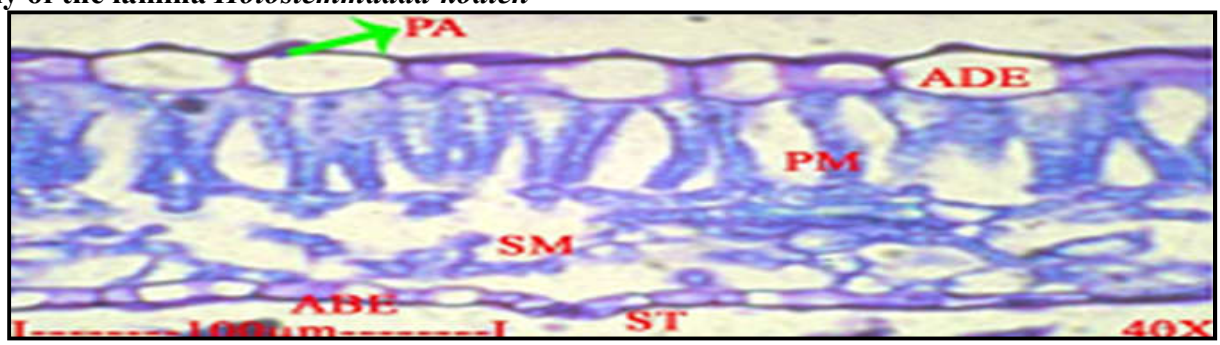

Figure No.5: T.S of lamina

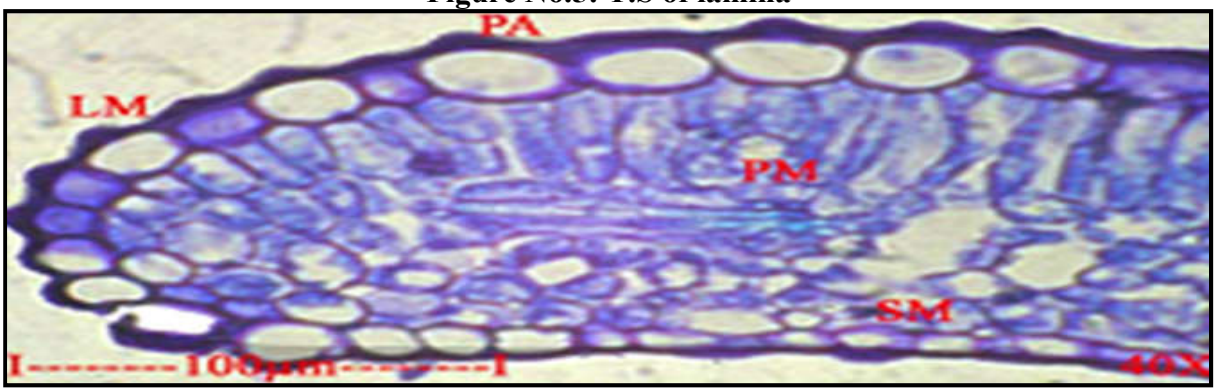

Figure No.6: T.S of leaf margin

ABE-Abaxial epidermis; ADE-Adaxial epidermis; ADS-Adaxial side; LM-Leaf margin; MT-Mesophyll tissue; PA-Papillae; PM-Palisade mesophyll; SM-Spongy mesophyll; ST-Stomata

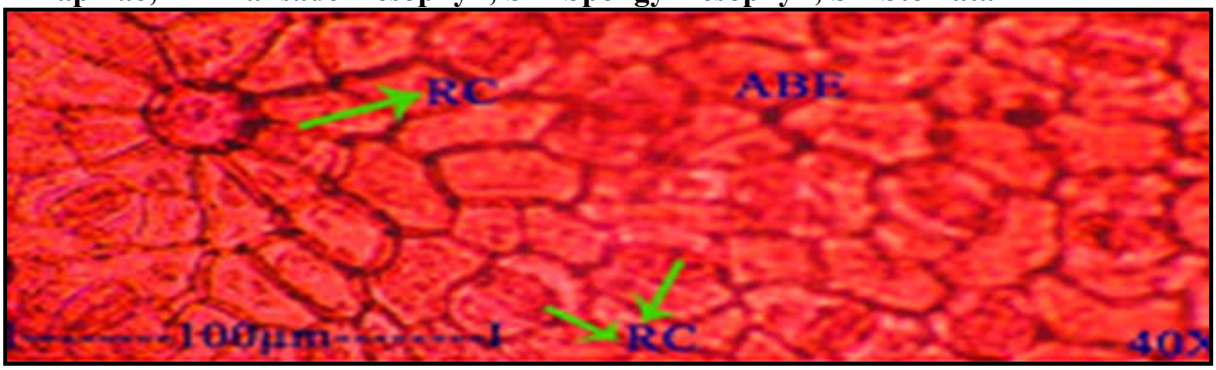

Figure No.7: Abaxial epidermis with stomata

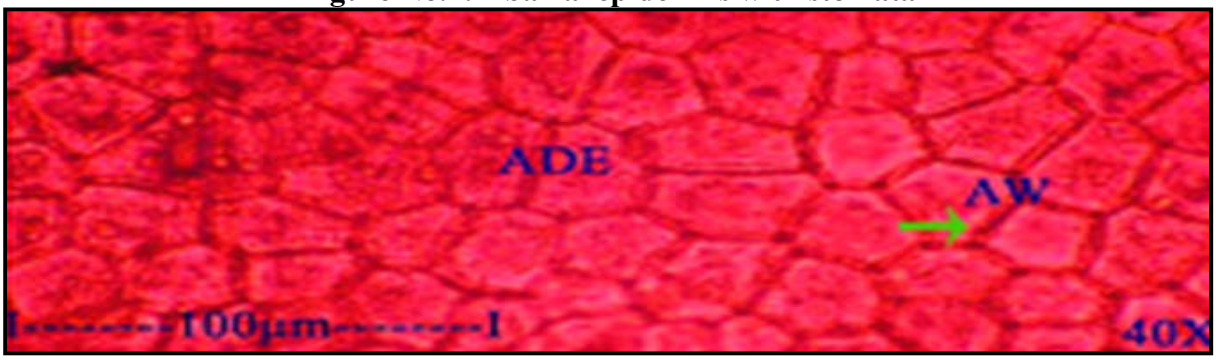

Figure No.8: Adaxial epidermis

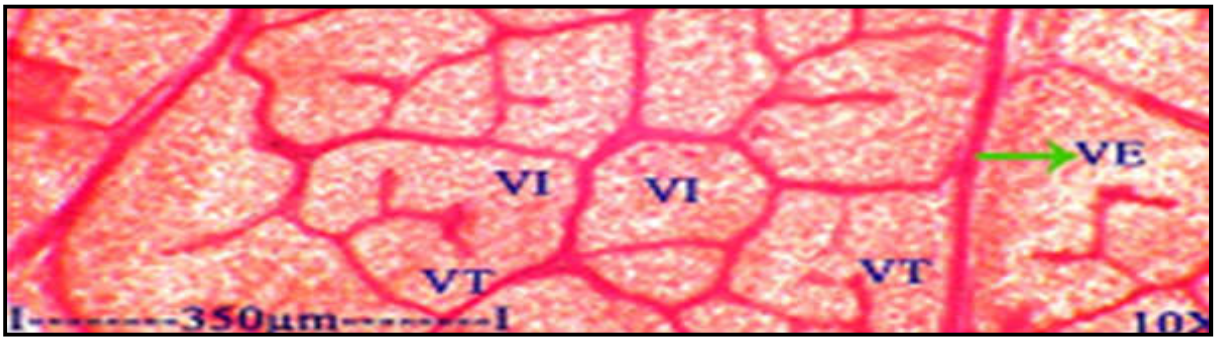

Figure No.9: Cleared leaf showing vein-islets and vein-termination

ABE-Abaxial epidermis; ADE-Adaxial epidermis; AW-Anticlinical wall; RC-Rosette cell; ST-Stomata; VE-Vein; VI-Vein-islets; VT-Vein-termination

Anatomy of young stem *Holostemmaada -kodien*

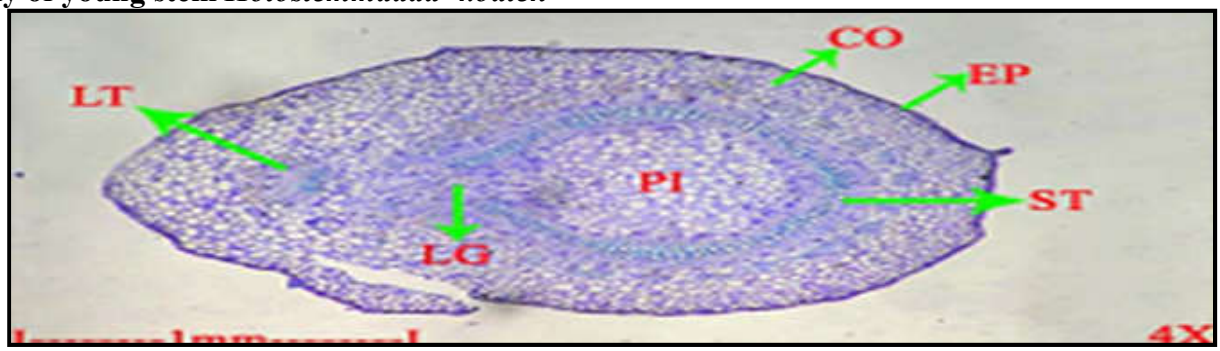

Figure No.10: T.S of stem entire view

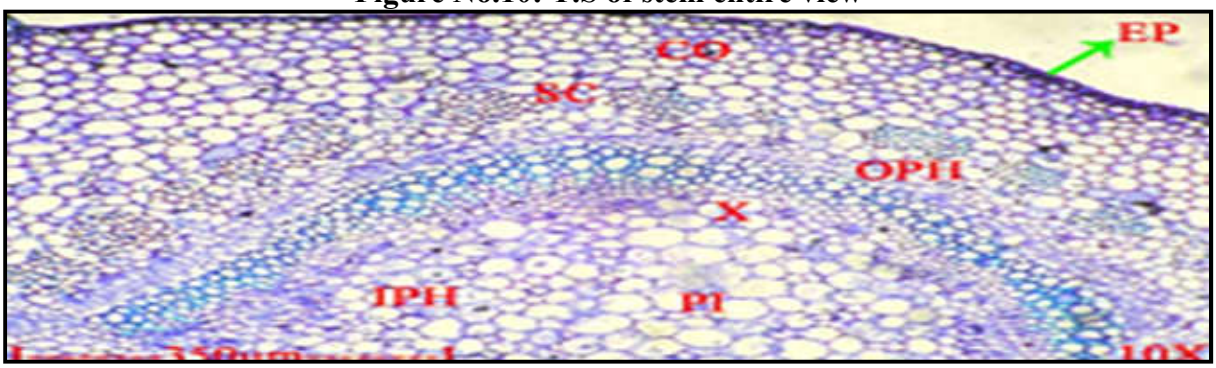

Figure No.11: T.S of stem a sector enlarged

Anatomy of the old stem *Holostemmaada-kodien*

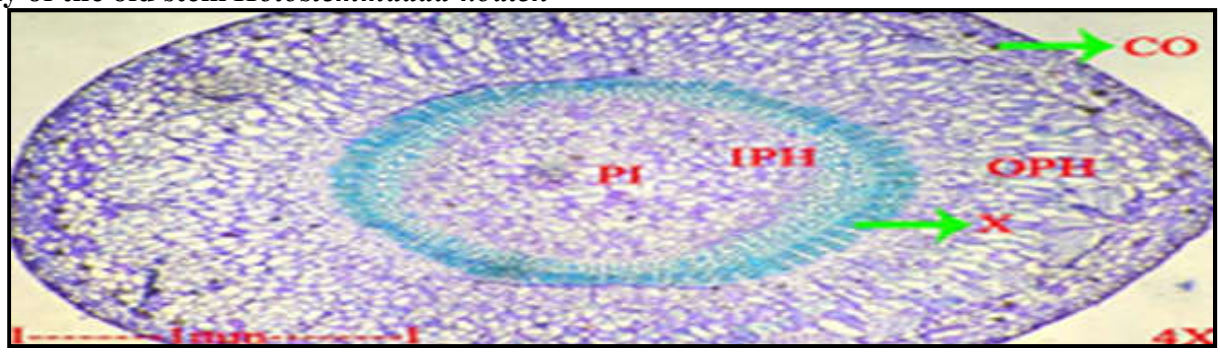

Figure No.12: T.S of stem ground plane

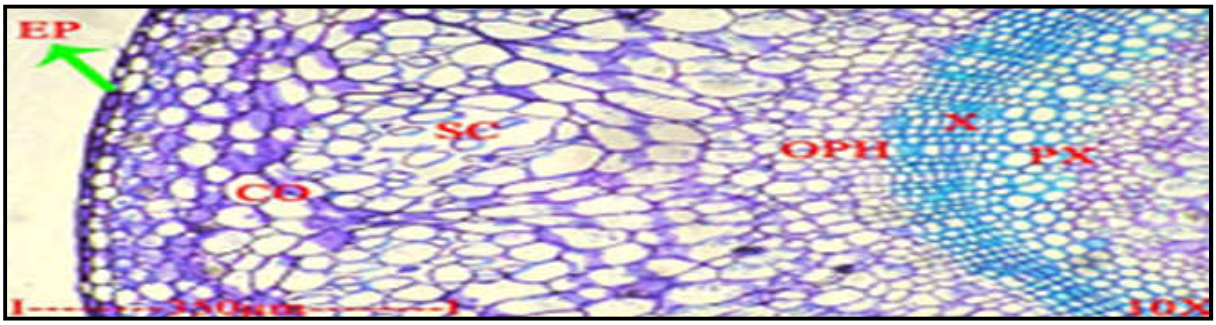

Figure No.13: T.S of stem a sector enlarged

Co-Cortex; EP-Epidermis; IPH-Inner phloem; OPH-Outer Phloem; PI-Pith; PX- Primary xylem; SC-Sclerenchyma; ST-Stele; X-Xylem

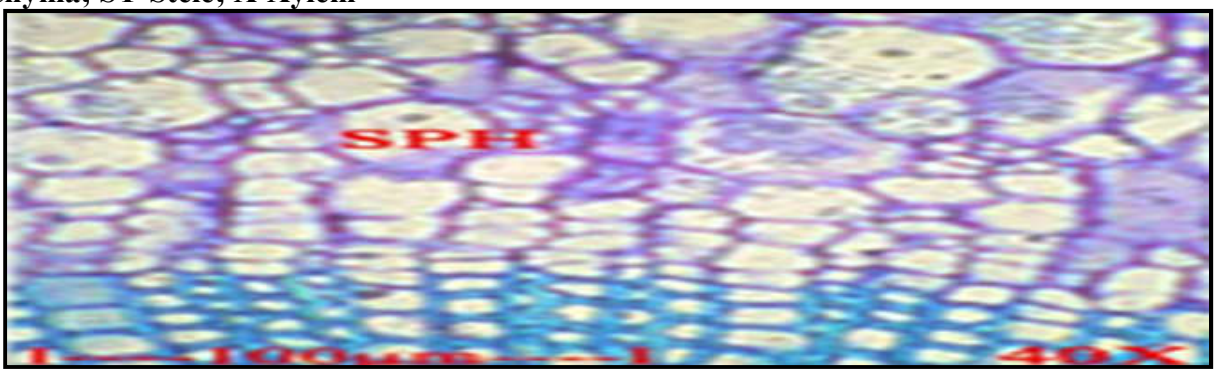

Figure No.14: T.S of old stem secondary phloem

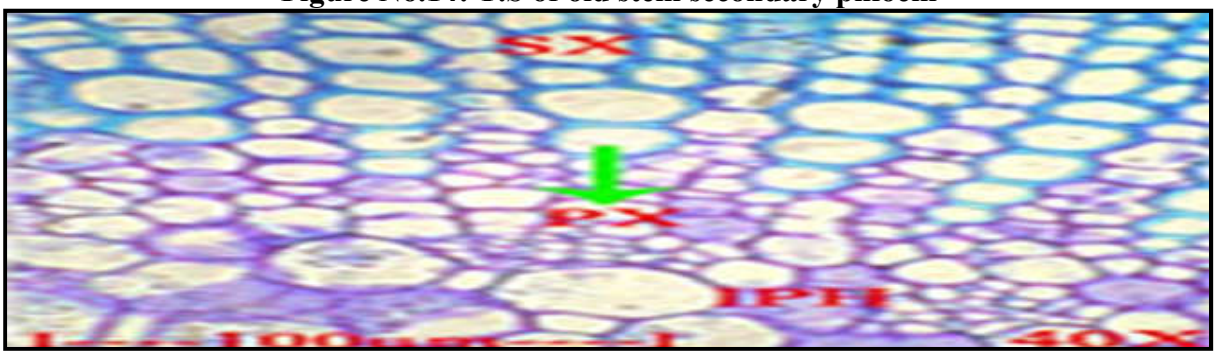

Figure No.15: T.S of old stem secondary xylem with inner phloem

IPH- Inner phloem; PX-Primary xylem; SPH- Secondary phloem; SX-Secondary xylem

Distribution of the druses (Crystals) (Under polarized light microscope)

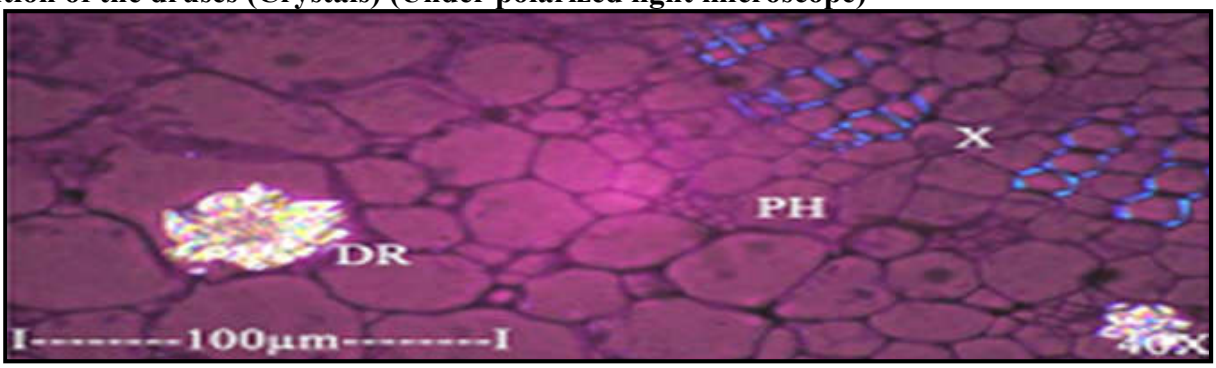

Figure No.16: T.S of showing druces in the ground tissue

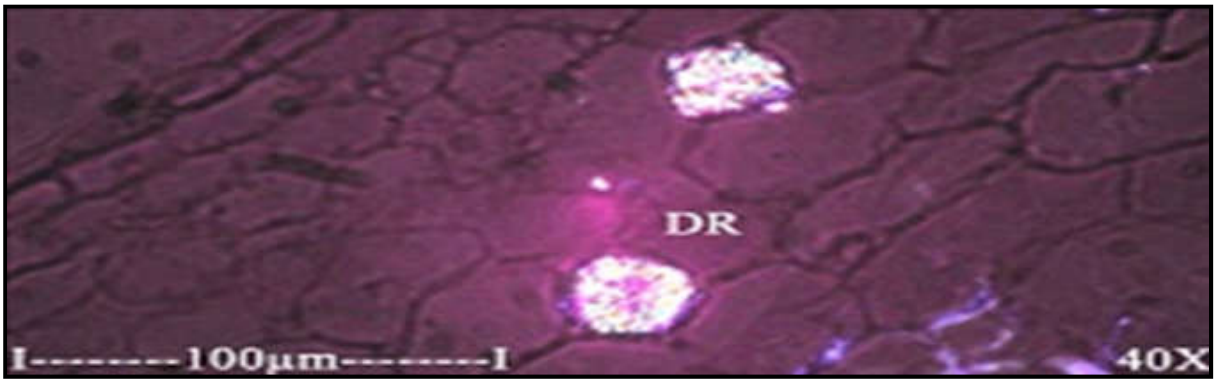

Figure No.17: T.S of showing druces in the cortical tissue

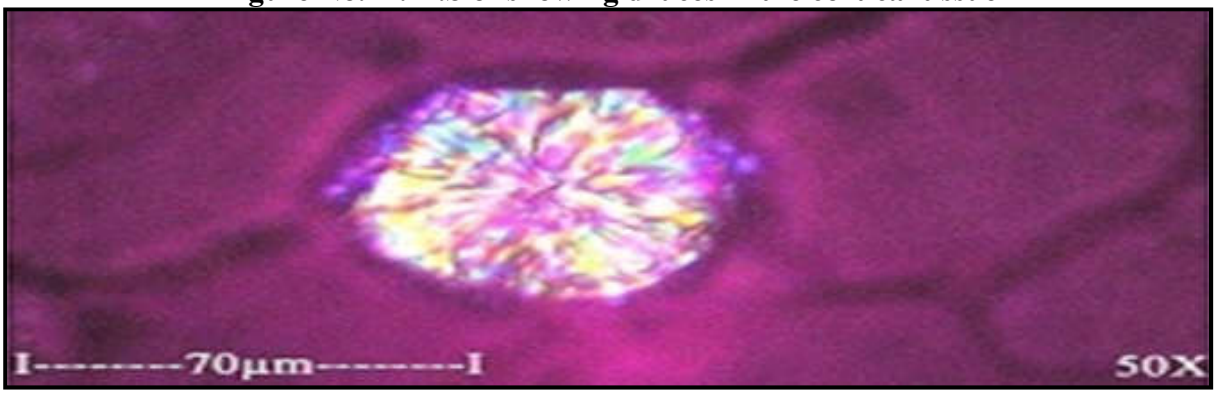

Figure No.18: Druses enlarged

DR-Druse; PH-Phloem; X-Xylem

CONCLUSION

In conclusion, the present study on pharmacognostical evaluation of *Holostemmaada-kodien* will be providing useful information in regard to its correct identity and help to differentiate from the other closely related species. The other parameters observed may be useful for the future identification of the plant.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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