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# PHARMACOGNOSTICAL EVALUATION OF AERIAL PARTS OF *DECALEPIS HAMILTONII*

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**ABSTRACT**

In this study, the pharmacognostical parameters for the aerial portions (leaves and stem) of the plant *Decalepis hamiltonii* were evaluated (Asclepiadaceae). The plant has historically been used to treat diabetes, blood problems, gout, jaundice, thirst, haemorrhage and urticaria. 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 legitimacy of *Decalepis hamiltonii*. This research aids in separating this medication from its other species.

**KEYWORDS**

*Decalepis hamiltonii (Asclepiadaceae)*, Pharmacognostical, Leaf and Stem.

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## 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 created a variety of methods and tools for health care as a result of his quest for eternal health and longevity and his need to find relief from suffering[1]. 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 verifying the identity and level of purity of such materials, according to the world health organisation (WHO, 1998), and should be carried out before any tests are carried out[5,6]. The Asclepiadaceae family includes the species *Decalepis hamiltonii*[7-9]. Locally known as Akasagaruda and Nagadonda.

It is spread in Ceylon, Gujarat, Rajputana, Punjab, Sind, and Andhra Pradesh. The plant grows in lower hill slopes in the state of AP, particularly on hedges, in the Nagapatla Reserve Forest, and in the Talakona Hills in Tirumala. The plant has historically been used as an emetic, bitter, and anti-inflammatory (Ayurveda). Snake bite, anaemia, leprosy, eczema, diarrhoea, arthritis, rheumatism, chronic mucous enteritis, and diabetes are all treated with root tubers. stem for diabetes, goitre, emetic, wounds, and filariasis[7-9]. 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, *Decalepis hamiltonii*, 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 voucher specimens for *Decalepis hamiltonii* (PARC/2007/182). For the investigation of macroscopical and microscopical features as well as quantitative microscopy, the specimens (leaf and stem) were employed. The extractive 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 for microscopy examinations according to the method described by Johansen[11] and Wallis[12].

### Physico-chemical analysis

Physico-chemical analysis the official procedures outlined by the Indian Pharmacopoeia[13] and WHO standards on quality control methods for medicinal plant materials, i.e. percentage of ash values and

extractive values, were used. WHO/QCMMPM recommendations [14].

**Preliminary phytochemical screening**

Initial phytochemical screening was done following the established procedures outlined by Kokate[15] and Harborne[16].

## RESULTS AND DISCUSSION

**Macroscopical characters**

It is a twiner of Woody. Branchlets have terete roots and bulging, winged nodes. The leaves are subooriaceous, orbicular or ellipticobovate, whole, undulate, and longitudinally folded. Flowers in axillary peduncled cymes are yellow. Five rectangular, brown-tinged, valvate calyx lobes. Corolla camanulate, having five valvate, villous and asymmetrical lobes. Connivent Stamen 5, five. Oval, lanceolate, or cylindrical folicles. Fruit epicarp is wrinkly and thick. Oval seeds with a long, white, silky coma at the tip. Fruits and flowers from June to January.

**Microscopic characters of *Decalepis hamiltonii***

**Microscopy of the *Decalepis hamiltonii* leaf**

The leaf has thick, prominently projecting midrib;

**Lamina:** It is thin and dorsiventral.

**Midrib**

It comprises a large, semicircular basal section and a widely conical adaxial part. The adaxial hump is 50metres high and 250metres wide, and the abaxial portion is 350metres wide. The midrib is 350metres long in the vertical plane.

Thin, round cells with cuticular papillae make up the epidermal layer. The remaining ground tissue of the midrib is parenchymatous, round to angular, less compact, and thin walled. One or two sub epidermal layers of the abaxial epidermis are identical to the epidermis. The ground parenchyma has many cells with high tannin concentrations (Figure No.1 and No.2).

The vascular system is made up of a phloem and vast xylem strand arc. The xylem is made up of several parallel rows of angular, thick-walled, three- to five-element structures (Figure No.2).

The mid-vein and the lateral vein are comparable (Figure No.3). It is 480m tall and has an adaxial cone and an abaxial semicircular body. It possesses a parenchymatous, tannin-filled ground parenchyma and a shallow arc of collateral vascular thread. The veinlet has a rather straightforward structure. It has a small elevation on both the adaxial and abaxial sides (Figure No.4). It has a top-shaped tiny vascular bundle with bundle sheath parenchyma extensions on both the adaxial and abaxial sides. Its thickness is 250m. The vertically oblong, broad, thick-walled epidermal cells at the leaf margin have a thick cuticle (Figure No.5). 80m thick in the margin. In the subepidermal region, it has a tiny cluster of cells with thick walls, and in the centre of the lamina, it differentiates into palisade-spongy mesophyll.

**Lamina: (Figure No.6 and No.7)**

130m thick is the lamina. It has a broad adaxial epidermis made up of cylindrical or circular cells with small papillae on the outside. Adaxial epidermis measures 20-25m in thickness. The abaxial epidermis is 10-15m thick and has barrel-shaped cells that are also rather broad.

An adaxial wide band of palisade cells and an abaxial zone of spongy parenchyma make up the mesophyll tissue. The 30 m tall, narrowly cylindrical palisade cells are arranged in a single row. The cells of the spongy parenchyma are lobed and loosely organised; it has four or five layers. The majority of the cells are tanniniferous (Figure No.6). Large calcium oxalate druses or sphaerocrystals were visible when the slices were studied with a polarised light microscope imbedded within the spongy parenchyma (Figure No.7). They have a 20m thickness.

**Adaxial epidermis (Figure No.8)**

The upper, or adaxial, epidermis, is apostomatic. The cells are tiny, square, rectangular, or polygonal in surface view, and their anticlinal walls are thick and gently undulating. Within the boundary of the epidermal cells, faint cuticular striations can be seen as thin parallel lines.

**Abaxial epidermis (Figure No.9)**

It has stomata of the anomocytic type and is stomatiferous. The guard cells range from being elliptical to circular. The anticlinal walls of the

abaxial epidermis are thick and wavy, giving the cells an amoeboid appearance. The epidermal walls do not exhibit cuticular marks.

**Venations (Figure No.10)**

The leaf exhibits a large reticulate venation system with lateral veins that are uniformly thick and wavy. The noticeable, wide vein-islets of diverse shapes and sizes are produced by the reticulate veins. The islets may be small and rectangular, or fairly wide and oblong or polygonal in outline. Vein-terminations are seen only in certain islets. The terminations are short, stumpy and straight, they are unbranched and simple.

**Microscopy of the *Decalepis hamiltonii* stem (Figure No.11-14)**

The stem has a smooth, uniform, spherical contour. The thickness is roughly 3mm. It has a wide cortex, narrow phloem, hollow thin xylem cylinder, and wide pith. It also contains an epidermal layer (Figure No.11).

The epidermis is thin and is made up of tiny, cir-cular cells with thick walls and slightly elevated tangential walls (Figure No.12 and No.13). Epidermis thickness is 10m. The outer cortex, which is located deeper within the epidermis, is made up of three to four layers of collenchyma and six to seven layers of broad, compact parenchyma (Figure No.13). Large, distinct masses of less lignified fibres are arranged in a cylinder along the cortex's inner margin; these sclerenchyma masses are spaced at regular tangential intervals. The thin-walled, delicate cells that make up secondary phloem are narrow and shatter when processed for sectioning (Figure No.14).

**Xylem cylinder**

The Xylem cylinder is a continuous cylinder with a consistent thickness; it is thin and hollow. The cylinder has thick-walled, angular cells arranged in regular radial packed rows. The vessels and fibres cannot be easily distinguished. The vessels are wider nevertheless, with a diameter of 20-30m. The fibres are angular and no wider than 15 micrometres (Figure No.14).

**Inner phloem or intraxylary phloem**

A fairly distinct cylinder of inner phloem, also known as intraxylary phloem, runs the length of the inner circumference of the xylem cylinder. Intraxylary phloem is the name given to this internal phloem. It is made up of phloem parenchyma cells and sieve components (Figure No.11).

**Root**

Thin and thick tuberous roots of the root system were examined.

**Table No.1: Quantitative microscopy (leaf constants) of *Decalepis hemiltonii***

| 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) | 5 | 4 | 3 | 5 |
| 4 | No. of epidermal cells / sq. mm (E) | 18 | 22 | 14 | 17 |
| 5 | Stomatal Index S I= (S/E+S)x100 | 21.73 | 15.38 | 17.64 | 22.72 |
| 6 | Average Stomatal No. | 4.25 per sq. mm | | | |
| 7 | Average Stomatal Index | 19.36 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) | 30 | 37 | 38 | 32 |
| 12 | Palisade ratio | 7.5 | 9.25 | 9.5 | 8 |
| 13 | Average Palisade Ratio | 8.56 | | | |
| 14 | Parameter → | | | | |
| 15 | No. of Vein-Islet per 4 sq.mm | 60 | 56 | 64 | 56 |
| 16 | No. of Vein-Islet per 1 sq.mm | 15 | 14 | 16 | 14 |
| 17 | Average Vein-Islet No. | 14.75 | | | |
| 18 | No. of Veinlet-Terminations per 4 sq. mm | 40 | 32 | 28 | 28 |
| 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 *Decalepis hemiltonii***

| S.No | Parameter → | Ash values (% w/w) |
|---|---|---|
| 1 | Parts used → | Aerial parts |
| 2 | Total ash | 7.25 |
| 3 | Water soluble ash | 5.50 |
| 4 | Acid insoluble ash | 4.50 |
| 5 | Sulphated ash | 6.50 |
| 6 | **Parameter →** | **Extractive values (% w/w)** |
| 7 | Ether soluble | 1.60 |
| 8 | Alcoholic soluble | 5.65 |
| 9 | Water soluble | 6.67 |

**Table No.3: Physical characteristics of extracts of *Decalepis hemiltonii***

| 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 | Sticky | Dark brownish green | 5.65 |
| 4 | Aqueous | Sticky | Brown | 6.67 |

**Table No.4: Qualitative chemical tests for phytoconstituents of *Decalepis hamiltonii***

| **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) LibermanBurchards 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) LibermanBurchard 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 thleaft C. *Decalepis hamiltonii***

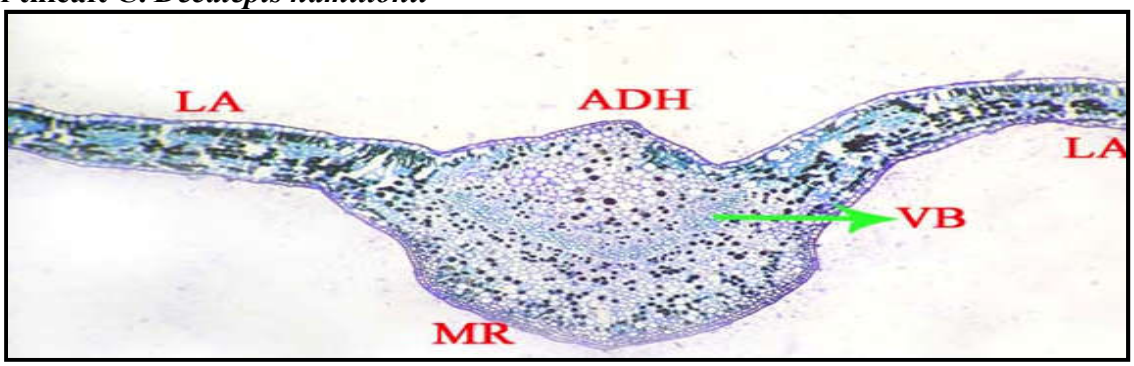


**Figure No.1: T.S of leaf through midrib with lamina**

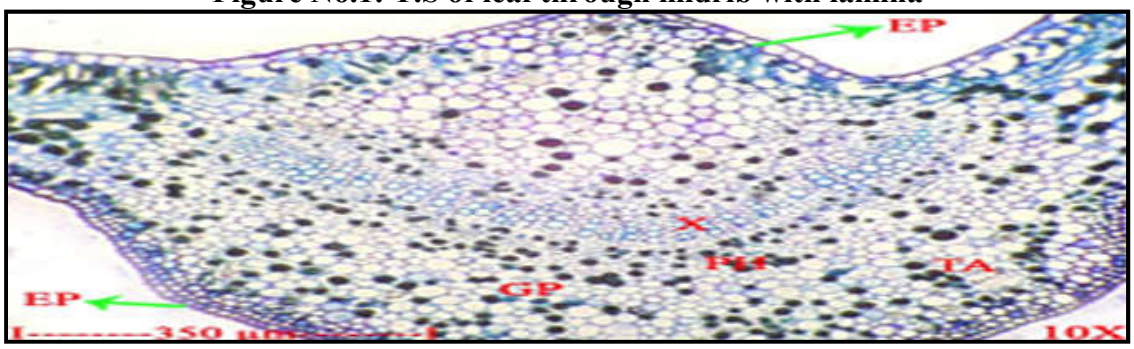


**Figure No.2: T.S of leaf through midrib with lamina**

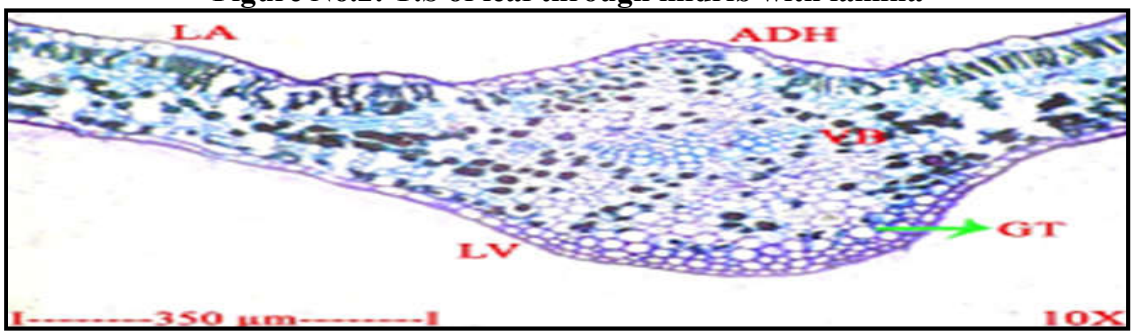


**Figure No.3: T.S of leaf through lateral vein with lamina**

**ADH-Adaxial hump; EP-Epidermis; GP-Ground parenchyma; GT-Ground tissue; LA-Lamina; LV-Lateral vein; MR-Midrib; PH-Phloem; TA-Tanniniferous; VB-Vascular bundle; X-Xylem**

**Anatomy of the lateral vein and leaf margin *Decalepis hamiltonii***

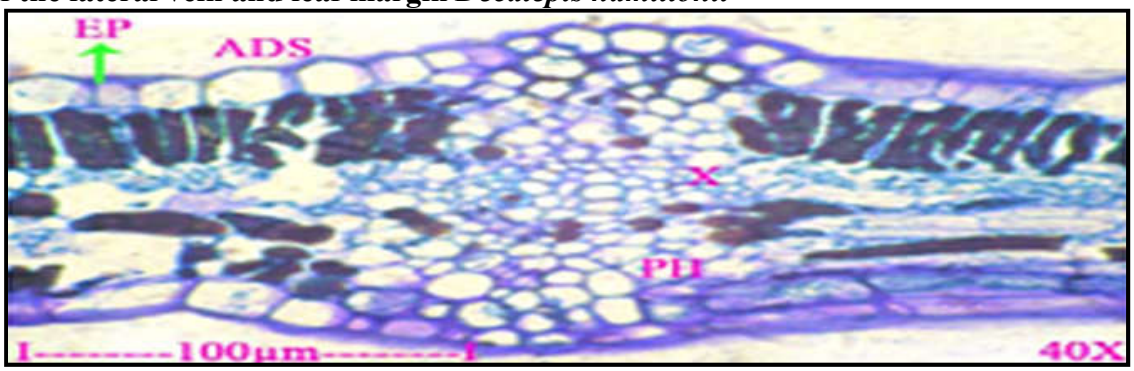


**Figure No.4: T.S of lateral vein-enlarged**

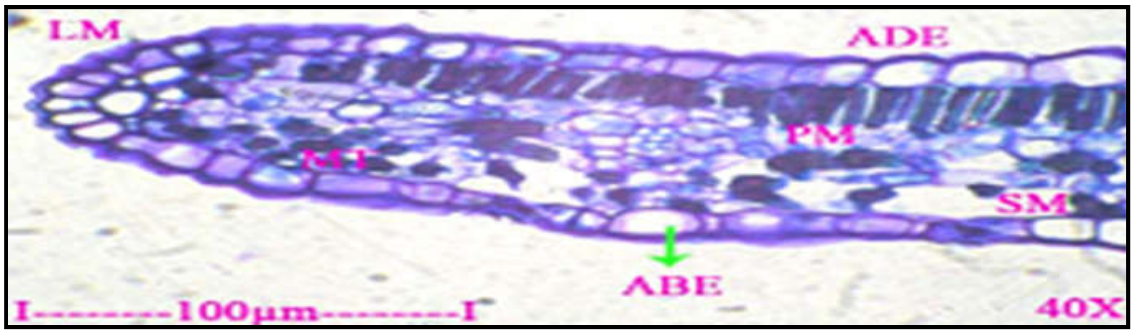


**Figure No.5: T.S of leaf margin-enlarged**

**ABE-Abaxial epidermis; ADE-Adaxial epidermis; ADS-Adaxial side; EP-Epidermis; LM-Leaf margin; MT-Mesophyll tissue; PH-Phloem; PM-Palisade mesophyll; SM-Spongy mesophyll; X-Xylem**

**Anatomy of the lamina *Decalepis hamiltonii***

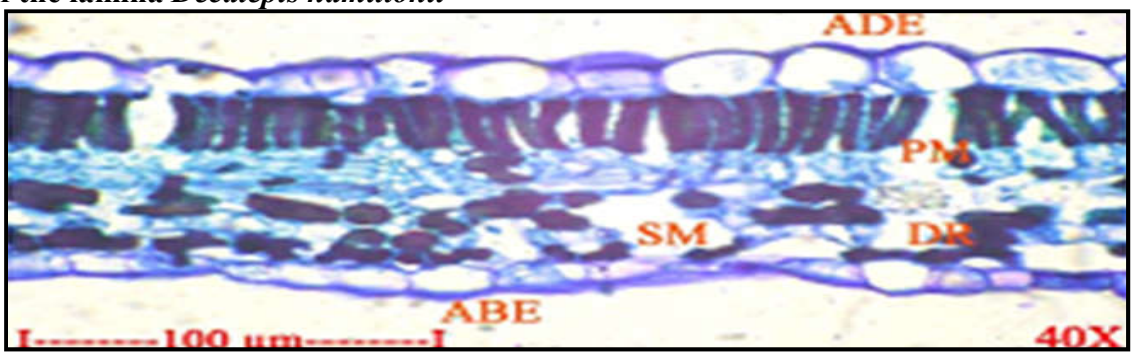


**Figure No.6: T.S of lamina (Under bright field microscope)**

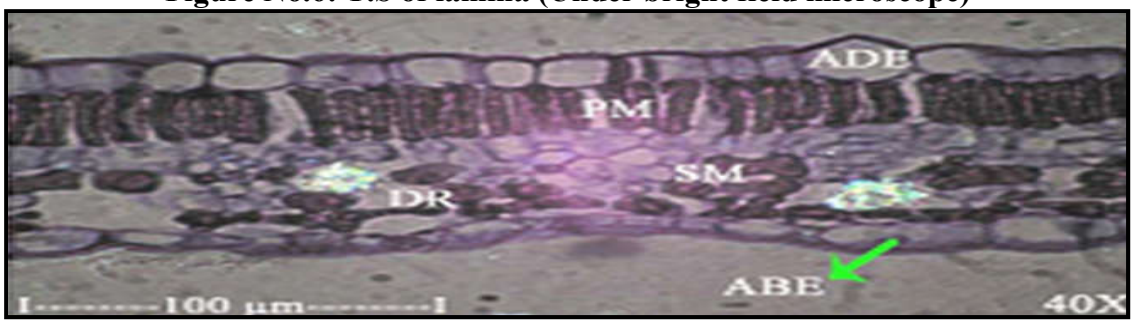


**Figure No.7: T.S of lamina showing druses in the spongy mesophyll tissue (Under polarized light microscope)**

**ABE-Abaxial epidermis; ADE-Adaxial epidermis; DR-Druses; PM-Palisade mesophyll; SM-Spongy Mesophyll**

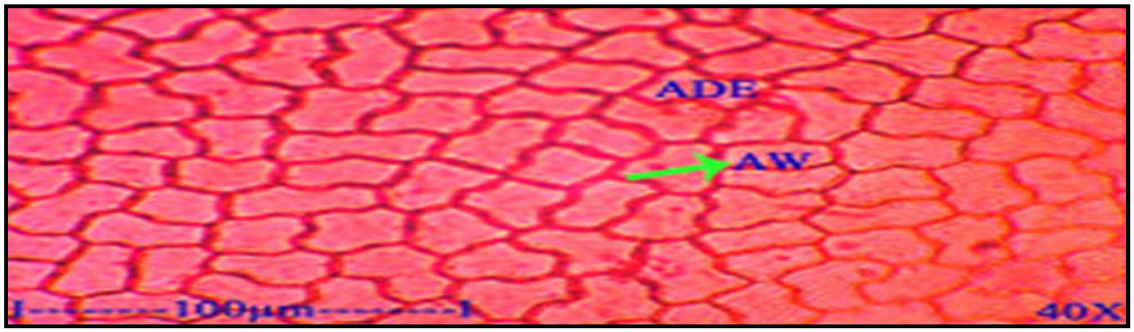


**Figure No.8: Adaxial epidermis**

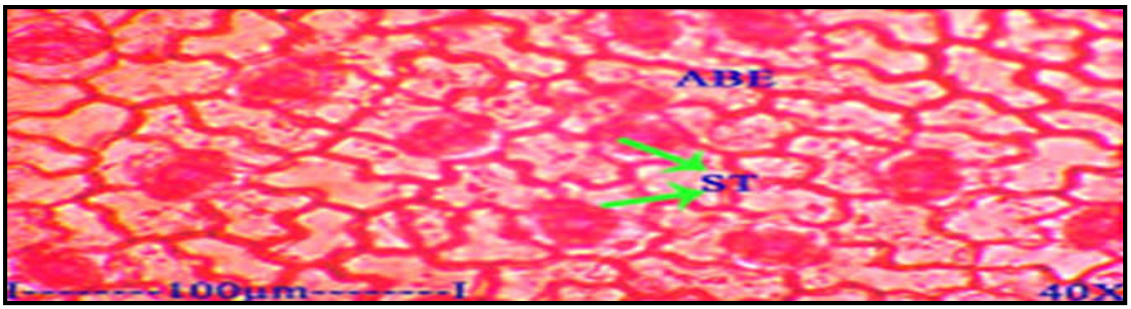


**Figure No.9: Abaxial epidermis with stoata**

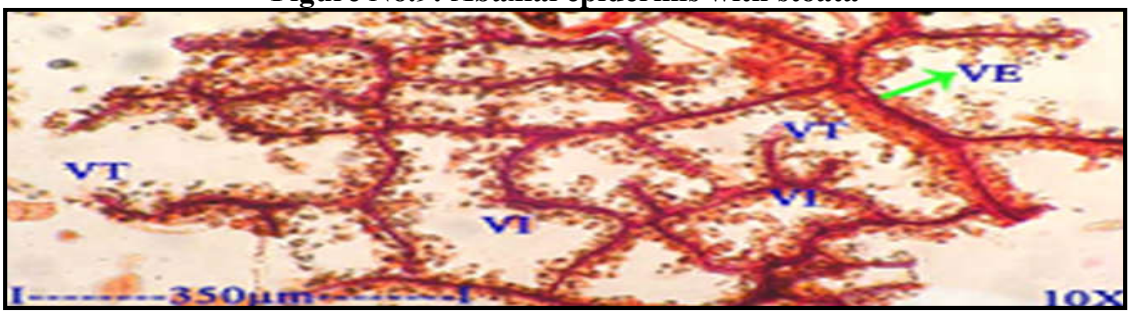


**Figure No.10: Cleared leaf showing vein-islets and vein-termination**

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

**Anatomy of the stem *Decalepis hamiltonii***

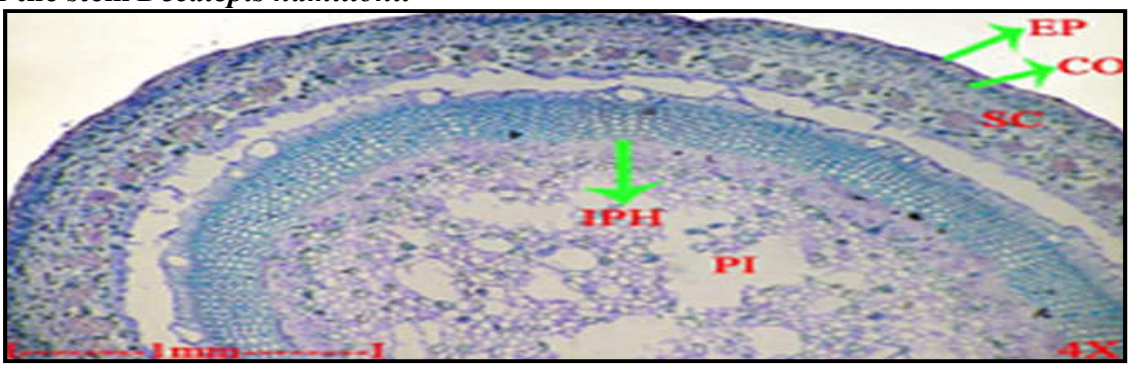


**Figure No.11: T.S of stem half portion enlarged**

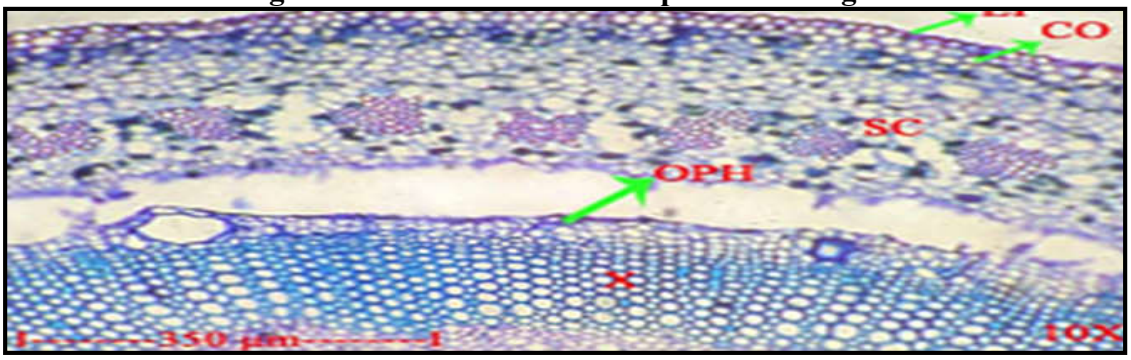


**Figure No.12: T.S of stem a sector magnified**

**Co-Cortex; EP-Epidermis; IPH-Inner phloem; OPH-Outer Phloem; PI-Pith; SC-Sclerenchyma; X-Xylem**

**The stem structure of *Decalepis hamilatonii***

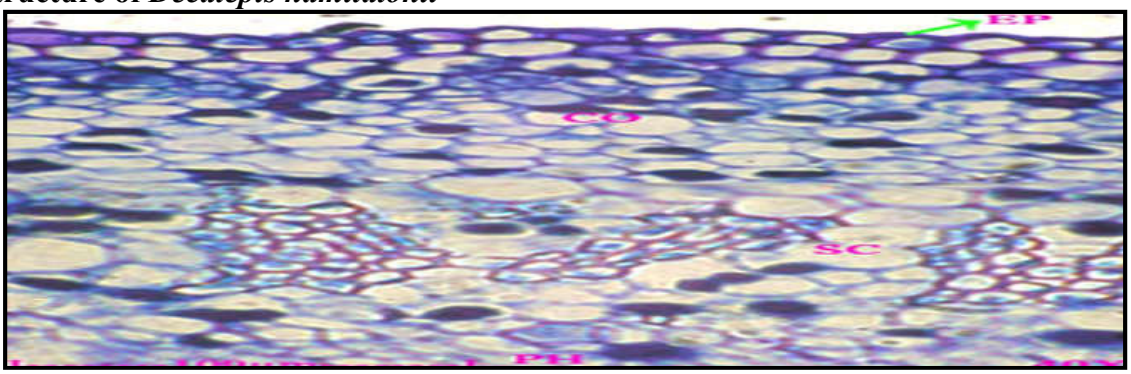


**Figure No.13: T.S of stem showing outer epidermis, cortex and sclerenchyma cells**

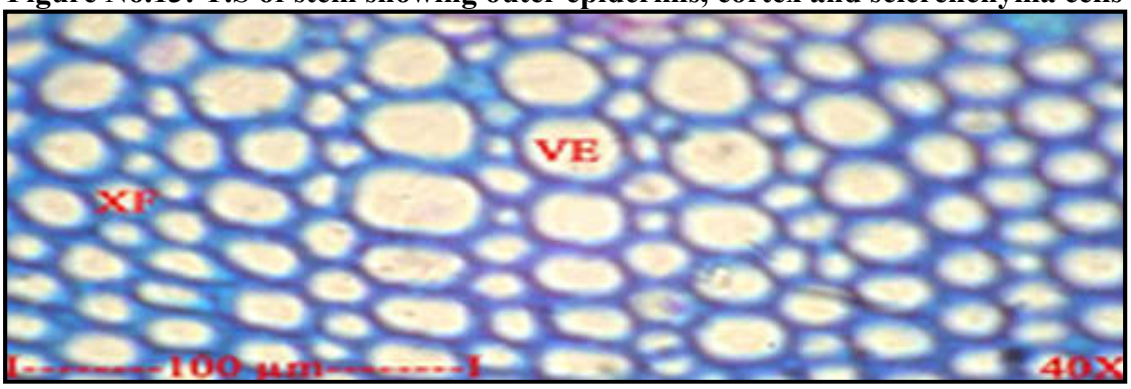


**Figure No.14: T.S of stem showing secondary xylem-enlarged**
**Co-Cortex; EP-Epidermis; PH-Inner Phloem; SC-Sclerenchyma; VE-Vessel; XF-Xylem fibres**

## CONCLUSION

In conclusion, the present study on pharmacognostical evaluation of *Decalepis hemiltonii* will be providing useful information inregard to its correct identity and help to differentiate from the other closely related species. The otherparameters observed may be useful for the future identification of the plant.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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