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PHARMACOGNOSTIC STUDIES ON AERIAL PARTS OF *Antigonon leptopus* Hook. & Arn. (Polygonaceae)

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ABSTRACT

Antigonon leptopus Hook. & Arn. (Polygonaceae) is a robust climbing vine commonly known as coral vine. It has been reported to have hepatoprotective, anti-diabetic, anti-thrombin, analgesic, anti-inflammatory and lipid peroxidation inhibitory properties. Pharmacognostical studies on aerial parts of *Antigonon leptopus* Hook. & Arn. has been carried out in the present study. The study includes macroscopical and microscopical evaluation along with estimation of its physico-chemical parameter such as ash values, extractive values, moisture content and fluorescence analysis of aerial parts powder of the plant. The present study reveals standardization profile for the plant *Antigonon leptopus* Hook. & Arn., which would be of immense value of authentication and botanical identification of the plant drug and may also help us in preventing its adulteration.

Keywords: *Antigonon leptopus* Hook. & Arn., Pharmacognostic studies, Standardization, Fluorescence.

INTRODUCTION

Plants and herbal preparations have been used as medicine since ancient time. With an increasing demand in the field of herbal medicines and cosmetics, it has become necessary and pertinent to probe into the area of systemic knowledge about herbal medicines. For the efficacy and safety of herbal products quality control is of paramount importance [1]. Therefore, it is very important to evaluate various quantitative and qualitative parameters, which may be helpful in setting standards for particular medicinal plant or parts of the plant. These standards can help in identify and characterized an individual drug, which may play a major role in maintaining purity and quality of that particular drug and its formulation and also prevent it form being adulterated by drug of same or other genus having low potency [2]. Standardization of herbal products is a complex task due to their heterogeneous composition, which is in the form of whole plant/plant part or extracts obtained thereof. To ensure reproducible quality of natural products, proper control of starting material is essential. Despite the modern techniques, identification of herbal medicines by pharmacognostical study is more reliable [3]. Thus, the present investigation deals with pharmacognostical studies of the aerial parts of *Antigonon leptopus* Hook. & Arn (Polygonaceae).

Antigonon leptopus Hook. & Arn. (Polygonaceae) is a robust climbing vine that holds via tendrils; and is able to reach 25 feet or more in length with tuberous roots, angled stems. It is commonly grown in gardens and often run wild throughout India [4]. It is native to Mexico and commonly found in tropical Asia, Africa, the Caribbean and the Americas [5]. Literature survey of this plant indicates its high medicinal values. Studies have reported that the plant *Antigonon leptopus* Hook. & Arn. exhibits hepatoprotective[6], anti-diabetic[7], anti-thrombin[8], analgesic and anti-inflammatory[9] and lipid peroxidation inhibitory[10] activities. Proper and detailed pharmacognostical studies have not been reported so far. Therefore, an attempt was made to standardize the drug on the basis of botanical, macroscopical, microscopical and physico-chemical parameters.

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MATERIALS AND METHODS

Plant Material

The aerial parts of *Antigonon leptopus* Hook. & Arn. (Polygonaceae) was collected from forest area of Srinivasa Mangapuram, Tirupati, Andhra Pradesh in the month of August, 2009. Care was taken to select healthy fully grown plant and normal organs. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, Chennai. The voucher specimen (PARC/2009/350) of the plant was deposited at the college, for further reference.

Macroscopical Studies

Macroscopical features of the aerial parts was studied directly in the field and photographed under original environment. The organoleptic features of powder prepared from aerial parts (stems, leaves and flowers) were also evaluated.

Microscopical Studies

The fresh leaves were cut into small pieces and fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 70% ethyl alcohol 90ml). After 24hrs of fixing, the specimen was dehydrated with graded series of tertiary butyl alcohol (TBA) as per the standard procedure [11]. After complete dehydration, the specimens were embedded in paraffin wax.

The paraffin embedded specimens were sectioned with the help of Rotary Microtome (thickness of 10-12µm). The dewaxing of the sections was carried out as per the procedure [12]. The sections were stained mostly with toluidine blue as per the method [13]. All permanent slides, after staining was dehydrated by using graded series of ethanol + Xylol and mounted in DPX. Photomicrographs were done on NIKON Lab Photo-2 Microscopic unit, using Konica colour film (100ASA). For normal observations, bright field was used. For the study of crystals, starch grains and lignified cells, the sections were photographed under polarized light. Magnifications of the figures are indicated by scale bars [14].

Physicochemical Parameters

Physicochemical parameters of the powdered drug such as Ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash), Extractive values (alcohol and water soluble) and Moisture content (Loss on Drying) were determined according to the procedure mentioned in Indian Pharmacopoeia [15].

Fluoresence Analysis

The powder prepared from aerial parts (stems, leaves and flowers) were treated with various solvents like 1N NaOH (alcoholic and aqueous), 1N H2SO4, 1N HNO3, 1N HCl, Acetic acid, Iodine, Ferric chloride, Ammonia and Water to evaluate the fluorescence analysis in visible/day light, long and short UV light. Various extracts of aerial parts were also subjected to fluorescence analysis. These parameters were carried out according to the standard procedures [16-17].

RESULTS AND DISCUSSION

Macroscopical Studies

It is assumed that macroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almost entirely on macroscopical characters. The macroscopical description of a crude drug includes size, shape, nature of outer and inner surfaces, type of fracture and organoleptic characteristics like colour, odour, taste, consistency, etc.

The macroscopical features of the fresh aerial parts (stem, leaves and flower) as well as the powder of dried aerial parts of *Antigonon leptopus* Hook. & Arn. were studied and the results shown in table 1. The photographs of whole plant and its aerial parts are shown in figures 1-3.

Microscopical Studies

Microscopical study of the plant drug either in entire or powdered form is one of the important aspect of its histological evaluation. Certain microscopical parameters like stomata, trichomes, calcium oxalate crystals, starch grains, stone cells, palisade ratio, vein islet number, vein termination number, etc, are important anatomical characteristics of organized drugs.

The microscopical studies of *Antigonon leptopus* Hook. & Arn. were carried out and it shown following features.

Anatomy of the leaf

The leaf has quite prominent abaxially protruding midrib and thin lamina. In transectional view, the midrib is more or less rectangular with more or less flat adaxial side and major part hanging abaxially. The midrib is 1.05mm thick and 900µm wide (Fig.4). The epidermal layer of the midrib is thin comprising small spindle shaped of squanish thin walled cells (Fig.5). The ground tissue is homogeneous and parenchymatous; the cells are polyhedral, compact and variable in size.

The vascular system of the midrib is unique and characteristic. It consists of one larger, semicircular abaxial vascular strand and a smaller, somewhat triangular adaxial vascular strand. The abaxial strand is 450µm wide and 300µm thick; the adaxial strand is 250 µm wide and 160µm thick. The vascular strands are collateral, having wide, circular, thick walled diffusely distributed xylem elements and a wide phloem abutting the metaxylem elements. The wide xylem elements are 40µm in diameter (Fig. 5).

Lamina: The lamina is even on both abaxial and adaxial sides, it is 90µm thick. The adaxial epidermal layer is

thick comprising diated, circular or barrel shaped cells (Fig. 6). The adaxial epidermis is 20µm thick. Unicellular, unbranched, short conical trichomes are often seen on the adaxial epidermis. The abaxial epidermis is comparatively narrow with rectangular or squanish cells. The mesophyll is differentiated into adaxial zone of the two layers of cylindrical palisade cells and 3 or 4 layers of spherical or lobed spongy parenchyma cells.

Leaf Margin: The leaf-marginal is slightly narrow, conical, blunt and is bent down. It is 60µm thick. The marginal portion consists of semicircular, thick epidermal cells and undifferentiated parenchymatous tissue (Fig. 7).

Epidermal Cells and Stomata: The epidermal cells of the lamina when viewed in surface view of the paradermal sections are thin walled and smooth, their anticlinal walls are undulate and the cells appear amoeboid in outline. The stomata occur on the lower epidermis. They are mostly aniso type; the stomata have three unequal subsidiary cells in circling the guard cells (Fig. 8). Some of the stomata also have four subsidiary cells. The stomata are wide and elliptical or circular. The stomatal pore is wide and narrowly elliptical. The guard cells are 15×20µm in size.

Venation Pattern: The venation system is densely reticulate. The lateral veins are thin slender, straight or slightly wavy. A thin parenchymatous sheath is seen all along the veins. The vein islets are distinct fairly wide and polygonal in outline. The vein-terminations are less frequent. When present, they are long, simple (unbranched) and straight (Fig. 9).

Cell Inclusions: In paradermal sections, large calcium oxalate druses here seen. The druses occur in wide circular mesophyll cells. They are random in distribution. The druses range in size from 10-20µm (Fig. 10).

Powder Microscopy

Powder sample of the aerial parts exhibits the following components.

Glandular Trichomes: Large numbers of glandular trichomes are seen in the powder. They are subsessile trichomes with unicellular, dilated spherical head. It had outer thick circle which is the cell wall of the secretory body. In the centre of the body is seen a circular, darkly staining portion, which represents the short, thick stalk of the trichome (Fig. 11).

Non-Glandular Trichomes: Multicellular, unbranched epidermal covering types of trichomes are common in the powder (Fig. 12, 13 and 14). The trichomes are two or three celled, unisereiate and thick walled. The outer surface is rough and echinate. The trichome has large basal cells which is burried in the epidermis (Fig. 13). They are either curved or straight. The trichomes are up to 330µm long and 20µm thick at the base.

Cell inclusions: Calcium oxalate druses and starch grains are common in the powder (Fig. 15 & 16). The druses are variable in size (as seen under polarized light microscope). They are up to 20µm in diameter (Fig. 15). Starch grains are prominausly visible when stained with IKI. They are spherical, ovoid or cylindrical in shape. The spherical grains are 30×20µm in size; the cylindrical ones are 30×50µm (Fig. 16).

Physicochemical Parameters

Physicochemical constant is an important parameter in detecting adulteration on improper handling of the drug. In the evaluation of crude drug, ash values, extractive values and moisture contents are important parameters. The estimation of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy matter. The determination of extractive values with a range of solvents gives information about extractable non-polar and polar as well as total extractable plant constituents. Determination of moisture content indicates the percentage of active chemical constituents in crude drugs mentioned on air-dried basis. The moisture content of a plant drug should be minimized in order to avoid decomposition of crude drugs either due to microbial contamination or chemical change.

The various physiochemical parameters of powdered drug of *Antigonon leptopus* Hook. & Arn. aerial parts was evaluated and the values are tabulated in table 2.

Fluorescence Analysis

Many phytochemicals fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent [18]. Hence, it is useful in detecting the adulterants and substituents.

The fluorescence studies of the aerial parts powder of *Antigonon leptopus* Hook. & Arn. showed the behavior and colour with different chemical reagents. These behavioral changes in different light showed different colours and mentioned in table 3. The powder shows the characteristic fluorescent colour when treated with alcoholic 1N NaOH and acetic acid exhibited the fluorescent orange colour under long UV light and aqueous 1N NaOH exhibited the fluorescent green colour under short UV light. The fluorescence analysis of various extracts were evaluated and the results shown table 4. In petroleum ether extract, fluorescent orange colour and in chloroform extract, fluorescent yellow colour was observed.

Table-1: Macroscopical Features of *Antigonon leptopus* Hook. & Arn. (Polygonaceae) aerial parts

Characteristics	Observations			
	Stem	**Leaves**	**Flowers**	**Powdered plant material**
Colour	Green with Brownish or purplish tinge	Green	Pink/Rose	Pale green
Odour	Odourless	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Slightly bitter	Characteristic	Slightly bitter
Texture	Slightly rough	Slightly rough	Smooth	---
Shape	Cylindrical; Angled	Cordate ovate / hastate ovate with acute apex	Pedicellate, Tepals-ovate to elliptic	---
Size	Up to 15m long	11.5-14.5cm long, 8.5-12.5cm wide	4-8mm long, 2-6mm wide	---

Table-2: Physicochemical parameters of *Antigonon leptopus* Hook. & Arn. (Polygonaceae) crude powder of aerial parts

Parameters	Determined values (% w/w)
Ash values	
Total ash	4.26±1.20
Acid insoluble ash	1.13±0.33
Water soluble ash	1.09±0.14
Sulphated ash	2.85±1.11
Extractive values	
Alcohol soluble	16.00±0.25
Water soluble	12.15±0.10
Moisture content	
Loss on drying	3.25±0.57

Values are expressed as Mean of triplicate determination ± SEM

Table-3: Fluorescence analysis of *Antigonon leptopus* Hook. & Arn. (Polygonaceae) crude powder of aerial parts

Treatments	Observations		
	Day light	**Long UV (365 nm)**	**Short UV (254 nm)**
Powder as such	Pale green	Pale green	Green
Powder + 1N NaOH (aqueous)	Orange	Dark green	Green Fluorescence
Powder + 1N NaOH (alcoholic)	Green	Orange Fluorescence	Brown
Powder + 1N H_2SO_4	Pale yellow	Pale yellow	Light green
Powder + 1N HNO_3	Pale yellow	Light green	Light green
Powder + 1N HCl	Pale yellow	Pale yellow	Light green
Powder + Ammonia	Brown	Dark green	Green
Powder + Acetic acid	Light green	Orange Fluorescence	Green
Powder + Iodine	Pale yellow	Green	Light green
Powder + $FeCl_3$	Dark green	Dark green	Light green
Powder + water	Pale yellow	Light green	Light green

Table-4: Fluorescence analysis of *Antigonon leptopus* Hook. & Arn. (Polygonaceae) extracts of aerial parts.

Extract	Day light	Long UV (365 nm)	Short UV (254 nm)
Pet. ether	Pale yellow	Orange Fluorescence	Light green
Chloroform	Green	Yellow Fluorescence	Green
Methanol	Dark green	Greenish yellow	Green
Dist. water	Orange	Orange	Light green

Fig. 1: *Antigonon leptopus* Hook. & Arn. Whole plant

Fig. 2: *Antigonon leptopus* Hook. & Arn. Flowers

Fig. 3: *Antigonon leptopus* Hook. & Arn. Leaf

Fig. 4: Transverse Section of leaf through midrib with lamina

Fig. 5: Transverse Section of midrib enlarged

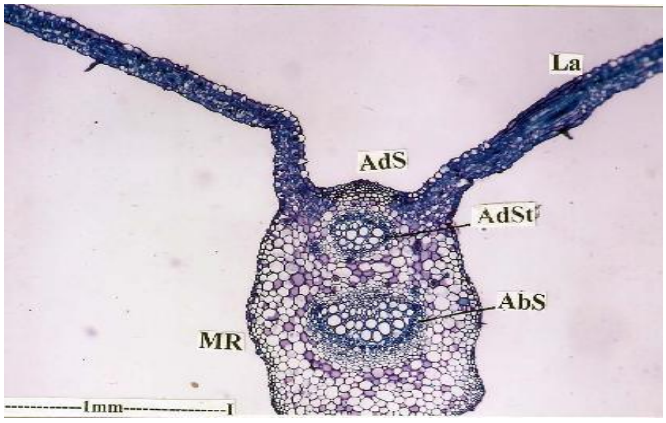


Fig. 6: T.S. of lamina

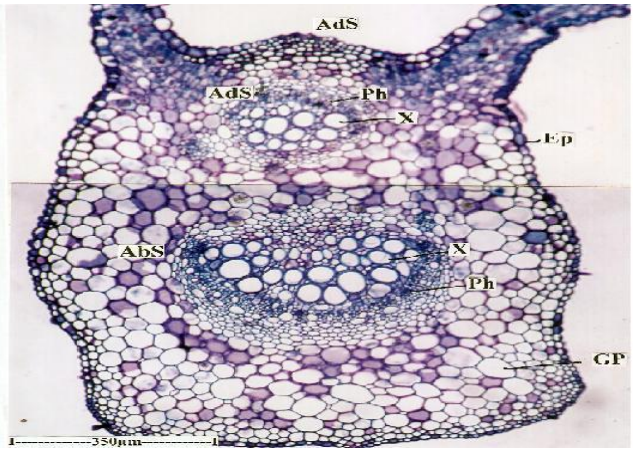


Fig. 7: T.S. of leaf margin

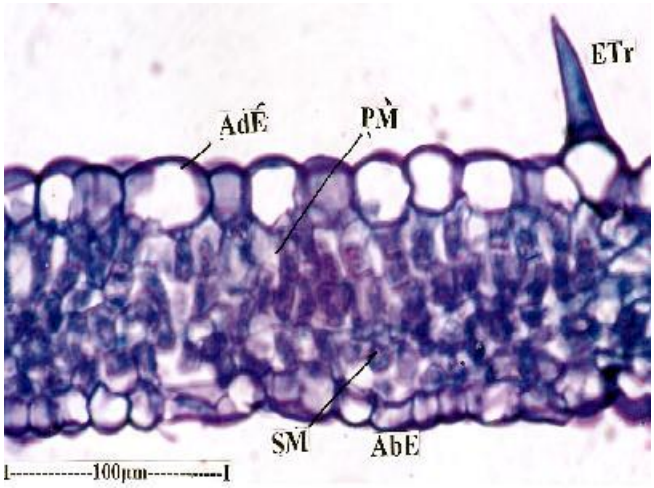


Fig. 8: Paradermal section showing abaxial epidermis with stomata

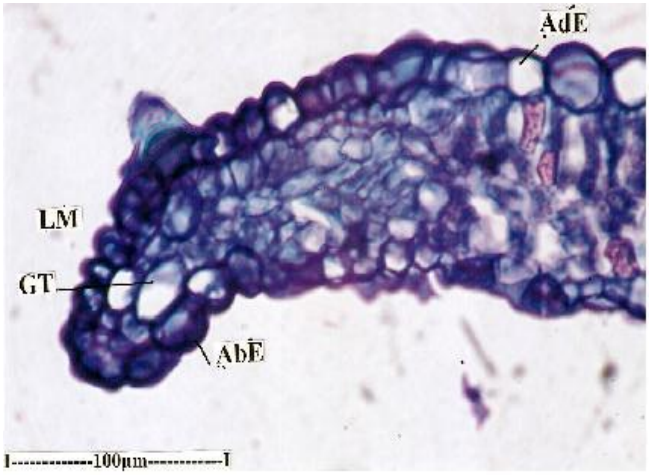


Fig. 9: Vein-islets and vein-terminations

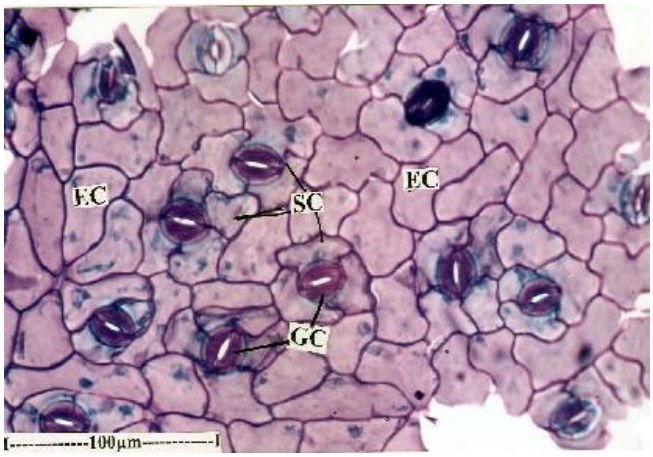


Fig. 10: Druses in the leaf mesophyll tissue

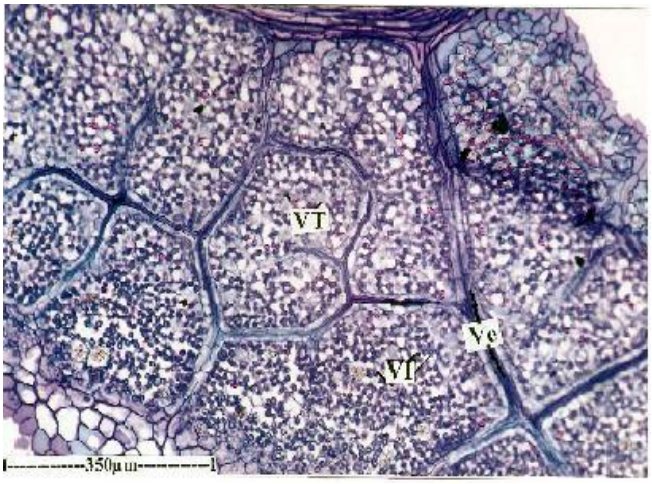


Fig. 11: Fragment of lamina showing glandular trichomes

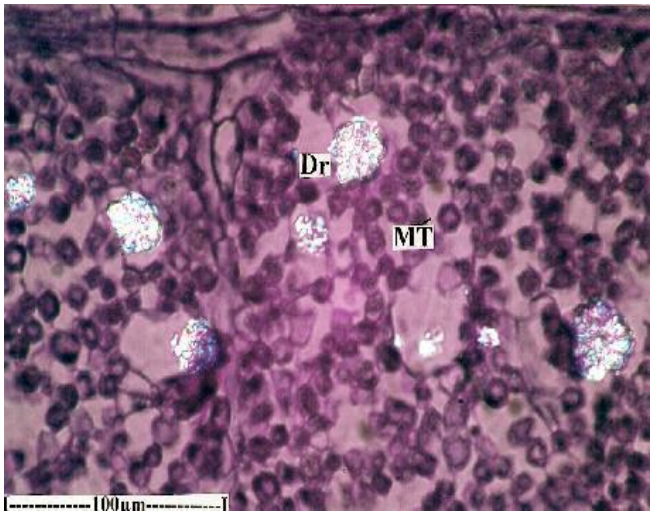


Fig. 12: Short covering trichome (strained with safranin)

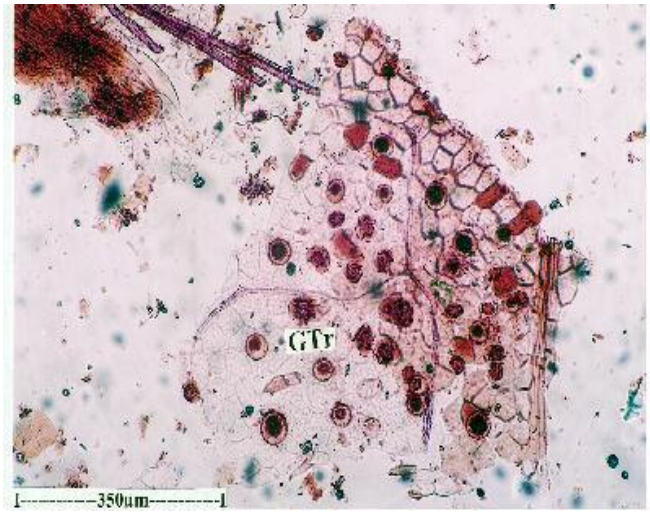


Fig. 13: Narrow longer covering trichome (strained with safranin)

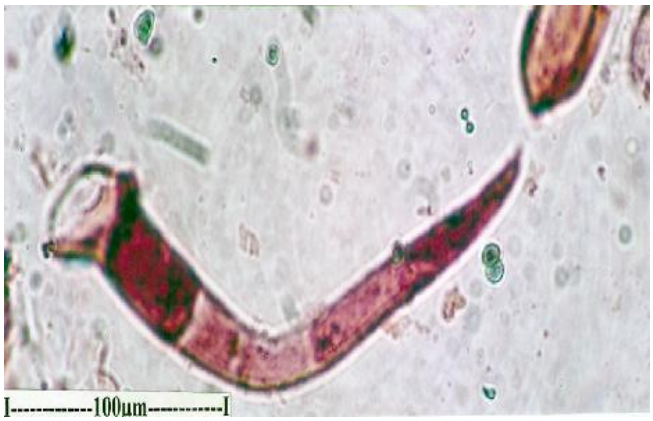


Fig. 14: Non-glandula trichomes
(Un-stained)

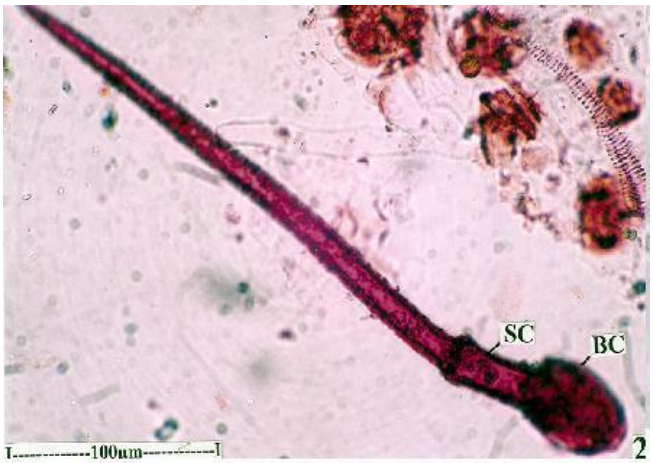


Fig. 15: Crystals and mesophyll tissue

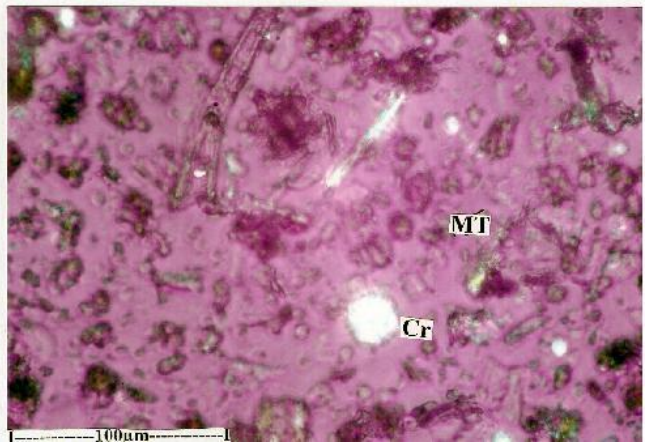
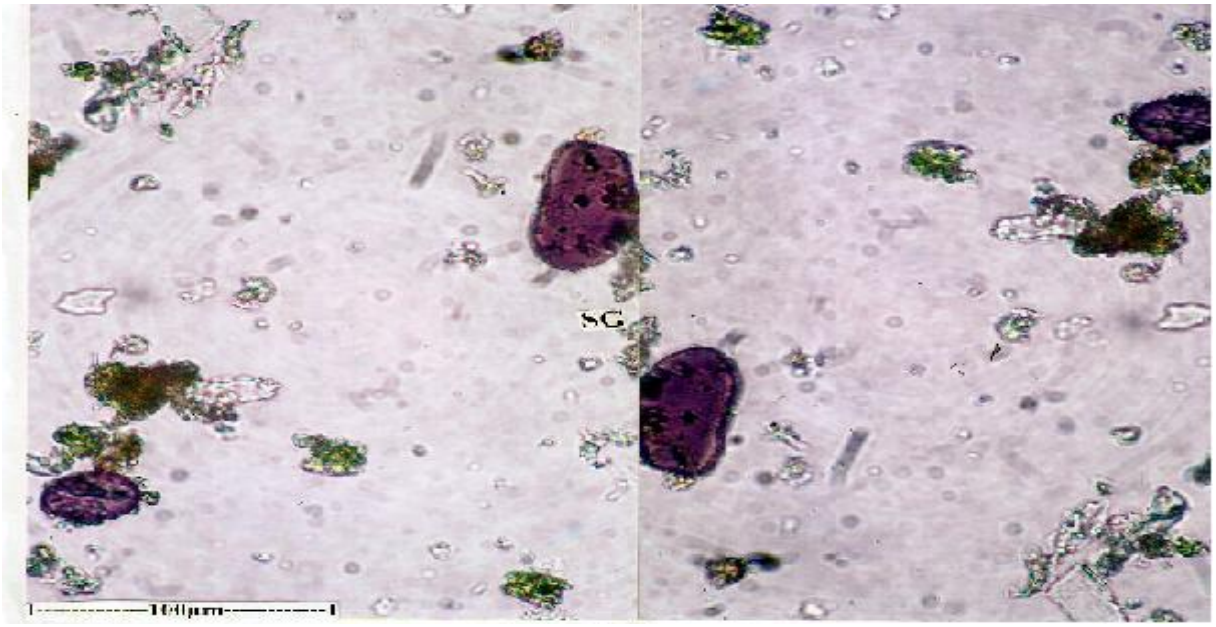


Fig. 16: Starch grains in the powder



(Cr – Crystals; MT – Mesophyll tissue, SG – Starch Grains)

CONCLUSION

The results obtained from present study may play a major role in setting particular standards for the

plant *Antigonon leptopus* Hook. & Arn. (Polygonaceae), which might broaden its pharmacognostical, pharmacological, botanical and economical importance. These parameters may also prove beneficial in identification of the plant. Thus, with the help of these standards we identify the adulteration of *Antigonon leptopus* Hook. & Arn. which will be of great use for the future workers in selecting the correct herbal specimen.

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