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Macro microscopic fingerprints of panchanga of Ishwari- *Aristolochia indica* Linn.

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Abstract

The drug Ishwari or Nakuli is botanically equated to *Aristolochia indica* Linn. The drug has been mentioned in Samhitha Granthas in different form for the condition like sheetha jwara (fever with rigor), sarpa visha (snake bite poisoning), vrana (wound), ekanga shopha (local oedema), unmada (schizophrenia), apasmara (epilepsy) etc. Substitution and adulteration due to similar morphological features, same classical vernacular name, and presence of similar active principles affect the therapeutic activity of medicinal products. Systematic macro-microscopy identity of herbal raw drug is becoming increasingly important to produce standardized herbal products. In this study, macro-microscopic and powder characters were recorded for five different parts (panchanga) of raw herb Ishwari using standard methodology. Both root and stem showed the presence of oil globules, stone cells, and starch grain. The midrib region showed a prominent vascular bundle, while there are few trace bundles distributed in the mesophyll tissue. The portion of perianth enlarged showed lower mesophyll tissue formed by parenchyma with intercellular space having a little chlorophyll in it. TS of fruit stalk is circular in outline, shows an epidermis covered with a cuticle. TS of fruit show an epicarp having 8 to 10 layers of parenchyma forming mesocarp. In the parenchyma cells below the endocarp there is a seed having testa, endosperm parenchyma. The powder microscopy showed the important cells of individual part of the plant. These diagnostic features can be used as a fingerprint for the identification and differentiation of their substitute and adulterants of the plant.

Keywords: *Aristolochia indica*, Ishwari panchanga, Macro-microscopy.

Introduction

The drug *Ishwari* also known *Nakuli* in *Samhitha Granthas* and the source of which is *Aristolochia indica* Linn. In *Caraka samhita* the drug has been prescribed for the preparation of *taila* (medicated oil) for *sheetha jwara* (fever with rigor) and *ghrta* (medicated ghee) for *jwara* (fever), *unmada* (schizophrenia) and *apasmara* (epilepsy).¹ In *Sushruta samhita* it has been mentioned for the preparation of *lepa* for *Sarpa visha*.² *Acharya Vagbhata* used this drug as *lepa* for *ekanga shopha* and preparation of oil for *sheetaja jwara*.³ *Guna Karma* of the drug is mentioned in *hareetakyaadi varga of Bhavaprakasha* where the drug is said to be effective in wound healing process.⁴ The plant root is said to be useful in the management of intermittent fever, children's bowel complaint and most extensively the root and leaf is used in the treatment of snake bite poisoning.⁵

Medicinal plant materials are being adulterated in commerce due to many reasons such as similar morphological features, same vernacular or classical name, presence of similar active principles etc. The practice of substitution and adulteration will badly affect the therapeutic activity of herbal products. Therefore systematic drugs identification of drugs is an essential step while producing standardized herbal products. There are many species of *Aristolochia* such as *A. tagala* and *A. bracteata* are used as substitutes for the root of *A. indica*. On the contrary *A. indica* root are employed as good substitutes for imported serpentaria i.e. *A. serpentaria* Linn.^{6,7}

Evaluation of crude drug is identification and determination of its purity and quality. The macroscopic or external morphological description of a crude drug includes size, shape, nature of outer and inner surface, type of fracture and organoleptic characteristic like color, taste, consistency etc. Microscopic or histological studies are important part of crude drug evaluation where the drug is studied in entire or in powder form. Arrangement of tissue in transverse and longitudinal section is made and types of cell and their contents are investigated with the help of microscope. Certain microscopical characteristics like stomata, trichomes, calcium oxalate crystals, starch grains, stone cells, fibers and vessels etc are important anatomical characteristic of organized drug.⁸

In this study an attempt is made to document the detailed atlas of macroscopy and microscopy of entire and powdered drug of whole plant parts like root, stem, leaf, flower and fruit of *Aristolochia indica* Linn.

Materials and Methods

Plant materials

Authenticated whole plant parts of *Aristolochia indica* Linn. were collected from herbal garden of SDM College of Ayurveda Udupi for macroscopic and microscopic study.⁹ A specimen of the samples is deposited in Pharmacognosy department of XYZ (255/13051008). Fresh sample preserved in Formalin-Alcohol-Acetic acid solutions (FAA) was used for histological studies. For powder microscopy the above parts were dried in shade, powdered and sift through mesh 40; the powder was stored in glass vials until microscopic evaluation.

Instrumentation and techniques

Microscopy

Sample was preserved in FAA fixative solution. FAA was prepared from formalin 5 ml, acetic acid 5 ml and 70% ethyl alcohol 90 ml). The materials were left in FAA for more than 48 hours. The preserved specimens were dehydrated with graded series of tertiary-butyl alcohol. The dehydrated specimens were sectioned with the help of 7° clock platinum blades using hands. The sections were stained with safranin.¹⁰ Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine.¹¹ Slides were observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Results

Macroscopy

Habitat: It is a greenish or pale to dark colored perennial climber with woody stem.

Root: Tap root, long, cylindrical, branched, irregularly bent, about 3 to 10 mm in diameter, grayish-brownish externally, smooth, finely wrinkled, inner surface white, fracture short and splintery, taste strongly bitter, odor characteristic.

Stem: Twining, slender, woody at the base, grooved, glabrous, brownish externally, smooth, finely wrinkled, inner surface white, fracture short and splintery, taste strongly bitter, odor characteristic.

Leaf: Simple, alternate, linear-oblong to obovate oblong or sub-panduriform, obtusely acuminate, entire with somewhat undulate margin, glabrous, cuneate at base, rounded, sub-truncate or sub-cordate, 3.5 to 12.5 cm long and 1.5 to 7.5 cm broad, taste bitter, odor characteristic.

Flower: In few flowered axillary racemes, bracts small, ovate, acuminate, opposite to the pedicels, pedicel long, thickened above, perianth greenish white, up to 4.5 cm long with globose inflated base, bent at right angle and suddenly narrowed into a cylindrical tube with oblique trumpet-shaped mouth gradually passing into a long narrow, linear-oblong, obtuse brownish lip. Anthers 6, style 6-lobed.

Fruit: A capsule, 4 to 5 cm long, oblong or globose-oblong opening from below upwards, 6 valved, the pedicel split into 6 filaments.

Seed: Deltoid, ovate, acute, flat, winged (Figure 1).



Figure 1: Macroscopy of panchanga of *Ishwari*

Microscopy

Root: Diagrammatic TS of root is circular in outline with irregular surface. There is an outer layer of cork followed by cortex, and a thin discontinuous pericycle surrounding 4 'V' shaped vascular bundles at the centre (Fig 2.1).

Detailed TS shows 8 to 10 layers of cork composed of tabular thin-walled cells, except the outer most layer which is composed of thick-walled cells with brownish content; secondary cortex 15 to 17 layered composed of thin-walled, rounded to isodiametric cells in the outer region and tangentially elongated in the inner region; plenty of round to oval simple starch grains measuring 5 to 18 μm in diameter, and 2 to 4 compound starch grains having components measuring 10 to 15 μm in diameter, and few oil globules present in some cells; in the middle region there is a pericycle made up of round, rectangular, oval or elongated stone cells in small irregular patches having simple pits and radiating lumen; centre of the section is occupied by xylem, split into strips of radiating arms by wedge shaped masses of parenchyma; each xylem arm is capped by thin patches of phloem consisting of sieve elements and phloem parenchyma, phloem fibres, and occasionally stone cells also found in this region; there is a ring of cambium present between phloem and xylem; xylem consists of large vessels, tracheids, fibres tracheids and parenchyma, all being lignified; in older roots, tyloses seen in vessels; medullary rays 8 to 10 in number, multiserial and dilating towards periphery and alternating with radiating arms of xylem; scattered group of stone cells present in a few wider rays; micro-crystals with a few appearing as elongated small prisms are present in a few cortical and ray cells (Fig 3).

Stem: Diagrammatic TS of root is heptagonal in outline with 7 wing-like projections. There is an outer layer of epidermis, often turning to cork tissue in mature stem, followed by cortex, a pericycle almost continuous surrounding 7 'U' to 'V' shaped vascular bundles having a obliterated pith (Fig 2.2).

Detailed TS shows single layered epidermis with thick cuticle, the epidermis often dividing and forming 3 to 4 layers of cork composed of tabular thin-walled cells, excepting the outer most layer, having thick-walled cells; cortex shows 5 to 7 layers of thin-walled, rounded to isodiametric chlorenchyma cells; under the winged projections there are 3 to 4 layers of collenchyma; few oil globules present in some cells; inner to cortex there is a pericycle made up of round, rectangular, oval or elongated fibers in the form of a cap having a vascular bundle underneath it, in the regions where there is no vascular bundles underneath the pericyclic fiber group is interrupted by polygonal, rectangular, oval or elongated stone cells having simple pits; centre of the section is occupied by xylem, split into radiating arms by wedge shaped masses of parenchyma; each xylem arm is capped by thin patches of phloem consisting of sieve elements; cambium is indistinct between phloem and xylem; xylem consists of large vessels, tracheids, fibers tracheids and parenchyma, all being lignified; medullary rays 7 to 8 in number, multiseriate and dilating towards periphery and alternating with radiating patches of xylem, in the centre there is compressed pith parenchyma, formed due to pressure exerted by the developing xylem tissue, the parenchyma forms a line of demarcation between xylem patches (Fig 4).

Petiole: Diagrammatic TS of petiole is urn shaped with two raised wing like projection on the upper lateral sides, wings being studded with multicellular trichomes. In the centre there are 5 patches of vascular bundles embedded in ground tissue (Fig 2.3).

Detailed TS of petiole shows an outer epidermis bearing uniseriate multicellular trichomes with few collapsed cells. Inner to epidermis there is a compact 2 to 3 layered region formed by collenchyma while that under the projection is completely made up of collenchyma. The ground tissue is parenchymatous often with chlorophyll. There are 5 patches of vascular bundles having phloem tissue on the lower side, and is devoid of any fibrous tissue, protoxylem vessels are distributed towards inner side. The parenchyma embedded inside these vascular bundles are often crushed, and are not round or oval as seen in the outer ground tissue (Fig 5).

Midrib and Lamina: Diagrammatic TS of leaf passing through midrib shows a midrib having broad elevation on the lower side and small conical elevation at the upper end. Lamina shows chlorophyll tissue slightly penetrating into the midrib region. Patches of vascular bundles are suspended towards the upper half of the transverse section (Fig 2.4), (2.5)

Detailed TS of midrib is covered with upper and lower epidermii having thick cuticle. Tissue beneath the epidermis of upper elevation is composed of 2 to 5 layers of collenchyma and that of the lower elevation is formed by 2 to 3 layers of collenchyma. The ground tissue is made up of compactly arranged parenchyma without any intercellular space. Chlorenchyma of the lamina is protruded into the midrib in the upper region. There is a continuous patch of xylem, with phloem tissue surrounding it, formed majorly by vessels and few fibers. Cells of the ground tissue are comparatively larger towards outer side and are reduced in size towards phloem and hypodermal collenchyma (Fig 6.1 and 6.2)

Detailed TS of lamina shows upper and lower epidermii, the former being larger in size, are covered with thin cuticle. The upper epidermis is devoid of stomata, shows single layer of short palisade tissue. The mesophyll is 4 to 5 layered, having air spaces. Few vascular strands are distributed in the mesophyll tissue (Fig 6.3 to 6.5).

Flower: Diagrammatic TS through perianth lobe of flower is 'V' shaped in outline, shows upper and lower epidermii embedding mesophyll tissue, few trace bundles are enclosed in the mesophyll tissue (Fig 2.6).

Detailed TS of perianth shows upper and lower thick-walled epidermal cells, the former being comparatively larger in size. The layers of tissue beneath the upper epidermis are thick-walled, 3 to 4 layered and are compactly arranged without any intercellular space. The lower mesophyll tissues are formed by parenchyma with intercellular space having little chlorophyll in it. The midrib region shows a prominent vascular bundle having xylem and phloem tissue, there are few trace bundles distributed here and there in the mesophyll tissue (Fig 7).

Fruit: TS of stalk is circular in outline, shows an epidermis covered with a cuticle. Cortex is formed with parenchyma with few chlorophyll pigments. Pericycle curved, discontinuous, formed with thick and thin walled fibers. Underneath each pericyclic fibers group there is a smaller vascular bundle with phloem on a patch of xylem. There is a wide pith in the centre which contain X shaped xylem strand (Figure 8).

TS fruit shows an epicarp having 8 to 10 layers of parenchyma forming mesocarp having few vascular bundles with usual elements. Mesocarp ends with few layers of thick walled cells forming endocarp. In the parenchyma cells below the endocarp there is a seed having testa and parenchymatous endosperm (Figure 9).

Powder: showed characters such as cells of the cork in multiple layers in surface and obliquely cut views; transversely cut epidermis of stem with thick walled cells having prominent cuticle; tubular epidermal cells of floral parts with thin wavy wall; epidermis of leaf in surface view bearing trichomes and stomata with underlying mesophyll parenchyma cells; multicellular covering trichomes with 3 to 4 cells; epicarp formed by polygonal thick walled cells in surface view; bundle of thick walled fibres; thin-walled parenchyma associated with different tissue of the plants from different parts of plant, often with some pale contents; pitted thick walled stone cells and sclereids in groups; and fragments of pitted, annular, and spiral vessels. (Figure10).



Fig 2.1 TS of root



Fig 2.1 TS of stem

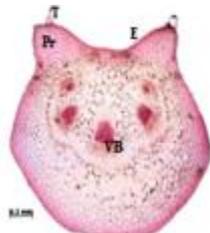


Fig 2.3 TS of petiole

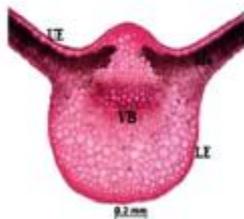


Fig 2.4 TS of midrib



Fig 2.5 TS of lamina



Fig 2.6 TS of petal

Ck, cork; **Ct**, cortex; **E**, epidermis; **LE**, lower epidermis; **Me**, mesophyll; **Pal**, palisade; **Per**, pericycle; **Ph**, phloem; **Pr**, projection; **SP**, spongy parenchyma; **T**, trichome; **UE**, upper epidermis; **VB**, vascular bundle; **W**, wing; **Xy**, xylem.

Figure 2: Microscopy of panchanga of *Ishwari*



Fig 3.1 TS of root



Fig 3.2 A portion of TS of root

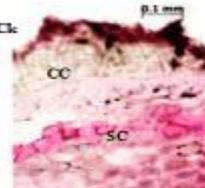


Fig 3.3 Bark region enlarge



Fig 3.4 Pericyclic fibres



Fig 3.5 Phloem



Fig 3.6 Starch grains

CC, cork cambium; **Ck**, cork; **Ct**, cortex; **Per**, pericycle; **Ph**, phloem; **PP**, phloem parenchyma; **SC**, stone cell; **SG**, starch grains; **Ve**, vessel; **XP**, xylem parenchyma.

Figure 3: Detailed microscopic features of root of *Ishwari*



Fig 4.1 TS of stem

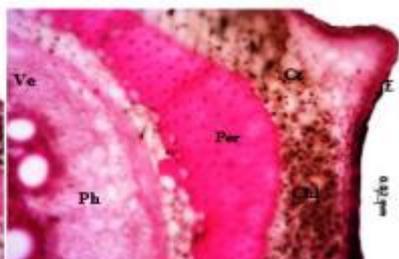


Fig 4.2 A portion of TS of stem

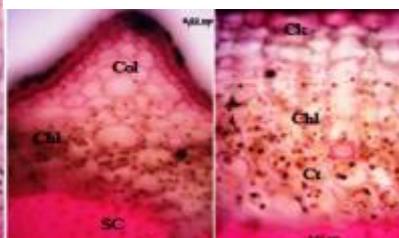


Fig 4.3 Outer region

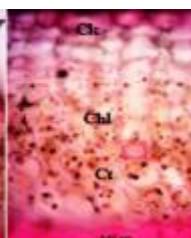


Fig 4.4 Cortex



Fig 4.5 Pericycle

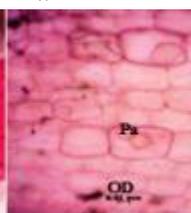


Fig 4.6 Oily content

Chl, chlorenchyma; **Ck**, cork; **Col**, collenchymas; **Ct**, cortex; **E**, epidermis; **OD**, oil drop; **Pa**, parenchyma; **Per**, pericycle; **Ph**, phloem; **Pi**, pith; **SC**, stone cell; **Ve**, vessel; **W**, wing; **XF**, xylem fibre.

Figure 4: Detailed microscopic features of stem of *Ishwari*

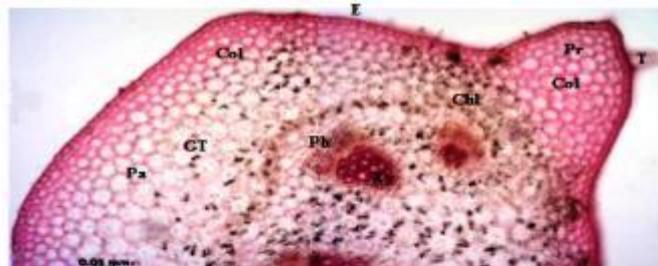


Fig 5.2 A portion of TS of petiole

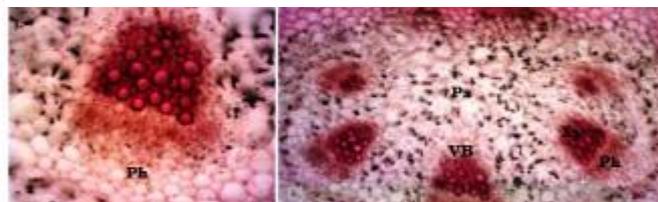


Fig 5.3 Vascular bundle

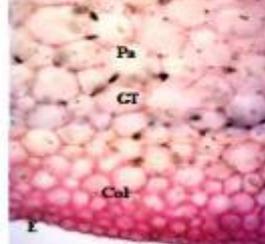


Fig 5.2 TS of petiole

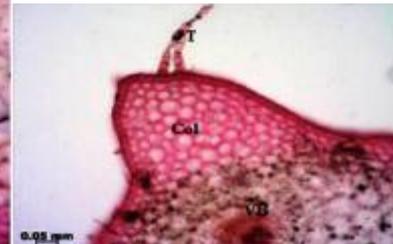


Fig 5.4 TS through wing

Chl, chlorenchyma; **Col**, collenchymas; **Chl**, chlorenchyma; **E**, epidermis; **GT**, ground tissue; **Pa**, parenchyma; **Ph**, phloem; **Pr**, projection; **T**, trichome; **VB**, vascular bundle; **Xy**, xylem.

Figure 5: Detailed microscopic features of petiole of *Ishwari*

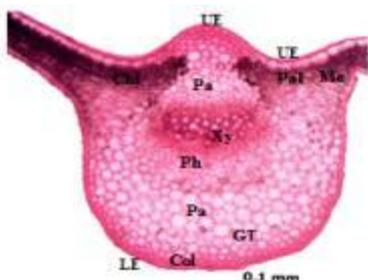


Fig 6.1 A portion of TS of midrib with lamina

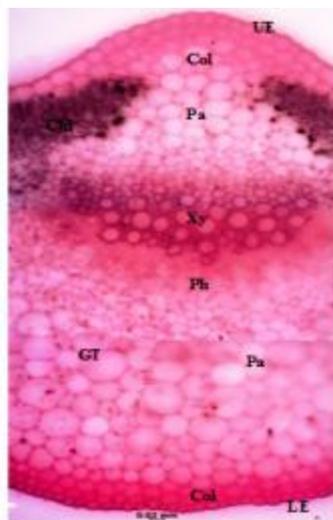


Fig 6.2 Detailed TS of midrib



Fig 6.3 TS of lamina



Fig 6.4 Upper epidermis

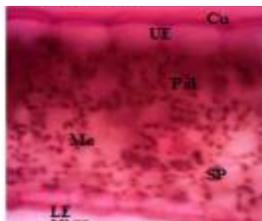


Fig 6.5 Detailed TS of lamina

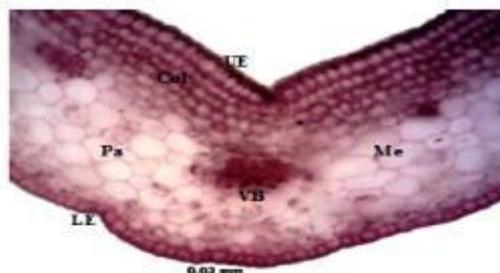


Fig 7.1 TS of petal

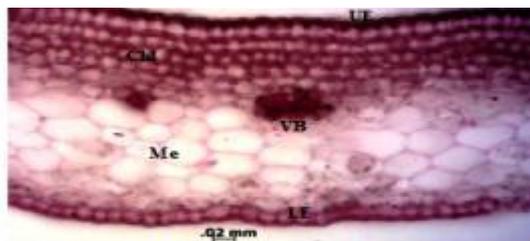


Fig 7.2 A portion of TS of petal enlarged

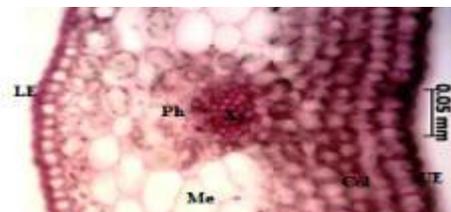


Fig 7.3 A portion of TS of petal showing vascular bundle

Col, collenchymas; Cu, cuticle; Chl, chlorenchyma; GT, ground tissue; LE, lower epidermis; Pa, parenchyma; Pal, palisade; Ph, phloem; SP, spongy parenchyma; UE, upper epidermis; Xy, xylem.

Figure 6: Detailed microscopic features of midrib and lamina of *Ishwari*

Col, collenchymas; LE, lower epidermis; Pa, parenchyma; Ph, phloem; UE, upper epidermis; VB, vascular bundle; Xy, xylem.

Figure 7: Detailed microscopic features of flower of *Ishwari*



Fig 8.1 Detailed TS of midrib



Fig 8.2 Entire section

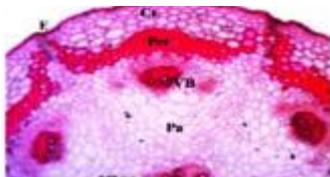


Fig 8.3 A Portion enlarge

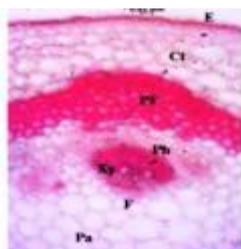


Fig 8.4 Outer Portion of TS

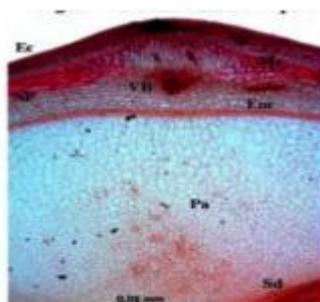


Fig 9.1 TS of fruit wall

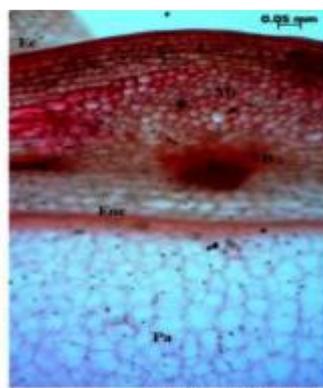
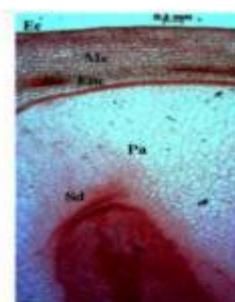


Fig 9.2 Portion of TS of fruit wall, TS enlarge



Ct, cortex; E, epidermis; Pa, parenchyma; PF, pericyclic fibres; Ph, phloem; VB, vascular bundle; Xy, xylem.

Figure 8: Detailed microscopic features of fruit stalk of *Aristolochia indica*

Ec, epicarp; Enc, endocarp; Mc, mesocarp; Pa, parenchyma; VB, vascular bundle.

Figure 9: Detailed microscopic features of fruit of *Aristolochia indica*

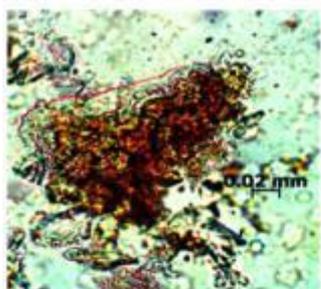


Fig 10.1 Transversely cut epidermis

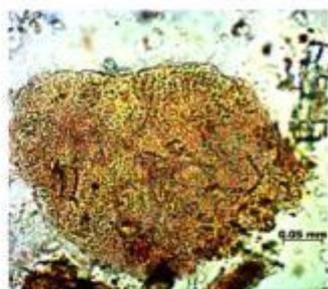


Fig 10.2 Phloem tissue

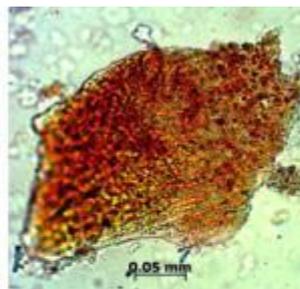


Fig 10.7 Mesophyll tissue

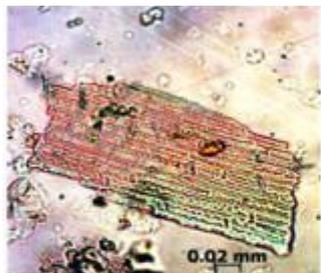
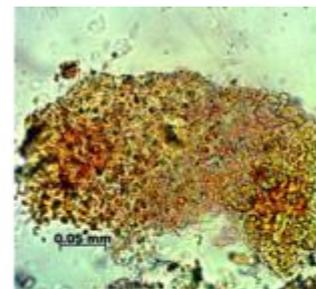


Fig 10.3 Epidermis of floral parts

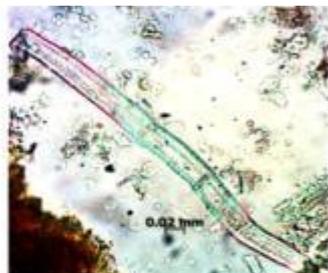


Fig 10.4 Trichome

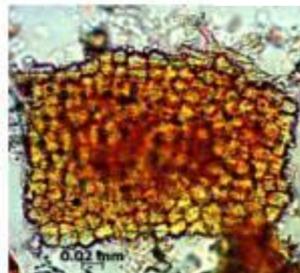


Fig 10.8 Epicarp in surface view



Fig 10.9 Cork in surface view

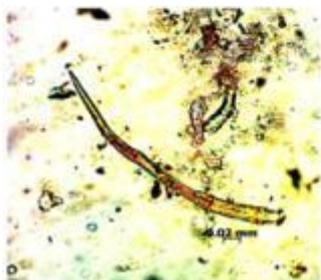


Fig 10.5 Trichome

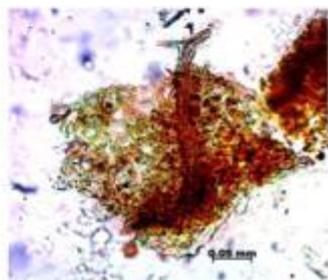


Fig 10.6 Epidermis in surface view with mesophyll

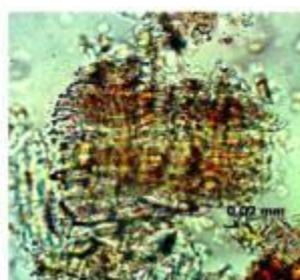


Fig 10.10 Obliquely cut cork cells

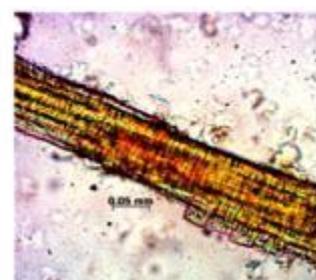


Fig 10.11 Fibre bundle

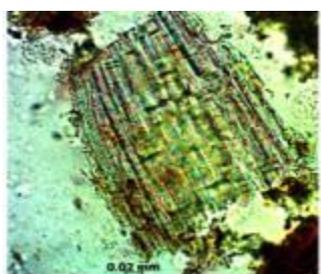


Fig 10.12 Parenchyma associated with different tissues

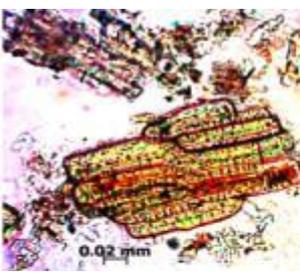
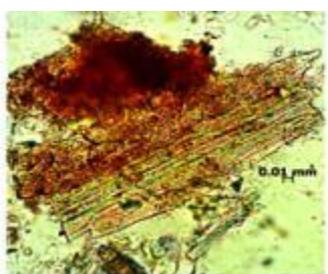


Fig 10.13 Pitted sclereids

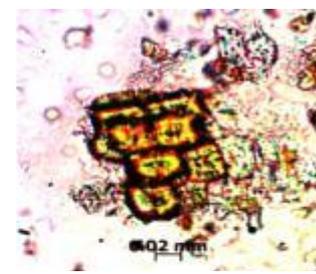


Fig 10.14 Stone cell group

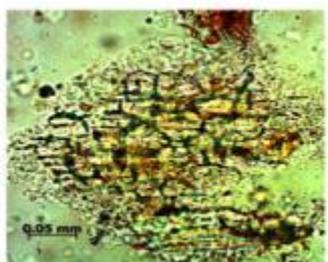
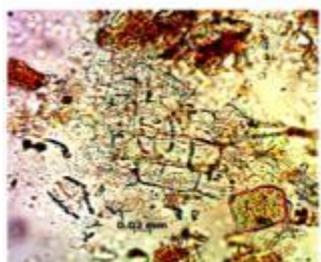


Fig 10.15 Different types of vessels

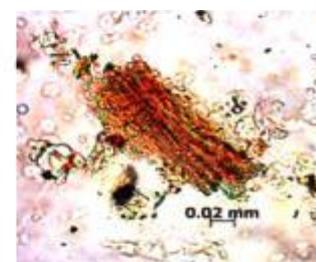


Figure 10: Powder microscopy of panchanga of *Aristolochia indica*

Discussion

The drug *Ishwari* also known as *Nakuli* in *Samhitha Granthas* and the source of which is *Aristolochia indica* Linn. Though *Nakuli* is having other source, still here the term *Ishwari* is given importance and source of which is confined to *Aristolochia indica* Linn.

In the present investigation the descriptions of external features of *Ishwari* have been discussed followed by histology. Being a part of quality control the plant parts such as root, stem, leaf, flower and fruit is studied in detail for macro-microscopic features. Certain microscopical characteristics like stomata, trichomes, calcium oxalate crystals, starch grains, stone cells, fibers and vessels etc are important anatomical characteristic of organized drug. Powder microscopy of panchanga of *Ishwari* also has been documented using standard methodology. The characters documented can be used for the identification in powder form also.

Conclusion

In present examination the detailed atlas of macro microscopic features of whole plant of *Ishwari* is made. The diagnostic features can be used as a fingerprint for the identification of the plant in fresh as well as dry form.

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