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Original Article

Pharmacognostical Evaluation of Selected Seeds from Fabaceae Family

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ABSTRACT

Background: Indigenous medicines regularly suffer with quality controversies because of similar species or varieties, substituent, adulterants wrong identification and lack of availability of original herb. **Objectives:** In the search for authenticity of traditional medicine, seeds of four *Fabaceae* species, *Abrus precatorius* L., *Pongamia pinnata* (L.) Pierre, *Trigonella foenum-graecum* L. and *Senna tora* L. were investigated in order to obtain characteristic patterns and medicinal potentiality by the way of pharmacognostical analysis. Each drug posse's excellent potential in Ayurvedic medicinal system. **Materials and Methods:** The essential parameters studied up for the pharmacognosy of given seeds were macroscopical study, histochemical evaluation, including transverse section, powder microscopy and physicochemical parameters. **Results:** The organoleptic and macroscopic profiles of seeds revealed the common characters like smooth surface and bitter taste. From the microscopical evaluation, oil globule, starch grains and prismatic type of calcium oxalate crystals were also found in the seed *of P. pinnata*. In *A. precatorius*, starch grains and yellowish cell content are present, but these are completely absent in other *Fabaceae* members, only mucilage content and oil globules present in *Trigonella foenum-graecum* and anthraquinone pigment cells and rosette type calcium oxalate crystals present in *Senna tora*. **Conclusion:** The current study report on pharmacognostical analysis will provides important diagnostic tool for future identification, authentication and evidence in correct identification.

Keywords: Indigenous medicines, Fabaceae species, Seed part, Pharmacognosy.

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INTRODUCTION

Ayurveda is an indigenous medicinal system of India since premedieval period. Several Indian indigenous medicines have been facing over exploitation for treatment and management of various diseases in human beings. As 'tradition to trend' recently many drugs have been developed and practiced from Ayurveda. Drug standardization factors like identity, purity, safety, drug content, physical and biological properties of Ayurvedic herbs will enhance the therapeutic efficacy of Ayurvedic medicinal system. Now a days the recent researchers are more focusing their research on standardization through the scientific validation and the documentation of Ayurvedic drugs. These are very essentially need for its quality evaluation and global acceptance.[1]

Seed part of *Abrus precatorius* L., *Pongamia pinnata* (L.) Pierre, *Trigonella foenum-graecum* L. and *Senna tora* L. are actively used in Ayurveda medicinal system from old age period for various therapeutical uses. Ayurveda recommends the use of Abrus *precatorius* seeds after the Shodhana process (purification),[2] *Pongamia pinnata* seed has been possess various therapeutical efficacy like anti-inflammatory, anti-nociceptive, antioxidant, anti-diarrheal, anti-fungal, anti-plasmodial, anti-ulcer, anti-hyperglycemic, anti-oxidative, analgesic and anti-hyperammonemic and functions.[3] *Trigonella foenum-graecum* has also been reported to exhibit therapeutical properties like anti-viral, anti-microbial, hypotensive, antioxidant, anti-inflammatory, anti-tumor activity. Its seed oil pointing to exhibit potent cytotoxicity against cancer cells.[4] In traditional Ayurvedic literature has been described that *Senna tora* as an antioxidant, anti-microbial, anti-hepatotoxic, anti-diuretic, anti-diarrhoeal and anti-mutagenic plant.[5]

Plants *A. precatorius, P. pinnata, T. foenum-graecum* and *S. tora* belongs to the family Fabaceae. Fabaceae is broadly defined by their distinct flower structure, characteristic podded (legume) fruit and the ability to symbiotically fix nitrogen as form of nodules with rhizobia. The pods of plants in the botanical family Fabaceae are commonly known as legumes and are approximately 80% of the total storage protein in the mature seeds of fruits in *Fabaceae*.[6-9]

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In this study, in the search for characteristics, valid for *Fabaceae* in general, seeds of four *Fabaceae* species, *A. precatorius, P. pinnata, T. foenum-graecum* and *S. tora* (Figure 1) were investigated to obtain characteristic patterns and medicinal potentiality by pharmacognostical analysis.

MATERIALS AND METHODS

Collection Drying and Preservation

Fresh Seed parts of four Fabaceae species, *A. precatorius, P. pinnata, T. foenum-graecum* and *S. tora* have been collected from Trans-Ganga plain Region of India. Trans Ganga plain Regions of India covers areas included in the States of Punjab, Haryana, Plain region of Rajasthan as well as the Union Territories of Chandigarh and Delhi. Trans Ganga plain Regions of India covers areas included in the states of Punjab, Haryana, Plain region of Rajasthan as well as the Union Territories of Chandigarh and Delhi. Geographically, this zone of agroclimatic encompasses the foothills of Shivalik, the plains area which is the basin of the Indus and the Ganga and the arid region is characterised by the rich diversity of evergreen, deciduous and semi-deciduous forests. The region has an average elevation of 700 msl. The climate is arid, semi-arid and sub-humid in different districts. The temperature ranges from 26 to 42°C and average rainfall varies from 190 mm to 1,150 mm. Plants were authenticated by BSI (Botanical Survey of India) and herbarium were prepared and deposited in the AIIA. Plant parts were separately cleaned and washed with tap water then left for shade drying and dried sample were powdered with a mixer grinder and sieved through 60 mesh and kept in air tight containers for further analysis.

Morphological and Microscopical analysis

Morphological analysis carried out by adopting the method of Wallis T.E, 2009. Microscopical evaluation has been conducted by section cutting. Dried samples were soaked in the water for softening their cells after that for thin free Transverse sections with the help of sharp blade. Each section stained with safranine stains, dehydrated with alcohol series of different concentration (10, 20, 30, 40, 50, 60, 70, 80 and 90%) and were mounted with glycerine. Various tissue and cell contents observed through trinocular microscope with 4x, 10x and 40x resolution and documented. Photomicrographs were taken with the Carl Zeiss camara attached Trinocular microscope.

Physicochemical Parameters

The various physicochemical parameters like foreign matter, total ash, acid-insoluble ash, alcohol soluble extractive, water soluble extractive and loss of drying that were determined as per the Ayurvedic Pharmacopeia of India.[10]

OBSERVATIONS AND RESULTS

Abrus precatorius L.

Syst. Nat., ed. 12. 2: 472 (1767)-Type: Sri Lanka, Hermann, Fl. Zelan. 284; LT designated by Breteler, Blumea 10: 607-624 (1960) (Lectotype: BM).

Synonym: Glycine abrus L., Sp. Pl. 2: 753 (1753).

Woody climber, sparsely adpressed stem. Petiole 0.5-1.8 cm long. Leaves 16-34-foliolate, 0.6-2.7 cm long, 3-10 mm wide, base rounded or subcordate, margin glabrous above, apex obtuse to acuminate. Inflorescence cymose, peduncle 1.5-6 cm. long, bracts 0.5-1 mm. Subsessile flower in dense fascicles, Corolla yellow, white, pink or mauve, Calyx sparsely puberulous, 3 mm long, denticulate. Fruit Pods oblong with scarlet with black area around the hilum.

Distribution: occurs in tropical, subtropical and warm temperate countries; native to India.

Habitat and ecology: The species grow on common roadside or human disturbed areas.

Examined Specimens: India, Haryana, (AIIA/RRDR/093).

Flowering and Fruiting: January-August, April-December.

Part used: Seeds (Ayurvedic Pharm. of India (1999-2011), Med. Pl. Braz. Pharm. (Brandão *et al.*, 2006)), Root (Unani Pharmacopoeia India (2007-2008), Siddha Pharmacopoeia India, Vol. 1 (2008)).

Pongamia pinnata (L.) Pierre

Fl. Forest. Cochinch. Sub t. 385 (1899)-Type: L. Plukenet, Phytographia 2: t. 104; LT designated by AC Smith, Fl. Vit. Nova 3: 170 (1985) (Lectotype).

Synonyms: *Pongamia pinnata* (L.) Merr., Herb Amboin. 271 (1917) (Isonym).

Tree, branches erect, pendulous. Petiole 65-220 mm long. Leaves puberulent becoming glabrous, leaflets 3-20 mm long, 1.3-12 mm wide, minute hairs becoming glabrous, shiny, base attenuated and cuneate, margin entire and often undulate above, apex acuminate. Inflorescence raceme, pedicle sericeous or puberulent, bracts 1-7 mm, broadly ovate, lanceolate. Calyx 3-5 mm long reddish-brown or purplish-brown stamen numerous, anther ovate or cordate, style apex incurved with sparse hairs, stigma capitate. Fruit pod with sparse minute hairs. Rounded-reniform seeds.

Distribution: Tropical and Subtropical Asia to W. Pacific; native to India.

Habitat and ecology: In Delhi, mostly found as avenue tree, best grow in sandy loamy soil.

Examined Specimens: India, Haryana, (AIIA/RRDR/023).

Flowering and Fruiting: March-April.

Table 1: Showing the macroscopical observation.

Plant Names	Shape	Dimensions (mm)	Colour	Texture	Odour	Taste
S. tora	Oblong to rhomboidal	3-5x2-7x2-3.5	Dark brown	Smooth surface	None	Bitter
T. foenum-graecum	Oblong, flattened, irregularly rhomboidal	3-5x2-3x2	Yellowish brown	Smooth surface	Characteristic	Bitter
A. precatorius	Elliptic and ovoid to somewhat globose	6-8x5.5-6	Sub median surface is occupied by red in color, other part black throughout	Smooth	None	Bitter
P. pinnata	Elliptical or reniform centrally swollen	28-30x12-20x4.7-5	Pale yellowish white cotyledons	Smooth	None	Bitter

Table 2: Result of Physicochemical Parameters.

Parameters	*Abrus precatorius*	*Pongamia pinnata*	*Trigonella foenum-graecum*	*Senna tora*
Foreign Matter	0.75±0.01	0.86±0.01	0.89±0.01	0.80±0.01
Total Ash	3.41±0.03	12.14±0.11	3.04±0.03	4.6±0.03
Acid-insoluble Ash	0.1±0.01	1.69±0.02	0.31±0.01	Negligible
Alcohol soluble extractive	7.34±0.08	5.14±0.03	9.53±0.10	11.46±0.12
Water soluble extractive	16.55±0.10	20.31±0.20	32.50±0.22	35.6±0.23
Loss of Drying (LOD)	8.05±0.10	9.01±0.10	9.28±0.10	9.6±0.16

Part used: Seeds (Ayurvedic Pharm. of India (1999-2011), Unani Pharmacopoeia India (2007-2008), Root bark (Siddha Pharmacopoeia India, Vol. 1 (2008)).

Trigonella foenum-graecum L.

Sp. Pl. 2:777 (1753)-Type: LINN-932.16; LT designated by Westphal, agric. Res. Rep. Center Agric. Publishing Doc. 815: 199, 202 (1974) (Lectotype).

Synonyms: *Buceras foenum-graecum* (L.) All. In Fl. Pedem. 1: 313 (1785).

Annual Herbs, rootstock fibrous and stalk. Stem erect, terete, branched. Stipule entire, membranous, petiole 6-15 mm long, spreading. Leaves oblong-elliptic, acute apex, base cuneate, margin dentate. Inflorescence axillary, solitary or in pairs, peduncle dichotomous, bracts numerous, small, hyaline, persistent, oblong, ciliate, pedicels and young ovary with minute sparsely pubescence. Flowers sessile, Calyx 7-8 mm long, villous. Corolla creamy or pale yellow, 13-18 mm long. Ovary puberulent; ovules numerous. Staminate flower: tepals 4, stamens ca. 20, monadelphous, anthers narrow-obovoid, connective not produced. Pistillate flower: tepals 4, inner two obovate; styles 3, stigmas lunate. Fruit Legume with long *beak*. Seeds 10-20, brown, oblong-ovoid shape.

Distribution: Iraq to Northern Pakistan; mostly cultivated in India.

Habitat and ecology: its cultivation is found in Punjab region which is somewhere dried land.

Examined Specimens: India, Haryana, (AIIA/RRDR/089).

Flowering and Fruiting: April-July; July-September.

Part used: Seeds (Ayurvedic Pharm. of India (1999-2011),Unani Pharmacopoeia India (2007-2008), Siddha Pharmacopoeia India, Vol. 1 (2008), Pharmacopoeia of China (2005, 2010), Trad. Drugs in Israel at End C20th (Lev and Amar, 2000), U.S. Pharmacopoeia USP 39 (2016), Taiwan Herbal Pharmacop. 3rd Chinese ed. (MOHW, 2018), Farmacopea Herbolaria Mexico (2001)).

Senna tora (L.) Roxb.

Fl. Ind. (Roxburgh) 2:340 (1832)-*Type:* LINN-528.9 (Holotype).

Synonyms: Cassia tora L. in Sp. Pl.: 376 (1753) (basionym).

Annual Herbs. Stipule 10-15 mm long, caducous, linear; petiole 1.5-2 mm long. Leaves obovate or obovate-oblong, base cuneate

to rounded and oblique. Inflorescence Racemes axillary, bracts linear, acute, pedicels 1-1.5 cm. Stamens 7. Calyx 5-8 mm long, ovate-oblong, membranous, Corolla yellow, unequal, obovate, 12-15 mm long, shortly clawed, *Ovary sessile, stigmas glabrous. Fruit Legume with both ends acuminate,* Seeds 20-30, shiny, rhomboid.

Distribution: Central America; cultivated to India.

Habitat and ecology: found in dry or marshy region.

Examined Specimens: India, Haryana, (AIIA/RRDR/089).

Flowering and Fruiting: October-February.

Part used: Seeds (Ayurvedic Pharm. of India (1999-2011),Unani Pharmacopoeia India (2007-2008), Korean Pharmacopoeia, 10th edn. (MFDS, 2012), Pharmacopoeia of China (2010), Taiwan Herbal Pharmacop. 3rd Chinese ed. (MOHW, 2018), Japanese Pharmacopoeia 15th edn. (2006) (Table 1).

Anatomical/Microscopical study

Abrus precatorius

T.S of seed (Figure 4) almost circular in outline; Outermost testa differentiated in epidermis and endodermis. In epidermis the large portion of the testa made of randomly arranged radially elongated palisade cells. Inner portion of the testa is comprised of thin collapsed cells forming a hyaline layer and followed by the group of palisades like cells of testa. Followed by endodermis starch grains and plenty of calcium oxalate crystals are scattered throughout the cotyledons.

Pongamia pinnata

TS of the seed (Figure 5) contain outermost testa region, made up of three-sided thickened dome shaped cells, covered with thin cuticle, followed by 4 rows of thin-walled parenchymatous cells, followed by narrow, thick-walled, cells filled with dark brown

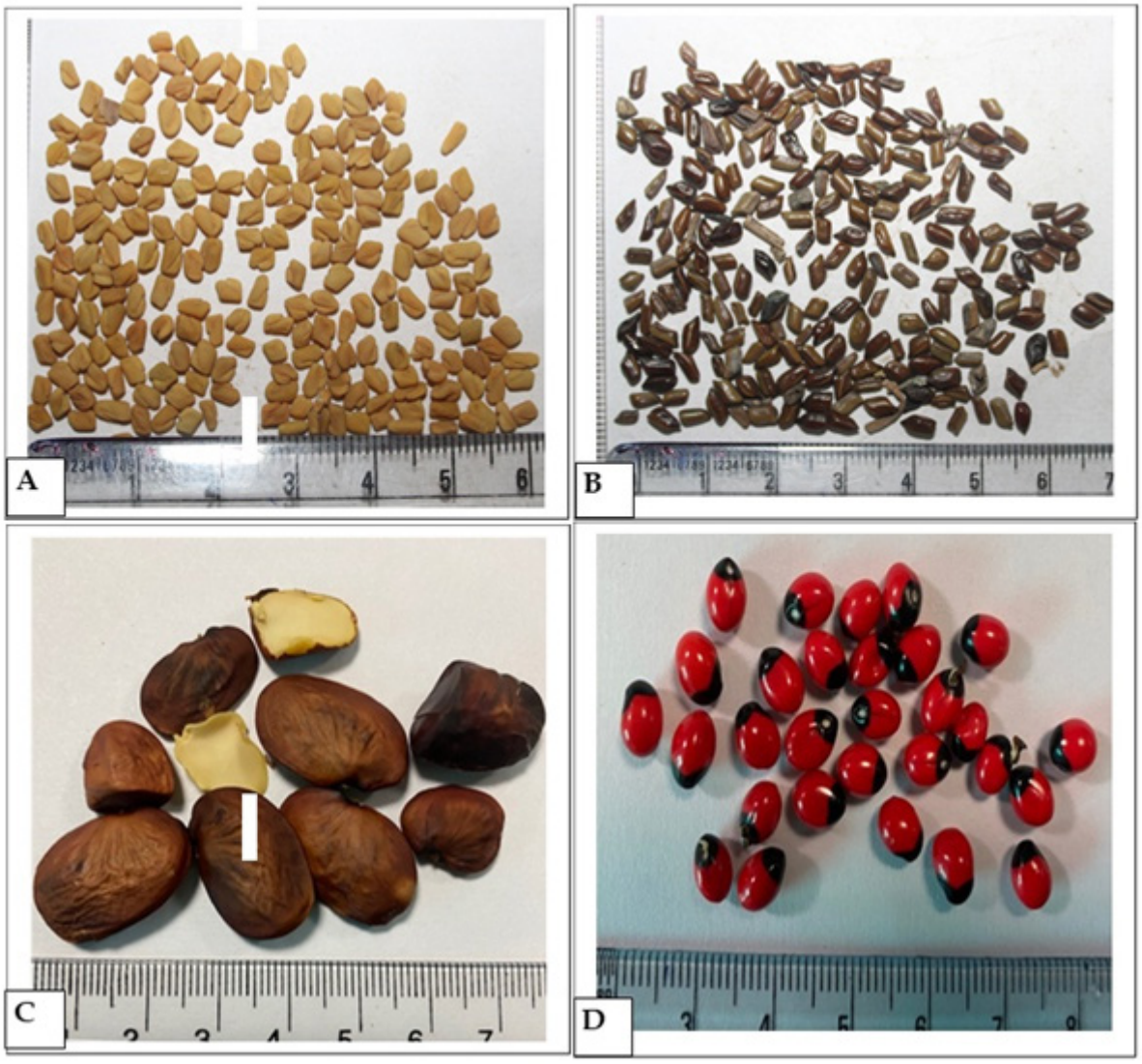

Figure 1: A: Seeds of *Trigonella foenum-graecum*, B: Seeds of *Senna tora*, C: Seeds of *Pongamia pinnata*, D: Seeds of *Abrus precatorius*.

pigment. The epidermis of cotyledon is made up of rectangle shaped cells; most of the sections are composed of oval, radially arranged big sized parenchymatous cells filled with oil globules, simple starch grains and prismatic calcium oxalate crystals.

Trigonella foenum-graecum

T.S of the seed (Figure 3) shows, outermost testa composed of a layer of thick walled palisade like lignified cells, underneath this layer lies sub epidermis with thin walled cells of parenchyma; underneath of this a row of rectangular to polygonal thin walled cells of endosperm, loaded with aleurone grains and filled with mucilage; two cotyledons are present in the testa, the cells of both the epidermis of the cotyledons are thick-walled; underneath the upper epidermis lie 3 to 4 rows of palisade cells with lot of oil globules followed by few rows of spongy tissue. Aleurone grains are present throughout the cell embryo and endosperm.

Senna tora: T.S of the seed shows (Figure 2), outermost cuticle composed of a layer of thick-walled palisade like cells thickened columnar cells underneath this layer lies sub epidermis with thin walled cells of parenchyma cells containing few clusters of small sized prisms of calcium oxalate crystals followed by a row of rectangular to polygonal thin walled cells of endosperm, rectangular to irregular shaped cells embedded with anthraquinone pigments, their secondary thickening being mucilaginous get dissolve readily in water. Few layers of collapsed cells present between endosperm cells and cotyledons. Cotyledons consists of outer epidermis covered with thin cuticle; the cells are isodiametric and radially elongated. Underneath the lower epidermis lies two layers of palisade the one adjacent to its being longer in size. Cluster crystals of calcium oxalate and oil deposited in the cotyledon.

Drug powder microscopy

In *Abrus precatorius* seed powder, palisades like cells from the cotyledon, fragments of hyaline layer, cotyledonary cells with aleurone grains, fragments of cotyledonary cells were identified (Figure 4). In *Pongamia pinnata* seed powder, fragments of cotyledonary cells with prismatic crystals, fragments of cotyledonary cells with oil globule, starch grains up to 10 µ in length, prismatic type of calcium oxalate crystals upto 15 µ in length and oil globules are upto 40 µ in dia, (Figure 5) are present. In *Trigonella foenum-graecum* seed powder, fragments of cotyledons, fragments of testa with lignified cells, mucilaginous cells, oil globules and fibre fragments of epidermis are present (Figure 3). In *Senna tora* seed powder, fragments of sub epidermis, fragments of endosperm, palisade cells of testa in surface view, group of anthraquinone pigment cells are observed in powder (Figure 2 and Table 2).

DISCUSSION

As a part of pharmacognostical standardization, the macroscopical examination of seed parts of four *Fabaceae* species, *A. precatorius, P. pinnata, T. foenum-graecum* and *S. tora* were studied. Macroscopically evaluation is a technique of qualitative evaluation based on the study of morphological and bodily profiles of drugs. The macroscopic characters of the Fabaceae seeds can be served as diagnostic parameters. Macroscopic parameters like shape, dimension, colour, texture, odour and taste have been carried out. The results showed common characters like smooth surface and bitter taste. From this study the morphological diversity of the four *Fabaceae* members analysed then subjected to microscopic investigation. Microscopic analysis plays a vibrant role in the standardization of the herbal drug material. The microscopic examinations are carried out to understand the whole anatomical structure of the plant and to describe the cell contents. In this research histochemical evaluation, including transverse section and powder microscopy were carried out for seed parts of four *Fabaceae* species, *A. precatorius, P. pinnata, T. foenum-graecum* and *S. tora*. Results revealed the cellular arrangement, cellular shapes, cell sizes, cell contents of seed parts of four *Fabaceae* species. Oil globule, starch grains and prismatic type of calcium oxalate crystals are also present in the seed powder of *P. pinnata*. In Abrus precatorius seed powder, starch grains and yellowish cell content are present, but these are completely absent in other *Fabaceae* members, only mucilage content and oil globules present in *Trigonella foenum-graecum* seed powder and anthraquinone pigment cells and rosette type calcium oxalate crystals present in *Senna tora* seed powder. Lack of starch storage of *Fabaceae* seeds from this study has been highlighted a high degree of genetic diversity within the Fabaceae. In this study cell depositions like oil globule, starch grains, mucilage content, aleurone grains, anthraquinone pigments, brownish cell content, yellowish cell content, rosette type calcium oxalate crystals and prismatic type of calcium oxalate crystals are observed in Fabaceae seed part.

According to Ayurvedic Pharmacopeia of India, this study finds out similar characters and certain new findings also. In *Abrus precatorius* seeds resulted the presence of starch grains are scattered throughout the cotyledons were not mentioned in the API. In *Pongamia pinnata* seeds; cell depositions like oil globules and dark brown pigments are reported but the presence of simple starch grains and prismatic calcium oxalate crystals are new findings. In *Trigonella foenum-graecum* seeds; cell depositions like aleurone grains and oil globules are correlating with API. In *Senna tora* seeds; depositions of cluster crystals of calcium oxalate, is reported in API.

The physicochemical parameters are also used to determine quality and purity of crude drugs.[10] Each parameters shows specific significance to standardize the crude drugs. Excess Foreign matters in crude plants may affect the quality of crude drug and leads to create severe health issues in human. Loss on

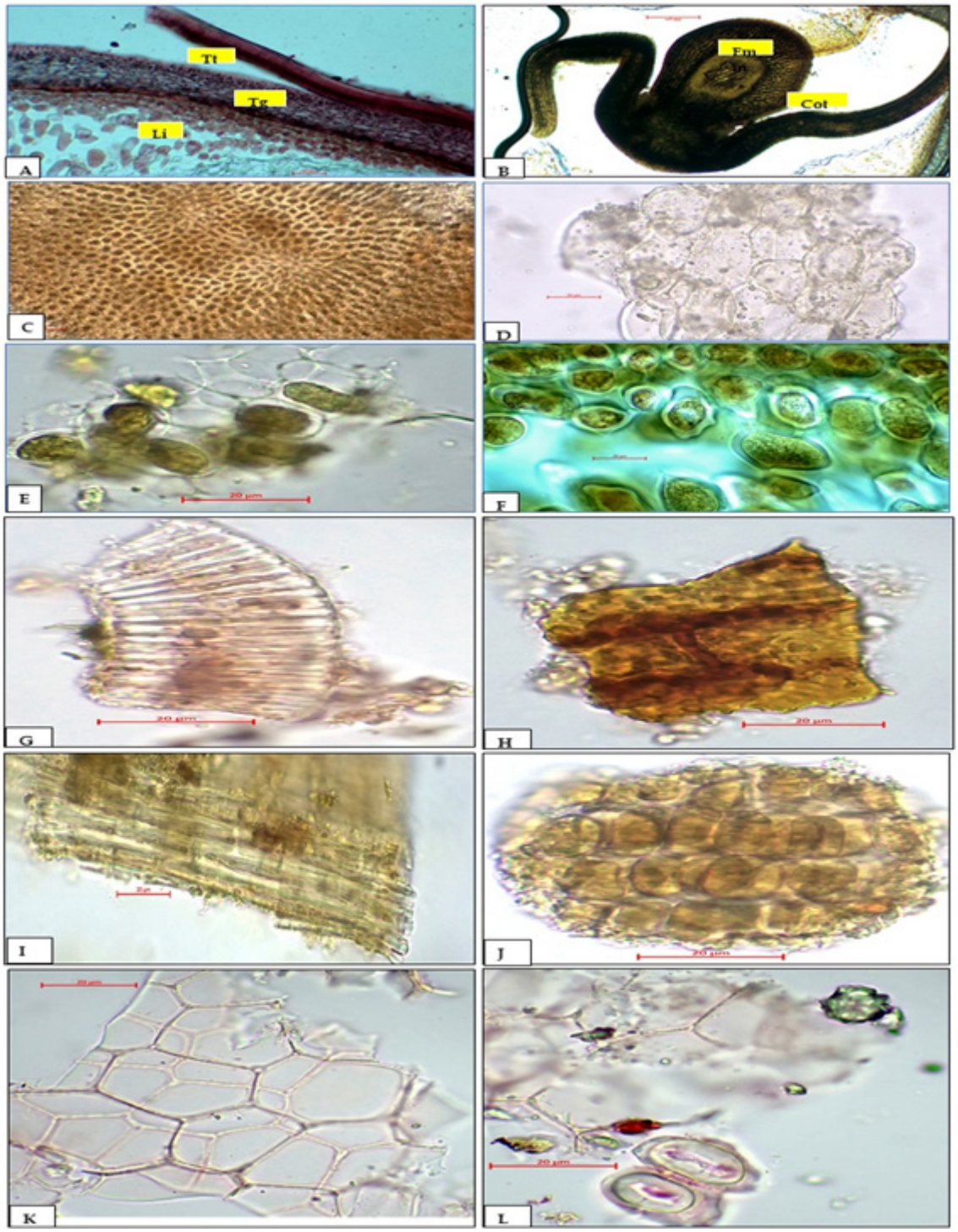

Figure 2: *Senna tora* Seed T.S and Powder, A: T.S of seed, B: T.S of cotyledon, C: Fragments tegmen, D: Fragments of endosperm, E: Cotyledonary cells with cell contents, F: Group of anthraquinone pigment cells, G: palisade like cells of testa in surface view, H: Group of yellowish brown content, I: Group of fibres, J: Group of stone cells, stone cell size upto 16 µ in dia, K: Fragment of cuticle, K: Outer layer of cuticle, L: Calcium oxalate crystals and stone cells (Tt: Testa, Li: Linea lucida, Tg: Tegmen, Em: Embryo, Cot: Cotyledon, Cr: Crystal, St: Stone cells).

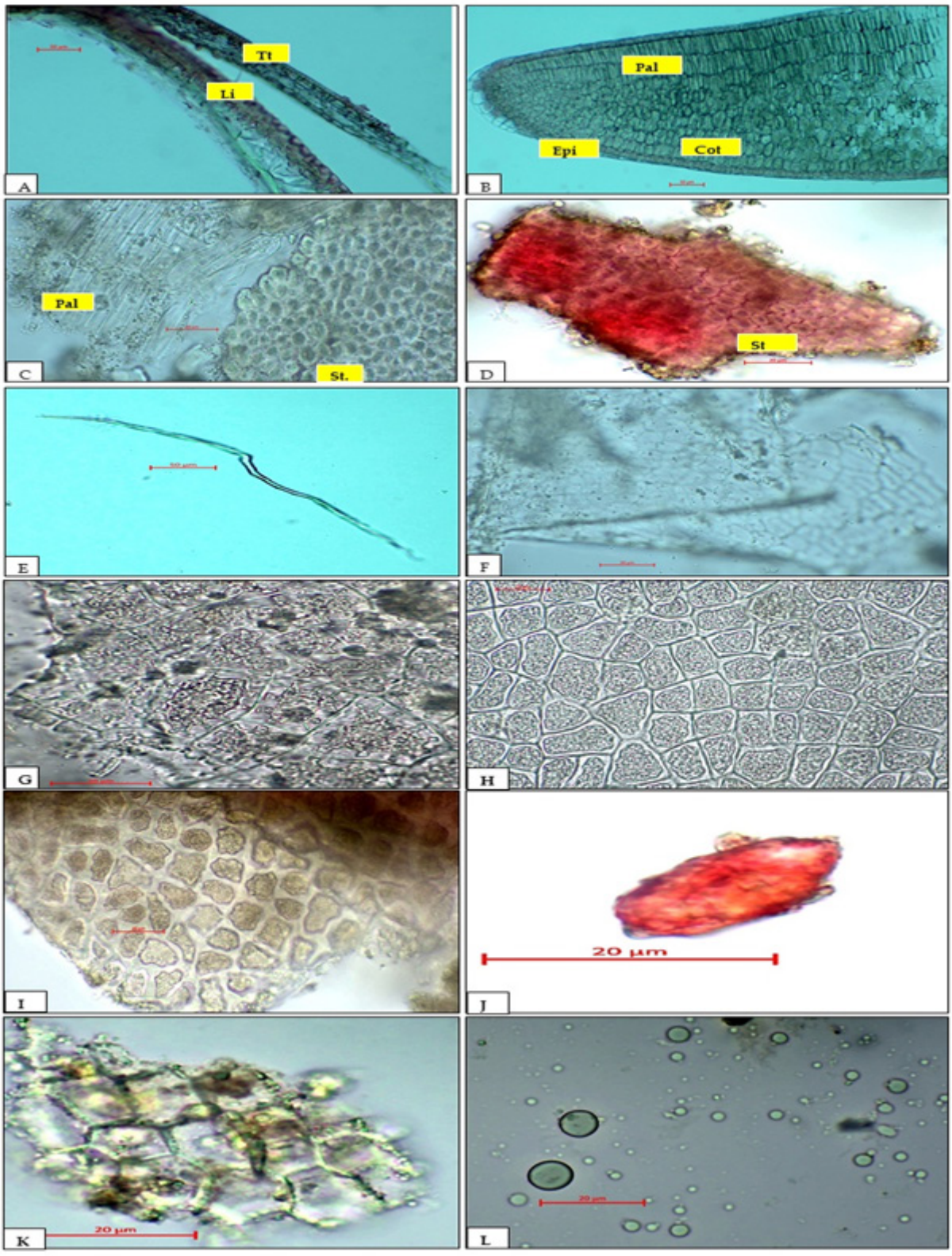

Figure 3: *Trigonella foenum-graecum* Seed T.S and Powder, A: T.S of testa, B: T.S of cotyledon, C: Fragments of cotyledons, D: Fragments of testa, E: Fibre, F: Fragments of epidermis, G, H and I: Different kind of thick-walled cells from seed coat, J: Mucilage cell, K: Group of parenchyma cells, L: Oil globules (Tt: Testa, Li: Linea lucida, Cot: Cotyledon, pal: Palisade cells, tegmen, St: Stone cells).

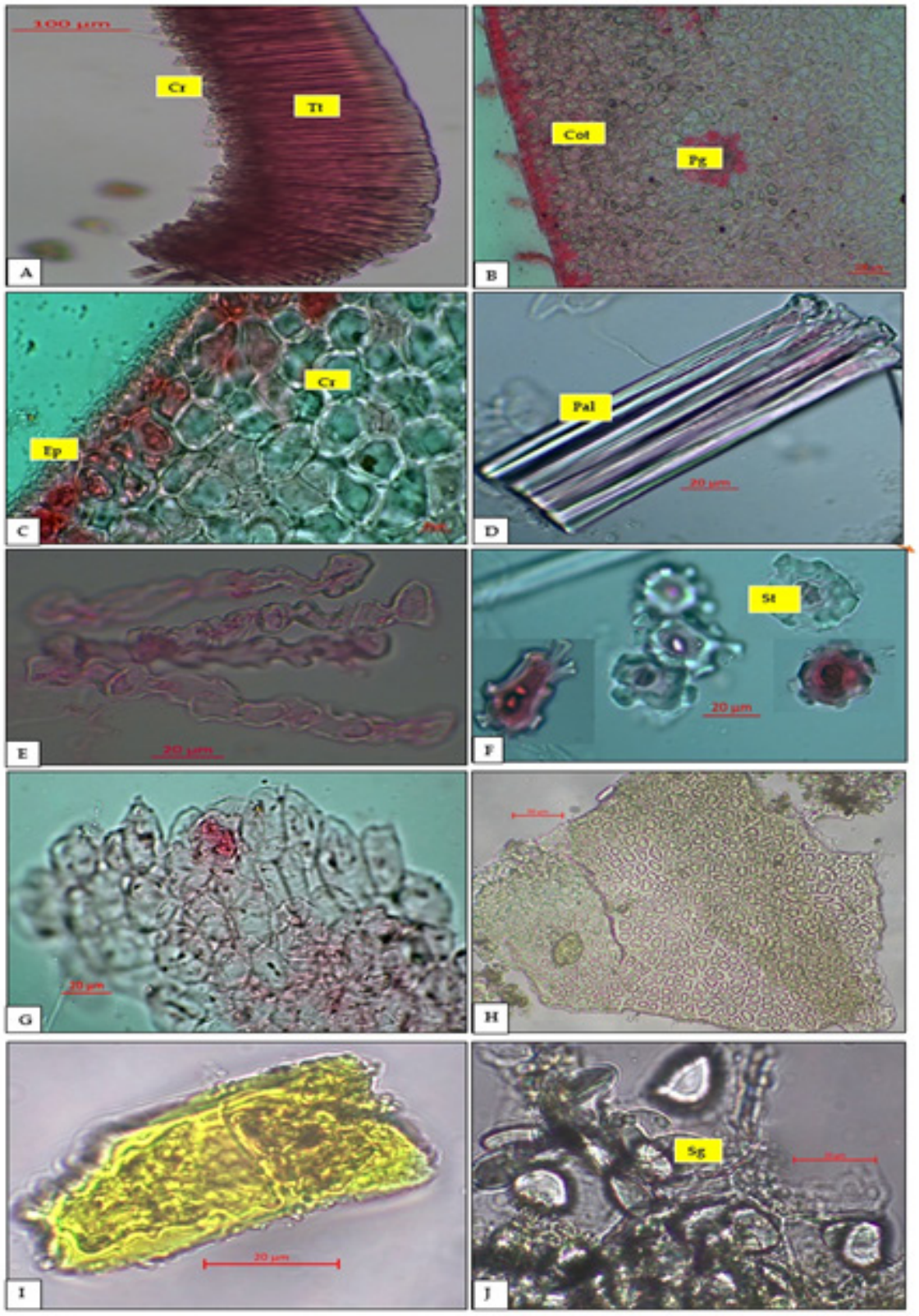

Figure 4: *Abrus precatorius* - Seeds, A: T.S of testa, B: T.S of cotyledons, C: T.S of cotyledons-enlarged view, D: palisade like cells, E: Fragments of hyaline layer, F: stone cells, G: Cotyledonary cells, H: Fragments of seedcoat, I: Yellowish content, J: Group of starch grains (Tt: Testa, Cot: Cotyledon, Pal: Palisade cells, Pg: Pigment cells, Cr: Calcium oxalate crystals, Ep: Epidermis, St: Stone cells, Sg: Starch grains).

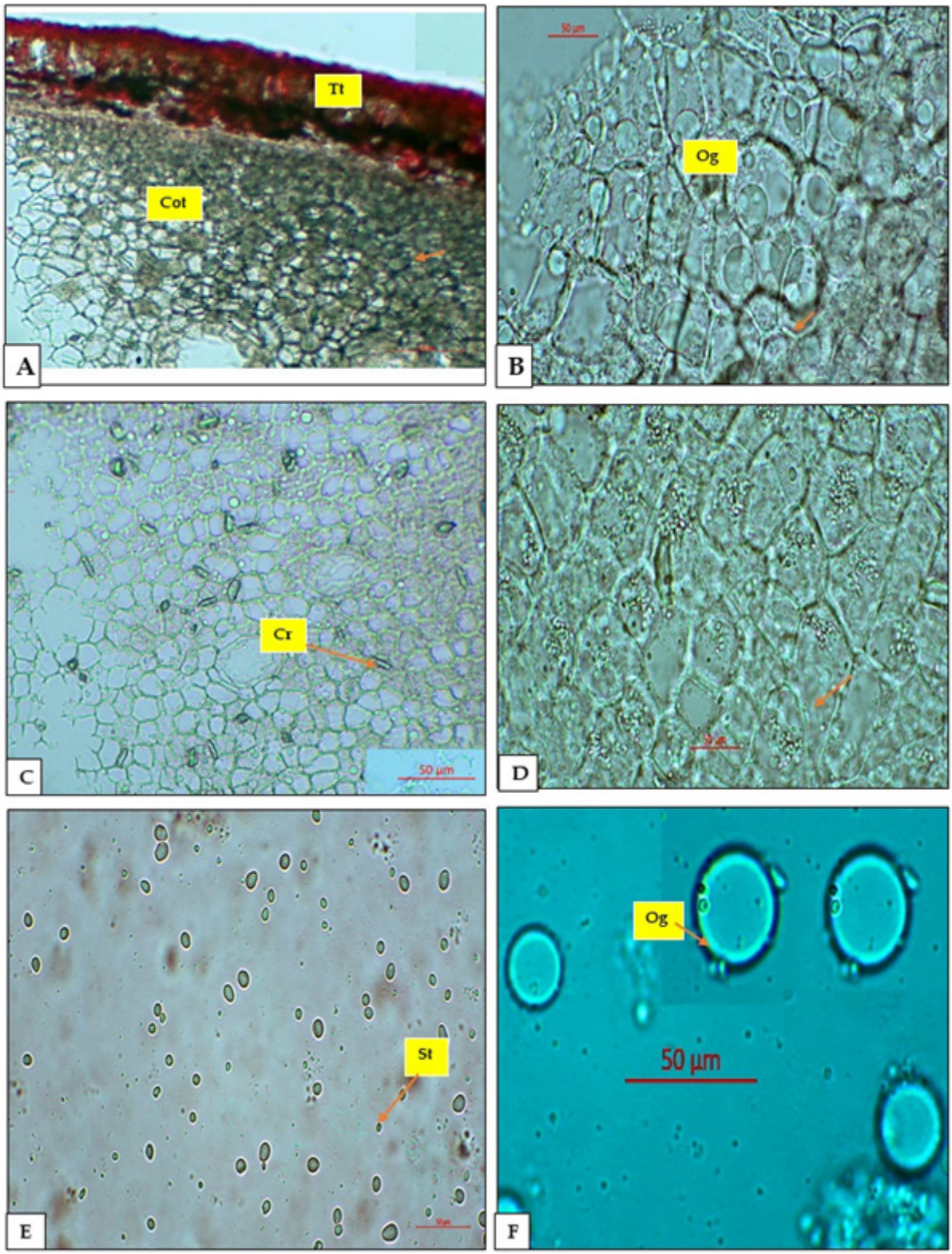

Figure 5: *Pongamia pinnata* seed T.S and powder, A: T.S of seed, B: starch filled parenchymatous cells, C: fragments of cotyledonary cells with prismatic crystals, D: fragments of cotyledonary cells with oil globule, E: starch grains, F: oil globules (Tt: Testa, Cot: Cotyledon, Cr: Calcium oxalate crystals, Og: Oil globule, Sg: Starch grains).

drying (Moisture Content) of drugs should be in minimal range to lower the risk of microbial growth and enzymatic activity, which accelerate spoilage.[11,12] Less value of moisture content could prevent bacterial, fungal and yeast growth this can be find out through the loss of drying test and ash value indicates the minerals compounds present in the drugs other than that impurities like sand soil particles.[13] The acid insoluble ash indicates the amount of sand and adhering dirt present in drugs. The water-soluble extractive values indicate the quantity of total amount of water soluble or alcohol soluble active constituents present in drugs.[14]

CONCLUSION

In conclusion, the discussed parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry/ trade and this can be included as microscopic standards. On pharmacognostical analysis will provide important diagnostic tools like characters of different cell structures, arrangements and various cells depositions which are reported in this study for future identification and authentication. Such type of analyses very helpful for understanding the presents of cell contents that evolved during ancient times for the preparation of various Ayurvedic medicines and also possible to relate the requirement and efficacy of these processes on human health in terms of currently available scientific knowledge.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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