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Comparative Anatomical and Histochemical Characterization of the Source Plants of the Ayurvedic Drug *Rasna*

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ABSTRACT
Rasna is an ayurvedic drug. *Alpinia galanga*, *Alpinia calcarata* and *Pluchea lanceolata* are used as source plants of *Rasna*. The two species of *Alpinia* are used in Kerala and in North India *Pluchea lanceolata* is used as the source of *rasna*. This paper deals with the morphological, anatomical and histological comparisons of rhizomes of the two species of *Alpinia* and root of *Pluchea lanceolata*. For anatomical comparison, histological and histochemical details were carried out. The study reveals how these plants can be easily identified using these markers for quality control and standardisation.

Keywords: *Alpinia galanga, Alpinia calcarata, Pluchea lanceolata*, Rasna, Zingiberaceae

1. Introduction
The drug *Rasna* is capable of maintaining the youthful vigour and strength. In Ayurveda it is also a popular remedy for rheumatism, intermittent fevers, dyspepsia, cough, bronchitis, asthma and other respiratory ailments. It is bitter, hot and aromatic and helps to overcome *kapha* and *vata*, poison, intestinal and biliary colic. It stimulates digestion, purifies blood and improves voice. The official part is the rhizome which forms a major ingredient of preparations like *Rasnadi kashayam*, *Rasnadi curnam*, *Rasnadi tailam*, *Asvagandharishtam*, etc. [1]. But the source plants used are different in different parts of the country. The greater part of North West India use *P. lanceolata* as *rasna* and so it is equated as the real source plant [2, 3]. As per ancient texts, *nakuli* and *gandha nakuli* are often treated as synonyms of *rasna* [4]. The Vaidyas of South India consider *A. galanga* Willd. to be *rasna*, as one of the synonyms of *rasna* is *elaparni* that is the plant bearing leaves resembling those of *ela*. It's another synonym *sugandha* indicates the fragrant rhizomes of *Alpinia galanga* [5]. The Kerala publications like *Osadhinigantu*, *Ayurvedavisvakosam* etc. give the Malayalam term *cittaratta* and *peraratta* for *nakuli* and *gandhanakuli* respectively. Though there is such a controversy, the rhizome of *A. galanga* is widely used as *rasna* in kerala. Both *A. galanga* and *A. calcarata* are available in the Kerala market as *rasna* [6]. In South Indian markets *A. galanga* is locally called *peraratta* and *A. calcarata* is *cittaratta* or *aratta*. The former is having more aromatic odour than the latter [7].

2. Materials and methods
Plant materials for the present study were collected from the Herb Garden, Arya Vaidya Sala, Kottakkal and raw drug markets of kerala. Materials were authenticated and voucher specimens of all the materials employed in the study are preserved in the herbarium of Centre for Medicinal Plants Research, Kottakkal. The materials for anatomical study were fixed in Formaldehyde- Acetic acid Alcohol mixture. Histological and histochemical staining was carried out according to standard procedure [8]. Photomicrographs were taken using Canon G3 camera attached to Zeiss microscope. Polarization microscopic studies were highly useful to locate and distinguish the types of crystals and minerals present in the useful parts. The characters were observed under Motic BA 400 polarization microscope. Fluorescent microscopic studies of the useful parts were done with the help of UV light. Observations were done under Leica DM 1000 LED fluorescent microscope and photographs were taken with the help of a digital camera.

3. Results & Discussion

3.1 Morphological characters

Rhizomes of *A. galanga* are cylindrical, branched, 2 to 8 cm in diameter, longitudinally ridged with prominent rounded warts marked with fine annulations; scaly leaves arranged circularly. Externally reddish- brown, internally orange yellow in colour, fracture hard and fibrous, fractured surface rough; odour pleasant and aromatic, taste spicy and sweet (Fig 1. A-B). In the case of *A. calcarata* rhizomes are horizontal and branched; individual pieces torturous, deep brownish orange externally, pale buff colour internally; prominently marked with wavy annulations at the nodes with scaly leaf bases; cut end circular in outline and light brown in colour and are covered by the dried leafy bracts; fracture is tough, uneven and fibrous. Odour pleasant, aromatic, taste pungent (Fig 1. C-D). In *Pluchea lanceolata* root is deeply penetrating, 0.5 to 2 cm in diameter, somewhat twisted and gradually tapering. The external surface of the young root is white, while the mature one is light brown to dark brown in colour and the internal surface is brownish. Odour is indistinct and the fracture is short, taste slightly bitter (Fig 1. E-F).

3.2 Anatomical characters

Transverse section of rhizome in *A. galanga* shows an outer cortical region and an inner stelar region. The outer cortex is 1 to 1.1 cm wide and the central stele is 1.2 cm in diameter. Cortex consists of an epidermis, which is composed of a single row of tangentially elongated cells. Below the epidermis, in the remaining part of the outer zone consists of numerous vascular bundles in scattered manner and each bundle is surrounded by lignified layer (Fig 2. A). Vascular bundles are nearly circular in shape and consists of groups of xylem elements and small patch of phloem. Xylem consists of parenchyma, vessels, tracheids & fibers. Phloem is seen above the xylem and consists of sieve elements, companion cells (Fig 2. C). The cells of the cortex are thin walled, polygonal and arranged with small intercellular spaces. Cortical and stellar region demarcated by a continuous ring of endodermoidal layer (Fig 2. A). The stelar region is composed of thin walled parenchymatous cells with numerous vascular bundles. Small vascular bundles are radially arranged xylem elements are present beneath this layer. These parenchymatous cells are slightly smaller than that of the cortical region and polygonal to circular in shape and compactly arranged with small intercellular spaces. Smaller bundles are towards the peripheral region and larger ones occupies the central part. Oleoresin cells are present near to each bundle. Sclerenchymatous sheath partially encircling the bundle is less thickened. Starch grains are simple, elongated and are more in number. Numerous oleoresin cells are scattered throughout the rhizome (Fig 2. A-C).

In *A. calcarata* TS of rhizome is about 1-2 cm in diameter and consists of outer cortical region and an inner stelar region (Fig 2. E). Cortical region consists of an outer single layered epidermis. The cells of the epidermis are tangentially elongated and their outer wall is highly thickened. The epidermis is followed by a parenchymatous cortex with intercellular spaces (Fig 2. D). Numerous starch grains are present in the cortical region and a few in the stelar region. Starch grains are of simple, oval or oblong, and rounded type. The number of vascular bundles in the stelar region is comparatively more than that of the cortical region. Numerous oleoresin cells are scattered both in the cortex and in the stelar region. Cortical and stellar regions are demarcated by a single endodermoidal layer (Fig 2. E). Vascular bundles are scattered without any definite arrangement and each bundle is surrounded by a prominent sclerenchymatous bundle sheath, it is a characteristic feature (Fig 2. D-F). Both small and large vascular bundles are present in the cortical region. Each vascular bundle is nearly circular or oblong in shape. The cells of the bundle sheath are small, variously shaped and compactly arranged. In each bundle the vessel members are seen as a group and occupies the major half of the bundle and the rest being occupied by the phloem. In a small bundle the vessel members are 2 or 3 and in a large bundle the vessel members are 7-9 in number (Fig 2. F). Xylem consists of vessels, tracheids and parenchyma. Phloem consists of sieve tubes, companion cells, parenchyma and few fibers (Fig 2. D-F).

In *P. lanceolata* TS of root is about 2 cm in diameter and consists of outermost 8-20 layers of thin and thick-walled tangentially elongated cells arranged in storied manner (Fig 2. G). Most of the cells contain small starch grains. Some cavities are also seen in the cortical region. This is followed by a broad zone of oval or rounded thick walled cells. Most of the cells are filled with large spherical or ovoid starch grains. Rosette crystals of calcium oxalate are plenty throughout the section (Fig 2. G). Resin containing cells are also present. Many vascular bundles are seen arranged in a ring. Vascular bundles are seen solitary or in groups of 3-5. Phloem region is seen in the form of a conical strip, upper portion of this strip is thick walled. Cambium is distinct and composed of 2-3 layers. Vascular bundles are wedge shaped. Strip of xylem core is bordered by a wide parenchymatous interfascicular region (Fig 2. H). Xylem vessels are more rounded and are arranged in radial strips. Central portion is very wide and composed of oval or rounded thick walled parenchymatous cells with starch grains. Starch grains are oval, rounded or large in size (Fig. 2. G-I).

3.3 Histochemical studies

Histochemical studies of *A. galanga* show that lignified cells are seen only in the xylem elements. Starch grains are simple, elongated and are more in number; numerous oleoresin cells are scattered throughout the rhizome (Fig 2. B). In *A. calcarata*, numerous starch grains are present in the cortical region and few starch grains are present in the stelar region. Starch grains are of simple, oval or oblong, and rounded type. Numerous oleoresin cells are scattered both in the cortex and in the stelar region. The lignified xylem elements are vessels, tracheids and few fibres (Fig 2. E). In *P. lanceolata*, the histochemical studies shows that some of the cells of outer region contain small starch grains. Most of the cells of inner region are filled with large spherical or ovoid starch grains. Rosette crystals of calcium oxalate are present. Resin containing cells and lignified xylem elements are also present (Fig 2. H).

In *A. galanga* powder consists of vessels with reticulate and spiral thickening, pitted tracheid, parenchyma cells with oleoresin cells, starch grains are of simple, oval or oblong, and rounded type, (Fig 3. A-E). In *A. calcarata*. Powder shows fragments of vessels with reticulate and spiral thickening, cross sectionally cut view of parenchyma cells with starch grains, fragments of xylem tracheids (Fig 3. F-J). Powder microscopy of *P. lanceolata* shows most of the cells filled with large spherical or ovoid starch grains. Rosette crystals of

calcium oxalate are plenty. Vessels are with reticulate thickening (Fig 3. K-N).

Polarization microscopic studies revealed the presence, position and shape of crystals and lignified cells. In *A. galangal*, outer and inner vascular bundles, sandy crystals and lignin show polarization (Fig 4. A-D). In *A. calcarata* outer and inner vascular bundles and sandy crystals showed polarization (Fig 4. E-H). In *P. lanceolata* rosette crystals observed throughout the cortical cells and lignified cells of vascular bundle also showed polarization (Fig 4. I-J).

Fluorescent microscopic studies showed auto fluorescence and gives a dark yellowish colour. In *A. galanga* the lignified cells produced fluorescence, outer and inner vascular bundles, sandy crystals and oleoresins show yellow fluorescence (Fig 5. A-C). In *A. calcarata* the lignified cells produced bright yellow fluorescence, outer and inner vascular bundles, sandy crystals and oleoresins and bundle sheath showed yellow bright fluorescence (Fig 5. D-F). In *P. lanceolata* cork and vascular bundle produced yellow fluorescence (Fig 5. G-I).

There are reports regarding the comparative morphology of plant groups as well as individual species [1, 3]. Studies were done on the morphology, histology and pharmacognosy of *P. lanceolata* [5, 9, 10]. Pharmacognostic studies of *A. galanga* and *A. calcarata* have been done earlier [11]. Some equated *A. galanga* with the name *Kulanjan* instead of *rasna* [12] whereas others described it as *Malayavaca* [13]. The plant *A. calcarata* is reported as Granthimula [14]. Though this much reports are there in this group, there is no clear cut view for comparing thee three plants. The present study carried out a comparative approach to distinguish these three plants with the help of recent anatomical tools such as fluorescent and polarisation microscopy.

In *A. galanga* cortical cells are thin walled, polygonal and parenchymatous. Sandy crystals are present, bundle sheaths are not much lignified and it partially encircles the bundles, which are present in the inner region of the endodermoidal layer, vascular bundles are scattered and oleoresin cells are present. Oleoresin cells are more in number in *A. galanga*. Starch grains are simple, elongated and oval, comparatively more in number, but in the case of *A. calcarata*, thin walled, polygonal and parenchymatous cortical cells are seen, bundle sheath cells are highly lignified and sclerenchymatous which completely encircles the bundle, present on both sides of the endodermoidal layer. Vascular bundles are scattered and oleoresin cells are less in number in *A. calcarata* when compared to *A. galanga*. Starch grains are simple, rounded or oval, less in number. In *P. lanceolata* the outer most region consists of thin and thick walled cells arranged in a storied condition. Large rosette crystals are present in *P. lanceolata*. Schizogenous cavities are seen in the cortical region, bundle sheath absent. Most of the vascular bundles are arranged in a ring, some bundles are seen solitary or in groups of 3-5. Resin containing cells are more in number. Starch grains are large spherical or ovoid type. Detailed anatomical characters of *A. galanga*, *A. calcarata* and *P. lanceolata* are shown in Table 1.

3.4 Tables and Figures

Table 1: Comparative Rhizome and Root anatomical characters of *A. galanga*, *A. calcarata* and *P. lanceolata*

Characters	***A. galanga***	***A. calcarata***	***P. lanceolata***
Shape in TS	Circular	Circular	Nearly circular with wavy
Diameter in TS	2 to 3 cm diameter	1 to 2 cm diameter	1.5 to 2 cm diameter
Nature of epidermis	Epidermis is single layered and the cells are small and arranged tangentially. Epidermal cells contain red coloured content.	Epidermis is single layered and the cells are small and oval in shape	Outermost region is cork and composed of 8-20 layers of thin and thick walled and tangentially elongated cells arranged in a storied manner
Nature of cortex	Epidermis is followed by thin walled, polygonal parenchymatous ground tissue with numerous fibro vascular bundles. Inter cellular spaces are present in this region. Many of the cells of this region contain reddish content	Epidermis is followed by parenchymatous ground tissue with numerous fibro vascular bundle Many of the cells of this region contain reddish content	Just inner to the cork is a broad zone of cortex composed of oval or rounded thick walled cells. Cavities are seen in the cortical region
Nature of bundle sheath	Bundle sheath cells are not much lignified and partially encircles the bundles, present inner region of the endodermoidal layer.	Bundle sheath cells are highly lignified and sclerenchymatous which completely encircles the bundle, present both in and outside of the endodermoidal layer.	Bundle sheath not present
Nature of vascular bundles	Vascular bundles are scattered both in the cortex and stelar region, without any definite arrangement	Vascular bundles are scattered both in the cortex and stelar region, without any definite arrangement	Most of the vascular bundles are arranged in a ring some bundles are seen solitary or in groups of 3-5.
	Sandy crystals of calcium oxalate and numerous oleoresin cells are present throughout the rhizome.	Numerous oleoresin cells are present throughout the rhizome.	Rosette crystals of calcium oxalate and resin containing cells are present.

Nature of cell inclusions	Colour containing cells are more in number	Colour containing cells are lesser in number	Colour containing cells are more in number
Nature of starch grains	Starch grains are simple, elongated and oval comparatively more in number.	Starch grains are simple, rounded or oval, less in number.	Starch grains are large spherical or ovoid type
Nature of xylem	Xylem consists of parenchyma, tracheids, fibers and vessels	Xylem consists of tracheids, parenchyma, fibers and vessels	Xylem consists of vessels, parenchyma and fibers. Vessels are wide and are arranged in long radial strips.
Nature of phloem	Phloem consists of sieve tubes, companion cells and fibers.	Phloem consists of sieve tubes, companion cells and fibers	Phloem region is seen in the form of a conical strip. upper portion of this strip is thick walled

Fig. 1. A. *Alpinia galanga* habit B. Pieces of dried rhizome C. *Alpinia calcarata* habit D. Pieces of dried rhizome E. *Pluchea lanceolata* habit F. Pieces of dried root

Fig 1: Morphological comparison of three plant species.

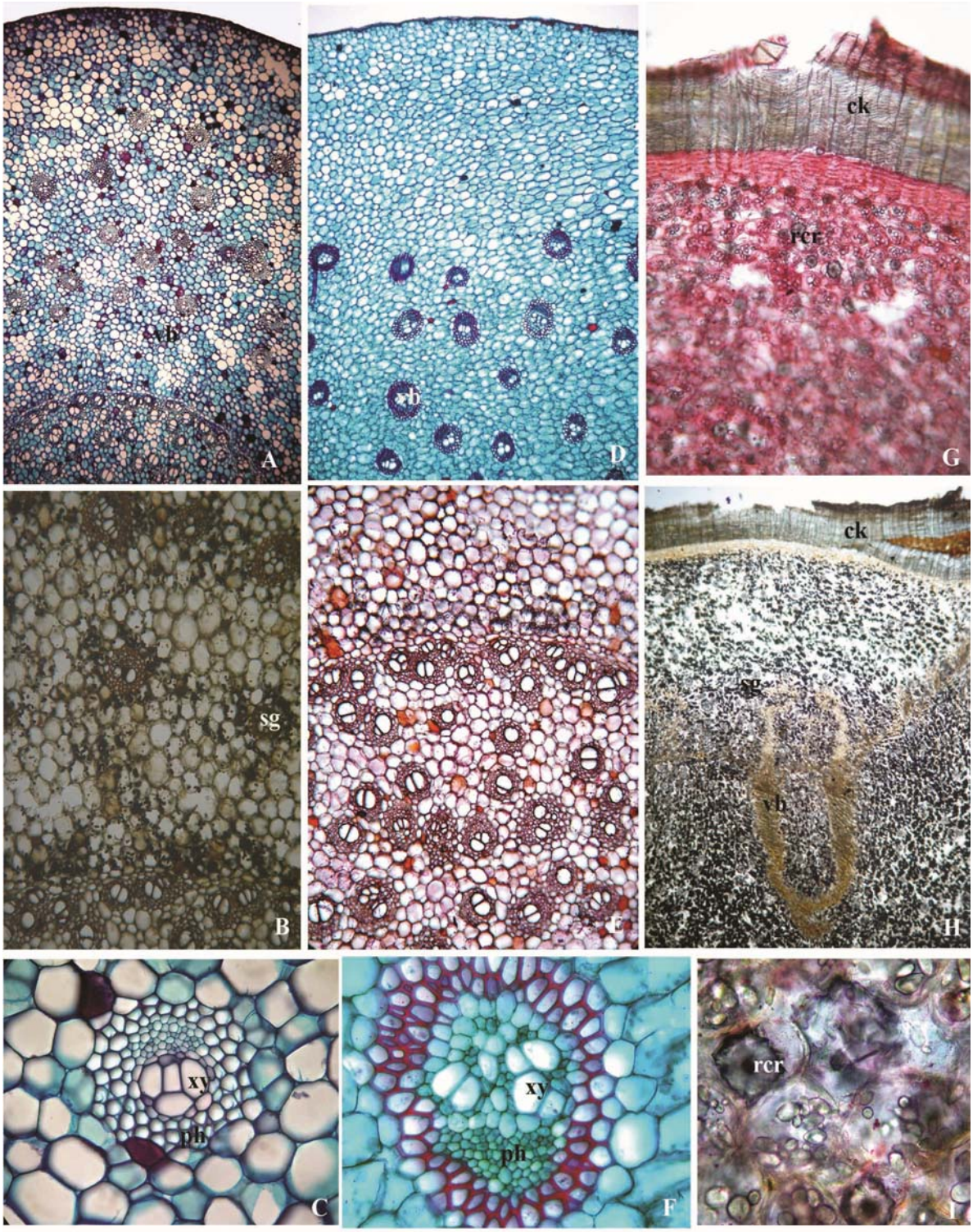

Fig. 2. (A-C). Microscopy of *A. galanga* rhizome. (A). TS of rhizome x 40, (B). Histochemical localization of starch x 200, (C). Vascularbundle enlarged x 400, (D-F). Microscopy of *A. calcarata* rhizome. (D). TS of rhizome x 40, (E). Histochemical localization of starch x 200, (F). Vascular bundle enlarged x 400, (G-I). Microscopy of *P. lanceolata* root. (G). TS of root x 40, (H). Histochemical localization of starch x 200, (I). Rosette crystals of calcium oxalate x 400. ck, cork; ph, phloem; rcr, rosette crystals of calcium oxalate; sg, starch grains; vb, vascular bundle; xy, xylem.

Fig 2: Histochemical comparison of three plant species.

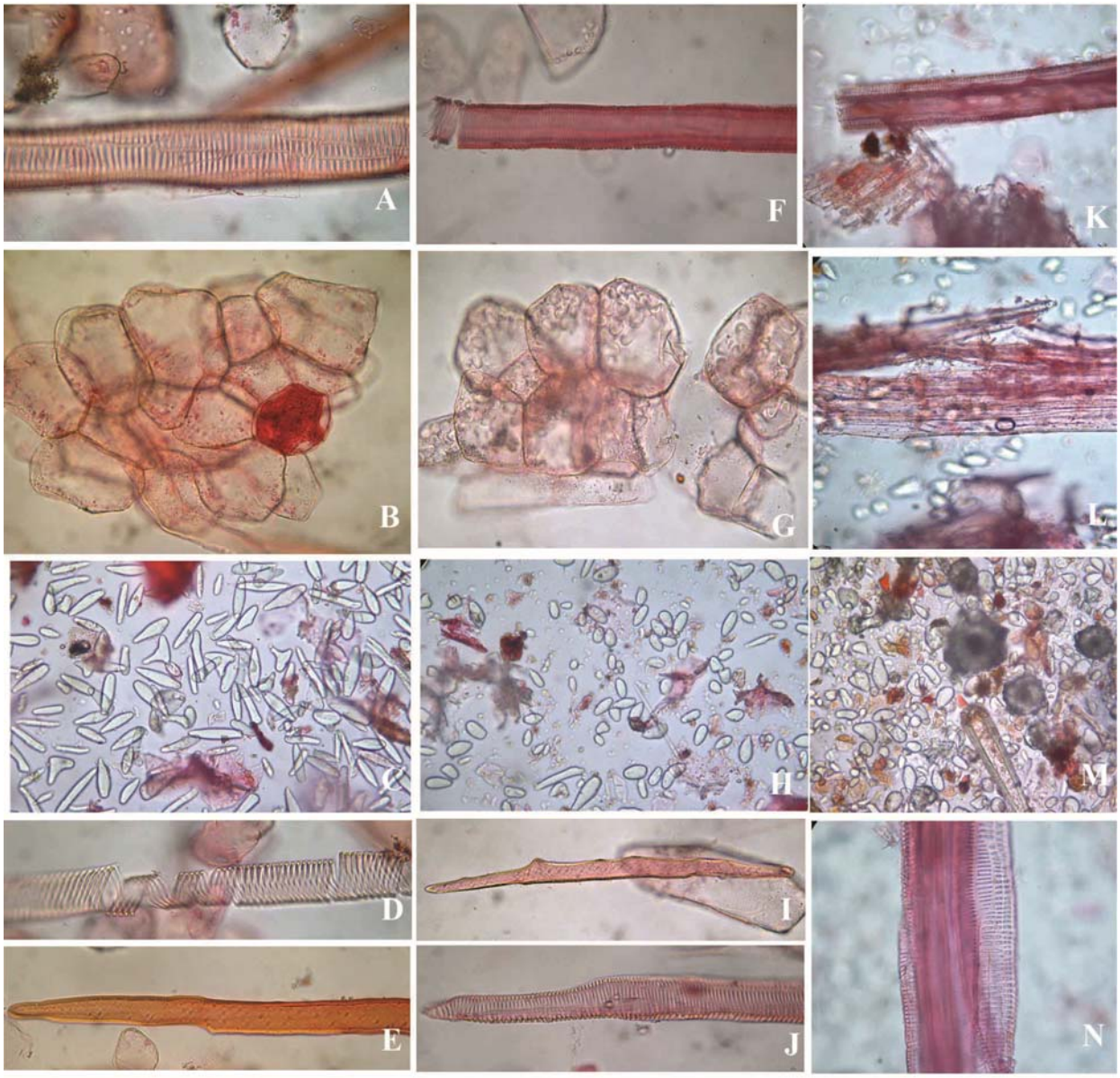

Fig. 3. (A-E). Powder characters of *A. galanga* rhizome. (A). Xylem vessel with reticulate thickening x 400, (B). Parenchyma cells with oil cell x 400, (C). Starch grains x 400, (D). Xylem vessel with spiral thickening x 400, (E). Tracheid x 400. (F-J). Powder characters of *A. calcarata* rhizome. (F). Xylem vessel with reticulate thickening x 400, (G). Parenchyma cells with starch x 400, (H). Starch grains x 400, (I). Tracheid x 400, (J). Xylem vessel with spiral thickening x 400. (K-N). Powder characters of *P. lanceolata* root. (K&N). Vessels with reticulate thickening x 400, (L). Reddish brown deposits x 400, (M). Rosette crystals of calcium oxalate and starch grains x 400.

Fig 3: Powder microscopic studies of three plant species.

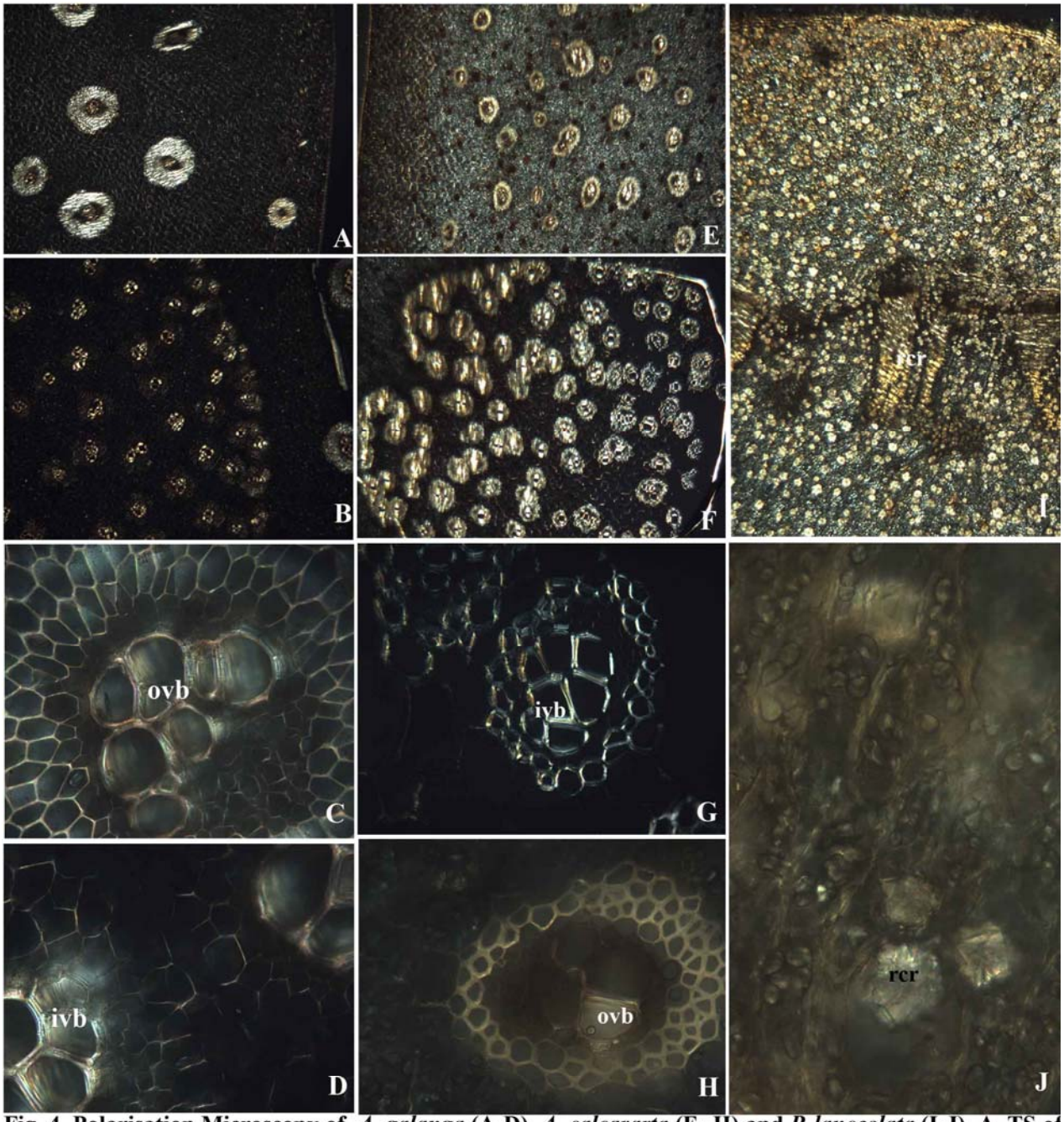

Fig. 4. Polarisation Microscopy of *A. galanga* (A-D), *A. calcararta* (E -H) and *P. lanceolata* (I-J). A, TS of rhizome showing outer portion x 40; B. TS of rhizome showing inner portion x 40; C. Outer vascular bundle x 400; D. Inner vascular bundle x 400. E. TS of rhizome showing outer portion x 40; F. TS of rhizome showing inner portion x 40; G. Outer vascular bundle x 400; H. Inner vascular bundle x 400. I. TS of root showing cork, cortex and wood region x 40; J. Rosette crystals of calcium oxalate x 400.ivb, inner vascular bundle; ovb, outer vascular bundle; rcr, rosette crystals of calcium oxalate.

Fig 4: Polarisation microscopic studies of three plant species.

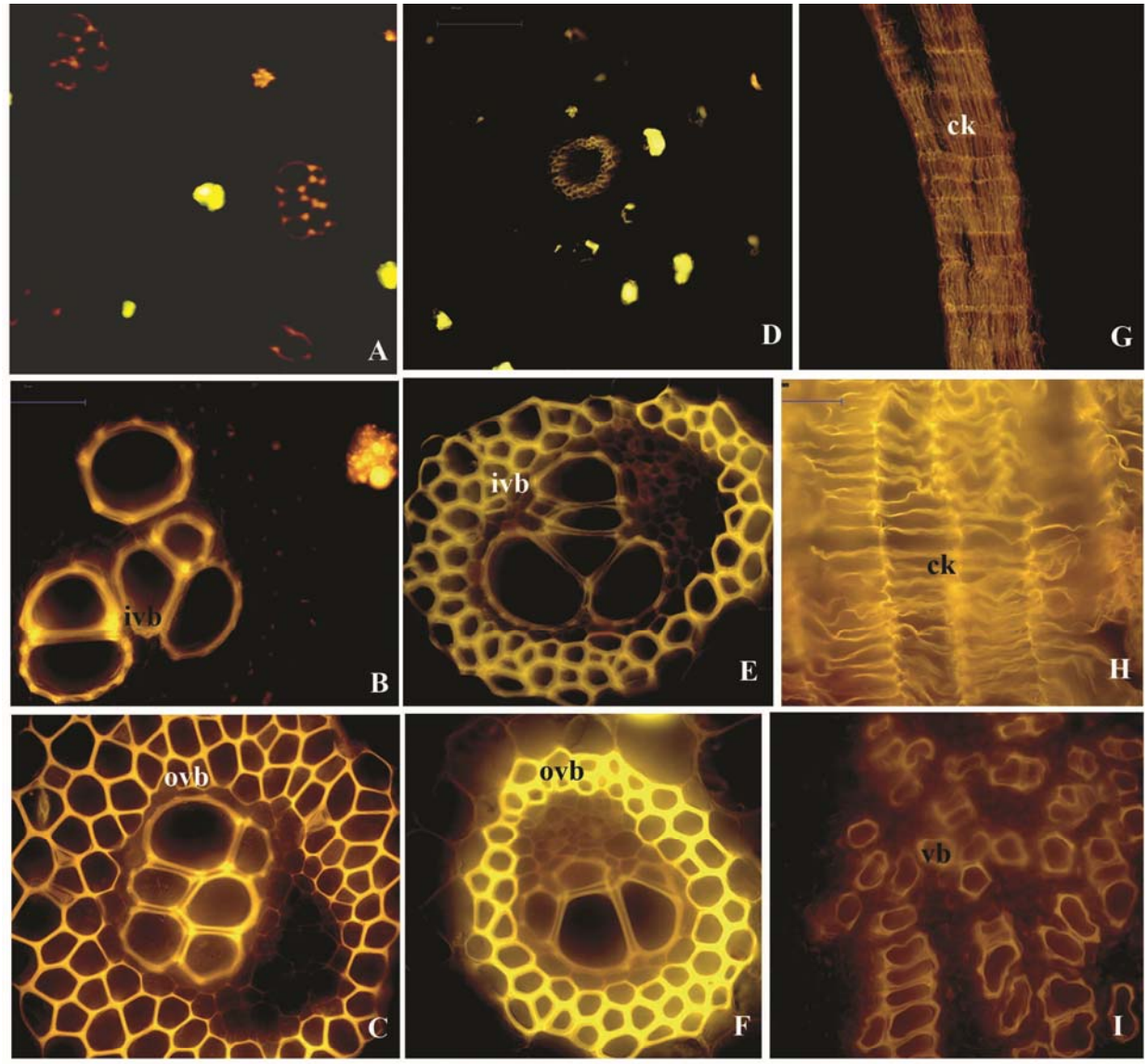

Fig.5. Flourescent Microscopy of *A. galanga* (A-C), *A. calcarata* (D-F) and *P. lanceolata* (G-I). A. TS of rhizome showing vascular bundle and oleoresin x 100; B. Inner vascular bundle x 400; C. Outer vascular bundle x 400. D. TS of rhizome showing outer portion and sandy crystal x 100; E. TS of rhizome showing inner vascular bundle x 400; F. Outer vascular bundle x 400.G. TS of root showing cork x 100; H. Cork x 400; I. Vascular bundle x 400. ck, cork; ivb, inner vascular bundles; ovb, outer vascular bundle; vb, vascular bundle.

Fig 5: Fluorescent microscopic studies of three plant species.

4. Conclusions

A thorough histological, histochemical, and powder studies of *A. galanga*, *A. calcarata* and *P. lanceolata* were carried out. *P. lanceolata* belongs to Asteraceae and it comes under dicots and the root structure is specific and vascular bundles are arranged in ring with a central large parenchymatous zone. Whereas, the other two plants come under Zingiberaceae, a monocot plant. The rhizome TS shows specific characters and vascular bundles are scattered in the cortex and stellar region without any definite arrangement. Among these two rhizomes, the distinguishing characters are in *A. galanga*, vascular bundles are partially encircled by the bundle sheath where as in *A. calcarata* highly lignified sclerenchymatous bundle sheath completely encircles the vascular bundle which is present in both inner and outer region. The shape of the starch grains is specific to *A. galanga*, *A. calcarata* and *P. lanceolata*. Plenty of rosette crystals of calcium oxalate are present in *P. lanceolata* whereas only sandy crystal is observed in *A. galanga* and *A. calcarata*. Reticulate and spiral vessels are a common feature. The powder characters are specific to *P. lanceolata* than the other two plants. The polarization and fluorescent microscopic studies gives a clear idea of calcium oxalate crystal and lignified cells present in these three plants. All the three plants are being used as *Rasna*. *Alpinia galanga* and *Alpinia calcarata* are used in Kerala and in Northern side *Pluchea lanceolata*. In the rhizomes of *A.galanga* the vascular bundles are large and the number of vessel members is much more but in *A. calcarata* the vascular bundles are considerably small in size and the vessel members are also fewer in number. Absence of starch grains in the stele as wells as the difference in the shape of the starch grains are the features that differentiate *A. galanga* from *A. calcarata*. In

P. lanceolata vascular bundles are arranged in the form of a ring and calcium oxalate crystals are present.

From the present study distinguishing features for these three plants using histological, histochemical and powder studies were developed for checking the identity of the raw drug *Rasna*.

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