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Pharmacognostical standardization of *Artabotrys Zeylanicus* and *Artabotrys Sahyadricus*

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Abstract
Objective: To establish the standardization parameters for complete pharmacognostic evaluation of leaf and stem bark of *Artabotrys zeylanicus* and *Artabotrys sahyadricus* are medicinally important liana.
Materials and Methods: Macro and microscopical, HPTLC profiles were carried out for phytochemical evaluation of ethyl acetate extracts of leaf and stem bark extract of *Artabotrys zeylanicus* and *Artabotrys sahyadricus.*
Results: Macro and micro the HPTLC revealed the presence of rich secondary metabolites in ethyl acetate leaf and stem bark extract of *A. zeylanicus* and *A. sahyadricus*. The HPTLC fingerprinting profile developed for ethyl acetate leaf and stem bark extracts will lead to the identification and quantification of marker compounds.
Conclusions: Pharmacognostical studies proved the presence of secondary metabolites and rich content fibre and ethyl acetate leaf and stem bark extract of *A. zeylanicus* and *A. sahyadricus* have the potential to formulated newly isolating and identifying marker compounds and new drugs.

Keywords: Macroscopic, microscopic, ethyl acetate leaf and stem bark extract of A. zeylanicus and A. sahyadricus, HPTLC finger printing

Introduction
The *Artabotrys* genus is well known for the medicinal important and species have a long history of traditional use for a wide range of medical conditions, particularly malaria, scrofula, and cholera and some other diseases like analgesic, antiplasmodial, anti-inflammatory, hepatocarcinoma, glandular swellings, fungal infection, infertility, food poisoning, general weakness, intestinal worms, snake bites, stomach ache, asthma and cough, venereal diseases and decoction of the leaves used against cholera also cured by this genus members [1, 2] and Flower part decoction of *A. zeylanicus* used to treat vomiting [3]. Demand for plant drugs increasing throughout the world because of their safety and efficacy. Today's folklore medicine is rechecked by pharmacognostical research on different plant species and their therapeutic principles.
In plants, a complex network is formed with the places of water intake, sites of food synthesis, growth areas, development and storage [4]. In vascular plants, morphological features and anatomical characters help to the identification of botanical knowledge and phytochemical analyses of possible active compounds [5]. High performance thin layer chromatography (HPTLC) can provide an automated and sophisticated electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample [6, 7]. In the present study, plants *Artabotrys sahyadricus* and *A. zeylanicus* performed for the morphological and anatomical evaluation and was to describe HPTLC analysis (fingerprint and densitometry) for the determination of secondary metabolites in plant extracts.

2. Materials and Methods
2.1. Collection and authentication of the plant material
These species were identified and authenticated by Dr. Prabhukumar KM, Senior Scientist and Head, Plant Systematics and Genetic Resources Division and 'CMPR' Herbarium, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram - 676 503, Kerala, India (*A. zeylanicus* 8680 and *A. sahyadricus* 8693) and voucher specimen has been deposited in 'CMPR' Herbarium, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram - 676503, Kerala, India.
Artabotrys zeylanicus Hook. f. & Thoms were collected from Muthikulam, Palakkad district, Kerala, India and *A. sahyadricus* Rabi *et al.* were collected from Kuttampuzha Forest Range, Ernakulam district, India. *A. zeylanicus* is a very vigorous, evergreen climbing shrub.

Generally, found in the tropical, semi-evergreen, evergreen forests and plains of India and Sri Lanka. Local distributions in Karnataka, Kerala, Maharashtra, Tamil Nadu and Global Distribution in Asia: India and Sri Lanka. *Artabotrys sahyadricus* endemic to Kerala parts of Western Ghats, India. Distributed in moist deciduous and semi-evergreen forests at altitudinal range between 100 and 500 m. Different population have been observed from Parambikulam of Palakkad district, Agasthyamalai hills of Thiruvananthapuram district, Kakkayam forest of Kozhikode district and Kunjippara forests of Eranakulam district [8]. Since *A. sahyadricus* a new species, no recorded reports on its use is available in any ailments. However, the species might have used as the medicine, like other species in their common name.

2.2 Pharmacognostic standardization
Aerial parts of *Artabortrys zeylanicus* and *A. sahyadricus* were subjected to morphological evaluation for parameters like color, shape, size, taste and texture. Macroscopical observation was carried out the freshly collected aerial parts and to the powder and anatomical characterization of samples done by stained hand/microtome sections with diluted aqueous safranin and Fast green, washed thoroughly and mounted in 40% glycerin and observed under the microscope. Anatomical microphotographs were transferred using the computer controlled microscopic system and camera. Images are examined thoroughly and compared the anatomical characteristics.

2.3 High Performance Thin Layer Chromatography (HPTLC)
HPTLC is a remarkable simple, quick and accurate method for analyzing plant material. It possesses better resolution, and estimation of active constituents is done with reasonable accuracy in a shorter time. It can provide the outcome of chromatographic fingerprints that can be visualize and stored as electronic images [9]. Qualitative densitometric HPTLC analysis was executed to resolve the phytochemical compounds such as alkaloids, flavonoids, glycosides, saponins and terpenoids as marker compound in the ethyl acetate leaf and stem bark

2.3.1 HPTLC finger printing analysis
HPTLC studies were carried out by following the method of [10-12].

2.3.2 Sample preparation
The ethyl acetate leaf and stem bark extracts of 100 mg each was dissolved in 1 ml HPTLC grade ethyl acetate and centrifuged at 3000 rpm for 5 min. These solutions were used as test solution for HPTLC analysis.

2.3.3 Developing solvent system
The leaf and stem bark extract (Ethyl acetate extract – 1 µl) of *A. zeylanicus* and *A. sahyadricus* were applied in TLC aluminium sheet silica gel 60 F 254 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (8.7:1.3:0.1). After development the plate was allowed to dry in air and examined under UV-254 nm, 366 nm and visible light (Vanillin-sulfuric acid).

2.3.4 Sample application
2 µl of test solution and 2 µl of standard solution were spotted in the form of bands of 5mm length using Hamilton syringe on silica gel 60F254 (precoated on aluminum plate 4x10 cm) with the help of CAMAG LINOMAT 5 applicator, spray gas is N_2 and deuterium and tungsten lamp was used.

2.3.5 Development of chromatogram
After the application of spots, the chromatogram developed in ascending order with a CAMAG twin trough glass chamber (20x10 cm) was pre-saturated with respective mobile phase for 15 min; the length of each run was 8 cm (90 mm). The TLC runs were performed under laboratory conditions (temperature: 25±2 ºC and relative humidity: 60±5). The plates were then air dried by hot air to evaporate solvents.

2.3.6 Photo-documentation
The air-dried plates were kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 366 nm and UV 254 nm.

2.3.7 Derivatization
The developed plate was sprayed with respective spray reagent for alkaloids, flavonoids, glycosides, saponins and terpenoids and dried at 100 ºC in hot air oven.

2.3.8 Scanning
After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm. The peak numbers with their height and area, peak presentation and peak densitograms and Rf values were programmed through WIN CATS software (1.3.4 version).

3. Results and Discussion
Leaf and wood anatomy play an important role as sub disciplines of systematic botany [13]. In *Annonaceae* several Asiatic genus *Uvaria, Cyathostemma, Rauwenhoffia, Ellipeia, Artabotrys, Fissistigma, Pyramidanthe, Mitrella* and *Friesodielsia* are Iianas. The woody nature of the *Annonaceae* have continuous, concentric tangential bands of parenchyma. The xylem vessels are small, mostly solitary or in 2-4-celled groups [14]. The finding of the present study confirmed the anatomical characters of leaf, petiole and stem of the *A. sahyadricus* and *A. zeylanicus.*

Morphological evaluation: Large woody climbing habit, 12-15 ft. long producing flowers and fruits in *A. sahyadricus*. 7-10 ft. long producing flowers and fruits in *A. zeylanicus*. Color of shrub is greenish and leaf is not shining, simple, alternate, smell pleasant fragrant, slightly bitter taste. Flowers are yellow, 2-4 in inflorescence in *A. sahyadricus*. 1-2 in inflorescence in *A. zeylanicus.* These are an important parameter for evaluation of plant parts and plant powder.

Anatomical evaluation

Artabortrys sahyadricus

TS of leaf: Transverse section of the leaf passing through the midrib is convex on the lower side, a collenchymatous layer underneath both upper and lower the epidermis, bicollateral vascular bundle located in central. Detailed T.S shows a layer of upper and lower epidermis, closely packed palisade cells with chloroplast below the upper epidermis, perpendicular to it 2-3 rows of spongy parenchymatous tissue with lots of air spaces. Lower epidermis has paracytic stomata and guard cells surrounded usually by two subsidiary cells having a triangular shape (Fig 1).

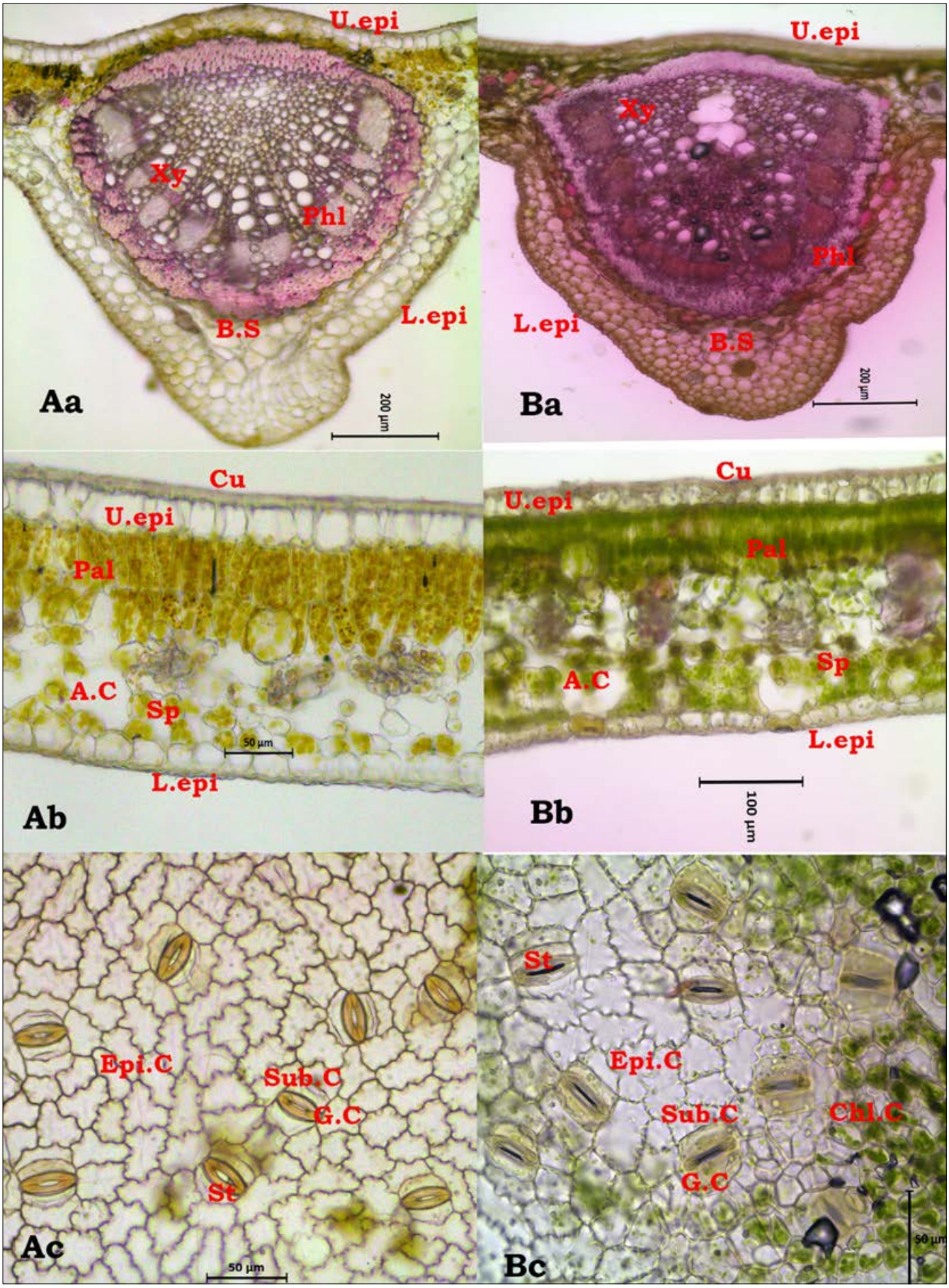

Fig 1: Aa: T.S of *A. sahyadricus* leaf- midrib view, Ba: T.S of *A. zeylanicus* leaf- midrib view, Ab: T.S of *A. sahyadricus* leaf- laminal view, Bb: T.S of *A. zeylanicus* leaf- laminal view, Ac: Stomatal view of *A. sahyadricus*, Bc: Stomatal view of *A. zeylanicus* [A.C: Air cavity, B.S: Bundle sheath, Chl.C: Chlorenchyma cells, Cu: Cuticle, Epi. C: Epidermal cells, G.C: Guard cells, L.epi: Lower epidermis, Pal: Palisade cells, Phl: Phloem, Sp: Spongy parenchyma cells, St: Stomata, Sub. C: Subsidiary cells, U.epi: Upper epidermis, Xy: Xylam]

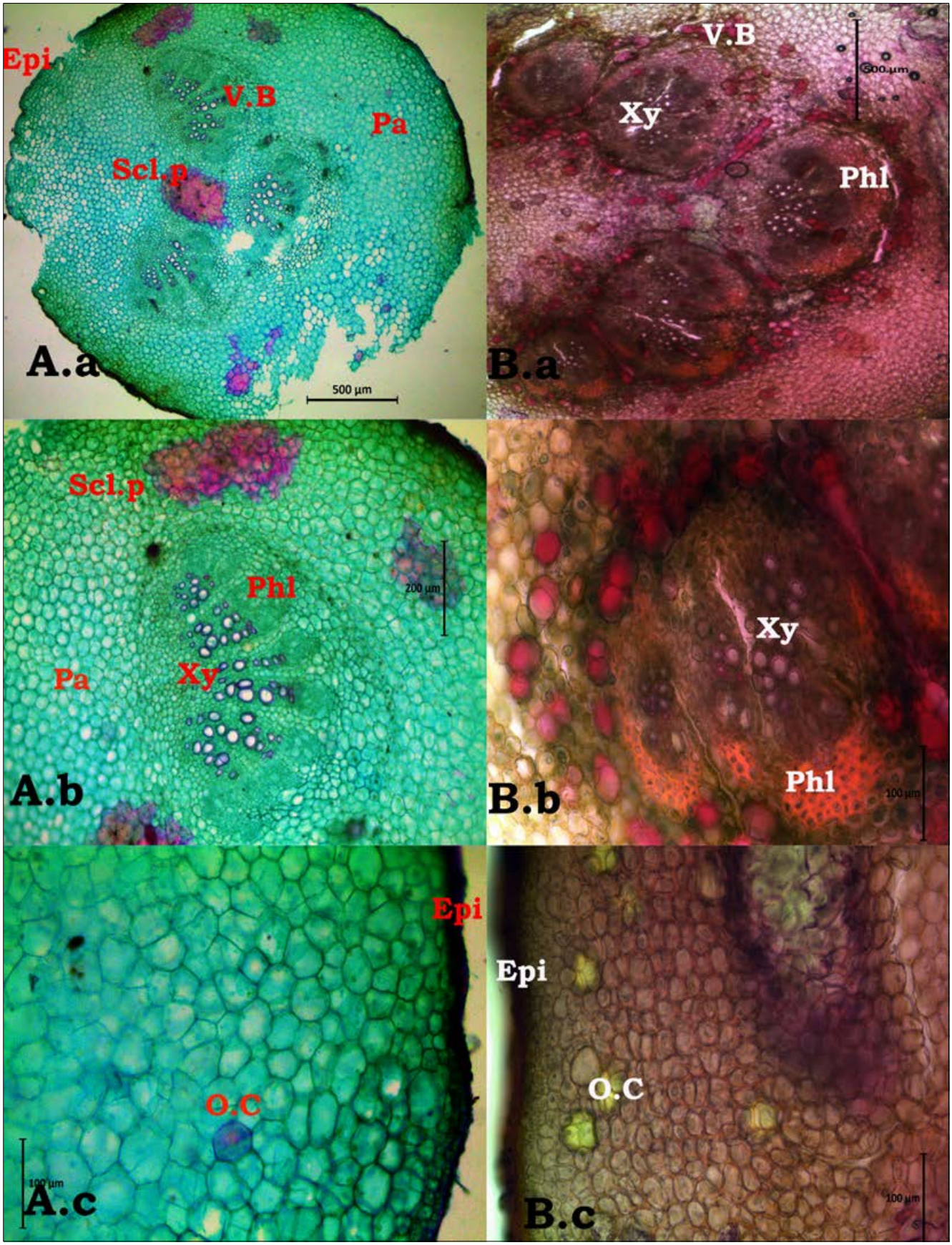

Fig 2: Aa: T.S of *A. sahyadricus* petiole, Ba: T.S of *A. zeylanicus* petiole, Ab: Enlarged view Vascular bundle in *A. sahyadricus* petiole, Bb: Enlarged view of vascular bundle in *A. zeylanicus* petiole, Ac: Enlarged upper view of *A. sahyadricus* petiole, Bc: Enlarged upper view of *A. zeylanicus* petiole [Epi: Epidermis, O.C: Oil cells, Pa: parenchyma cells, Phl: Phloem, Scl.p: Sclerenchyma patches, V.B: Vascular bundle, Xy: Xylam]

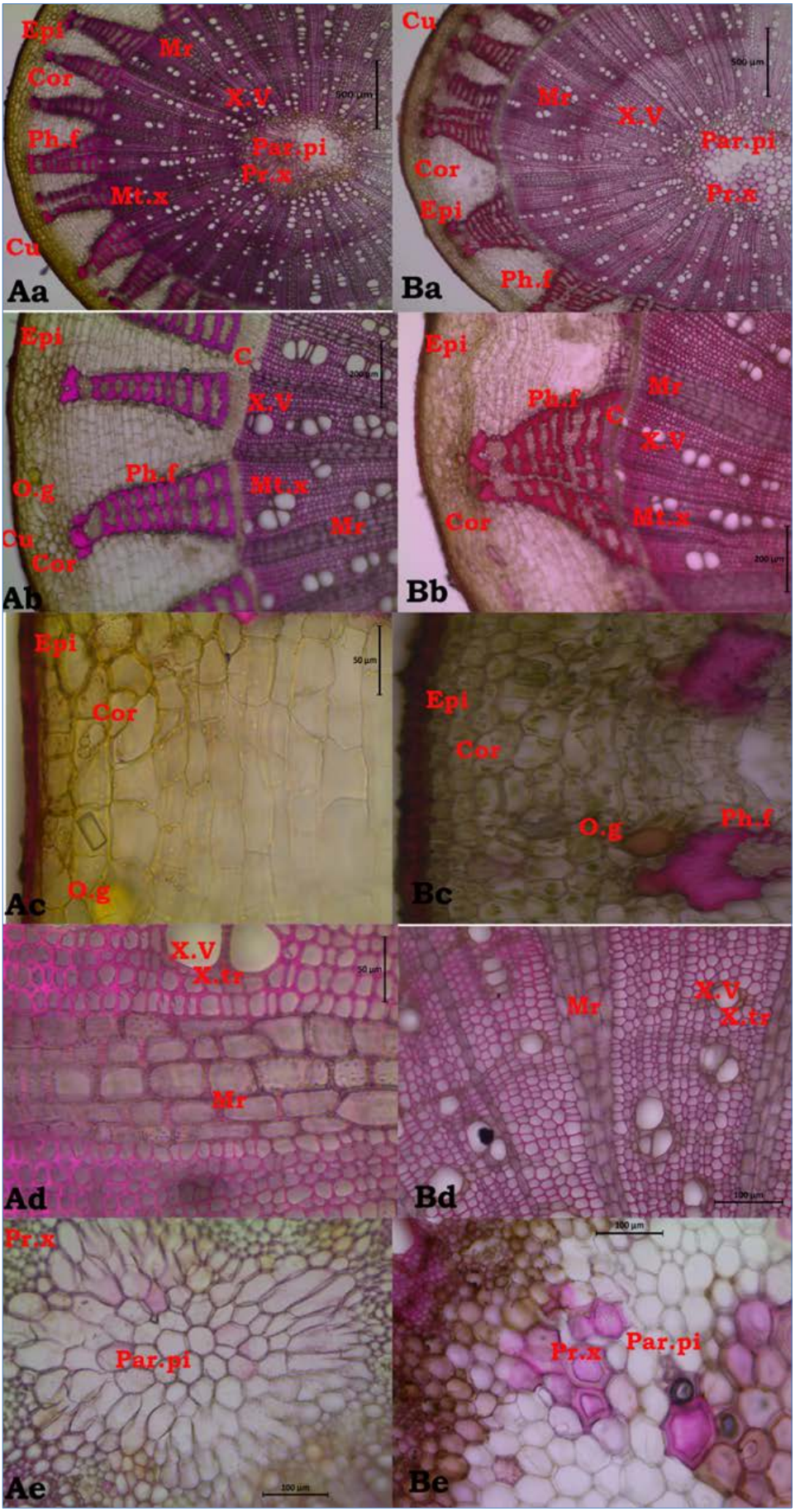

Fig 3. Aa: T.S of *A. sahyadricus* stem, Ba: T.S of *A. zeylanicus* stem, Ab: Enlarged view of *A. sahyadricus* stem, Bb: Enlarged view of *A. zeylanicus* stem, Ac: Enlarged upper view of *A. sahyadricus* stem, Bc: Enlarged upper view of *A. zeylanicus* stem Ad: middle view of *A. sahyadricus* stem Bd: Enlarged upper view of *A. zeylanicus* Ae: central view of *A. sahyadricus* stem Be: Central view of *A. zeylanicus* stem [C: Cambium, Cor: cortex, Cu: cuticle, Epi: Epidermis, Mr: medullary rays, Mt.x: Meta xylem, O.G: Oil globules, Pa: parenchyma cells, Par. pi: parenchymatous pith, Ph. F: Phloem fibre, Pr. x: Protoxylem, Scl. p: Sclerenchyma patches, Xy: Xylam, Phl: Phloem, X.tr: Xylem tracheids, X.V: xylem vessels]

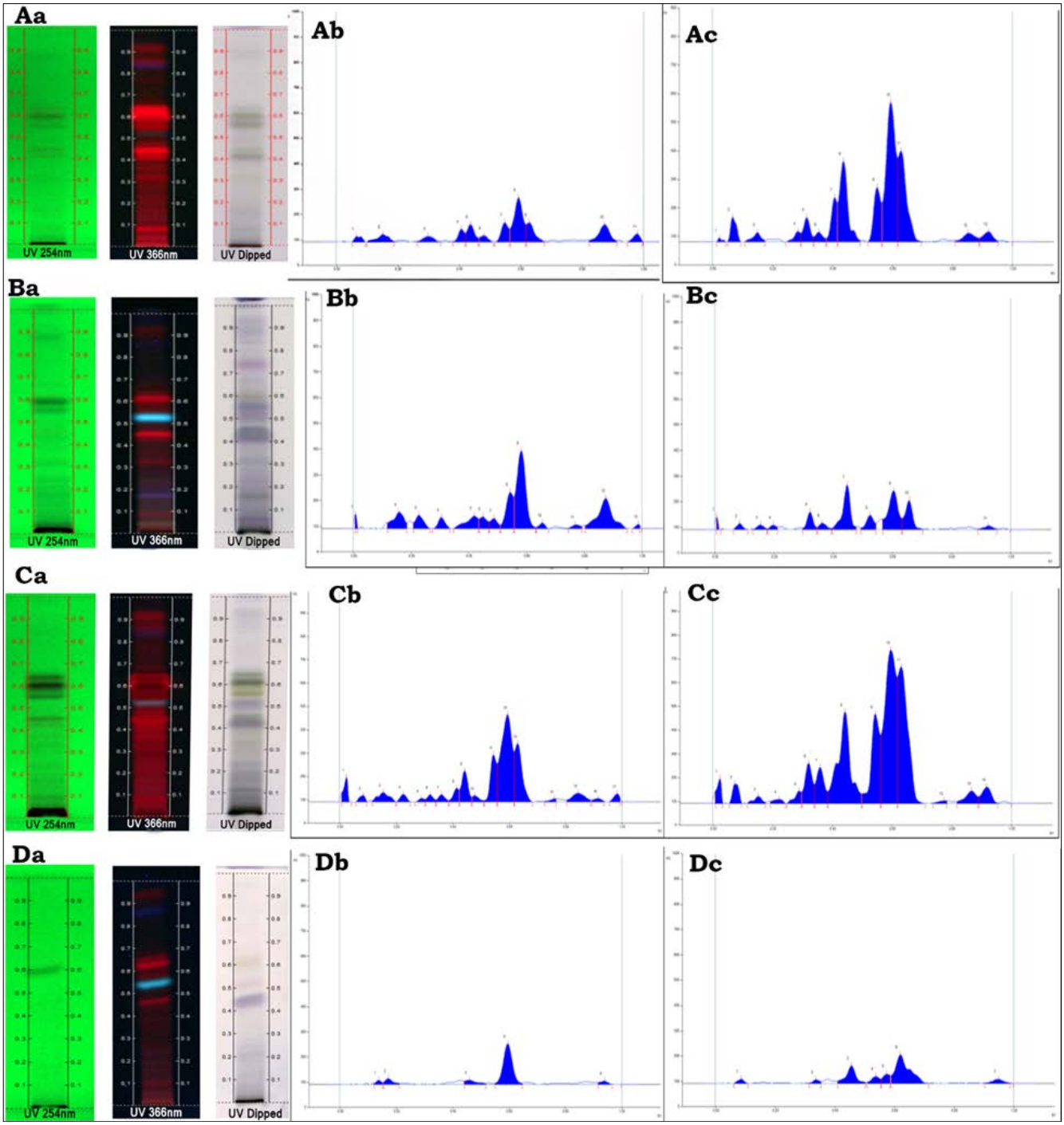

Fig 4. Aa: HPTLC analysis for ethyl acetate leaf extract of *A. sahyadricus*, Ab: HPTLC finger print for ethyl acetate leaf extract of *A. sahyadricus* at 254nm, Ac: HPTLC finger print for ethyl acetate leaf extract of *A. sahyadricus* at 366 nm, Ba: HPTLC analysis for ethyl acetate stem bark extract of *A. sahyadricus* Bb: HPTLC finger print for ethyl acetate stem bark extract of *A. sahyadricus* at 254 nm, Bc: HPTLC finger print for ethyl acetate stem bark extract of *A. sahyadricus* at 366 nm, Ca: HPTLC analysis for ethyl acetate leaf extract of *A. zeylanicus.* Cb: HPTLC finger print for ethyl acetate leaf extract of *A. zeylanicus* at 254 nm. Cc: HPTLC finger print for ethyl acetate leaf extract of *A. zeylanicus* at 366 nm, Da: HPTLC analysis for ethyl acetate stem bark extract of *A. zeylanicus*, Db: HPTLC finger print for ethyl acetate stem bark extract of *A. zeylanicus* at 254 nm, Dc: HPTLC finger print for ethyl acetate

TS of petiole: Transverse section of the petiole is circular in shape. 3-4 sclerenchymatous patches occurred inside. Parenchymatous ground tissues and vascular strand located in central region. 3 vascular bundles located in middle of the petiole (Fig. 2).

TS of stem: Transverse section of the stem of *A. sahyadricus* is circular in shape, shows cuticle layer in outer, single layered epidermis followed by cortex region. Number of oil depositions located in the outer cortex region. Well distinct secondary phloem containing phloem fibers and phloem parenchyma cells. The phloem parenchymatous cells surrounded by two distinct stack of fiber. In additionally the phloem cells converted to fibers help in elongation and flexibility to the climbing nature of plant. Cambium located in between the secondary phloem and secondary xylem and tracheid, vessels and medullary rays were arranged clearly in underneath part medullary rays running in continuation with protoxylem surrounded with parenchymatous cells. Pith was widely arranged with parenchymatous cell and attached to the xylem ring (Fig. 3).

Artabotrys zeylanicus

TS of leaf: Transverse section of the leaf passing through the cuticle, upper epidermis, two layers of palisade cells, in central portion located large vascular bundles, surrounded by spongy parenchymatous layers with stomatal region and followed by lower epidermis. Paracytic type of stomata containing two guard cells surrounded by rectangular shaped two subsidiary cells (Fig. 1).

TS of petiole: Transverse section of the petiole is circular in shape. Parenchymatous epidermis followed ground tissues and vascular strand located in the middle. 5 set of vascular bundles located in middle of the petioles (Fig. 2).

TS of stem: Transverse section of the stem of *A. zeylanicus* is circular in shape, shows outer most layer of cuticle, parenchymatous epidermis layer followed by cortex region. Few oil depositions located in the outer cortex region. Well distinct secondary phloem containing phloem fibers and phloem parenchyma cells and phloem parenchymatous cells surrounded by single or mixed distinct stack of fiber. Furthermore, cell types of the phloem converted as fibers may be for the elongation and flexibility to the climbing nature of plant species. Cambium located in between the secondary phloem and xylem. Xylem tracheid's, xylem vessels and medullary rays were arranged clearly in underneath part, medullary rays running in continuation with protoxylem surrounded with parenchymatous cells. Broadly, arranged pith made by parenchymatous cells and attached at the xylem ring (Fig. 3).

In leaf anatomy the plant, *A. sahyadricus* has two triangular shaped subsidiary cells in paracytic stomata and in *A. zeylanicus* has two rectangular shaped subsidiary cells in paracytic stomata. In petiole, three set of vascular bundles in *A. sahyadricus* and five set of vascular bundles in *A. zeylanicus.* Well distinct secondary phloem containing in bark portion of both plants. Phloem parenchyma cells surrounded by single or mixed distinct stack of phloem fibre in *A. zeylanicus* and two distinct stack of phloem fibres and phloem parenchyma occurred in *A. sahyadricus.*

Subsequent transport of food and minerals taking place through phloem cells [15]. Cell depositions observed in the parenchymatous cortex region of stem bark, many of secondary metabolites accumulated in the cell depositions [16, 17]. Therefore, these plant species have possibility to more phytochemical and pharmaceutical potential in stem bark part than leaf. Plant *A. haxapetalous* revealed the presence of collenchyma, parenchyma cells, palisade cells and presence of parasitic stomata [18]. From ancient period plant species *A. zeylanicus* and *A. odoratissimus* popular as useful fiber plant of the world [19] and same plant flowers used for the manufacture of perfume also [20]. This led to chance for the presence of aromatic terpenoid in *Artabotrys* genus.

HPTLC suggested for identification of the medicinal plants and finds solution for the taxonomical issues [21, 22]. The extraction yield calculated using petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of leaf and stem bark of *A. zeylanicus* and *A. sahyadricus*. Ethyl acetate extract gave higher percentage of yield than other solvent extracts. So that, the HPTLC profile was prepared by ethyl acetate extracts to identify secondary metabolites for these plants. High quantity of secondary metabolites was detected in ethyl acetate leaf and stem bark extracts of *A. zeylanicus* and *A. sahyadricus* by HPTLC technique (Fig 4). Phytochemicals like alkaloids, flavonoids, phenols, tannins, glycosides, steroids, terpenoids and cardiac glycosides in the plant extracts revealed presence of various secondary metabolites in both *A. zeylanicus* and *A. sahyadricus* such as alkaloids, flavonoids, phenols, tannins, glycosides, steroids, terpenoids and cardiac glycosides are highly indicated in ethyl acetate extract of leaf and stem bark extracts of *A. zeylanicus* and *A. sahyadricus* [23]. Better quantity of secondary metabolites were detected in ethyl acetate leaf and stem bark extracts of *A. zeylanicus* and *A. sahyadricus* by HPTLC technique.

4. Conclusion

From this study, the presence of aromatic nature and fibre dominance gives a hope for perfume manufacturing fibre extracting in both plants. The well-resolved HPTLC profile showed the presence of secondary metabolites, which hold up the traditional therapeutic uses of plants *A. zeylanicus* and *A. sahyadricus.*

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