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Taxonomic value of the petiole anatomy in the genus *Cinnamomum* (Lauraceae) found in Sri Lanka

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Abstract. We assessed the petiole anatomy of 8 species of Sri Lankan *Cinnamomum* (*C. capparu-coronde*, *C. citriodorum*, C. *dubium*, *C. litseaefolium*, *C. ovalifolium*, *C. rivulorum* and *C. sinharajaense* and *C. verum*) belonging to the family Lauraceae using light microscopy in the petiole anatomy for identification and classification of species. This is the first report on comprehensive petiole anatomy in *Cinnamomum* species. The distinctive, important and key taxonomic characteristics of the petiole include the shape and outline of the petiole, the presence of winged extensions, surface grooves on upper surface, presence or absence of trichomes, structure of vascular bundle and stone cell characters (shape, structure, pits and fissure size). These identified petiole characters provide the additional information for the identification of *Cinnamomum* species. Almost all the characters studied had little difference within a species but vary among the species. However, shape of epidermal cells was relatively constant for all species. All polymorphic characters were used for Principal Component Analysis (PCA) and species were divided into three main clusters. The PCA revealed that acicular crystals, cluster of crystals, trichomes and trichome abundance contribute to the first component that account for 31.76% total variance. Shape of the petiole, upper surface wings and raphides are the major loading characters to the second component. Therefore, anatomical structures of the petiole are useful in the identification and may help for crop improvement, conservation, management and future usefulness of germplasm resources of *Cinnamomum* species.

Keywords. *Cinnamomum*, Lauraceae, PCA, petiole anatomy, taxonomy

1 Introduction

Lauraceae is a large family represented by about 50 genera and approximately 2,500 to 3,000 species in the world (Nishida and van der Werff 2007). In Sri

Lanka, the family is represented by 41 species in 9 genera (Senaratna 2001) The genus *Cinnamomum* includes eight species (*C. capparu-coronde* Blume, *C. citriodorum* Thw., *C. dubium* Nees, *C. litseaefolium* Thw., *C. ovalifolium* Wight, *C. rivulorum* Kosterm, *C. sinharajaense* Kosterm and *C. verum* Presl) (Sritharan 1984). Many species of Lauraceae in the world are used for medicinal and food purposes (Khan *et al*. 2003, Faixov and Faix 2008, Smerq and Sharma 2011, Pugazhenthi and Suganthi 2013, Wang *et al*. 2013, Nwokwa *et al*. 2014). These include bay (*Laurus nobilis*) leaves for flavoring foods and medicinal purposes (Pugazhenthi and Suganthi (2013), camphor (*Cinnamomum camphora*) for moth repellant and medicinal purposes (Nwokwa *et al*. 2014), *C. verum* for food flavouring (Wang *et al*. 2013), myrtlewood (*Umbellularia californica*) and white stinkwood (*Celtis africana*) for furniture, sassafras (*Sassafras albidum*) for tea (Jackson 2005) and avocado as edible fruits (*Persea americana*). *Cinnamomum verum* is one of the most economically important spices in Sri Lanka which produces about 70% of the world bark production of cinnamon (EDB 2015). *C. capparu-coronde*, *C. citriodorum, C. dubium*, *C. ovalifolium* and *C. sinharajaense* are considered as vulnerable species while *C. litseaefolium* and *C. rivulorum* are endangered (MOE 2012). The morphology of *Cinnamomum* species found in Sri Lanka has been studied and they show high variation in gross morphology of leaves among species and also within species, which precludes diagnosis and species identification (Sritharan 1984). Flowers are rare and flowering times of the different species are dissimilar. Therefore, identification of *Cinnamomum* species is difficult using only vegetative and floral morphological characters. Anatomical features are widely used in plant taxonomy in families. For instance, for infra-generic classification of Fabaceae (Mıchael and Soladoye 1982), Rubiaceae (Martínez-Cabrera *et al*. 2009), Anacardıaceae (*Mangifera indica* L.), Cruciferae (Brassicaceae), (Gorovoy *et al*. 2011), Lamiaceae (Akçin *et al*. 2011) and Rutaceae (Vun *et al*. 2015), to delineate the taxa even at the species level for *Momordica*, Cucurbitaceae (Aguoru and Okoli 2012) and *Mangifera indica* varieties (Sharma *et al*. 2012) especially when the floral parts or fruits are not available (Ayomipo *et al*. 2015), identification and authentification of medicinal plants (Ingole and Kaikade, 2015). Moreover, the anatomical characteristics of the petiole have been used to describe the fossil genus *Heleophyton* (Matias *et al*. 2007). Although the classification of plants is mainly based on morphological and anatomical concepts, there is no comprehensive information regarding the anatomy of *Cinnamomum* species found in Sri Lanka and anatomical descriptions of *Cinnamomum* species are scarce. Therefore, the study described the petiole anatomy of eight *Cinnamomum* species using light microscopy (LM) and discussed the possibility of utilizing these features among the investigated species.

2. Materials and methods

The first fully expanded three to five new leaf samples from three individuals of 8 species of *Cinnamomum* were collected from sun-exposed branches from healthy individuals of more or less similar age from the Cinnamon Research Station, Department of Export Agriculture, Palolpitiya, Matara, Sri Lanka and preserved in FAA (Huang and Yeung, 2015). After preserving them for one week, samples were transferred to 70% alcohol and the fixed samples were rehydrated in graded series (Retamales *et al*. 2015). Hand sections of median portion of the petioles from preserved sampled were taken using a sharp blade. Three or five petiole samples per each species were observed under Nikon eclipse 50i compound microscope and images captured using the Nikon NIS-Elements imaging software (Nikon Instruments, Amsterdam, The Netherlands). Polymorphic characters were observed using the taken images and recorded (Table 1). All petiole anatomical features presented in Table 1 were coded for cluster analysis. A data matrix (Table 2) was generated for the calculation of the similarity among the species using the data present in Table 1. Cluster analysis of the species was carried out using Minitab 17 software package using the Euclidean distance treating with the complete method and unweighted pair-group method with arithmetic averages (UPGMA) as the sorting strategy. In this study, principal components analysis (PCA) was used to analyze the 18 variables to reduce the dimension of factors.

3. Results and discussion

Anatomical evidence has been exploited in delimiting taxa: Asteraceae (Mabel *et al.* 2013), *Salvia* species (Özdemir *et al.* 2016) and *Nepeta* species of Lamiaceae (Shahri *et al.* 2016) and petiole anatomy is one of the important biomarkers (Ingole and Kaikade, 2015). However, no data were available for petiole anatomy of Sri Lankan *Cinnamomum* species. Furthermore, no recent literature is available on the taxonomic revision of *Cinnamomum* species found in Sri Lanka except Abeywikrama (1973). In Australia, a revision of family Lauraceae has been conducted using only morphological characters (Hyland 1986). Our comprehensive study of petiole anatomy of the *Cinnamomum* species showed marked variations which were of systematic and taxonomic significance. Polymorphic anatomical features studied are summarized in Table 1. Character number is preceded by # and states of the same character are assigned serial numbers in the parenthesis. On the basis of studied characters, wing extension, shape and outline of the petiole in a cross section, trichomes (presence, abundance and number of cells), grooved margins in upper surface, vascular bundle (shape, structure, and arrangement), pits and pore size of the stone cells were unique to some species (Figures 1 and 2). The results will be presented in three parts, (1) Survey of the petiole anatomical and micromorphological characters, (2) Cluster analysis, and (3) an identification key of studied species using polymorphic anatomical and micromorphological characters.

3.1 Survey of the petiole anatomical and micromorphological characters

Petiole shape

The research on petiole structure of *Cinnamomum* species has allowed us to reveal species differences. Petiole shapes showed distinct differences among the examined samples. All the petioles were bifacial, each petiole has its own structral properties. The petiole has seven different shapes of the entire petiole outline (circular/sub-circular to somewhat reniform) in cross section and they can be catergorized as Type I, II, III, IV, V, VI and VII (Table 1). Each petiole has its own structral properties. Petioles with a shallow adaxial groove/s can be seen on all the petioles except *C. citriodorum* (somewhat flat) while *C. litseaefolium*, *C. rivulorum* and *C. sinharajaense* have highly concave upper surface while in *C. capparu-corindo* and *C. verum* has slight concave shape. Apart from the adaxial groove, secondary ridges accompanying shallow grooves may be observed in sections but these were not constant between specimens of the same species. Petiole shapes show distinct differences among the examined types. However, *C. citriodorum* and *C. ovalifolium* have mostly similar round shape. *C. dubium* and *C. litseaefolium* have more or less oval and nonsymmetrical shape (along the vertical axis) while *C. cappru-corindo*, *C. citriodorum C. ovalifolium*, *C. sinharajaense* and *C. verum* have symmetrical shape. *C. cappru-corindo*, *C. citriodorum* and *C. ovalifolium* have more or less flat adaxial surface. *C. capparu-corindo* shows the winged extensions of the adaxial side. *C. litseaefolium*, *C. rivulorum* and *C. sinharajaense* have highly concave upper surface and in *C. verum* has slight concave upper surface. The upper surface of *C. capparu-corindo* and *Ocotea duckei*, a member of family Lauraceae has a very much similar structure with winged extensions (Coutinho *et al*. 2006). Similar dorsiventral structure has been observed in *Cinnamomum pauciflorum* leaf anatomy (Baruah and Nath, 2006). However, the petiole anatomy of *Cinnamomum* species is scanty.

Cuticle and upper surface

The adaxial and abaxial epidermis are covered with undulate thick film of epicuticular waxes. The cuticle may be smooth or undulated (Figure 1a and 1b), cuticular striations occur frequently (*C. dubium*, *C. litseaefolium*, *C. rivulorum*, *C. sinharajaense* and *C. verum*), or both can be found in all species.

Table 1: Comparison of the polymorphic qualitative characters of petiole of the studied *Cinnamomum* species. Character number is preceded by # and states of the same character are assigned serial numbers within parenthesis.

Character Number	Type	Type I	Type II	Type III	Type IV	Type II	Type V	Type VI	Type VII
	Shape and species Character (code)	 *C. capparu-corondo*	 *C. citriodorum*	 *C. dubium*	 *C. litseaefolium*	 *C. ovalifolium*	 *C. rivulorum*	 *C. sinharajaense*	 *C. verum*
#1	Symmetry (SY)	Symmetrical (1)	Symmetrical (1)	Not symmetrical (2)	Not symmetrical (2)	Symmetrical (1)	Symmetrical (1)	Symmetrical (1)	Symmetrical (1)
#2	Shape of the entire petiole in cross section (SH)	Round (1)	Round (1)	Oval shaped (2)	Reniform (3)	Round (1)	Roundish (4)	Roundish (4)	Roundish (4)
#3	Upper surface wings (USW)	Present (1)	Absent (2)	Absent (2)	Absent (2)	Absent (2)	Absent (2)	Absent (2)	Absent (2)
#4	Upper surface groove (USG)	Slight (2)	No groove (1)	Undulate (3)	Broad grove (5)	Slight (2)	deep grove (5)	Medium (4)	Medium (4)
#5	Trichomes (T)	Absent (2)	Absent (2)	Present (1)	Absent (2)	Present (1)	Present (1)	Absent (2)	Absent (2)
#6	Trichome abundance (TA)	Absent (2)	Absent (2)	High (3)	Absent (2)	Low (1)	High (3)	Absent (2)	Absent (2)
#7	Trichome no. of cells (NT)	Absent (2)	Absent (2)	Unicellular (1)	Absent (2)	Unicellular and bicellular (3)	Unicellular and bicellular (3)	Absent (2)	Absent (2)
#8	Shape of the trichomes (ST)	Absent (2)	Absent (2)	Straight or bent (1)	Absent (2)	Straight (3)	Straight (3)	Absent (2)	Absent (2)

Table 1. Continued…

	Type	Type I	Type II	Type III	Type IV	Type II	Type V	Type VI	Type VII
#9	Vascular bundle (VB)	Partially dissected into 3-segments (3)	Simple open arc (1)	Clearly separated into three (4)	Partially dissected into 3-segments (3)	Simple open arc (1)	Simple open arc (1)	Simple open arc with slightly curved ends (2)	Simple open arc with slightly curved ends (2)
#10	No. of collateral vessels (CV)	~20-25 collateral vessels (2)	~17-20 collateral vessels (1)	~ 25-30 collateral vessels (3)	~ 25-30 collateral vessels (3)	~ 25-30 collateral vessels (3)	~20-25 collateral vessels (2)	~40-45 collateral vessels (4)	~20-25 collateral vessels (2)
#11	Sand crystals (SC)	Present (1)	Absent (2)	Present (1)	Absent (2)	Absent (2)	Present (1)	Absent (2)	Absent (2)
#12	Elongated shaped crystal (ESC)	Present (1)	Present (1)	Present (1)	Present (1)	Present (1)	Present (1)	Absent (2)	Present (1)
#13	Acicular shaped crystals (AC)	Present (1)	Absent (2)	Present (1)	Absent (2)	Present (1)	Present (1)	Present (1)	Present (1)
#14	Rectangular shaped (RS)	Present (1)	Absent (2)	Present (1)	Present (1)	Present (1)	Absent (2)	Absent (2)	Absent (2)
#15	Box-shaped (BL)	Present (1)	Absent (2)	Present (1)	Present (1)	Absent (2)	Present (1)	Present (1)	Absent (2)
#16	Shape of the stone cells - slerides (SS)	Elongated (1)	Elongated (1)	Isodiametric (2)	Isodiametric (2)	Isodiametric (2)	Isodiametric (2)	Elongated (1)	Isodiametric (2)
#17	Porosity of the stone cells (PC)	Finely porous (1)	Strongly porous (2)	Finely porous (1)	Strongly porous (2)	Small pore (3)	Small pore (3)	Finely porous (1)	Small pore (3)
#18	Pits of stone cells (PS)	Remarkable (3)	Not observable (2)	observable (1)	Not observable (2)	Remarkable (3)	Observable (1)	Observable (1)	Observable (1)

Cuticle and epidermal cells were observed to be mostly similar in all materials studied. İn *O. duckei*, thick and smooth cuticle have been observed by Coutinho *et al*. (2006). In this study, *Cinnamomum* species were collected from the fully sun exposed plants. Therefore, thick cuticle helps to prevent the water loss efficiently, as well as the leaf from wilting when the cells dehydrate (Metcalfe and Chalk, 1972). Simple, unicellular/bicellular, falcate (*C. dubium*, Figure 1c) unicellular/multicellular (*C. rivulorum* 1d) trichomes are present only on adaxial surface (Figures 1). All trichomes are non glandular and are absent in other species. As we observed similar results have been obtained for leaf epidermis and thick-walled erect hair on the abaxial surface of the leaf of *Cinnamomum* species by Bakker *et al.* (1992). Kamel and Loutfy (2001) have identified the unbranched trichomes of some members of the family Lauraceae.

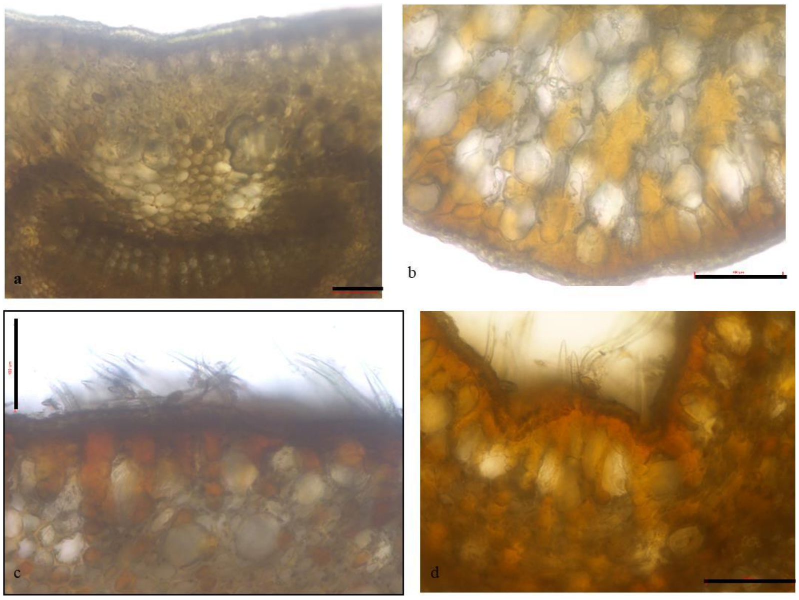

Fig 1. Cuticle structure, a) upper surface of *Cinnamomum citriodorum* b) lower surface of *C. rivulorum* c) bicellular/non glandular trichomes of *C. dubium* d) *C. rivulorum* with unicellular/multicellular trichomes. Scale bar is 100 nm.

Vascular tissues

The vascular system has received the most attention in the study of petiole anatomy (Kocsis and Borhidi 2003, Noraini *et al*. 2016, Talip *et al*. 2017, Long and Oskolski 2018). According to the pattern of vascular tissue arrangement, the petiole is classified into eight types and is shown in Figures 2a- 2h. Different shape of vascular bundle of *Cinnamomum* species have allowed us to reveal species differences. The shape of the vascular tissues in all the species studied has only an open arc with no lateral bundles. Figure 2 shows the petioles with vascular bundles based on shape and bundle arrangements in the petioles of each species. *C. cappru-corindo*, *C. sinharajaense* and *C. verum* which has an open arc with slightly curve ends towards the centre (incurving), thereby presenting a wide arc-shaped appearance. In *C. dubium*, the shape of the vascular bundle is quite characteristic; the open arc has clearly separated into three segments (multi-lobed, Figures 2c) while in *C. capparu-corindo* and *C. litseaefolium*, open arc has partially dissected into 3 segments (Figures 3a, 3d). *C. citriodorum* (Figures 3b) and *C. rivulorum* (Figures 3f) has more or less similar structure; 'C' shaped open arc and a large number of collateral vessels are arranged vertically. *C. verum* has slightly curved ends (Figures 3h).

Vascular bundle is open and consists of more than ten collateral vesseles. The number of collateral vessels differs among the species; *C. capparu-corindo* (±24), *C. citriodorum* (±17), *C. dubium* (±29), *C. litseaefolium* (±28), *C. ovalifolium* (±29), *C. rivulorum* (±22), *C. sinharajaense* (±42), and *C. Verum* (±27). Different patterns of vascular bundle of *Cinnamomum* species have allowed to reveal species differences. Kamel and Loutfy (2001) have observed the crescent shape vascular strands in some members of Lauraceae. The vascular bundle is enclosed by parenchyma cells with distinctive dark-staining secondary compounds (tannin and phenolic compounds) in all studied species but the color and the density of the deposits vary depending on the species (Figure 2). We also observed the pigmented cells in all the species in ground tissues (Figures 2a-2h) which has been observed in worldwide genera of the family Lauraceae (Kamel and Loutfy, 2001).

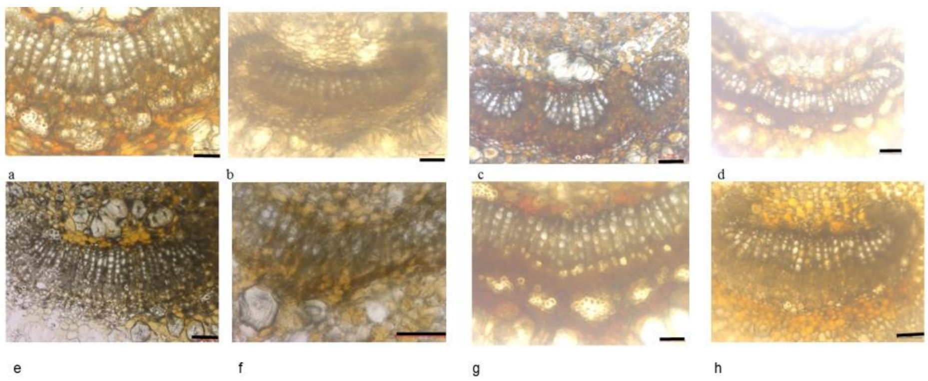

Fig. 2 Different patterns of vascular bundle a) partially dissected into 3 segments of *Cinnamomum cappru-corindo*, b) simple open arc of *C. citriodorum*, c) clearly separated into three of *C. dubium*, d) partially dissected into 3 of *C. litseaefolium*, e and f) simple open arc of *C. ovalifolium* and *C. rivulorum*, g and h) simple open arc with slightly curved ends of *C. sinharajaense* and *C. verum.*

Therefore, the research on petiole anatomical structure has allowed us to reveal species differences. A similar tissue arrangement; cortex with fundamental parenchyma cells, discontinuous layers of sclerenchymatic cells surround the vascular bundle, open arch vascular bundle, collateral type xylem tissues have been observed in *O. duckei* (Coutinho *et al*. 2006). Supporting tissues such as fibers and sclerotic cells are present in the petioles. Tissues of support in the form of a cluster of sclenchymatous cells are observed mainly around the vascular tissues (Figure 2a, b, c). The pattern of the vascular tissues in all the species studied has only an open arc with no lateral bundles (Figure 2a-h), with a discontinuous sclerenchymatous sheath with dark-colored deposits including the cell lumina (Figure 2). Different kinds of vascular patterns are present in the speceis studied (Table 1 and Figures 2a-h). The presence or absence of perivascular tissues appears to be not consistant in all species (Figure 2).

Table 2: Data matrix generated for the calculation of the similarity index among the *Cinnamomum* species.

Species	Symmetry (SY)	Shape (SH)	Upper surface wings (USW)	Upper surface groove (USG)	Trochome **(T)**	Trichome abundance (TA)	No. of cells of trichome (NT)	Shape of trichome (ST)	Vascular bundle (VB)	No. collateral vessels (CV)	Crystal sands (CS)	Elongated shaped crystals (ESC)	Acicular crystals (AC)	Rectangular shaped crystals (RS)	Box-like bundles (BL)	Shape of the stone cells –sclerides (SS)	Porosity of the stone cells (PC)	Pits of stone cells (PS)
C. cappru-corindo	1	1	1	2	2	2	2	2	3	2	1	1	1	1	1	1	1	3
C. citriodorum	1	1	2	1	2	2	2	2	1	1	2	1	2	2	2	1	2	2
C. dubium	2	2	2	3	1	3	1	1	4	3	1	1	1	1	1	2	1	1
C. litseaefolium	2	3	2	5	2	2	2	2	3	3	2	1	2	1	1	2	2	2
C. ovalifolium	1	1	2	2	1	1	3	3	1	3	2	1	1	1	2	2	3	3
C. rivulorum	1	4	2	5	1	3	3	1	1	2	1	1	1	2	1	2	3	1
C. sinharajaense	1	4	2	4	2	2	2	2	2	4	2	2	1	2	1	1	1	1
C. verum	1	4	2	4	2	2	2	2	2	2	2	1	1	2	2	2	3	1

Stone cells (sclereids)

In the cortex, all the species frequently contain different shapes and different sizes of stone cells (sclereids) which lie mainly around the vascular bundle. Although branched stone cells were absent in all studied *Cinnamomum* species, stone cells have different types depending on the wall structure (whether pitted, non-pitted or rarely pitted, thickness of the wall, size of cell cavity, presence or absence of color deposits in cell contents, etc.). In *C. capparu-coronde*, brachysclereids were greatly elongated, rectangular shaped, the walls were yellowish white and finely porous. The central cavity is narrow and is marked with branched pits (Figure 3a). In *C. citriodorum*, different sized, elongated shaped light yellowish, irregularly thickened with prominent cavity of the stone cells were observed (Figure 3b). The stone cells of *C. dubium*, different shapes and sizes of brachyssclerides were yellowish white, less porous and have branched pits. The central cavity is not prominent. In *C. litseaefolium*, different shapes of stone cells were concentrated above the vascular bundle and scattered below the middle part the vascular bundle and beneath the epidermis (Figure 2d), mostly round in form, pores were visible. The diagnostic stone cell of *C. ovalifolium* (Figure 2e), round in outline, the walls were yellowish and the central cavity has pits. In *C. rivulorum*, brachysclereids have different shapes and sizes (Figure 2f), the pits were prominent, central cavity is observable. The different shapes and sizes stone cells of *C. sinharajaense* were elongated shaped, the walls were pitted, finely porous and contain tannins (Figure 2g).

A very few stone cells of *C. verum* were scattered around the the bundle (Figure 1h), vary in shape from square to nearly round, similar to the stone cells of *C. ovalifolium*, the walls were yellow and very thick, equally thickened, the central cavity is small which is filled with masses of reddish-brown tannin. Therefore, the color of the walls of the different stone cells varies. The width of the cell cavity varies considerably in the stone cells of the studied species. In *C. citriodorum* and *C. litseaefolium* the cell cavity is greater than the thickness of the cell wall. The cavity of *C. cappru-corindo*, *C. dubium* and *C. sinharajaense* is finely porous. The pits were prominent in *C. cappru-corindo, C. dubium, C. ovalifolium* and *C. sinharajaense*. The cavity of many stone cells contains colored deposits such as tannins. The porosity and the pits (presence or absence) of stone cells were the different and diagnostic characters of the *Cinnamomum* species. Moreover, they were different in abundance; *C. capparu-coronde* ~50, *C. citriodorum* ~30, *C. dubium* ~15, *C. litseaefolium* ~10, *C. ovalifolium* ~25, *C. rivulorum* ~8, *C. sinharajaense* ~25 and *C. verum* ~ 8 (Figure 3).

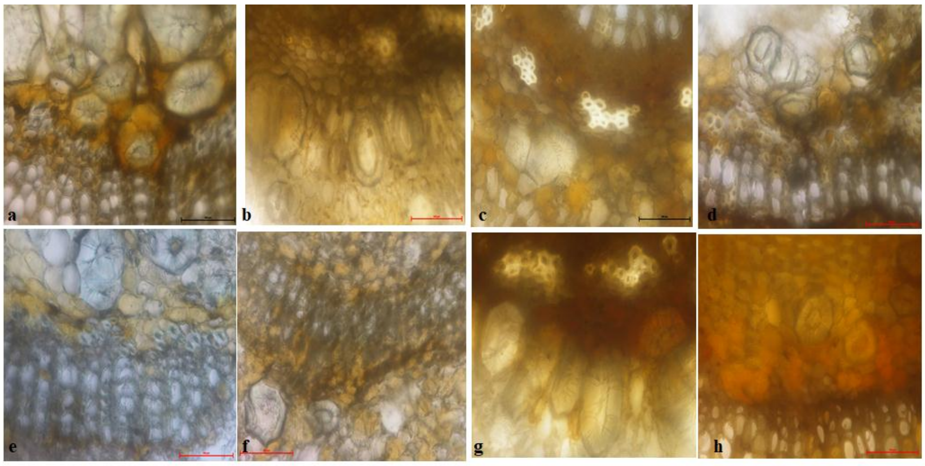

Fig. 3 Different sclereids of studied species. a) brachysclereids, or stone cells of *C. cappru-corindo*, b) macro-sclereids, elongated and columnar (rod-like) cells of *C. citriodorum*, c) brachysclereids, or stone cells of *C. dubium*, d) brachysclereids with thin cell wall of *C. litseaefolium*, e) brachysclereids of *C. ovalifolium* f) brachysclereids, or stone cells of *C. rivulorum* g) macro-sclereids, elongated and columnar (rod-like) cells of *C. sinharajaense* h) brachysclereids, or stone cells of with thin cell wall of *C. verum* (100 µm scale bar).

Crystals and secondary compounds

Crystals were present in the parenchyma and collenchyma cells in all the species. In general, we found crystals as prism, styloids (elongated shaped), sand crystal, acicular shaped crystals, cluster of crystals, isolated crystals, rectangular shaped and box-shaped of CaO_x crystals in *Cinnamomum* species. Crystals found in petioles were divided as groups (agglomeration - same shape or different shape crystals) and single crystals. Styloid crystals which were lined up neatly were generally found in groups.

Although the crystals were present, both druses and raphides were absent. The staining reaction of ruthenium red confirmed the presence of pectin in the mesophyll of all the species studied. In petiole, idioblasts are rich in tannins and polyphenols mainly in parenchyma cells contained big vacuoles, xylem and phloem cells. Those compounds were easily observed due to the red-brown color with the ferric chloride staining. The presence of crystals, stone cells and dark - stained deposits to particular *Cinnamomum* species suggests the potential taxonomic value of these features.

3.2 Cluster analysis

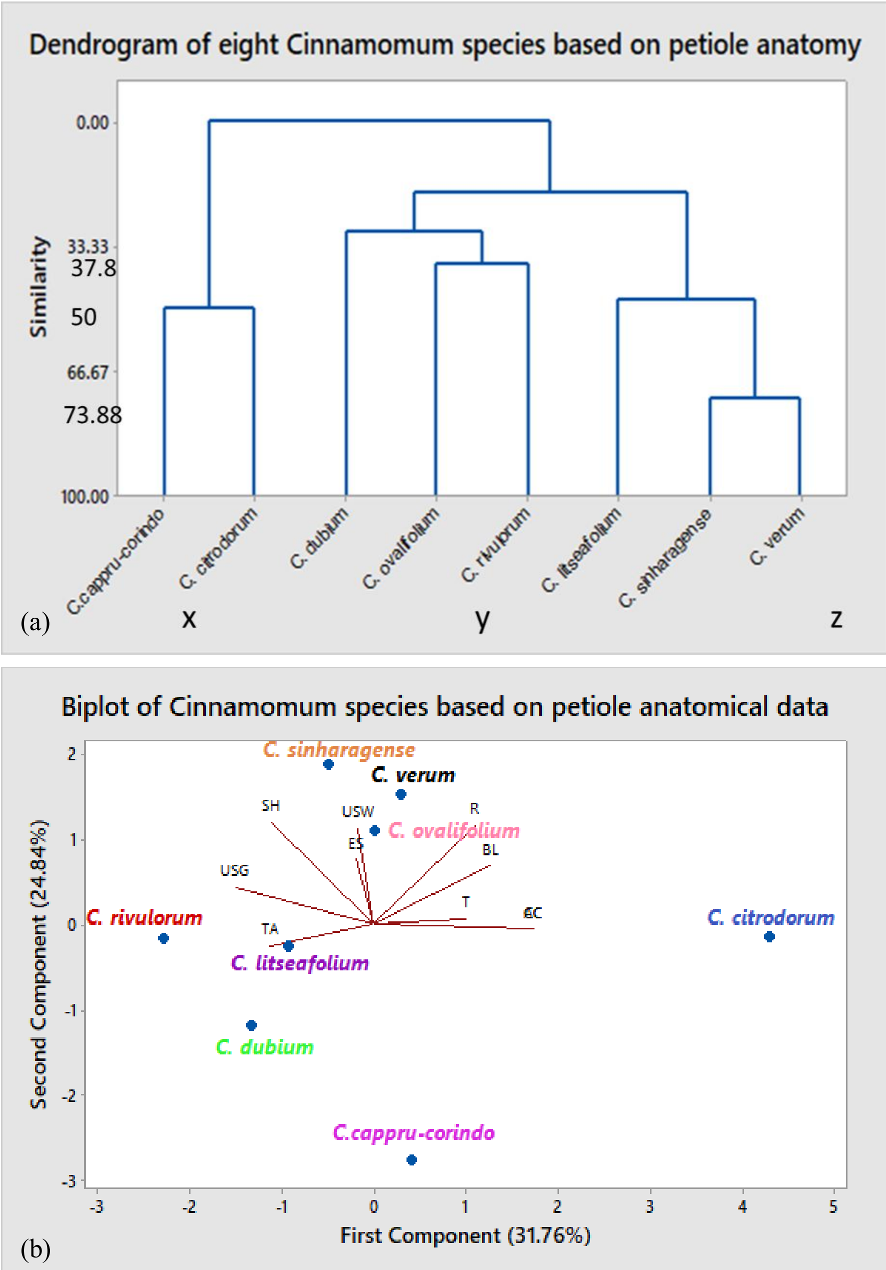

Fig. 4 a) Cluster analysis of *Cinnamomum* species based on all anatomical characters b) Biplot on the first and second eigenvectors of principal components (PC) of 10 informative characters out of all (18) anatomical characters of *Cinnamomum* species sampled from Sri Lanka.

In an attempt to study the relationship of these species, the petiole anatomical characters were analyzed. In order to get some insight in the overall similarities of the individual species of *Cinnamomum*, cluster analyses were applied. Based on the data matrix (Table 2) prepared using the polymorphic characters, cluster analysis was carried and resulted the three main clusters (Figure 4a); x) *C. capparu-corindo* and *C. citriodorum* y) *C. dubium*, *C. ovalifolium*, *C. rivulorum* and z) *C. litseaefolium* and *C. sinharajaense* and *C. verum*. Further, *C. sinharajaense* and *C. verum* have clustered very closely and clearly shows the higher similarity between two species. *C. sinharajaense* and *C. verum* (z) have the highest similarity value, 73.8 for all studied characters and *C. sinharajaense* and *C. verum* have clustered with *C. litseaefolium* at the similarity value ~50. Moreover, *C. capparu-corindo* and *C. citriodorum* have clustered (x) at the similarity value ~50 and *C. ovalifolium* and *C. rivulorum* have clustered (y) together with similarity value of ~33. *C. dubium* is clustered with *C. ovalifolium* and *C. rivulorum* cluster with similarity value at ~28. *C. capparu-corindo* and *C. citriodorum* cluster (x) is linked with other two clusters y and z with the least similarity values of 19. Figure 4b shows the score plot of *Cinnamomum* species based of petiole anatomy and the first and second Principal Components explain 31.76% and 24.84% of total variations respectively. In general, the PCA revealed a high distribution of characters but without correlation among the species, which indicates a morphological diversity of plants among the species.

PCA showed that the accumulative contribution rate of the three principal components were 58.38%. Thus the first, second and third principal components had more than 0.637. The PCA revealed that acicular crystals (AC), cluster of crystals (CC), trichomes (T), box-like bundles (BL) and trichomes abundance (TA) contribute to the first component (F1) that account for 31.76% total variance (Figure 4b) Shape of the petiole (SH), upper surface wings (USW) and raphides (R) are the major loading characters to the second component (F2) that explained 24.84% total variance and showed the high variation (Figure 4b). Some characters such as upper surface groove (USG) and elongated shaped crystals (ES) are the loading factors for third component. These variables, therefore, are predicted to affect the distribution patterns of petiole anatomy of all species. These data will contribute to enhance the petiole anatomy of *Cinnamomum* species.

3.3 Identification key

It was possible to build an indented taxonomic (diagnostic) key based on petiole anatomical characters observed of the *Cinnamomum* species for the Sri Lankan members of the genus *Cinnamomum*.

1a. Non-symmetrical along the vertical axis of the petiole (in LS) ….. go to 2

b. Symmetrical along the vertical axis of the petiole (in LS) ………. go to 3

2a. Presence of unicellular trichomes on the adaxial surface, oval shaped, vascular bundle clearly separated into three segments, stone cells were isodiametric shape, observable pits, finely porous ………………………………………………………… *C. dubium*

2b. Absence of trichomes on the adaxial surface, reniform shape of the petiole, vascular bundle partially dissected into 3 segments, stone cells were isodiametric, observable pits, strongly porous stone cells ………………………………….................................. *C. litseaefolium*

3a. Presence of trichomes …………………………………….……. go to 4
3b. Absence of trichomes …..……………………………………... go to 5

4a. Unicellular and bicellular trichomes, round shaped, simple open arc vascular bundle, stone cells were isodiametric shape, remarkable pits, small pore and slight grove on the adaxial surface …. *C. ovalifolium*

4b. Unicellular and bicellular trichomes, roundish shaped, simple open arc vascular bundle stone cells were isodiametric shape, observable pits, small pore and deep grove on the adaxial surface ...…………………………………………………….. *C. rivulorum*

5a. Wing extensions were absent, vascular bundle partially dissected into 3 segments, stone cells were elongated shape, remarkable pits finely porous ……………………………………… *C. appru-corindo*

5b. Wing extensions were present, simple open arc vascular bundle ……………………………………………………………….……. go to 6

6a. Stone cells were elongated shape, strongly porous, pits not observable ………………………………………………...… *C. citrodorum*

6b. Simple open arc with slightly curved ends ………………..…………………………………………………... go to 7

7a. Stone cells were elongated, remarkable pits, finely porous …………………………………..………….…………….. *C. sinharagense*

7b. Stone cells were isodiametric, observable pits, small pore ……………………………………………………………….. *C. verum*

4 Conclusions

The present study revealed the polymorphism of petiole anatomy of the genus *Cinnamomum*. Presence of upper surface wings, upper surface grooves, trichome characters, shape and structure of vascular bundle, stone cell characters (shape, structure, pits and pore size) were some of the key characters for identification of *Cinnamomum* species. Upper surface wings were observed only on petiole of *C. capparu-corindo*. Vascular bundle is clearly separated into three segments and partially dissected into 3 segments in *C. dubium* and *C. litseaefolium* respectively. Trichomes were absent on the adaxial surface of the *C. capparu-corindo*, *C. citrodorum*, *C. sinharagense* and *C. verum*. Moreover, these data may be helpful to fill the gap on the petiole anatomy of Laurals. The application of cluster analysis revealed that *Cinnamomum* species mainly grouped into three main clusters. The present findings could provide valuable anatomical information for future research in identifying the *Cinnamomum* species.

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