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Leaf anatomical characters variation of *Strobilanthes* s.l. from Sumatra, Indonesia and its taxonomic implications

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Abstract. *Suratman, Suranto, Muzzazinah, Purnomo. 2022. Leaf anatomical characters variation of* Strobilanthes *s.l. from Sumatra, Indonesia and its taxonomic implications. Biodiversitas 23: 3705-3720.* The objective of this study was to evaluate leaf anatomical character variation among species of *Strobilanthes* s.l. from Sumatra. A total of 35 species from four genera previously recognized within the subtribe *Strobilanthineae* (*Strobilanthes, Hemigraphis, Sericocalyx, Semnostachya*) were used for leaf paradermal section observations. A total of seventeen leaf anatomical characters consisting of ten quantitative and seven qualitative characters were selected for leaf anatomical comparison. The quantitative and qualitative character analysis showed great variability in the majority of tested leaf anatomical characters. This result indicates the large diversity of leaf anatomical characters occurs among species of *Strobilanthes* s.l. A similarity dendrogram among species of *Strobilanthes* s.l. based leaf anatomical character data revealed two major clusters. The first cluster consists of eleven species with regular epidermal cell shape and straight cell wall with a similarity coefficient of 0.67. The second cluster consists of twenty four species that differed from species in the first cluster due to their irregular epidermal cell shape and undulate anticlinal cell wall with a similarity coefficient of 0.59. This study indicates that leaf anatomical characters are valuable taxonomic features in taxa delimitation at the species level. The results of this study also support earlier works through molecular studies that all members of the *Strobilanthinae* then accepted as *Strobilanthes* s.l. group which could not be easily split into smaller component genera.

Keywords: Leaf anatomical characters, *Strobilanthes* s.l., Sumatra, variation

INTRODUCTION

Strobilanthes Blume s.l. (sensu lato) is one of a large and variable genus of the family Acanthaceae. *Strobilanthes* s.l. includes the genus *Strobilanthes* and its closest allied genera in the subtribe *Strobilanthineae* (sensu Bremekamp 1944) such as *Hemigraphis*, *Aechmanthera*, *Stenosiphonium*, *Sericocalyx*, etc. The number of species in *Strobilanthes* s.l. is estimated to be more than 400 species (Mabberley 2008). *Strobilanthes* s.l. is native and distributed in the tropical and subtropical regions such as southern Asia, eastern Asia, southeastern Asia, Australia, and Pacific. *Strobilanthes* s.l. is also introduced into Western Africa and Middle America (POWO 2022).

Some species of *Strobilanthes* s.l. have economic uses such as ornamental plants, natural dye producers and medicinal plants. For medicinal purposes, they have been used in the treatment of wound healing (Sreekumar et al. 2021), ulcers, anemia, gall stones, diabetes (Skaar et al. 2014), kidney stones, arthritis, rheumatism, cerebrospinal meningitis, encephalitis B (Preethi and Suseem 2014), dermatoses, hemorrhage (Yu et al. 2021), infections by respiratory viruses, such as influenza viruses, mumps virus, severe acute respiratory syndrome (Gu et al. 2015; Zhou et al. 2017), human coronavirus (Tsai et al. 2020), against helminthiasis and spider bite poison (Baby and Raphael 2018). As natural indigo dyes producer, their extracted leaves are commonly used to dye clothes (Liu et al. 2014; Fan et al. 2018; Li et al. 2019).

The taxonomic status especially generic circumscription and species delimitations based on morphological characters in *Strobilanthes* s.l. however remain problematic (Moylan et al. 2004a). Based on Carine and Scotland (2002), the complex pattern of morphological variation in this group has contributed to problems and resulted in three radically proposed different classifications of the group i.e. Anderson (1867), Bremekamp (1944) and Terao (1983). Anderson (1867) recognized four genera in this group i.e. *Strobilanthes*, *Aechmanthera*, *Stenosiphonium* and *Hemigraphis* based on their ovule number as diagnostic characters (Moylan 2004a). *Strobilanthes* can be distinguished from the latter three genera in having an ovary with 2 or 4 ovule while the other genera possess the multiovulate (four to many ovules) ovary (Moylan et al. 2004a; Yunfei 2019). Bremekamp (1944) divided the subtribe *Strobilanthinae* into 54 genera (including *Strobilanthes* and its genera allies) and arranged them into 27 informal groups based on seed coat and pollen morphology (Carine and Scotland 2002; Bennet and Scotland 2003; Yunfei 2019). Terao (1983) then proposed a broadly circumscribed *Strobilanthes* comprising all species of the subtribe *Strobilanthinae* (sensu Bremekamp

1944) and all multiovulate genera recognized by Anderson (1867) based on pollen and gross morphology (Yunfei 2019). Finally, the classification of *Strobilanthes* s.l. based morphological characters clearly still remains confused so additional characters are then required to support the taxa delimitation in this group.

The use of anatomical features in the identification and delimitation of plant groups is widely accepted (Aworinde et al. 2013; Awomukwu et al. 2015; Syaheera et al. 2015; Nwachukwu et al. 2016; Budel et al. 2018; Migacz et al. 2018; Nikmah et al. 2020; Paul and Chowdhury 2021; Zakaria et al. 2022). The leaf anatomy characters are considered the second most important characters after flowers and fruits in taxonomic studies (Chauhan and Daniel 2011; Muzzazinah et al. 2021). They will be important to solving taxonomic problems such as identification, delimitation, classification, and arranging interrelationships among the plant groups (Wang et al. 2015; Khan et al. 2017; Wetzel et al. 2017; Cheryatova 2020; Nikmah et al. 2020). The leaf anatomical characters such as foliar epidermis especially epidermal cells, stomata and trichomes are useful in the identification and delimitation of many plant groups (AbdulRahaman et al. 2014; Chaudhari et al. 2014; Uka et al. 2015; Haron et al. 2015; Khan et al. 2018; Zhang et al. 2018; Steyn and Van Wyk 2021).

Leaf anatomical characters of *Strobilanthes* s.l. have been explored globally (Patil and Patil 2014; Fernandes and Krishnan 2019; Khonkayan et al. 2019). However, investigation in detail about leaf anatomical characters variation of *Strobilanthes* s.l. from Indonesia, especially Sumatra, their contribution to taxonomic problem solving has never been published before. For this reason, this study aims to evaluate the leaf anatomical character variation of *Strobilanthes* s.l. from Sumatra (Indonesia). This is the first study reported for this region and hopefully this works useful to support the previous classification of *Strobilanthes* s.l. based on morphological characters.

MATERIALS AND METHODS

Plant Material

This study was based on observations of voucher specimens of *Strobilanthes* s.l. collected from a wide range of collection sites from Sumatra and kept in Herbarium Bogoriense (BO) (Table 1, Figure 1). A total of 35 species from four genera previously recognized within the subtribe *Strobilanthineae* (*Strobilanthes*, *Hemigraphis*, *Sericocalyx*, *Semnostachya*) were used for leaf anatomical characters observation. Information about habitat (including altitude) for each species was noted on the specimens label.

Procedures

Leaf anatomical characters were observed from paradermal sections. The preparation of microscope slides of the leaf paradermal section was carried out according to Dilcher (1974) with some modifications. Place the dried leaves from the specimens studied (1 cm square size from the median area of the leaf) in a petri dish or beaker glass. Add commercial bleach (sodium hypochlorite ($NaHClO_2$) 5.25 %) for 3-6 hours (depending the specimens, thin leaves may require less time) and remove when the leaves become cream or white colored. The bleaching leaves were then dried in an oven for 2-3 hours until the leaves became dry. Place the dried leaves in aquades and then stain with solution of 1% safranin for 5 minutes, afterward wash the stain out in aquades until the desired level of staining is obtained. The leaf sample was placed on a glass slide and then mounted in glycerin jelly. The transparent nail polish was also deposited along the margins of glass slides with cover glass to make it semi-permanent (Raza et al. 2020). For observations, light microscopic (Model: Nikon Eclipse E100, magnification of 10 x for ocular and a 40 x for objective) were used. Random observations were conducted at three different microscopic fields on both the abaxial and adaxial epidermis leaf surfaces (Nikmah et al. 2020). There were ten measured quantitative leaf anatomical characters included cystolith density adaxial, cystolith length adaxial, cystolith width adaxial, cystolith length:width ratio adaxial, glandular trichomes density adaxial, cystolith density abaxial, cystolith length abaxial, cystolith width abaxial, cystolith length:width ratio abaxial, and glandular trichome density abaxial. All measurements of these characters were averaged and averages for each species were then used in further analyses (Suratman et al. 2016; Pitoyo et al. 2018). The observation of seven qualitative leaf anatomical characters included shape and anticlinal cell wall of the epidermal cell, stomata position, stomata type, non-glandular trichomes type, cystolith type and presence of multicellular foot cell. The terminology used for these characters follows Dilcher (1974).

Data analysis

Analysis of variance was performed by Duncan's multiple range test ($p < 0.05$) using SPSS 20.0 version software for measured quantitative leaf anatomical characters data in order to test the significance of variation among species (Suratman et al. 2016; Pitoyo et al. 2018). The quantitative and qualitative leaf anatomical characters then subjected to principal component analysis in order to evaluate the importance of the tested characters for the discrimination of the taxa. A software of PAST 4.03 version was used to perform principal component analysis (Muzzazinah et al. 2021). A cluster analysis within a computer programme, NTSYS ver. 2.00, by applying SAHN (Sequential Agglomerative Hierarchical and Nested) approach was conducted with an Unweighted Pair Group Method with Arithmetic Averages (UPGMA) procedure in order to group the species based on leaf anatomical characters similarity (Suratman et al. 2016; Pitoyo et al. 2018).

Table 1. Voucher specimens of *Strobilanthes* s.l. species studied from Sumatra, Indonesia

Species	Voucher specimens	Collection sites	Altitude (m a.s.l.)
Strobilanthes alboviridis J. B. Imlay	Lorzing 14491	Karo, North Sumatra	600
Strobilanthes atropurpurea Nees	Steenis 9349	Gayo, Aceh	1.600
Strobilanthes axilliflora Clarke ex Moore	de Wilde 12219	Gunung Leuser, Aceh	200-400
Strobilanthes backeri (Bremek.) J. R. Benn.	Poulsen et al. 11	Gunung Singgalang, West Sumatra	1.800
Strobilanthes barisanensis (Bremek.) J. R. I. Wood	Tokuoka et al T-0694	Gunung Sinabung, North Sumatra	1.676
Strobilanthes bibracteata Blume	Afriastini 2390	South Tapanuli, North Sumatra	1.300
Strobilanthes bunnemeyeri J. R. I. Wood	Bunnemeijer 5546	Gunung Talang, West Sumatra	1.700
Strobilanthes capillipes C. B. Clarke ex Ridl.	Jacobson 113	Rimbo Pengadang, Bengkulu	1.000
Strobilanthes cernua Blume	Yates 1295	East Coast, Riau	-*
Strobilanthes cruciata (Bremek.) Terao	Lorzing 14110	Gunung Sibayak, North Sumatra	1.100
Strobilanthes cusia (Nees) Kuntze	Djarwaningsih TD 2573	North Tapanuli, North Sumatra	-*
Strobilanthes echinata Nees	Steenis 3594	Gunung Raja, South Sumatra	1.400
Strobilanthes hamiltoniana (Steud.) Bosser & Heine	Wyssling 104	Gunung Kerinci, Jambi	1.300
Strobilanthes hossei C. B. Clarke	Steenis 6030	Takengon, Aceh	1.290
Strobilanthes inflata T. Anderson	Steenis 8363	Gayo, Aceh	2.500
Strobilanthes involucrata Blume	Lorzing 15645	Toba, North Sumatra	1.550
Strobilanthes multiflora Ridl.	Lorzing 5201	Sibolangit, North Sumatra	1.000
Strobilanthes ovatifolia (Bremek.) J. R I. Wood	Bunnemeijer 4156	Gunung Malintang, West Sumatra	1.150
Strobilanthes palawanensis Elmer	Afriastini 773	Bukit Pelalawan, Riau	1.450
Strobilanthes parabolica Nees	Bunnemeijer 9159	Gunung Kerinci, Jambi	2.000
Strobilanthes pateriformis Lindau	de Voogd 10	Danau Ranau, South Sumatra	-*
Strobilanthes pedunculosa Miq.	Rappard K-19	Gunung Kaba, Bengkulu	900
Strobilanthes polybotrya Miq.	Bunnemeijer 371	Gunung Talamau, West Sumatra	600
Strobilanthes pubescens (Bremek.) J. R. I. Wood	de Voogd 1437	Bukit Daun, Bengkulu	1.600
Strobilanthes ramosissima J. R. I. Wood	Bunnemeijer 9447	Gunung Kerinci, Jambi	2.020
Strobilanthes rufopauper C.B.Clarke	Lorzing15500	Habinsaran, North Sumatra	1.200
Strobilanthes speciosa Blume	Steenis 9799	Gunung Kemiri, Aceh	1.100
Strobilanthes sumatrana Miq.	Bunnemeijer 8043	Gunung Kerinci, Jambi	1.100
Strobilanthes tonkinensis Lindau	Nagamasu 3779	Gunung Sago, West Sumatra	1.200
Strobilanthes violascens Ridl.	Bunnemeijer 408	Padang, West Sumatra	400
Sericocalyx crispa (L.) Bremek.	Lorzing 11833	Sibolangit, North Sumatra	500
Hemigraphis alternata (Burm. f.) T. Anderson	Lorzing 14014	Medan, North Sumatra	20
Hemigraphis blumeana (Nees) Boerl.	Teysmann 4484	Lampung	-*
Hemigraphis serpens (Nees) Boerl.	Steenis 3357	Palembang, South Sumatra	500
Semnostachya benculensis Bremek.	de Voogd 1329	Gunung Kaba, Bengkulu	1000

Note: * : No information about altitude from specimens label

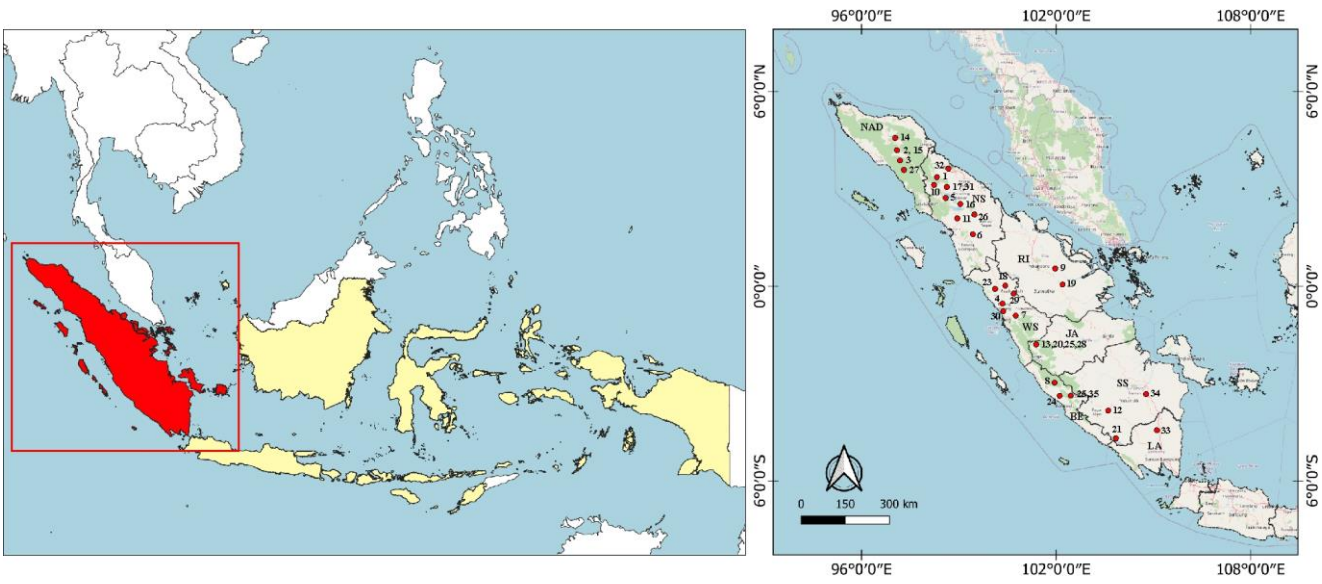

Figure 1. Map of the collection areas for *Strobilanthes* s.l. species studied in Sumatra based on label information of voucher specimens. The number (1 to 35) indicated the location of each collected species. Note: NAD: Nangroe Aceh Darussalam; NS: North Sumatra; WS: West Sumatra; RI: Riau; JA: Jambi; BE: Bengkulu; SS: South Sumatra; LA: Lampung

RESULTS AND DISCUSSION

Results

A total of seventeen leaf anatomical characters consisting of ten quantitative and seven qualitative characters were selected for leaf anatomical comparison among species of *Strobilanthes* s.l. The quantitative leaf anatomical characters of *Strobilanthes* s.l. species studied in Sumatra are summarized in Table 2 and Table 3. The analysis of variance showed that there was significant to highly significant difference among species of *Strobilanthes* s.l. for all tested quantitative leaf anatomical characters. Cystolith density adaxial varied highly significant and showed wide variation and ranging from 8.49/mm^2 (*S. rufopauper*) to 171.55/mm^2 (*S. parabolica*) with an average 37.17/mm^2. Cystolith density abaxial values also exhibited highly significant variation and displayed a range from 3.4/mm^2 (*S. barisanensis*) to 69.64/mm^2 (*S. ovatifolia*) with an average 33.43/mm^2. Glandular trichomes density abaxial displayed highly significant variation and ranged from 10.19/mm^2 (*S. atropurpurea*) to 71.34/mm^2 (*S. palawanensis*) with an average 27.12/mm^2. Glandular trichomes density adaxial although exhibited narrower differences among species but they varied high significantly with a range from 0 (*S. hamiltoniana, S. violascens*) to 32.27/mm^2 (*S. palawanensis*) with an average 10.09 /mm^2. Cystolith length adaxial varied highly significant and showed wide variation and ranging from 71.90 µm (*S. palawanensis*) to 325.11 µm (*S. alternata*) with an average 137.28 µm. Cystolith width adaxial values also exhibited highly significant variation with an average 21.51 µm and displayed a range from 12.19 µm (*S. backeri*) to 46.3 µm (*S. hamiltoniana*). Cystolith length : width ratio adaxial only revealed significant variation with an average 6.78 and showed a range from 4.11 (*S. alboviridis*) to 9.25 (*S. cruciata*). Cystolith length abaxial, cystolith width abaxial, cystolith length:width ratio abaxial values also showed highly significant variability among all tested species. *Strobilanthes echinata* displayed the lowest value of cystolith length abaxial (76.91 µm) whereas the highest value of cystolith length abaxial (205.51 µm) can be found in *S. bunnemeyeri* with an average 125.47 µm. The lowest cystolith width abaxial value was distributed in *S. cusia* (11.79 µm) whereas the highest one can be found in *S. violascens* (26.56 µm) with an average 17.45 µm. Cystolith length:width ratio abaxial values showed an average 7.53 and displayed a range from 4.54 (*S. echinata*) to 13.46 (*S. bunnemeyeri*).

The cystolith density adaxial had the highest coefficient of variance (84.96%) then followed by glandular trichomes density adaxial (76.55%), cystolith density abaxial (48.62%), glandular trichomes density adaxial (45.92 %), cystolith width adaxial (36.98 %), cystolith length adaxial (32.69%), cystolith length:width ratio abaxial (25.61%), cystolith length abaxial (22.85%), cystolith width abaxial (21.49%), and cystolith length:width ratio adaxial (20.38%).

Qualitative leaf anatomical character variation among species of *Strobilanthes* s.l. are summarized in Table 4. Leaf anatomical characters observation showed that majority species of *Strobilanthes* s.l. had irregular epidermal cells with slightly wavy anticlinal cell wall (*S. axilliflora, S. backeri, S. barisanensis, S. bibracteata, S. bunnemeyeri, S. capillipes, S. cernua, S. cruciata, S. hamiltoniana, S. hossei, S. involucrata, S. ovatifolia, S. polybotrya, Sm. benculensis*), sinusoid (*S. palawanensis, S. parabolica, S. pateriformis, S. violascens, Se. crispa, H. blumeana*) and deeply sinusoid anticlinal cell wall (*S. echinata, S. pedunculosa, S. speciosa, H.alternata*). A few species had straight cell wall with tetragonal epidermal cell (*S. inflata*), pentagonal (*S. alboviridis, S. cusia, S. multiflora, S. ramosissima, S. sumatrana*) and hexagonal (*S. atropurpurea, S. pubescens, S. rufopauper, S. tonkinensis, H. serpens*) (see Figure 2.).

Table 2. Estimates of the variability of leaf anatomical characters among species of *Strobilanthes* s.l. from Sumatra, Indonesia

Leaf anatomical characters	Mean	Min	Max	Standard deviation	Coefficient of variance (%)	P*(F-test)
Cystolith density adaxial (unit/mm^2)	37.17	8.49	171.55	31.58	84.96	0.00**
Glandular trichomes density adaxial (unit/mm^2)	10.09	0	32.27	7.72	76.55	0.00**
Cystolith length adaxial (µm)	137.28	71.9	325.11	45.05	32.69	0.00**
Cystolith width adaxial (µm)	21.51	12.19	46.3	7.95	36.98	0.00**
Cystolith length:width ratio adaxial	6.78	4.11	9.25	1.38	20.38	0.015*
Cystolith density abaxial (unit/mm^2)	33.43	3.4	69.64	16.25	48.62	0.00**
Glandular trichomes density abaxial (unit/mm^2)	27.12	10.19	71.34	12.45	45.92	0.00**
Cystolith length abaxial (µm)	125.47	76.91	205.51	28.67	22.85	0.00**
Cystolith width abaxial (µm)	17.45	11.79	26.56	3.75	21.49	0.00**
Cystolith length:width ratio abaxial	7.53	4.54	13.46	1.93	25.61	0.00**

Note: **P < 0.01= highly significant; * 0.05 <P < 0.01 = significant

Table 3. Quantitative leaf anatomical character variation among species of *Strobilanthes* s.l. from Sumatra, Indonesia

Species	ADCyD	ADGtD	ADCyL	ADCyW	ADCyR	ABCyD	ABGtD	ABCyL	ABCyW	ABCyR
S. alboviridis	32.27±2.94bcdefghijk	16.99±7.78fgh	101.26±8.33abcde	25.40±5.81bc	4.11±0.86a	50.96±8.82ijkl	49.26±12.82i	162.59±22.67defg	21.52±2.06defgh	7.53±0.34abcde
S. atropurpurea	30.57±13.48abcdefghijk	3.40±5.88abc	118.32±32.53abcdefghi	20.23±5.95abc	6.00±1.64abcdefg	45.86±8.82fghijkl	10.19±5.09a	114.71±25.93abcdef	16.82±5.64abcdefg	7.10±1.85abcde
S. axilliflora	35.67±13.48defghijk	16.99±7.78fgh	108.53±9.96abcdefg	16.07±0.26abc	6.75±0.65abcdefg	18.68±7.78abcde	39.07±15.56ghi	117.04±10.47abcdef	19.27±3.08abcdefgh	6.20±1.28abcde
S. backeri	44.16±12.82hijk	23.78±2.94h	98.60±2.14abcd	12.19±1.01a	8.13±0.81bcdefg	37.37±14.7defghij	28.87±2.94cdefgh	109.61±16.11abcde	18.35±1.51abcdefgh	5.99±0.88abcd
S. barisanensis	10.19±0abc	11.89±7.78cdefg	170.65±46.19ghij	41.38±4.78d	4.25±1.68a	3.40±2.94a	15.29±5.09abcd	142.45±0.10bcdef	20.53±3.09cdefgh	7.06±1.17abcde
S. bibracteata	39.07±7.78fghijk	16.99±7.78fgh	128.69±12.01abcdefghi	18.28±2.42abc	7.07±0.32abcdefg	27.18±2.94abcdefghi	20.38±5.09abcdef	118.10±6.88abcdef	16.08±2.47abcdefg	7.42±0.80abcde
S. bunnemeyeri	37.37±21.21efghijk	6.79±2.94abcde	133.33±17.22abcdefghi	21.36±6.81abc	6.57±1.54abcdefg	56.05±13.48jkl	44.16±5.88hi	205.51±32.84g	15.34±2.40abcdefg	13.46±1.85h
S. capillipes	49.26±10.6jk	5.10±0abcd	145.88±42.30bcdefghi	22.29±3.74abc	6.55±1.42abcdefg	45.86±18.37fghijkl	23.78±12.82abcdefg	165.35±58.56efg	22.46±2.36fgh	7.24±1.89abcde
S. cernua	25.48±5.09abcdefghi	1.70±2.94ab	105.93±16.63abcdefg	15.68±0.90abc	6.81±1.44abcdefg	22.08±22.97abcdefg	27.18±2.94bcdefg	82.28±20.94ab	13.58±0.66abcde	6.04±1.40abcd
S. cruciata	13.59±2.94abcde	11.89±2.94cdefg	110.51±21.42abcdefgh	12.36±1.87a	9.25±3.18g	32.27±16.37cdefghij	28.87±2.94cdefgh	129.93±27.28abcdef	13.50±3.67abcde	9.91±2.06efg
S. cusia	45.86±5.09ijk	6.79±2.94abcde	120.58±38.99abcdefghi	23.25±2.64abc	5.12±1.22abcde	33.97±7.78defghij	39.07±7.78ghi	97.23±16.08abc	11.79±3.28a	8.67±2.82bcdefg
S. echinata	42.46±5.88ghijk	20.38±5.09gh	91.08±19.35abc	14.47±1.89abc	6.25±0.51abcdefg	42.46±2.94efghijk	30.57±8.82defgh	76.91±11.09a	17.41±2.38abcdefg	4.54±1.34a
S. hamiltoniana	30.57±5.09abcdefghijk	0.00±0a	209.51±53.61j	46.30±3.23d	4.49±0.90ab	5.10±5.09ab	13.59±5.88abc	113.32±26.13abcdef	11.98±0.44ab	9.42±1.79cdefg
S. hossei	20.38±5.09abcdefgh	13.59±2.94defg	155.29±46.53cdefghij	26.99±9.82c	5.89±1.32abcdefg	35.67±8.82defghij	30.57±5.09defgh	170.78±3.24fg	19.82±2.49abcdefgh	8.69±0.92bcdefg
S. inflata	25.48±5.09abcdefghi	23.78±2.94h	116.00±16.15abcdefghi	15.75±2.60abc	7.47±1.38abcdefg	25.48±5.09abcdefghi	33.97±5.88fghi	88.55±19.95abc	12.43±3.26abc	7.40±2.30abcde
S. involucrata	20.38±5.09abcdefgh	6.79±2.94abcde	158.26±76.81defghij	24.75±11.83abc	6.44±0.66abcdefg	6.79±2.94abc	20.38±0abcdef	94.34±28.77abc	13.53±4.55abcde	7.09±1.08abcde
S. multiflora	91.72±15.28m	1.70±2.94ab	149.61±23.26cdefghij	20.64±4.85abc	7.51±1.98abcdefg	42.46±37.55efghijk	37.37±10.6ghi	148.59±20.72cdef	13.16±2.11abcd	11.39±1.49fgh
S. ovatifolia	103.61±16..37m	5.10±0abcd	111.39±10.14abcdefgh	16.17±3.12abc	6.99±0.81abcdefg	69.64±15.56l	28.87±2.94cdefgh	101.23±6.68abc	16.00±2.83abcdefg	6.45±1.17abcde
S. palawanensis	71.34±20.38l	32.27±7.78i	71.90±3.69a	15.58±1.75abc	4.64±0.39abc	22.08±15.56abcdefg	71.34±18.37j	148.87±19.53cdef	15.29±1.22abcdefg	9.74±1.16defg
S. parabolica	171.55±41.5n	5.10±0abcd	83.16±8.29ab	13.35±1.81ab	6.26±0.33abcdefg	50.96±10.19ijkl	32.27±16.37efgh	102.53±5.04abcd	13.76±2.24abcde	7.54±0.82abcde
S. pateriformis	18.68±7.78abcdefg	8.49±2.94abcdef	154.48±17.53cdefghij	20.43±9.89abc	8.48±2.99defg	64.54±22.97kl	13.59±10.6abc	89.80±5.02abc	16.36±2.58abcdefg	5.56±0.72ab
S. pedunculosa	35.67±5.09defghijk	8.49±2.94abcdef	144.54±7.54bcdefghi	23.71±3.75abc	6.16±0.66abcdefg	47.56±16.37ghijkl	27.18±2.94bcdefg	146.32±14.91cdef	18.36±3.94abcdefgh	8.32±2.50abcdefg
S. polybotrya	11.89±2.94abcd	6.79±2.94abcde	159.85±0.26defghij	17.99±2.03abc	8.96±0.97fg	23.78±2.94abcdefgh	15.29±5.09abcd	122.31±16.57abcdef	13.47±8.99abcde	11.49±5.82gh
S. pubescens	44.16±15.56hijk	1.70±2.94ab	152.32±28.60cdefghij	18.63±4.11abc	8.24±0.95cdefg	50.96±5.09ijkl	16.99±2.94abcde	134.07±49.07abcdef	15.95±5.20abcdefg	8.57±2.23bcdefg
S. ramosissima	50.96±18.37k	6.79±2.94abcde	174.54±71.53hij	21.23±11.68abc	9.01±2.47fg	30.57±5.09bcdefghij	15.29±5.09abcd	143.27±64.76cdef	22.31±4.90fgh	6.22±1.73abcde
S. rufopauper	8.49±7.78ab	8.49±5.88abcdef	90.73±19.16abc	12.96±1.86ab	7.18±2.21abcdefg	20.38±5.09abcdef	18.68±2.94abcdef	112.33±7.74abcdef	18.30±6.50abcdefgh	6.76±2.64abcde
S. speciosa	18.68±2.94abcdefg	1.70±2.94ab	180.36±9.19ij	22.75±8.79abc	8.54±2.38efg	30.57±10.19bcdefghij	11.89±5.88ab	137.77±64.46bcdef	17.49±4.25abcdefg	7.76±2.38abcde
S. sumatrana	27.18±2.94abcdefghij	1.70±2.94ab	165.98±20.96efghij	22.58±1.12abc	7.39±1.31abcdefg	28.87±5.88abcdefghi	28.87±7.78cdefgh	119.63±19.18abcdef	20.78±5.68cdefgh	5.87±0.62abc
S. tonkinensis	15.29±5.09abcdef	16.99±7.78fgh	151.63±10.76cdefghij	24.26±3.19abc	6.35±1.21abcdefg	22.08±2.94abcdefg	37.37±11.76ghi	110.15±15.43abcde	20.70±2.51cdefgh	5.41±1.19ab
S. violascens	23.78±12.82abcdefghi	0.00±0a	111.56±3.23abcdefgh	20.52±1.68abc	5.47±0.54abcdef	49.26±32.75hijkl	23.78±7.78abcdefg	137.73±68.75bcdef	26.56±7.48h	4.97±1.17ab
Se. crispa	18.68±5.88abcdefg	13.59±2.94defg	128.39±45.36abcdefghi	16.51±4.2abc	8.33±4.58defg	5.10±5.09ab	13.59±5.58abc	97.71±15.03abc	15.11±2.06abcdef	6.48±0.70abcde
H.alternata	33.97±10.6cdefghijk	16.99±7.78fgh	325.11±36.93k	43.53±3.72d	7.55±1.42abcdefg	25.48±5.09abcdefghi	33.97±7.78fghi	123.28±28.81abcdef	18.75±3.03abcdefgh	6.86±2.62abcde
H. blumeana	16.99±2.94abcdef	10.19±5.09bcdef	104.15±25.71abcdef	20.81±5.34abc	5.01±0.14abcde	33.97±12.82defghij	23.78±10.6abcdefg	116.53±20.74abcdef	20.29±4.65bcdefgh	5.84±1.00abc
H. serpens	10.19±0abc	15.29±5.09efgh	160.35±56.12defghij	22.56±13.00abc	7.67±1.51abcdefg	27.18±7.78abcdefghi	27.18±2.94bcdefg	139.12±30.62bcdef	17.57±2.79abcdefg	7.90±0.90abcdefg
Sm. benculensis	25.48±17.65abcdefghi	5.10±5.09abcd	131.52±22.02abcdefghi	21.90±6.17abc	6.51±2.87abcdefg	35.67±10.19defghij	16.99±2.94abcde	171.73±35.88fg	26.08±5.56h	6.74±1.71abcde
Average	37.17	10.09	137.28	21.51	6.78	33.43	27.12	125.47	17.45	7.53

Note: * ADCyD: cystolith density adaxial (unit/mm^2); ADGtD: glandular trichomes density adaxial (unit/mm^2); ADCyL: cystolith length adaxial (µm); ADCyW: cystolith width adaxial (µm); ADCyR: cystolith length:width ratio adaxial; ABCyD: cystolith density abaxial (unit/mm^2); ABGtD: glandular trichomes density abaxial (unit/mm^2); ABCyL: cystolith length abaxial (µm); ABCyW: cystolith width abaxial (µm); ABCyR: cystolith length:width ratio abaxial. ** Values are means ± standard deviation. Values followed by the different letters in the same column indicate significant differences at Duncan Multiple Range Test ($p < 0.05$

Based on the position of the stomata, most of the studied taxa have stomata located on the abaxial leaf surface (hypostomatic) as found in *S. alboviridis, S. atropurpurea, S. axilliflora, S. backeri, S. barisanensis, S. bibracteata, H. blumeana, S. bunnemeyeri, S. cernua, Se. crispa, S. cruciata, S. cusia, S. echinata, S. hamiltoniana, S. hossei, S. inflata, S. involucrata, S. multiflora, S. ovatifolia, S. palawanensis, S. parabolica, S. pateriformis, Sm. benculensis, S. polybotrya, S. pubescens, S. ramosissima, S. rufopauper, H. serpens, S. speciosa, S. sumatrana, S. tonkinensis,* and *S. violascens.* A few species of *Strobilanthes* s.l. have stomata located on both abaxial and adaxial leaf surface (amphistomatic), such as in *H. alternata, S. capillipes*, and *S. pedunculosa*. Based on the type of stomata, there are two types, namely diacytic (a guard cell surrounded by two equal size neighboring cells) and anisocytic (a guard cell surrounded by three unequal size neighboring cells) (see Figure 3.). Majority species of *Strobilanthes* s.l. from Sumatra have a diacytic stomata type only such as in *S. atropurpurea, S. axilliflora, S. backeri, S. barisanensis, S. bibracteata, H. blumeana, S. bunnemeyeri, S. capillipes, S. cernua, S. cruciata, S. echinata, S. hamiltoniana, S. hossei, S. involucrata, S. ovatifolia, S. palawanensis, S. parabolica, S. pateriformis, S. pedunculosa, Sm. benculensis, S. pubescens, S. ramosissima, S. rufopauper, H. serpens, S. speciosa, S. sumatrana, S. tonkinensis,* and *S. violascens.* It was also observed that some species such as *S. alboviridis, H. alternata, Se. crispa, S. cusia, S. inflata, S. multiflora* and *S. polybotrya* bear more than one stomata type (diacytic and anisocytic) on the same leaf surfaces together.

There are two types of trichomes i.e. glandular and non-glandular trichomes. All species of *Strobilanthes* s.l. have glandular trichomes on both adaxial and abaxial leaf surfaces, which are peltate glandular trichomes with an apical head. Non-glandular trichomes are usually found in the majority species of *Strobilanthes* s.l. but they are not found in some species such as *H. alternata, S. barisanensis, S. capillipes, S. hamiltoniana, S. multiflora, S. ovatifolia, S. palawanensis, S. multiflora, S. pateriformis, S. pedunculosa, Sm. benculensis, S. polybotrya, S. ramosissima,* and *S. sumatrana*. Non-glandular trichomes are usually simple, cone-shaped and consist of many cells (multicellular) (see Figure 4) such as 1-3 cells (*S. alboviridis, S. atropurpurea*), 2 cells (*H. blumeana, S. tonkinensis*), 2-3 cells (*S. cusia*), 2-4 cells (*S. inflata, Se. crispa*), 3 cells (*S. involucrata, S. violascens*), 3-4 cells (*S. axilliflora, S. bunnemeyeri*), 3-5 cells (*S. echinata, S. speciosa*), 3-6 cells (*S. backeri*), 4 cells (*S. rufopauper, H. serpens*), 4-5 cells (*S. bibracteata, S. parabolica*), 4-6 cells (*S. hossei*), 5-6 cells (*S. cernua, S. cruciata*), 5-7 cells (*S. pubescens*). The majority species of *Strobilanthes* s.l. have no multicellular foot cell (9-10 cells) surrounding non-glandular trichomes but this structure can be found in some species such *S. palawanensis* and *S. parabolica.*

The cystolith is easily observed and visible on dried herbarium specimens. They can be found on both adaxial and abaxial leaf surfaces. During the present observation, there are three types of cystolith namely one end pointed, both ends pointed and both end obtuse (see Figure 5). The one end pointed cystolith type is widely distributed in most species of *Strobilanthes* s.l. Both end pointed cystolith type is only found in one species (namely *S. speciosa*), but both end obtuse cystolith type can be found in some species such as *S. backeri, S. cruciata, S. involucrata, S. palawanensis, S. parabolica,* and *H. blumeana.*

Principal Component (PC) analysis was used to determine the highly loaded leaf anatomy characters that can be considered to delimit the tested taxa. The quantitative and qualitative leaf anatomy characters data set subjected to PC analysis revealed seven components with eigenvalues greater than 1 (Table 5). The first seven principal components (PC) accounted for about 79.43 % of the total variation. The first principal component (PC1), with an eigen value of 3.12 and explained 18.35 % of the total variation, was dominated by the highly loaded characters such as cystolith density adaxial, glandular trichomes density adaxial, cystolith density abaxial, glandular trichomes density abaxial, non-glandular trichomes type, multicellular foot cell and cystolith type but some characters such as cystolith length adaxial, cystolith width adaxial, cystolith length abaxial, cystolith width abaxial and stomata position showed negative values. The second PC (PC2) with an eigen value of 2.36 and 13.91% variation was positively associated with cystolith density adaxial, cystolith length adaxial, cystolith width adaxial, cystolith density abaxial, glandular trichomes density abaxial, cystolith length abaxial, cystolith length:width ratio abaxial, anticlinal cell wall, stomata position and stomata type but negatively associated with cystolith length:width ratio adaxial, epidermal cell shape and non-glandular trichomes type.

The third PC (PC3) exhibited 11.34 % variation and eigen value of 1.93 with the dominance of characters such as glandular trichomes density adaxial, cystolith length adaxial, cystolith length:width ratio adaxial, glandular trichomes density abaxial, cystolith length:width ratio abaxial, epidermal cell shape and stomata type but negatively associated with cystolith density abaxial, cystolith length abaxial, cystolith width abaxial, anticlinal cell wall and stomata position. The fourth PC (PC4) with 10.44 % variation and eigen value of 1.78 showed glandular trichomes density adaxial, cystolith width adaxial, glandular trichomes density abaxial, cystolith length abaxial, cystolith width abaxial and non-glandular trichomes type as highly loaded characters but cystolith density adaxial, cystolith length adaxial, cystolith length:width ratio adaxial, cystolith density abaxial, anticlinal cell wall, stomata position and cystolith type as the negatively loaded characters.

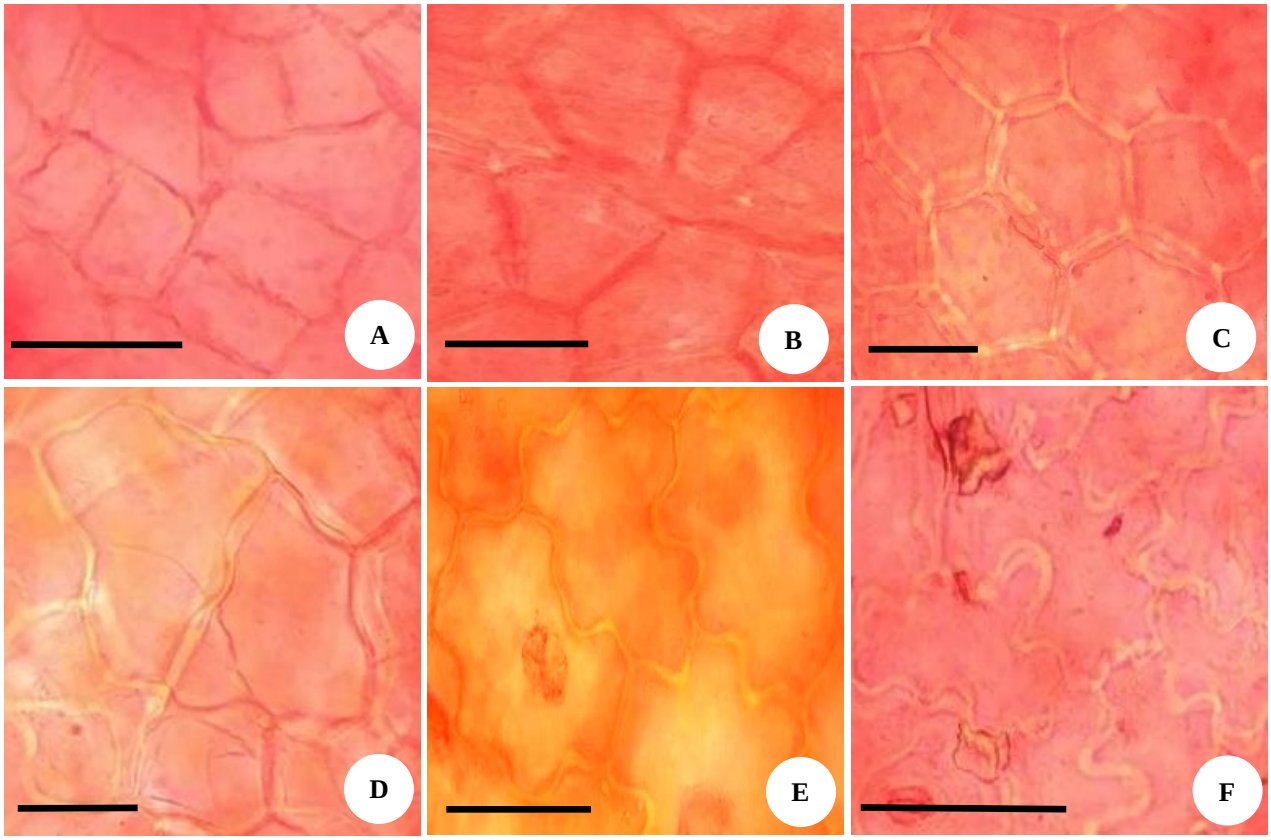

Figure 2. Variation of shape and anticlinal cell wall epidermal cell of *Strobilanthes* s.l. A. tetragonal and straight cell wall (*S. inflata*); B. pentagonal and straight cell wall (*S. sumatrana*); C. hexagonal and straight cell wall (*H. serpens*); D. irregular and slightly wavy cell wall (*S. violascens*); E. irregular and sinusoid cell wall (*S. crispa*); F. irregular and deeply sinusoid (*S. echinata*). Scale bars = 5 µm

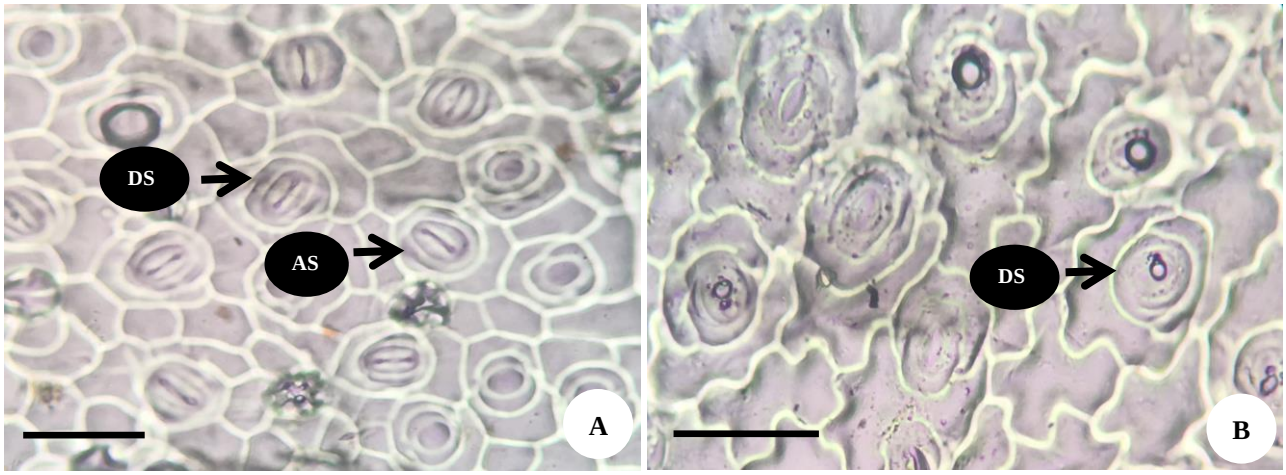

Figure 3. Variation of stomata type of *Strobilanthes* s.l. A. diacytic and anisocytic stomata (*S. cusia*). B. diacytic stomata (*S. violascens*). Scale bars = 5 µm. Note : DS: Diacytic Stomata; AS: Anisocytic Stomata

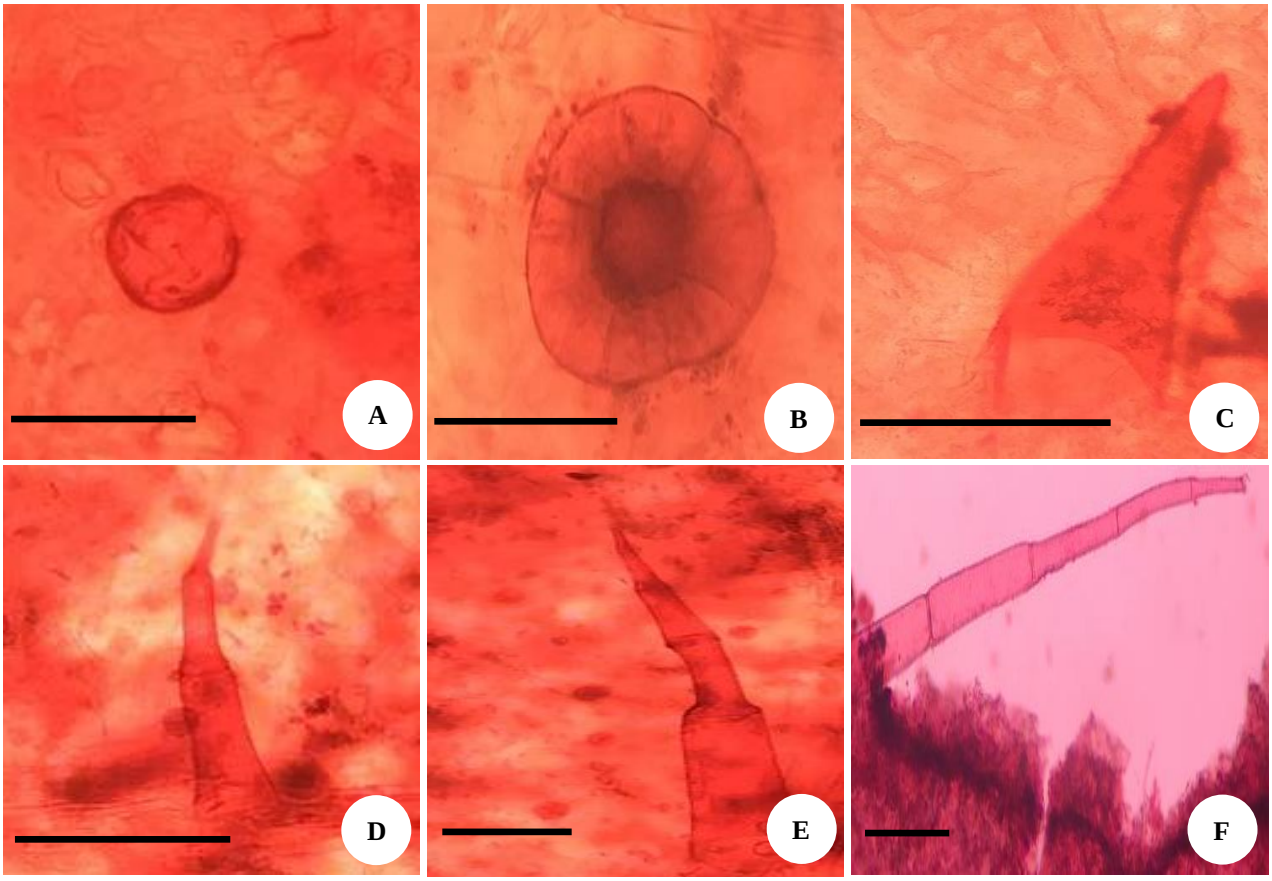

Figure 4. Glandular trichomes and non-glandular trichomes of *Strobilanthes* s.l. A. glandular trichomes (*S. alboviridis*). B. Multicellular foot cell surrounding non-glandular trichomes (*S. palawanensis*); C. 1-celled non-glandular trichomes (*S. atropurpurea*). D. 3-celled non-glandular trichomes (*S. violascens*). E. 4-celled non-glandular trichomes (*S. rufopauper*). F. 5-celled non-glandular trichomes (*S. pubescens*). Scale bars (A, D) = 4 µm; Scale bars (B, C, E, F) = 5 µm

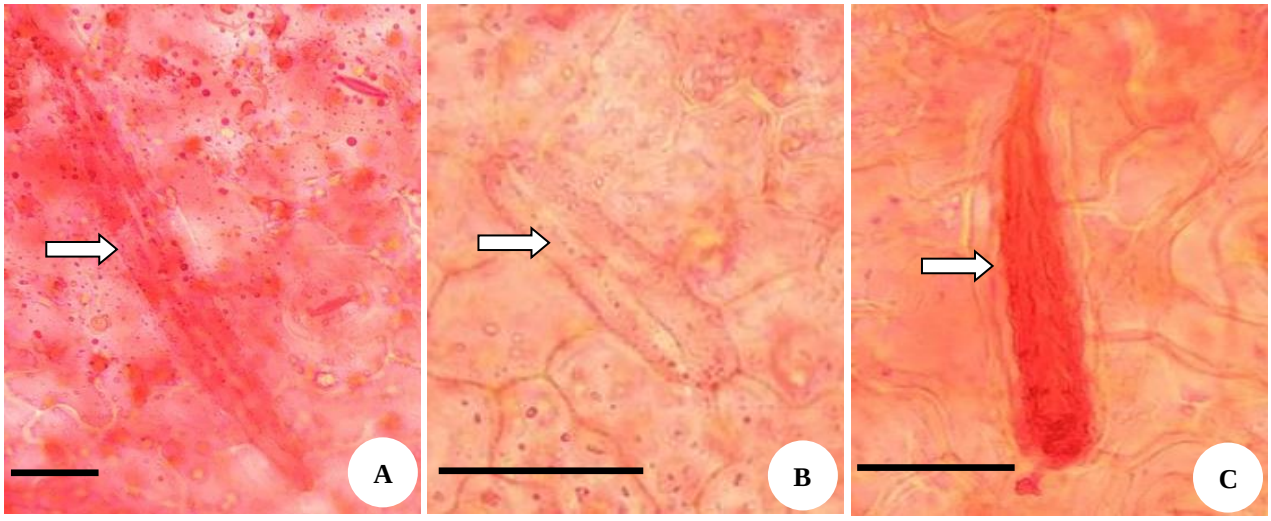

Figure 5. Variation of cystolith type of *Strobilanthes* s.l. A. elongated, both ends pointed (*S. speciosa*). B. elongated, both ends obtuse (*S. palawanensis*); C. elongated, one end pointed and other end obtuse (*S. violascens*). Scale bars = 5 µm

Table 4. Qualitative leaf anatomical character variation among species of *Strobilanthes* s.l. species from Sumatra, Indonesia

Species	Epidermal cell shape	Anticlinal cell wall	Stomata position	Stomata type	Non-glandular trichomes type	Multicelluar foot cell of non-glandular trichomes	Cystolith type
S. alboviridis	Pentagonal	Straight	Hypostomatic	Diacytic, anisocytic	1-3-celled	Absent	One end pointed
S. atropurpurea	Hexagonal	Straight	Hypostomatic	Diacytic	1-3-celled	Absent	One end pointed
S. axilliflora	Irregular	Slightly wavy	Hypostomatic	Diacytic	3-4-celled	Absent	One end pointed
S. backeri	Irregular	Slightly wavy	Hypostomatic	Diacytic	3-6-celled	Absent	Both end obtuse
S. barisanensis	Irregular	Slightly wavy	Hypostomatic	Diacytic	3-celled	Absent	One end pointed
S. bibracteata	Irregular	Slightly wavy	Hypostomatic	Diacytic	4-5-celled	Absent	One end pointed
S. bunnemeyeri	Irregular	Slightly wavy	Hypostomatic	Diacytic	3-4-celled	Absent	One end pointed
S. capillipes	Irregular	Slightly wavy	Amphistomatic	Diacytic	Absent	Absent	One end pointed
S. cernua	Irregular	Slightly wavy	Hypostomatic	Diacytic	5-6-celled	Absent	One end pointed
S. cruciata	Irregular	Slightly wavy	Hypostomatic	Diacytic	5-6-celled	Absent	Both end obtuse
S. cusia	Pentagonal	Straight	Hypostomatic	Diacytic, anisocytic	2-3-celled	Absent	One end pointed
S. echinata	Irregular	Deeply sinusoid	Hypostomatic	Diacytic	3-5-celled	Absent	One end pointed
S. hamiltoniana	Irregular	Slightly wavy	Hypostomatic	Diacytic	Absent	Absent	One end pointed
S. hossei	Irregular	Slightly wavy	Hypostomatic	Diacytic	4-6-celled	Absent	One end pointed
S. inflata	Tetragonal	Straight	Hypostomatic	Diacytic, anisocytic	2-4-celled	Absent	One end pointed
S. involucrata	Irregular	Slightly wavy	Hypostomatic	Diacytic	3-celled	Absent	Both end obtuse
S. multiflora	Pentagonal	Straight	Hypostomatic	Diacytic, anisocytic	Absent	Absent	One end pointed
S. ovatifolia	Irregular	Slightly wavy	Hypostomatic	Diacytic	Absent	Absent	One end pointed
S. palawanensis	Irregular	Sinusoid	Hypostomatic	Diacytic	Absent	Present	Both end obtuse
S. parabolica	Irregular	Sinusoid	Hypostomatic	Diacytic	4-5-celled	Present	Both end obtuse
S. pateriformis	Irregular	Sinusoid	Hypostomatic	Diacytic	Absent	Absent	One end pointed
S. pedunculosa	Irregular	Deeply sinusoid	Amphistomatic	Diacytic	Absent	Absent	One end pointed
S. polybotrya	Irregular	Slightly wavy	Hypostomatic	Diacytic, anisocytic	Absent	Absent	One end pointed
S. pubescens	Hexagonal	Straight	Hypostomatic	Diacytic	5-7-celled	Absent	One end pointed
S. ramosissima	Pentagonal	Straight	Hypostomatic	Diacytic	Absent	Absent	One end pointed
S. rufopauper	Hexagonal	Straight	Hypostomatic	Diacytic	4-celled	Absent	One end pointed
S. speciosa	Irregular	Deeply sinusoid	Hypostomatic	Diacytic	3-5-celled	Absent	Both end pointed
S. sumatrana	Pentagonal	Straight	Hypostomatic	Diacytic	Absent	Absent	One end pointed
S. tonkinensis	Hexagonal	Straight	Hypostomatic	Diacytic	2-celled	Absent	One end pointed
S. violascens	Irregular	Sinusoid	Hypostomatic	Diacytic	3-celled	Absent	One end pointed
Se. crispa	Irregular	Sinusoid	Hypostomatic	Diacytic, anisocytic	2-4-celled	Absent	One end pointed
H. alternata	Irregular	Deeply sinusoid	Amphistomatic	Diacytic, anisocytic	Absent	Absent	One end pointed
H. blumeana	Irregular	Sinusoid	Hypostomatic	Diacytic	2-celled	Absent	Both end obtuse
H. serpens	Hexagonal	Straight	Hypostomatic	Diacytic	4-celled	Absent	One end pointed
Sm. benculensis	Irregular	Slightly wavy	Hypostomatic	Diacytic	Absent	Absent	One end pointed

On the fifth PC (PC5), the important contributing characters to 10.13 % of total variation such as glandular trichomes density adaxial, cystolith length adaxial, cystolith width adaxial, anticlinal cell wall, stomata position, stomata type, presence multicellular foot cell and cystolith type. The sixth PC (PC6) showed 8.74% of the total variation which is determined by characters such as glandular trichomes density adaxial, cystolith density abaxial, glandular trichomes density abaxial, cystolith width abaxial, epidermal cell shape, anticlinal cell wall, stomata position and stomata type. The seventh PC (PC7) exhibited 6.52% of the total variation and eigen value of 1.10. The variation was mainly contributed by characters such as glandular trichomes density adaxial, cystolith length:width ratio adaxial, glandular trichomes density abaxial, cystolith length abaxial, cystolith width abaxial, cystolith length:width ratio abaxial, anticlinal cell wall, stomata position and cystolith type.

Plotting the species of *Strobilanthes* s.l. based on their leaf anatomical characters across the first 2 PCs (PC1 and PC2) through PCA analysis showed separation of groups across the PC1 axis (Figure 6). Species with higher values for PC1 (*S. palawanensis* and *S. parabolica*) had higher glandular trichome density and cystolith density on both surfaces (abaxial and adaxial) than species with lower PC1 values such as *H. alternata, S. hamiltoniana* and *S. barisanensis*. Species with higher values for PC2 (*S. palawanensis, H. alternata, S. multiflora, S. pedunculosa)*

had a larger size of cystolith length on both surfaces (adaxial and abaxial), a larger size of cystolith width on both surfaces (adaxial and abaxial) and a larger cystolith length:width ratio on abaxial leaf surface than species with lower PC2 values such as *S. rufopauper, S. atropurpurea* and *S. cernua*.

A similarity dendrogram among species of *Strobilanthes* s.l.from Sumatra based on leaf anatomical character data is presented in Figure 7. The cluster analysis through UPGMA methods revealed two major cluster of *Strobilanthes* s.l. The first cluster consist of eleven species (*S. sumatrana, S. ramossisima, S. pubescens, S. multiflora, H. serpens, S. tonkinensis, S. rufopauper, S. atropurpurea, S. inflata, S. alboviridis,* and *S. cusia*). This cluster can be distinguished from species in the second cluster in having regular epidermal cell shape and straight cell wall with a similarity coefficient of 0.67. This cluster then can be separated into four sub-cluster i.e. sub-cluster A (pentagonal epidermal cell sub-cluster with diacytic stomata type, consist of *S. multiflora, S. ramosissima, S. sumatrana*) with a similarity coefficient of 0.72, sub-cluster B (hexagonal epidermal cell sub-cluster with diacytic stomata type, consist of *S. atropurpurea, S. rufopauper, S. tonkinensis, H. serpens*) with a similarity coefficient of 0.74, sub-cluster C (tetragonal epidermal cell sub-cluster with diacytic and anisocytic stomata, consist of *S. inflata*) with a similarity coefficient of 0.72, and sub-cluster D (pentagonal epidermal cell sub-cluster with diacytic and anisocytic stomata type, consist of *S. alboviridis* and *S. cusia*) with a similarity coefficient of 0.84. The second cluster consist of twenty four species (*Sm. benculensis, S. capillipes, S. hossei, S. barisanensis, S.polybotrya, S. hamiltoniana, S. involucrata, S. axilliflora, S.cernua, S. ovatifolia, S. bibracteata, S. echinata, S. bunnemeyeri, S. cruciata, S. backeri, S. pateriformis, Se. crispa, S. violascens, H. blumeana, S. speciosa, S. pedunculosa, H. alternata, S. palawanesis, S. parabolica*) which differed from species in the first cluster mainly due to their irregular epidermal cell shape and undulate anticlinal cell wall with a similarity coefficient of 0.59. This cluster then can be separated into five sub-cluster i.e. sub-cluster E (consist of species had irregular epidermal cells with slightly wavy anticlinal cell wall and lower glandular trichome density and cystolith density on both leaf surface such as *Sm. benculensis, S. capillipes, S. hossei, S. barisanensis, S. polybotrya, S. hamiltoniana, S. involucrata*) with a similarity coefficient of 0.72, sub-cluster F (consist of species had irregular epidermal cells with slightly wavy anticlinal cell wall and smaller size of cystolith length on both leaf surface, smaller size of cystolith width on both leaf surface and smaller cystolith length:width ratio on abaxial leaf surface such as *S. axilliflora, S. cernua, S. ovatifolia, S. bibracteata, S. bunnemeyeri, S. cruciata, S. backeri*) with a similarity coefficient of 0.75, sub-cluster G (consist of species had irregular epidermal cells with sinusoid anticlinal cell wall and lower glandular trichome density and cystolith density on both leaf surface such as *S. pateriformis, Se. crispa, H. blumeana, S. violascens*) with a similarity coefficient of 0.74, sub-cluster H (consist of species had irregular epidermal cells the with deeply sinusoid anticlinal cell wall and larger size of cystolith length on both leaf surface, larger size of cystolith width on both leaf surface and larger cystolith length:width ratio on abaxial leaf surface such as *S. speciosa, S. pedunculosa, H.alternata*) with a similarity coefficient of 0.68, sub-cluster I (consist of species had irregular epidermal cells with sinusoid anticlinal cell wall and higher glandular trichome density and cystolith density on both leaf surface such as *S. palawanensis* and *S. parabolica*) with a similarity coefficient of 0.84.

Table 5. Eigenvectors, eigenvalues, the individual and cumulative percentage of variation explained by the first seven principal components after assessing leaf anatomical characters of *Strobilanthes* s.l. from Sumatra, Indonesia

Leaf anatomical characters	Principal components (PC)						
	PC 1	**PC 2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
Cystolith density adaxial	**0.318**	**0.300**	-0.057	-0.286	-0.080	0.029	-0.375
Glandular trichomes density adaxial	**0.215**	0.056	**0.143**	**0.307**	**0.391**	**0.141**	**0.368**
Cystolith length adaxial	-0.452	**0.193**	**0.120**	-0.102	**0.144**	-0.128	0.054
Cystolith width adaxial	-0.397	**0.238**	0.047	**0.231**	**0.127**	-0.230	-0.270
Cystolith length:width ratio adaxial	-0.069	-0.192	**0.145**	-0.508	-0.093	0.065	**0.501**
Cystolith density abaxial	**0.141**	**0.135**	-0.297	-0.263	-0.283	**0.402**	-0.026
Glandular trichomes density abaxial	**0.315**	**0.333**	**0.119**	**0.307**	0.084	**0.203**	**0.105**
Cystolith length abaxial	-0.102	**0.338**	-0.158	**0.274**	-0.415	0.044	**0.360**
Cystolith width abaxial	-0.168	0.005	-0.554	**0.225**	0.030	**0.207**	**0.171**
Cystolith length:width ratio abaxial	0.032	**0.304**	**0.395**	0.034	-0.445	-0.128	**0.185**
Epidermal cell shape	0.006	-0.237	**0.239**	0.094	0.009	**0.456**	-0.059
Anticlinal cell wall	0.040	**0.155**	-0.146	-0.252	**0.492**	**0.127**	**0.108**
Stomata position	-0.237	**0.359**	-0.137	-0.126	**0.195**	**0.147**	**0.143**
Stomata type	-0.080	**0.145**	**0.490**	-0.060	**0.148**	**0.299**	-0.009
Non-glandular trichomes type	**0.199**	-0.340	-0.017	**0.314**	-0.017	-0.070	0.044
Multicellular foot cell of non-glandular trichomes	**0.400**	**0.302**	-0.029	-0.038	**0.130**	-0.177	-0.121
Cystolith type	**0.245**	0.004	-0.082	-0.132	**0.126**	-0.517	**0.371**
Eigenvalue	**3.12**	**2.36**	**1.93**	**1.78**	**1.72**	**1.48**	**1.10**
Individual percentage	**18.35**	**13.91**	**11.34**	**10.44**	**10.13**	**8.74**	**6.52**
Cumulative percentage	**18.35**	**32.26**	**43.60**	**54.04**	**64.17**	**72.91**	**79.43**

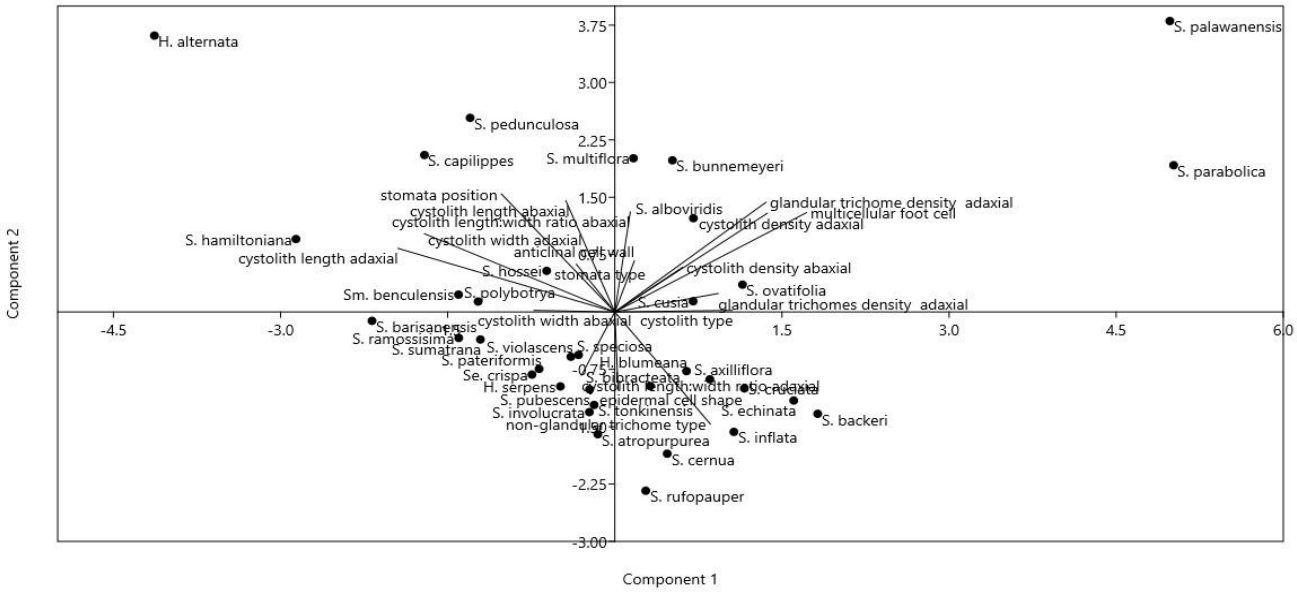

Figure 6. Scatter diagram of the species of *Strobilanthes* s.l. from Sumatra and their leaf anatomical characteristics when plotted against the first two PCs of the correlation matrix (explaining 32.26 % of the total variation)

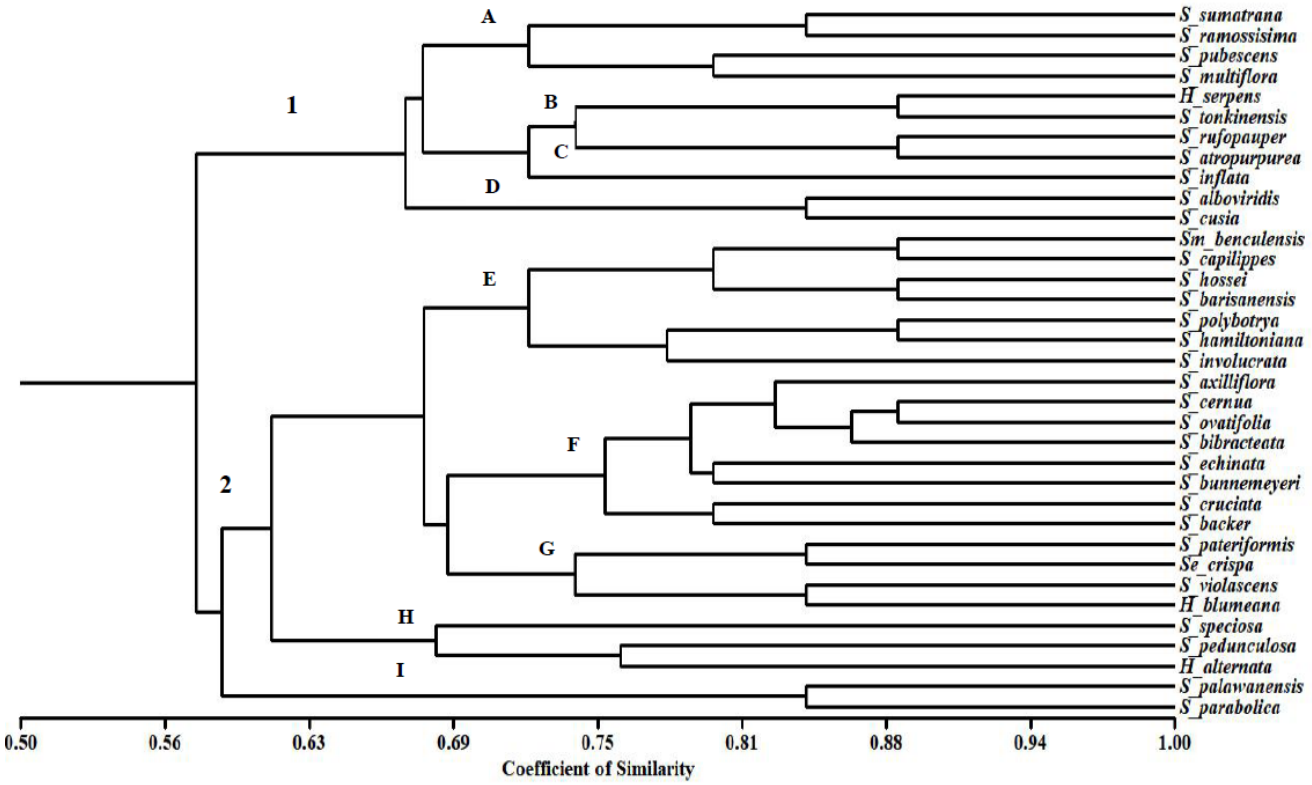

Figure 7. Similarity dendrogram among species of *Strobilanthes* s.l. from Sumatra based on leaf anatomical characters data

Discussion

Leaf anatomical characters variation

In order to evaluate the variation of leaf anatomical characters among species of *Strobilanthes* s.l., the observation was conducted on seventeen characters including ten quantitative characters and seven qualitative characters. The quantitative characters included cystolith density adaxial, cystolith length adaxial, cystolith width adaxial, cystolith length:width ratio adaxial, glandular trichomes density adaxial, cystolith length abaxial, cystolith width abaxial, cystolith length:width ratio abaxial, cystolith density abaxial, and glandular trichome density abaxial. Then, the observed qualitative leaf anatomical characters included shape and anticlinal cell wall of epidermal cell, stomata position, stomata type, non-glandular trichomes type, presence of multicellular foot cell, and cystolith type.

The observed leaf anatomical characters showed a good variation qualitatively and quantitatively. The analysis variance of quantitative leaf anatomical characters showed significant to highly significant variation among the all tested *Strobilanthes* s.l. taxa whereas a great variation were also observed in the qualitative leaf anatomical characters. In general, this study revealed that *Strobilanthes* s.l. showed great variability in the majority of tested leaf anatomical characters. This result indicates the large diversity of leaf anatomical characters occurs among species of *Strobilanthes* s.l.

Epidermal cell patterns (including epidermal cell shape and anticlinal cell wall) showed high variation among all the investigated taxa. There are two basic epidermal cell shapes namely irregular and regular cell shapes. The irregular epidermal cells are commonly found in among species of *Strobilanthes* s.l. These epidermal cells usually have various undulate anticlinal cell wall such as slightly wavy, sinusoid, and deeply sinusoid. These findings also support the investigation by Patil and Patil (2011a) that anticlinal cell wall of epidermal cells in Acanthaceae (including *Strobilanthes* s.l.) are usually undulate, rarely otherwise. The regular epidermal cell shape includes tetragonal, pentagonal, hexagonal and polygonal with a straight cell wall. The undulation of the anticlinal wall of the epidermal cell showed the differences among species in this study, therefore it can be used as a diagnostic character for distinguishing species (Nikmah et al. 2020). Hence, epidermal cell wall pattern serves as taxonomically imperative diagnostic characters (Tripathi and Mondal 2012; Zhang et al. 2018). The epidermal cells are also less subjected to modification, so they serve as good taxonomic characters. The leaf epidermal features could be said to be taxonomically significant if discontinuities occurred within and between species (AbdulRahaman et al. 2014). This observed variation in plant species provides evidence that can be used as a tool for accurate species delimitation and identification (Raza et al. 2020).

Stomata also display great variations in their distribution and types. Considering the occurrence of stomata in the leaves, hypostomatic leaves are commonly found in the majority species of *Strobilanthes* s.l. Only a few species exhibited amphistomatic leaves condition such as *H. alternata*, *S. capillipes*, and *S. pedunculosa*. Based on the type of stomata, there are two basic types, namely diacytic and anisocytic stomata. The stomata of *Strobilanthes* s.l. are generally diacytic type, whereas the anisocytic stomata type are occasionally observed on the same leaf surfaces together from the same species. These findings also support earlier works that diacytic stomata type (cross-walled) were generally observed in all the studied species of *Strobilanthes* (Fernandes and Krishnan 2019; Tajudin et al. 2022). The stomata are diacytic type when the subsidiary cells are consistently two, however, there can be other types of stomata on the same foliar surface. Nevertheless, other stomatal types were rarely mixed with the diacytic type. The anisocytic stomata type was usually observed mixed together with diacytic stomata type in some species of *Strobilanthes* (Fernandes and Krishnan 2019). Therefore, in this study, the diacytic stomata type generally found in majority of the all studied taxa, only a few species bear more than one stomata type (diacytic and anisocytic) on the same leaf surface such as *S. alboviridis*, *H. alternata*, *Se. crispa*, *S. cusia*, *S. inflata*, *S. multiflora* and *S. polybotrya*. However, in the latter stomata type, the number of diacytic stomata type is greater than that of anisocytic stomata type. Therefore species of *Strobilanthes* s.l. can be separated and distinguished based on their stomata type and distribution in this study. Thus, variation of stomata types and their distribution on the adaxial or abaxial leaf surface can be utilized as taxonomic features especially in Acanthaceae as reported by Amri et al. (2014). Hence, stomata served as a taxonomically significant parameter and helped to delimit the confusing systematic position of various taxa (Tripathi and Mondal 2012). Although the analysis variance of some quantitative stomata characters such as stomatal density abaxial, stomatal index abaxial, stomatal length abaxial and stomatal width abaxial showed a highly significant variation among species of *Strobilanthes* s.l. from Sumatra (data not published) but these characters can not be used as diagnostic characters in this study. However, some quantitative stomata characteristics such as frequency and dimensions can be affected by the type of species and environmental factors (Munir et al. 2011; Pitoyo et al. 2018; Cassola et al. 2019).

There are two basic types of trichomes, namely glandular and non-glandular trichomes. The peltate glandular trichomes with apical head can be found in all the tested taxa. This finding support previous works as reported by Fernandes and Krishnan (2019) that the majority species of *Strobilanthes* have peltate glandular trichomes. They are different among taxa in their density and different patterns of density were observed on the adaxial and abaxial surfaces. In this study, the glandular trichomes distributed in both adaxial and abaxial leaf surface but most of the studied species showed higher abundance on abaxial than on adaxial surface. The non-glandular trichomes were observed on both abaxial and adaxial leaf surfaces and distributed in the majority of the all studied taxa, whereas some species have no non-glandular trichomes. Non-glandular trichomes of *Strobilanthes* s.l. are simple, not branched, cone-shaped

and multicellluer. This result supports an earlier study that non-glandular trichomes of *Strobilanthes* s.l. are filiform, wide at base and tapering towards the apex, composed of multicellular cells of which the apical cells are the longest (Moylan et al. 2004b). Hence, trichomes can be used as a good diagnostic character to distinguish each plant taxa at species level because they show a great variation qualitatively and quantitatively (Muzzazinah et al. 2021). The occurrence of trichomes, variation in their morphological form and density on both leaf surfaces are then considered important for providing taxonomic relationships of the studied taxa (Juhari et al. 2014; Singh et al. 2020; Zakaria et al. 2022).

Cystoliths are large growths of cellulosic cell wall material and consist of amorphous calcium carbonate (Gabel et al. 2021). The cystolith is generally found in various vegetative parts of the plant including leaf blades, petioles (Pattil and Pattil 2011b), bracts, bracteoles and calyces (Bennet and Scotland 2003). In leaves, they can be found on both abaxial and adaxial leaf surfaces (Gabel et al. 2021). In this study, the cystolith character that was useful for distinguishing among species of *Strobilanthes* s.l. was the shape, size (length, width, ratio length:width), and distribution of cystolith on both leaf surfaces. In general, the cystolith of *Strobilanthes* s.l. is simple, linier and elongated. In some taxa, both ends of cystoliths are obtuse or acute, whereas in others one of the ends of cystolith is either obtuse or acute (Pattil and Pattil 2011b). Either obtuse or acute ends of cystolith type are commonly found in most species of *Strobilanthes* s.l. Only a few species which have both ends pointed cystolith type (in *S. speciosa*) and the both end obtuse cystolith type (in *S. backeri, S. cruciata, S. involucrata, S. palawanensis, S. parabolica,* and *H. blumeana*). Analysis variance of cystolith density on both adaxial and abaxial leaf surface revealed highly significant variation of these features. Although cystolith can be found in both abaxial and adaxial leaf surfaces, but they usually densely distributed and more prominent on adaxial surface. Cystolith size (length, width, ratio length: width) on both adaxial and abaxial leaf surface showed significant to highly significant variation among all investigated taxa. In general, cystolith dimension on adaxial leaf surface showed greater values than the abaxial ones. Thus, the presence of cystoliths in the leaves is one of the important characters that have been used to solve taxonomic problems, especially in Acanthaceae (Scotland and Vollesen 2000; Amri et al. 2018; Zakaria et al. 2020; Gabel et al. 2021). In this study, cystolith size, shape and distribution are variable among the species in the studied genera, their occurrence is also constant, and therefore they constitute an important character for a taxonomic purpose (Choopan and Grote 2015).

Principal component analysis (PCA) can be used to determine the relative contribution of each character to the total variation and to identify the most informative character to represent the existing variability using the corresponding factor loadings within the species (Arriel et al. 2007; Akinyele et al. 2020). PCA has also been used by various authors to evaluate the systematic importance of various attributes (Paul and Chowdhury 2021). In this study, PCA revealed that all leaf anatomical characters are diagnostic and valuable for differentiating taxa especially at the species level, although they are not working well yet at the genera level. Cystolith features such as cystolith type, cystolith density, cystolith length, cystolith width, cystolith length:width ratio on adaxial and abaxial leaf surface are the best descriptors that can be used for distinguishing and identification of species. The cystolith features are one of the important characters in taxa delimitation and classification at the species levels of members of the Acanthaceae family (Pattil and Pattil 2011b; Zakaria et al. 2020). Trichomes attributes such as density of glandular trichomes on adaxial and abaxial leaf surface, non-glandular trichomes type and presence of multicellular foot cell of non-glandular trichomes are diagnostic and can be used in separating the species. This finding support earlier works that the presence or absence of different types, sizes and numbers of trichomes as good diagnostic features for taxonomic studies (Juhari et al. 2014; Zakaria et al. 2022). Epidermal cell pattern such as shape and anticlinal cell wall of epidermal cell also affirms their strength in delimiting the taxa, especially at the species level. Stomata features such as stomata type and distribution also can be used to distinctly separate the species under study. Then, the combination of leaf epidermal data and stomatal data can further give information concerning species identification (da Silva et al. 2016).

Taxonomic implications

Generic circumscription within the tribe *Strobilanthinae* (sensu Bremekamp 1944) has long proven to be problematic (Moylan et al. 2004a). *Strobilanthes, Hemigraphis, Sericocalyx* and *Semostachya* are previously included in the subtribe *Strobilanthinae* and distinguished based on their morphological characters. *Hemigraphis* easily distinguishable from *Strobilanthes* in having multiovulate ovary (six to twelve ovules), and small, caducous, non-resupinates corolla (Moylan et al. 2002; Yunfei 2019). Based on Bremekamp (1944), *Semnostachya* and *Sericocalyx* resemble *Strobilanthes* in having four ovules per ovary but *Semnostachya* can be distinguished in having three-nerved bractea, long peduncle, and exareolate seeds. *Sericocalyx* also can be distinguished from *Strobilanthes* by its inside densely sericeous (silky) calyx, yellow corolla and multiovulate ovary (four to twelve ovules). Based on Moylan et al. (2004a), *Sericocalyx*, was described to accommodate species intermediate in morphology between *Strobilanthes* and *Hemigraphis.*

The relationship dendrogram of *Strobilanthes* s.l. based on leaf anatomical characters in this study showed that the genera *Hemigraphis, Sericocalyx* and *Semnostachya* nested together with *Strobilanthes*, indicating that these genera are probably monophyletic group. The previous studies also showed that *Strobilanthes* and *Hemigraphis* from Southern India and Sri Lanka were clustered as a monophyletic group (Carine and Scotland 2000a). The results of this study also support an investigation by Moylan et al. (2004a) through molecular studies that all members of the *Strobilanthinae* (sensu Bremekamp 1944) recognized as a

monophyletic group that could not be easily split into smaller component genera (Yunfei 2019). Thus, the division of members of this tribe into separate genera (eg *Hemigraphis*, *Sericocalyx*, *Strobilanthes*, etc) however should be neglected.

Therefore, the solution that is considered quite appropriate to solve the problem of generic delimitation and classification of the *Strobilanthineae* tribe members is to place all segregate genera (eg *Hemigraphis*, *Sericocalyx*, *Strobilanthes*, etc) in synonymy with one large and well-defined genus *Strobilanthes* s.l. within the tribe Ruelliinae (Scotland and Vollesen 2000) as proposed by Terao (1983). Therefore, the results of this study also support an investigation by Bennet and Scotland (2003) that all members of the genera *Hemigraphis* and *Sericocalyx* deserve to be used as synonyms for *Strobilanthes*. Consequently, Yunfei (2019) then transferred species of *Hemigraphis* in the Philippines to *Strobilanthes* in his account.

In conclusion, this study clearly shows that leaf anatomical characters are useful diagnostic features for the identification of the tested taxa, especially in forming a basic tool for distinguishing the species of *Strobilanthes* s.l. in their vegetative state. This study also indicates that leaf anatomical characters are valuable taxonomic features that can be used to solve the taxonomic problem within problematic taxa such as *Strobilanthes* s.l., especially in taxa delimitation at the species level. The species then can be distinguished based on their leaf anatomical features such as epidermal cells pattern, stomata type and distribution, trichomes type and distribution, cystolith type, dimension and distribution. However, leaf anatomical characters are not working well yet at genera level because the separation of *Strobilanthes* s.l. into smaller component, genera can not easily be conducted in this taxa.

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