Morphological and anatomical characteristics and chemical components of *Arisaema pierreanum*

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Abstract
Arisaema pierreanum Engl. is an endemic species in Ba Đen Mountain, Tay Ninh Province, Vietnam. The study provides the database of the morphological and anatomical characteristics and chemical components of this species. The results from the present study could be used in the determination of the medicinal plants and further application of A. pierreanum in the medicinal field and other fields.

Keywords: *Arisaema pierreanum*, anatomy, chemical components, GC/MS.

Introduction

Arisaema Martius, a large genus of the family Araceae, consists of about 200 accepted species widely found in tropical Asia, Himalaya, Australia and New Guinea[2,7,18,23]. Currently, twenty six species belonging to the *Arisaema* genus have been recorded in Vietnam[13,14,18,23-25].

Many species of this genus have been extensively used in ethnopharmacology in some Asian countries to treat various diseases including numbness of limbs, traumatic injury, rheumatic, labor pain, tuberculosis pain, rheumatoid arthritis, tetanus, traumatic injury, snake bite, mouth and eye deviation, arthralgia, stomachache and hemiplegia etc.[20] *Arisaema pierreanum* Engl. was first described by Engler[5] in 1923 based on the plant samples collected by Pierre from Ba Den Mountain, Tay Ninh Province, Vietnam in 1869.

This species was re-collected and fully described by Nguyen et al[16] and Van et al[24] whose specimens were also collected from the type location. *A. pierreanum* has been found in Ba Đen Mountain so far. The present study, thus, provides the morphological, anatomical characteristics and chemical components of *A. pierreanum* for the first time.

Material and Methods

Plant materials: The specimens of *A. pierreanum* were taken from Ba Den Mountain, Tay Ninh Province, Vietnam, about 438 m elevation, approximate coordinates $11^0$23'10" N and $106^0$09'41" E, on April 27, 2023. The vouchered specimens, NPN-BD025 and NPN-BD026, were deposited at the Herbarium of University of Science, Vietnam National University-HCMC (PHH).

Morphological characteristics: The guidelines of the Royal Botanic Gardens, Kew[4] were applied to identify species of studied samples. The taxonomic significance of morphological characters of *A. pierreanum* was taken using a Canon EOS 90D digital camera with macro lens. The reproductive and vegetative characteristics of *A. pierreanum* from previous studies were used to compare with those of studied species[13,14,18,23-25].

Anatomical characteristics: The leaf, petiole, root and rhizome of *A. pierreanum* were cut transversally into thin slices. The microscopic specimens were bleached using Javel water. The iodine green-carmine double staining method was used to stain these samples. The slices were washed with water several times and preserved in 10% glycerol[21]. The samples were observed under the Olympus BX53 Digital Upright Microscope.

Extraction procedures: The fresh aerial part of *A. pierreanum* was washed and dried at 50°C and ground into powder. 250 milliliters of acetone solution 99% (Thermo Fisher Scientific, USA) was used to immerse 50 grams of the powder for 72 hours. The Whatmann filter paper was used to filter the soaking liquid. The remaining residue was immersed in two more rounds of acetone extraction. The solvent was then eliminated in vacuum condition at a temperature of 45°C.

Gas chromatography/mass spectrometry assays: The chemical components of the acetone extract from *A. pierreanum* were identified using the TRACE 1310 gas chromatograph in conjunction with the ISQ 7000 mass spectrometer (Thermo Fisher Scientific, USA). The DB-5MS column (Agilent, USA) was used as stationary phase. GC/MS run parameters were configured as previously described by our prior work[26]. The acquired mass spectral data were compared to the NIST 2017 library to determine the chemical components in the examined extract.

Results

Arisaema pierreanum Engl., 1920. Pflanzenr. 73 (IV. 23F): 159 (Figure 1).

Anatomical characteristics

Roots: The cross-section of root is nearly circular and divided into 2 distinct regions, the cortical (2/3 of the radius) and pith (1/3 of the radius) areas.

Cortex: The piliferous layer consists of a layer of polygonal cells, irregular size, thin walls impregnated with phellem and the surface contains many root hairs. The exodermis layer includes 2-4 layers of cells, the outermost layer is rectangular, the walls are impregnated with phellem; The lower layers have polygonal shapes, cellulose walls. The cortical parenchyma is divided into 2 areas; the outer region is polygonal cells, sometimes nearly round, dented walls; the inner region consists of 8-9 layers of rectangular cells arranged in radial rows. The endodermis has a clear casparian strip.

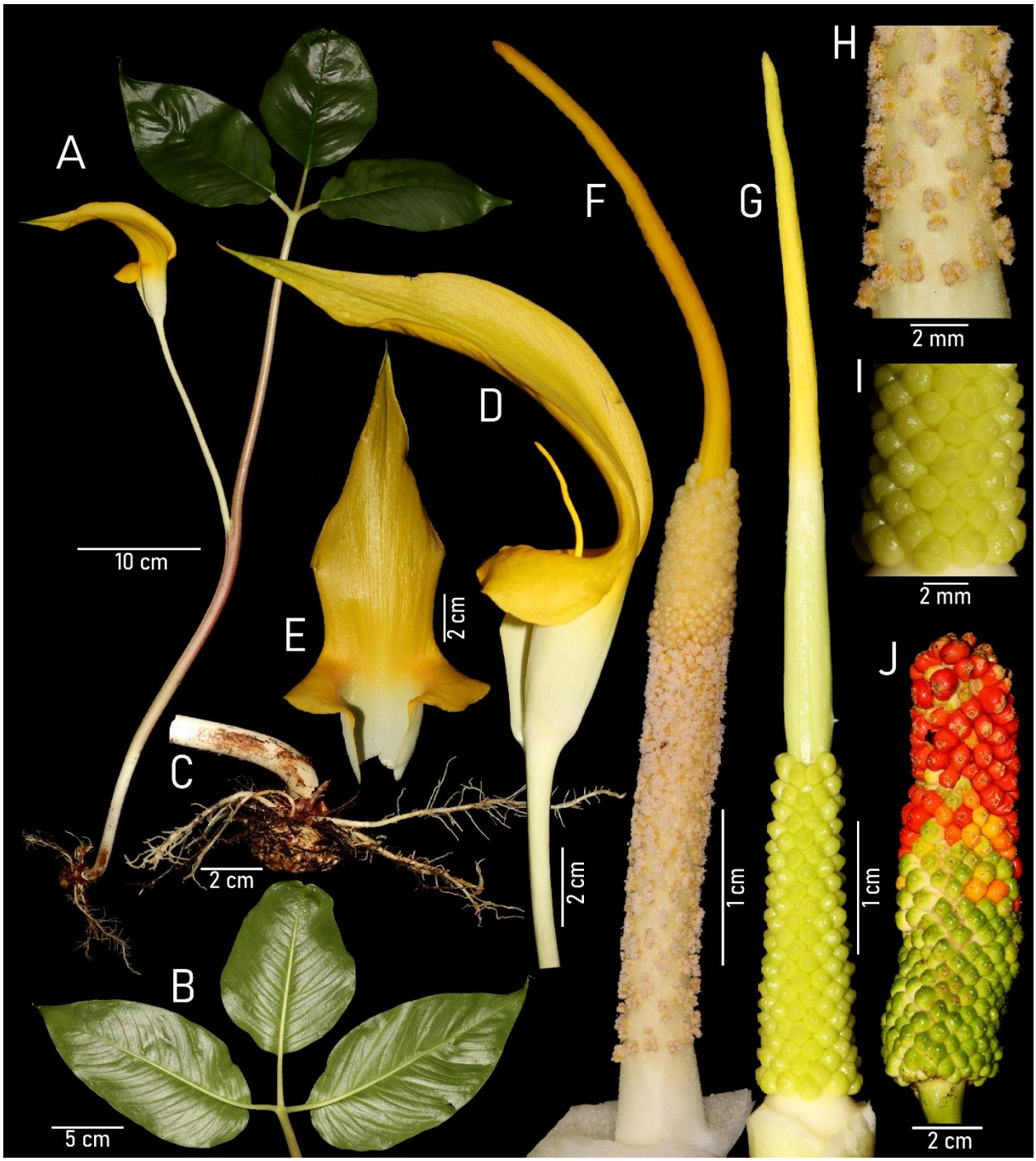

Figure 1: ***Arisaema pierreanum*** **Engl. A. The whole plant. B. Leaf blade. C. Tuber and roots. D. Inflorescence. E. Spathe limb. F. Male spadix. G. Female spadix. H. Synandria. I. Ovaries. J. Fruit spike**

Type: *Pierre sine num* (iso. P00141040, P001410401; holo: B_10_0279782), Ba Den Mountain, Tay Ninh Province, Vietnam, 500m in elevation, April 1869.

Habitat: The species found under tropical forest canopy, on humus soil on rocks

Distribution: Only in the type location.

Stele: The pericycle includes 1-2 layers of polygonal cells with cellulose walls, irregular size, arranged alternately with the endodermis. The conducting system consists of 7-8 phloem bundles 1 alternating with 7-8 protoxylem bundles arranged in a ring, separated by medullary rays. The phloem bundles form clusters of oval, polygonal, irregular cells. The protoxylem bundle includes 1-3 xylem vessels, polygonal shape, radially differentiated. The 9-10 metaxylem vessels may or may not contact the protoxylem bundles. Medullary rays consist of 1-2 series of horizontally flattened polygonal parenchyma cells with cellulose walls. The medullary parenchyma is polygonal cells, closely arranged, cellulose or lignin-impregnated walls, irregular size and haphazardly arranged.

Leaves - Midrib: The midrib is slightly concave on the upper side, convex on the lower side. The upper and lower epidermis includes a layer of polygonal cells, the outer side of epidermis is slightly cuticularized, scattered with air holes. The upper angular collenchyma consists of 3-4 layers while the angular collenchyma arranges in clusters, each cluster has 10-12 layers of polygonal cells, smaller than the cells of upper angular collenchyma. The medullary parenchyma consists of polygonal cells, some areas have cells with dented walls, irregular size. The vascular bundles with xylem bundles located above phloem are haphazardly arranged in the medullary parenchyma. The size of the vascular bundles gradually enlarges towards the center.

Leaf blade: The upper and lower epidermis consist of a layer of polygonal cells with cellulose wall. The upper epidermal cells are larger than the lower epidermis. The chlorenchyma contains a layer of rectangular cells, closely arranged and perpendicular to the upper epidermis. The spongy parenchyma has 6-8 layers of polygonal cells, irregular and haphazardly arranged, creating spaces between cells. The vascular bundles are small, arranged in a row in the mesophyll.

Petiole: The outermost epidermis contained a layer of polygonal cells with cellulose walls. The collenchyma cells arrange in clusters under the epidermis, each cluster includes 8-11 layers of polygonal cells, small, haphazardly arranged. The medullary parenchyma consists of polygonal, irregular cells, some cells have curved walls, haphazardly arranged. The outermost ring of vascular bundle has small phloem bundles arranged under collenchymatous clusters. Further inside, there are many vascular bundles haphazardly arranged throughout the medullary parenchyma.

Rhizome: The cross-section of rhizome is circular; the cortical area is smaller than the stele.

Cortex: The phellem consists of 3-4 layers of flattened, rectangular cells, radially arranged, the outer layers are sometimes peeled. The phelloderm consists of flattened oval cells with cellulose walls. Cortical parenchyma includes nearly round cells, arranged to leave small polygonal spaces. The secretory cells are scattered in the parenchyma.

Stele: There are a few vascular bundles scattered in the medullary parenchyma. The directions of differentiation of phloem and xylem are unknown. The medullary parenchyma has many cells containing spiny and needle-shaped calcium oxalate crystals and scattered with cells containing secretions.

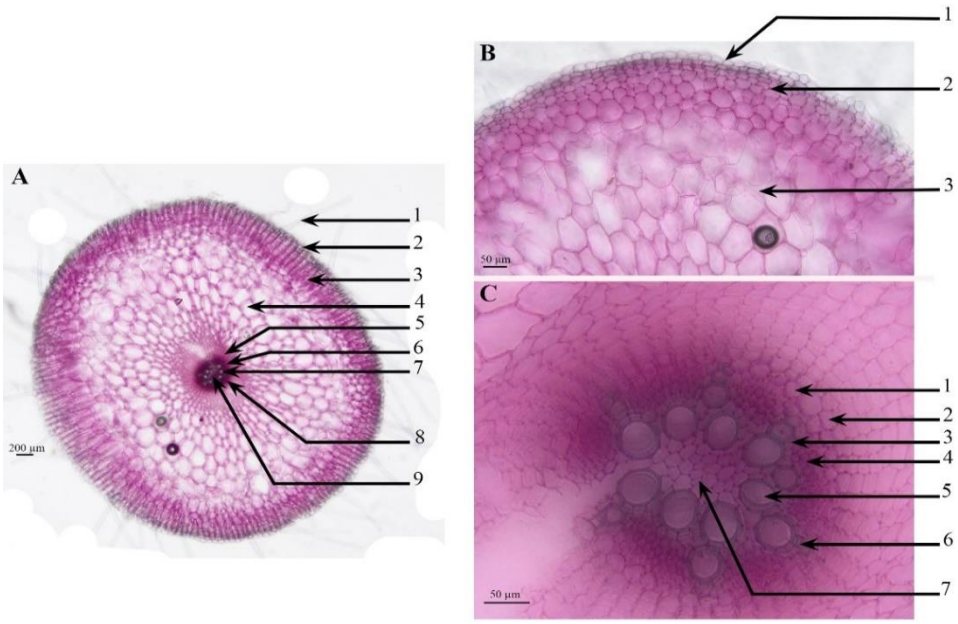

Figure 2: The cross-section of root. A. The whole cross-section (1: root hair, 2: piliferous layer, 3: exodermis, 4: cortical parenchyma, 5: endodermis with casparian *strip*, 6: pericycle, 7: xylem, 8: phloem, 9: medullary parenchyma). B. Cortex (1: piliferous layer, 2: exodermis, 3: cortical parenchyma). C. Stele (1: endodermis with casparian *strip, 2:* medullary ray, 3: pericycle, 4: phloem, 5: metaxylem, 6: Protoxylem, 7: medullary parenchyma)

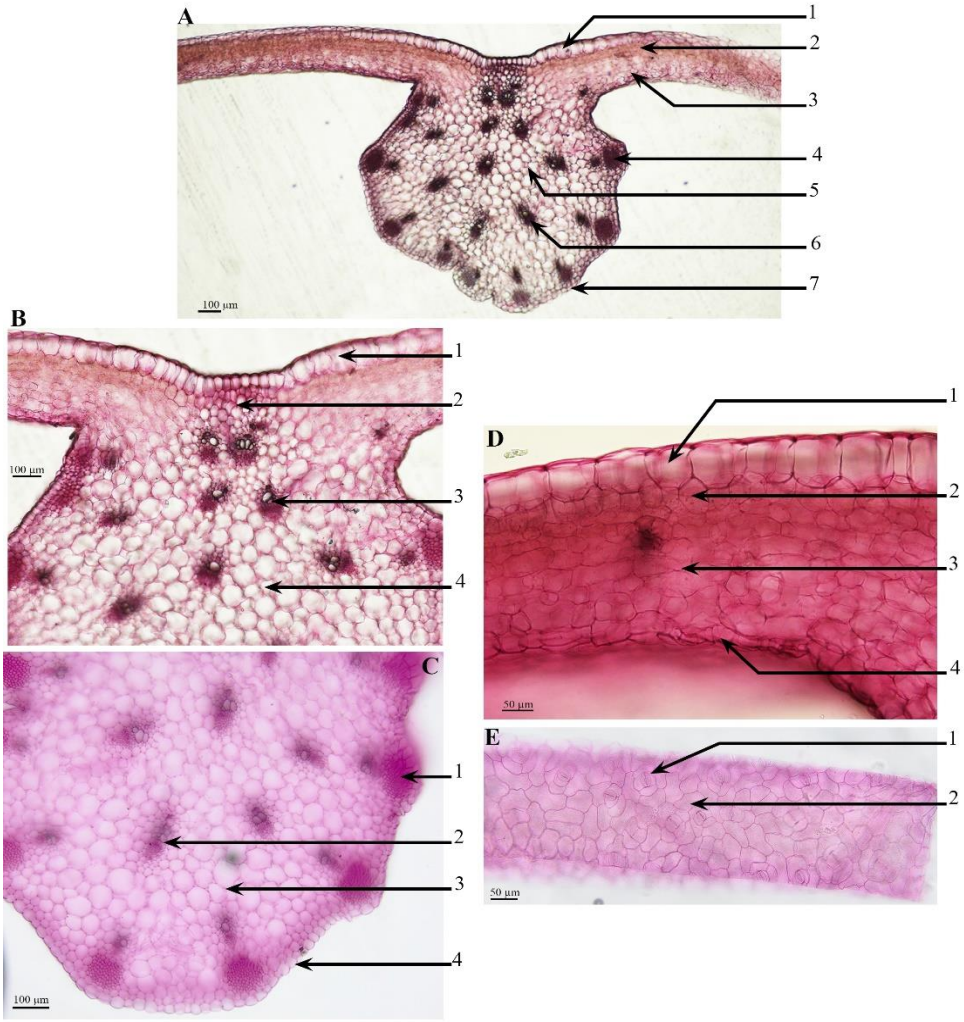

Figure 3: The cross-section of leaf. A. The whole cross-section of midrib (1: upper epidermis, 2: chlorenchyma cell, 3: spongy parenchyma, 4: collenchyma, 5: medullary parenchyma, 6: vascular bundle, 7: lower epidermis). B. Upper side of midrib (1: upper epidermis, 2: collenchyma, 3: vascular bundle, 4: medullary parenchyma). C. under side of midrib (1: collenchyma, 2: vascular bundle, 3: parenchyma, 4: lower epidermis). D. Leaf blade (1: upper epidermis, 2: chlorenchyma cell, 3: spongy parenchyma, 4: lower epidermis). E. Epidermis on the underside of leaf blade (1: stomata, 2: epidermis cell)

Chemical compositions of the acetone extract from *A. pierreanum*: Table 1 showed the chemical components of acetone extract isolated from *A. pierreanum*. A total of 29 compounds were identified in the studied sample of which neophytadiene (17.04%), 2-pentanone, 4-hydroxy-4-methyl- (11.14%), phytol (10.41%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.83%), n-hexadecanoic acid (7.62%), stigmasterol (6.41%) and 2-monolinolenin (6.36%) were the major constituents.

Discussion

The morphological traits of *A. pierreanum* is similar to those of *A. condaoense* and *A. liemiana* in characteristics with the yellow spathe[13,24]. However, *A. pierreanum* differs from others in having male part shortly stiped, densely arranged, light yellow and non-grouping stamens. Furthermore, according to the anatomical characteristics, the rhizome of *A. pierreanum* consisted of so many needle-shaped and spiny-shaped calcium oxalate crystals. Numerous calcium oxalate crystals have been discovered in Araceae species, leading to reports of swelling in the lips, throat and mouth when consumed in their raw state. Thus, the calcium oxalate crystals have been believed as an important indicator of the family Araceae[1,3,10,17,19]. Studies also provided various kinds of calcium oxalate crystals in the *Arisaema* plants. For instance, *A. leschennautii* contained mainly the needle-shaped calcium oxalate crystals[1] whereas the prismatic calcium oxalate crystals have been found in *A. propinquum*[15].

Studies provided the chemical components of different solvent extracts and essential oils isolated from so many species belonging to the genus *Arisaema* using GC-MS technique. For example, the chemical compounds from ethanol extract and its fractions such as chloroform and n-hexane isolated from the leaf of *A. tortuosum* were determined.

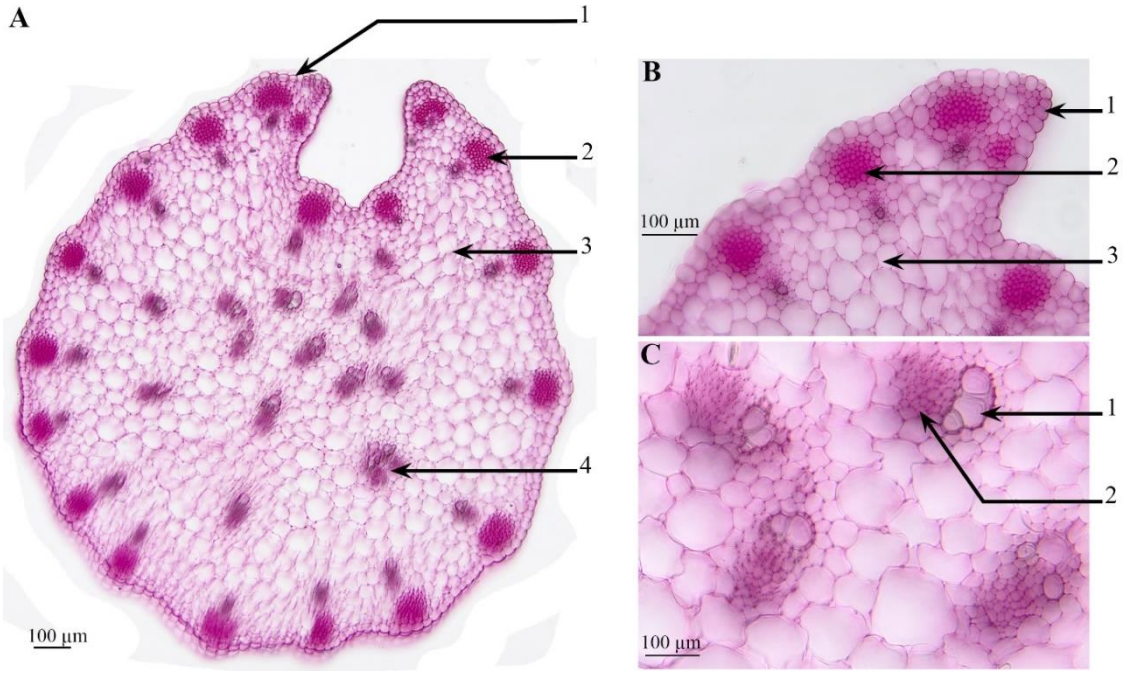

Figure 4: The cross-section of petiole. The whole cross-section (1: epidermis, 2: collenchyma, 3: parenchyma, 4: vascular bundle). B1: epidermis, B2: collenchyma, B3: parenchyma. C1: xylem, C2: phloem.

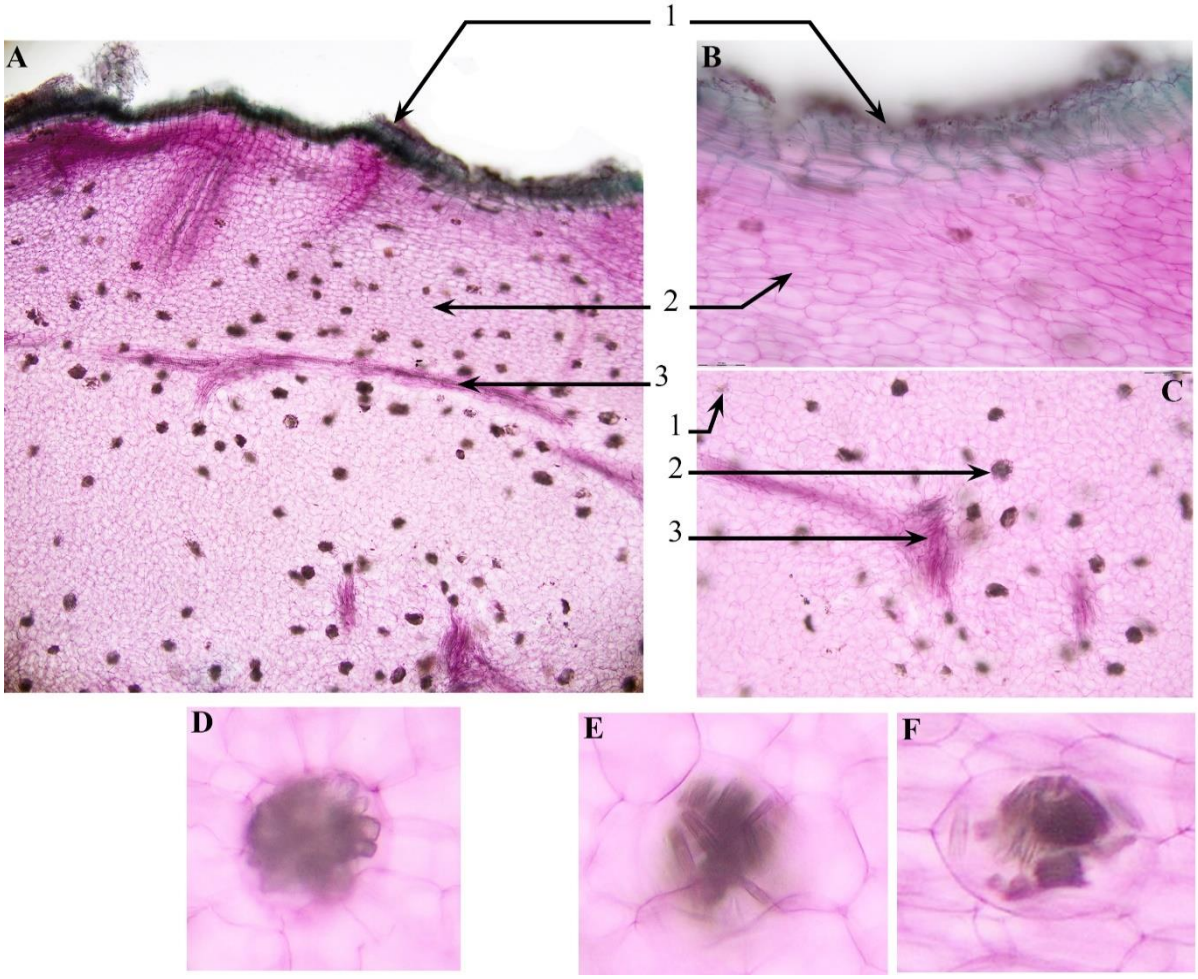

Figure 5: The cross-section of rhizome. A. Sector of cross-section (1: phellem, 2: spongy parenchyma, 3: phloem-xylem). B. Cortex (1: phellem, 2: spongy parenchyma). C. Stele (1: needle-shaped calcium oxalate crystals, 2: spiny-shaped calcium oxalate crystals, 3: phloem-xylem). D. Spiny-shaped calcium oxalate crystals. E and F. Needle-shaped calcium oxalate crystals

Table 1
Chemical composition of acetone extract from *A. pierreanum*

S.N.	RT	Compounds	%
1	2.59	2-Pentanone, 4-hydroxy-4-methyl-	11.14
2	3.50	2-Heptanol, acetate	0.28
3	4.48	Glycerin	0.26
4	7.82	Pyranone	0.84
5	8.66	Coumaran	2.54
6	8.73	5-Hydroxymethylfurfural	0.30
7	8.92	Acetin, 1-mono-	0.22
8	9.58	Phenol, 5-ethenyl-2-methoxy-	0.18
9	9.96	Benzaldehyde, 4-hydroxy-	0.08
10	10.57	2,6-Cresotaldehyde	3.37
11	11.42	Phenol, 2,6-dimethoxy-4-vinyl-	0.36
12	12.32	p-Coumaric acid, trans	0.52
13	13.03	Neophytadiene	17.04
14	13.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.83
15	13.69	n-Hexadecanoic acid	7.62
16	14.41	Phytol	10.41
17	14.56	9(E),11(E)-Conjugated linoleic acid	1.98
18	14.60	Linolenic acid	4.60
19	14.65	Stearic acid	0.50
20	16.40	Palmitin, 2-mono-	2.89
21	17.67	Linolein, 2-mono-	2.98
22	17.76	2-Monolinolenin	6.36
23	19.65	1-Hexacosene	0.17
24	22.75	17-Pentatriacontene	0.30
25	23.21	Vitamin E	1.22
26	25.31	Campesterol	2.18
27	25.80	Stigmasterol	6.41
28	26.69	γ-Sitosterol	5.82
29	26.86	Stigmasta-5,24(28)-dien-3β-ol, (Z)-	0.29
Total			98.69

Accordingly, the ethanol extract was found to be rich in 9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-; hexadecanoic acid, methyl ester and 9,12-octadecadienoic acid, methyl ester. The major components of chloroform fraction were dibutyl phthalate, 1,2-benzenedicarboxylic acid, bis(2- methylpropyl) ester and 1,2-benzenedicarboxylic acid whereas phytol, ethyl (9z,12z)-9,12-octadecadienoate and hexadecanoic acid, ethyl ester were the main compounds in the n-hexane fraction[11].

Similarly, the methanol extract from *A. tortuosum* leaf was characterized by the predominance of nonane, 6,10,14-trimethyl-2-pentadecanone and phytol[6]. More recently, the chemical compositions of the acetone extract from *A. liemiana* aerial part and rhizome were also investigated.

Accordingly, the major constituents of the tuber extract were 2-pentanone, 4-hydroxy-4-methyl- stigmasterol and γ-sitosterol while the aerial part was found to be rich in 2-pentanone, 4-hydroxy-4-methyl- n-hexadecanoic acid, 2,3-butanediol[22]. The chemical compounds of the essential oils obtained from the various parts of *A. amuremse* were also investigated by Li et al[12]. Accordingly, the fruit essential oil contained hexadecanoic acid methyl ester; (Z, Z)-9,12-octadecadienoic acid methyl ester and 13-octadecenoate as the major components while the leaf essential oil was found to be rich in hexadecanoic acid methyl ester; 6,10,14-trimethyl-2-pentadecanone and (Z) 9-octadecenoic acid methyl ester.

The petiole oils mainly consisted of 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, 1-hexadecanol acetate and 14-methylpentadecanoic acid methyl ester whereas 3-cyclohexyl-1-phenyl propane, perhydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone and 2-pentadecanone were the prominent compounds in the tuber oil[25]. The chemical compositions of the *A. lobatum* essential oil were also provided of which linalool, β-caryophyllene and limonene were the major compounds while *A. franchetianum* oil was found to be rich in linalool, limonene and β-selinene[27]. The oil obtained from *A. fargesii* aerial part was characterized by large quantities of linalool, carvacrol and eugenol[8]. Moreover, the aerial part oil of *A. anurans* contained asarone, cubenol and guaiol as the main constituents[9].

Conclusion

In the present study, chemical constituents, morphological and anatomical characteristics of *A. pierreanum* were first reported. A total of 29 components were identified in the studied sample of which neophytadiene (17.04%), 2-pentanone, 4-hydroxy-4-methyl- (11.14%) and phytol (10.41%) were the major compounds. These findings enhance the understanding of the pharmacological potential of *A. pierreanum* and its further application in the medical field and other related fields.

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