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Comparative Morphology, Anatomy and Phytochemistry of *Cyrtosperma senegalense* (Schott) Engl. and *Alocasia macrorrhizos* L. (Araceae)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Morphological, anatomical and epidermal studies were carried out on two species of Araceae, *Cyrtosperma senegalense* (Schott) Engl. and *Alocasia macrorrhizos* L. to investigate the taxonomic value of their similarities and differences. Morphological features were visually observed. Fresh specimens were dehydrated, wax embedded, mounted, microscopically observed and micrographed. Basic similarities were found in their leaf shape, venation, inflorescence and spathe, seeded fruits, scattered vascular bundles, possession of aerenchyma, and presence of stomata on the abaxial and adaxial leaf surfaces. Conversely, the presence of prickles and sparseness of raphide idioblasts containing a raphide bundle each in *C. senegalense* distinguishes it from *A. macrorrhizos which has abundant* raphides. Phytochemical screening shows differences in their

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alkaloids, saponin, triterpenoids, steroids and glycosides contents. Though these preliminary studies yielded data that revealed their relationship and phytochemistry, further investigations of their cytology using electron microscopy and molecular biology are needed for more diagnostic data to distinguish them from each other and add more incite to their potential in drug discovery.

Keywords: Aerenchyma; Alocasia macrorrhizos; Araceae; Cyrtosperma senegalense; raphide bundles.

1. INTRODUCTION

Araceae, also called the aroid, arum or cocoyam family comprises monocotyledonous flowering plants with palmately, pinnately or pedately dissected commonly arrow-shaped leaves with sheathing base petioles. Their unique characteristic is their inflorescence spadix, an internal spike of crowded flowers on fleshy axis, which is partially enclosed in a spathe, an external modified leaf [1]. They are pollinated by small flies that get temporarily trapped in the spadix. They bear yellow or red juicy berry fruits with seeds. Most of their body parts, especially leaves, petioles and tubers have milky saps with calcium oxalate crystals which make them irritable [2,3,1].

They diverged from the Order Alimastales, most of which got extinct during the Oligocene climate cooling and some reaching Africa, Australia, South America and South-East Asia [4, 5]. They are divided into subfamilies; Aroideae, Lasioideae, Zamioculcadoideae, Monsteroideae, Pothoideae, Lemnoideae, Orontioideae and Gymnostachydoideae. *Cyrtosperma senegalense* is in the subfamily Lasioideae while *A. macrorrhizos* is in Aroideae. The genera *Alocasia*, *Colocasia* and *Xanthosoma* species have monoecious flowers while *Amorphophallus* and *Cyrtosperma* species have hermaphrodite flowers [6,7].

Cyrtosperma senegalense (Scott) Engl. (homotypic synonym of *Lasimorpha senegalensis* (Scott); herbaceous and found in swamps) and *A. macrorrhizos* (herbaceous and commonly called the giant taro) represent Araceae species growing in different environmental conditions with similarities and differences in many parameters. *Cyrtosperma senegalense* is indigenous to Nigeria [8] and is noted for its possession of prickles on petioles, peduncles and underside of main leaf veins. It grows up to 6 – 12 ft (1.83 - 3.66 m) tall [9]. In southern Nigeria, swamp arum fruits are used to treat dysentery and gonorrhea while the young leaves are used as ulcer remedy and vegetable in Gabon [10], thus making it one important case of the medicinal biodiversity of wetlands in Africa [11]. According to Tarawou and Young [8], the powdered seeds proved effective in the removal of Mercury ions from aqueous solutions.

Alocasia macrorrhizos which gets as big as 15 feet (4.57 m) tall and 10 feet (3.05 m) wide in optimum terrestrial growth conditions is native to Phillipines and Asian mainland and can be found along roads, waste places and forests. Its elephant ear-like leaves, jute (tilting) skywards instead of drooping [2,12] Though it is regarded as a weed in Africa and tropical America, it is cultivated for its edible tubers in Malaysia, Sri Lanka and Bangladesh [13,14,15]. The tubers are thoroughly boiled to dematerialize the irritable calcium oxalate crystals before consumption [5]. It is frequently grown at home and outdoors as ornamental plant. The rootstock, which is seen as cooling and diuretic is a mild laxative useful in treatment of inflammations, piles, rheumatism, constipation, jaundice and as an astringent [16].

Vegetative and reproductive morphological information constitute important and diagnostic sources of evidence for classification [17]. Hence they are traditionally used as sources of discriminatory evidence at all taxonomic levels, particularly at the specific and generic ranks [18, 19] In the Araceae, most diagnostic morphological information include: floral and inflorescence forms, leaf shape and coloration pattern. Taia [20] and Osuji and Nwala [21] underscored the value of morphological and anatomical features in plant classification, especially the aroids. Architecture of the vascular system, idioblasts and epidermal structure [22] presence or absence of ergastic substances [23], trichomes, stomata, cuticles and leaf architecture [21,24,25] have been frequently used to draw systematic conclusions.

Calcium oxalate crystals, which irritate the skin, and inflame the oral cavity and mucous membranes are common in Araceae [22]. Though Prychid and Rudall [3] reported that the druse, a type of Calcium oxalate crystal, may function as main irritant in toxic organs of plants,

Konyar et al. [26] stated that the presence and type of calcium oxalate crystals is not absolutely correlated to the toxicity of plant organs. However, Osuji and Nsaka (2014) showed the presence of raphide bundles of calcium oxalate in the edible Nigerian aroids. This finding agrees with Prychid and Rudall [3] and Osuji [1] that crystals of calcium oxalate play a taxonomic role as their location, type, quantity, shape, frequency of occurrence and distribution may be used for both taxonomic classification and delimitation. These crystals have been found in specific tissues such as epidermis, airspaces, cortex, or distributed in all parts of the plant [27,26] and have been implicated as waste product in plants, objects of defensive mechanism, stored products or bye products of metabolism [27].

Considering the medicinal and ecological peculiarities of these two aroid species, morphological, anatomical, epidermal and phytochemical studies were conducted on them to gain clearer understanding of their taxonomic relationship (i.e. similarities and differences) and phytochemistry as these could contribute more insight into their potential drug discovery values.

2. MATERIALS AND METHODS

2.1 Plant Materials

Samples of *A. macrorrhizos* were collected from the Bioresources Conservation area in the University of Port Harcourt while samples of *C. senegalense* were collected along New Calabar River in Rivers State in the month of March when the swamp is dry enough to permit entry and access to the plants. Voucher specimens of both plants UPH/P/240 (*A. macrorrhizos*) and UPH/238 (*C. senegalense*) were deposited at the University of Port Harcourt Herbarium.

2.2 Morphology

Vegetative and reproductive morphology of the two species were carefully observed, photographed and described.

2.3 Epidermal Studies

Following the method of Osuji and Nwala [21], upper and lower epidermal membranes of leaves of the two species were peeled. The peeled epidermes were kept in absolute ethanol till needed. The epidermes were rinsed with distilled water and stained with 0.1 % safranin solution. The peels were then mounted with glycerin on clean glass slides, covered with cover slip and sealed with nail hardener. They were observed and micrographed under the microscope.

2.4 Anatomy

Following the modified method of Ekeke *et al.* [25], fresh parts (petiole, midrib, leaf and root) were fixed in formalin acetic acid (FAA) for 24 hours after which they were passed through alcohol series (30, 50, 70 and 95 % v/v) solution and stored in 100 % ethanol until use. The specimens were embedded in paraffin wax and sectioned. Thin sections of the petiole, midrib and root were obtained by free-hand sectioning using a new blade. Selected thin sections were de-waxed, stained with safranin, rinsed with distilled water and mounted on clean glass slides each with a drop of glycerin and covered with a cover slip. The slides were microscopically studied and micrographed.

2.5 Phytochemical Screening

Phytochemical screening was done on fresh leaves, roots and fruits of *C. senegalense* and leaves, roots and tubers of *A. macrorrhizos*.

2.6 Test for Alkaloids

A total of 5g of plant samples was pulverized and heated in 10 ml of 10 % H_2SO_4 for 5 minutes on water bath. The mixture was filtered, and to three different 2 ml of filtrate, 3 drops of Dragendorff"s reagent, Mayer's reagent and Hager's reagent were added respectively. Precipitation indicates presence of alkaloids.

2.7 Test for Saponin

A total of 2g of plant sample was pulverized and warmed in 5 ml of distilled water for about 5 minutes on water bath and filtered. The filtrate was shaken vigorously for 20 seconds and allowed to stand. Observation of persistent frothing indicates the presence of saponin.

2.8 Test for Carbohydrates (Molisch's Test)

Approximately 2g of plant sample was pulverized, warmed in 5 ml of water on a water bath for 5 minutes and filtered while warm. A total of 1 ml of alpha naphtol solution was added. The test tube was slanted and 1 ml of conc H_2SO_4 was added. A violet-purple-brown colour at the interface indicates the presence of carbohydrates.

2.9 Test for Triterpenoids (Liberman-Burchard's Test)

A total of 2g of plant sample was pulverized and macerated in 5 ml of chloroform and filtered. About 1 ml of acetic anhydride was added to the filtrate followed by 2 ml of conc. H_2SO_4 down the side of the test tube. The appearance of pink-red color at the interface indicates the presence of triterpenoids.

2.10 Test for Steroids (Salkowski's Test)

Plant sample weighing 2g was pulverized; macerated in 5 ml of chloroform and filtered; then 2 ml of conc. H_2SO_4 was carefully added down the side of the test tube. A brown color at the interface indicates the presence of steroid.

2.11 Test for Glycosides (Kedde's Test)

Plant sample weighing 2g of was pulverized, macerated in 5 ml of chloroform and filtered. A total of 3 drops of Kedde A solution followed by one drop of Kedde B solution were added. An immediate purple or violet color indicates the presence of glycosides.

3. RESULTS

3.1 Morphology

3.1.1 Leaf morphology

The two species have palmate leaves held by conspicuous sheathing base petioles, inflorescences that are enclosed in a spathe, and fruits borne in cross-pattern arrangements on their spadix. They share few leaf features but show distinct variation in leaf margin, base, colour and lamina orientation (Table 1). Whereas both *Alocasia macrorrhizos* and *Cyrtosperma senegalense* have entire leaf margin when young, the margin becomes undulate in *A. macrorrhizos* at maturity. Colour of the leaves are very distinct between the two species. The lamina of *A. macrorrhizos* is auriculate while that of *C. senegalense* is overlapped and acutely pointed. The leaves are yellow green in *A. macrorrhizos* but brown in younger and dark green in mature leaves of *C. senegalense*. The lamina of *A. macrorrhizos* is nearly vertical in orientation with leaf apex pointing upwards while that of *C. senegalense* is horizontal with apex bent downwards.

The petiole (leaf stalk) shows distinct variation between the two species. The petiole of *A. macrorrhizos* is tubular without prickles while that of *C. senegalense* is angular with numerous prickles arranged along the angles. Both species have variable leaf sizes on one plant. The leaves of *A. macrorrhizos* reach 30-90 cm in length, are yellow-green in colour, wavy along secondary veins, lance-ovate and are arranged in rosette ascending order (Plate 1a) while leaves of *C. senegalense* are horizontal and reach 20-86 cm in optimum growth. Some leaves of *C. senegalense* (Plate 1b) are sagitate, slightly hastate.

The fruits of both plants are similar in appearance to maize (*Zea Mays*) when stripped of the spadix. *A. macrorrhizos'* (Plate 4a) are milky green in colour, with pinkish star-like ornaments at the tips. The fruits were embedded in a whitish liquid. The fruits of *C. senegalense* (Plate 4b) are reddish brown and bigger. In the wild, the fruits of *C. senegalense* were observed to be eaten by animals. The seeds range from three to five in each fruit. At the time of plant collection for this study, seeds of *C. senegalense* were found to be light yellow and hardened while that of *A. macrorrhizos* had cream colour and were fragile. *A. macrorrhizos* has underground tubers while *C. senegalense* has rhizomes, with which they propagate.

3.2 Anatomy

3.2.1 Epidermal Anatomy

Epidermal structures were very prominent in both adaxial (upper) and abaxial (lower) epidermes of the leaves of both species (Plate 2). There was clear similarity in upper and lower epidermal features of each of the species; and clear distinction between the epidermes of the two species. The ordinary epidermal cells of the upper and lower epidermes of *A. macrorrhizos* were elongated with angular edges while those of *C. senegalense* were elongated but slightly more robust and rounded at the edges. There was clear absence of trichomes on the epidermal structures of both species. However, stomata in *A. macrorrhizos* were of various types which include: tetracytic, brachytetracytic and cyclocytic while stomata in *C. seenegalense* were mostly anomocytic.

3.2.2 Leaf Anatomy

The leaves of both species are clearly dorsiventral (Plate 3) with well-defined single layer of upper and lower epidermes. The leaf lamina of both species has 2-3 layers of palisade

mesophyll and 3-5 layers of spongy mesophyll cells. In transverse section, the leaf of *A. macrorrhizos* has less number and size of lacunae in the midrib (Plate 3). However, there are more laticifers in *A. macrorrhizos* than in *C. senegalense*. Several raphide bundles are present in *A. Macrorrhizos* where they are mostly jutting out from the interior walls of the laticifers than in *C. senegalense.* There are several vascular bundles scattered but larger and more numerous aerenchyma in *C. senegalense* than in *A. macrorrhizos* midrib.

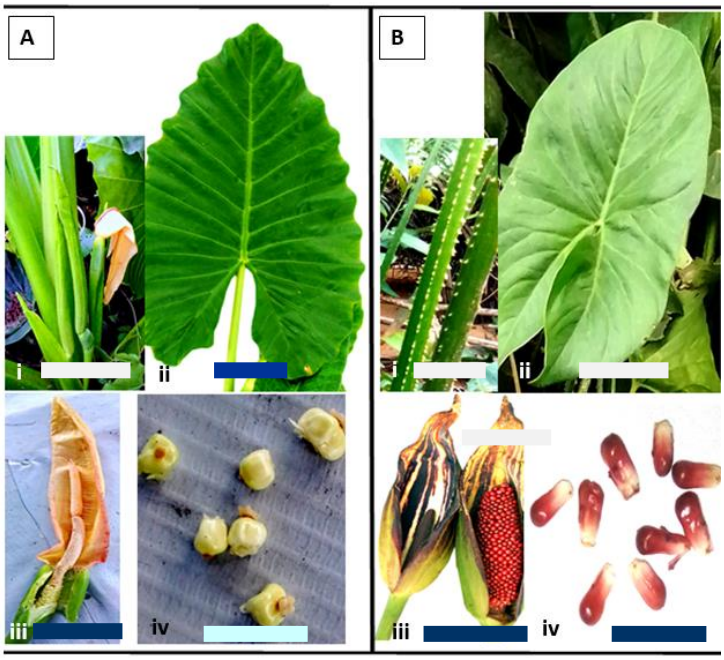

Plate 1. Morphological features of *A. macorrhizos* (A) showing: (Ai) leaf structure, (Aii) smooth petiole, (Aiii) inflorescence and infructescence and (Aiv) off-white-coloured fruits; and *C. senegalense* (B) showing (Bi) leaf structure, (Bii) prickly petiole, (Biii) infructescence and (Biv) maroon-coloured fruits. Scale bar represents 10-15 cm in Ai-iii and Bi-Biii; and 1 cm in Aiv and Biv

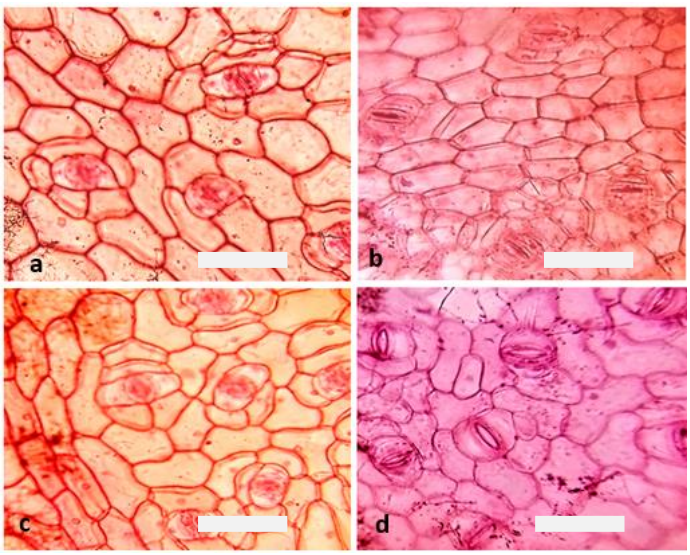

Plate 2. Epidermes of *A. macrorrhizos* and *C. senegalense*. a) Upper epidermis of A *macrorrhizos*, b) Upper epidermis of *C. senegalense*, c) Lower epidermis of *A. macrorrhizos* and d) Lower epidermis of *C. senegalense*. Scale bar represents 40 µm

Table 1. Morphological features of *A. macrorrhizos* and *C. senegalense*

Morphological Feature	*Alocasia macrorrhizos*	*Cyrtosperma senegalense*
Habitat	Mesophytic	Swamp
Foliar Features		
Leaf Type	Simple	Simple
Leaf Venation	Palmate	Palmate
Leaf margin	Undulate (older leaves) /entire (younger leaves)	Entire
Leaf Shape	Sagitate	Sagitate/ hastate
Leaf Apex	Acute	Acute
Leaf base	Auriculate	Overlapped, acutely pointed
Phylotaxy	Spiral/ whorled	Spiral/ whorled
Leaf Colour	Yellow Green	Dark-green (older leaves)/ brown (younger leaves)
Leaf Lamina	Vertical with apex pointing up	Horizontal with apex pointing down
Other Morphological Features		
Prickles	Absent	Present
Petiole shape	Tubular	Angular
Lacunae in Petiole	Present	Present
Underground System	Tubers	Rhizomes
Type of flower	Imperfect	Perfect
Colour of Inflorescence	Yellowish brown to white	Dark Purple
Number of inflorescence on one plant	3-5	1
Lacunae	Laticiferous	Non-Laticiferous
Milky sap around Fruits	Present	Absent
Fruit smell	Weak	Strong
Fruits colour	Milky Green	Reddish Brown
Accessories on Fruits	Present	Absent
Colour of Spathe	Green	Purple/white/green streaks
Nature of seeds in Fruit	Soft	Hard
Number of seeds in fruit	3-5	3-5

3.2.3 Petiole Anatomy

The petioles of *A. macrorrhizos* and C. *senegalense* (Plate 4) are uniseriate (Plate 4). However, the epidermal cells in the petioles of *A. macrorrhizos are more laterally extended than those of C. senegalense* which are more angular inwards. Their vasculature is collateral close. There are more vascular bundles in the outer ground tissue of *A. macrorrhizos* than *C. senegalense*. The vascular bundles in *A. macrorrhizos* are also closer distributed than in *C. senegalense.* Larger aerenchyma were found in the petiole and midrib of *C. senegalense*. In both plants, there are well defined schlerenchymatous tissues at the xylem but phloem fibre is isodiametric in cross-section and more massive in *A. macrorrhizos* while that of *C. senegalense* is like V-shape inverted inward. Plate 4d shows the prickle at the angle of the petiole of *C. senegalense*. The petioles have laticifers, which in *A. macrorrhizos* contain several raphide idioblasts attached to their inside walls but none in *C. senegalense*.

3.2.4 Root anatomy

The root, in transection, is round in both species. The roots of both plants show presence of epiblema, cortex, pith, meta xylem, proto xylem and phloem. A. macrorrhizos' (Plate 5) shows the presence of root hairs, casparian strip, higher number of meta- and proto -xylem.

3.3 Phytochemistry

Though leaves of both species contain saponins, carbohydrates and glycosides, C. senegalense has steroids in the leaves while A. macrorrhizos lacks steroids. A common phytochemical in their roots was triterpenoids. Saponins, carbohydrates and glycosides where present in the root of *A. macrorrhizos* and absent in *C. senegalense.* Based on this result, it is observed that the two plants show low mutuality in their chemistry.

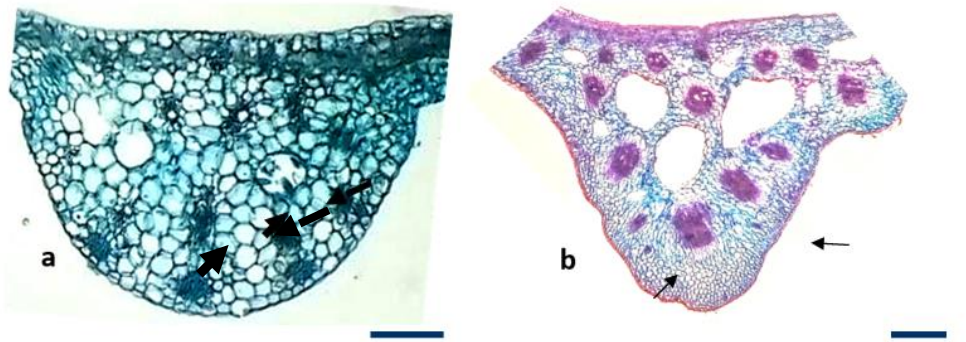

Plate 3. Midrib Anatomy of *A. macrorrhizos* and *C. senegalense.* a) T.S. midrib of *A. macrorrhizos* showing fewer laticifers with raphide bundles (see arrows) and b) T.S. midrib of *C. senegalense* showing three large lacunae running through the midrib without raphide bundles. Scale bar represents 40 µm

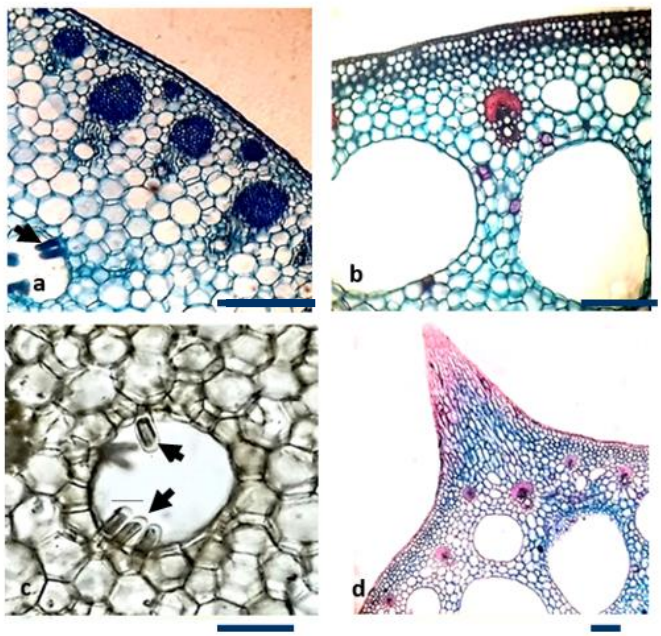

Plate 4. Anatomical features of the petioles and leaves of *A. macrorrhizos* and *C. senegalense*. a) Transverse section of *A. macrorrhizos* with the presence of raphide bundles in raphide ideoblasts (black arrow head), b) T.S. petiole of *C. senegalense*, c) T.S. petiole *A. macrorrhizos* showing presence of raphide bundles each in a raphide idioblast (black arrow heads) sitting on the inside wall of a laticifer, d) T.S petiole of *C. senegalense* showing angular edge of the petiole and absence of raphide bundles in lacunae. Scale bar represents 40 µm

4. DISCUSSION

A. macrorrhizos and *C. senegalense* are informally referred to as wild cocoyams due to shared morphological features with cocoyams, especially *Xanthosoma* and *Colocasia* spp. The similarities found are particular to the Araceae as outlined by Burkil [10], Petersen (1986), Ray [28], Nauheimer et al. [5] and Osuji [1]. A striking difference between the two species is that the leaves of *A. macrorrhizos,* though on the same plant, may differ in details. It is often the case that different species have characteristic leaf shapes which have been used to identify them.

Thermogenesis in the sterile appendix of the spathe, which helps to attract pollinators has been reported [14]. Reproductive mechanism is

different between the two species as *A. macrorrhizos* reproduces both sexually by seeds and vegetatively through tubers and basal offset divisions [29,30]. In cultivation, *A. macrorrhizos* rarely produces flowers hence the vegetative features are mostly used for identification [1]. Observation of hermaphroditic flowers with fruits formed all over the spadix of *C. senegalense* agrees with the reports of Nauheimer et al. [5] and Ivancic and Lebot [6]. Consumption of the fruits by animals in the wild can be a pointer to its medicinal or nutritive value.

Obvious presence of large lacunae in these species attest to long exposure to low oxygen levels, which can induce the formation of lacunae or large air spaces in roots, which also affects the anatomy. In large shrubs growing in marshland, transportation of oxygen from the shoot to the submerged roots is not effective through diffusion. For good aeration of these roots, pressurized internal gas flow is employed. Possession of hollowed midribs, petioles and stems by *C. senegalese* enforce easier transportation of oxygen to the roots. This, in addition to elongated petioles and presence of aerenchyma in roots and rhizomes is an adaptations which it uses to deal with the low oxygen and changing water levels of swamps.

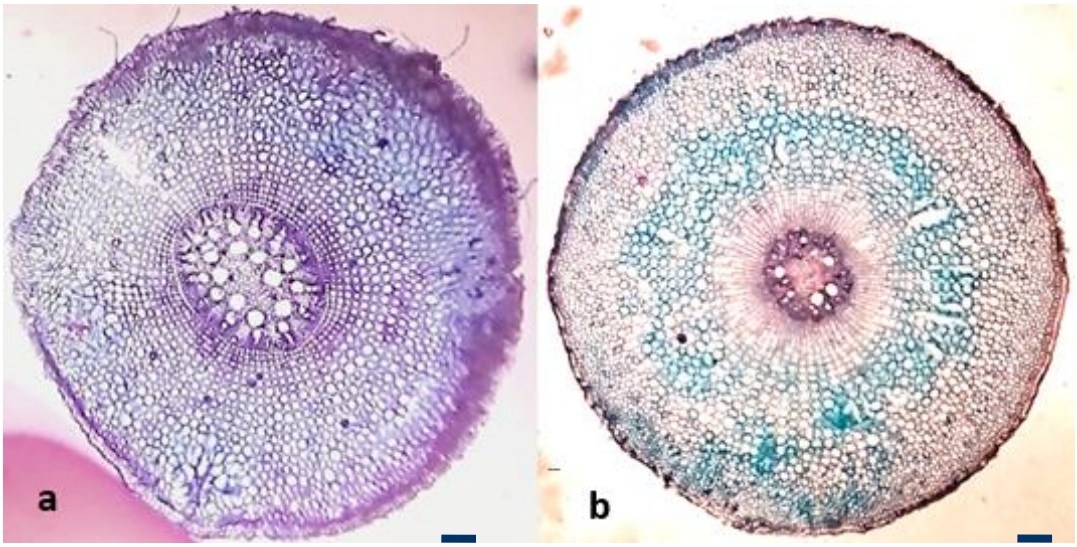

Plate 5. TS Root of *A. macrorrhizos* and *C. senegalense* showing the centralized vascular system and variation in their tissue outlay. Scale bar represents 40 µm

Table 2. Summary of phytochemical screening results of *A. macrorrhizos* and *C. senegalense.*

S/N	Phytochemical	Test	Species	Leaf	Root	Tubers
1	Alkaloids	Drangendoff's/ Meyer's/ Hager's	A.m.	-	-	+
			C.s.	-	-	-
2	Saponin	Frothing	A.m.	+	+	-
			C.s.	+	-	-
3	Carbohydrates	Molisch's/ Fehling's/ Charring	A.m.	+	+	+
			C.s.	+	-	+
4	Triterpenoids	Libermann-Buchard's	A.m.	-	+	+
			C.s.	-	+	+
5	Steroids	Salwoski's	A.m.	-	-	-
			C.s.	+	+	-
6	Glycosides	Kedde's	A.m.	+	+	-
			C.s.	+	-	-

A.m. = Alocasia macrorrhizos; C.s. = Cyrthosperma senegalense; + = presence and - = absence

The presence of anomocytic stomata has been reported among Araceae species [21]. Variation in stomatal types is of taxonomic significance as similarity of stomatal features in related taxa reflect shared genetic background [21]. Hence occurrence of paracytic and brachyparacytic stomata in *A. macrorrhizos* as in *Xanthosoma maffafa* indicate close relationship between them.

Though Konyar et al. [26] reported that the presence and type of calcium oxalate crystals is not absolutely correlated to the toxicity of plant organs, the observation of such ergastic oxalate substance in *A. macrorrhizos* explains its acridity and agrees with the report of Osuji, [1] that connects acridity to occurrence of calcium oxalates in edible aroids.

The midrib and petiole of *A*. *macrorrhizos* possess laticifers with raphide bundles attached to their inner walls. The laticifers are responsible for the secretion of irritable milky sap or latex, reported to contain calcium oxalate crystals in *A*. *macrorrhizos*. These are absent in *C. senegalense.* The presence of irritatable latex is a feature of the Araceae according to Mayo et al. [2] and Osuji [1]. It is notable that occurrence of raphide bundles inside the laticifers implicate the raphide structures as storage forms of calcium oxalate. This evidence supports the observation of Okoli and Green [31] that raphide bundles are storage facilities in which calcium is stored in the form of calcium oxalate. Similarly, Osuji and Ndukwu [27]) reported calcium oxalate as stored and useful products of metabolism, which are regularly translocated from old to young plant parts

Phytochemical contents such as: alkaloids, carbohydrates, saponin, and terpenes observed on crude extracts of *A. macrorrhizos* agrees with the findings of Moghal et al. [16]. According to Moghal et al. [16], the methanolic extract of *A. macrorrhizos* depicts good anthelmintic potential and could be used for prevention of free radical-mediated diseases. The presence of these bioactive secondary metabolites singly or in combination may imply defensive functionality against microorganisms and insects. Phytochemical screening of *C. senegalense* shows the presence of saponins, carbohydrates, terpenoids, steroids and glycosides. Observations in this study corroborate the claim of Onwukaeme et al. [32] of absence of tannins and presence of reducing sugar in *C. senegalense*. The presence of phenolic compounds and flavonoids in this plant suggests antioxidative property and explains the usefulness of this plant in herbal medicine [33, 34, 35].

5. CONCLUSION

It's expedient to conclude that habitats of the two species have, no doubt, elicited structural and biochemical response in them to develop characters that support their survival. Large aerenchyma in *C. senegalense* has very significant value in keeping it buoyant in its aquatic habitat whereas relatively smaller aerenchyma and slightly more schlerenchyma in *A. macrorrhizos* reflect adaptation to its mesophytic habitat that requires physical strength and less buoyancy. Their anatomical variations explain how they have uniquely adapted to their different ecologies for optimal use of solar energy, protective adaptation against herbivores and inter-specific competition by *C. senegalense*, unique storage of calcium oxalate in uniquely idioblasts and their seed variations. This work has also shown their constituent spectra of phytochemicals, which explain their potency as medicinal plants. Further investigations into their cytology, cytogenetics and genomics and time-bound phytochemical screening would reveal more data necessary for their improved exploitation and conservation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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