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Short Communication: Morpho-anatomical and histochemical characterization of four species of Cyperaceae in Rivers State, Nigeria

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Abstract. *Ajuru MG, Chinwe-Chikere L, Ibiye A, Elebachi BO. 2024. Short Communication: Morpho-anatomical and histochemical characterization of four species of Cyperaceae in Rivers State, Nigeria. Cell Biol Dev 7: 68-74.* This research investigated the morpho-anatomical and histochemical characteristics of four species of Cyperaceae (*Kyllinga erecta, Kyllinga bulbosa, Pycreus lanceolatus* and *Mariscus alternifolius*) in Rivers State University. They are perennial, monocotyledonous herbs commonly known as erect greenhead sedge, spikesedge, narrow-leaf flatsedge and umbrella sedge respectively. Hand sectioning method and light microscopy were used for both anatomical and histochemical studies, while meter rule was used for quantitative morphological characters and visual observation was used for qualitative characters. The results for anatomy indicated that epidermal cells are one layered and present in the four species studied, the vascular bundles are all scattered, collateral, conjoint and closed in the stems and are also surrounded by parenchyma cells. The results of histochemical studies showed that there were no calcium oxalate crystals in either the stem or rhizomes of the four species. Results from morphological studies showed that the four species have triangularly shaped stems and are tufted and herbaceous perennial sedges. The four species also have fibrous roots and their leaves are all simple, alternate, glabrous and tristichously arranged. The results also showed that the four species of sedges exhibited a wide range of morphological features that distinguish them from another group of plants, and the characters showed some level of similarities amongst themselves. There were no calcium oxalate crystals in both the stem and rhizomes of all four species studied. Also, the presence of fibrous roots and umbel inflorescence authenticated their classification as monocotyledonous plants. The results of this research would help to improve upon the already existing information about the four species.

Keywords: Cyperaceae, histochemistry, *Kyllinga*, *Mariscus*, morpho-anatomy, *Pycreus*

INTRODUCTION

The family Cyperaceae, commonly called the Sedge family is documented as the third largest monocotyledon family, with approximately 5000 species distributed into 109 genera (Pal and Choudhury 2017; Xu and Zhou 2017). Two hundred and thirty (230) species distributed into 23 genera have been identified in Nigeria. They can be found in different habitats, but more than 80% exist in damp or wet places (Goetghebeur 1998; Pal and Choudhury 2017). The genus *Carex* L. contains about 2000 species and is said to be the largest genus in the family followed by *Cyperus* L. (Bruhl 1995; Goetghebeur 1998; Muasya et al. 2002; Tantwai 2017).

Kyllinga erecta, which is a perennial, erect, tufted, glabrous sedge grows up to about 12-45 cm high, possess thick segmented rhizomes with numerous inter-twisted fibrous roots. It has tough stem made up of an underground rhizome that gives rise to above ground stem and shoots. In some regions, *K. erecta* is a source of nutrition for animals in form of fodder (Burkill 1985). It also plays a role in restoring degraded ecosystems (de Moraes and Sennikov 2021).

Kyllinga bulbosa, a vivacious herbaceous plant, possesses fairly long, creeping, slender rhizomes. It has a creeping stem, which holds the slender inflorescence, but it is swollen at the base in the form of bulbs or tubers, measuring up to 35 cm high. *Kyllinga bulbosa* has been reported to be used for heart problems (Bussmann and Sharon 2006). It is also a good candidate for groundcover, soil stabilization, or erosion control because of its rhizomes and ability to grow in different environments (Bryson and Carter 2008).

Mariscus alternifolius is commonly called umbrella sedge. It is a grass-like, erect, glabrous, tufted perennial plant that grows up to 60 cm high and reproduces from seed. The leaves, linear in shape and up to 30 cm long, arise from the base of the stem and have purplish red leaf sheaths. *Mariscus alternifolius* has attractive foliage and, therefore is used as an ornamental plant. It is also tolerant of wet conditions and it is used for erosion control along water bodies and in landscaping projects (Huxley 1999).

Pycreus lanceolatus is commonly called lance leaf umbrella-sedge. It is a densely tufted perennial sedge which grows up to about 20-60 cm high and reproduces from seeds and slender rhizomes. The linear shaped stem is 3-angled in cross-section, with a dark brown colored leaf sheath (Goetghebeur 1998). This plant provides food and serves as shelter for different wildlife species. It is also used in the treatment of stomach aches, diarrhea and skin

infections (Andrew 2007). Also, the plant is used as a forage for goats and cattle in some regions.

The study of morphological and anatomical structures of plants is highly essential and can be used in plant breeding programs and different agricultural activities. Knowledge of plant morphological Knowledge of plant morphological characteristics can help breeders select desirable characteristics for crop improvement and weed management strategies. Several studies have demonstrated the use of morpho-anatomical characters in the taxonomy of closely related species. Importance of morpho-anatomical study for taxonomy was emphasized by Standley (1990) and Hejazi et al. (2012), who used anatomy of leaves for the taxonomical and phylogenetic classification within the genus *Carex*. Based on the qualitative and quantitative morpho-anatomical characters, Plunkett et al. (2013) recognized a new species of Lepidosperma (Cyperaceae) from the mountain of Tasmania. Pashrizad et al. (2014) used morphological and anatomical data in identification of Iranian *Cyperus*. The four species of Cyperaceae in this study: *K. erecta, K. bulbosa, P. lanceolatus* and *M. alternifolius* are used extensively for different medicinal purposes, and several studies have shown that they all have triangularly shaped stems, fibrous roots and several other similarities. This may lead to drug adulteration if not properly identified. Also, literature review indicated that structural studies on this plant species are very scanty, making it difficult to easily differentiate among them. Therefore, this research was carried out to fill the gap by providing more morphological and anatomical characteristics that can aid in distinguishing these four species for taxonomic and medicinal purposes.

MATERIALS AND METHODS

Study area

This study was conducted on the Rivers State University (RSU) campus within the Port Harcourt metropolis, Rivers State, Nigeria, which was established in October 1980. Port Harcourt is an industrialized cosmopolitan city located in the health of Niger Delta. The study area RSU lies south-south of the Niger Delta within latitude 4° 31'-4° 40' N and longitude 70°1-7°10' E. It is on an elevation of about 10-15 m above sea level (Ubong et al. 2015).

Sources of sample collection, identification and preparation

The research study took place from June to August 2024. The samples of the four species of Cyperaceae were freshly collected from biology laboratory field of the University (4°31'-4°40' N, 7°01-7°10'), Nkpolu-Oroworukwo, Rivers State (Table 1). They were taken to the Department of Plant Science and Biotechnology in polythene bags with tags. They were identified and authenticated by Dr. M.G Ajuru, a plant taxonomist in the Department and given the Accession numbers RSUPbH0196-0199. The samples were rinsed in distilled water several times and placed in a sample bottle containing Formalin-Acetic Acid-Alcohol (FAA) mixture in the ratio of 1:1:18 percent of 70% ethanol in preparation for morphological, anatomical and histochemical studies.

Procedures

Morphological procedure

The morphological features of the four selected species, which are of taxonomic importance, were noted on matured living plant samples. Qualitative morphological studies include visual observation of the vegetative and reproductive parts of the plant species. Quantitative morphological traits include the measurement of leaf width, leaf length, petiole length and plant height using a meter rule and a measuring tape. Qualitative morphological plant features studied includes the leaf shape, leaf type, leaf arrangement, plant habit, stem size, color etc.

Anatomical procedure

This was done using the methods of Akomolafe et al. (2017). Plant samples where fixed in FAA to remove excessive pigments. Transverse sections were made by hand using commercial razor blades. They were stained using 2 drops of 1% safranin on clean slides and then rinsed with water. Thereafter, the specimens were mounted on 25% glycerol and observed using digital compound photomicroscope. The observed features include thickness of epidermis, thickness of cortex, diameter of vascular bundles, number of trichomes, length of trichomes and number of cells per mm.

Histochemical procedure

The specimens were fixed in FAA and were rinsed in distilled water before sectioning. The hand sectioning method was employed. Plants were sectioned with sharp razor blades held on the right hand horizontally while the plant part was held with the left hand. Thin sections were made and fixed in petri dish filled with distilled water. After sectioning, good sections were placed on clean slides with the use of a Carmel hair brush. The sections were stained with hydrogen peroxide and silver nitrate for 5 minutes in bright light supplied by 100Watt electric bulb following the method of Silver and Price as modified by Osuji et al. (2013). The stain sections were mounted in glycerin with a coverslip. The microscope slides were observed under a light microscope.

Table 1. Sources of collection of plant materials used for the study

Plant samples	Accession number	Date of collection	Location of collection
Kyllinga erecta	RSUPbH0199	15th-06-2024	Rivers State University
Kyllinga bulbosa	RSUPbH0196	15th-06-2024	Rivers State University
Pycreus lanceolatus	RSUPbH0198	15th-06-2024	Rivers State University
Mariscus alternifolius	RSUPbH0197	15th-06-2024	Rivers State University

RESULTS AND DISCUSSION

Morphological features of the four species of Cyperaceae studied

The morphological features of the four species of Cyperaceae studied are summarized in Figures 1 and 2, Table 2. *Kyllinga erecta, K. bulbosa, P. lanceolatus* and *M. alternifolius* are all herbaceous, perennial, tufted sedge plants with fibrous root systems. *Kyllinga erecta* and *K. bulbosa* are vivacious plants mostly found in the terrestrial habitat, while *P. lanceolatus* and *M. alternifolius* are found in marshland and swampy habitats respectively. They all possess underground stems modified to be rhizomes. The rhizome is slender and slightly long in *K. erecta* and *K. bulbosa*, robust and yellowish in *P. lanceolatus* and woody and aromatic in *M. alternifolius*. The stem of *K. erecta* measures up to 35-50 cm long, has sharp angles, smooth with a trigonal shape and is light green; *K. bulbosa* stem measures up to 35-49 cm long, smooth, thick at the base, with a trigonal shape and is light green; the stem of *P. lanceolatus* measures up to 40-52 cm long. It is trigonal in shape, erect and light green, while the stem of *M. alternifolius* is about 25-40 cm long, trigonal in shape, grooved and lignified in nature and purplish green. The leaves of the four species are all simple, alternate, tristichously arranged at flower base, narrow, glabrous, entire and sessile, with *K. erecta*, *K. bulbosa* and *M. alternifolius* having acute leaf apices, while the leaf apex in *P. lanceolatus* is acuminate. The inflorescence in *K. erecta* and *K. bulbosa* is a spike, and spikelets in *P. lanceolatus* and *M. alternifolius*; it is greenish in *K. erecta*, light green in *K. bulbosa*, golden green in *P. lanceolatus* and greenish to reddish in *M. alternifolius*.

Anatomical structures of the four species studied

The stem anatomical features of the four species of Cyperaceae studied are summarized in Figure 3. Stems of all the four species studied are triangularly shaped with epidermal cell that is one layered, followed by 1-2 layers of hypodermal schlerenchymatous cells in *K. bulbosa, P. lanceolatus*, and *M. alternifolius*, but 3-4 layers in *K. erecta*. The ground tissue in all the species is filled with parenchyma cells with large intercellular air spaces. There is no epidermis, pericycle and pith cavity. Vascular bundles are scattered through the ground tissue, with the larger ones towards the center and the smaller ones towards the periphery. The vascular bundles are collateral, conjoint and closed. Each vascular bundle is surrounded by a bundle sheath of parenchyma cells. The phloem tissue is towards the outside, while the xylem tissue is towards the center.

Figure 1. Morphological characters of the four species of Cyperaceae: A. *Kyllinga erecta*; B. *Kyllinga bulbosa*; C. *Pycreus lanceolatus*; D. *Mariscus alternifolius*

Figure 2. Morphological characters of the four species of Cyperaceae in the field: A. *Kyllinga erecta*; B. *Kyllinga bulbosa*; C. *Pycreus lanceolatus*; D. *Mariscus alternifolius*

Table 2. Morphological structures of the four species of Cyperaceae studied

Character	*Kyllinga erecta*	*Kyllinga bulbosa*	*Pycreus lanceolatus*	*Mariscus alternifolius*
Duration	Perennial	Perennial	Perennial	Perennial
Habit	Herbaceous	Herbaceous cespitose tuft	Herbaceous and tufted	Herbaceous and tufted
Growth form	Sedge	Sedge	Sedge	Sedge
Habitat	Terrestrial	Terrestrial	Marshland	Damp places, swamps
Biological cycle	Vivacious	Vivacious	Vivacious	Robust
Root type	Fibrous	Fibrous	Fibrous	Fibrous
Rhizome	Present, slender and slightly long	Compact underground stems	Present, yellowish in color	Present, woody and aromatic
Stem				
Size	Small (35-49 cm long)	35-50 cm	40-52 cm	25-40 cm
Shape	Trigonal	Trigonal	Trigonal	Trigonal
Nature	Thick at the base, smooth	Sharp angles, smooth	Erect	Grooved and lignified
Color	Light green	Light green	Light green	purplish green
Leaf				
Type	Simple	Simple	Simple	Simple
Arrangement	Alternate	Alternate	Alternate	Alternate
Nature of arrangement	Tristichously arranged at flower base	Tristichously arranged at flower base	Tristichously arranged at flower base	Tristichously arranged at flower base
Petiole	Absent, sessile	Sessile	Sessile	Sessile
Shape	Narrow	Linear	Linear	Linear
Size				
Length	10-23 cm	25-35 cm	30-42 cm	20-35 cm
Width	0.5-0.7 mm	0.3-0.8 mm	0.4-0.8 mm	0.2-0.9 mm
Apex	Acute	Acute	Acuminate	Acute
Surfaces	Glabrous	Glabrous	Glabrous	Glabrous
Margin	Entire	Scabrous	Rough	Scabrous
Color	Deep green	Light bright green	Deep green	Reddish brown purple
Leaf sheath	Deep green	Deep green	Deep green	Purple
Inflorescence				
Type	Spike	Spike	Spikelets	Spikelets
Nature	Dense	Dense	Dense	Subtended by leaf-like bracts
Shape	Ellipsoid to globular	Conical to globulose	Capitate cluster	Cluster
Size				
Length	3-5 mm	2-5 mm	2-4.5 mm	2-3.7 mm
Diameter	2-4 mm	8-10 mm	2-4.3 mm	2-4.1 mm
Color	Light green to whitish	Green	Golden green	Greenish to reddish

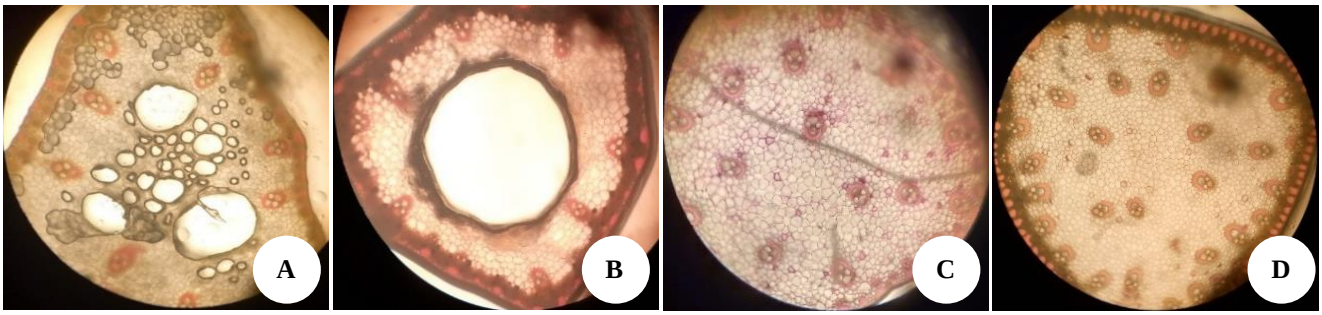

Figure 3. Stem anatomical structures of four species of Cyperaceae: A. *Kyllinga bulbosa*; B. *Kyllinga erecta* stem; C. *Pycreus lanceolatus*; D. *Mariscus alternifolius* (X100)

Rhizome anatomy

This is summarized in Figure 4 below**.** The epidermal cell in all the species is one layered, followed by their ground tissue filled with parenchyma cells. There are starch/ food droplets all over the parenchyma cells and intercellular air spaces. The central portion of the rhizome is filled with vascular bundles made up of phloem and xylem tissues, surrounded by bundle sheath of parenchyma cells. There are starch/ food droplets all over the parenchyma cells and intercellular air spaces.

Histochemical studies

Stem histochemistry

There were no calcium oxalate crystals in the stem of all the species, as shown in Figure 5.

Rhizome histochemistry

There were no calcium oxalate crystals in the rhizomes of all the species.

Discussion

The stems of the four species of Cyperaceae studied are triangularly shaped, tufted and herbaceous perennial sedge as stated by Govaerts et al. (2007). The stems of *K. bulbosa*, *K. erecta* and *P. lanceolatus* are light green, as reported by Gordon-Gray (2006) while the stem of *M. alternifolius* is purplish green. The four species of Cyperaceae studied all have fibrous root types, as stated by Simpson (2011). They are all vivacious sedges except *M. alternifolius* which is robust, and they all possessed underground rhizomes, which conforms with the study by Metcalfe and Gregory (1971), who stated that rhizome is the main underground stem of Cyperaceae.

Kyllinga erecta and *K. bulbosa* are found in terrestrial habitats, while *P. lanceolatus* is found in marshland habitat, which aligns with the study of Govaerts et al. (2007), who stated that Kyllinga species usually grow in dry, terrestrial environments while *P. lanceolatus* thrives in marshy places, and *M. alternifolius* is found in damp places and swamps. As stated by Metcalfe and Gregory (1971), that *M. alternifolius* can easily be found in swampy environment. The rhizome is present in all four species studies, and it aligns with the study of Govaerts et al. (2007).

Kyllinga erecta, *K. bulbosa*, *P. lanceolatus* have light green stem color, while the stem color of *M. alternifolius* is purplish green. Their leaves are all simple, alternate, tristichous arranged at the flower base, glabrous and thus are in conformity with the study of Holm et al. (1979) on morphological structures of some species of Cyperaceae.

Kyllinga erecta, *K. bulbosa*, *P. lanceolatus* and *M. alternifolius* have linear leaf shapes, while *K. bulbosa* has narrow leaf shape and this aligns with Govaerts et al. (2007) description in his research work on the vegetative morphology of Cyperaceae family. They all possess sessile petiole, and this aligns with the study of (Holm et al. 1979). They all have dense inflorescence except *M. alternifolius*, which has inflorescence subtended by leaf-like bracts.

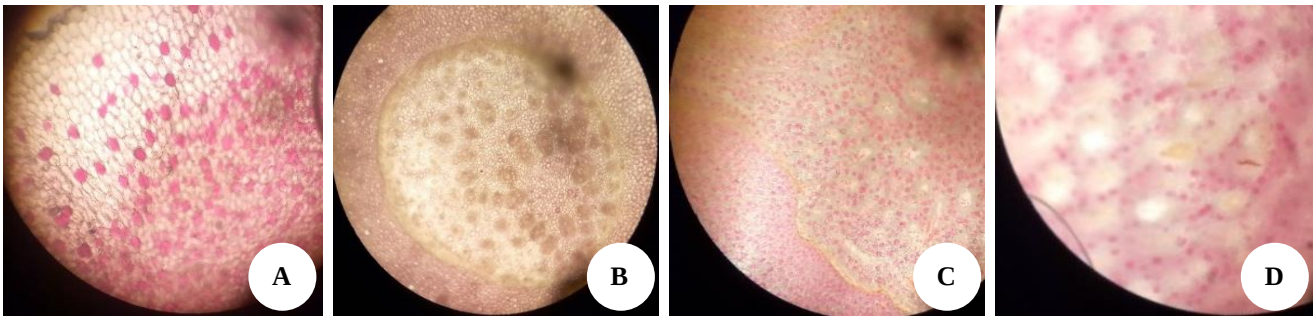

Figure 4. Rhizome anatomical structures of four species of Cyperaceae: A. *Kyllinga bulbosa*; B. *Kyllinga erecta* rhizome; C. *Pycreus lanceolatus*; D. *Mariscus alternifolius* (X100)

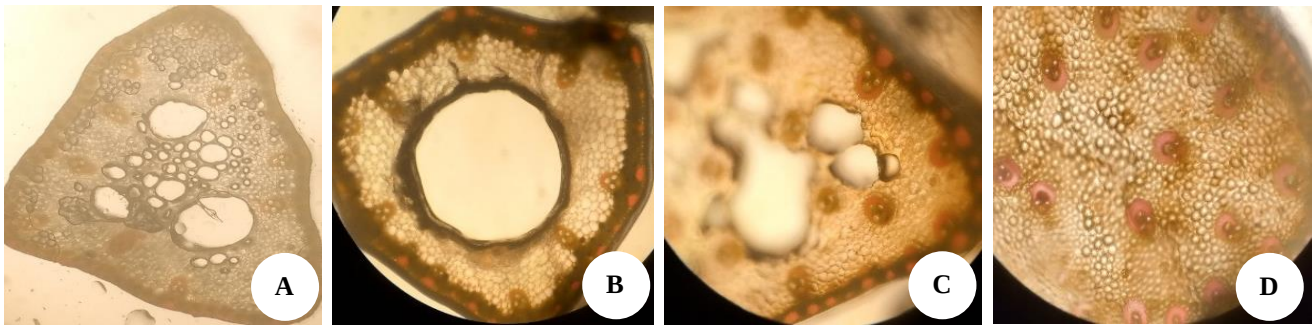

Figure 5. Histochemical localization of crystals in stems of four species of Cyperaceae: A. *Kyllinga bulbosa*; B. *Kyllinga erecta* stem; C. *Pycreus lanceolatus*; D. *Mariscus alternifolius* (X100)

The stem anatomical structures of the plants studied showed that the stem of the four species is triangularly shaped. Their epidermal cell is one layered followed by hypodermal cells which are made up of sclerenchyma tissues as stated by Govaerts et al. (2007) in his study of the anatomical features of Cyperaceae. They all have ground tissues, which are made of parenchyma cells. There is no endodermis, pericycle and pith cavity in *K. erecta, K. bulbosa* and *P. lanceolatus*, but there is in *M. alternifolius* which are not differentiated. The vascular bundles in all four species are collateral, conjoint, closed, scattered all over the ground tissues and are made up of xylem, phloem and lacking cambium. The phloem tissues in the four species are towards the periphery while the xylem is towards the center as reported by Metcalfe and Gregory (1971) and Dickson (2000), who stated that monocotyledons usually have numerous vascular bundles, with the xylem tissues facing the central portion of the stem, while the phloem tissues are usually towards the periphery.

In the rhizome, there is only one layered epidermal cell in the four species, followed by one ground tissue filled with parenchyma cells. There are starch/ food droplets all over the parenchyma cells and this is in alignment with the research of Singh et al. (2015), who stated that rhizomes in plants are modified food storage organs. Several authors like Rodrigue and Estellita (2002), Alonso and Moraes-Dallaqua (2004), made reference to the endodermis in rhizomes.

There was a total absence of calcium oxalate crystals in the stem and rhizomes of the four species of Cyperaceae studied. This may be as a result of environmental conditions which may affect the chemical contents in plants. This is in support of the findings by Fodor (2002), Krupa et al. (2002), and Friday and Ajuru (2014), who stated that toxic metals may cause deficiency of elements essential for plants. Calcium oxalate crystals are very important for plant's overall metabolism because the crystals are essential in calcium homeostasis, calcium storage and removal of excess oxalate, which might have a toxic effect when in excess (Franceschi and Horner 1980; Çalışkan 2000).

In addition, calcium oxalate crystals help support plant tissues and protect plants from attack by herbivores since they are associated with chemicals that cause irritation (Rupali 2012). Therefore, plants that grow in polluted environments will not thrive well as a result of little or absence of these crystals. Also, the presence of these crystals has been reported in leaves and stems of plants (Horner and Whitmoyer 1972; Genua and Hillson 1985; Doaigey 1991; Wu and Kuo-Huang 1997; Meric 2009; Aybeke et al. 2010).

In conclusion, the research study on the morphological and anatomical features of *K. erecta, K. bulbosa, P. lanceolatus* and *M. alternifolius* was carried out. The quantitative and qualitative morphological characters were studied, as well as the description of the transverse sections of stem and rhizomes of the species. The present study will help to improve the existing data for identification and taxonomic purposes, and might be further used to develop a regulatory document on these species for proper identification to avoid adulteration since the plants are essentially used for medicinal purposes. Further work on molecular analysis should be carried out to authenticate the classification of these species for taxonomic purposes.

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