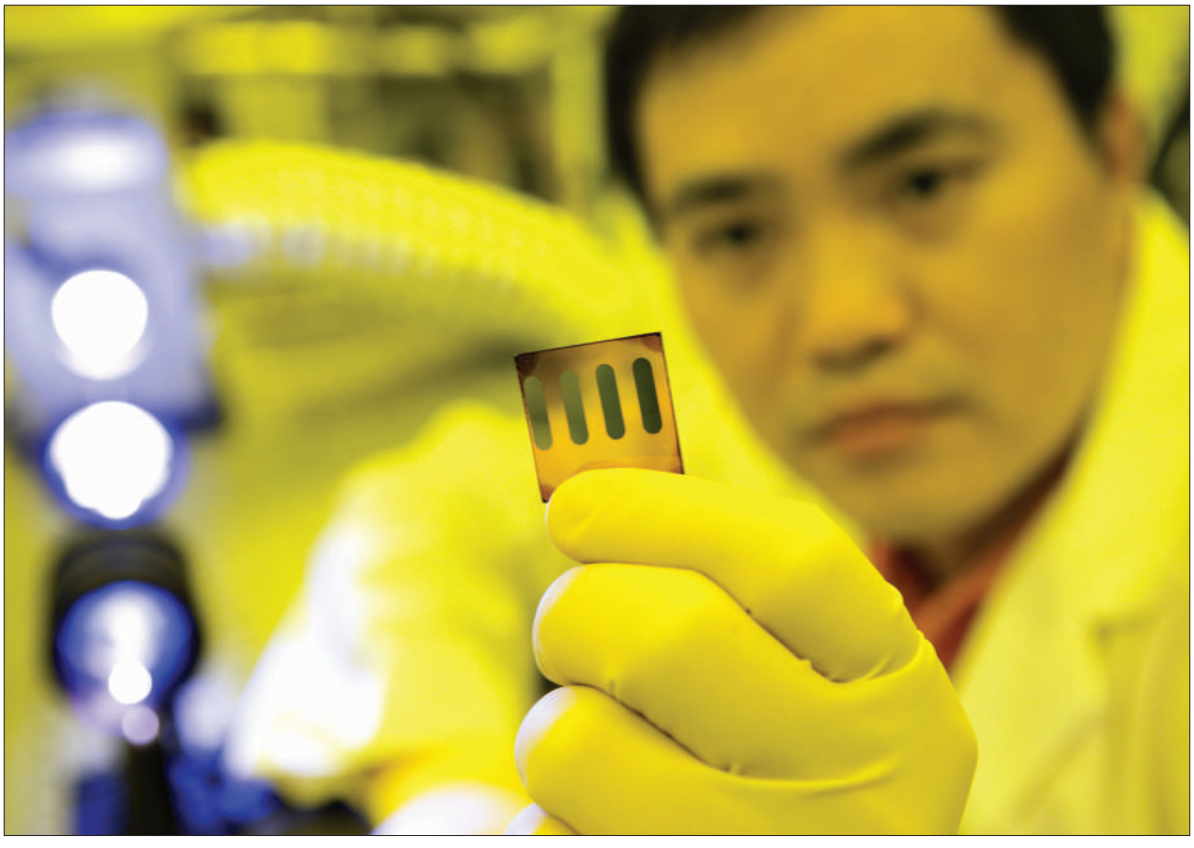

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Research Article
Comparative Anatomical Studies on *Amaranthus spinosus* L., *Celosia argentea* L. and *Gomphrena celosioides* Mart. (Amaranthaceae)

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Abstract

Background and Objective: Plants from the Amaranthaceae are used in folk medicine for their nutritional qualities and for the treatment of various disorders. To classify plants, taxonomists make use of anatomy, phytochemistry etc as taxonomic lines of evidence to determine their similarities and differences in order to group them into various taxa. An evaluation of comparative anatomical studies was carried out on various parts of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* to ascertain their anatomical characteristics that delimit them as members of the family Amaranthaceae. **Materials and Methods:** The transverse sections of roots, stem, leaves and petioles were carried out using microtomy while abaxial and adaxial surfaces of mature leaves were studied using epidermal peels technique. **Results:** Results revealed similar striking features among the species such as possession of anomocytic stomata on both abaxial and adaxial surfaces, collateral vascular bundle and pinkish pigment found in their parts. Differences were seen in number, arrangement and shape of the vascular bundles and number of layers present in various tissues. **Conclusion:** Study concludes that three species were related and this justified their placement under the same family Amaranthaceae while the differences among them suggest the reason for their separation into different genus.

Key words: Amaranthaceae, anatomical, comparative, taxonomy, *Amaranthus spinosus*, *Celosia argentea*, *Gomphrena celosioides*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amaranthaceae, family of flowering plants in the order Caryophyllales is commonly known as the amaranth family, in reference to its type genus *Amaranthus*. It is represented in West Africa by 14 genera and 37 species[1]. The genus *Amaranthus* is represented by 7 species in Nigeria[2]. The genus *Amaranthus* is well known for its weedy species. The monoecious species are largely annuals, self pollinated and successful weeds. Sauer[3] considered that the dioecious *Amaranthus* which are annual have succeeded as weeds not as a result of new gene combination due to hybridization but because of old adaptation of certain species with a long history of survival. The leaves of some members of this family are used as vegetable, examples are *Amaranthus hybridus, Amaranthus spinosus* and *Celosia argentea.*

Amaranthus spinosus is an annual herb up to 60 cm high and reproducing from seeds. The fruit is a one seeded capsule with shiny, lens-shaped reddish-brown seeds[4]. In tropical Africa and elsewhere *A. spinosus* leaves and young plants are collected for home consumption as a cooked, steamed or fried vegetable, especially during the periods of drought. *A. spinosus* is also forage and said to increase the yield of milk in cattle[5]. The plant is astringent, diaphoretic, diuretic, emollient, febrifuge and galactagogue[6]. It is used internally in the treatment of internal bleeding, diarrhoea and excessive menstruation[7]. It is also used in the treatment of snake bites[7]. *Celosia argentea L.* commonly known as plumed cockscomb is a leafy vegetable crop in the family Amaranthaceae. It is one of the most versatile herbaceous plants characterized with fast growth. It has erect stem with alternate leaves and few branches. It is propagated by seed, the seedlings emerge 5-7 days after sowing and flowering 6-7 weeks after sowing[8]. The leaves and tender stems are edible and cooked as vegetables, soups and stews with other ingredients. The young inflorescences are also eaten as potherb[8]. It has a high nutritive value and its grains and leaves are used and processed into many food items, supplements and additives[9]. In Ethiopia and Democratic Republic of the Congo, the seeds of *Celosia argentea* are used as medicine for diarrhea and in Ethiopia, the flowers are used to treat dysentery and muscle troubles[9]. In China and Japan, seeds extracts are used in traditional medicine against jaundice, gonorrhoea, wounds, fever, eye and hepatic diseases. The leaves possess anti-inflammatory and anti-itching activities. *Celosia* production provides a complementary source of income to small-scale farming households[9].

Gomphrena celosioides is an herbaceous annual or perennial and a cosmopolitan pioneer plant of disturbed areas. The fruits are indehiscent and one-seeded and the seeds are flat[4]. It has not been reported to be edible rather it is mostly used for medicinal purposes and as ornamental plant. In different parts of the world is used for various folkloric medicinal purposes. In Brazil, some species are employed in the treatment of bronchial infections, diarrhea and malaria fever, while others had found applications as analgesic, tonic, carminative and diuretics[10].

To classify plants, taxonomists make use of anatomy, phytochemistry etc as taxonomic lines of evidence to determine their similarities and differences in order to group them into various taxa[11]. Anatomy is essentially of great importance in plant taxonomy[12]. Anatomical characters of taxonomic importance include such characters as type of stomata, width of medullary rays, presence of bicollateral bundles, cortical and medullary bundles and anomalous secondary thickening, wood characters, sclerenchyma or pericycle etc.[11]. Anatomical characteristics play a vital role in the determination of relationship in different taxa and their distinctive traits have played a useful role in phylogenetic relationships[13]. Stace[14] discovered that trichome anatomy is very useful in classification at levels from the circumscription of the family down to the separation of species and even varieties. *Amaranthus spinosus* L., *Celosia argentea* L. and *Gomphrena* celosioides Mart grouped into the family Amaranthaceae based on their similarities and into different genus based on differences. Determination of these differences and similarities with regards to anatomy of the three species based on the outcome of the study were the objectives of this research.

MATERIALS AND METHODS

Area of study: The experiments were carried out between the years 2016-2018 in the Botany Laboratory Nnamdi Azikiwe University, Awka, Anambra State.

Procurement and identification of plant species: *A. spinosus, C. argentea and G. celosioides* were procured between the months of April-May, 2016 within the premises of Nnamdi Azikiwe University, Awka (6° 12 N', 7° 04 E') Anambra State. The samples were authenticated and given voucher number by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka.

Anatomical studies

Following materials were used for the anatomical studies: Photomicroscope, a staining jar, a wash bottle, a Reichert sledge microtome, a beaker, Carmel's hair brush, light B. Bran

microscope with the serial No. 4F8662206. The reagents and stains were 97% alcohol, absolute alcohol, 50/50 alcohol/ xylene, xylene, safranin, fast green and Canada balsam as mountant. This procedure is as outlined by Ilodibia and Okoli[11]. Transverse sections were made from middle part of fully grown leaves, midpoint of petiole, centre of an internode of a young and mature stem and mature root. This procedure is as outlined by Ilodibia and Okoli[11].

Procedure: Sectioning of leaf, stem, petiole and root of specimens was done using a sledge microtome. The sections were transferred into a staining jar and stained in safranin for 5 min. The safranin was drained off and the sections washed three times with distilled water. The sections were washed again with 97% alcohol and absolute alcohol for 2 min each and counter stained in 1% fast green for 5 min and then washed with absolute alcohol 4 times. The sections were transferred into a staining jar containing 50/50 alcohol/xylene and washed until they became very clear. Pure xylene was used to finally clear the sections in the staining dish. Canada balsam was then used to mount the specimens on slides. Each slide was carefully covered with a 22×22 mm cover- slip. The mounted specimens were observed under a light microscope and photomicrographs were made. This procedure is as outlined by Ilodibia and Okoli[11].

Leaf epidermal impression: Material used- Mature leaves of three species, alcian blue stain, sharp razor.

Procedure: The abaxial and adaxial surfaces of the leaf were torn to see the transparent area. The transparent areas were cut unto a clean slide, stained with alcian blue, covered with cover slip and examined to see the type of the stomata and the arrangement of the surrounding epidermal cells (termed subsidiary cells). Photographs of good slides were taken with 14.1mega pixels Sony digital camera. This procedure is as outlined by Ilodibia and Okoli[11].

RESULTS

The results of the study are presented in Fig. 1-10.

Anatomy of root: Observations on the transverse sections of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* roots (Fig. 1a-c) showed the following features: *A. spinosus* showed single-layered epidermis, thin cortex and small pith with some conjunctive parenchyma. The vascular bundle showed radial vessel arrangement. *C. argentea*

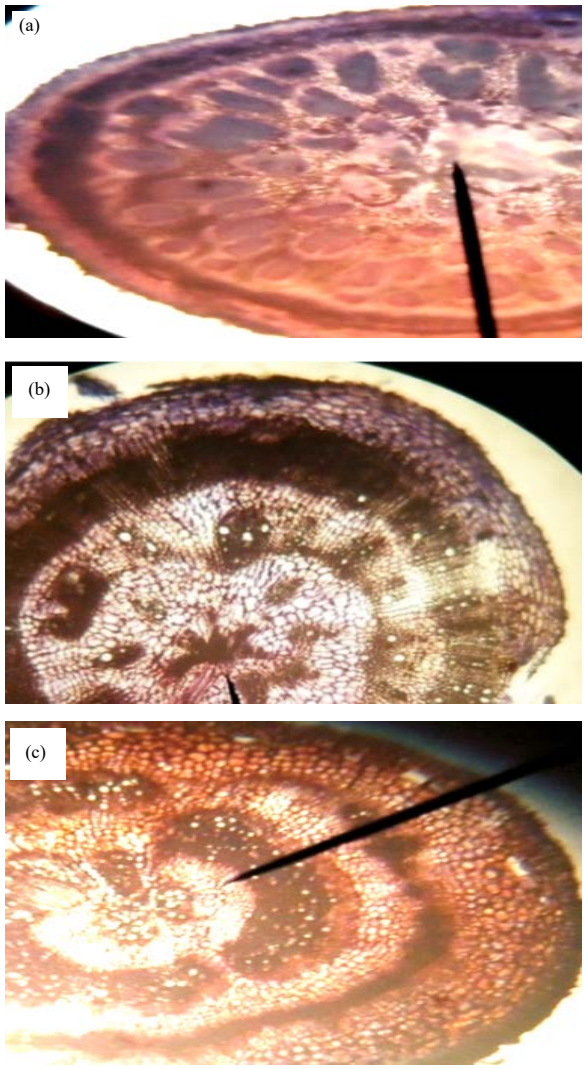

Fig. 1(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* root X40

showed single-layered epidermis, wide cortex and medium sized pith with some medullary vascular bundles. The vascular bundle showed radial vessel arrangement. While *G. celosioides* showed single-layered epidermis, a medium sized cortex and small pith with some medullary vascular bundles. The vascular bundle showed conjoint vessel arrangement.

Anatomy of stem: Observations on the transverse sections of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* primary stem (Fig. 2a-c) showed the following features: *A. spinosus* stem showed single-layered epidermis followed by 2-3 layers of collenchyma cells and 3-4 layers of parenchyma cells with wide pith. The vascular bundles are

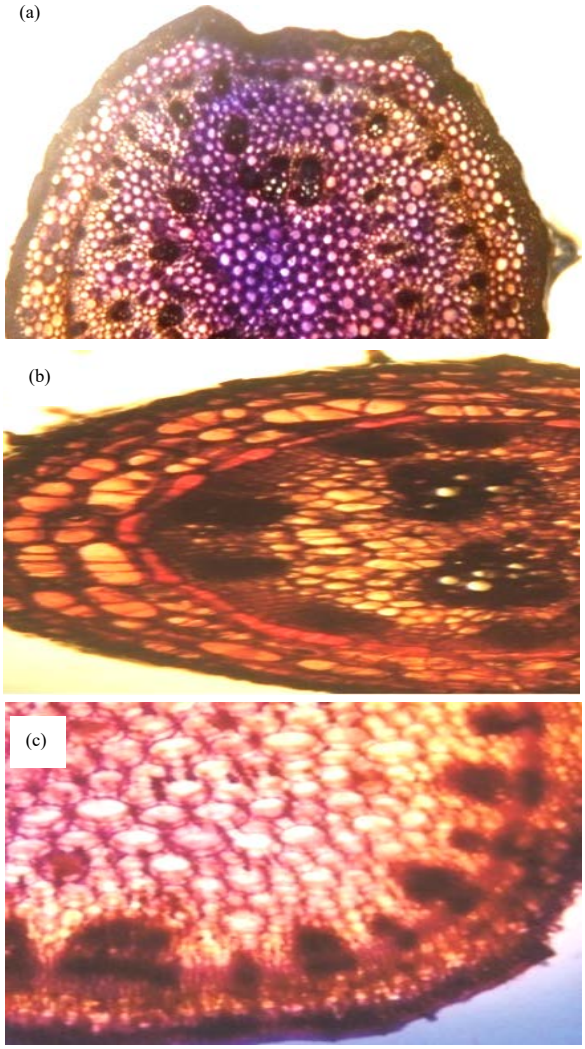

Fig. 2(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* primary stem X40

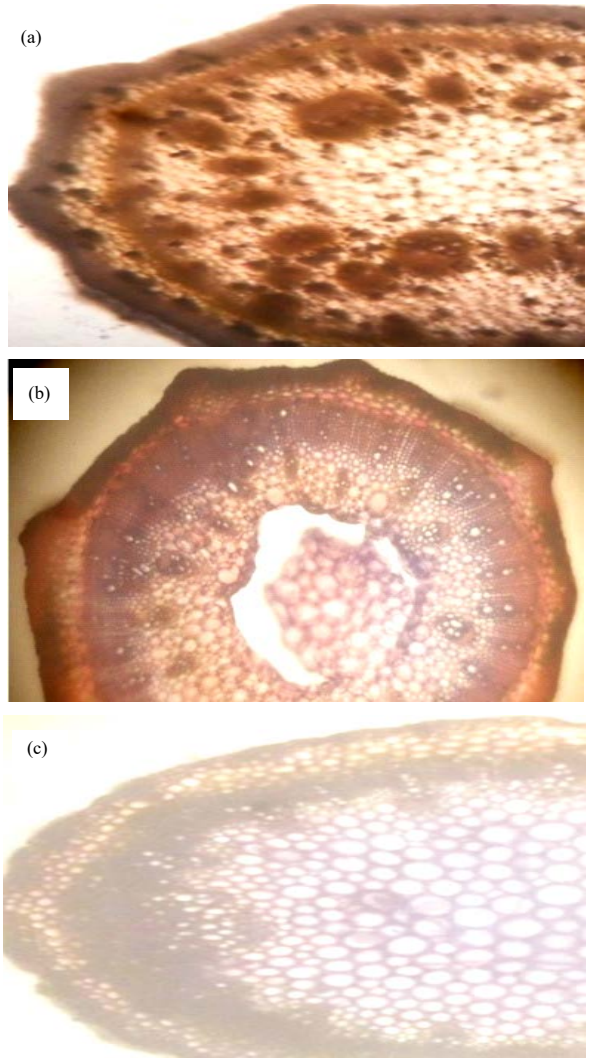

Fig. 3(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* secondary stem X40

conjoint and collateral. *C. argentea* showed single-layered epidermis, 2 layers of collenchyma, 2-3 layers of parenchyma, pinkish endodermis. The vascular bundles are conjoint and collateral with 2 medullary bundles at the centre. The pith was small with parenchymatous cells. *G. celosioides* showed single-layered epidermis, followed by 2 layers of collenchyma, 4 layers of parenchyma and a layer of endodermis on which the vascular bundles are conjointly arranged. There was also presence of wide pith filled with parenchymatous cells.

Observations on the transverse sections of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* secondary stem (Fig. 3a-c) showed the following features: *A. spinosus* showed single-layered epidermis, 4-5 layers of small collenchyma cells, 3-4 layers of parenchyma cells, a layer of endodermis on which conjoint collateral vascular bundles were arranged. Pith made of parenchymatous cells with medullary vascular bundles was also seen. *C. argentea* revealed single-layered epidermis, followed by 2 layered collenchyma cells, 3-4 layers parenchyma cells with blackish deposits. This was followed by pinkish endodermis on which the vascular bundles were arranged. Secondary vascular bundles were seen. The pith was filled with parenchymatous cells which were shrivelling to form a hollow at the center of the stem. There was also presence of deposits (Fig. 4). *G. celosioides* showed single-layered epidermis, 2 layers of collenchyma cells, 3 layers of parenchyma cells followed by endodermis on which vascular bundles were arranged. Also, wider pith filled with parenchyma cells.

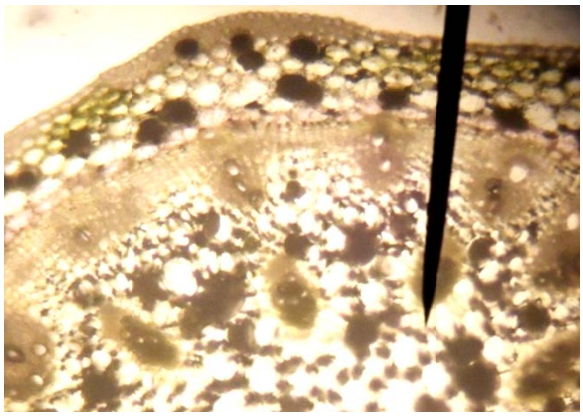

Fig. 4: T/ S of *C. argentea* stem showing deposits X40

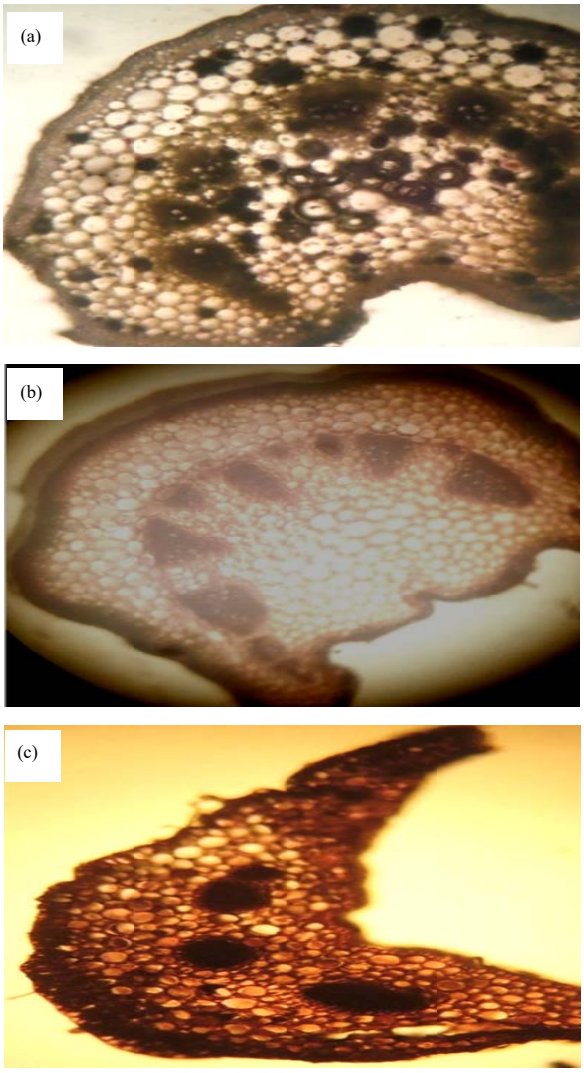

Fig. 5(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* petiole X40

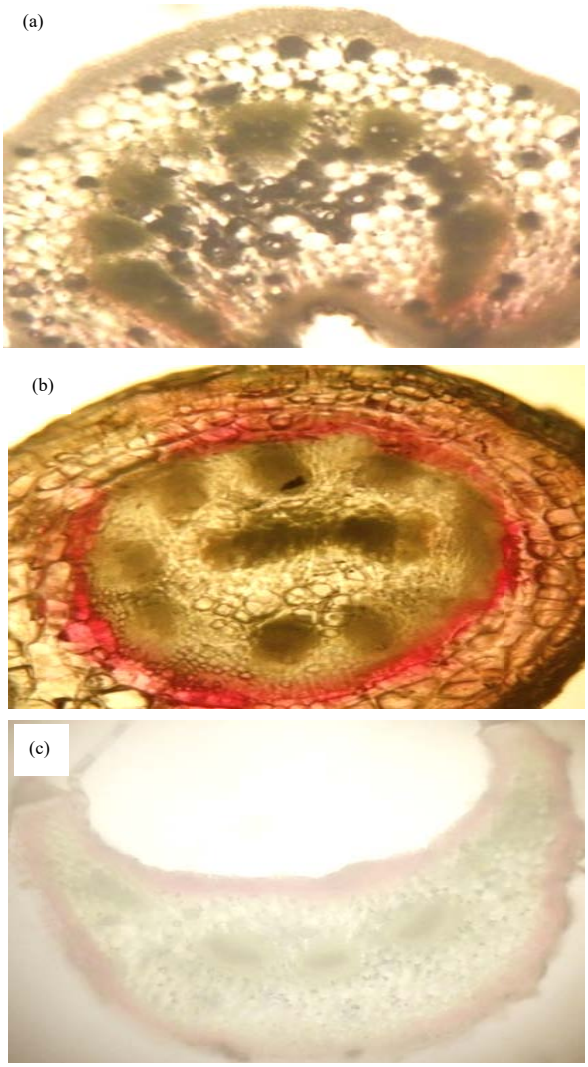

Fig. 6(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* petiole showing pinkish colour X40

Anatomy of petiole: Observations on the transverse sections of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* petiole (Fig. 5a-c, 6a-c) showed the following features: *A. spinosus* had heart shaped and single-layered epidermis, followed by 3 layers of collenchyma cells, 3-4 layers of spherical parenchyma cells. It had 8-11 vascular bundles arranged in cup-shape. It showed some pinkish pigment and black patches of deposits. *C. argentea* had cup-shaped and showed single-layered epidermis, 2-3 layers of collenchyma cells, 5 vascular bundles arranged in a crescent manner. *G. celosioides* had crescent shaped and showed single-layered epidermis followed by 2-3 pinkish layer of collenchyma cells. It had 3 vascular bundles very close to the upper part.

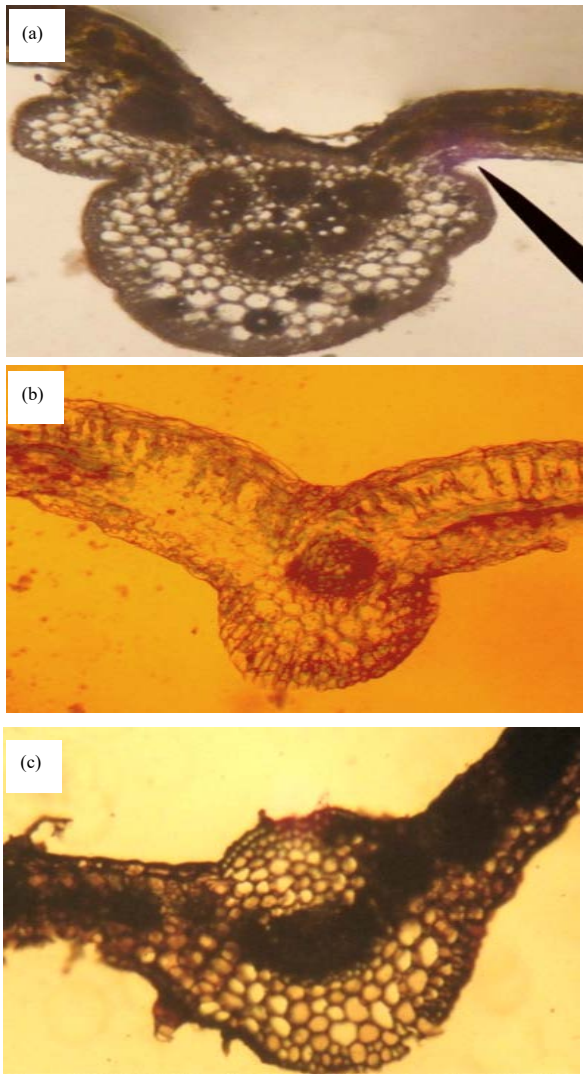

Fig. 7(a-c): T/S of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* leaf X40

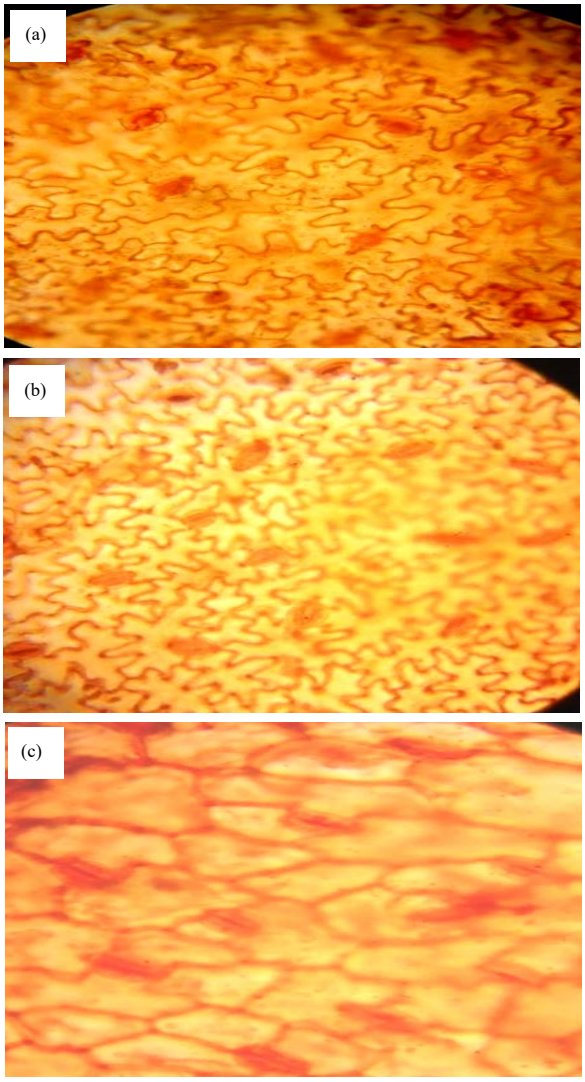

Fig. 8(a-c): Leaf epidermal peel of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* abaxial surface X40

Anatomy of leaf: Observations on the transverse sections of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* leaves (Fig. 7a-c) showed the following features: *A. spinosus* leaf showed single-layered epidermis, followed by 3 layers of collenchyma cells, 3 layers of big parenchyma cells and 2 layers of small parenchyma cells surrounding the five collateral vascular bundles arranged in a circular pattern. The structure also showed some deposits and smaller vascular bundles of the veins. *C. argentea* had single-layered epidermis, 2-3 layers of collenchyma cells and 2-3 layers of bigger parenchyma cells before the vascular bundles which were five in number, conjoint and collateral and were arranged in a semi-circular pattern. Smaller bundles of the veins were also seen. *G. celosioides* had single-layered epidermis with cut trichomes followed by 5 layers of parenchyma cells. It had 3 vascular bundles at the midrib and the other vascular bundles seen were those of the veins.

Leaf epidermal study: Observations on the leaf epidermal study of *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* leaves showed the following features: *A. spinosus, C. argentea* and *G. celosioides* had irregular cells and stomata on both abaxial and adaxial surfaces. *C. argentea* had more stomata, followed by *A. spinosus* and then *G. celosioides.* The stomata were superficial and anomocytic (Fig. 8a-c, 9a-c). The leaf epidermal peel of *G. celosioides* had non- glandular septate trichomes (Fig. 10).

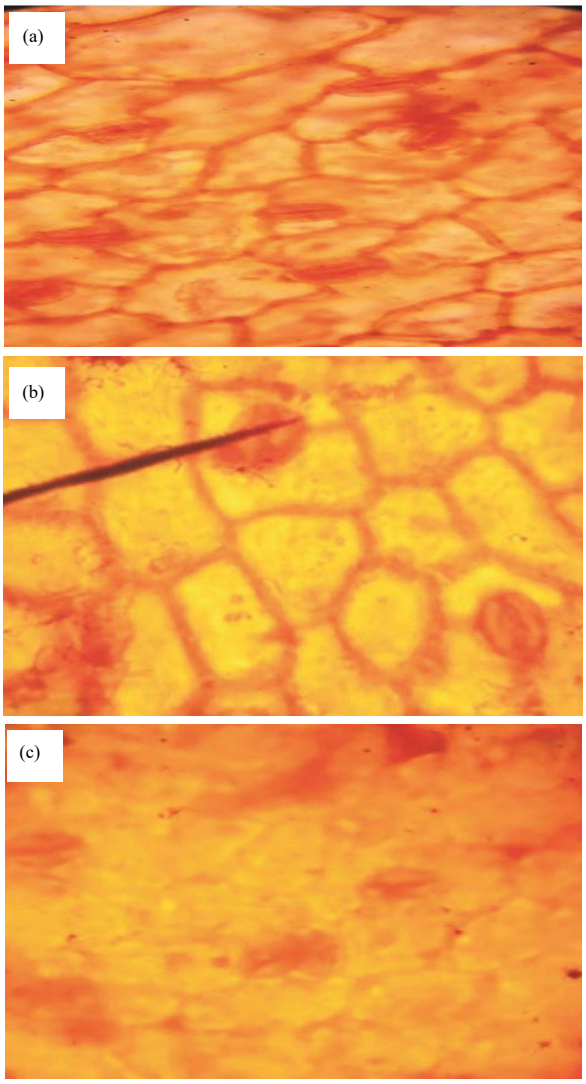

Fig. 9(a-c): Leaf epidermal peel of (a) *A. spinosus*, (b) *C. argentea* and (c) *G. celosioides* adaxial surface X40

DISCUSSION

The result obtained from this study revealed some similarities and differences in the Anatomical features of the three species (Fig. 1-10). Similarities were observed in their possession of uniseriate epidermis, anomocytic stomata on both abaxial and adaxial surfaces, collateral vascular bundle and pinkish pigment found in their parts. These similarities could be the reason for their placement in the same family Amaranthaceae. Differences were seen in number, arrangement and shape of the vascular bundles, petiole and number of layers present in various tissues. *Amaranthus* spinosus is heart-shaped, *Celosia argentea* cup-shaped and

Fig. 10: Trichomes on the epidermal peel of *G. celosioides* X40

Gomphrena celosioides crescent shaped petioles. The primary stem of *Amaranthus spinosus* showed anomalous growth, in that the vascular bundles remains scattered in the ground tissue. Only the leaf of *Gomphrena celosioides* showed cut trichomes. The differences could be the reason for the different genus. The variations in number, arrangement and shape of the vascular bundles, petiole and number of layers present in various tissues strengthen the reliability of anatomical characters in systematic botany as stated by Ayensu[15]. Anatomy is essentially of great importance in plant taxonomy. According to Esau[16], by the use of epidermal Characteristics, a general understanding of the similarities and differences between two related taxa of plants could be elucidated. Shaheen[17] reported variations in the number of secondary vascular bundles in the petioles of some species. And thus was also used as a distinguishing character among the taxa. *Gomphrena celosioides* possession of trichomes, is a feature that has been implicated in xeromorphy helps it to withstand drought and thrives all the year round[18]. The morphology and density of leaf trichomes vary considerably among plant species and may also vary among populations and within individual plants. Their possession of stomata on both surfaces showed that they are mesophytes. AbdulRahaman and Oladele[19] in their study on stomatal complex types, stomatal size, density and index in some vegetable species in Nigeria showed that in *Amaranthus cruentus*, the most frequent stomatal complex was anisocytic, followed by tetracytic, diacytic and anomocytic, while in *Celosia argentea*, the most frequent stomatal complex is anisocytic followed by tetracytic and diacytic. Stomatal density was highest in *Corchorus olitorius* and lowest in *Vernonia amygdalina,* while stomatal index was highest in *Amaranthus cruentus* and lowest in *Celosia argentea.* The results also tally with the work of Bibian *et al.*[20], who had similar results on leaf epidermal features of the seven varieties of *Vigna unguiculata*

(L.). Illoh *et al.*[21] in their study on comparative systematic foliar morphological and anatomical studies of three *Cleome* (Linn.) species in Nigeria showed that the exomorphology of the species, like structures of both the adaxial and abaxial epidermal surfaces, trichome types, presence and absence of kranz tissue, values of stomatal index were valuable in delimiting the species. Their overall result showed that the 3 species have morphological diversity though there were some similarities in their foliar anatomy. Characters like irregular shaped epidermal cells, anomocytic or anisocytic stomata were of diagnostic indices in the taxonomy of the three species as they appear to be generic characters. Anatomical characters have been proved very useful in the determination of relationship in orders and genera and their features have played an increasingly important role in phylogenetic relationships[13].

Information from this study is valuable for proper taxonomic characterisation and identification of the plant species in the family Amaranthaceae.

CONCLUSION

The comparative anatomical studies carried out on *Amaranthus spinosus*, *Celosia argentea* and *Gomphrena celosioides* have indeed provided some information that are useful in their delimitation as members of the family, Amaranthaceae. Nature of their vascular bundles was well developed for efficient transportation, the nature of their stomatal complexes were anomocytic and the possession of hairs or trichomes by *Gomphrena celosioides* which has been a feature implicated in xeromorphy and thus helps it to thrive all the year round and with that *Gomphrena celosioides* can be used as ornamental.

SIGNIFICANCE STATEMENT

This study discovered a new type of stomata (anomocytic stomata) which was present on both abaxial and adaxial surfaces of the leaves of the three species. Pinkish pigment was also discovered in some parts of the three species. This may be an indication that vitamin A may be present in the plants. This study is beneficial to taxonomist by providing anatomical features of the three species which when supplemented with additional characters would guarantee the level of sensitivity needed to correctly identify and delimit species into the family Amaranthaceae. Their possession of pinkish pigment in any of the parts shows that they can be a source of vitamin A since lycopene responsible for that pinkish character has the same structure as carotene. This may be beneficial to food and drug industries in the manufacture of food and drug supplements. This study will help researchers to uncover the critical areas of taxonomic problems that many researchers were not able to explore. Thus strengthens the interspecific relationship for proper taxonomic characterization and identification of the plant species in the family Amaranthaceae.

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