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LEAF EPIDERMAL ANATOMY OF *Ipomoea carnea* JACQ SAMPLED FROM SELECTED AREAS IN GOMBE STATE, NIGERIA

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ABSTRACT

Leaf epidermal microscopy of *Ipomoea carnea* Jacq was studied to investigate the structure of the stomata and epidermal features which may be used for delimitation of the specie. Fresh leaves of *Ipomoea carnea* were obtained from five different LGA,S (Gombe, Y/deba, Balanga, Funakaye and Dukku) in Gombe State, Nigeria. The specimens were studied under light microscope to examine the Stomatal features, Epidermal cell shapes and Anticlinal cell-wall patterns. It had the presence of amphistomatic leaves; one type of Stomatal complex type namely Cyclocytic. Accession 1 had the highest Stomatal Density ($40.00 \pm 1.00\text{mm}^2$) with lowest Stomatal size ($51.13 \pm 7.47\mu\text{m}$) on the Abaxial leaf surfaces while Accession 2 possessed lowest Stomatal density ($23.40 \pm 7.67\text{mm}^2$) with highest Stomatal Size ($88.68 \pm 1.95\text{mm}^2$) on the Adaxial leaf surfaces. Curved anticlinal cell wall patterns with polygonal epidermal cell shapes were also observed. It was concluded that the presence of Cyclocytic type of stomata, with large stomatal sizes greatly helped in the delimitation of the plant and could also be used for classification/identification of the plant and some of the features such as trichomes could also be used for adaptation purposes.

Key words: Epidermal, Stomata, *Ipomoea carnea*, Cyclocytic, Trichomes.

INTRODUCTION

Ipomoea carnea belongs to the family Convolvulaceae and is popularly known as Besharam, Behaya in India, and Bush Morning glory in English, Kashe Kori or Kafi- Kancela in Gombe State, Nigeria. Most members of the family are twining and erect herbs, with a few woody vines, trees and shrubs. The family is widespread in both tropical and temperate areas. Bhattacharyya and Midya (1979) and Frey (1995) indicated wide ecological amplitude for this plant as much as they observed it growing in xeric and hydric conditions. Originally from the tropics of South America, this evergreen, flowering shrub has spread to Asia particularly in Chhattisgarh and Madhya Pradesh as reported by Indian Herbal pharmacopoeia, (Mumbai, 2002), Pakistan, Srilanka, North America including American tropics, Argentina, Brazil, Bolivia, Africa, and Gombe in Nigeria . It invades fallow land and shallow wetlands. It is extremely hardy and is resilient to several forms of chemical and biological control. As a result, there have been numerous attempts to find a use for the plant. It has been reported to have medicinal properties and is used in traditional medicine in several countries. This plant can be used for various purposes such as a recent attempt at using the plant to produce bio-gas seems promising. It could also be used in farm lands as a source of green manure/ improving the soil fertility in tropical countries like India and elsewhere where it is prevalent which may be due to the addition of essential nutrients to the soil by the leaf incorporation which finally helped in the increase of grain yield (Kondap *et al.*, 1981). Aqueous flower extracts at 5% or higher of this plant showed the greatest nematicidal properties against the second stage of *Meloidogyne incognita* (Nikure and Lanjewar, 1981), aqueous extracts of dried and powdered corolla,

senescent leaves and roots of *Ipomoea carnea* inhibits the shoot and root growth of wheat, sorghum, rice and kidney bean (Jadhav *et al.*, 1997), The leaves has poisoning effects on the nervous system (Idris *et al.*, 1973, Tirkey *et al.*, 1987) and toxicity in goats (Indrajit and Pathak, 1995). It is however palatable for various fish species (Frey, 1995). The latex of the plant has been used in traditional medicine as a topical antiseptic in lesions (Chowdhury *et al.*, 1997), extracts prepared from whole plant in hot, not boiling, water seem to be widely used as antirheumatic remedy in Bolivia. Frey (1995) also reported a new use of entire *Ipomoea carnea* subsp. *fistulosa* as a raw material for paper-bag production in the surroundings of Tiruchirapalli and along the Eastern Ghats in India and it can be applicable elsewhere in the world. He reported a rare use of dried stem material as fire- wood in Rajasthan, because of its yellow flame. Van den Berg (1982) reported the use of plant against dermatoses without referring to any sources. Also the wood of the stems can be used in turneries, which seems doubtful considering the low amount of lignin in the soft wood (Sharma *et al.*, 1989). It can also cause some problems such as obstruction and difficulties in the proper use of the irrigation, navigation, and fisheries (Chaudhuri *et al.*, 1994), In Egypt, the farmers use *I. carnea* as ornamental and hedge plant along the banks of irrigation and drainage canals (Eid, 2002). It is in view of the importance of this plant that this research is being carried out. The present work, which forms a part of the investigation of stomatal and epidermal studies of *Ipomoea carnea*, is an attempt to fill in the gap in our knowledge and to provide a base for better under accessionsing of epidermal characters which might be useful to delimit the species.

MATERIALS AND METHODS

Microscopic Examination For Epidermal Study

Fresh leaves of *Ipomoea carnea* were obtained from five different LGA's (Gombe, Y/deba, Balanga, Funakaye, Dukkuin) in Gombe State, Nigeria. The specimen was identified at University Herbarium, Department of Biological Sciences, Gombe State University, Gombe State, Nigeria. Leaf samples were then fixed in Formalin Acetic Acid (FAA) and then preserved in 70% ethanol. Epidermal peels of the leaf surfaces of the plant were made using the method of (Zhigila *et al.*, 2015). The adaxial and abaxial surfaces of the leaves were carefully sectioned from the median portion of the leaves with razor blade (free hand section) and placed on a microscope clean glass slide. The preparation were stained with 1% aqueous solution of safranin for 4 to 8 minutes, and rinsed carefully in water to remove excess stain and then mounted in 10% glycerol and observed under a light microscope and the leaf epidermal features were then examined using 35 fields of view at × 40 objective as quadrats. The numbers of subsidiary cells per stomata was noted to determine the frequency of the different stomatal complex types and was expressed as percentage occurrence of such complex types based on all occurrences (Obiremi and Oladele, 2001). Terminologies for naming stomatal complex types followed (Dilcher, 1974). The Stomatal size of a species was determined as the product of length and breadth. The mean stomatal sizes/ guard cell area were determined by Francos constant method (Guard cells area = length × breadth (width) × 0.7854) of guard cells using an ocular eye-piece micrometer and finally converted by the ocular constant with respect to the power with which they were taken. Samples of 35 stomata were used. The method followed those of (Franco, 1939; Wilkinson, 1979).

The stomatal densities were determined by counting the number of stomata per square millimeter based on the entire leaf surface. Stomatal Index was determined as number of stomata per square millimeter divided by number of stomata plus number of epidermal cells per square millimeter multiplied by 100 (Dilcher 1974). The stomata observed were viewed with the light microscope and were calculated

in unit area using the stomatal Index (S.I) formulae as determined according to Metcalfe and Chalk (1979) as shown below.

$$S.I = \frac{S}{S+E} \times 100$$

Where S.I = Stomatal Index

S = Number of stomata per unit area

E = Number epidermal cells in the same unit area.

Statistical Analysis

All data were processed using analysis of variance (ANOVA). Computer software used was Minitab^(c) V. 17 (State College PA). A probability value of 0.05 was used as bench mark for significant differences between parameter

RESULTS

Table 1 shows the results of stomatal features of *Ipomoea carnea* for the five accessions. Stomata were observed on both leaf surfaces i.e leaves were amphistomatic. Only one type of stomatal complex type was observed in all the five accessions i.e Cyclocytic type on both leaf surfaces. The frequency was only of one type i.e 100%. There were variations in the stomatal sizes ($51.13 \pm 7.47^b - 88.68 \pm 13.95^a$), Stomatal densities ($23.40 \pm 7.67^b - 40.00 \pm 1.00^a$) within the same plant of *Ipomoea carnea* and the Stomatal index ($62.80 \pm 15.92^a - 84.39 \pm 7.36^a$) was also variable between the Adaxial and Abaxial leaf surfaces. It however has the highest number on the Abaxial leaf surface (84.39 ± 7.36^a) in Accessions 5, and lowest on the Adaxial leaf surface (62.80 ± 15.92^a) in Accessions 2.

Table 2 shows the presence of polygonal shapes of epidermal cells on both leaf surfaces of *I. carnea*, with curved anticlinal cell wall pattern (Table 2, Plate 1-10). The highest average epidermal cell density was recorded on the Adaxial surface (13.40) and the lowest was recorded on the Abaxial surface (6.60). There was no wide range of epidermal cell wall pattern among the studied accessions. Trichomes were observed.

Table 1: Stomatal features in accession of *Ipomoea carnea*

| Accessions | LS | Stomatal complex type | Frequency (%) | SD (mm ²) | SLe(µm) | Stomatal breadth (µm) | Stomatal Size (µm) | Stomatal Index (%) |
|------------|----|-----------------------|---------------|-------------------------|-------------------------|------------------------|---|--------------------------|
| 1. | Ad | Cyclocytic | 100.00 | 35.40±2.88 ^b | 11.80±0.84 ^a | 9.20±1.64 ^a | 86.31± | 78.90±8.01 ^a |
| | Ab | Cyclocytic | 100.00 | 40.00±1.00 ^a | 9.00±1.23 ^b | 7.20±0.45 ^b | 21.31 ^a | 80.32±4.22 ^a |
| 2. | Ad | Cyclocytic | 100.00 | 23.40±7.67 ^b | 12.20±0.45 ^a | 9.20±1.30 ^a | 51.13±7.47 ^b | 62.80±15.92 ^a |
| | Ab | Cyclocytic | 100.00 | 33.20±3.77 ^a | 10.40±0.55 ^b | 8.01±0.69 ^a | 88.68±13.95 ^a | 76.04±7.52 ^a |
| 3. | Ad | Cyclocytic | 100.00 | 28.80±4.09 ^b | 11.00±1.41 ^a | 8.40±1.67 ^a | 66.59±7.41 ^b | 76.50±9.64 ^a |
| | Ab | Cyclocytic | 100.00 | 35.00±3.08 ^a | 9.40±0.55 ^b | 7.40±0.55 ^a | 73.70±22.60 ^a | 82.63±4.96 ^a |
| 4. | Ad | Cyclocytic | 100.00 | 29.40±2.41 ^a | 11.60±1.14 ^a | 8.80±1.30 ^a | 54.92±5.59 ^a | 77.19±11.16 ^a |
| | Ab | Cyclocytic | 100.00 | 33.40±4.04 ^a | 9.40±0.89 ^b | 7.00±0.00 ^b | 81.27±19.44 ^a | 80.87±3.71 ^a |
| 5. | Ad | Cyclocytic | 100.00 | 28.40±1.14 ^b | 11.80±1.09 ^a | 9.20±1.64 ^a | 51.92±4.94 ^b | 72.73±7.08 ^b |
| | Ab | Cyclocytic | 100.00 | 35.80±4.82 ^a | 10.20±0.45 ^b | 7.60±0.89 ^a | 84.58±18.59 ^a 61.07±6.54 ^b | 84.39±7.36 ^a |

Mean followed by the same letter in a column are not significantly different at 0.05% probability level using Multiple Range Test.

Ad = Adaxial, Ab = Abaxial, Cy = Cyclocytic, SD = Stomatal Density = SL = Stomatal length, LS = Leaf Surface

Table 2: Epidermal cell characteristics in accession of *Ipomoea carnea*

| Accessions | Taxa | Leaf Surface | Epidermal cell shape | Anticlinal cell wall pattern | Epidermal cell Density | Trichome |
|------------|------------------|--------------|----------------------|------------------------------|-------------------------|----------|
| 1. | <i>I. carnea</i> | Adaxial | Polygonal | Curved | 9.60±4.04 ^a | Present |
| | | Abaxial | Polygonal | Curved | 9.20±1.30 ^a | Present |
| 2. | <i>I. carnea</i> | Adaxial | Polygonal | Curved | 13.40±5.41 ^a | Present |
| | | Abaxial | Polygonal | Curved | 10.60±3.91 ^a | Present |
| 3. | <i>I. carnea</i> | Adaxial | Polygonal | Curved | 8.80±3.49 ^a | Present |
| | | Abaxial | Polygonal | Curved | 7.40±2.30 ^a | Present |
| 4. | <i>I. carnea</i> | Adaxial | Polygonal | Curved | 9.20±5.36 ^a | Present |
| | | Abaxial | Polygonal | Curved | 8.00±2.24 ^a | Present |
| 5. | <i>I. carnea</i> | Adaxial | Polygonal | Curved | 11.00±4.00 ^a | Present |
| | | Abaxial | Polygonal | Curved | 6.60±3.05 ^a | Present |

Mean followed by the same letter in a column are not significantly different at 0.05% probability level using Multiple Range Test.

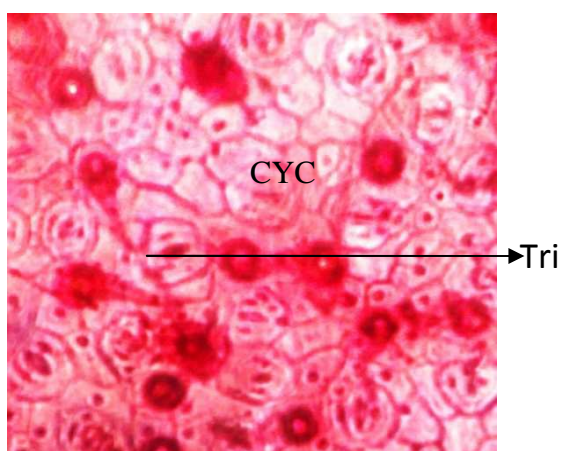


Plate 1: Adaxial surface of *I. carnea* showing Cyclocytic stomata X400



Plate 2: Abaxial surface of *I. carnea* showing Cyclocytic stomata X400



Plate 3: Adaxial surface of *I. carnea* showing Cyclocytic stomata X400

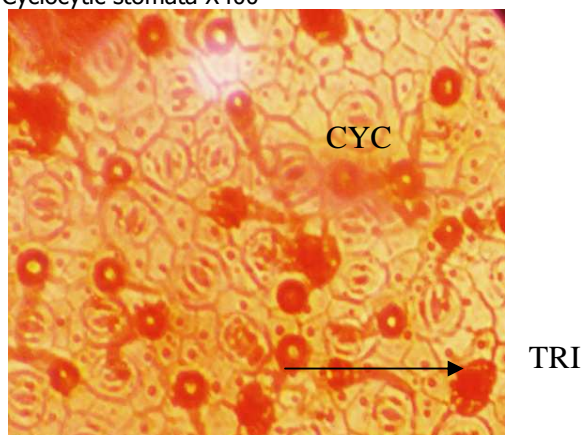


Plate 4: Abaxial surface of *I. carnea* showing Cyclocytic stomata X400

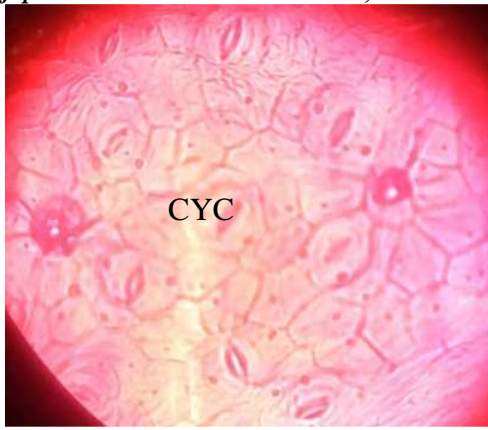


Plate 5: Adaxial surface of *I. carnea* showing Cyclocytic stomata X400

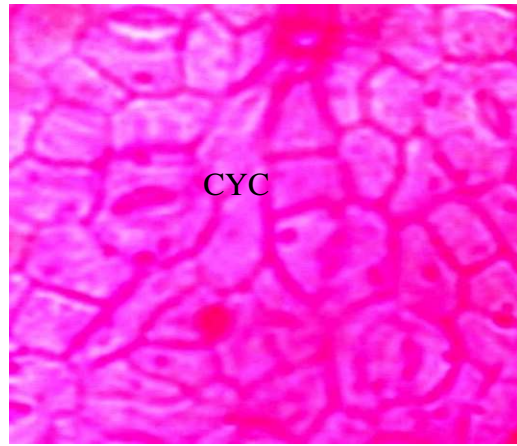


Plate6: Abaxial surface of *I. carnea* showing Cyclocytic stomata X400

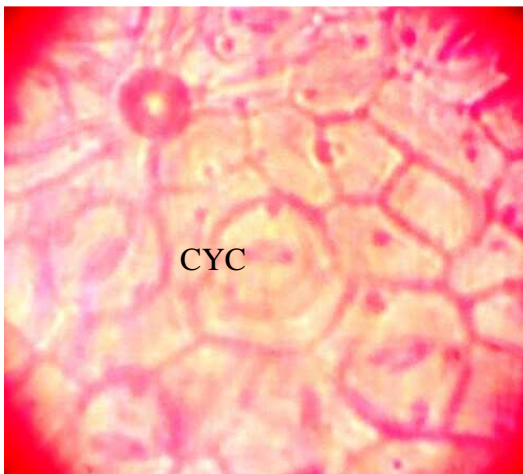


Plate 7: Adaxial surface of *I. carnea* showing Cyclocytic stomata X400

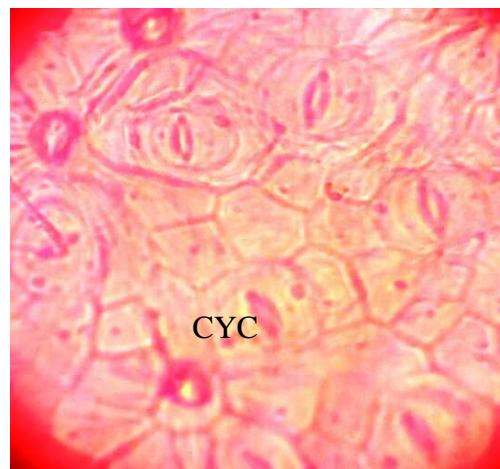


Plate 8: Abaxial surface of *I. carnea* showing Cyclocytic stomata X400

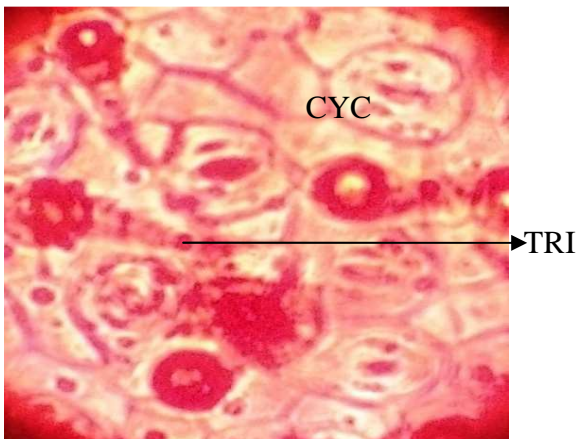


Plate 9: Adaxial surface of *I. carnea* showing anomocytic stomata X400



Plate10: Abaxial surface *I. carnea* showing anomocytic stomata X400

KEY

CYC = Cyclocytic stomata
TRI = Trichome

DISCUSSION

The results showed that the leaves of the studied specie were amphistomatic (stomata present on both surfaces) (Plate 1-10). This was similar to the findings of Zhigila *et al.*, 2015 in his study of *Capsicum annum*. Stomatal complex type (SCT) was exclusively Cyclocytic within all the accessions in the species. This distinguishes the plant from all other species in the same genus. The homogeneous nature of the SCT on the leaves is responsible for its 100% occurrence. The 100% frequency of cyclocytic stomata in *I. carnea* is noteworthy. This suggests that the stomatal system of *I. carnea* in having two or more subsidiary cells which form one or two narrow rings around the guard cells coupled with amphistomatic feature is likely to be water-conserving. A similar finding was reported by (Abdurrahman and Oladele, 2003; Abdul Rahaman *et al.*, 2014) in their study of *Talinum triangulare* in stomatal complex types, stomatal size, density and index of some vegetable species in Nigeria and in some Six species of *Anacardiaceae* and *Lannea* species. Carr and Carr (1990) reported that large number of subsidiary cells per stoma may be responsible for a more precise and rapid regulation of stomatal opening. This view was corroborated by Obiremi and Oladele (2001) in some *Citrus* species, in which species with few subsidiary cells are more water –conserving than those with many subsidiary cells. The presence of non- glandular trichomes on both surfaces of the plant was also observed in the study. This is of great diagnostic interest. The long unicellular hairs in them serve to reduce the rate of transpiration in the plant .The importance of trichomes has been highlighted by Stace (1980), Illoh and Inyang (1998) and Obute and Ndukwu (2006). Corsi (1999) suggested that the primary function of stinging trichomes might be the regulation between the plant and environment. It is consistent with findings of (Ramayya, 1969). The variations in stomatal index observed in this study can be reasonably employed in delimiting the *Ipomoea* species (Table 1). The stomatal index which indicates the proportion of stomata relative to leaf surface is also a reliable taxonomic character. It is highly constant for any given species and the value is more uniform upon the abaxial than the adaxial surface except in isobilateral leaves (Metcalf and Chalk, 1979; Olatunji, 1983; Adedeji and Jewoola, 2008). This is because (Abdurrahman and Oladele, 2004) reported that stomatal index is independent of the changes in epidermal cells size brought about by environmental factors. The role of stomatal index in systematic work to separate species has also been reported by Abdul Rahman and Oladele (2003); Isawumi (1989) and Aworinde *et al.*, (2009). For this study, the Stomatal index was highest (84.39 ± 7.36^a) on the Abaxial surface of Accessions 5, and lowest (62.80 ± 15.92^a) on the Adaxial surface of Accessions 2. This shows that stomata occupied larger proportion of the leaf surface in the abaxial surface and smaller proportion on the adaxial surface (Table I). Duncan Multiple Range Test (DMRT) - one-way ANOVA revealed that

there was significant difference between the Stomatal densities of all the 5 Sites. The stomatal density was highest on the abaxial surface of Accessions 1 (40.00mm^2) and lowest on the adaxial surface of Accessions 2 (23.40mm^2). DMRT of the Stomatal sizes in the five accessions were shown in Table 1. However, the stomatal sizes of adaxial surface of Accessions 2 was the highest ($88.68 \mu\text{m}$), while abaxial surface of Accessions 1 showed the lowest ($51.13 \mu\text{m}$). Pataky (1979) had earlier reported that stomata whose guard cells are less than $15\mu\text{m}$ long are designated 'small' while those that are more than $38\mu\text{m}$ long are termed 'large'. In this study all the stomatal sizes were large. This could probably imply that the stomata have the tendency of opening the guard cells more than some other plants without large sizes of guard cells. Stomatal size (guard cell area) and stomatal index provide values that would serve as parameters for comparison among taxa which can be useful for identification of the studied taxa. (Essiet *et al.*, 2011). Stomatal size is often correlated with stomatal density such that small stomata give high density and large stomata give low density (Metcalf and Chalk, 1988; Abdulrahman and Oladele, 2004). This statement is in line with the findings from this work where abaxial surface of Accessions 1 had the smallest Stomatal size with highest Stomatal density while adaxial surface of Accessions 2 had the largest stomatal size with lowest stomatal density. Chen *et al.*, (2009); Alege *et al.*, (2013) reported that Stomatal density may depend on the environmental factor. Contrary to their observation, Stomatal Index, Stomatal density, and Stomatal Size on both surfaces may be inherent characters as the plants were subjected to the same treatment. This may have adaptive implication for water conservation in the plant since the leaf surfaces are responsible for loss of water through the stomata (Zhigila *et al.*, 2015). The functions of epidermis are water regulation, protection against sunlight and defense to other organisms (Mauseth,1998). No variability existed within the epidermal cell shape pattern within all the sites. According to Alege *et al.*, (2013), epidermal cell characteristics are under strong genetic control, hence are stable traits and therefore proved to be a better tool for the delimitation of *Ipomoea carnea* than stomatal traits. Similar findings were made by Bhattacharyya and Midya (1979) based on leaf epidermal characteristics. These findings suggest that to a certain extent and with verification from other taxonomic characters, and epidermal cell density can make contribution in delimiting this species. The present study of *I. carnea* Jacq. also revealed that plant exhibits certain anatomical adaptations for surviving in the xeric and hydric habitats it occupies. The xeric adaptation Then it was concluded that the presence of non- glandular trichomes, large stomatal sizes, Cyclocytic type of stomata, 100% frequency which indicates that the plant shows xeromorphic features in their stomatal structure, greatly helped in the delimitation of the plant and could also be used for classification/identification of the plant and some of the features could also be used for adaptation purposes.

RECOMMENDATION

It is recommended that since the plant can adapt successfully to the gullies in Gombe State, and it is abundantly found throughout the State, it could be exploited judiciously by the government using the plant as a source of green manure, as a fish feed for various fish species, as a raw material for paper bag production to mention but a few.

Contributions of Authors

This work was carried out in collaboration among all authors. Authors AHM and AA designed the study, wrote the protocol and interpreted the data. They also

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managed the literature searches and produced the initial draft. Author UY anchored the field study, gathered the initial data and performed preliminary data analysis.

Conflict of Interest

All authors read and approved the final manuscript. There are however no conflict of interest.

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