Journal of Global Biosciences
Peer Reviewed, Refereed, Open-Access Journal
ISSN 2320-1355
Volume 10, Number 3, 2021, pp. 8478-8487
Website: www.mutagens.co.in
URL: www.mutagens.co.in/jgb/vol.10/03/100309.pdf

SCAN ME

Research Paper

CHEMOTAXONOMIC CHARACTERISTICS AND PHYTOCHEMICAL CONSTITUENTS OF *Cyperus esculentus* Linn. A MEMBER OF CYPERACEAE

Wahua, C. and J. Ukomadu

Department of Plant Science and Biotechnology,
University of Port Harcourt, Choba, P.M.B. 5323,
Nigeria.

Abstract

The present study is set to investigate the chemotaxonomic characteristics of *Cyperus esculentus* Linn. evergreen perennials belonging to Cyperaceae. It is a tall tuber-bearing rhizomatous moncotyledon commonly called yellow nut sedge or tiger nut. It grows up to 70±20cm in height having umbel of flower spikes which forms 4 to 10 ranks of floral spikelets, yellow to golden-brown in color surrounded with 4-6 flat leaf bracts and leaves are attenuate and have alternate phyllotaxy, reddish brown at base and light green towards the tip which measures up to 45±17cm in length and 3±0.46cm in width. Venation is parallel. Roots are fibrous but the edible tubers or nuts arise from the rhizomes. Epidermal study revealed graminaceous stomata which is amphistomatic and more numerous on the abaxial foliar regions. Anatomical sections showcased scattered vascular bundles in ground tissues of stem presenting the xylem towards the centre while the phloem towards the periphery. Qualitative phytochemical study showed the presence of alkaloids, terpenoids, saponins, steroids and carbohydrate.

Key words: Chemotaxonomy, phytochemistry, sedges, *Cyperus*.

INTRODUCTION

The Cyperaceae are monocotyledonous Graminoid flowering plants known as sedges which resemble grasses and rushes, containing about 113 genera and 5,732 species (1). They are widely distributed with the centres of diversity occurring in Tropical Asia and Tropical South America (2). They are regarded as sedges because of their triangular cross section and spirally arrange leaves in three ranks, unlike grasses that have alternate leaves forming two ranks. *Cyperus esculentus* is commonly known as tiger nut, yellow sedge nut, earth nut, rush nut and edible galingale (3) which has three varieties:

the black, brown and yellow cultivars. Several hundreds to thousands of tubers can be produced from one plant during a single growing season. Seedlings readily emerge from a depth of 1.3cm and slowly from that of 2.5cm. They can sprout to several years later (4). *Cyperus esculentus* grow best with sandy-moist soil with pH range of 5.0 to 7.5. In micro-morphological study of the genus Cyperaceae, leaves in outline crescentiform, V-shaped (sometimes flanged), or inversely W-shaped; median adaxial groove generally absent. Epidermal cells of adaxial surface are much longer than abaxial foliar epidermal cells. Except sometimes over adaxial sclerenchyma and near leaf margins (5). Sclerenchyma present in hypodermal strand (6 and 5). Large pith in the root and vascular bundles numerous are scattered in the ground tissues more in peripheral ground tissues (5).

The major constituents of *Cyperus esculentus* starch and dietary fiber, the starch component is almost twice that of sweet potato (7). The roots are edible. Tiger nut has been reported to be used in the treatment of flatulence, indigestion, diarrhea and dysentery (8). The relevance of the study is to enhance information on the existing literature and taxonomic properties of *Cyperus esculentus* Linn. Thus the objective is aimed at considering the chemotaxonomic characteristics of the plant, *Cyperus esculentus* Linn.

MATERALS AND METHODS

Geographic Location

The location of the parent plant studied was Port Harcourt, Rivers, Nigeria.

Morphological Studies

The meter rule was used to ascertain the plant height from the root-collar to the terminal bud while leaf length from the leaf tip to the petiole base. The leaf width is measured across the leaf lamina, from one margin to another at the widest region.

Micro-morphological (Epidermal) Studies

Fresh leaves and young stem collected for this study were peeled and subjected to alcohol solutions in the ratio of 50%, 75% and absolute alcohol respectively. The cleared epidermal layers obtained were stained with safranin for 5 minutes washed and counter stained with Alcian blue for same time interval, washed and temporarily

mounted in aqueous glycerol solution. Photomicrographs were taken from good preparations. The stomatal index [S.I.] was obtained using the formula:

$$S.\ I. = \frac{S}{S + E} \times \frac{100}{1}$$

Where *S* and *E* are mean numbers of stomata and epidermal cells respectively within the particular area under investigation. Likewise trichome Index (T.I) was obtained using:

$$T.\ I. = \frac{T}{T + E} \times \frac{100}{1}$$

Where T and E are trichomes and epidermal cells respectively within the study area.

Anatomical Study

The plants were harvested from the wild for the secondary anatomy. The harvested stems, leaves, petioles, flowers, fruits and roots were dehydrated in alcohol solutions of 50%, 75%, absolute alcohol and thereafter subjected through alcohol chloroform series in the ratio of 3:1 of alcohol chloroform series, 1:1, 1:3 and pure chloroform respectively for five minutes in each. They were there after rehydrated following same procedure to 50% alcohol before staining with safranin for 2 to 5 minutes, counter stained with Alcian blue for same time interval. Free hand section was done using a systematic arrangement of 5 razor blades as described by (9) was also adopted. Microphotographs were taken from good preparations using Sony camera of 7.2 Mega pixels having 2.411 LCD monitor and High sensitivity ISO 1250.

Phytochemical Study

Leaves of *Cyperus esculentus* studied were sun dried for 72 hours (3 days) and weighed. Fifty grams (50g) of the dried leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter filtered and evaporated to dryness (constant

weight) using a rotary evaporator set at 45^0C. Residue yields were noted and a portion was used for the phytochemical screening.

Test for alkaloids

This was carried out using 0.5g of the plant extract which was stirred with 5ml of 1 percent aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. The third 1ml was treated with Wagner's reagent. Turbidity or precipitation with any of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (10 and 11). A modified form of the tin-layer chromatography (TLC) method as described by (12) was used. One gram (1g) of the extract was treated with 40 percent calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. The extracts were combined and concentrated to 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvent systems were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatograms (indicated by an orange or darker colored spot against a pale yellow background) was confirmatory evidence that the plant extract contained alkaloid.

Test for flavonoids

Shinoda reduction test: 5g of the pulverized sample was boiled in 5ml of distilled water for 5 minutes on water bath and filtered while hot. Magnesium (Mg) was added to the filtrate and few drops of conc.H_2SO_4 were carefully introduced into the mixture. The formation of orange, red, crimson or magenta was taken as evidence of preliminary presence of flavonoid.

Lead acetate test: 5g of pulverized sample was boiled in 5ml of distilled water for 5 minutes in water bath and filtered while hot. 2ml of 10% lead acetate was added to the filtrate and observed. Yellow precipitate indicated presence of flavonoids.

Test for tannins

Ferric chloride test *$FeCl_3$

5g of the pulverized sample was boiled in 5mls of distilled water for 5 minutes on water bath. This was filtered while hot. 1ml of 5% $FeCl_3$ was added to the filtrate and observed. Blue-black, green or blue-green precipitate was taken as the presence of tannins (11)

Test for anthraquinones

Borntrager's test as used. Five grams (5g) of each plant extract was shaken with 10ml benzene, filtered and 5ml of 10 per cent ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones.

Test for combined anthraquinones

Five (5g) of each plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10 per cent ammonia solution added. A pink, red or violet coloration in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract (11).

Test for phlobatannins

The deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1 percent aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (11).

Test for cardiac glycosides

Lieberman's test was used in which 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled in ice. One milliliter (1ml) of Sulphuric acid was carefully added in drops until a color change from violet to blue to green indicated the presence of a steroidal aglycone portion of the cardiac glycoside (13).

Test for Saponins

Frothing tests was done following the method described by (14). The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. 0.5g of the plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. The disc was then washed in ether, dried and placed on a 7 percent blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was taken as further evidence of presence of saponins.

RESULTS AND DISCUSSION

The Geographic location

The geographic location of the parent plant is Choba community in Obio-Akpor Local Government Area of Rivers State of Nigeria.

Morphological Study

Morphological studies revealed Plates 1a, 1b, 1c and 1d.

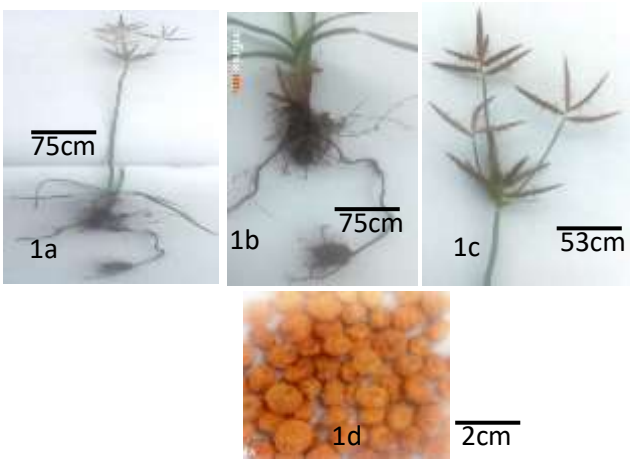

Plate 1: Morphology of *Cyperus esculentus* Linn. 1a: Stem bearing flower inflorescence, 1b: Stem with rhizome at base, 1c: The attenuate leaf, 1d: The edible tuber.

Epidermal Study

Epidermal studies are graminaceous which is amphistomatic. Lower epidermis has more stomata than the upper one. See plates 2a, 2b and 2c.

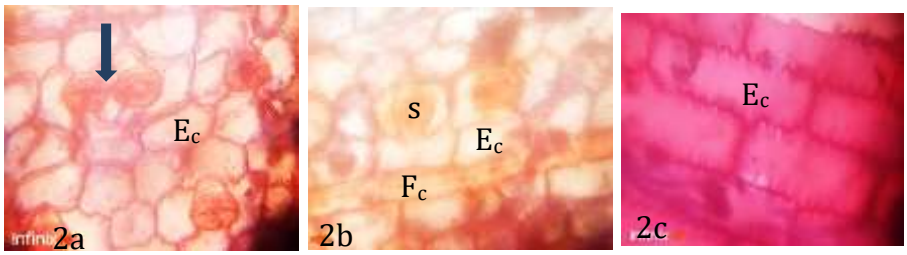

Plate 2: *Cyperus esculentus* Linn. foliar epidermis. 2a and 2b: Adaxial foliar surfaces and 2c: Abaxial surface. Arrow revealed contiguous cells. 'S' stands for stoma, E_c is epidermal cells while F_c represents foliar fibre cells. Stomatal type is graminaceous.

Leaves in outline crescentiform, V-shaped (sometimes flanged), or inversely W-shaped; median adaxial groove generally absent. Epidermis: adaxial much longer than abaxial foliar epidermis.

Anatomical Study

Anatomical studies revealed numerous vascular bundles in stem, mid-ribs and root, but absence of pith in the stem while the root anatomy has large central pith. The hypodermis is preoccupied with sclerenchyma and vasculation is closed type. Plates 3a and 3b.

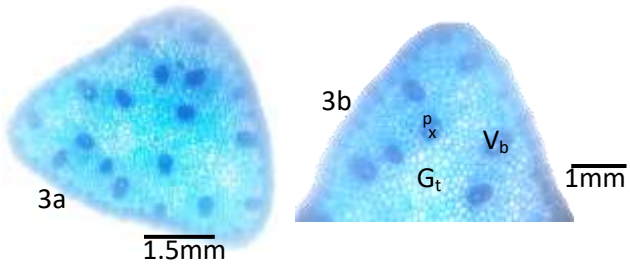

Plate 3: Stem anatomy of *Cyperus esculentus* Linn. 3a: *Cyperus esculentus* stem anatomy triangular shaped. 3b: Stem of one region of the 3 sided polygon magnified. V_b represents vascular bundle and G_t- Ground tissue. 'P' is phloem while 'x' is xvlem

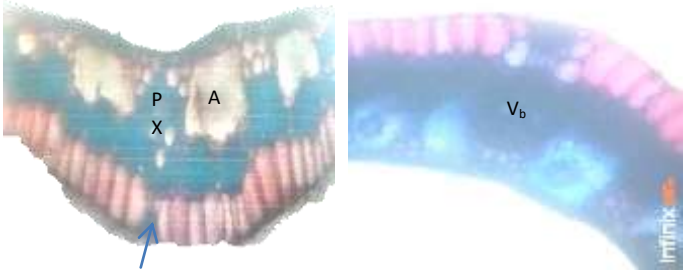

Plate 4: *Cyperus esculentus* Linn. Mid-rib anatomy. 'P' represents Phloem, 'X' is the xylem; 'A' showed the Air chamber, V_b. Leaf V-shaped. Arrow revealed sunken stoma

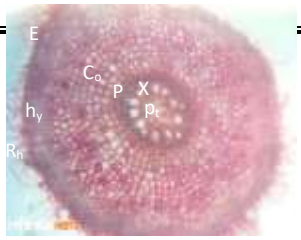

Plate 5: *Cyperus esculentus* Linn. root anatomy. C_o revealed the general cortex, h_y -hypodermis, E – epidermis, P – Phloem, X is the Xylem while P_t is

Phytochemical Study

Phytochemical studies showed the presence of alkaloids, saponins, alkaloids, and flavonoids are present in. This may be the reason why. See table 1.

Cyperus esculentus Linn. has numerous irregular-shaped radiant cells in the cortex also have been reported by (5). The leaf anatomy revealed V-shaped structure and sclerenchyma present in hypodermal strands as also reported by (6), and (5). Large pith observed in the root section and vascular bundles numerous are scattered in the ground tissues more in peripheral ground tissues and air-chambers are revealed in the mid-rib anatomy as also reported by (5).

Table 1: Qualitative phytochemical studies on *Cyperus esculentus* Linn.

Phytochemical Constituents	Presence or Absence
Alkaloids	
Mayers test	+ve
Hagers test	+ve
Wager's test	+ve
Dragendorff test	+ve
Flavonoids	
Shinda test	-ve
Lead acetate test	-ve
$AlCl_3$	-ve
Tannins	
$FeCl_3$	-ve
Anthraquinone	
Free anthraquinone	-ve
Combined anthraquinone	-ve
Triterpenoids/Steroids	
Salwoski	+ve
Carbohydrates	
Molish test	+ve
Fehling test	+ve
Cyanogenic glycosides	-ve
Saponins	
Frothing test	+ve

Key: +ve represents presence while -ve shows absence

CONCLUSION AND ACKNOWLEDGEMENT

The milk and fibre contents are useful in nutrition dietary. It is likely that the Mutualistic relationship involved in the roots that resulted in the formation of the root tubers could be used to instigate the tuberosis in other related species within the same genus. In as much as morphological, anatomical and phytochemical studies on *Cyperus esculentus* may not be new, proximate analysis, the karyotypes, quantitative aspect of phytochemistry and DNA barcodes may be of future interest.

The effort of Miss Abraham, Blessing Nkeruiruka who did the initial field collection of *Cyperus esculentus* Linn. and assisted in some of the laboratory research is immensely commended.

REFERENCES

1. Milne, L.J., Milne, M.J.G. (1975). Living plants of the world. Random House. P. 301.
2. Hipp, A.L. (2007). "Non-uniform processes of chromosome evolution in sedges (Carex: Cyperaceae)". *Evolution.* 61(9): 2175-2195.
3. Oderinde, R.A. and Tairu, O.A. (1988). Evaluation of the properties of yellow nut sedge (*Cyperus esculentus*) tuber oil. Food Chemistry, 28"233-237.
4. Stroller, E.W. (1981). *Yellow Nut Sedge: A Menace in the Corn Belt.* US Department of Agriculture, Agricultural Research Service. No. 1642.
5. Amini Rad, M. and Sonboli, A. (2008). Leaf and Stem Anatomy of the Cyperus subgenus Cyperus in Iran. *Rostaniha*, Vol. 9 (1) 2008.
6. Metcalfe, C.R. and Chalk, L. (1971). *Anatomy of monocotyledons*. Vol.5 Cyperaceae. 597 pp. Oxford.
7. Coskuner, Y., Ercan, R., Karababa, E., Nazlican, A. N. (2002). Physical and chemical properties of chufa (*Cyperus esculentus* L.) tubers grown in the Çukurova region of Turkey. *Journal Science Food Agriculture,* 82:625-631.
8. Bixquert-Jiménez, M. (2003). Horchata y Salud: Propiedadessaludables y de prevención de enfermedadesdigestivas In: *Fundación Valenciana de*

EstudiosAvanzdos, editor. Jornada Chufa y Horchata: Tradición y Salud. Valencia, Spain: Conselleria de Agricultura, Pesca y Alimentación. P. 71-85.

9. Wahua, C. (2020). Free-hand Sectioning Machine Invented for Anatomical Studies of Biological Materials. *Scientia Africana*, Vol. 19[no.1] pp. 159-162.
10. Harborne, J.B. (1973). *Phytochemical Methods: A Guide to modern Techniques of Plants Analysis*. Chapman and Hall London. 279pp.
11. Trease, G.E. and Evans, I.N.C. (1989). *A Textbook of Pharmacognosy* 3rd ed. Boilliere Tinall LTD. London
12. Farnsworth, N.R. and Euer, K.L. (1962). An Alkaloid screening procedure utilizing thin-layer chromatograph. *Lyiodia,* 25-186.
13. Shoppee, C.W. (1964). *Chemistry of the Steroids*, 2nd Ed. Butterworths, London. 56pp.
14. Wall, M.E., Eddy, C.R., McClenna, M.L. and Klump, M.E. (1952), Detection and estimation of steroid Sapogenin in plant tissues. *Anal Chem.* 24:1337