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COMPARATIVE CHEMOTAXONOMIC INVESTIGATIONS ON AMARANTHUS HYBRIDUS L. AND AMARANTHUS SPINOSUS L.

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ABSTRACT

The present study is set to investigate the comparative chemotaxonomic investigations on Amaranthus hybridus L. and Amaranthus spinosus L. which belong to the family Amaranthaceae. They are dicots pre-dominantly found in the Niger Delta Tropics, Nigeria. The species are annual erect herbs with flower inflorescences as elongated spikes which are mostly paniculate occurring at ends of branches in globose fashion in axils of leaves.The nodes often have pair of axillary spines. Flowers are small, greenish with male ones at the top while the female ones below the clusters and stem is greenish but often reddish with one-seeded capsule as fruit in Amaranthus spinosus which attains up to 80 ± 20cm in height whereas A. hybridus differ in absence of a pair of axillary spines, the stems are greenish or slightly pinkish which grows up to 100 ± 10cm in height. A. hybridus is more of a vegetable and has alternate phyllotaxi and narrow cuneate base. Fruits from both species are circumscissile capsules and their inflorescences are terminal racemes positioned at their axils with female perianth segments of five. Epidermal studies revealed amphistomatic stomata which is anisocytic type for both species. The stomatal index for A. spinosus adaxial foliar epidermis is 20% and the abaxial 20% whereas for A. hybridus adaxial is 20% and abaxial foliar stomatal index of 20%. Anatomical studies revealed open vascular system, collenchyma dominating the hypodermis while parenchyma occupied the general cortex and pith regions. A. hybridus has more vascular bundles and trichomes, and wider pith than A. spinosus. Phytochemical studies showed the presence of tannins, saponins, alkaloids, and flavonoids are present in A. spinosus while alkaloids were absent only in A. hybridus. This may be the reason why A. spinosus is used more in tradomedicine than A.hybridus which served more as vegetable.

Key Words: Morphology, Anatomy, Phytochemistry, *Amaranthus*, Amaranthaceae.

INTRODUCTION

Amaranthus species belong to the Order Caryophyllales, commonly known as pigweed and amaranthes.

They are annual to perennial herbs, rarely shrubs or climbers, characterized with simple, alternate leaves with opposite arrangement and the female perianths segments are 5 in number, Hutchinson and Dalziel (1954]. Members of this genus and the family as a whole have betacyanins in place of anthocyanins in their floral and vegetative parts (Nyananyo, 2006). *Amaranthus spinosus* is believed to have originated from lowland tropical South and Central America and was introduced into other warmer parts of the world (Holm *et al.*, 1991). *Amaranthus hybridus* Linn. has no spine (Akobundu and Agyakwa, 1998). Their fruits are circumscissile capsules (Hutchinson and Dalziel, 1954; Akobundu

and Agyakwa, 1998). In Nigeria, *Amaranthus hybridus* leaves with condiments are used to prepare soup. In Congo, the leaves are eaten as spinach or green vegetable (Dhellot *et al*., 2006). These leaves boiled and mixed with groundnut sauce are eaten as salads in Mozambique and in West Africa (Martins and Telek, 1979). *Amaranthus hybridus* is highly hybridized, (Akobundu and Agyakwa). *Amaranthus* diversities within same species in anatomical properties do exist due to environment especially among *Amaranthus spinosus, Amaranthus viridis* and *Amaranthus hybridus*. Variation in number of vascular bundles in the mid-ribs, petioles, and stems differ from collections from Rivers East, Rivers West and Rivers South of Nigeria respectively (Ozimede *et. al*., 2019). *Amaranthus spinosus, Amaranthus viridis* and *Amaranthus hybridus*. They are used as medicine and can reduce cholesterol level in the blood (Smith, 2000).

The relevance of the study is to add more information to the chemotaxonomic knowledge of *Amaranthus spinosus* and *Amaranthus hybridus*. Thus, the objectives focus on investigating the comparative chemotaxonomic investigations on *Amaranthus hybridus* L. and *Amaranthus spinosus* L. (Amaranthaceae). They are dicots pre-dominantly found in the Niger Delta Tropics, Nigeria.

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:

$$\text{S. I.} = \frac{S}{S+} \quad X \quad \frac{100}{1}$$

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:

$$\text{T. I.} = \frac{T}{T+} \quad X \quad \frac{100}{1}$$

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

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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 Wahua (2020) 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 *Amaranthus spinosus* and *Amaranthus hybridus* 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.

Qualitative Test for Saponin

Frothing tests was done following the method described by Wall *et al.* (1952)). 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.

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. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (Harborne, 1973 and Trease *et al.*, 1989). A modified form of the tin-layer chromatography (TLC) method as described by Farnsworth *et al.* (1962) 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 tannins

Five grams (5g) of each portion of plant extract was stirred with 10ml of distilled water, filtered, and 5% ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Shoppee, 1964).

Test for anthraquinones

Borntrager's test was 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 (Trease and Evans, 1989).

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 (Trease and Evans, 1989).

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 (Shoppee, 1964).

RESULT

The Geographic location

The geographic location of the parent plants ($4^0$52'44" North and $6^0$55'20" East).

Morphological Study

Morphological studies revealed both species as annual erect herbs with flower inflorescences as spikes which elongates and mostly paniculate occurring at ends of branches in a globose flower clusters in leaf axils, nodes often with a pair of very sharp axillary spines, flowers are small, greenish with male ones at the top while the female ones below the clusters and stem is greenish but often reddish with one-seeded capsule as fruit for *Amaranthus spinosus* which grows up to 80 ± 20cm in height. *A. hybridus* differ in absence of a pair of axillary spines, and stems are greenish or slightly pinkish, grows up to 100 ± 10cm in height. *A. hybridus* grows and serves as vegetable with alternate phyllotaxy. Fruits from both species are circumscissile capsules and their inflorescences are terminal racemes positioned at their axils with female perianth segments of five. The leaves are simple measuring up to 9 ± 3cm long and 4 ± 1cm wide for *A. hybridus* with petiole length of 5.5 ± 2cm while A. spinosus 6 ± 1cm long and 3 ± 1cm wide for *A. spinosus* with petiole length of 3 ± 1cm. Tap root system with shallow rooting observed for both species. Plates 1 and 2.

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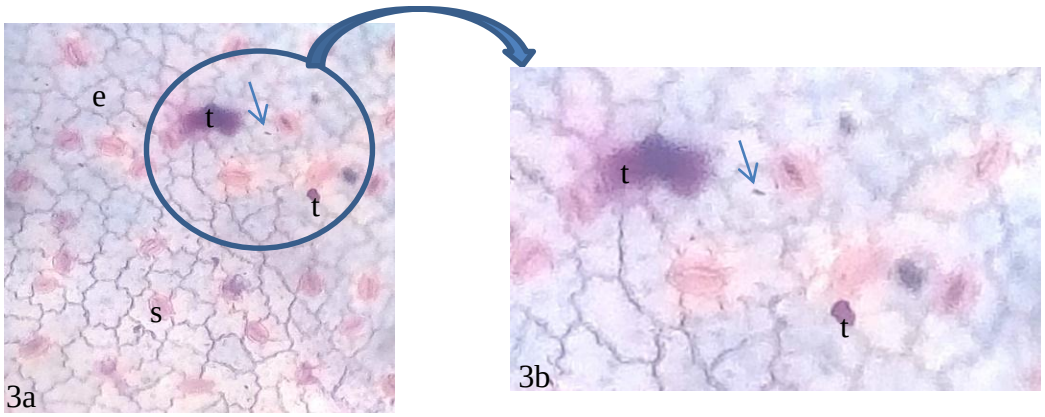
Plates 1: *Amaranthus spinosus* Linn. Plate 1: *Amaranthus spinosus* Linn.
Arrow revealed terminal inflorescences in both species

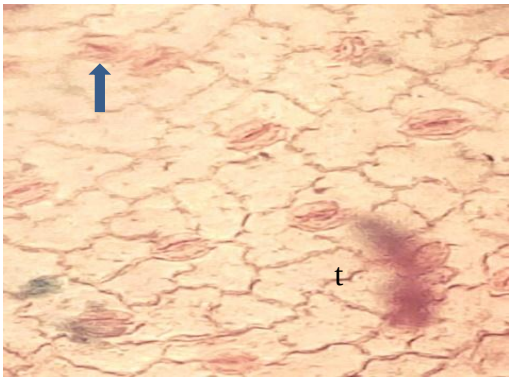
Epidermal Study

Epidermal studies showcased anisocytic stomata for both species which is amphistomatic. The stomatal index for *A. spinosus adaxial* foliar epidermis is 20% and the abaxial 20% whereas for *A. hybridus adaxial* is 20% and abaxial foliar stomatal index of 20%. Lower epidermis has more stomata than the upper one for both species. See plates 3, 4, 5 and 6.



Plates 3: *A. hybridus* Linn. Abaxial foliar surface.

Arrow showed presence of calcium oxalate deposits, ‘t’ revealed trichomes, ‘S’ represents anisocytic stoma, ‘e’ represents epidermal cells. ‘3b’ is the enlarged part of ‘3a’ circled, done for clarity.



Plates 4: *Amaranthus hybridus* adaxial foliar Epidermis. Arrow revealed contiguous cells While‘t’ showed trichome.

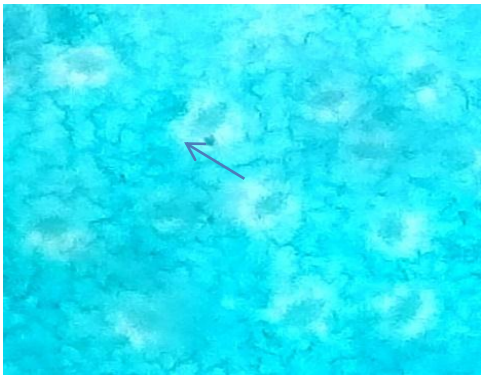


Plate 5: *Amaranthus spinosus* Abaxial foliar epidermis. Arrow revealed deposit of calcium oxalate.

Plate 6: *Amaranthus spinosus* adaxial foliar epidermis

Anatomical Study

Anatomical studies revealed pattern of cell types from epidermis, hypodermis, general cortex to the pith are similar in mid-ribs, petioles, stems and nodes. *A. hybridus* has more vascular bundles and wider pith in the stem while the root anatomy revealed more vessel elements for *A. hybridus* and no pith for both species. The roots have piths and vasculation is closed type. Plates 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

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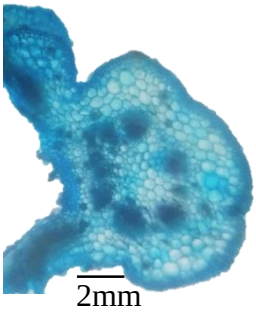

Plate 7: Anatomy of *A. hybridus* mid-rib. T.S.

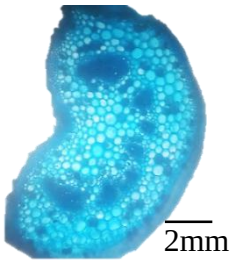

Plate 8: Anatomy of *A. hybridus* petiole. T.S.

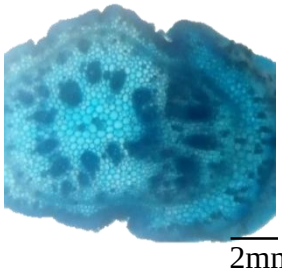

Plate 9: Anatomy of *A. hybridus* node. T.S.

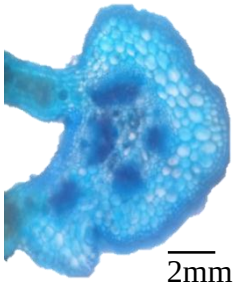

Plate 10: Anatomy of *A. spinosus* mid-rib

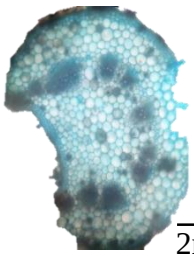

Plate 11: Anatomy of *A. spinosus*

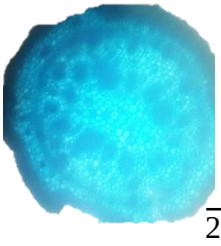

Plate 12: Anatomy of *A. spinosus* node.T.S.

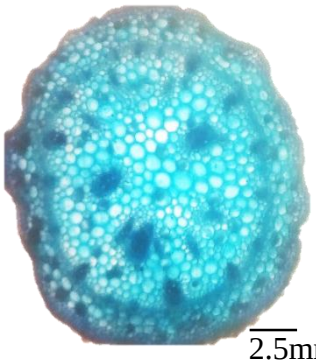

Plate 13: Anatomy of *A. hybridus* stem .T.S.

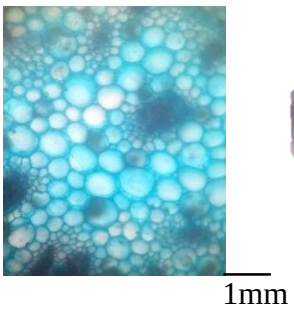

Plate 14: Anatomy of *A. hybridus* stem pith.

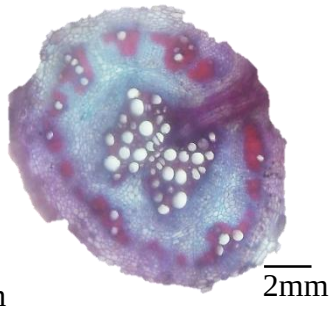

Plate 15: Anatomy of *A. hybridus* root. T.S.

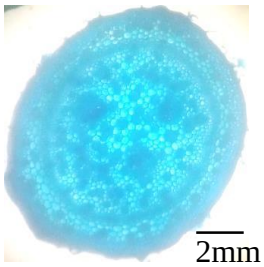

Plate 16: Anatomy of *A. spinosus* stem. T.S.

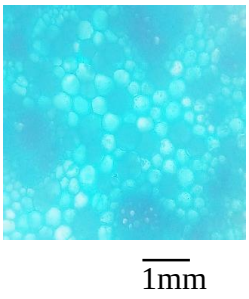

Plate 17: Anatomy of *A. spinosus* stem pith.

Plate 18: Anatomy of *A. spinosus* root. T.S.

Amaranthus hybridus is highly hybridized not only morphologically but anatomically also. Vasculation is open system; roots have no pith while large piths are observed in mid-ribs, petioles, nodes and stems of both species respectively.

Phytochemical Study

Phytochemical studies showed the presence of tannins, saponins, alkaloids, and flavonoids are present in *A. spinosus* while alkaloids were absent only in *A. hybridus.* This may be the reason why *A. spinosus* is used more in tradomedicine than *A.hybridus* used as vegetable. See table 1.

Table 1: Qualitative phytochemical studies on *Amaranthus spinosus* and *Amaranthus hybridus*

	Amaranthus spinosus		*Amaranthus hybridus*	
Phytochemical constituents	Methanol extract	Aqueous Solution	Methanol extract	Aqueous Solution
Akaloids	+	_	_	_
Flavonoids	+	_	+	_
Tannins	+	+	_	+
Saponins	+	+	_	+

DISCUSSION

Amaranthus hybridus and *Amaranthus spinosus* differ by the absence of a pair of axillary spines in the former which is eaten as vegetable, this observation has been highlighted by Akobundu and Agyakwa (1998], and Hutchinson and Dalziel (1954). *Amaranthus hybridus* is highly hybridized in nature; this is also revealed by Akobundu and Agyakwa (1998). Variations due to the environment in number of vascular bundles in mid-ribs, petioles and stems has been treated by Ozimede *et al*. (2019) and corresponds to that revealed in *Amaranthus spinosus* which is within 6 to 19 in number but for *Amaranthus hybridus* number of vascular bundles with species collected in Choba community in Obio-Akpor Local Government Area, there were those with 22 vascular bundles in stems transverse sections. Their use in tradomedicine is due to the presence of phytochemicals observed present in them. Both species are amphistomatic and stomatal type is anisocytic.

CONCLUSIONS

Amaranthus spinosus and *Amaranthus hybridus* are both useful in tradomedicine. Variations due to environmental implications in anatomical sections are becoming very obvious. In as much as morphological, anatomical and phytochemical studies on these plants may not be altogether new, proximate analysis, the karyotypes, quantitative aspect of phytochemistry and DNA barcodes may be essential area of future interest.

REFERENCES

Akobundu, I.A. and Agyakwa, (199). *A Handbook of West African Weeds*. INTEC Printers. Ibadan. Pp. 148-149.

Farnsworth, N.R. and Euer, K.L. (1962). *An Alkaloid screening procedure utilizing thin –layer Chromatography*. Lioydia. Pp. 25-186.

Harborne, J.B. (1973). *Phytochemical Methods: A Guide to modern Techniques of Plants Analysis*. Chapman and Hall London. 279pp.

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 91-100 *https://dx.doi.org/10.4314/sa.v20i1.8*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Nyananyo, B.L. (2006). *Plants from the Niger Delta.* Onyoma Research Publications. Port Harcourt. Pp. 21-23.

Ozimede, C.O., Obute, G.C. and Nyananyo, B.L. (2019). Morphological and Anatomical Diversity in the Study on 3 species of *Amaranthus* namely: *A. hybridus* L., *A. viridis* L. and *A. spinosus* L. from Rivers South, Nigeria. *J. Appl. Sci. Environ. Management*. Vol.23 (10) 1875-1880.

Shoppe, C.W. (1964). *Chemistry of the Steroids*, 2nd Ed. Butterworths, London. 56pp.

Smith, T.J. (2000). Squalene; Potential Chemo preventive Agents. *Expert Opin. Invest. Drugs*. 1841-1848.

Trease, G.E. and Evans, I.N.C. (1989). *A textbook of Pharmacognosy* 3rd ed. Boilliere Tinall LTD. London

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

Wahua, C. (2020). Free-hand Sectioning Machine Invented for Anatomical Studies of Biological Materials. *Scientia Africana*, Vol. 19 19 (1): 159 - 162.