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RESEARCH ARTICLE

MICRO-MORPHOLOGICAL PROPERTIES AND PHYTOCHEMICAL CONSTITUENTS OF *TERMINALIA IVORENSIS* A. CHEV. AND *TERMINALIA CATAPPA* LINN.

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ABSTRACT

This work is set to investigate the micro-morphological characteristics and phytochemical constituents of *Termina liacatappa* and *Terminalia ivorensis*, members of the family Combretaceae. *Teriminalia catappa* has two varieties: the red fruit and the yellow fruit varieties. The epidermal study revealed amphistomatic with anomocytic stomatal type in all three types investigated. These *Terminalia* species have relatively same phytochemical composition, however *Termina liacatappa* (yellow fruit variety) has the highest flavonoid concentration on the stem bark, hence a good source of anti-oxidant for therapeutic purpose in tradomedicine.

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INTRODUCTION

The genus *Terminalia* L. belongs to the family Combretaceae which comprises twenty genera and about four hundred and seventy-five species. (Thiombiano *et al.,* 2006). *Terminalia* L. is the second largest of the family. The genus *Taminalia* L. has about 250 species. About 25 species are present in West Africa(Hutchinson and Dalziel, 1954).

Distribution: *Terminalia catappa* L. is a native species of Southeast Asia, it is presently found in many countries of the tropics including Northern Australia, Pakistan, India, Sri. Lanka and many African countries (Jayaweera, 1992). *Terminalia ivorensis* (Black afara) is a large deciduous forest tree which belongs to the family Combretaceae. It is found in the rainforests of Central Africa but is predominantly a tree of seasonal forest zones. *Terminalia ivorensis* is found in China, Guinea, Ivory Coast, Liberia, Nigeria and Serra Leone. *Terminalia* has been reported to be a large genus consisting of over 200 Species of very large trees that occur extensively in the tropical regions of the World. It is usually found in forest and transition zones (Lamb and Ntima1991).

Medicinal values: *Terminalia ivorensis* showed great promise as a trypanocidal agent (Adewunmi *et al.,* 2001). Biological activities associated with the plant include anti-inflammatory and anti-arthritis (Iwu and Anyanwu, 1992) also antibacterial activity (Malcom and Sofowora, 1969). The powdered bark of *Terminalia ivorensis* (commonly called black afara) is used to treat ulcers, cuts, sores and wounds (Etukudo, 2003). In ethnomedicine, *Terminalia ivorensis* forms part of the treatment for syphilis among the Jukuns. The pulverized leaves are used as poultice to treat burns and bruises. Similarly the decoction of the bark is used as lotion for sores. The ashes of the leaf gall (common on the tree) is mixed with the roasted bulb of Crinum, made into an ointment with fresh cow butter is rub on rheumatic and other swollen joints for relief. The tree exudes gum while the bark yields black dye (Jayaweera, 1992). The bark of *Terminalia catappa* is used against bilious diarrhea, gastric fevers and dysentery. A decoction of the bark is recommended for curing gonorrhea and leucorrhoea. The young leaves are used for curing leprosy, scabies and other cutaneous diseases; (Jayaweera, 1982). In Taiwan, the fallen leaves are used as herbal drugs in the treatment of liver related diseases. The macro-morphology of the leaves and fruits vary considerably interms of size. There is little or n o information on the micro-morphology. The two species are used in traditional medicine, and the interest here is to acertain what kind of phytochemicals are present in them.

Aims and objectives: This research work is aimed at investigating themicro-morphological characteristics and the relationship in terms of the phytochemical constituents of the species. The research is expected to add to existing knowledge about the plants in plant biosystematics and taxonomy.

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MATERIALS AND METHODS

Study Area: Fresh green leaves of *Terminalia ivorensis*A. Chev. and *Terminalia catappa* (yellow and red fruit varieties) were collected from a botanical garden along East-West road Choba, Obio-Akpo L.G.A Rivers State; and identified by a plant taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

Epidermal Studies: Fresh leaves and stems were peeled and bleached using sodium hypochlorite for about 2 minutes following the method of Cutler (1978). For other fresh leaves whose epidermal layers were difficult to peel, chemical frictions or scraping method were used, usually the surface to be examined was placed on a glass slide while the other surface was carefully cleared by flooding with 5% sodium hypochlorite and scraping with cork material or razor. The cleared epidermal layers obtained were then washed in several changes of distilled water and stained with Alcian blue or safranin and temporarily mounted in aqueous glycerol solution (Cutler, 1978) modified. The epidermal peels were, thereafter, passed through series of 30%, 50%, 70%, 95%, 100% ethanol and then allowed to rehydrate in water for about 5 minutes. The treated peels were then stained with safranin and counter stained with methylene blue, the excess stain is washed off in distilled water and then placed on a glass slide where glycerin is applied on it before placing cover slip and sealant to avoid drying and insect attack. Microphotographs were taken from good preparation Digital Sony camera.

Phytochemical Screening Test

Test for alkaloid: Five grams (5gm) of the plant sample was pulverized and boiled with 10ml of 10% H_2S0_4 for 5 minutes on water bath. The mixture was filtered, and 2ml of the filtrate, 3-4 drops of the following reagents were added and observed: with Dragendorff's reagent, a reddish precipitate indicates the presence of alkaloids while with Mayer's reagent, a turbidity or cream precipitate indicates the presence of alkaloids and with Hager's reagent, a yellow precipitate indicates the presence of alkaloids. (Sofowora, 1993). Test for anthraquinone glycosides: Borntrager's test was done using 2gm of plant sample which was pulverized and macerated with 5ml of chloroform. The mixture was filtered and 2ml of 10% NH_3 was added to the filtrate and shaken vigorously and allowed to stand for few minutes and observed. A pink colour at ammoniacal layer indicates the presence of free hydroxyanthraquinone. (Trease and Evans, 2002). Borntrager's test for combine anthraquinone: Two grams (2gm) of plant sample was pulverized and boiled with 10ml of 10% H_2S04 on water bath for 10minutes. The mixture was filtered while hot and 5ml of chloroform was added to the filtrate and shake vigorously and then allowed to stand so as to fully partitioned. The chloroform layer was collected and 2ml of 10% NH_3 was added to the organic fraction with vigorous shake, then it was allowed to stand. A pink colour at ammoniacal layer indicates the presence of C-glycoside of anthraquinone glycoside. Borntrager's test for anthracene; Two grams (2gm) of plant sample was pulverized and boiled with 2ml of 5% $FeCl_3$ and 10ml of 10% H_2S0_4 for 10 minutes. The mixture was filtered while hot and 5ml of chloroform was added to the filtrate and it was shaken. Then it was allowed to stand for better partitioning. The chloroform layer was collected and 2ml of 10% NH_3 was added to organic fraction and it was vigorously shaken, and then allowed to stand. A pink, red or violet coloration in the ammoniacal layer indicate the presence of anthracene a characteristic anthraquinone derivatives. (Sofowora, 1993). Test for saponin glycoside: Frothing test: Two grams (2gm) of plant sample was pulverized and boiled with 5ml of distilled water for about 5 minutes on water bath and filtered. The filtrate was shook vigorously for 20 seconds and was allowed to stand. Observation of persistent frothing indicates the presence of saponin glycosides (Sofowora, 1993).

Emulsion test: Two grams (2gm) of plant sample was pulverized and boiled in 5ml of distilled water for 5 minutes on water bath and filtered. About 3-4 drops of olive oil was added to the filtrate and was shaken vigorously for few minutes and allow standing undisturbed. Presence of soluble emulsion indicates the presence of saponin glycosides. Sodium bicarbonate test: Two grams (2gm) of plant was pulverized and boiled in 5ml of distilled water for about 5 minutes on water bath, and filtered while hot. 1ml of 5% Na_2CO_3 and 1ml of Fehling's A and B solutions was added to the filtrate and boiled for 10-15minutes on water bath. Presence of brown or reddish brown coloration indicates the presence of saponin glycosides (Trease and Evans, 1989). Test for flavonoids: Shinoda Reduction test was carried out using 2gm of plant, pulverized and boiled in 5ml of distilled water for 5 minutes on water bath, and filtered while hot. Few pieces of magnesium metal were added to the filtrate and few drops of concentrated H_2S0_4 were carefully introduced to the mixture. The formation of orange, red, crimson or magenta coloration shows the presence of flavonoids (Trease and Evans, 1989). Sodium hydroxide test: Two grams (2gm) of plant sample was pulverized and boiled in 5ml of distilled water for 5 minutes on water bath, and filtered while hot. 1ml of 10% NaOH was added in drops and excess. The presence of yellow coloration on addition of NaOH and subsequent decolorization of the mixture on introduction of HCl to colourless indicates the presence of flavonoids.

Lead acetate test: Two grams (2gm) of plant sample was pulverized and boiled in 5ml of distilled water for 5 minutes on water bath, and filtered while hot. 2ml of 10% $Pb(CH_3COO)_2$ was added to the filtrate and observed. The presence of yellow precipitate indicates the presence of flavonoids.

Tests for tannins: Ferric chloride test was done using 2gm of plant sample which was pulverized and boiled in 5ml of distilled water for 5 minutes on water bath and filtered while hot. 1ml of 5% $FeCl_3$ was added to the filtrate and observed. The presence of blue black, green or blue green coloration indicates the presence of tannins. (Trease and Evans, 1989).
Test for phlobatannins: Two grams (2gm) of plant sample was pulverized and boiled in 5ml of distilled water for 5minutes on water bath and filtered while hot. 2ml of 5% HCl was boiled with the filtrate for 15 minutes on water bath. The presence of red colour precipitate indicates the presence of phlobatannins (Sofowora, 1993).

Test for carotenoids: Five grams (5gm) of plant sample was pulverized and macerated with 10ml of chloroform and filtered. The filtrate was divided into two, 2ml each and 1ml of concentrated H_2SO_4, concentrated HCl were added respectively. The presence of blue layer coloration in any of them indicates the presence of carotenoid. Test for steroid and triterpenoids: Salkowski's test was used whereby 2gm of plant sample was pulverized and macerated in 5ml of chloroform,

and filtered. 2ml of concentrated H_2SO_4 was carefully added to the filtrate and observed. A reddish brown colour at the interface indicates the presence of steroidal nucleus of aglycone in the cardiac glycosides. Lieberman-Burchard's test: Two grams (2gm) of plant sample was pulverized and macerated in 5ml of chloroform and filtered. 1ml of acetic anhydride was added to the filtrate followed by 2ml of concentrated H_2SO_4 to form a layer.

A colour change from violet to blue to green indicates the presence of steroidal nucleus, then, the existence of pink-red colour at the interface indicates the presence of triterpenoids nucleus. Test for cardiac glycoside (cardenolide): Kedde's test for lactone ring was used, 2gm of plant sample was pulverized and macerated in 5ml of chloroform and filtered. 1ml of 2% 3, 5-dinitrobenzoic acid was added to the filtrate followed by 2ml of 10% Na0H. An immediate purple or violet colour indicates the presence of cardiac glycosides.

Test for fixed oil: Five grams (5gm) of plant sample was vigorously but carefully rubbed with filter paper. The filter paper was allowed to air- dry for 5-10 minutes and then observed. Translucence on the filter paper proves the presence of fixed oil.

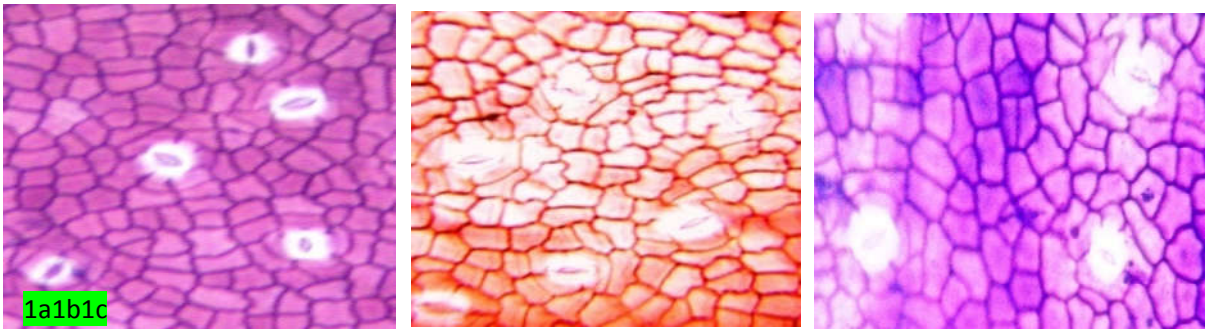

Plate 1a.Adaxial foliar of *T. catappa* (red); 1b: Adaxial of foliar *T. catappa* (yellow); 1c: Adaxial foliar of *T. ivorensis*

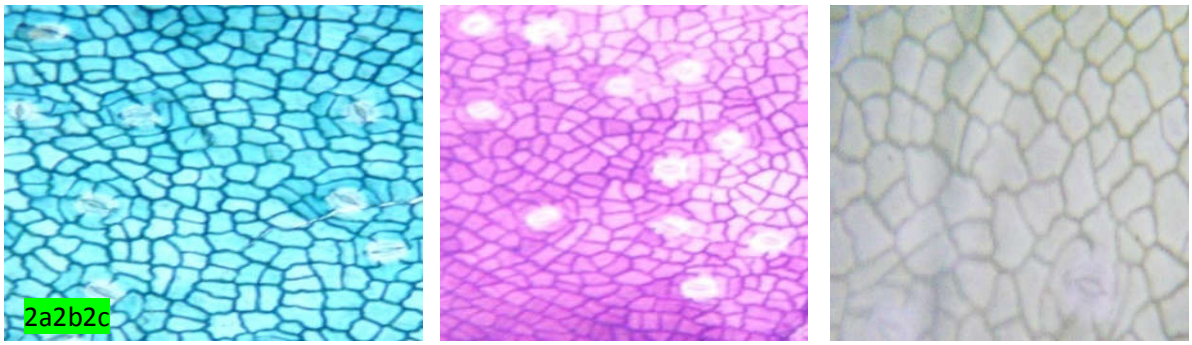

Plate 2a. Abaxial epidermis of *T. catappa* (red);2b. Abaxial epidermis of *T. catappa* (yellow); 2c. Abaxial epidermis of *T. ivorensis*

Table 3. Summary of the Qualitative Phytochemical Screening of *T. catappa*, (Red and Yellow varieties) and *T. ivorensis*

S/N	Phytochemicals/Test	*T.catappa* (Red)	*T. catappa* (Yellow)	*T. ivorensis*
A	Alkaloids			
i.	Drangedorff's Test	-ve	-ve	-ve
ii.	Mayer's Test	-ve	-ve	-ve
iii.	Hager's Test	-ve	-ve	-ve
B	Flavonoids			
i.	Shinoda Test	+ve	++ve	+ve
ii.	Lead acetate Test	+ve	++ve	+ve
iii.	$AlCl_3$	+ve	++ve	+ve
C	Tannins			
i.	$FeCl_3$ Test	+ve	+ve	+ve
ii.	Phlobatannins	-ve	-ve	-ve
D	Anthraquinone (Bontrager;S Test			
i.	Free Anthraquinone	-ve	-ve	-ve
ii.	Combined Anthraquinone	-ve	-ve	-ve
E	Triterpenoid /steroid Test			
i.	Liebermann-Buchard Test	+ve	+ve	+ve
ii.	Salwoski Test	+ve	+ve	+ve
F	Fixed Oils	-ve	-ve	-ve
i.	Kedde Test	+ve	+ve	+ve
I	Saponins Test			
i.	Frothing Test	+ve	+ve	+ve
ii.	Emulsion Test	+ve	+ve	+ve
	Carotenoid Test	-ve	-ve	-ve

Legend- present (+ve), absent (-ve).

RESULTS

Epidermal studies: The species of *Terminalia* studied indicates relatively little or no difference in their epidermal features; the cells of the epidermis of the species are polygonal, isodiametric or elongated in various directions with irregular arrangement (anomocytic). The anticlinal epidermal are

sinuous, undulating, straight or arched. Combretatious glandular trichomes were observed on the abaxial surface of the leaves alongside with amphistomatic stomatal distribution. However, the abaxial has greater number of pavement cells compared to the adaxial epidermis.

DISCUSSION

Extract of the stem bark from the three species show the presence of tannins, flavonoid, Triterpenoid, Cardenolide and Saponin with the yellow fruit variety having the highest concentration of flavonoid.

Conclusion

The species studied also show relationship in such features as phytochemical composition, other areas of interest need are anatomical descriptions, DNA barcoding, Palynology and quantitative aspect of phytochemical analysis.

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