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Phyto-anatomical characteristics of the West African {Umbrella tree} *Musanga cercropioides* M.Smithii R. Br. (Moraceae)

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Abstract: Two complimentary studies of anatomy and phytochemistry used in pharmacognostic drug research have been conducted on *Musanga cercropiodes* using light microscopy and phytochemical methods. Diagnostic anatomical features of the plant include bulbous trichome bases, and flaky strand-like waxes. Other distinguishing features of the plant are hypostomatic leaf, anomocytic stomata, abaxially restricted simple unicellular and non glandular trichomes as well as crescentiform and hairy petiole. The bioactive compounds which are present in both leaf and bark are alkaloids, flavonoids, tannins, free and bound anthraquinone, saponin and cardiac glycosides but anthocyanosides and cyanogenic glycosides are absent. *Musanga cercropioides* is a popular plant in folkloric medication in West Africa.

Keywords: *Musanga cercropioides, phytochemistry, anatomy.*

Introduction

Musanga cercropioides M.Smithii R. Br. is found mostly in the tropical forests of Africa (Kamanyi *et al*., 1992). It is a quick growing soft wooded tree with straight stem, stilt roots and an umbrella- like crown, up to about 60cm high. Branchlets are very stout and pithy. The large stipular sheaths enclosing the bud and inflorescence are wine-red, densely silky inside and deciduous. It is a pioneer colonizer constituting the first phase of succession leading to the rebuilding of rain forest (Hutchinson & Dalziel, 1954). The leaf is digitately divided into 12-15 spreading segments; segments entire, narrow, shortly acuminate, up to 45cm.long and 10cm.broad, covered with grayish indumentum beneath; lateral nerves numerous and very conspicuous beneath. Stipules large, connate, 15-20cm.long densely pubescent; male flowers in numerous small round heads about 4mm.diameter, but female inflorescence is about 2cm long on a peduncle that is up to 12cm long. Fruit is yellowish green and succulent. The wood of the plant can be sourced for production of match stick and paper making. The medicinal uses of the plant include promoting menstruation, inducing labour, lowering elevated blood pressure and high blood sugar, as dehydrant, expectorant, anthelminthic, antidysenteric and analgesic, For treating asthenia in infants, for restoration of appetite, bark macerate for treating toothache and as a decoction for pulmonary troubles. It is also useful as cough medicine, for dressing wounds and sores. Bark scrapings can be used as aphrodisiac (Gill, 1994; Irvine, 1961; Kamanyi *et al*., 1992). The aerial stilt-roots and also the younger branches are noted for their capacity of yielding a large amount of potable sap. 'Half a bucketful' is said to be obtainable from a single tree overnight (Irvine, 1961). The sap is colourless, odourless and of an insipid sweetish taste. The sap is drunk as blood-purifier, for cleansing stomach, for blenorrhoea, cough and chest infections, as a galactogogue, and commonly as a wash for persons with sleeping sickness, leprosy and fevers to relieve aches and pains and rheumatism. The wood is also used for construction works (Kamanyi *et al*., 1992; Lontsi *et al*., 1998).

The chemical properties of the plant have been reported (Kamanyi *et al*., 1992; Trease & Evans, 2001). The plant sap contains estrogen, galactogen lactogen etc. Isovitexin, vitexin, cholorogenic acid, catechin and procyanidins have been isolated from the leaf. Other phytochemical studies have also reported the presence of kalaic acid in the stem bark and some other triterpenoid acids in the leaves, stem bark and the root (Lontsi *et al*., 1998). Previous studies established uterotonic effects of leaf in rats (Kamanyi *et al*., 1992), the hypotensive effects of the water extracts of the leaf and stem bark (Dongmo *et al*., 1996; Ayinde *et al*., 2003) as well as antihyperglycaemic activities of the leaf extract in laboratory animals.

Table 1. Qualitative and quantitative leaf and petiole anatomical characteristics of Musanga cercropioides

Anatomical characters	Adaxial leaf surface	Abaxial leaf surface
Epidermal cell shape	Polygonal	Polygonal
Anticlinal wall pattern	Straight - curved	Straight - curved
Stomatal type	Absent	Anomocytic
Trichome type	Simple, unicellular non-glandular	Absent
Other epidermal appendages	Trichome bases present	Waxes present
Epidermal cell no. per mm²	25(35±3)42	20(32±3)40
Mean epidermal cell size (µm)	45.0 x 23.6	35.0 x 18.5
Stomatal no. per mm²	---	10(12±2)15
Mean stomatal size (µm)	---	36.0 x15.4
Petiole shape	Convex	Crescentiform
Trichome type	Simple, unicellular non-glandular	Simple, unicellular non-glandular
Trichome length (µm)	85- 200	85-200

M. cecropioides in West Africa is also known for its oxytocic, hypotensive and antidiabetic activities. The investigation of plant anatomical characters is an intrinsic aspect of pharmacognostic drug research. It is an approach that exposes the location areas of the bioactive compounds which present those features that may be diagnostic and useful in separating the plant from close allies. There are good contributions on the phytochemistry of the plant (Ayinde *et al*., 2003; 2006; Buniyamin *et al*., 2007; Dongmo *et al*., 1996; Lontsi *et al*., 1998), but the anatomical data of the plant is scanty. Metcalfe and Chalk (1950;

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Table 2. Phytochemical analysis of bark extract of M. cecropioides

Alkaloids	Test	Observation	Inference
A	2ml of extract + Dragendorff's reagent	Dark orange-red ppt	Alkaloid present
B	2ml of extract + Mayer's test	Dark yellowish ppt	Alkaloid present
C	2ml of extract + Wagner's reagents	Dark turbid brown ppt	Alkaloid confirmed
Flavonoids			
A	2ml of extract + 2ml of fecl3	Wooly brownish ppt	Flavonoid confirmed
B	2ml of extract + 2ml of 10% Lead acetate	Yellowish green ppt	Flavonoid confirmed
C	2ml of extract + 2ml of dil. NaoH	Golden reddish ppt	Flavonoid confirmed
Tannins			
A	2ml of extract + 2ml of fecl3	Wooly brownish ppt	Catehol Tannins present
B	2ml of extract + 2ml of Bromine H2O	Turbid brownish reaction	Condensing Tannins present
Anthraquinone glycosides			
A	BORNTRAGERS TEST. 2ml of extract + 10ml of Benzen flittered + 5ml of 10% ammonia soln.	Reddish colour confirmed	Anthraquinones confirmed
B	COMBINED ANTHRAQUINONES. 2ml of extract + dil. H2SO4 filtered + Benzene+ ammonia soln.	Red coloured in ammonia phase	Anthraquinone Derivatives confirmed

Table 3; Phytochemical analysis of bark extract investigating anthraquinone glycosides, anthocyanosides cyanogenic glycosides and saponins

	Test	Observation	Inference
Anthraquinone glycosides A	BORNTRAGERS TEST 2mls of extract + 10mls of Benzene flittered + 5ml of 10% ammonia soln.	Reddish colour confirmed	Anthraquinones confirmed
B	COMBINED ANTHRAQUINONES 2mls of extract + dil. H2SO4 filtered + Benzene + ammonia solution	Red coloured in ammonia phase	Anthraquinone Derivatives confirmed
Anthocyanosides A	2ml of extract + 2ml of dil. HCl	No offensive reaction for the test	Free anthocyanosides
Cyanogenic Glycosides A	2ml of extract + sodium pictrate paper	No brownish colour was found reacted to the sodium pictrate paper	Free from cyanide
Saponins A	BENEDICTS TEST 2mls of extract + 2ml of Benedicts reagents	Blue black ppt.	Saponin present
B	FROTHING TEST 2mls of extract was shaken vigorously to observe the reaction	Frothing persist.	Saponin present
C	EMULTION TEST 2ml of extract + 2ml of Arachies oil	Turbid stable emulsion in the sample	Positive to emulsion reaction
D	HAEMOLYSIS OF RBC 2ml of extract + 2ml of 20% blood in saline mix well and centrifuge	Blood haemolyzed after the test	Saponin present

1979) documented the anatomical characteristics of the family with very limited mention of *M. cercropioides*. Therefore, the present study was undertaken so as to contribute more data on the phytochemistry and leaf anatomy of the plant. These features can be useful in distinguishing the plant from other related species. These studies are complementary for pharmacognostic drug research.

Materials and methods

The leaf and bark of *Musanga cecropioides* collected from fields across southern Nigeria were used for the study. Specimen authenticity was determined in the herbarium of University of Lagos (LUH) and Forestry Research Institute of Nigeria (FHI). Herbarium abbreviations follow Holmgren *et al.* (1990). For anatomical study, the leaf epidermis and petiole were investigated. About 3-5cm² leaf portions which were cut from the standard median area of the leaf lamina near the mid-rib were used. Dried leaves were revived by boiling in water for thirty minutes and they were either soaked in concentrated trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8-24 hours to macerate the mesophyll or irrigated in sodium hypochlorite solution (commercial bleach) for 30-120 minutes to bleach the leaf portions. Tissue disintegration was indicated by bubbles and the epidermides were transferred into Petri dishes containing water for cleansing and then, separated with forceps and mounting needle. In case of fresh leaves, they were scraped with razor blade to separate epidermides. Tissue debris were cleared off the epidermides with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol: 50% - 100% were added in turns to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five

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Table 4. Phytochemical analysis of bark extract investigating cardiac glycosides

	Test	Observation	Inference
Cardiac glycosides A	LEGAL TEST 2ml of extract + 2ml of pyridine and a few drops of 2% sodium nitropruside + 20% NaOH	Brownish colour seen	Cardenolides present
B	LIEBERMAN'S BURCHARDS TEST. 2ml of extract + 2ml of acetic acid + H_2SO_4 (conc.) carefully added and cool	Brownish green observed	A steroidal nucleus present
C	SALKOWSKI TEST 2ml of extract was dissolved with 2ml of chloroform + conc. H_2SO_4 carefully added	Deep reddish brown colour at the interface a steroid ring seen	A glycone portion of the cardiac glycosides present
D	KEDDE TEST 2ml of extract + 3.5 dinitrobenzoic acid in methanol + NaoH	Reddish brown ring	Lactone ring in cardenolides present
E	KELLER-KILIANI TEST 2ml of extract + 2ml of glacial acetic acid + $FeCl_3$ + conc. H2SO4	Brownish green ring seen	A de-oxy sugar character of cardenolids present

minutes before mounting in glycerine on the glass slide with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration.

The transverse sections of the petiole were obtained by free hand sectioning using a sharp razor blade. The thin sections were bleached in commercial bleach to remove chlorophyll and these were mounted in glycerin after staining with acidified phloroglucinol. Preparations were later observed at x40 and x100 under Zeiss light microscope. Photomicrographs were taken using Motic camera attached to the light microscope and observed on Pentium IV computer.

Phytochemical studies:

For phytochemistry, pieces of the bark and leaves were spread on sterilized work bench for about 4-5 days. They were .oven dried at 60°C for 24hrs, after which they were milled into powder and then kept in air-tight containers.

The bioactive compounds were extracted with 96% ethanol using soxhlet extractor for 6 hours and the extracts were further evaporated to dryness with the aid of vacuum rotary evaporator machine. The extracts were screened for the presence of plant secondary metabolites such as alkaloids using Mayer's, Drangendorff's and Wagner's reagents, flavonoids using lead acetate, ferric chloride and sodium hydroxide tests. Tannins were investigated with the aid of ferric chloride and bromine water, anthraquinone (free and bound) using chloroform and dilute ammonia solution, and hydrolysis, anthocyanosides, cyanogenic glycosides using sodium picrate papers, saponin using frothing and haemolysis tests, cardiac glycosides using legal, Kedee's Liebermenn-burchard's, Salkowski's and Keller-Keliani's tests and reducing compounds by the use of Fehling's solutions A & B. The extraction procedures are presented in Tables 2- 7.

Table 5. Phytochemical analysis of leaf extract investigating alkaloids, flavonoids, and tannins

	Test	Observation	Inference
Alkaloids A	2ml of extract + Dragendorff's reagent	Light orange turbid coloured	Alkaloid confirmed
B	2ml of extract + Mayer's test	Dark yellow ppt.	Alkaloid confirmed
C	2ml of extract + Wagner's reagents	Light turbid brownish ppt.	Alkaloid confirmed
Flavonoids			
A	2ml of extract + 2ml of FeCl3	Wooly brownish ppt	Flavonoid confirmed
B	2ml of extract+ 2ml of 10% Lead acetate	Light Yellowish green ppt.	Flavonoid confirmed
C	2ml of extract + 2ml of dil. NaOH	Reddish golden colour ppt.	Flavonoid confirmed
Tannins			
A	2ml of extract + 2ml of FeCl3	Dirty brownish ppt.	Catehol Tannins present
B	2ml of extract + 2ml of Bromine H_2O	Light turbid brownish	Condensing Tannins present

Results

The synopses of the results are presented in Figs.1 & 2 and Tables 1-8. The leaf is hypostomatic, the epidermal cell shape is irregular to polygonal in shape but the anticlinal wall pattern is straight to curved (Figs. 1& 2, Table 1). Other leaf anatomical characteristics include simple unicellular and non-glandular trichomes (Fig. 2A, Table 1), copious deposition of flaky waxes and marks of previous trichome existence otherwise called trichome bases (Fig. 1A). The cell number varies from 25 – 40 on both surfaces. Mean cell sizes are 45.0 x 23.6µm and 35.0 x 18.5µm on the adaxial and abaxial surfaces respectively (Table 1). The stomatal number ranges from 10 – 15 whereas mean stomatal size is 36.0 x 15.4µm. The petiole is crescentiform on the abaxial and convex on the adaxial pubescent surface (Fig. 2B). Flavonoids, anthraquinone, saponin and cardiac glycosides are present but anthocyanosides and cyanogenic glycosides are absent.

Discussion

The anatomical features, both

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Table 7. Phytochemical analysis of leaf extracts investigating cardiac glycosides

	Test	Observation	Inference
Cardiac glycosides A	LEGAL TEST 2ml of extract + 2ml of pyridine and a few drops of 2% sodium nitropruside + 20% NaOH	Brownish colour seen	Cardenolides present
B	LIEBERMAN'S BURCHARDS TEST 2ml of extract + 2ml of acetic acid + H2SO4 (conc.) carefully added and cool	Light brownish green seen	A steroidal nucleus present
C	SALKOWSKI TEST 2ml of extract was dissolved with 2ml of chloroform + conc. H2SO4 carefully added	Deep reddish brown colour, at the interface a steroid ring seen	A glycone portion of the cardiac glycosides present.
D	KEDDE TEST 2ml of extract + 3.5 dinitrobenzoic acid in methanol + NaOH	Reddish brown ring	Lactone ring in cardenolides present
E	KELLER-KILIANI TEST 2ml of extract + 2ml of glacial acetic acid + FeCl3 + H2SO4 conc.	Greenish brown ring	A de-oxy sugar character of cardenolids present

Table 6. Phytochemical analysis of leaf extracts investigating anthraquinone glycosides, anthocyanosides cyanogenic glycosides and saponins

Anthraquinone glycosides	Test	Observation	Inference
A	BORNTRAGERS TEST 2mls of extract + 10mls of Benzene flittered + 5ml of 10% ammonia solution	Reddish coloured confirmed	Anthraquinones confirmed
B	COMBINED ANTHRAQUINONES 2mls of extract + dil. H2SO4 filtered + Benzene + ammonia solution	Red coloured in ammonia phase	Anthraquinone Derivatives confirmed
Anthocyanosides A	2ml of extract + 2ml of dil. HCl	No offensive reaction for the test	Free anthocyanosides
Cyanogenic Glycosides A	2ml of extract + sodium pictrate paper	No brownish colour was found (with sodium pictrate)	Free from cyanide
Saponins A	BENEDICTS TEST 2ml of extract+ 2ml of Benedicts reagent	Blue black ppt.	Saponin present
B	FROTHING TEST 2mls of extract was shaken vigorously to observe the reaction	Frothing persist	Saponin present
C	EMULTION TEST 2ml of extract + 2ml of Arachies oil	Turbid stable emulsion in the sample	Positive to emulsion reaction
D	HAEMOLYSIS OF RED BLOOD CELLS 2ml of extract + 2ml of 20% blood in saline mix well and centrifuge	Blood haemolyzed after the test	Saponin present

quantitative and qualitative of the leaf can be employed in distinguishing the species from other members of Moraceae even when the leaves are fragmentary. These characteristics can also be used in solving the problem of herbal adulteration and substitution (Inamdar & Gangadhara, 1977; Olowokudejo, 1993; Rejdali, 1991; Singh & Dube, 1993; Kadiri & Ayodele, 2003; Ogundipe & Wujek, 2004). Useful anatomical features of the plant which can be used for identification are bulbous trichome bases, flaky strand-like waxes, hypostomatic leaf, anomocytic stomatal type, abaxially restricted simple unicellular and non glandular trichomes as well as crescentiform and hairy petiole. Plants that are used in folkloric medicine should be screened for their chemical properties in order to source them for drugs (Odebiyi & Sofowora. 1978; Trease & Evans, 2001). The chemical substances of plants are stored up in different parts of the plant. In *Mussanga cercropioides,* the bioactive substances were found in the bark as well as leaf; alkaloids, tannins, flavonoids, anthraquinone, saponin and cardiac glycosides are present in them but anthocyanosides and cyanogenic glycosides are lacking. Cyanogenic glycosides when under certain conditions, have been reported to yield prussic hydrocyanic acid which is a deadly poison. It further confirms that the plant can be harnessed for consumable drug production and it is also one good reason for its wide application in the West African folkloric medicinal application.

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Table 8. Phytochemical properties of Musanga cercropioides

Chemical constituent	Leaf	Bark
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Anthraquinone (Free)	+	+
Anthraquinone (Bound)	+	+
Anthocyanosides	-	-
Cyanogenic glycosides	-	-
Saponins	+	+
Cardiac glycosides	+	+
(i) Legal test	+	+
(ii) Keller Killiani	+	+
(iii) Salkowski test	+	+
(iv) Liebermers test	+	+
(v) Keddle test	+	+

+ = Positive, - = Negative

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Fig.1. Epidermal characteristics of M. cecropioides. A: Adaxial surface. Note Polygonal epidermal cell shape with crystal-like cell inclusions and circular glandular trichome bases (arrowed). B: Abaxial surface covered by wax deposits which occur as interlocking flakes and long unicellular trichomes obscuring other epidermal features. Note stomata with thick rim and narrow to wide aperture. Stomata are arrowed. Scale bar is 50µm.

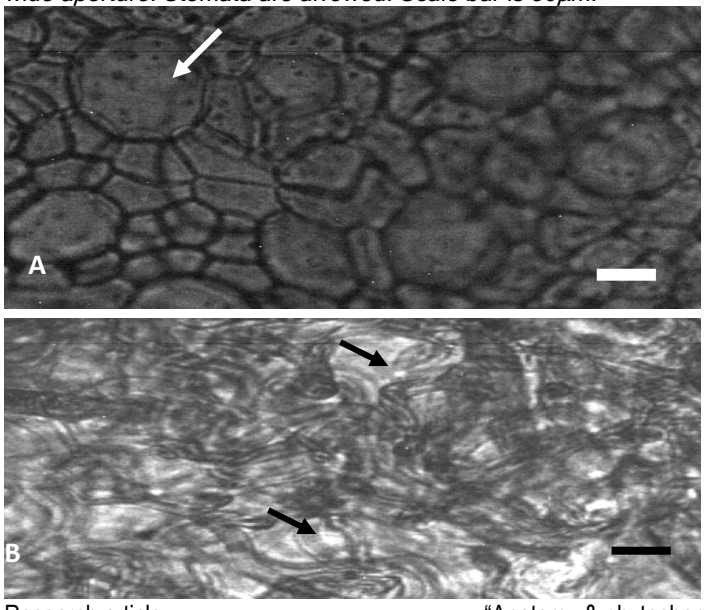

Fig. 2. Petiole anatomy of Musanga cercropioides. Petiole is crescentiform on the abaxial surface and convex on the adaxial surface. The surface is pubescent. Scale bar= 50µm