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PHARMACOGNOSTIC EVALUATION OF THE LEAF OF ANTIARIS TOXICARIA (FAMILY: MORACEAE)

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ABSTRACT: Background: Antiaris toxicaria is a tree in the mulberry and fig family of Moraceae. The leaf is used to remedy various ailments but the preparation depends on individuals. There is a need to set a standard for its preparation that would ensure optimum therapeutic benefits. Medicinal plants are generally reputed as cheaper and safer alternatives to synthetic drugs but these are often adulterated with inferior materials thereby affecting the overall therapeutic advantage. Pharmacognostic studies on such medicinal plants are therefore necessary to set standards for the preparation of herbals. Materials and Methods: Macroscopic and microscopic studies, including anatomical sectioning, chemo-microscopic, fluorescent analysis, and numerical and quantitative leaf microscopy were evaluated according to standard and reported method. **Results:** The leaf is simple, lanceolate or oblong, alternate, with reticulate venation, acute apex and cuneate base. Types of calcium oxalate crystals found were rosettes and prisms. Stomata are anomocytic with epidermal cells radiating around the stoma. Leaf is isobilateral. It has cellulose, tannins (condensed), calcium carbonate, fats and fatty oils, proteins, lignin, starch. Numerical and quantitative features are within standard range. **Conclusion:** The diagnostic features established from this research are a useful guide for correct identity and quality assurance in the preparation of A. toxicaria leaf for curative purposes.

INTRODUCTION: Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of poor communities and local therapy is the only means of medical treatment for such communities ¹. As more people return to herbal remedies, adulteration and substitution of these herbals are on the increase.



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This is further worsened by the complexity in composition and diversity in various constituents of the plant due to different geographical sources for these plants. Antiaris toxicaria is one of such medicinal plants with a remarkably wide distribution in tropical regions. It is commonly referred to as "bark cloth tree" in English and often described as "false iroko" in Igbo because of its striking morphological resemblance to Iroko tree (Milicia excelsa). The wood of A. toxicaria resembles that of Triplochiton scleroxylon K.Schum and can be used as a substitute for the latter. It can also be substituted for Terminalia superba Engl. & Diels and for the wood of Pterygota macrocarpa K.Schum². It is a large tree in the mulberry and fig family, growing to 25-40 m tall, with a trunk up to 40 cm diameter, often buttressed at the base, with pale grey bark 3 . In Africa, there are three varieties clearly distinguished by habitat and their juvenile forms, one is confined mainly to wooded grassland, the other two are found in wet forests, riverine forest and semi-swamp forests 2,4 . It is valued as a source of wood, bark cloth, and pharmacological or toxic substances 5,6 .

In Africa the latex is applied to cuts, wounds and skin complaints such as eczema and leprosy, and is taken internally as a purgative ⁵. The active principles of the latex are cardiac glycosides, which have digitalis-like effects on the heart ^{7, 8}. In larger amounts they lead to cardiac arrest and secondary effects such as vomiting and convulsions. The latex is one of the principal components of most dart and arrow poisons in South-East Asia 9. It is thus used medicinally and as a hunting poison. The bark has a high concentration of tannins that are used in traditional clothes dyeing and paints. It is used as an anodyne, vermifuge and in the treatment of hepatitis ⁵. A decoction of the inner bark is drunk as a treatment for cancer, leukaemia and spleenomegaly ¹⁰.



FIG. 1: A TWIG OF ANTIARIS TOXICARIA

An aqueous ethanol extract of the bark is reported to have cytotoxic activity against tumour cell lines ¹¹. In Indonesian traditional medicine, the leaves

and root are used to treat mental illness ¹². In Africa and various parts of Asia, the leaves and bark are used as astringent and febrifuge ¹³. Added to this, the seeds are also used as a treatment for dysentery ¹⁴. Against the rampant cases of adulteration and resemblance of *Antiaris toxicaria* to a nember of other plants, establishment of the pharmacognostic profile of the leaves of *Antiaris toxicaria* will assist in standardization, which can guarantee quality, purity and identification of samples ¹⁵.

MATERIALS AND METHODS: Fresh leaves of *Antiaris toxicaria* were collected from a tree along Nru junction road near University of Nigeria, Nsukka new gate in the month of July, 2015. The leaves were carefully washed in water to exclude dusts and other extraneous materials. Identification and authentication were done by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development (Inter CEDD) Nsukka, where voucher specimens were deposited with the number INTERCEDD/ Mora./ 082015.

Macroscopy: The following macroscopic and organoleptic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste ^{16, 17}.

Fluorescence Study: A small quantity of the dried plant powder was placed on a grease-free microscopic slide and 1-2 drops of freshly prepared reagent solution was added, mixed by gentle tilting the slide and waited for 30 minutes. The prepared slide was placed inside an ultraviolet chamber and the colour exhibited was observed in visible light: short (254 nm) and long (365 nm) UV radiations. The colour observed by application of different reagents in different radiations was recorded ¹⁸.

Chemomicroscopic Examination: Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques ¹⁷.

Microscopy: The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence / absence of the following were observed: epidermal cells,

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stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed ¹⁹.

Quantitative Investigation: Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number were carried out on epidermal strips. Other parameters determined for the powdered leaves were moisture content, total ash, acid – insoluble ash, water – soluble ash, alcohol (90 % ethanol) and water soluble extractive values ²⁰.

RESULTS: Macroscopically, the leaf is simple in composition, alternate in arrangement, apex is acute, base is cuneate, margin is crenate, venation is reticulate, shape is lanceolate or oblong and average leaf size is 11.63 cm length x 6.20 breadth. Fresh leaves are green in colour; odourless and tasteless (**Table 1**).

TABLE 1: MACROMORPHOLOGICAL AND ORGANOLEPTIC FEATURES OF A. TOXICARIA

S.no.	Character	Observation
1	Colour	Adaxial: dark green
		Abaxial: light green
2	Odour	Characteristic
3	Taste	Bland
4	Length	11.63 cm
	Width	6.20 cm
5	Shape	Lanceolate or Oblong
6	Texture	Very rough
7	Surface	Scabrous
8	Apex	Acute
9	Base	Cuneate
10	Margin	Crenate
11	Leaf arrangement	Alternate
12	Venation	Reticulate
13	Presence/absence of petiole	Petiolate
14	Lamina	Intact
15	Presence/absence of stipule	No stipulate
16	Leaf type	Simple

Micro morphological features revealed rosettes and prisms of calcium oxalates (**Fig. 2** and **5**), numerous similar epidermal cells radiating about the stoma (anomocytic arrangement) as shown in **Fig. 3** below. Uniseriate covering trichomes are present on both epidermal surfaces and are triangular with a very pointed tip and a broad base (**Fig. 4** and **5**). It has Isobilateral leaf arrangement.

The transverse section of the mid-rib shows wide parenchymatous pith against pericyclic fibres in the petiole (**Fig. 5**). Chemomicroscopic examination of the leaves revealed the presence of cellulose, tannins (condensed), calcium carbonate, fats and fatty oils, proteins, lignin, starch. The fluorescence analysis shows various colours exhibited by the powder in different solvents (**Table 3**). The numerical and quantitative values are presented in **Tables 4** and **5**.

TABLE 2: CHEMO MICROSCOPIC FEATURES OF A. TOXICARIA

IOXICARIA				
Chemicals	Reagents	Observations	Inference	
Cellulose	N/50 Iodine +	Blue-black	Present	
	80 % Sulphuric	colour		
	acid			
Tannins	70 % Methanol	Blue-black	Present	
Condensed	+ dil. Ferric	colour	Present	
tannins	chloride More	Olive green		
	ferric chloride	colour		
Calcium	Acetic acid +	Effervescence	Absent	
carbonate	50 % Sulphuric	+needle-		
	acid	shaped crystals		
		separated		
Fats and	Sudan IV + heat	Orange-red or	Present	
fatty oils		brick red		
		substances		
Proteins	Few drops of	Blue colour	Present	
	Ninhydrin +			
	gentle warming			
	for 5 min			
Lignin	Few drops of	Pink or cherry	Present	
	phloroglucinol	red colour		
	+ stand for 2 –			
	$3 \min + \text{drop of}$			
	con. HCl			
Starch	Few drops of	Deep blue to	Present	
	N/50 Iodine	pinkish colour		

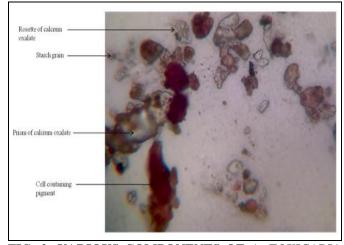


FIG. 2: VARIOUS COMPONENTS OF A. TOXICARIA POWDERED LEAF

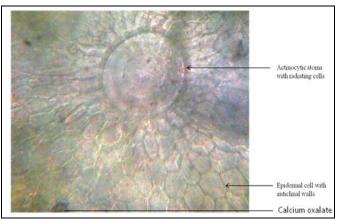


FIG. 3: EPIDERMAL LAYER OF A. TOXICARIA SHOWING ANOMOCYTIC STOMATA

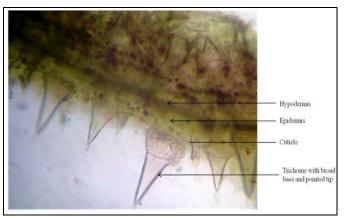


FIG. 4: SECTION OF A. TOXICARIA LEAF SHOWING VARIOUS COMPONENTS OF THE EPIDERMAL LAYER

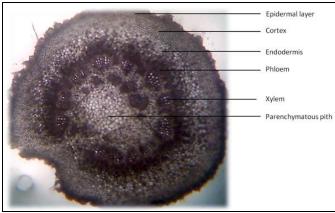


FIG. 5: PETIOLE OF A. TOXICARIA IN T.S.

TABLE 3: FLUORESCENCE ANALYSIS OF LEAVES OF ANTIARIS TOXICARIA

Detection reagents	UV wavelength / Color exhibited	
	254 nm	365 nm
Petroleum ether	Brick red	Dark green
Methanol	Brick red	Dark red
50 % HCl	Dark brown	Deep brown
50 % H ₂ SO ₄	Greenish	Brown
Ammonia	Dark green	Greenish black
Ethyl acetate	Brick red	Brown

TABLE 4: PHYSICOCHEMICAL FEATURES OF LEAVES OF ANTIARIS TOXICARIA

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S.no.	Parameters	Leaf powder
1	Moisture content (LOD) (% w/w)	08.01 ±1.02
	Ash Values	
2	Total ash (% w/w)	16.00 ± 0.29
3	Acid-insoluble ash (% w/w)	11.00 ± 0.29
4	Water soluble ash (% w/w)	02.50 ± 0.03
	Extractive values	
5	Water soluble extractive value (% w/w)	03.83 ± 0.26
6	Alcohol soluble extractive value (% w/w)	02.33 ±0.33

TABLE 5: QUANTITATIVE LEAF MICROSCOPY OF ANTIARIS TOXICARIA

Parameter	Mean
Palisade ratio	35
Stomata number Upper surface	1
Stomata number Lower surface	15
Stomatal index Upper surface	9.76
Stomatal index Lower surface	28.85
Vein islet number	8
Veinlet termination number	3

DISCUSSION: A. toxicaria leaf is used for curative purposes by traditional herbal practitioners and there is a need to ensure a standard guide to preparation. This would forestall adulteration, which can render a medicinal plant poisonous due to the nature of adulterants. The pharmacognostic parameters established in this research would facilitate the creation of a monograph. A. toxicaria is a plant that can be confused with Milicia excelsa due to macro-morphological resemblance. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. Key macro— and micro— morphological features of the leaf described are distinctive and analytical.

Chemo microscopy, numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization. The veinlet termination, vein islet numbers and stomatal index are relatively constant for a particular leaf ¹⁵ and therefore particularly diagnostic.

CONCLUSION: Data obtained from this pharmacognostic studies are useful diagnostic profile for *Antiaris toxicaria* and could be used for quality control of the plant in terms of purity and authenticity.

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