Trends in
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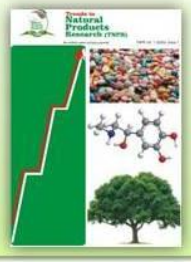

Standardization of *Combretum platypterum* (Welw) Hutch & Dalziel (Combretaceae) Leaf

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Abstract: Standardization is the investigation and documentation of unique and peculiar characters and properties which identify and separate a plant from others even members of the same family. *Combretum platypterum* (Welw.) Hutch. & Dalziel belongs to the family Combretaceae which is known to contain many secondary metabolites and have many pharmacological activities. While there are reports on some members of the genus C*ombretum*, there is virtually none on the plant *Combretum platypterum* (Welw) Hutch. & Dalziel. This research aimed to provide information on standardization parameters of the leaves of *Combretum platypterum* (Welw.) Hutch. & Dalziel. The fresh leaf was used for quantitative microscopy studies while a part of the collection was dried under shade, powdered and used for powder microscopy, physico-chemical, phytochemical, mineral content and acute toxicity analysis. The leaf is hypostomatic with paracytic stomata on the abaxial face. The total ash value (10.5 %), water-soluble ash value (5.53-7.5 %), acid insoluble ash value (0.50-1.01 %), sulphated ash 3.7-6.0 %), moisture content (LOD) (9.33-9.67 %), water extractive value (5.13 %), ethanol extractive value (0.8 %) were obtained. Phytochemical evaluation revealed that the 70 % methanol extract contained 43-50 % total alkaloids. The acute toxicity evaluation showed that the plant is non-toxic at doses up to 5000 mg/kg. These study have provided basic and fundamental information on the plant, and justification for its safe use in ethno medicine.

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INTRODUCTION

Members of *Combretum* are mostly deciduous or semi-deciduous trees, shrubs, scandent shrubs, sub-shrubs with woody rootstocks or woody climbers, sometimes with spine-tipped lateral shoots (*C. imberbe* Wawra). The bark on young stems is often flaking and peeling in stringy strips or threads in most species or in large ± cylindrical or hemicylindrical pieces revealing an exposed cinnamon-red surface (*C. psidioides* group). The leaves are opposite, sub-opposite, sometimes 3- or 4-whorled, exstipulate, simple and the margins are always entire, rarely crenulate, or sometimes undulate (*C. elaeagnoides* Klotzsch, *C. petrophilum* Retief and *C. tenuipes,* Engl& Diels) Indumentum on leaves, flowers and fruit consists of unicellular, compartmented or combretaceous hairs (sharp-pointed, thick-walled with a bulbous base), multicellular stalked glands and multicellular scales (Jordan *et al*, 2011).

The usage of *Combretum* genera in folk medicine includes the treatment of a broad range of diseases, such as abdominal pain, back pain, cough, cold, conjunctivitis,dysmenorrhea, earache, fever, headache, infertility in women, leprosy, pneumonia, scorpion stings and snake bite (McGaw *et al*, 2001). *C. platypterum* is ethno-therapeutically used to treat lower back-ache, eye problems, malaria, swellings, lumps, sexually transmitted diseases, helminthiasis and diarrhoea. It is also used as tonic, febrifuge and to stop post-partum bleeding (Idu, *et al*., 2016), febrifuge (Bongers *et al*, 2005), tonic, (Chukwuma *et al*, 2015). *Combretum* species are prepared for herbal remedies as hot water decoctions, cold water extracts or mixed with food, such as maize porridge. Sometimes fresh leaf sap is used as such. The remedies made from *Combretum* species are used both internally and externally. Sometimes the active compounds of the plants are inhaled through fumes of steam baths of hot water extracts or from the smoke of burnt plant material. (Googen, 2018) Species of Combretaceae have also been used as food supplement for babies (Yé *et al*, 2008) It has also been reported to have the following activities, antidiarrhoeal (Jiofack *et al.*, 2009, Muganza *et al.*, 2012), antiretroviral (Mbaveng *et al.*, 2011, Leteaneb *et al.*, 2012), antimalarial (Dhooghe *et al*., 2010, Abiodun *et al.*, 2011, Tsabanga *et al* 2012, Maregesi *et al* 2014).

Standardization is the investigation and documentation of unique and peculiar characters and properties which identify and separate a plant from others, even members of the same family. This will avert the consequences of mistakenly identifying medicinal plants and combat criminal adulteration of herbal medicinal products as well as ensure product quality consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes (Waldesch *et al*, 2003). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility (Kunle, *et al*., 2012).

Evaluation of crude drugs either in the whole or the powdered condition involves both macroscopic and microscopic assessments. Microscopy is the assessment of those features or character identifiable to the naked eye-size, shape, fracture and texture, while microscopic procedures uses both human and non-human devices. However, since these *Combretum platypterum* characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis (WHO, 2011). Standardisation may involve pharmacognostic, organoleptic, phytochemical, pharmacological and toxicological assessments

Some members of the genus Combretum have been the subject of pharmacognostic evaluation, standardization, phytochemical and pharmacological evaluations. No such investigation was found in literature for *C. platypterum* (Welw.) Hutch. & Dalziel. This study was aimed to evaluate pharmacognostic profile and standardization parameters of the leaf of *Combretum platypterum*.

MATERIALS AND METHODS

Collection of Plant Samples

Combretum platypterum (Welw.) Hutch. &Dalziel leaves were collected from Obukpa, Nsukka LGA in Enugu State, South East Nigeria, between April and May 2017, and identified by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. Herbarium specimens were deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. (Voucher number: PCG/UNN/0096).

Pharmacognostic Profile

Determination of Foreign Matter

Three 250 g potions of the leaves were weighed and spread in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6× or 10×).The content of each group was calculated in grams per 100 g of air-dried sample (WHO, 2011).

Chemomicroscopic Examination of the Powdered Leaf

The presence of various substances such as tannins, starch grains, lignin and calcium oxalate crystals were investigated according to the methods of (Evans, 2005) Physico-chemical evaluation was according to WHO, (2011) and Evans (2009). Parameters evaluated were moisture content total ash, water-soluble ash, acid-insoluble ash**,** sulphated ash, water soluble extractive, ethanol soluble extractive, foaming index, bitterness value and swelling index..

Fluorescence Analysis

A small quantity of the dried plant powder was placed on a grease-free microscopic slide and 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.

Qualitative and Quantitative Phytochemical

Phytochemical analysis of the plant material was performed according to the methods of Evans (2009), Harborne (1998) and Sofowara (2008) with slight modifications.

Total Alkaloid Determination as Marker Substance

This was according to the method described by Evans (2009) with slight modification. A 100 g of the powdered plant material was extracted by cold maceration using 70% methanol. The material was extracted by straining and filtration with Whatmann No. 1 filter paper for three days with fresh solvent added every day. The filtrates were combined and solvent removed by placing it in an oven set at 40 ^{o}C. The dried extract (0.1 g) was macerated with 10 % acetic acid filtered and the pH reduced to 10 with ammonia and extracted (three times) with chloroform by partitioning. The chloroform layers were combined in a previously weighed beaker and evaporated to dryness and the beaker weighed again. The difference in weight of the beaker was considered the total alkaloids.

Mineral Content Analysis

Sodium and potassium were analysed on samples digested with hydrochloric acid, using Flame Photometry according (WHO, 1994), Calcium was estimated using EDTA titration methods (Mohawk, 2008). Spectrophotometric methods were used to determined cupper, and phosphorus (AOAC (1990). Selenium, mercury, arsenic, magnesium, manganese, zinc, iron, and phosphorus were analysed using atomic absorption spectrometry (AAS).

Acute Toxicity Determination

Acute toxicity was determined as described by Sennin (2006).

Microscopy

Micromorphological Study of the Foliar Epidermis

Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in commercial bleach containing 3.5 % sodium hypochlorite for 18 hr. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip. The slides were viewed under a light Olympus Tokyo (Japan No.271961) microscope at x40, x100 and x400 magnifications and photomicrographs were taken with a Motican Camera 2.0.

Quantitative Microscopy - Determination of Leaf Constants

The method described by Evans (2009) was slightly modifies and used for the quantitative microscopy of the leaves. Fresh leaves of *Combretum platypterum* were selected for the microscopical studies. Microscopic sections of the part of the leaf between the mid-rib and the lamina were cut with the help of micrometer. Numerous temporary and permanent mounts of the microscopical sections of the leaf specimen were made and examined microscopically for its stomatal structure, epidermal pattern, vein islet pattern, vein termination pattern and palisade ratio. Histochemical studies using staining reagents on transverse sections and on leaf powder were undertaken.

RESULTS

Physicochemical Properties

The physiochemical properties of the powder were; Moisture content (LOD) 9.50 ± 0.1 (% w/w), Total Ash value 10.50 ± 0 (% w/w), Acid-insoluble ash value 0.75 ± 0.15 (% w/w, Water-soluble ash value 6.51 ± 0.57 (% w/w, Ethanol extractive value, 0.80 ± 0.0 (% w/w), Water extractive value 5.13± 0.55 (% w/w) and Sulphated Ash 5.07 ± 0.1

Chemo microscopy

Chemo microscopy showed the presence of cellulose, condensed tannins, lignin, fats and oil, calcium carbonate, starch and proteins.

Fluorescent Analysis

The results of the fluorescent analysis is shown in Table 1

Bitterness Value

Bitterness value was 0.667 IU/g while the swelling and foaming index were insignificant

Mineral Contents.

The mineral contents (Table 2) were within the permissible limits. The phosphorus content was 86 mg/kg

Qualitative Phytochemical Analysis

Qualitative phytochemical screening revealed the presence of various secondary metabolites (Table 3)

Acute toxicity

During the first one hour the rats were mildly sedated (restricted to a corner of the cage with raised hair. There was no lacrimation, salivation, diarrhoea, convulsion, or urination. There was no death in both groups of rats given 300 and 2000 mg/Kg.

Macroscopy

The leaf is oblong-elliptic in shape with acuminate apex, rounded or cuneate base and entire margin. It was glabrous, deep shiny green on the upper surface and lighter on the lower surface. It measured 9-15 cm in length and 4-7 cm in width (Figures 2 and 3).

Epidermal cells were polygonal in shape with straight anticlinal cell walls on the adaxial (upper surface) and irregular in shape with undulate/wavy anticlinal walls on the abaxial (lower) surfaces (Figure 4). The leaf were hypostomatic (stomata only occur on the lower surface) with paracytic type of stomata.

Both glandular and covering trichomes were absent. The stomatal number was 3 ± 0.91, stomata density-241.18 $mm^{-2,}$ and stomata index 38.32 %. The stomata length was 31.77 ± 0.51 µm, stomata width 22.59 ± 0.27 µm, stomata size 717.84 ± 16.19 $µm^{2,}$ palisade ratio 11.25 ± 2.39, vein islet number 4.68 mm^{-2} and veinlet termination number 4.22 mm^{-2} (Figure 5).

The transverse section of the leaf showed the presence of the outermost covering tissues - the upper and the lower epidermises, which were uniseriate and lacked chloroplasts. There was presence of closely packed palisade mesophyll cells with numerous chloroplasts (the main photosynthetic organ) and scattered spongy mesophyll cells that were loosely fitted to leave air spaces. The midrib beard the vascular bundle which comprises the phloem (exteriorly located) and the xylem (interior located) - the main conducting organs. Some mass of parenchymatous cells formed the pith at the centre (Figure 6). Three types of calcium oxalate crystals (large prism shaped, medium cylindrical and crystal sand) occurred in the plant. There were lignified vessels, and cystoliths.

Table 1: Fluorescent Analysis

Detection reagents	**Colour exhibited**		
	Normal light	254 nm	365 nm
Petroleum Ether	Army green	Cherry red	Purple
Methanol	Army green	Pink	Purple
50 % Hydrochloric Acid	Army green	Brown	Black
50 % Sulphuric Acid	Army green	Very bright green	Black
Ammonia	Yellow	Green	Purple
Ethyl Acetate	Army green	Cherry red	Pink

Table 2: Metal Content (ppm)

Metal	Concentration (%)
Ca	0.14
Mg	12.09
Mn	1,78
Cu	0.18
Na	1.34
K	4.49
Hg	2.00
As	0.90
Fe	5.01
Se	0.36
Zn	0.51

Table 3: Qualitative Phytochemical Results

Phytoconstituents	Amount
Phenolics	+
Saponins	-
Flavonoids	+++
Tannins	+
Terpenoids	+
Steroids	+
Resins	++
Glycosides	+
Carbohydrates	+
Reducing sugars	+
Cyanogenic glycosides	-

+ = present: - = absent

Figure 1: Habit Photograph of *C. platypterum*

Figure 2. A twig Showing Leaf Arrangement of *C. platypterum*

Figure 3. Showing veination of *C. Platypterum* leaves

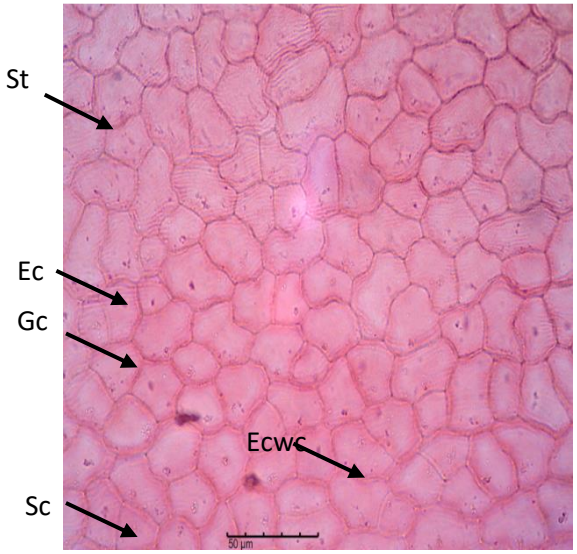

Figure 4. Abaxial surface showing paractic stomata at x400, Adaxial surface showing absence of stomata. Ec = epidermal cell; Ecw = epidermal cell wall, Ec = epidermal cell; St = stoma, Gc = guard cell; Sc = subsidiary cell

Figure 5. Showing Stomatal Dimensions, Type (Paracytic), And Wavy Margin Epidermal Cells.

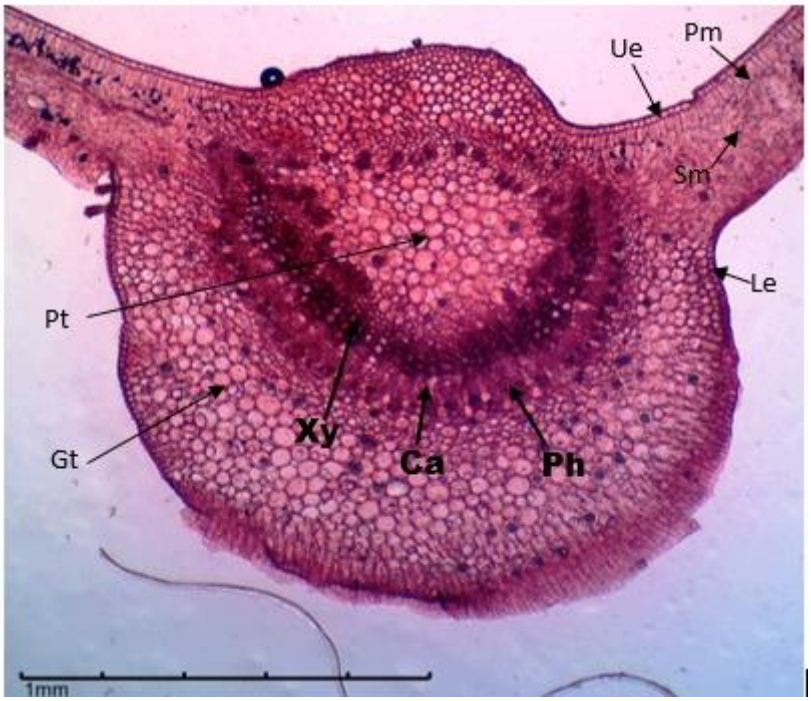

Figure 6. Transverse Section of Leaf of *C. platypterum* X400
Ue = upper epidermis; Sm = spongy mesophyll; Pm = palisade mesophyll; Le = lower epidermis; Pt = pith; Xy = xylem; Ca = cambium; Ph = phloem; Gt = ground tissue

DISCUSSION

The pharmacological activities of plants are derived from their phytochemical contents. Therefore the presence of many significant secondary metabolites will confer a wide range of activities on the plant. The high level of alkaloids as demonstrated by the result of the total alkaloid content (Marker substance) is a good pointer to this, considering the pharmacological activities of alkaloids. The acute toxicity result which shows that the extract belong to GHS category 5 with LD_{50} cut-off of 5000 mg/kg means that the plant extract is safe up to a dose of 5000 mg/kg. The extractives values of powdered leaves with different solvents, n-hexane 2.8±0.06, methanol 1.7 ± 0.06, ethanol 0.8 ± 0.00, water 5.13 ± 0.55, is an indication that the leaf contains both low polar and polar compounds that were extracted by both non-polar n-hexane and polar methanol and water. The pH of the aqueous solution 6.1 to 6.4 can be interpreted as non-acidity, hence, safety of the aqueous preparation while the low bitterness value 0.667 IU/g will make the preparation considerably acceptable. The low swelling and foaming index will make preparation less difficult. The low levels of mercury and arsenic are indications that the site where it was collected is minimally contaminated by heavy metals and the plant may be considered not to be accumulating heavy metals. The levels of magnesium, manganese, selenium and zinc (0.511 ppm), are hints of an antioxidant activity. The leaf is hypostomatic (stomata only occur on the lower surface) with paracytic type of stomata. This is of pharmacognostic importance since it will aid correct identification of the plant. Some of the quantitative macroscopy and microscopy results are in line with that of some earlier works (Akinsulire *et al*, 2018). The leaf is hypostomatic (stomata only occur on the lower surface) with paracytic type of stomata. Stomata length and width were similar to thos reported by Obembe, (2015). The types of calcium oxalate crystals and their location in the spongy mesophyll are similar to that reported by Ekeke and Agbagwa, (2014).

The leaf arrangement (opposite) is in line with the earlier observation (Stace (2007) that the Combretaceae, family is characterized by mainly opposite leaves and the absence of stipules or stipu1es being rudimentary. The total ash (10.0 %) may be explained by the fact that the plant is a woody climbers (lianas). The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical changes or microbial growth. So the moisture content of a drug should be determined and controlled. A moisture content of 9.50 ±0.1 (% w/w) by loss on drying method implies that the plant contains a lot of water or other volatile substances, thus a lot of attention must be given to this dying process to discourage microbial infestation.

Plants make calcium oxalate crystals in an intriguing variety of defined shapes. In higher plants, the distribution of crystals, like their morphology, follows species-specific patterns, indicating regulation over the sites and modes of calcium oxalate accumulation (Webb, 1999). This can be of help in identification of plants.

CONCLUSION.

This study has provided basic and fundamental information that have been previously unavailable on the plant, and justification for its safe use in ethno medicine, and provides more information on the properties and usefulness of the plant.

Conflict Of Interest:

The authors declare no conflict of interest

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