PHYTOCHEMICAL AND PHARMACOGNOSTIC STUDIES OF THE LEAF OF *DETARIUM MICROCARPUM*

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ABSTRACT

Detarium microcarpum is a shrub that grows wildly in Nigeria. There are several ethnomedicinal claims on its activity. This has necessitated the need to establish its pharmacopoeia standards that could be used as reference guide for the identification and assessment of its quality and purity. This study aims to evaluate the pharmacognostic characters of *D. microcarpum* leaf and determine the phytochemical composition of the methanolic leaf extract. WHO guideline was used to establish the pharmacognostic standards of the leaf and preliminary phytochemical screenings, physical constants and elemental analysis were conducted using established protocols. The macroscopical analysis of the leaf showed that it is green in colour, odourless, tasteless, with thickly leathery glabrescent surface and an ovate to elliptical shape. The base is rounded and the apex is largely emarginated. Microscopical observation revealed the presence of anomocytic stomata on the lower surface which were largely absent on the upper surface. The moisture content, total ash value, acid insoluble and water soluble ash values were 6.4±0.05, 5.15±0.351.68±0.53 and 3.93±0.08 respectively. This shows that the inorganic matter content adhered to the leaf were within approved limit. The physical constants were within the limits of reference standards. Macro and microelements were present in the sample with toxic levels of mercury and arsenic observed. The macroscopical, microscopical and physical parameters of the leaf of *D. microcarpum* observed have revealed information that could be used in its standardisation.

Keywords: *Detarium microcarpum*, Phyto-chemicals, Pharmacopoeia, Standardisation, Elemental

INTRODUCTION

The plant kingdom has from pre-historic time served as source of food and medicines to man. The developing countries depend a lot on wild and cultivated plants for the treatment of various diseases. According to the World Health Organisation (WHO), more than 80% of the healthcare needs of persons from developing countries are provided by Traditional Medicine Practitioners (TMP) (Sumner, 2000; Akerele, 1984). A renewed global interest in the use of herbal medicines even among developed nations has necessitated the need for the standardisation of herbal products. The World Health Assembly has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. Standardisation is necessary to mitigate the incidence of lack

of efficacy, poor quality, adverse effects and toxicity (WHO, 1998; WHO, 2007).

Tallow tree, *Detarium microcarpum* (Fabaceae) is wide-spread in tropical West Africa in dry Savannah habitat (Vautier *et al.*, 2007). It is a shrub or small tree up to 10 m high which grows in dry savannah woodland. It is mainly found on shallow, stony and lateritic soils and on hills. The plant is deciduous in nature shedding its leaves in November and producing new ones in March. Flowering takes place during the rainy season and fruits mature from December to April (Contu, 2012). The plant is considered an underutilised species medicinally even though there are reports of its valuable ethnomedicinal properties, nutritional edible fruit and exploitation for hard wood.

Dalziel has reported the use of the bark and leaves in dressing wounds, ulcers and fresh cuts (Dalziel, 1955). The fresh bark or leaves are applied to wounds, to prevent and cure infections (Kouyate et al., 2006). The bark, leaves and roots of the plant are widely used due to their diuretic and astringent properties. When made into infusions or decoctions, they are used for the treatment of rheumatism, venereal diseases, urogenital infections, haemorrhoids, caries, biliousness, stomach-ache, intestinal worms and diarrhoea including dysentery. A decoction prepared from the powdered bark is widely taken to alleviate headache and pains related to the back, menstruation and sore throat. There is also reported folkloric use of the plant to treat impotence, malaria, and leprosy. In a survey conducted in North-eastern Nigeria, the roots and stems of *Detarium microcarpum* were found to be used in the treatment of cancer (Ngulde et al., 2015). Pharmacological investigations have revealed that it possesses antioxidant, antidiabetic and antimicrobial properties (Semde *et al.*, 2018; Akinsanmi *et al.*, 2019**;** David *et al.*, 2017a; David *et al.*, 2017b**;** Uchenna *et al.*, 2014). In a study, the fruit pulp extracts of *D. microcarpum* effectively inhibited a fungus, *Cladosporium cucumerinum* and acetylcholinesterase enzyme thereby demonstrating potential anti-Alzheimer activity (Cavin et al., 2006). The seed flower has been shown to be useful in water treatment by coagulating fibre cement effluent (Ani et al., 2012).

Phytochemical screening of the leaf extract has revealed the presence of anthraquinones, flavonoids, saponins, steroids, tannins and trace metals such as zinc, iron and calcium (Mariod et al., 2019; David *et al.*, 2017; Uchenna *et al.*, 2014**;** Iful, 2008). Terpenoids have been identified from the volatile oil extracted from the leaf **(**Semde *et al.*, 2018**).** High phenolic content was shown to be present in the methanolic leaf extract (Meda *et al.*, 2017).

Due to scarcity of information on its standardisation parameters, this study aims to establish and ascertain some physicochemical and phytochemical features of *Detarium microcarpum* that could serve as a basis for its proper identification, collection and investigation.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant *Detarium microcarpum* was identified by Malam Muhammad Namadi in its natural habitant around Kudingi bush in Ahmadu Bello University, Zaria. The plant (leaves) was collected around 10 am in April,

2018 and specimen deposited at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and a voucher number (901451) was subsequently issued.

Macroscopical Evaluation

Macroscopical (organoleptic) Analysis: The leaf size, shape, margin, apex, odour, taste, colour, surface texture, presence or absence of petiole, base, lamina, and venation were all observed and recorded (Wallis, 1985; Evans, 2009).

Microscopical Analysis

Fresh leaves were collected and placed in a glass jar containing fixative agent; formalin, glacial acetic acid, 70 % ethanol (10:5:85). The preserved leaves were then cleared in the laboratory using chloral hydrate solution, mounted with glycerin and observed under a compound microscope. The presence/absence of the following were observed: epidermal cells, stomata (type and distribution) and trichomes (types of and distribution). Transverse sections of fresh leaves were also prepared through the lamina and the midrib, mounted on glass slide and observed under compound optical microscope (Brendler *et al*., 2010).

Chemomicroscopy

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques (Evans, 2006).

Physicochemical Analysis

Leaves were washed clean, air dried and pulverised. The powdered samples were then subjected to the tests for moisture content (loss on drying), total ash content, acid insoluble ash, water soluble ash, water extractive value, alcohol extractive value according to WHO (1998).

Phytochemical Screening

Powdered leaf sample of about 500 g was macerated with distilled water at room temperature for 72 hours with frequent agitation. The liquid extract was filtered *in vacuo* and solvent evaporated by gentle heating (50°C) over water bath. The aqueous extract was then subjected to quantitative and qualitative phytochemical screening.

This was carried out as described by Sofowora (1993) and Evans (2002).

Elemental Analysis

About 5 g of powdered leaf sample was used to carry out Energy-Dispersive X-ray Fluorescence (EDXRF) analysis (Bruker S2 Ranger, USA) with a 28 capacity sample tray. Analyses were conducted in supplied sample cups prepared as reported by Guild et al. (2017).

Microwave Plasma Atomic Emission Spectrometry (MP-AES) was also used to analyze the heavy metal composition of digested sample using the Agilent 4200 MP-AES (USA) according to the method of Poirier et al. (2017).

RESULTS

The organoleptic features of the leaf are as shown in table 1 below.

Table 1: The macroscopy of *Detarium microcarpum* leaf

Parameter	Observation
Condition	Fresh
Colour	Green
Shape	Ovate
Size: Length	5.4 cm
Margin	Entire
Venation	Reticulate
Petiole	Short
Surface	Glossy
Phyllotaxy	Alternate
Odour	Odourless
Taste	Neutral

The transverse section of the leaf shows the presence of paracytic stomata type and unicellular uniserrate covering trichomes. Translucent crater-like glands were present on the abaxial surface with projecting trichome while pitted polygonal thick-walled epidermal cells were seen on the adaxial surface.

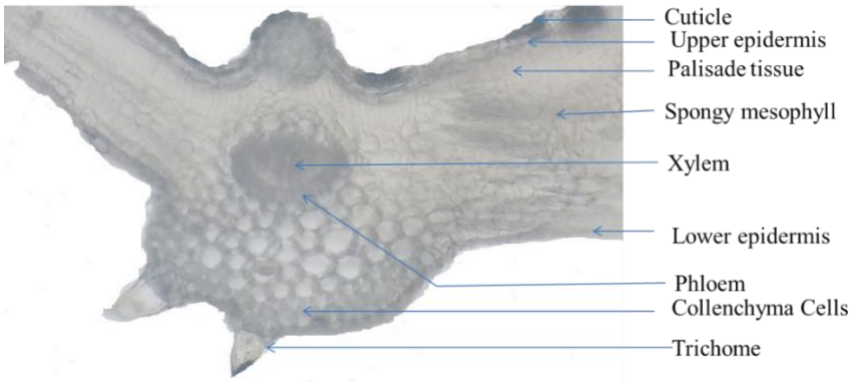


Figure 1: The transverse section of the leaf of DM showing its tissue arrangement

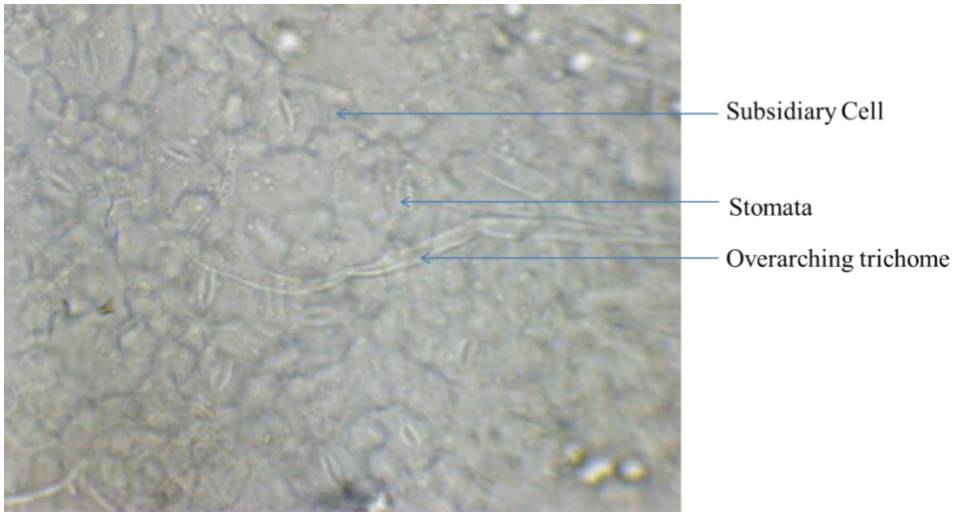


Figure 2: Lower epidermis of the leaf in surface view showing anomocytic stomata

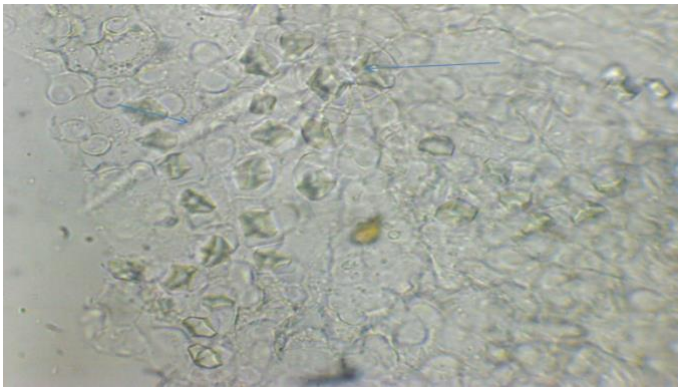


Figure 3: Translucent crater-like gland on abaxial surface with projecting trichome
The palisade ratio, stomata number, stomata index, vein-islet number and veinlet termination number were 2-2.75, 13-14, 9.35-9.79, 7-8 and 3-4 respectively.

Table 2: Quantitative microscopic parameters of *Detarium microcarpum* leaf

Parameter	Range	Mean	Standard Deviation
Palisade Ratio	2.0-2.75	2.42	0.38
Stomata number	13-14	13.33	0.58
Stomata Index	9.35-9.79	9.58	0.22
Vein islet number	7-8	7.7	0.58
Veinlet termination	3-4	3.7	0.58

n=3

The physical constants analysed showed the moisture content, total ash content, acid insoluble ash, water soluble ash, water extractive value, alcohol extractive were 6.4, 5.15, 1.68, 3.93, 25.70, and 20.10 respectively.

Table 3: The physical parameters of *Detarium microcarpum* leaf

Experimental Studies	**Observations (Percentage)**
Moisture Content	6.4±0.05
Total Ash Value	5.15±0.35
Acid Insoluble Ash	1.68±0.53
Water Soluble Ash	3.93±0.08
Alcohol Soluble Extractive Value	25.70±4.5
Water Soluble Extractive Value	20.10±0.7

Values of percentage composition shown are Mean ± SEM, n = 3

The chemomicroscopy of the plant as shown in table 3 shows that there is absence of inulin and mucilages.

Table 4: Chemomicroscopy of *Detarium microcarpum* leaf

Test	Observation	Inference
Starch grains	Blue-black colour on addition of Iodine solution	Positive
Lignin	Cherry-red colour on addition of Phlorogucinol	Positive
Suberin/Cutin	Faint orange colour on addition of Sudan red	Positive
Gum/Mucillage	No pink colour on addition of Ruthenium red	Negative
Calcium Oxalate/Calcium Carbonate Crystals	Crystals that slowly dissolve without effervescence on addition of acetic acid	Positive for Calcium Oxalate
Inulin	No colour change after addition of 1-naphtol and sulphuric acid	Negative
Tannins	Greenish black colour on addition of ferric chloride solution	Positive
Cellulose	Bluish colour colour on addition of Chlor-zinc Iodine	Positive

The preliminary phytochemical screening reveals the presence of all the major phytochemical classes except the anthraquinones.

Table 5: Qualitative phytochemical screening of *Detarium microcarpum* leaf

Test	Methanol Extract	
	Observation	**Inference**
Alkaloid (a) Mayer's	No formation of precipitate	Negative
(b) Dragendoff	No formation of precipitate	Negative
(c) Wagner's	No formation of precipitate	Negative
Terpenoid/Steroid		
(a) Liebermann-Burchard	Brownish colour seen at interphase	Positive
(b) Salkowski	Brownish colour seen at interphase	Positive
Tannins (Ferric Chloride)	Blue black precipitate	Positive
Flavonoids		
(a)Sodium Hydroxide	Yellow solution formed	Positive
(b) Shinoda's	Orange colour forms slowly	Positive
Anthraquinones (Borntrager's)	No bright pink colour formed at upper aqueous layer	Negative

Cardiac glycosides (Keller-Killiani)	Purple brown ring formed at interphase	Positive
Saponins(a) Frothing Test	Honeycomb froth formed persist after 15 minutes	Positive
(b)Haemolysis Test	Erythrocytes settle at the base of test tubes	Positive

The elemental analysis of DM leaf indicated that it contains some pharmacological important trace elements like Iron, 4.21 mg/100g, manganese, 4.70 mg/100g, zinc, 0.09mg/100g and chromium, 0.73mg/100g etc. (Table 5).

Table 6: Elemental composition of *Detarium microcarpum* leaves sample

Element	**Concentration (mg/100g)**
Calcium (Ca)	40.52
Potassium (K)	19.84
Magnesium (Mn)	9.90
Phosphorous (P)	6.04
Manganese (Mn)	4.70
Sulphur (S)	4.53
Iron (Fe)	4.21
Silicon (Si)	3.46
Sodium (Na)	2.7
Chlorine (Cl)	1.51
Aluminium (Al)	0.90
Chromium (Cr)	0.73
Titanium (Ti)	0.40
Cerium (Ce)	0.16
Strontium (Sr)	0.16
Zinc (Zn)	0.09
Barium (Ba)	0.05

The heavy metal composition of the powdered leaf sample is indicated in table 7: Chromium, 1.48 ppm, Arsenic, 141.28 ppm, and Mercury, 88.85 ppm, Nickel 1.93 ppm and Copper 14.89 ppm.

Table 7: Heavy metal composition of *Detarium microcarpum* leaves sample

Element	Concentration (ppm)
Arsenic (As)	141.28*
Cadmium (Cd)	ND
Chromium (Cr)	1.48
Copper (Cu)	14.89
Cobalt (Co)	ND
Lead (Pb)	ND
Manganese (Mn)	527.82
Mercury (Hg)	88.85*
Nickel (Ni)	1.93
Zinc (Zn)	76.67

*****Values above WHO recommendation, ND=Not detected

DISCUSSION

Tallow tree has several ethnomedicinal and pharmacological claims. It is therefore necessary to establish its pharmacognostic parameters for the purpose of standardization. These standards are prerequisite for the inclusion of the plant in an herbal pharmacopoeia. The macroscopical analysis of the leaf showed that it is green in colour, odourless, tasteless, with thickly leathery glabrescent surface and an ovate to elliptical shape. The base is rounded and the apex is largely emarginated. Microscopical observation revealed the presence of anomocytic stomata on the lower surface which were largely absent on the upper surface. This is common to non- tropical plants in order to conserve water from transpiration. The presence of cuticle on both surfaces also prevents the loss of water as well. Chemomicroscopic examination of the leaves revealed the presence of starch, cellulose, lignin, suberin, and calcium oxalate crystals. Ugwoke et al. (2017) has also reported the presence of anomocytic stomata in another study.

The WHO and Pharmacopoeias have set a limit of 15% moisture content to guarantee long storage period and prevent deterioration from mould and bacterial growth, hydrolysis and other physical interactions (Sena *et al*., 1998). In this study, the moisture content of DM was

6.4±0.05 which is within the approved limit but higher than that reported by a similar study (Ugwoke *et al*., 2017). The total ash value is low (5.15±0.35) indicating that there is little amount of inorganic matter adhered to the powdered sample. The acid insoluble and water soluble ash values (1.68±0.53 and 3.93±0.08 respectively) were within the limits in the standard crude drug monograph and this gives evidence of the presence of minute earthy matters (African Pharmacopoeia, 1986). The physical parameters reported were different from those of another study which may be due differences in the geographical location of the sample collected (Ugwoke et al., 2017). The chemical contents of the leaves were very soluble in both water (20.10±0.7) and alcohol (25.70±4.5) with the highest solubility being in the latter. This shows that the drug would be better extracted using alcohol than water.

Phytochemical screening of the extract revealed the presence of terpenoids, steroids, flavonoids, saponins, tannins, alkaloids and cardiac glycosides. Ugwoke et al. (2017) has also reported the presence of tannins, flavonoids, saponins and steroids in methanol extract of the leaf. The presence of these phytochemicals have also been reported in previous studies (Akinsanmi et al., 2019). Clerodane diterpenes have been isolated from the fruit pulp extract of the plant (Cavin et al., 2006). There was absence of anthraquinones. The presence of these phytochemical constituents has been reported in *D. microcarpum* ((Iful, 2008)). Knowledge of the phytochemicals present in a sample extract help to identify the bioactive molecules that may be responsible for its biological activity as well as the plant's medicinal value.

The plant leaf contains macro and microelements that are useful in the body's metabolism. However, toxic levels of arsenic and mercury have been detected. The WHO recommends that, the level of in organic arsenic should not exceed 0.01 ppm (Jones, 2007). Thus, 141.28 ppm of arsenic detected in the sample is exceedingly high. This calls for serious concern especially if it exists in the inorganic form since the organic form has been reported to be far less toxic (Sambu & Wilson, 2008). Symptoms from arsenic toxicity include vomiting, bloody watery diarrhoea, abdominal pain, malignancy, encephalopathy and thickened thin (Ratnaike, 2003). The possible explanation for the high amount of arsenic may be due ecological accumulation from the oil as result of pre-existing arsenic deposits or arsenic containing waste disposal (Ngwoke et al., 2015).

The toxic levels of mercury in the sample may cause debilitating nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes (Bernhoft, 2012; EEN, 2006)**.** It has been recommended that mercury consumption above 0.02 ppm is harmful to health (WHO, 2019). The high mercury level (88.85 ppm) in the sample may be existing in the organic form, with methylmercury as the most toxic. Ethylmecury which is used as vaccine preservative (thiomersal) is easily excreted from the body with less tendency for accumulation (Offit, 2007). There is need to investigate the source of these heavy metals in the sample and prevent possible fatalities that may result from ingesting the sample.

CONCLUSION

The macroscopical, microscopical and physical parameters of the leaf of *Detarium microcarpum* observed have revealed

information that could be used in its standardisation. The methanol extract of the leaf has shown the presence of several phytochemical constituents that could be of medicinal value.

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