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Standardization of stem bark of *Pseudocedrela kotschy* (Meliaceae)

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Abstract

Pseudocedrela kotschy (Meliaceae) stem bark has been used for the treatment of numerous diseases but without standardisation. The study is aimed at evaluating Pharmacognostic parameters of *P. kotschy* stem bark. Standard methods of evaluating crude drugs were used to establish the pharmacognostic parameters. The microscopy study revealed the presence of cambium, pith, epidermis, cortex, vascular bundles, calcium oxalate crystals and long lignified fibres. The chemo-microscopy revealed the presence of some cell inclusions and contents. The averages of physico-chemical parameters values obtained (moisture content 5.22%, total ash 11.23%, acid insoluble ash 3.20%, water soluble ash 7.23%, alcohol extractive 15.83% and water extractive 24.68%) were within permissible limits. The micrometry of the calcium oxalate crystals gave averages of length and width as $35.13\mu\text{m}\pm 4.14$, $17.57\mu\text{m}\pm 2.62$ respectively while those of fibres were $824.25\mu\text{m}\pm 36.01$, $14.05\mu\text{m}\pm 1.44$, respectively. The elemental analysis established the presence of some macro and micro elements. The powdered stem bark of *P. kotschy* under daylight and UV- visible at short (254 nm) and long wave (365 nm) lengths emitted medium range of diagnostic colours. The pharmacognostic parameters of *P. kotschy* stem bark that have been established can be used in future studies for ensuring identity, purity and quality.

Keywords: Pharmacognostic, *Pseudocedrela kotschy*, extractive value, microscopy, physico chemical

Introduction

There is a surge in the use of herbs and herbal products round the globe for the treatment of various of diseases in recent time. The major problem that is associated with the use of traditional medicines is lack of pharmacognostic parameters for identification and authentication of plants in the events of substitution or adulteration. Standardisation of these herbs will go along away in ascertaining their quality and purity. World Health Organisation states that macroscopy and microscopy study of medicinal plants is the first step that is targeted at establishing the identity and extent of purity such materials, and should carried out before further tests are undertaken (WHO, 2011) [33].

Pseudocedrela kotschy (schweinf) Harms is a small deciduous tree that belongs to the family of Meliaceae. It is widely spread in the savanna zone from east Senegal to western Ethiopia, Uganda and Nigeria (Burkill, 1997) [11]. It is commonly called Dry-zone cedar, hard cedar-hogany and locally known as *Emi gbegi* among the Yoruba's and *Tuna* among the Hausa's (Neuwinger, 1996) [22]. *Pseudocedrela kotschy* is a deciduous and monoecious small tree that is up to (12–20) m tall with bole branchless of up to 7.5 m, straight, cylindrical and up to 70 cm in diameter (Burkill, 1997) [11]. Its leaves are alternate but often clustered at the ends of branchlets, the flowers are unisexual, male and female that are very similar in appearance, the fruits are narrowly club-shaped capsule of 7–14.5 cm long, brown and dehiscent and the seeds are 4–6 cm long, pale brown and with winged at the apex (Burkill, 1997) [11].

The roots, leaves and stem bark of *P. kotschy* are used for various medicinal purposes. The root and stem bark are used for the treatment of fever, malaria, diarrhoea, worm infestation and oral infection (Oliver-Bever, 1958; Okunade *et al.*, 2007; Tapsoba and Deschamps, 2006) [26, 25, 29]. *Pseudocedrela kotschy* wood is also used as a chewing stick for dental cleaning in western Nigeria (Akande and Hayashi, 1998) [3]. The decoction of the leaf is used traditionally in the folk medicine in Nigeria for the treatment of a number of diseases and health conditions, including, fever, pains, diabetes and convulsion (Anuka *et al.*, 1999; Georgewill and Georgewill 2009; Akuodor *et al.*, 2013 and Akuodor *et al.*, 2015) [6, 14, 5, 4]. The plant is used traditionally in Ghana to treat leprosy and epilepsy (Neuwinger, 2000) [23], malaria and stomach aches (Adekunle, 1998; Asase *et al.*, 2005) [1, 8].

Hay *et al.*, 2007 [17] reported that limonoids, 7-desacetoxoy-7- oxogedunin and pseudrelones A, B and C isolated from *P. kotschy* displayed good antiprotozoal activity.

Limonoid 7-deacetoxy-7-oxogedunin isolated from *P. kotschy* showed anti-HIV activity (Taiwo *et al.*, (2017) [28]. The leaves of *P. kotschy* contain 3-O-rhamnosides of myricetin and quercetin, and 3-O-glucosides (or galactosides) of these aglycones (Asase *et al.*, 2008) [7]. The limonoid that was previously isolated from *P. kotschy* stem bark (Niven and Taylor, 1988) [24] has been reported to have several activities including antiplasmodial and anti-HIV (Miranda-Júnior, 2012) [21]. Stigmasterol was reported to be isolated from the crude hexane extract of *P. kotschy* stem bark (Atinga *et al.*, 2018) [9].

2. Material and Methods

2.1 Collection and Authentication of Plant Material

2.2 Plant Collection and Identification

The stem bark of *P. kotschy* was collected from Dajin Kudingi area, Samaru district of Sabon Gari Local Government, Kaduna State. The plant was identified by Malam Namadi Sanusi at the Department of Botany, Ahmadu Bello University, Zaria-Nigeria with a voucher number 900243. A voucher specimen was deposited for future reference.

2.3 Preparation of plant sample

The Stem bark of the plant was cleaned, air dried and ground to coarse powder using grinding machine (3.5 KVA). The powder was stored in airtight containers for further use.

2.4 Pharmacognostic Studies of *Pseudocedrela kotschy* stem bark powder

2.4.1 Macroscopical Examinations

Morphological features of the stem bark sample which include the shape, mean dimension, size range of pieces, the appearance of both inner and outer surfaces and the form of the fracture was examined in accordance with WHO (2011) [33].

2.4.2 Microscopic Studies

sample of the plant was cut into the transverse section (T.S.) and longitudinal section (L.S) with razor blade, followed by clearing with 70% hypochlorite, the material was mounted in alcohol and dilute glycerol and observed for the presence of vascular bundles, cork, pith, epidermis under the microscope as described by Evans (2009) [13].

2.4.3 Micrometric Evaluation

This involved measurements of dimensions (length and width) of the various diagnostic microscopic characters of the stem bark of the plant. It was carried out by using binocular microscope with the aid of graticles (Kokate, 1994) [20].

2.4.4 Determination of Physicochemical Constants of the Stem bark

Some physicochemical parameters of the powdered sample of the plant such as moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol and water extractive values were determined as described in the updated edition of quality control methods for medicinal plant materials (WHO, 2011) [33].

2.4.5 Chemo-microscopic studies on the stem bark of *P. kotschy*

Powdered stem bark of *P. kotschy* was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube

containing 70% chloral hydrate solution. It was then boiled on a water bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance to WHO (2011) [33] guidelines.

2.4.6 Fluorescence Analysis on the stem bark of *P. kotschy*

A small quantity of dried and finely powdered stem bark of *P. kotschy* was placed on a clean microscope slide and 1-2 drops of freshly prepared reagents solutions at different instances were added and mixed by gentle tilting of the slides and allow to stand for 1-2 minutes and viewed in visible day light. It was then placed inside the UV viewer chamber and observed under short (254 nm) and long (365 nm) ultraviolet radiations. The different colours detected were recorded (Kokashi *et al.*, 1958; Gupta *et al.*, 2006) [19, 16].

Table 1: Organoleptic/macrosopic Features of *P. kotschy* stem bark

Features	Description
Length	7.0-9.0 cm
Width	4.1-6.0 cm
Thickness	0.4-0.5 cm
Colour	Outer: Rusty brown Inner: Reddish brown
Texture	Slightly rough
Fracture	Fractures with little resistance along the transverse section
Odour	Characteristic
Taste	Intensely bitter



Plate 1: Image of the *Pseudocedrela kotschy* stem bark



Plate 2: Photomicrograph of Transverse section of *P. kotschy* stem showing some morphological features (Mag. x100)

Key: P= Pith, X= Xylem, C= Cambium, Ph= Phloem, Co= Cortex, Ep= Epidermis

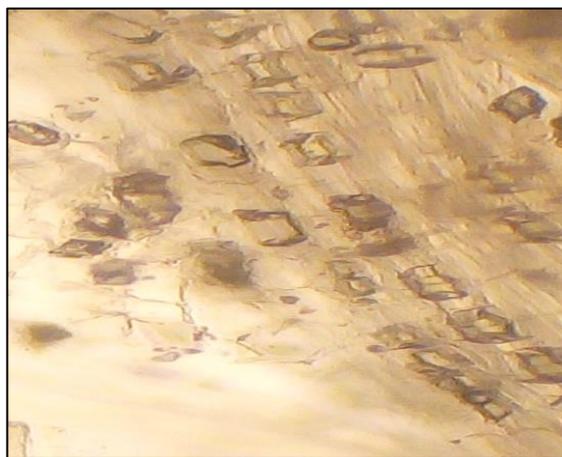


Plate 3: Photomicrograph of powder of *P. kotschy* stem bark showing some Anatomical features (Mag. $\times 100$) Key: CO= Calcium Oxalate, Fb= Fibre

Micrometric Evaluation of *Pseudocedrela kotschy* Powdered stem bark

Measurement of dimension of some microscopic features of stem bark expressed as Mean \pm SEM from five observations revealed the averages of lengths / widths of calcium oxalate crystals and fibres as $35.13 \mu\text{m} \pm 4.14$ / $17.57 \mu\text{m} \pm 2.62$ and $824.25 \mu\text{m} \pm 36.01$ / $14.05 \mu\text{m} \pm 1.44$, respectively.

Table 2: Chem microscopical Features of *P. kotschy* Powdered stem bark

Constituent	Detecting reagent	Observation	Inference
Starch	N/50 iodine	Blue-black colour on grains within the cell.	Starch present
Lignin	Phloroglucinol and Hydrochloric acid	Red-pink colour on the walls of lignified cell.	Lignin present
Tannins	5% Ferric Chloride	Greenish-black colour in some parenchyma cells.	Tannins present
Mucilage	Ruthemium red	Dilated fragments	Mucilage present
Calcium oxalate	Hydrochloric acid	Dissolution of crystals on the cell wall with no effervescence.	Calcium oxalate present
Calcium carbonate	Hydrochloric acid	No effervescence in the cell.	CaCO ₃ absent
Cellulose	Chlor-Zinc-Iodine	Blue coloration of the cell wall	Cellulose present
Suberin	Sudan red	Orange red colour on cell wall.	Suberins present
Aleurone grains	Iodine in ethanol	Yellowish brown	Aleurone grains present
Inulin	1-naphthol and H ₂ SO ₄	Reddish colouration	Inulin present

Table 3: Physicochemical Constants of *P. kotschy* Powdered stem bark

Parameters	Values (%w/w) \pm SEM*
Moisture content	5.33 \pm 0.11
Total Ash value	11.25 \pm 0.83
Acid Insoluble ash	3.20 \pm 0.833
Water Soluble ash	7.23 \pm 0.60
Alcohol soluble extractive	15.83 \pm 0.88
Water soluble extractive	24.68 \pm 0.88

*Average values of five determinations

Table 4: Fluorescence Features of *P. kotschy* Powdered stem bark

Reagent/solvent	Daylight		Short Wavelength (254 nm)	Long Wavelength (365 nm)
	Immediately	After an hour		
Conc. HCl	Deep brown	Dark brown	Green	Violet
Dil. HCl	Brown	Dark brown	Light green	Reddish brown
Conc. H ₂ SO ₄	Dark brown	Dark brown	Black	Black
NaOH	Brown	Dark brown	Light green	Reddish brown
Dil. NH ₄ OH	Brown	Brown	Dark brown	Dark brown
Conc. NH ₄ OH	Brown	Brown	Brown	Dark brown
Water	Brown	Brown	No reaction	No reaction
Ferric chloride	Dark green	Dark green	Dark green	Black
Dil. Nitric acid	Reddish Brown	Reddish Brown	Yellow	Light brown
Conc. Nitric acid	Brown	Brown	Green	Reddish brown
Methanol	Dark brown	Dark brown	Deep brown	Reddish brown
Iodine	Brown	Brown	Green	Purple
Picric acid	Yellowish brown	Yellowish brown	Light green	Brown

Discussion

The studies carried out on the stem bark of *Pseudocedrela kotschy* have established some pharmacognostic standards that will guide its utilization as crude drug in pharmacy and other fields. The organoleptic studies on the stem bark showed very important parameters (Table 1) which are unique to *P. kotschy* as compared to other members of the Meliaceae family. The outer and inner layers have rusty and reddish brown colours respectively, its texture was found to be rough and fractures with little resistance along the transverse section. The dimension of the length, width and thickness of the stem bark were 7.0-9.0 cm, 4.1-6.0 cm and 0.4-0.5 cm respectively. It has characteristic odour and was intensely bitter taste. We observed that most of the species of the Meliaceae family have bitter taste of varying intensity (e.g. *Azadirachta indica*, *Khaya senegalensis*, *Cedrela odorata*, *Melia azedarach* etc.).

The photomicrograph of *P. kotschy* stem bark along the transverse section revealed features namely cortex, pith, epidermis and vascular bundles (plate 2). The photomicrograph of powder of *P. kotschy* showed calcium oxalate crystals and long lignified fibres (plate 3).

The anatomical features of the plant drugs provide salient diagnostic characteristics for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990) [15]. Macro and microscopical evaluation of crude drugs are targeted at identification of precise variety and search for contaminants in plant materials (WHO, 1996 c) [32].

Chemo-microscopical examination of the powdered stem bark of *P. kotschy* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent (Table 2). The measurement of dimension of lengths and widths of calcium oxalate crystals and fibres showed the averages of lengths and widths of calcium oxalate crystals and fibres as $35.13 \mu\text{m} \pm 4.1$, $17.57 \mu\text{m} \pm 2.62$ and $824.25 \mu\text{m} \pm 36.01$, $14.05 \mu\text{m} \pm 1.44$, respectively. The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the

identification and authentication of crude drugs especially in powdered plants (Chanda, 2011) [12].

The physicochemical constants of *P. kotschy* stem bark determined include the moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractives value (Table 3). These values are useful as criteria to evaluate the identity and purity of crude drugs (Evans, 2009; WHO, 1996 a and b) [13, 31, 30]. They also indicate the presence of various inorganic materials like carbonate, oxalate and silicate in plant materials.

The average moisture content of the powdered plant material using loss on drying method was found to be 5.22%, and this value was within the permissible limits because WHO, (2011) [33] recommend the percentage of moisture content in any crude drug to be within 12-14%. Low or permissible moisture in crude drugs may discourage the growth of bacteria, yeast, mould and fungi and will stand for long period of time during storage without spoilage or suggesting better stability against degradation of product (WHO, 1996 b) [30]. Ash values obtained include total ash as 11.25%, acid insoluble ash 3.20% and water soluble 7.23%. These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials (Kaner and Chanda, 2011) [18].

From the ash values mentioned above, the total ash value represented both the physiological and non-physiological ash from the crude drugs upon incineration. The non-physiological ash is the inorganic residues in water soluble ash after the plant drug was burnt while the acid insoluble ash value indicated that the plant was in good physiological condition and it contained little extraneous matters compared to the total ash content. The total ash value is used as a standard to assess the identity and purity of crude drugs (WHO, 1996 c, WHO, 2011) [32, 33].

The alcohol and water extractive values were 15.83% and 24.68%, respectively (Table 3). It was observed that water had a higher extractive value (24.68%). This is because water is a universal solvent that has high polarity and was able to extract more phytochemical constituents than alcohol that has less polarity. This verified why water is mostly used as solvent by traditional medical practitioner and individuals in preparation of dosage forms (Ajazuddin and Shailendra, 2010) [2]. We observed that despite alcohol's low extraction capacity, it is sometimes preferred to water especially in researches that deal with natural products because it serves as preservative against microbial growth and easy to evaporate and handle. Fluorescence is the phenomenon that exhibit various colour due to chemical constituents present in the plant material. The fluorescence colour is precise for each phytocompound. Some compounds do not fluoresce but when mixed with impurities are able to fluoresce. The fluorescent method is adequately sensitive and enables the precise and accurate determination of pharmaceutical samples (Banoti, 1980) [10]. The powdered stem bark of *P. kotschy* when treated with various acids, alkalis and other reagents and observed under daylight and UV- visible at short (254 nm) and long wave (365 nm) lengths, numerous colours were emitted depending on the reagents and wave length used (Table 4). This gives a clue if powdered plant samples are adulterated; thus can be used as a diagnostic tool for testing purity and identity (Sumitra, 2014) [27]. Fluorescence procedure is serving as a pre-requisite before going for detailed photochemical investigation.

Conclusion

The pharmacognostic standards of *P. kotschy* stem bark established from this study are within permissible limits and are reported for the first time. These standards will serve as diagnostic tools for correct identification and detection of adulteration of samples of *P. kotschy* stem bark during use. It will also ensure that authentic plant materials are selected for researches, drug production and home usage in managing diseases. The outcome of this study may be incorporated into official books for future reference.

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Conflict of Interest

There is no conflict of interest among the authors.

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