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Pharmacognostic studies and elemental analysis of the aerial parts of *Cassytha filiformis* Linn. (Family: Lauraceae)

Adamu A. Ambi[1*], Garba F. Nuru[2], Ahmed T. Mora[3] and Abubakar Ahmad[1]

[1]Department of Pharmacognosy and Drug Development, [2]Department of Biological Sciences, [3]Department of Clinical Pharmacy and Pharmacy Practice, Ahmadu Bello University, Zaria. Nigeria.

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Abstract
Cassytha filiformis Linn. (family Lauraceae) a leafless and perennial vine with small scales as a replacement of the leaves is currently being used in the treatment of various disease conditions including jaundice. The aim of this work is to study the pharmacognostic characters of *C. filiformis* Macroscopic/organoleptic characters, microscopic, chemo-microscopic characters, numerical standards, and elemental analysis were determined from the whole plant of *C. filiformis.* Findings from this study revealed the presence of some diagnostic microscopical features such as paracytic stomata, unicellular covering trichomes with cystoliths, prismatic calcium oxalate crystals and annular xylem vessels. Quantitative physical constants include moisture contents (5.5 %), ash value (17 %), acid insoluble ash value (1 %), total tannins (27.3 %), swelling index (165 %), water, alcohol and oil extractive indices (20.6 %, 13.6 % and 1.6 % respectively). Trace metals detected in *C. filiformis* such as Fe (165.4279 ppm), Mn (14.4093 ppm) and Ni (2.7933 ppm) which are essential were higher than FAO/WHO (1984) permissible limit for edible plants. While others: Pb (0.0568 ppm) Zn (0.1094 ppm), Cd (0.0103 ppm) and Cu (0.0535 ppm) were found to be within the safety limit.

Keywords: *Cassytha filiformis*; *Rumfar gada*; Lauraceae; Pharmacognostic characters

INTRODUCTION

Plants of the Lauraceae are nearly all woody trees and shrubs comprising 32 genera and about 2000 – 2500 species. An exception is the vining, leafless, parasitic genus *Cassytha* [1]. This genus is considered to be unique in the family of Lauraceae as it is a parasite. The genus derived its name, *Cassytha*, from the Greek name of *Cuscuta* (meaning dodder). The vine has several common names in the regions of the tropics. For example, South Sea Islanders called this vine as *"tentanini"* which has the meaning "to go round and round," and this seems to be a true descriptive adjective for the plants entwining habit [2]. Hausas in northern Nigeria called the plant "Rumfar Gada".

Cassytha filiformis is a plant used for its various ethnomedical purposes in Nigeria. The plant is used in traditional treatment of many diseases e.g. it is used as a vermifuge and also in the suppression of lactation after still birth, by several tribes in Nigeria [3]. The plant (stem and leaves) is boiled in water and administered for varying lengths of time to treat jaundice (Personal communications). Men were also reported to use it in love potions while women use the extracts of the vine as a colouring agent or as a dye to provide a black color for the fabrics [4]. In the

* Corresponding author. *E-mail*: aaambi@abu.edu.ng *Tel*: +234 (0) 8037012464
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traditional Ayurveda, *Cassytha filiformis* is used as the major substitute for *Cuscuta* [5]. The brown colour of the stem is used as the colouring agent and hence possesses a major application in the dyeing industries. Several aporphinoid alkaloids were isolated from the samples originating from Taiwan, Brazil, Australia and New Guinea but compositions were found to be quite variable among the different origins. Six aporphines from *C. filiformis* were shown to have *in vitro* cytotoxic properties out of which actinodaphnine, cassythine, and dicentrine, also show *in vitro* antitrypanosomal properties against *Trypanosoma brucei brucei* [6]. Aqueous and alcoholic extracts of *C. filiformis* were tested for their diuretic activity in Wistar rats. Total urine output volume and the concentration of Na^+, K^+ and Cl^- ions excretion in the urine were finally estimated. Aqueous and alcoholic extracts of *C. filiformis* were found to exhibit significant diuretic activity by causing a marked increase in the Na^+ and K^+ excretion [**7**]. This study was designed to determine some of the pharmacognostic standards of diagnostic importance for smooth and easy identification of *Cassyttha filiformis.*

EXPERIMENTAL

The chemicals used during the study were of analytical grade. The instruments were well calibrated before use [8].

Plant material. The plant *C. filiformis* was collected from ABU dam area, identified and confirmed by a Taxonomist at the Department of Biological Sciences, Ahmadu Bello University, Zaria. The voucher specimens were preserved at the Department's herbarium (No. 2314).

Macroscopy. The following macroscopic characters of the fresh leaves were observed: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture [9].

Microscopy. The free hand thin transverse and longitudinal sections of the fresh plant material were treated with different staining agent and observed for the general and specific microscopic characteristic. Furthermore, small quantity of the powdered plant material was cleared, mounted and observed for diagnostic powder characteristics [10].

Physicochemical investigations. The fresh and dried leaf powder material were used for the determination of numerical standards to include ash values, extractive values, swelling index, bitterness value, crude fibre etc. The chemomicroscopical examination of the powder using chemical reagents was also studied [10].

Analysis of metals of the powdered *C. filiformis* using Atomic Absorption Spectrophotometry. Macro and micronutrients of *C. filiformis* were obtained using. atomic absorption spectrophotometer (AAS, Shimadzu 2010, Japan) available at National Research Institute for Chemical Technology (NARICT), Zaria.

RESULTS

Macroscopic and organoleptic properties of *C. filiformis.* Stem of *C. filiformis is* green to orange, filiform, and glabrous. Leaves are reduced to minute Scale 1mm long, near the tips of stem. Flowers are sessile and borne in small panicles. (Plate I). The organoleptic characters include dark greenish colour, distinct odour and a slightly bitter taste of the powdered plant material. The fracture is fibrous and the texture is smooth and hairy.

Microscopic examination of *C. filiformis*

The diagnostic features identified from the aerial parts of *C. filiformis* include; unicellular covering trichome that is conical in shape, with thick walls. Scattered paracytic stomata, appearing on the aerial stem. The parenchyma cells which are rectangular in shape, the trichomes which contain cystoliths, conducting elements e.g. annular xylem

vessel were observed. A lignified fibre with medulary rays crossing the fibres at right angles were detected (Plates II, III, and IV).

Chemomicroscopical examination of powdered *C. filiformis.* The chemo-microscopical features identified were starch, and calcium oxalate crystals, (cell inclusions), tannins, and calcium carbonate (cell constituents), and cellulose and mucilage, suberin and cuticles (cell wall materials). (Table 1).

Numerical standard of *C. filiformis.* The numerical standard of powdered *C. filiformis* determined in this work include; moisture content, total ash value, acid insoluble ash value, total tannins, swelling index, bitterness value, alcohol and water soluble extractive values, oil content and crude fibre (Table 2).

Analysis of metals detected in powdered *C. filiformis.* Elemental analysis was also carried out on the powdered *C. filiformis.* Some of the analysed metals include; copper, chromium, iron, manganese, potassium, calcium, sodium, nickel, cadmium, zinc and lead (Table 3).

DISCUSSION

Cassytha filiformis is used in many cultures for the treatment of various disease conditions including jaundice without standardization. Crude form of *C. filiformis* has been evaluated with the view to providing useful and diagnostic parameters for the standardization of the drug. The parameters obtained include microscopical features (Plates II, III, IV), chemomicroscopical features (Table 1) and numerical standards (Table 2).

The leafless plant *C. filiformis* was found to have paracytic stomata located in between thin and thick walled parenchymatous cells in the epidermis. This result is in conformity with the previous work done by Sharma *et al.*, [7]. The presence of stomata promotes heat dissipation by water loss, maximizing the control of water loss by leaf and increases photosynthetic potential [11], these features are essential for the plant as the whole of its aerial part is involved in photosynthesis. Prismatic calcium oxalate were found to be scattered in ground parenchymatous cells as important parameters for identification and standardization of *C. filiformis* an indication that the plant might be rich in oxalic acid with which higher plants synthesize the crystals and deposit them in specialized organ or tissue [12,13].

Plate I: The plant *Cassytha filiformis*

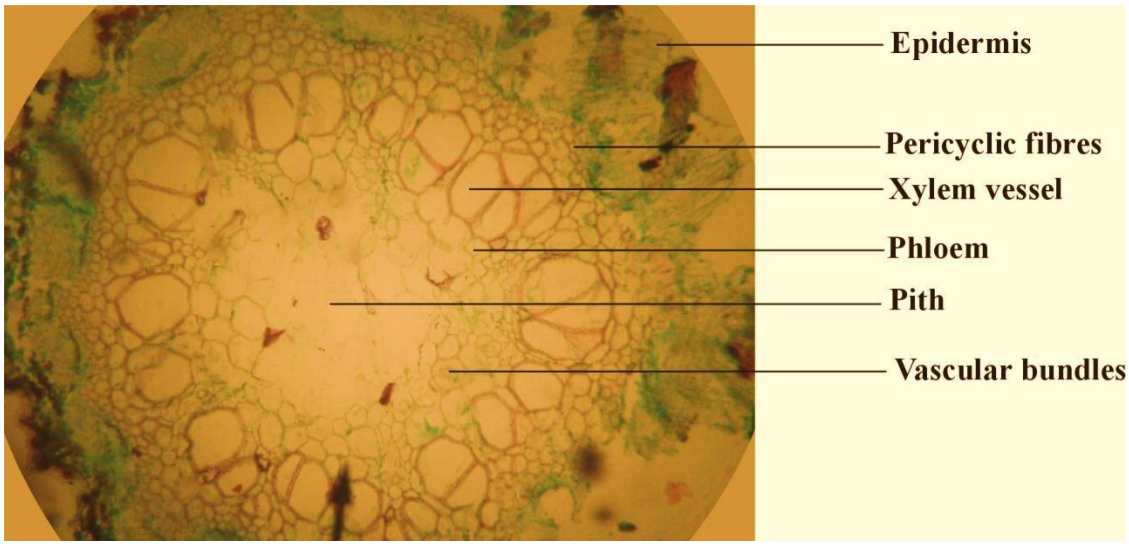


Plate II: Transverse section of *C. filifomis* stem
(Safranin and Fast green Stain. X 200)

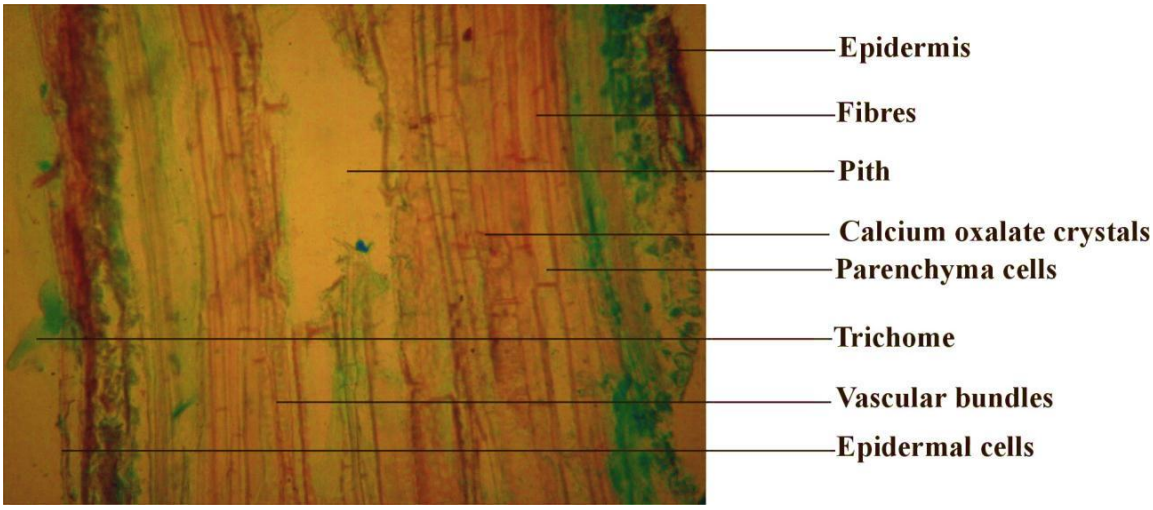
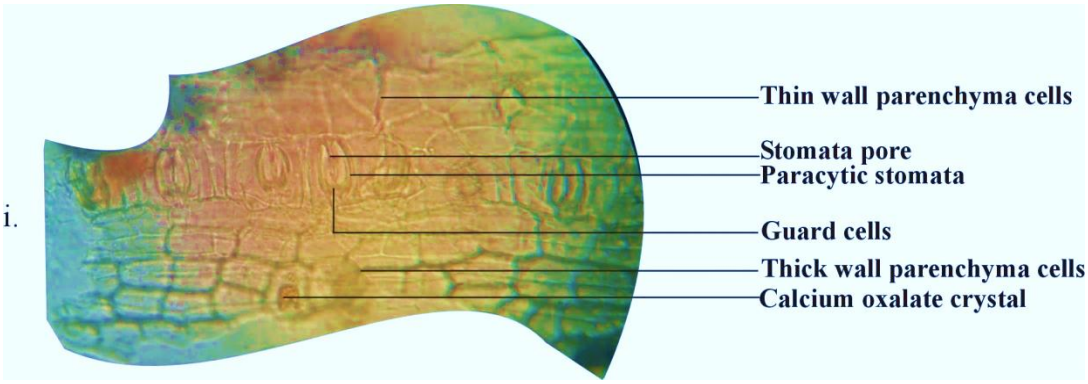
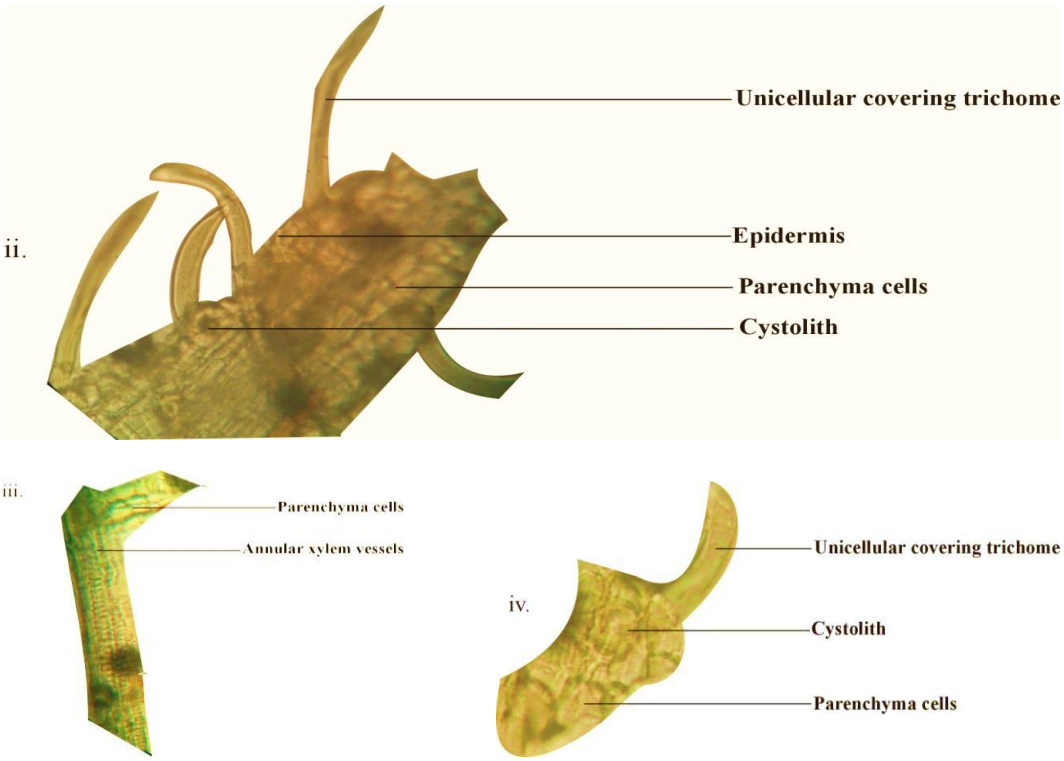


Plate III: Longitudinal section of *C. filifomis* stem
(Safranin and Fast green Stain. X 200)





Plates IV: Microscopical features of powdered whole plant of C. *filifomis* (X 200)

Table 1: Chemomicroscopical features from the powdered *C. filiformis*

	Constituents	Reagents	Observation	Inference
Cell wall materials	Cellulose	Chlor-zinc-iodine + Conc. HCl	Blue-violet	Cellulose
	Suberin/Cuticle	Sudan iv red	Orange red	Suberin/Cuticle
	Lignin	Phloroglucinol + Conc. HCl	Pink	Lignin
Cell constituents	Tannins	5 % Ferric chloride	Greenish black	Tannins
	Starch	N/50 Iodine	Blue	Starch
	Protein	Millon's reagent	Red colouration	Protein
	Calcium oxalate crystals	80 % H_2SO_4	Shiny crystals dissolves	Calcium oxalate crystals
	Calcium carbonate	5% acetic acid	Crystals dissolves with effervescence	$CaCO_3$

Table 2: Numerical Standards of *C. filiformis*

Numerical Standards	*C. filiformis* (%)
Moisture content	5.50
Ash value	17.00
Acid insoluble ash	1.00
Water soluble extractive value	20.60
Alcohol soluble extractive value	13.60
Total tannins	27.30
Bitterness value	0.23
Swelling index	165.00
Crude fibre	22.40
Oil	1.60

n =4 - 5

Table 3: Elemental analysis of powdered *C. filiformis*

Elements	Concentration (ppm)	FAO/WHO (1984) limit*(ppm)
Na	5.1735	-
Mg	9.3911	-
Ca	84.3993	-
Cr	7.7940	-
Cu	0.0535	3.0
Fe	165.4279	20
K	0.8313	-
Mn	14.4093	2.0
Zn	0.1094	27.4
Pb	0.0568	0.43
Cd	0.0103	0.21
Ni	2.7933	1.63
Co	0.4621	-

* For edible plants; ppm = parts per million

There is also presence of unicellular covering trichomes with cystoliths, (calcium carbonate deposit) which can be an excellent diagnostic feature for *C. filiformis*. The presence of cystoliths in the base of the trichomes found by this study is reported for the first time. Trichomes are epidermal outgrowths of considerable value for taxonomic purposes for some plants. These outgrowths play a role in plant defense especially with regard to phytophagous insects [14]. They may also be involved in the regulation of temperature and water repellency as well [15]. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for extractive values. This study found the extractive value of water (20.60 %) to be the highest followed by alcohol (13.60 %) then lipid (oil content) or diethyl ether extract (1.6 %). This is expected as water extracts most polar compounds such as carbohydrates which are the commonest in most plants. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired [16].

Studies of numerical standards can serve as a valuable source of information and are usually employed in judging the purity and quality of the drug [17]. The moisture content of *C. filiformis* is exceptionally low compared to the pharmacopoeia [8] limit (10 - 12 %). This may not be unconnected to the absence of the leaves and could be essential in preventing decomposition of the crude drug either due to chemical change or microbial contamination during drying and storing. The ash value and acid insoluble ash value of *C. filiformis* were found to be 17 %, and 1 %, w/w respectively. The acceptable (WHO) limits for total ash and acid insoluble ash vary according to the vegetable drug. For example, the total ash and acid insoluble ash values of *Centella asiatica* should not be more than 19 % and not less than 6% respectively [18]. Similarly, in *Pericarpium granati* the total ash should not be more than 4 % and the acid insoluble acid should not be less than 1 % [19]. The ash value is a measure of the earthy matter or inorganic composition and/or other impurities present along with the drug such as carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium [20]. The low values of ash in *C. filiformis* are indications that these minerals occur only in trace quantities. The bitterness value of *C. filiformis* was found to be 0.23. The acceptable limit varies according to the vegetable drug. Plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric

juice. The total tannins of *C. filiformis* was found to be 27.30 %. Other pharmacognostic parameters found by this study include swelling index, and crude fibre. These parameters are characteristic and are been reported for the first time on this plant. The parameters can be considered as additional indices for the authenticity of the drug. The swelling index of *C. filiformis* found was 165 % of the original volume of the plant material. Many plant materials are of specific therapeutic or pharmaceutical necessity because of their swelling properties – especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose. Therefore, swelling index gives an idea on the mucilaginous and pectin content of crude drug. The crude fibre content of *C. filiformis* was found to be 22.40 %. Determination of crude fibre is useful in distinguishing between similar drugs or in the detection of adulteration [21]. It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination.

Concentrations of minerals in *C. filiformis* determined by this study include iron (165.429 ppm), manganese (14.4093 ppm) and nickel (2.7933 ppm) as against the permissible limit set by FAO/WHO [18, 22] for edible plants (Table 3) However, for medicinal plants, the WHO [23] limits has not yet been established for Fe, Mn and Ni The literature, [24] shows similarity in Fe content (between 261 and 1239 ppm), and wide differences in Mn. (44.6 and 339 ppm) content in selective medicinal plants of Egypt. Trace elements with lower concentration in *C. filiformis* include Zn (0.1094 ppm), Cu (0.0535 ppm), lead (0.0568 ppm), and cadmium (0.0103 ppm) which are below the permissible limit, Zn (27.4 ppm), Cu (3.00 ppm), Cd (0.21 ppm) as set by FAO/WHO [22] for edible plants. However, these results are within the permissible limits for Cu set by China and Singapore as 20 and 150 ppm, respectively and the limit for lead (10 ppm) as set by China, Malaysia, Thailand [23]. The overall results indicated clearly the contents of the essential metals such as iron, manganese and nickel were within acceptable limits of the toxic metals such as lead are within safe limit (Table 3). Therefore *C. filiformis* can also be beneficial sources of appropriate and essential trace elements.

Conclusion. The pharmacognostic studies of C. *filiformis* yielded a set of qualitative and quantitative parameters that are useful in ascertaining the identity of the plant and to determine the quality and purity of the drug materials for future studies. The parameters which are reported here especially the bitterness value, total tannins, swelling index and crude fibre which are reported for the first time in this study can be considered distinctive enough to identify and decide the authenticity of the drug.

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