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# Pharmacognostic studies and elemental analysis of *Cassytha filiformis* Linn

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***Cassytha filiformis,*** **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 such as jaundice. 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, ethanol and oil extractive indices (20.6, 13.6 and 1.6%, respectively). Trace metals such as Fe (165.4279 ppm), Mn (14.4093 ppm) and Ni (2.7933 ppm) detected in *C. filiformis* 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. The aim of this work was to study the pharmacognostic, characters, elemental analysis and numerical standard of *C. filiformis*.**

**Key words:** Atomic absorption spectrophotometer (AAS), Rumfar gada, Lauraceae, pharmacognosy.

## INTRODUCTION

Plants of the Lauraceae are nearly all woody trees and shrubs comprising 32 genera and about 2000 to 2500 species. An exception is the vining, leafless, parasitic genus, *Cassytha* (Watson and Dallwitz, 1993). 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, *Cuscuta* (meaning dodder). The vine has several common names in the regions of the tropics. For example, South Sea Islanders called this vine *"tentanini"* which has the meaning "to go round and round," and this seems to be a true descriptive adjective for the plants entwining habit (Mythili et al., 2011). Hausas in northern Nigeria call the plant “Rumfar Gada”.

*Cassytha filiformis* is a plant used for its various ethnomedical purposes in Nigeria. The plant is used in

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traditional treatment of many diseases e.g vermifuge and also in the suppression of lactation after still birth by several tribes in Nigeria (Burkill, 1995). 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 the love magic while women used the extracts of the vine as a colouring agent or as a dye to provide a black color for the fabrics (Schroeder, 1967). In the traditional Ayurveda, *C. filiformis* is used as the major substitute for *Cuscuta* (Sakshy et al*.,* 2010). The brown colour of the stem is used as the colouring agent and hence have a major application in the dyeing industries (Sharma et al., 2009). 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* (Quetin-Leclercq et al., 2004). Aqueous and alcoholic extracts of *C. filiformis* were tested for their diuretic activity in Wister 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 (Sharma et al*.,* 2009).

The design of this study was to determine some of the pharmacognostic standards of diagnostic importance, elemental content and numerical standarda for smooth and easy identification of *C. filiformis.*

## MATERIALS AND METHODS

All chemicals and reagents used during the study were of analytical grade purchased from Sigma Aldrich chemical company and Merck (From Distributors, Lagos, Nigeria). The instruments were well calibrated before use.

The plants, *C. filiformis* were collected from Ahmadu Bello University (ABU) Dam area in the month of August, 2016. The plant was identified and authenticated as *C. filiformis* (Family: Lauraceae) by U. S. Gallah, a Taxonomist at the Department of Biological Sciences, Ahmadu Bello University, Zaria, the voucher specimens (No. 2314) were preserved at the Department herbarium library.

### Macroscopy

The following macroscopic characters of the fresh aerial parts were noted: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina and texture (Evans, 2009).

### Microscopy

The free hand thin transverse and longitudinal sections of the fresh aerial stem of the plant material were treated with different staining reagents 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 (WHO, 2011).

### Physicochemical investigations

The fresh and dried aerial parts powdered plant material was used for the determination of numerical standards e.g ash values, extractive values, swellimg index, bitterness value, crude fibre, etc. The chemomicroscopic examination powder with chemical reagents were also studied (WHO, 2011).

### 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 yellow, filiform and glabrous. Leaves are reduced to minute Scale 1 mm long, near the tips of stem. Flowers are sessile and borne in small panicles (Plate 1). The organoleptic characters include, dark greenish colour of the dried powdered plant material, it has a distinct odour, a slightly bitter taste. The fracture of the bark is fibrous and the texture is smooth and hairy.

### Microscopic examination of *C. filiformis*

Diagnostic features identified from the aerial stem parts of *C. filiformis* include: unicellular covering trichome that is cornical in shape, with thick walls. Scattered paracytic stomata, appearing on the aerial stem. Some parenchyma cells which are oval in shape, the trichomes which contain cystoliths, conducting elements e.g annular xylem vessel were observed (Plates 2, 3 and 4).

### Chemomicroscopical examination of powdered *C. filiformis*

Some of the chemo-microscopical features identified were starch, and calcium oxalate crystals, (cell inclusions), tannins and calcium carbonate (cell constituents), and cellulose, suberin and cuticles (cell wall materials) (Table 1).

### Numerical standards of *C. filiformis*

Some of the numerical standards of powdered *C. filiformis*

**Plate 1.** The plant, *Cassytha filiformis.*

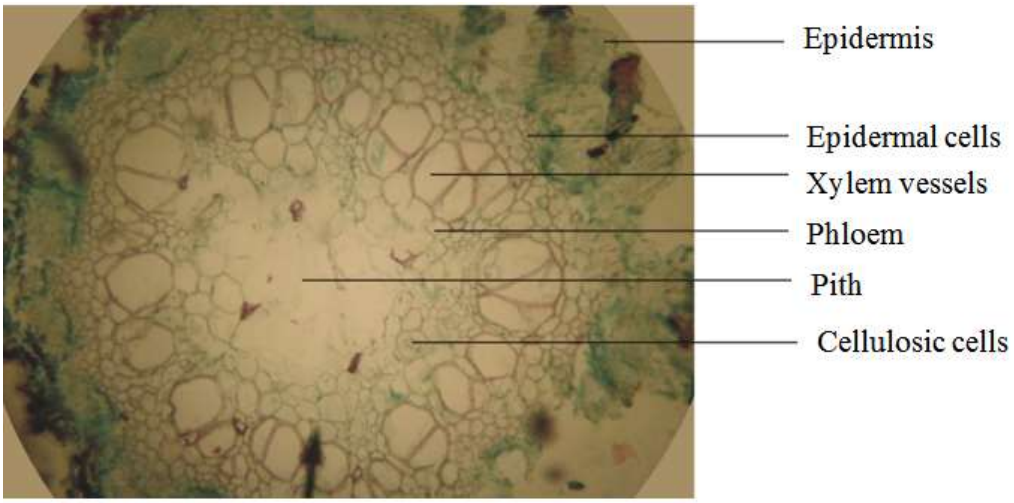


**Plate 2.** Transverse section of *C. filifomis* stem (Safranin and Fast green Stain. X 200).

determined under 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 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

*C. filiformis* is used in many cultures for the treatment of various disease conditions including jaundice without standardization. In this study, crude form of *C. filiformis* has been evaluated with the view to provide useful and diagnostic parameters for the standardization of the drug.

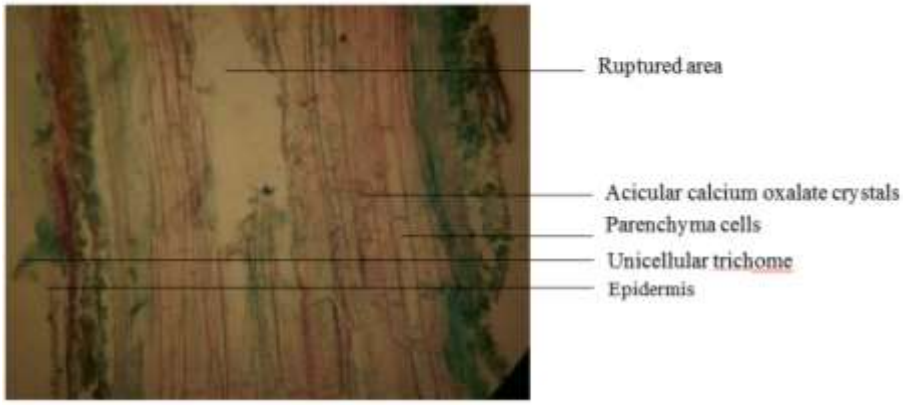


**Plate 3.** Longitudinal section of *C. filifomis* stem (Safranin and Fast green Stain. X 200).

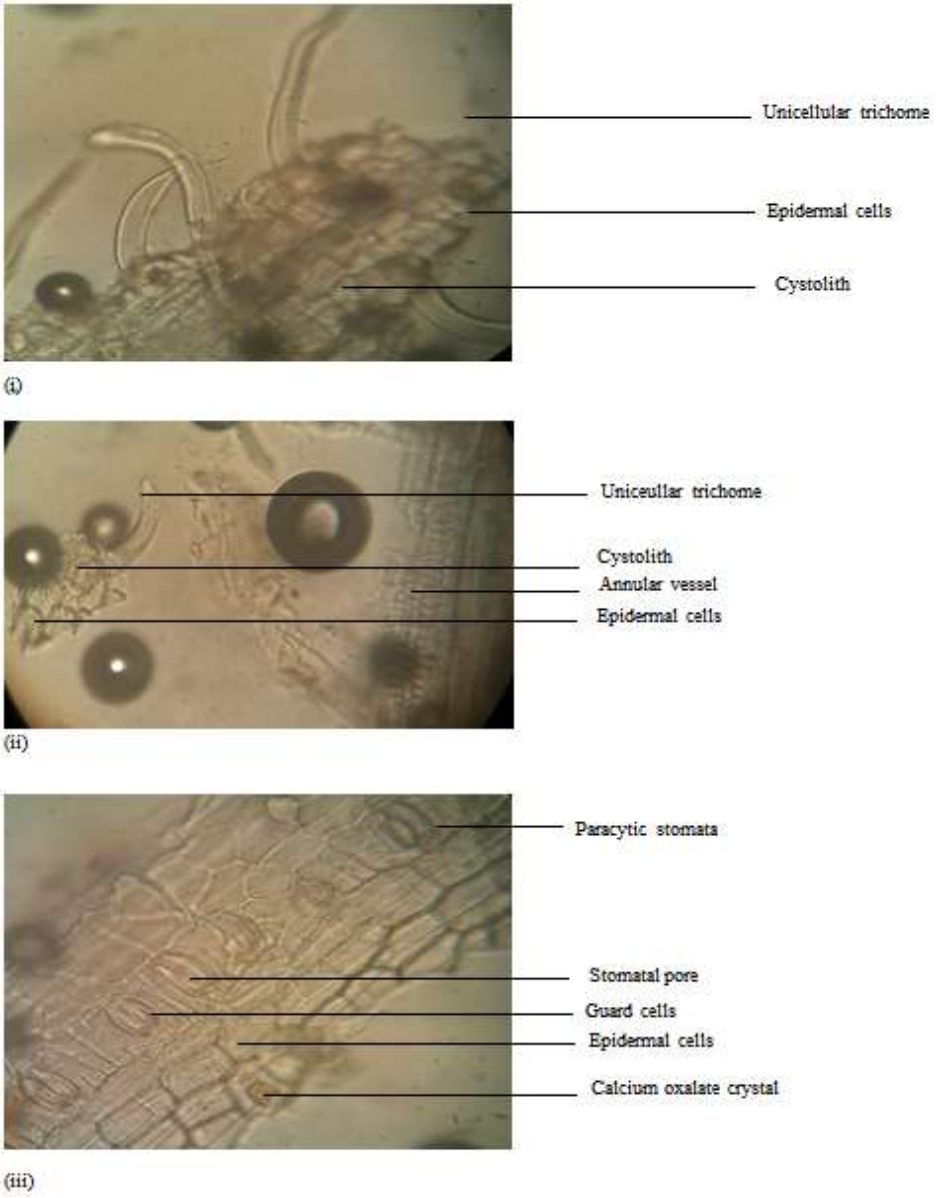


**Plate 4.** Microscopical features of powdered whole plant of C. *filifomis* (200X).

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

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

**Table 2.** Numerical standards of *C. filiformis.*

| Numerical standards | *C. filiformis* |
|---|---|
| Moisture content | 5.50±0.82 |
| Ash value | 17.00±1.08 |
| Acid insoluble ash | 1.00±0.41 |
| Water soluble extractive value | 20.60±0.77 |
| Ethanol soluble extractive value | 13.60±0.69 |
| Total tannins | 27.30±6.81 |
| Bitterness value | 0.23±0.01 |
| Swelling index | 165.00±10.00 |
| Crude fibre | 22.40±0.10 |
| Fixed oil | 1.60±0.16 |

n =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.

The parameters obtained include microscopical features (Plates 2, 3 and 4), 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 by Sharma et al. (2009). The presence of stomata promotes heat dissipation by water loss, maximizing the control of water loss by leaf and increases photosynthetic potential (Woodward, 1998), these features are essential for the plant as the whole of its aerial part is involved in photosynthesis. Acicular calcium oxalate (Plate 3) found scattered in ground parenchymatous cells are important parameters for identification and standardization of *C. filiformis* and it is a clear indication that the plant is rich in oxalic acid with which higher plants syntheses the crystals and deposit them in specialized in any organ or tissue (Nakata, 2003; Webb, 1999). Presence of unicellular covering trichomes with cystoliths (calcium carbonate deposit) is 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 (Metcalfe and Chalk, 1988). They may also be involved in the regulation of temperature and water repellency as well (Neinhuis and Barthlott, 1997).

Taking into consideration, the diversity in chemical nature and properties of contents of drugs, various solvents are used for extractives 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 of 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 (Kokate et al., 2009).

Studies of numerical standards can serve as a valuable source of information and are usually used in judging the purity and quality of the drug (Nisharaj and Radhammany, 2012). The moisture content of *C. filiformis* is exceptionally low as compared to the pharmacopoeia (EP, 2011) limit (10 to 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. Some typical examples include the total ash and acid insoluble ash values of *Centella asiatica* which should not be more than 19% and not less than 6%, respectively (WHO, 1999), similarly, in *Pericarpium granati*, the total ash should not be more than 4% and the acid insoluble acid should not be less than 1% (WHO, 2009). 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 (Wallis, 2005). 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* were found to be 27.30%. Other pharmacognostic parameters found by this study include swelling index, crude fibre and bitterness value. These parameters are diagnostic characteristic of *C. filiformis* and are been reported for the first time in 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 utility 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 (Thomas et al., 2008). 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 (165.4279 ppm), manganese (14.4093 ppm) and nickel (2.7933 ppm) as against the permissible limit set by FAO/WHO (1984) for edible plants (Table 3). However, for medicinal plants, the WHO (2005) limits has not yet been established for Fe, Mn and Ni. Sheded et al. (2006) showed 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 (1984) 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 (WHO, 2005). 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 which 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 in determining

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.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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