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Original article

Pharmacognostic and Elemental Analysis of the Leaves of *Tapinanthus globifer* (A. Rich). Tiegh

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Abstract

Background and objectives: *Tapinanthus globifer* is a semi-parasite plant that mostly grows on the branches of the host tree species of the genera *Vitellaria*, *Kola*, *Citrus*, *Combretum*, *Acacia*, and *Terminalia*. The leaf is known for its use in traditional medicine to treat inflammations, malaria, headaches, bacterial infections, ulcers and diabetes mellitus. The current study was aimed to establish standards on purity, identity and quality control of *T. globifer*. **Methods:** The pharmacognostic standardization of the leaf was assessed to determine the macroscopic/organoleptic features, microscopical and chemo-microscopical analysis as well as physico-chemical parameters, fluorescence analysis and elemental content. **Results:** The leaf was found to be simple, petiolated 10.0 cm long and 4.4 cm wide. Microscopically, the leaf had irregularly shaped epidermal cells with numerous paracytic stomata on the lower surface. It was devoid of trichomes of any kind; however, it possessed clusters of calcium oxalate crystals. The vascular bundles were of concentric type with fitted xylem vessels. Fluorescence analysis of the powdered leaf revealed the presence of yellow colorations under long wavelength. Elemental analysis showed the presence of magnesium, sodium, potassium, phosphorus, zinc, manganese, iron, nitrogen, sulphur, calcium and copper. Physico-chemical parameters (%w/w) such as moisture content (5.53), total ash content (8.40), water soluble ash content (2.10), acid insoluble ash content (1.20), ethanol extractives (25.60) and water soluble extractives (32.4) were also determined. **Conclusion:** It is hoped that these findings will be useful towards establishing standards on identity, purity, quality and preparation of monogragh of the drug obtained from the leaves of *T. globifer*.

Keywords: elemental analysis; microscopy; pharmacognostic standardization; physico-chemical standards; *Tapinanthus globifer*

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Introduction

During the years much attention has not been given to the adulterants in herbal medicines. This is because of nature of herbal parts, ingredients and different phytochemicals present in the plants [1]. To ensure the quality of herbal medicines, proper control of starting raw material is very important. Authentication of medicinal plants is of paramount importance in ensuring quality and safety of crude drugs [2]. The sources of drugs, their histological and morphological characters, chemical constituents, their qualitative and physicochemical tests and pharmacological studies are parameters used to study the Pharmacognostic characters of plant drugs [3]. It also includes the commercial varieties, substitutes, adulterants and any other quality control of the drugs. Authentication and quality assessment of herbal materials deals with pharmacognosy that is based on macroscopic and microscopic characters [4].

Tapinanthus globifer (A. Rich) Teigh. is a semi-parasite with glabrous pendulous stems up to 1.2

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m long with presumable roots that mostly grow on the branches of a large number of tree species of the genera *Vitellaria* (C.F. Gaertn), *Kola* (Schott & Endl.), *Citrus* (L.), *Combretum* (Loefl.), *Acacia* (Mill.), *Aloe* (L.) and *Terminalia* (L.) as hosts [5]. *Tapinanthus globifer* is locally known as mistletoe (English), "Kauchi" (Hausa), "Eme-emiafomo" (Yoruba), and "Osisi/Okwumaosa" (Igbo) in Nigeria, and belongs to the family of Loranthaceae [6]. *Tapinanthus globifer* has been used for long time in traditional medicine of Nigeria ana Cameron to treat inflammations, diabetes mellitus, stroke, malaria, bacterial infections, ulcer, headaches, stomach problems, and also to relieve pains. Recent studies have revealed that the plant exhibits a variety of pharmacological activities including anti-trypanosomal [7], antimicrobial [8], analgesic [9] and is rich in antioxidants [10]. The present study was intended to establish standards on purity, identity, quality and preparation of monograph of the drug obtained from the leaves of *T. globifer*.

Matrial and Methods

Collection, identification and preparation of leaves of *T. globifer*

Tapinanthus globifer was first identified and collected from *Citrus* species in May 2016 from the fields around Yankarfe village, Sabon gari Local Government Area, Kaduna State, Nigeria. It was identified and authenticated by a taxanomist at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria where a specimen (voucher number 1449) has been deposited. The leaves were cleaned, dusted and all foreign matters were removed. They were air dried under shade, comminuted to powder using pestle and mortar and stored in a sterile airtight container for further use.

Macroscopical examination

Macroscopical features diagnostic to *T. globifer* leaves were described using the previously outlined method [1,11].

Microscopical examination

The microscopical evaluation of the anatomical section and powdered sample of the leaves was carried out using standard methods [12,13]. The prepared sections were cleared using 70% chloral hydrate solution and boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol and observed under a microscope. Appropriate images were taken and documented. The micrometric evaluation of some of the diagnostic feature was also carried out.

Quantitative leaf microscopy analysis

Quantitative leaf microscopy analysis to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number was carried out on epidermal peelings and examined under microscope with the aid of Camera Lucida [12,14].

Chemomicroscopic examination

The histochemical detection of cell walls and contents of the powdered leaves such as cellulose cell wall, lignin, starch, cutin, tannins and calcium oxalate, calcium carbonate etc. was carried out using standard methods [1,13].

Physicochemical parameters

Powdered sample was subjected to physicochemical analysis such as water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content [1,13].

Determination of flourescence analysis

The fluorescent analysis of the leaves of *T. globifer* was carried out according to the methods previously described [15].

Determination of elemental analysis

The elemental composition of the leaves was determined using Atomic Absorption Spectrophotometry (AAS) [16].

Results and Discussion

Macroscopically, the leaf was elliptic in shape, apex obtuse phyllotaxy opposite, with smooth upper surface and rough lower surface. The length and width were 8 - 12 cm and 3.0 - 6.4 cm, respectively. The leaves were found to be greenish in color, with distinct odor and bitter taste.

The transverse section and powdered sample of *T. globifer* leaves revealed diagnostic features which included paracytic stomata, epidermal cells which were polygonal in shape, calcium oxalate crystals which were of prism type and vascular bundles as shown in figure 2, 3 and 4.

(A) **(B)**

Figure 1. (A): *Tapinanthus globifer* growing on *Citrus sinensis;* (B): the leaves and flowers of *T. globifer* in its natural habitat at Yankarfe village, Sabo-Gari Local Government Area of Kaduna State, Nigeria; photographed by Steven Madi.

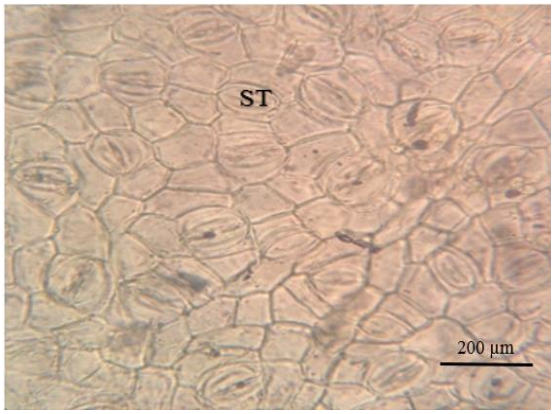

Figure 2. Photomicrograph of the lower surface of *Tapinanthus globifer* leaf showing paracytic stomata (ST)

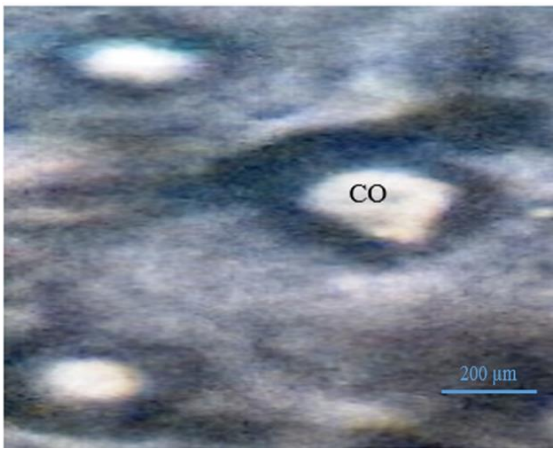

Figure 3. Photomicrograph of the leaf powder of *Tapinanthus globifer* showing calcium oxalate (CO) deposited

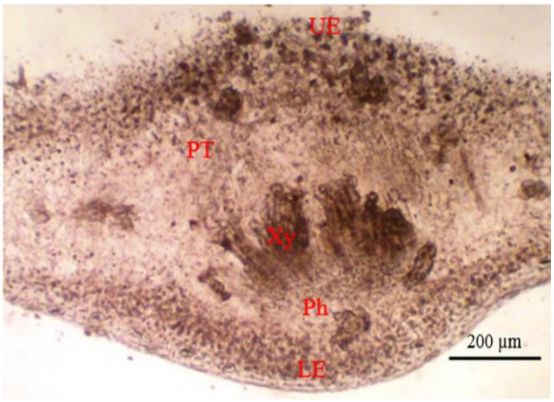

Figure 4. Photomicrograph of the transverse section through the midrib of *Tapinanthus globifer* leaf showing upper epidermis (UE), palisade tissue (PT), xylem (Xy), phloem (Ph) and lower epidermis (LE)

The micrometric evaluation of some of the diagnostic features such as, epidermal cells, calcium oxalate crystals and stomata have been shown in table 1.

The result for the numerical standards (quantitative microscopy) of the leaf such as stomata number, stomata index, palisade ratio, vein-islet and vein termination are shown in table 2. Chemo-microscopic examination of the powdered leaves of *T. globifer* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, mucilage, calcium oxalate and cutin while calcium carbonate was absent.

Table 1. Micrometric evaluation of some of the diagnostic features of *Tapinanthus globifer*

Parameters	Lower range (µm) *	Upper range (µm) *	(Mean µm) *
Lower epidermis			
Length	33.17 ± 0.23	42.80 ± 1.60	37.66 ± 0.43
Width	19.26 ± 1.51	27.82 ± 0.56	23.54 ± 0.91
Upper epidermis			
Length	34.24 ± 0.85	47.08 ± 0.33	39.23 ± 0.73
Width	21.40 ± 0.66	32.10 ± 0.82	26.39 ± 0.39
Stomata (paracytic)			
Length	32.10 ± 0.99	40.66 ± 0.21	37.09 ± 2.01
Width	17.12 ± 0.01	20.33 ± 0.88	18.55 ± 0.58
Calcium oxalate			
Length	1.07 ± 0.89	3.21 ± 1.17	2.14 ± 0.39
Width	1.01 ± 0.67	2.57 ± 0.16	1.81 ± 0.86

*Average values of 5 counts

Table 2. Quantitative microscopy of *Tapinanthus globifer* leaves

Parameters	Values (%) ± SEM*
Vein islet number	10.0 ± 0.71
Vein termination number	33.0 ± 0.95
Palisade ratio	5.0 ± 0.00
Stomata number	38.6 ± 2.32
Stomata index	36.7 ± 1.79

*****Average values of five determinations

The result of average moisture contents using loss on drying method was calculated to be 5.53 % and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 8.40 %, 1.20 % and 2.10 %, respectively. The extractives obtained were 25.60 % and 32.40 % for alcohol and water solvents, respectively (table 3).

Table 3. Physicochemical constants of *Tapinanthus globifer* leaves powder

Parameters	Values (%w/w) ± SEM*
Moisture content	5.53 ± 0.08
Total ash value	8.40 ± 0.24
Acid insoluble ash	1.20 ± 0.12
Water soluble ash	2.10 ± 0.10
Ethanol extractives	25.60 ± 0.24
Water extractives	32.40 ± 0.24

*****Average values of five determinations

The results showed different color changes under daylight and in the UV chamber (table 4). The results of the elemental analysis showed the presence of some macro and micro elements which has been recorded in table 5. Calcium was recorded to show the highest value (27400.00 ppm).

In this report, various macroscopical, microscopical, physicohemical and elemental standards have been developed that will assist in proper identification and standardization of *T. globifer*. The macroscopic and microscopic evaluation is a reliable, cost effective and time saving method for establishing the right identification of herbal materials [1,17].

These morphological description of *T. globifer* were in line with the morphological description of *T. bangwensis* (Engl. & K. Krause) Danser [18].

Microscopic examination of the leaves of *T. globifer* revealed the presence of some important diagnostic features such as polygonal epidermal cells with straight - wall on both adaxial (39.23 µm x 26.39 µm) and abaxial (37.66 µm x 23.54 µm) epidermal layers. There were paracytic stomata or rubiaceous (parallel celled) types of stomata (39.09 x 18.55 µm) on both epidermal layers while trichomes were absent. The transverse section of *T. globifer* leaf revealed some prominent features like the vascular bundle (which consisted of xylem and phloem) and the lower and upper epidermal layers. Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials [2].

Chemo-microscopical features of powdered leaf of *T. globifer* revealed the presence of cellulose cell wall, lignified cell wall, mucilage, tannins, starch, suberin and calcium oxalate crystals while calcium carbonates were found to be absent. The microscopic structures 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 [19]. The physicochemical parameters including the moisture content, acid insoluble ash, water soluble ash, alcohol extractives value, water extractives and total ash values were determined from the powdered leave of the plant which has been shown in table 3 [9,14].

Table 4. Fluorescence analysis of *T. globifer* leaves powder

Reagents/Solvents	Daylight		Short wavelength	Long wavelength
	Immediately	After an hour		
Conc. HCl	Light yellow	Yellow	No reaction	Yellow
NaOH	Light green	Brown	No reaction	No reaction
Sulphuric acid	Dirty green	Brown	No reaction	Yellow
n-Hexane	Green	Green	No reaction	No reaction
NH_4OH	Light green	Yellow	Yellow	Yellow
Water	Colorless	Colorless	Colorless	Colorless
Glacial acetic acid	Light green	Faint yellow	No reaction	Yellow
Nitric acid	Brown	Dark brown	No reaction	Yellow
Methanol	Light green	Yellow	No reaction	Yellow

Table 5. Elemental analysis of *T. globifer* leaves powder

Elements	Concentration (ppm)
Calcium (Ca)	27400.00
Magnessium (Mg)	2395.00
Potassium (K)	16000.00
Iron (Fe)	1220.00
Sodium (Na)	675.00
Nitrogen (N)	2160.00
Phosphorous (P)	1305.00
Manganese (Mn)	34.81
Copper (Cu)	12.00
Zinc (Zn)	68.00
Lead (Pb)	155.45
Sulphur (S)	12.00

These are values that are very important as basis to judge the identity, purity and in detecting adulterants in a crude drug [1,13,14].Moisture content (5.53 %) was not high which indicated less chances of microbial degradation of the drug during storage. The general requirement of moisture content in crude drug is recommended not to be more than 14 % [20] and the value obtained in this research work was within the accepted range. Determination of the moisture content helps preventing degradation of drug during storage. The lower the value, the less likelihood of degradation of drug and suggests better stability of the product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of moisture promotes mold and bacterial growth [21,22].

Total ash value (8.4 %) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated. The acid insoluble ash values (1.2 %) obtained in this study indicated that the plant was in good physiological condition and contained little extraneous matter such as sand, silica and soil. The total ash value is used as criteria to judge the identity and purity of drugs [1,21].

Extractive value is determined when a given amount of plant material is extracted with a particular solvent. When the crude drug is extracted with a particular solvent, it produces a solution that contains several constituents [9,10,13,16,20]. The nature of the crude drug and the solvent used determines the constitution of the phyto-constituents present [10, 16, 20, 22]. It also helps to determine if the crude drug is debilitated or not [9, 10, 16, 23]. This study indicated that ethanol gave lower extractive value (25.6 % w/w) compared to water which had extractive value of 32.4 % w/w. Fluorescence analysis is a very important and useful tool for the identification of different constituents present in natural products [15]. The use of different chemical reagents to carry out fluorescent analysis under day light and ultraviolet light revealed various colors from light green to yellow [32,33]. For example, concentrated hydrochloric acid was applied to a portion of the powdered leaves on a clean slide and was viewed under day light, it immediately gave light yellow color and showed yellow color after one hour while yellow color was observed under ultra violet light. This analysis suggested that, the leaves extract of *T. globifer* probably contain active agent(s) which has provided the basis for its folkloric use as a cure for some human ailments.

Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases [24]. Minerals are of critical importance in the diet, even though they compromise only 4-6 % of the human body. However, lack of full understanding of the amount and type of elements found in medicinal plants can cause a lot of danger to consumers as some of these plants may contain toxic elements in high quantities [6]. Again, proper dose rate of many of these medicinal plants is not established and makes it difficult for users to take them appropriately. The probability of taking overdose to facilitate healing processes is high and these can cause serious problems for users because

they are ignorant of the dangers involved [6,8,17,27]. Thus, the elemental composition of *T. globifer* was screened in the present study. The mineral element concentrations in milligram per kilogram of the leaf powder of *T. globifer* revealed that it contains both the macro (Ca, Mg, Na, K, S among others) and micro elements (Fe, Zn, Mn among others). Calcium content (27,400 ppm) was observed to be the element with the highest concentration. High content of Calcium is required for mineralization and enhancement of the qualities of bones and teeth [25]. By stimulating the release of thromboplastin from the blood platelets, calcium is essential for the normal clothing of blood [1,2,3]. It is essential for proper functioning of the heart, nervous systems, relief of stress and normal function of muscle system [8]. The health of the muscles and the nerves depends on calcium. It is needed for the synthesis of the neurotransmitter acetylcholine, the activation of enzymes such as the pancreatic lipase and the absorption of dietary vitamin B [6,8,25]. It also aids in regulating the heart, the activity of skeletal muscle, and many other tissues [25,26]. Therefore, high content of calcium in *T. globifer* is an advantage in the use of *T. globifer* in treating different ailments. Recommended daily dietary allowance of Calcium for children and adolescent is between 500 and 1,300 mg, while for adults is 1,200 mg [27]. An essential component of bone, cartilage and the crustacean exoskeleton is Magnesium (2,395ppm). Magnesium is an activator of several key enzyme systems including kinases, mutases, muscle ATPases, and the enzyme cholinesterase. Along with its role in enzyme activation, magnesium (like calcium) stimulates muscle and nerve irritability (contraction), is involved in the regulation of intracellular acid-base balance, and plays an important role in carbohydrate, protein and lipid metabolism. Recommended daily intake for children and adolescent is between 240-360 mg [27]. The main function of sodium (675 ppm) is to control the volume of fluid and to maintain the acid-base equilibrium in human body [26], sodium also has an effect on muscle irritability, and plays a specific role in the absorption of carbohydrates. Recommended daily intake of sodium for children and adults is between 2,300 mg and 3,400 mg, respectively [27]. Potassium (16,000 ppm) regulates intracellular osmotic pressure and acid-base balance, plays a vital role as electrolyte in the blood and for the smooth flow of communication signals from cell to cell. Like sodium, potassium has a stimulating effect on muscle irritability; it is also required for glycogen and protein synthesis, and the metabolic breakdown of glucose [28]. Recommended intake is 4,700 mg per day [27] while for breastfeeding mothers, it is about 5,100 mg a day. Iron (1220 ppm) is an essential component of respiratory pigments haemoglobin and myoglobin and also of various enzyme systems including the cytochromes, catalases, peroxidases, and the enzymes xanthine and aldehyde oxidase, and succinic dehydrogenase. As a component of the respiratory pigments and enzymes concerned in tissue oxidation, iron is essential for oxygen and electron transport within the body [29]. Iron deficiency is the most prevalent nutritional deficiency in humans [30]. The recommended daily in-take for iron is 13.7 - 6.5 mg/day for children and 19.3 - 20.5 day for adults [27,34]. The results showed the presence of various elements detected with different concentrations and its medicinal importance with regards to life processes and the probable significant roles of the plant in metabolism in the human body. This work has established some pharmacognostic standards which could be useful in setting some diagnostic indices for the proper identification, quality control and compilation of a suitable monograph on *T. globifer*.

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Author contributions

Celestine Jeremiah designed the study and wrote the protocol for M.Sc. thesis; Umar Adam Katsayal and Hadiza Dijie Nuhu were supervisors of the M.Sc. thesis; Aliyu Nuhu managed the literature searches and interpretation of results. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

CO: calcium oxalate; LE: lower epidermis; Ph: Phloem; PT: palisade tissue; SEM: standard error mean; ST: stomata; UE: upper epidermis; UV: ultraviolet; Xy: xylem