



Anatomical characterization and physicochemical standardization of *Gongronema latifolium* Benth. (Apocynaceae)

Adedapo Adedayo ADENIRAN^{1,2*}, Emmanuel ADEYANJU², Nneka Kingslena OKEKE²

¹Department of Pharmacognosy and Natural Medicine, University of Calabar, Calabar. Nigeria.

²Department of Pharmacognosy, Madonna University, Elele. Rivers State, Nigeria.

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Abstract

Gongronema latifolium Benth belongs to the family Apocynaceae. Generally, the leaves and stems of *G. latifolium* are used as medicine and spices for sauces, soups and salads in Southern Nigeria. In view of its medicinal uses, the present study aimed at documenting anatomical characters and physicochemical properties for its standardization. The leaf epidermis, transverse section of the midrib, petiole and stem were prepared using standard procedures. Physicochemical properties of the powdered plant were conducted following standard methods. The leaf epidermal layer revealed anomocytic stomata, calcium oxalate crystals (rosette), uniseriate multicellular trichome (non-glandular). The midrib has an abundantly non glandular trichome with numerous rosette crystals. The percentage extractive values for ethanol, distilled water and petroleum ether in the leaf powder were 16.71±0.53, 13.24±0.71 and 2.08±0.13%, respectively, while that of stem were 12.51±0.15, 10.90±0.53 and 1.10±0.21, respectively. The moisture content and total ash values for the leaf powder were 11.45±0.53 and 9.70±0.17%, respectively while for the stem were 11.07±0.38 and 7.50±0.05, respectively. In the light of frequent consumption and use of *G. latifolium* as medicine, some of the pharmacognostic standards provided herein may be useful for its proper identification and subsequent compilation in a monograph.

Keywords: *Gongronema latifolium*; Medicine; Monograph; Physicochemical properties; Standardization

INTRODUCTION

Gongronema latifolium Benth. is a tropical rainforest plant which belongs to the family Apocynaceae. It is propagated by seed or stem cuttings [1]. It is widely distributed across the West Africa region - Nigeria, Guinea-Bissau, Cameroon, Senegal, Ghana, Sierra Leone [2]. Morphologically, *G. latifolium* is a twinning perennial shrub with a simple, opposite leaf and a hollow stem

(Figure 1). *G. latifolium* represents one of the important medicinal aromatic plants in Nigeria [3]. In Southwestern Nigeria, it is commonly called 'arokeke' while it is referred to as 'Utazi' in the South-Eastern region. Nutritionally, the leaves are rich source of protein, minerals and vitamins, hence its application as a spice in indigenous brewed beer [4] and also used in food preparation especially local soups. The leaves of *G.*

*Correspondence. E-mail: adedapo.adeniran@unical.edu.ng Tel: +234-8057324457.

latifolium have been reported as vermifuge in some indigenous communities [5]. The leafy vegetable of *G. latifolium* is commonly consumed as spices in the Southern part of Nigeria. It is commonly consumed as spice with roasted plantain on the streets. In this regard, the health benefits derived from *G. latifolium* cannot be overemphasized coupled with its diverse use in managing ailments by indigenes from Southern Nigeria.

Pharmacologically, *G. latifolium* has been reported as hypoglycemic [6]; hypolipidemic [7]; nephroprotective [8]; antifertility [9]; anti-inflammatory [10]; antiulcer [2]; anticancer [11] and antimicrobial [12, 13]. The pharmacological activities of *G. latifolium* have been attributed to some of the chemical constituents such as alkaloids, flavones, sterols, saponins, tannins, flavonoids [5, 14, 15].

Despite its varied utilization as medicine, there are no records from the literature on diagnostic characters for anatomical identification and physicochemical properties of *G. latifolium*. Hence the need to document this medicinal plant which has gained wide popularity as spices. Given the aforementioned, this research aimed to compile anatomical characters and physicochemical properties of *G. latifolium* thereby contributing to its proper identification as a crude drug.

EXPERIMENTAL METHODS

Plant collection and authentication.

Gongronema latifolium was collected in August, 2019 at Boki, in Cross River State, Nigeria. The geographical location of the point of collection lies within Latitude 6.2500 N, and Longitude 9.0333 E. Fresh collected samples of *G. latifolium* was identified and authenticated at the herbarium of the Department of Plant and Ecological Studies, University of Calabar, where a voucher specimen was prepared and deposited. The

voucher number of *G. latifolium* is Bot/Herb/UCC/0719.

Plant preparation. The leaves of *G. latifolium* were dried under shade between 27-30°C. They were oven-dried at 40°C for 1 h before pulverizing with a milling machine. Similarly, the stem was chopped into pieces and treated as mentioned in the leaves. Powdered samples were stored in an airtight container until use.

Epidermal layer and midrib preparation

The epidermis and transverse section of the midrib were prepared using standard methods [16, 17].

Petiole and stem preparation. Thin sections of the middle region of petiole and stem were obtained by using an unripe pawpaw tissue as support. The obtained sections were cleared, rinsed, stained with safranin O and dehydrated with graded series of ethanol [18].

Quantitative measurement of anatomical character.

Quantitative measurement was achieved by calibration of an eyepiece and stage micrometer on a light microscope following standard methods [19].

Stomatal number and stomatal index.

Stomatal number and stomatal index were determined using a square graticule following standard procedures [20].

Determination of physicochemical parameters.

The powdered leaf and stem of *G. latifolium* were evaluated for its extractive values, moisture content, total ash value, acid insoluble ash value and water-soluble ash value using standard methods [21, 22].

RESULTS

Anatomical characters from the leaf epidermal layer.

The observed diagnostic characters in the epidermal layer of *G. latifolium* include: uniseriate multicellular trichome (a non-glandular), straight epidermal walls, rosette (calcium oxalate crystals), sinuous epidermal wall, anomocytic stomata as shown

in Figure 2. The epidermal layer also showed amphistomatic stomata (stomata on both the adaxial and abaxial surfaces).

Transverse sections of midrib, petiole and stem. Interesting diagnostic characters from the midrib section showed numerous non glandular trichomes, clusters of rosette crystals and vascular tissues comprising the phloem and xylem cells (Figure 3a). The vascular tissue of the petiole is arc-shaped (Figure 3b). The collenchyma cells of the petiole and stem are circular in shape and compactly arranged (Figure 3b and 3c).

Quantitative measurement of diagnostic characters. The trichome dimension is larger in the adaxial surface ($194.5 \pm 14.09 \mu\text{m}$ by $15.06 \pm 0.64 \mu\text{m}$) of epidermis of *G. latifolium* compared to the abaxial surface ($113.75 \pm 7.43 \mu\text{m}$ by $11.25 \pm 0.52 \mu\text{m}$) (Table 1). The mean stomatal number and stomata index per mm^2 obtained from the epidermis of the abaxial layer of *G. latifolium* are 8.25 ± 0.52 and 17.60 ± 0.95 , respectively (Table 1).

Physicochemical parameters. The highest extractive values for leaf ($16.71 \pm 0.35\%$) and stem ($12.51 \pm 0.15\%$) powder was recorded in ethanol (Table 2). The moisture content and ash value of the leafy *G. latifolium* were 11.45 ± 0.53 and 9.70 ± 0.17 , respectively (Table 2).

DISCUSSION

Anatomical characters from leaf epidermal layer (epidermal cell shape, epidermal cell wall, stomata type, trichome type and crystals) have been utilized in the time past as a taxonomical tool for plant identification and distinguishing marker for genuine crude drug and their adulterants [18, 23-25]. In the same vein, microscopic evaluation of medicinal plants has been reported as a veritable marker for herbs identification which essentially forms an

integral part of modern monograph [26]. The microscopic technique has also taken a central stage in identifying powder crude drugs using specific diagnostic characters in the event of substitution in the herbal market.

In the present study, anatomical characters observed from the leaf epidermal tissue of *G. latifolium* include straight epidermal walls on the adaxial surface with deeply wavy (sinuous) epidermal wall on the abaxial surface; abundant calcium oxalate crystals (rosette) on the abaxial surface only; presence of uniseriate multicellular trichome (non-glandular) predominantly on the abaxial surface; and anomocytic stomata type abundantly present on the abaxial surface. Interestingly, the rosette crystal observed in *G. latifolium* has been reported in medicinal plants such as *Cannabis indica* Lam, *Eucalyptus globulus* Labil, *Gymnema sylvestre* (Retz.) R.Br.ex sm., *Ricinus communis* L. and *Hillieria latifolia* (Lam.) H. Walt [27, 28].

In addition to qualitative diagnostic characters, quantitative documentation has significant role in compiling set of standards and distinguishing closely related species which can be used as substitute [24, 28, 29]. The stomatal number can be significant but can be varied due to environmental conditions in which the plant is grown. However, stomatal index is a more useful value as it is less subjected to variations with external conditions [30]. In the present study, the stomatal number (8.25 ± 0.52) and stomatal index (17.60 ± 0.95) on the abaxial surface of *G. latifolium* could complement a set of standards for its identification.

Physicochemical properties such as moisture content, extractive value, ash value, water-soluble ash value and acid insoluble ash value are quantitative sets of standards for crude drug evaluation. These parameters expose improper handling of drugs during packaging and also aids in detecting adulterants.



Figure 1: Habit picture of *Gongronema latifolium* showing leaf and stem

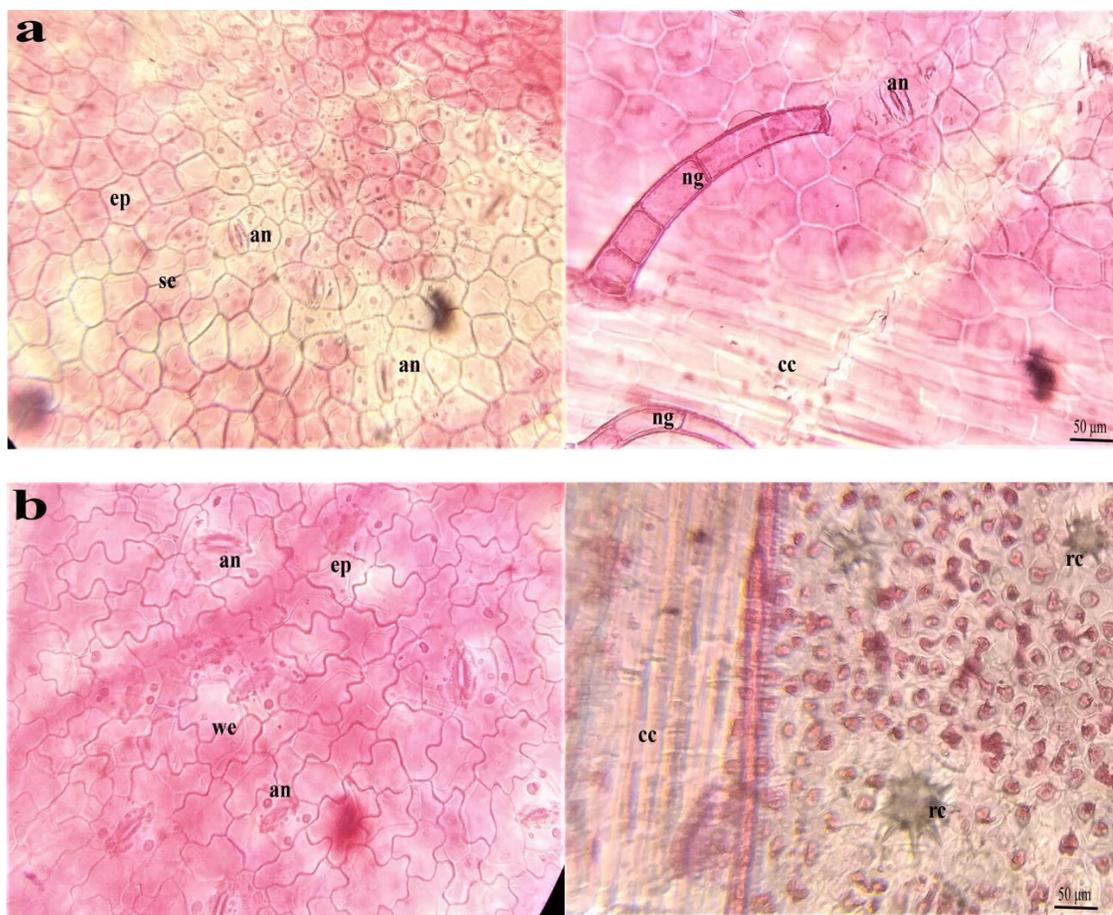


Figure 2: Epidermal layer of *Gongronema latifolium* (a) Adaxial surface (b) Abaxial surface an- anomocytic stomata; ep- epidermal cell; cc- coastal cell; ng- non glandular trichome (multicellular uniseriate trichome); rc- rosette crystals; se- straight epidermal wall; s- starch grain; we- wavy epidermal wall (sinuous).

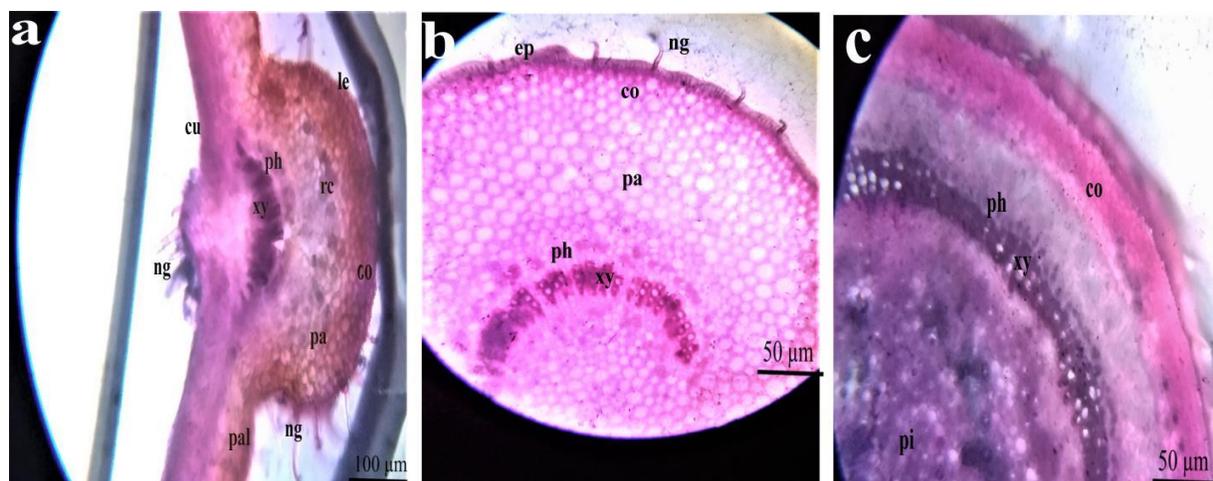


Figure 3: *Gongronema latifolium* (a) Transverse section of mid rib (b) Transverse section of Petiole (c) Transverse section of Stem

CO- Collenchyma cells; Cu- Cuticle; Pa- parenchyma cells; Pal- Palisade cells; Ph- Phloem cells; lower epidermis; ep- epidermis; ng- non glandular trichome; rc- rosette crystals, xy- xylem cells; Pi- Pith

Table 1: Quantitative measurement of diagnostic characters from the leaf epidermal layer of *Gongronema latifolium*

Diagnostic characters	Dimension (adaxial) μm	Dimension (abaxial) μm
Trichome length	194.5 \pm 14.09	113.75 \pm 7.43
Trichome width	15.06 \pm 0.64	11.25 \pm 0.52
Stomata length	18.75 \pm 0.95	18.25 \pm 1.33
Stomata width	3.25 \pm 0.26	3.13 \pm 0.32 Per mm^2
Stomatal number	-	8.25 \pm 0.52
Stomatal number range	-	4.00-8.25-12.00
Number of epidermal cells	-	38.40 \pm 1.10
Stomatal index	-	17.60 \pm 0.95
Stomatal index range	-	9.09-17.6-23.53

Table 2: Physicochemical parameters of *Gongronema latifolium*

Parameter	Extract	GL(l)%	GL(s)%
Extractive value	Petroleum ether	2.08 \pm 0.13	1.10 \pm 0.21
Extractive value	Distilled water	13.24 \pm 0.71	10.90 \pm 0.53
Extractive value	Ethanol	16.71 \pm 0.35	12.51 \pm 0.15
Moisture content		11.45 \pm 0.53	11.07 \pm 0.38
Total ash value		9.70 \pm 0.17	7.50 \pm 0.05
Acid insoluble ash value		4.1 \pm 0.54	2.53 \pm 0.18
Water soluble ash value		5.73 \pm 0.20	6.07 \pm 0.14

GL- *Gongronema latifolium* leaf, (l)- leaf, (s)-stem

Specifically, according to British Pharmacopoeia, moisture content in packaged crude drugs should not be more than 14% [20] to deactivate enzymatic reactions within the cells and minimize degradation by microorganisms during storage. In the present study, the percent moisture of the leaf (11.45 \pm 0.53) and stem (11.07 \pm 0.38) of *G. latifolium* is within the

range of acceptable Pharmacopoeia standard of not more 14%.

The percent extractive values in various solvents have been reported to indicate the quantity and nature of constituents in extracts [31]. In both the powder leaf and stem of *G. latifolium*, the extractive value was highest with ethanol followed by distilled water and petroleum ether. This suggests ethanol as most preferable solvent for the

extraction of bioactive constituents from the plant. This is in agreement with [32] wherein alcohol and water yielded high extractive values in the extraction of leaf of *Fadogia cienkowskii*, thereby suggesting it as a better choice for the extraction of secondary metabolites present in the plant. The ash content indicates extraneous inorganic matter like metallic salts of carbonates, phosphates, and silica, which could have been added inadvertently or deliberately during packaging of crude drugs. The ash value aids in detecting quality and purity of crude drugs. The ash value of the leaf (9.70 ± 0.17) was higher than the stem (7.50 ± 0.05). This could be due to numerous calcium oxalate crystals observed on the leaf surface, which was conspicuously absent from the stem. This same observation has been reported by [25] wherein stem bark and root of *Spondias mombin* had higher ash value due to the abundance of calcium oxalate crystals compared to the leaf without crystals.

Considering the medicinal importance of *G. latifolium* and its use as spicy, these sets of diagnostic characters represent first documentation to the best of our knowledge. According to Adeniran *et al.* [25], compilation and standardization of crude drugs patronized as food/herbal medicine are paramount for global compliance and also aids in restoring consumers' confidence in traditional medicine. Adeniran *et al.* further pointed out that accurate pharmacognostic information is important in identifying medicinal plants for their biological diversity, sustainability, utmost utilization and detection of adulterants. In the wake of substituting herbal products with close substitutes among herb sellers and food vendors on the street, this set of diagnostic characters can help checkmate such sharp practices, thereby gaining consumers' confidence.

Conclusion. Anatomical characters and physicochemical properties are part of standards required in the compilation of

monograph for medicinal plants profiling. The physicochemical properties, qualitative characters such as anomocytic stomata, rosette crystals and uniseriate multicellular trichome with their quantitative measurements could be helpful in the identification of *Gongronema latifolium* even in fragmented form.

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