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RESEARCH ARTICLE

MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL STUDIES ON *ZANHA GOLUNGENSIS* HIERN. (FAMILY: SAPINDACEACE)

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ABSTRACT: *Zanha golungensis* Hiern. is one of the commonly used species of Sapindaceae in Nigeria owing to its medicinal properties; however, taxonomic data on the species are limited. In this study, morphological, anatomical and phytochemical characters of the leaf and stem of *Z. golungensis* were assessed with a view to add to existing literature on the species. Vegetative morphology, light microscopy as well as phytochemical examinations were performed. Taxonomically useful characters were recorded which can be applied in the identification of *Z. golungensis* in pharmacognostic crude drug research without ambiguity. This is a contribution to already existing literature on the species.

Key words/phrases: Dodonaeoideae, Epidermal cells, Medicinal plants, Pharmacopeia, Taxonomic characters.

INTRODUCTION

Since ancient times, plants have been used in the treatment of various health disorders. However, plant remedies are prone to adulteration thereby limiting their acceptability and authenticity. It is therefore, imperative to provide standards that can ensure proper identification and ascertain the quality of medicinal plants (Awotedu and Ogunbamowo, 2019). The genus *Zanha* Hiern comprises of three species in Africa, namely *Z. africana* (Radlk.) Exell., *Z. golungensis* Hiern. and *Z. suaveolens* Capuron. In Nigeria, the most common species is *Z. golungensis* Hiern – a small tree species of 6–25 m in height (Maroyi, 2019). *Zanha golungensis* occurs at an altitude of 300–1700 m usually in deciduous forest, sometimes in evergreen forest or around riverine forests (Maroyi, 2019). Several chemicals have been isolated from the plant including triterpenoids zanhic acid, zanhic acid-γ-lactone, prosapogenins zanhin and medicagin from the root bark (Lavaud *et al*., 2015) as well as medicagenic acid and triterpenoids (Zanhic acid)

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(Dimbi *et al*., 2010). Also, Lavaud *et al*. (2015) reported the presence of saponins and oleanane-type triterpenes in the root bark of the plant. *Zanha golungensis* is one of the most common medicinal plants in Africa (Bruschi *et al*., 2011). It is useful in construction (timber), medicine (chew-sticks) and nutrition (fodder and food) (Bosch, 2011). Hence it is over exploited, threatened with extinction and categorized as being in need of conservation in various countries (Maroyi, 2019). Also, according to Maroyi (2019), detailed pharmacognostic and toxicological evaluations of *Z. golungensis* should be done to correlate its medicinal uses with its activities. This study seeks to assess and document the morphological, anatomical and phytochemical characters of the leaf and stem of *Z. golungensis* with a view to add to the existing literature on the species.

MATERIALS AND METHODS

Source of plant material

Young mature leaves and stem of *Z. golungensis* were collected from Forestry Research Institute of Nigeria (FRIN), Fiditi-Oyo road, Oyo state, at latitude 7.71362 and longitude 3.9172 between 9.00 and 11.00 h on 26th July 2019. Twenty different samples were collected for evaluation. Photographs were taken; the plant was authenticated at the University of Lagos Herbarium (LUH) and a voucher specimen was deposited.

Morphological studies

This was done following Adeyemi *et al*. (2013). Qualitative and quantitative characters of *Z. golungensis* were both observed and assessed using twenty different samples. The qualitative characters include leaf type, shape, surface, apex, base, margin, venation, arrangement, petiole surface, stem type, surface and colour. Descriptive terminologies followed Radford *et al*. (1974). The quantitative characters on the other hand include leaf length, width, petiole length and stem girth.

Anatomy of *Zanha golungensis*

For the anatomical studies, slides prepared include: abaxial and adaxial surfaces of the leaf, transverse section of the petiole, and transverse section of the stem following Ogundipe *et al*. (2008) and Onuminya and Adediran (2018).

Epidermal preparation: This was done following Onuminya and Adediran (2018). Median portion (2−3 cm) of the lamina near the mid-rib was used for the study. The cut portion of the dried leaves of *Z. golungensis*

were placed in boiling water for 5 min and were carefully rolled into small McCartney bottle containing concentrated nitric acid (HNO_3). This was allowed to soak for 5 h in order to macerate the mesophyll. The specimen was then removed using forceps and placed in a petri dish containing distilled water. The specimen was cleansed using soft camel brush in order to remove any form of dirt present and this was rinsed in distilled water. The adaxial and abaxial surfaces were carefully separated and a portion of it was cut and placed on a clean glass slide. The sample was dehydrated using 70% ethanol, stained with safranin O and excess stain was removed by adding drops of ethanol. Then few drops of glycerin were added on the slide and covered with 0.2 mm cover slips. The slides were labeled and viewed under the microscope to observe the leaf epidermal features. All measurements were carried out using a calibrated micrometer eyepiece with x40 objectives. Quantitative characters (number of epidermal cells, anticlinal wall, width and length of the leaf epidermis and number of stomata) were also recorded.

Petiole and stem anatomy: This was done following Ogundipe *et al*. (2008). Dried petiole and stem of *Z. golungensis* were boiled for 10 min. A transverse section of each was obtained with the aid of razor blade until very thin sections were obtained. These were soaked in sodium hypochlorite solution (bleach) for about 5 min until they were totally bleached and then transferred into petri dishes containing distilled water. The specimens were dehydrated on a slide using 70% ethanol, stained with safranin O for 2 min and excess stain was removed using drops of ethanol. They were mounted in glycerin, covered with 0.2 mm cover slips, labeled and viewed under the microscope.

The stem bark was macerated using mortar and pestle then a very thin section was obtained and soaked in sodium hypochlorite solution for about 5 min until totally bleached. It was then transferred to a petri dish containing distilled water, mounted on a slide, dehydrated using 70% ethanol, for 2 min, stained with phloroglucinol, and covered with 0.2 mm cover slip. The slide was then ringed with nail polish, labeled and viewed under a microscope.

Photomicrography

The photomicrographs of the leaves showing diagnostic characters on the adaxial and abaxial surface, section of the petiole and stem and were taken using Amscope image plus version 2.0 mm with MC camera and a PIV computer system.

Data analyses

Descriptive statistics such as mean, range and standard error were calculated for twenty plant samples. Also, stomata index was calculated for the specimen using the formula below:

$$\text{Stomata index} = \frac{S}{S+E} \times 100$$

where: S represents the number of stomata per unit area and E represents the total number of epidermal cells

Preparation of extracts

Extracts from the two plant parts were prepared following Sofidiya *et al*. (2012), with minor modifications. Fresh leaves and stem were weighed separately and oven dried at 50–60°C for 4–5 days. The dried plant parts were pulverized and weighed. Then, 120 g of each part was soaked in different specimen bottles with distilled water for 24 h and another set in ethanol for 72 h for the leaves and stem, respectively. The aqueous extract was boiled for 30 min, allowed to cool and then filtered. The filtrate was poured in a pre-weighed beaker and concentrated on a water bath to dryness. The resultant extract was transferred into sample bottles, labeled, weighed and kept inside a refrigerator. The samples, soaked in ethanol, were filtered after 72 h and concentrated using a rotary evaporator. The concentrated samples were then weighed and transferred into sample bottles, labeled and kept in the refrigerator for further screening.

Phytochemical screening

Phytochemical screening of extracts was carried out to check for the presence of secondary metabolites. The alcohol and aqueous extracts of the leaves and stem were used to test for alkaloids, saponins, flavonoids, phenolics, reducing sugar, tannins, terpenoids, phlobatannins and cardiac glycosides as described by Omoruyi *et al*. (2012), Asekun *et al*. (2013) and Onuminya *et al*. (2017).

Alkaloids

The alkaloid content was determined using Mayer's test following Omoruyi *et al*. (2012). The extract was heated with 2% hydrochloric acid in a boiling water bath. The mixture was cooled and a few drops of Mayer's reagent 10 were added. Presence of turbidity or yellow precipitation was recorded.

Saponins

Saponin content was determined using frothing test as described by Asekun *et al*. (2013). Here, 1 ml of distilled water was added to 1 ml of the extract and shaken vigorously. The presence of saponin was indicated by a stable persistent froth.

Phenolics

Phenolic content was determined using the lead test as described by Asekun *et al*. (2013). One ml each of the extract and iron (III) chloride were mixed and change in colour of the mixture to a deep bluish green solution was taken as an indication of the presence of phenols.

Flavonoids

Flavonoid content was determined using alkaline reagent test as described by Asekun *et al*. (2013) with modifications. Two (2.0) ml of diluted sodium hydroxide was added to 2 ml of the extract. The appearance of a yellow colour indicated the presence of flavonoids.

Reducing sugar

Reducing sugar content was determined using Fehling's test as described by Onuminya *et al*. (2017). Approximately 5–8 drops of Fehling's solution and 1 ml of water was added to 0.5 ml of extract solution and heated. A brick red precipitate indicated presence of reducing sugar.

Tannins

Tanin content was determined using ferric chloride test as described by Onuminya *et al*. (2017). A volume of 1ml of water and 1–2 drops of ferric chloride solution were added to 0.5 ml of extract solution. Formation of blue coloration indicated the presence of gallic tannins while greenish black coloration showed the presence of catecholic tannins.

Terpenoids

Terpenoid content was determined using Liebermann-Burchard's test as described by Onuminya *et al*. (2017). Approximately 0.5 ml of acetic anhydride was mixed with 1 ml of sample extract and a few drops of concentrated H_2SO_4. A bluish green precipitate indicates the presence of terpenes.

Phlobatannins

Phlobatannin content was determined using HCl test as described by Onuminya *et al.* (2017). A volume of 1ml of extract was boiled in few drops of 1% HCl. Formation of a red precipitation indicates the presence of phlobatannins.

Cardiac glycosides

Cardiac glycoside content was determined using Keller Kiliani's test as described by Asekun *et al*. (2013) and Onuminya *et al*. (2017). About 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 1 drop of ferric chloride solution. To this, 2 ml of concentrated sulphuric acid was added to underlie the mixture. Formation of a brown ring at the inter phase indicates the presence of de-oxy sugar.

RESULTS

Assessment of the vegetative morphological, anatomical and phytochemical features of *Z. golungensis* revealed valuable characters of taxonomic importance. These are shown in Tables 1 and 2 as well as Figures 1–3.

Morphological characters

Assessment of the vegetative morphological features of *Z. golungensis* revealed paripinnately compound leaf type, leaflets with a cuneate base, entire margin, alternate arrangement, acuminate apex, ovate shape, glabrous texture and reticulate venation. The stem surface is glabrous and colour is grey; the bark has a dark brown colour with thick and rough scale (Fig. 1). The leaflet length is 4.5–11.0 cm; leaflet width is 2.1–4.5 cm while leaflet circumference is 11.2–24.8 cm. The number of leaflet on each stem is 6–10, petiole length is 4.5–16.5 cm and stem girth ranges between 1.2 cm and 3.2 cm.

Fig. 1. *Zanha golungensis* Hiern. (A) Specimen showing the leaves and bark (B) Voucher specimen.

Anatomical characters

On the abaxial surface of the leaf, striations and wax deposits were observed. The cell shape is polygonal, stomata type is paracytic, anticlinal wall pattern is straight (Fig. 2a-b). Stomata number ranges from 94–247. Stomata size is 4–8 x 3–7 µm. Epidermal cell number ranges between 172 and 695 but cell epidermal size is 5–8 µm x 3–9 µm. Cell thickness is 0.6–1.0 µm (Table 1).

On the adaxial surface of the leaf of *Z. golungensis*, striations are present, the cell shape is polygonal, stomata are absent and the anticlinal walls are straight (Fig. 2c-d). Epidermal cell number ranges between 301 and 452, but cell size are 7–13 x 6–12 µm. Cell thickness is 0.5–1 µm. (Table 1).

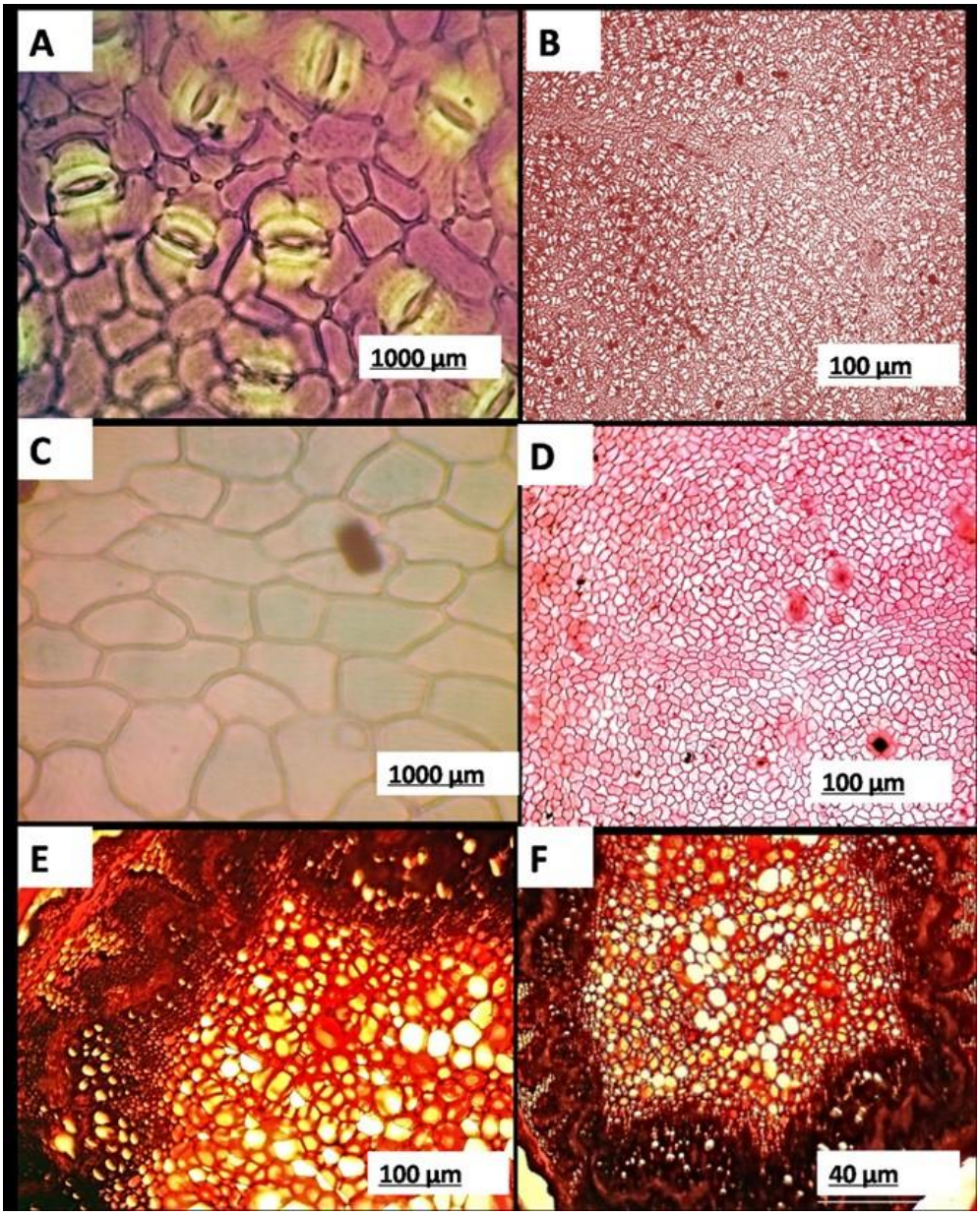

Fig. 2. Anatomical features of *Zanha golungensis.*
A-D: Foliar epidermal surfaces (A-B) abaxial surface showing stomata type and number of stomata per unit area; (C-D) adaxial surface showing epidermal cell shape and number of cells per unit area, E-F: Transverse section of the petiole showing concentric arrangement of the vascular tissues and cortical cells.

Table 1. Foliar anatomical characters of *Zanha golungensis*.

Characters	Adaxial surface	Abaxial surface
Cell shape	Polygonal	Polygonal
Anticlinal wall pattern	Straight	Straight
Stomata type	Absent	Paracytic
Striation	Present	Present
Cell number min (π±S.E) max	301 (396.4±13.1) 452	172 (474.7±51.8) 695
Cell length min (π ±S.E) max	7.0 (10.7±0.7) 13.0	5.0 (6.7±0.5) 8.0
Cell width min (π ±S.E) max	6.0 (8.7± 0.7) 12.0	3.0 (5.8±0.5) 9.0
Cell thickness min (π ±S.E) max	0.5 (0.9±0.01) 1.0	0.4 (0.8±0.1) 1.0
Stomata number min (π ±S.E) max	-	94 (149.9±13.2) 247
Stomata length min (π ±S.E) max	-	4.0 (6.2±0.4) 8.0
Stomata width min (π ±S.E) max	-	3.0 (5.6±0.4) 7.0
Stomata index (%)	-	20.3%

The anatomy of the petiole as seen in the transverse section revealed that crystals are present in the parenchyma cells and vascular bundles are concentric (Fig. 2e-f). The transverse section of stem showed paratracheal vessels, a situation whereby the axial parenchyma cells lie close to the vessels. The vessels diameter is noticeably of varied dimension. The parenchyma cells are oval to polyhedral, while the axially arranged ones are by the sides of the vessels (Fig. 3c-d). The macerated stem bark showed axial parenchyma cells with copious rays (Fig. 3a-b).

Phytochemical characters

The phytochemical screening of the aqueous extract of the leaves and stem of *Z. golungensis* revealed the following: in the leaves, bioactive compounds such as alkaloids, saponins, phenols, reducing sugar, terpenoids and cardiac glycosides are present while flavonoid, tannins and phlobatannin are absent. In the stem, alkaloids, saponins, phenols, reducing sugar and tannins while flavonoids, terpenoids, phlobatannins and cardiac glycosides are absent. The ethanol extracts on the other hand showed the presence of alkaloids, phenols, flavonoids, reducing sugars, terpenoids, tannins and cardiac glycosides in both plant parts tested. However, phlobatannin are absent in the leaves and stem (Table 2).

Description of *Z. golungensis* Hiern.

Morphology: A small tree, bark dark brown, scale thick and rough, stem grey, surface glabrous, girth 1.2−3.2 cm. Leaves compound paripinnate, alternate, petiole 4.5−16.5 cm, leaflets 3−5 pairs, opposite, glabrous, 4.5−11.0 x 2.1−4.5 cm in size, shape ovate, margin entire, apex acuminate, base

cuneate, venation reticulate.

Anatomy: Leaf hypostomatic, stomata paracytic, size 4−8 x 3−7 µm, epidermal cell shape polygonal, surface with striations and wax deposits, anticlinal wall pattern straight, thickness 0.6−1.0 µm, abaxial cell 94−247, size 5−8 µm x 3−9 µm, adaxial cells 301−452, size 7−13 x 6−12 µm. Vascular bundles concentric in both stem and petiole, vessels paratracheal, parenchyma oval to polyhedral, with copious rays, crystals present; in parenchyma cells.

Phytochemistry: Leaf and stem contain alkaloids, saponins, phenols, reducing sugars, terpenoids, and cardiac glycosides.

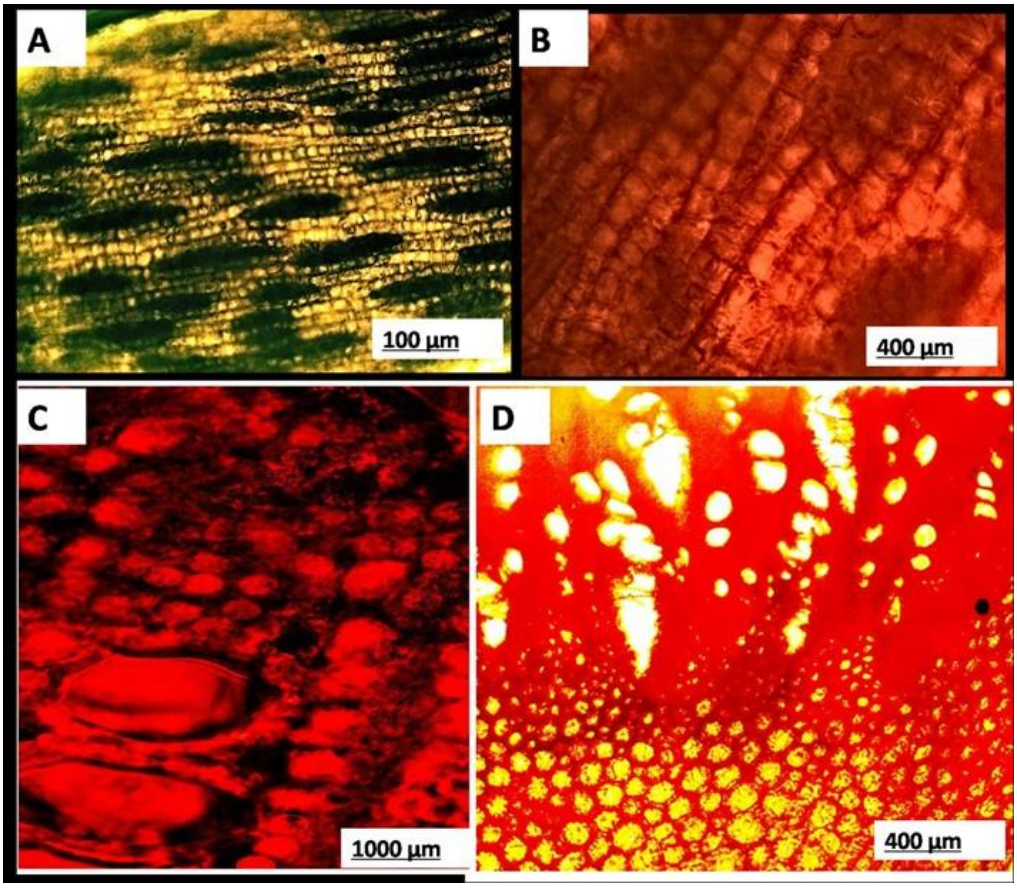

Fig. 3. Wood anatomical features of *Zanha golungensis.* A-B: macerated bark showing (A) ray cells (B) axial parenchyma cells. C-D: transverse section of the stem showing (C) paratracheal parenchyma cells (D) arrangement of vessels in the vascular bundles.

Table 2. Phytochemical composition of extracts of leaf and stem of *Zanha golungensis*.

Tests	Aqueous extract		Ethanol extract	
	Leaves	Stem	Leaves	Stem
Alkaloids	++	+	+	+
Saponins	+	+	+	+
Phenols	++	+	++	++
Flavonoids	-	-	++	+
Reducing sugars	+	+	+	+
Tannins	-	+	++	++
Terpenoids	+	-	++	+
Phlobatannins	-	-	-	-
Cardiac glycosides	+	-	++	++

\+ Represents the presence of phytochemical compound in small quantity
++ Represents the presence of phytochemical compound in large quantity
\- Represents the absence of phytochemical compound

DISCUSSION

According to Awotedu and Ogunbamowo (2019), pharmacognostic and micro-morphoplogical characters are useful tools in identification and authentication of medicinal plants. These characters were used in our assessment of *Z. golungensis* in this study and data obtained proved to be useful in the identification of the species. Morphological evaluation of the vegetative characters of the plant revealed characters which were seen to be consistent with reports given by Adeyemi *et al*. (2013), Onuminya and Ogundipe (2014) as well as Hyde *et al*. (2020a) on the description of the species. However, they are distinct from those recorded in *Z. africana* where even though the leaves are compound pinnate with 3–5 pairs of leaflets as in *Z. golungensis*, the leaflets are pubescent at maturity, sub-sessile, round to cordate at the base, elliptic-oblong in shape and obtuse at the apex (Hyde *et al*., 2020b). Several researchers have documented the use of epidermal features for the purpose of taxonomy and pharmacognosy including Kadiri *et al*. (2013), Onuminya and Adediran (2018) as well as Awotedu and Ogunbamowo (2019). Our observations on the foliar epidermal characters corroborate the findings of Onuminya and Adediran (2018). Although, previous research has been on the foliar epidermal morphology of *Z. golungensis*, this research further studied the anatomy of the petiole and stem and the characters observed were consistent with the report of Metcalfe and Chalk (1950). Our observations are also like Mojeremane (2011) on the wood anatomy of *Z. africana* where parenchyma cells, vessels and fiber cells were recorded.

Phytochemical analysis revealed the presence of alkaloids, saponins, phenols and reducing sugar in the aqueous extract of the leaves and stem of *Z. golungensis*. Additionally, flavonoids, terpenoids, tannins and cardiac

glycosides were observed in the ethanol extracts of both plant parts. These observations are similar to the reports of Dimbi *et al*. (2010) who found triterpenoids in the extracts of the leaves and findings of Sofidiya *et al*. (2012) who reported presence of flavonoids and polyphenols, as well as Lavaud *et al*. (2015), who reported the presence of saponins in the stem bark of *Z. golungensis*. Furthermore, Awotedu and Ogunbamowo (2019) reported that alkaloids are potent antimicrobial and anti-inflammatory agents, flavonoids protect against oxidative stress while saponins are effective in treating hypoglycemia. All of these were recorded in this study and hence validated the use of *Z. golungensis* in management of such diseases. The observations and summaries given on *Z. golungensis* in this study are of taxonomic significance and would be useful in identification of the species.

CONCLUSION

The diagnostic features of *Z. golungensis* that were observed in this study can be used in recognition and identification of the plant in pharmacognostic crude drug research without ambiguity. This study is a contribution to existing literature on the taxon.

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