

Assessment of the foliar micromorphology, phytochemical and mineral composition of *Justicia secunda* Valh leaves

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Resumen

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Evaluación de la micromorfología foliar, fitoquímica y composición mineral de las hojas de Justicia secunda Valh

Justicia secunda es una planta medicinal que pertenece a la familia Acanthaceae, es muy utilizada en la medicina tradicional para el tratamiento y manejo de una amplia gama de dolencias. En este estudio, la selección/análisis de fitoquímicos, minerales y características micro-morfológicas de las hojas de *J. secunda* se evaluaron utilizando procedimientos estándar. Los datos generados para el contenido fitomineral y fitoquímico de las hojas así como la prueba quimio-microscópica y los rasgos anatómicos foliares de las hojas que revelaron rasgos característicos distintos serán de utilidad en la sistemática, adecuada identificación, autenticación y estandarización de esta planta para fines medicinales propósitos.

Palabras clave: Quimio-microscopía; *Justicia secunda*; Fitominerales; Micromorfología; Fitoquímicos; Anatomía de la hoja.

Abstract

Justicia secunda is a medicinal plant that belongs to the family Acanthaceae, it is widely used in traditional medicine for the treatment and management of a wide range of ailments. In this study, the phytochemicals screening/analysis, minerals and micro-morphological characteristics of the leaves of *J. secunda* were evaluated using standard procedures. The data generated for the phytomineral and phytochemical contents of the leaves as well as the chemo-microscopic test and foliar anatomical features of the leaves which revealed distinct characteristic features will be useful in the systematics, proper identification, authentication and standardization of this plant for medicinal purposes.

Key words: Chemo-microscopy; *Justicia secunda*; Phytominerals; Micro-morphology; Phytochemicals; Leave anatomy.

Introduction

Justicia secunda Vahl belongs to the family Acanthaceae, which is one of the many species of *Justicia* L. genus commonly used in African traditional medicine. *J. secunda* originated from South America, but has fully been domesticated in tropical regions of Africa including Nigeria (Koffi *et al.* 2013; Kitadi *et al.* 2019). It is a perennial herb that grows up to 90 cm with purplish green stem, evergreen leaves and pink flowers. It is an upright shrub that grows well in humid soils and colonies around river banks and creeks. *J. secunda* has simple leaves and the flowers are contained in terminal panicles (Heywood & Moore 1978). It is commonly known as blood leaf or blood root in Barbados (Carrington *et al.* 2012). The leaf decoction is reportedly consumed in different regions of Africa including Nigeria for the management of anaemia (Mpiana *et al.* 2010a). The leaf decoction is known to produce a purplish/blood red colouration which may suggest why through the doctrine of signature the plant is used traditionally used as hematinic to boost the blood. Its aqueous decoction is normally served as a tea drink to anaemic patients in some communities. The activities of *J. secunda* plant parts such as the anti-sickling and hematinic have been demonstrated in previous studies (Mpiana *et al.* 2010a; Mpiana *et al.* 2010b; N'guessan *et al.* 2010; Kone *et al.* 2012). Various ethno-medicinal importance of this plant have been reported in studies seeking to provide scientific bases for the traditional uses of the plant. The potential of the plant in the treatment of diabetes mellitus was demonstrated through the isolation of alpha glucosidase inhibiting compounds from *J. secunda* leaves (Theiler *et al.* 2016), while its biological activity against certain gram positive bacteria were reported by Herrera-Mata *et al.* (2002). The antihypertensive activity (Manda *et al.* 2011), the hypoglycemic and anti-hyperglycemic effect (Mea *et al.* 2017) of the plant has been demonstrated in experimental animals, while the antioxidant, anti-inflammatory and antinociceptive activities have also been reported (Onoja *et al.* 2017). Most of these activities have been attributed to the bioactive substances present in the plant.

Although phytochemicals, also called secondary metabolites are naturally present in various parts of medicinal plants including *J. secunda*,

they have protective mechanism and defend plants from various stress, they are non-nutritive compounds that are needed for sustaining life; they confer flavour, colour and protection to plants and humans (Agbafor & Nwachukwu 2011). The foliar micro-morphological characters of medicinal plants offer an in-depth evidence to assist in the proper identification of a wide range of species; these features are vital taxonomic characters that help in minimising the misrepresentation and misidentification of medicinal plants. Hence, phytochemical analysis and micro-morphological evaluation are some of the ways medicinal plants utilized in traditional medicine are typically standardized and authenticated (Chanda 2014). In spite of the diverse range of chemical compounds reported in the leaves of some *Justicia* genus, little data is available on the basic chemical composition and foliar micro-morphology of the *J. secunda*. This information, together with studies on the epidermal features of the leaves is of significance in the proper identification and standardization during the evaluation of medicinal plants. The aim of this study was to evaluate the phytochemical profile, mineral composition, chemo-microscopic and micro-morphological features of the leaves of *J. secunda*.

Materials and methods

Collection and preparation of plant materials

Fresh leaf samples were harvested at the herbal garden of Forestry Research Institute of Nigeria (FRIN) (7°23'48.287.5N, 3°51'48.96.0E). The leaves were identified and authenticated by a taxonomist at the Forest Herbarium Ibadan. The leaf samples for phytochemical determination, mineral estimation and chemo-microscopic examination were dried at room temperature for two weeks after which it was milled to powdered using a milling machine. The powdered sample was kept in an air tight container for further use.

Phytochemical evaluation

Qualitative screening and quantitative phytochemical analysis were done to identify and quantify the chemical components present in the leaves of *J. secunda*. The chemical components determined include: alkaloids, saponins, flavonoids, tannins, phenolic compounds, terpenoids, and cardiac glycosides. The phytochemical screening was evalu-

ated using the standard procedures as described by Boye *et al.* (2012) and Omoruyi *et al.* (2012), while the quantitative analysis was done using the procedures described by Mbaebie *et al.* (2012).

In the qualitative test, frothing test was done to test the presence of saponins, the presence of tannins was ascertained using the ferric chloride test, flavonoids presence was tested using ethyl acetate and dilute ammonia, terpenoids were tested using chloroform and acetic anhydride test, alkaloids were tested using Dragendorff's, Mayer's and Wagner's reagent, while cardiac glycoside was tested using the Keller-Killani test. In the quantitative estimation, alkaloids were determined by gravimetry after extraction with 10 % acetic acid in ethanol for 4 hours followed by precipitation using concentrated ammonium hydroxide, flavonoids were determined spectrophotometrically with aluminium chloride, saponins were determined by gravimetry after repeated extraction with 20 % ethanol, followed by diethyl ether, and then butan-1-ol, tannins were determined spectrophotometrically using Folin-ciocalteu reagent with tannic acid standard, phenolic compounds were also determined similarly as tannin but using gallic acid standard while terpenoids was determined by gravimetry after extraction with ethanol followed by petroleum ether.

Mineral evaluation

Elemental analysis was done according to the method described by Isaac & Korber, (1971). Powdered sample (0.5g) was digested with a diacid mixture of HNO₃:HClO₄ (2:1) after which the digest, adjusted to 50 mL mark was read on Atomic Absorption Spectrophotometer for all the mineral elements except sodium and potassium which were determined using a flame photometer.

Chemo-microscopic evaluation

Powdered samples of *J. secunda* leaves were cleared by soaking in hypochlorite solution, then pieces of the cleared samples was mounted on a new microscopic slide for each test, then detecting reagents for the detection of lipids (3 drops of sudan IV reagent was added), calcium oxalate (2 drops of dilute HCl), lignin (2 drops of phloroglucinol followed by the addition of concentrated HCl), mucilage (1 drop of ruthenium red was added to the sample on a microscope slide) and starch (2 drops of iodine was added to the sample

on the slide) were added (Evans 2005).

Leaf micro-morphology

The micro-morphological features of the *J. secunda* leaves were evaluated by viewing the epidermal and transverse sections under a light microscope; the former was obtained by careful peeling of the abaxial and adaxial surfaces and the latter was obtained from the central vein using a microtome from matured fresh leaves of *J. secunda*. The epidermal sections obtained were cleared in sodium hypochlorite, washed in ethanol and then stained with safranin after which it was mounted in glycerine on a microscopic slide. The anatomical structures were viewed, analysed and photomicrographed using a light microscope (Amscope) (Verdam *et al.* 2012; Ohikhen *et al.* 2017).

Results

Phytochemical Analysis

The results of the phytochemical screening of the leaves of *J. secunda* confirmed the presence of a variety of secondary metabolites such as saponins, tannins, flavonoids, terpenoids, alkaloids and cardiac glycosides (Table 1).

The results of phytochemical composition confirmed that *J. secunda* leaves contain phytonutrients in varying amounts where tannin had the highest concentration followed by alkaloids and flavonoids was the lowest constituent (Table 2).

Phytochemical Test	Observation	Leaves
Saponins (Frothing test)	Persistence foaming	+
Tannins (Ferric chloride)	Greenish-brown precipitate	+
Flavonoids (Ethyl-acetate test)	Intense yellow colouration	+
Terpenoids (Chloroform and acetic anhydride test)	A grey colouration	+
Alkaloid •Dragendorff's reagent	Red precipitate	+
•Mayer's reagent	Creamy-white precipitate	+
•Wagners reagent	Reddish-brown precipitate	+
Cardiac Glycosides (Sulphuric acid test)	Brick-red precipitate	+

+ Present

Table 1. Examen fitoquímico cualitativo de las hojas de *J. secunda*

Table 1. Qualitative phytochemical screening of the leaves of *J. secunda*.

Phytochemical	Levels (mg/100g)
Alkaloids	2040±210
Flavonoids	130±80
Saponins	760±162
Tannins	2410±150
Phenols	1652±10
Terpenoids	980±112

*values are presented as Mean ± Standard deviation, n = 3

Tabla 2. Análisis fitoquímico cuantitativo de hojas de *J. secunda*.

Table 2. Quantitative phytochemical analysis *J. secunda* leaves.

Mineral composition

Results obtained for mineral analysis of the leaves of *J. secunda* revealed that macro-element ranged from 0.025 % (phosphorus) to 2.48 % (calcium) while the micro-elements ranged from 0.003 % (copper) to 0.12 % (iron) (Table 3).

Minerals	Concentration (%)
Macro Elements	
Sodium (Na)	0.037±0.03
Potassium (K)	0.29±0.12
Calcium (Ca)	2.48±0.07
Magnesium (Mg)	0.41±0.02
Phosphorus (P)	0.025±0.023
Micro Elements	
Iron (Fe)	0.12±0.02
Manganese (Mn)	0.005±0.00
Copper (Cu)	0.003±0.00
Zinc (Zn)	0.03±0.01

*values are presented as Mean ± standard deviation, n = 3

Tabla 3. Composición mineral e hojas de *J. secunda*.

Table 3. Mineral composition of *J. secunda* leaves

Chemo-microscopic properties

The results of the chemo-microscopic analysis of the powdered leaf samples of *J. secunda* showed that calcium carbonate and starch were the only chemo compounds present in the powdered samples while other properties like lipids, calcium oxalate, lignin and mucilage were absent (Table 4).

Property	Observation	Inference
Lipids	No pink colouration seen	Absent
Calcium carbonate	Effervescent was observed	Present
Calcium oxalate	No effervescent observed	Absent
Lignin	No visible colour change	Absent
Mucilage	No pink colouration	Absent
Starch	Blue-black colouration	Present

Tabla 4. Propiedades químico-microscópicas de hojas pulverizadas de *J. secunda*.

Table 4. Chemo-microscopic properties of *J. secunda* leaf powder.

Leaf micro-morphology

Results of the microscopic examination of the epidermal sections of *J. secunda* leaves as summarized in the table 5 shows that the shape of the epidermal cells are irregular, with anticlinal walls that are sinuate in both the abaxial and adaxial epidermis, also possessing non-glandular trichomes which are bristle-like and hairy that are also densely distributed in both upper and lower epidermis (Figs. 1A-D), the mean cell length was determined to be 26.95 and 25.21 μm , cell width (20.11 and 16.91 μm) and cell density (108.10 and 210.60 μm) for the abaxial and adaxial epidermis respectively. Meanwhile, the epidermis was also characterized by anomocytic stomata, with the stomata length, width, index and density were found to be 19.67 μm , 13.61 μm , 47.53 % and 98.65 for the abaxial epidermal surface respectively. The transverse section of the leaves is characterized by presence of the lower and upper epidermis, as well as the parenchyma cells, collenchyma cells, vascular bundles among the other common features (Fig. 2).

Epidermal Characteristics	Abaxial	Adaxial
Cell shape	Irregular	Irregular
Anticlinal walls	Sinuate	Sinuate
Cuticle	Present	Nil
Cell length (μm)	26.95 ±3.57	25.21 ±4.67
Cell width (μm)	20.11 ±2.40	16.91 ±3.04
Cell density	108.10 ±4.38	210.60 ±6.58
Stomata type	Anomocytic	Anomocytic
Stomata length (μm)	19.67±2.47	-
Stomata width (μm)	13.61 ±1.71	-
Stomata index (%)	47.53 ±1.43	17.33 ±2.09
Stomata density	98.65 ±4.04	34.24±2.02
Trichomes	Non-glandular hairs	Non-glandular hairs

*values are presented as Mean ± standard deviation, n = 20

Tabla 5. Características epidérmicas de las hojas de *J. secunda*.

Table 5. Epidermal features of *J. secunda* leaves.

Discussion

Results from this study revealed the presence of all the phytochemicals tested, this observation was similarly reported in earlier studies on various extracts of *J. secunda* leaves from different locations (Mora & Pesantes 2017, Osioma & Hamilton-Amachree 2017; Nnadozie *et al.* 2019) likewise, in other closely related species like *Justicia carnea* Lindl. (Onyeabo *et al.* 2017). The quantitative phytochemical composition of *J. secunda* leaves showed different levels of the measured

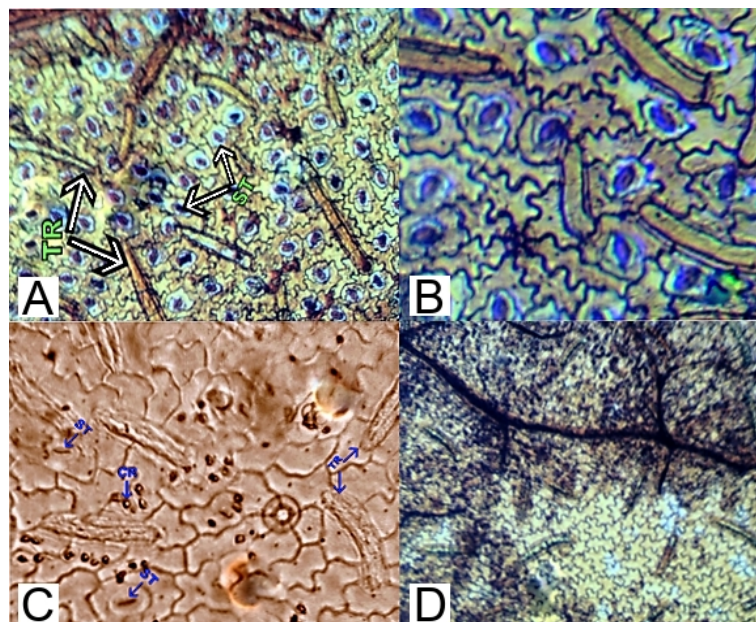


Figura 1. Fotomicrografías de secciones epidérmicas de hojas de *J. secunda*. **A:** superficie abaxial (x5); **B:** Superficie abaxial (x10); **C:** superficie adaxial (x10); **C:** superficie adaxial (x5). ST: estoma, TR: tricoma, CR: cristales.

Figure 1. Photomicrographs of epidermal sections of *J. secunda* leaves. **A:** abaxial surface (x5); **B:** abaxial surface (x10); **C:** adaxial surface (x10); **D:** adaxial surface (x5). ST: stomata, TR: trichome, CR: crystals.

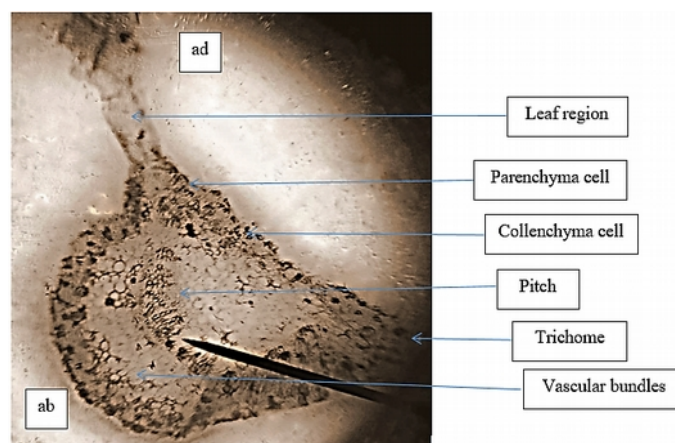


Figura 2. Fotomicrografía de la sección transversal hoja de *J. secunda*, mostrando la zona de la hoja, la pared celular, los haces vasculares, las células del colénquima y parénquima y la resina (x10).

Figure 2: Photomicrograph of transverse section of *J. secunda* leaf showing the leaf region, cell wall, vascular bundle, collenchyma and parenchyma cells and pitch (x10).

substances which are quite within the range similarly reported for *J. secunda* leaves (Nnadozie *et al.* 2019), this in essence shows the richness of the plant in important secondary metabolites that are responsible for the traditional medicinal effects. Phytochemicals, also referred to as secondary metabolites that are naturally present in plants are known to be responsible for the medicinal effect they produce when utilized as herbal drugs. Many studies have suggested that phytochemicals possess pharmacological activities that can reduce or prevent the risk of diverse human diseases (Zhang

et al. 2015, Shuruq *et al.* 2017, Forni *et al.* 2019). They help in the development of many therapeutic drugs that are utilized in the treatment and management of various diseases. Mostly, these plant chemicals are developed innately by plants to protect themselves against external hazards build-up in different parts of the plant parts, including leaves, roots, stem, bark, fruits and seeds (Costa *et al.* 1999, Zulak *et al.* 2006). Thus, *J. secunda* could serve as a rich source of phytochemicals such as alkaloids and tannins which are present in higher quantity in the plant. Alkaloids for in-

stance, have been reported to have a wide range of pharmacological effects including microbiocidal effect, analgesic, sedatives, antimalarial activity, antioxidant and anti-inflammatory activities (Alves de Almeida *et al.* 2017, Bribi 2018). Furthermore, the presence of tannins in medicinal plant suggests the ability of these plants to play major therapeutic roles some of which are constantly being identified (Yildirim & Kutlu 2015, Vu *et al.* 2017). Overall, all these phytochemicals have been reported for various pharmacological and therapeutic actions in various models. The biological activities of saponins (Yildirim & Kutlu 2015, Desai *et al.* 2009), flavonoids (Kumar & Pandey 2013, Agrawal 2011), cardiac glycosides (Morsy 2017, Schneider *et al.* 2018), and terpenoids are widely documented. The presence of these phytochemicals in *J. secunda* therefore, justifies its popularity as a very potent plant that could be used in the treatment and prevention of various diseases.

In addition, another important class of plant chemicals are minerals which play vital roles in nutrition and health (Soetan *et al.* 2010) and by virtue of the fact that most living organisms cannot synthesized them naturally, they need to be sourced for externally, most importantly through plants. However, it is also commonly known that minerals sourced from different plants also varies widely from various places, as a result, investigations on the mineral composition of different medicinal plants continue to provide much needed information on the mineral status of such plants and could also explain some of the pharmacological responses attributed to it. This study provides information on some mineral content in *J. secunda* leaves. The results of the mineral composition of *J. secunda* leaves revealed that among the macro elements determined, the leaves contain high concentration of calcium (2.48 %), which suggests that it could be a good source of calcium. Calcium is one of the essential minerals for human health. It plays active roles in the biological functions of many tissues, while also serving as cofactors in several enzyme reactions and the maintenance of homeostasis (Martínez-Ballesta *et al.* 2010). Higher concentration of calcium was previously reported in aqueous extracts of *J. carnea* leaves (Orjiakor *et al.* 2019) which may suggest an inherent property of the *Justicia* genus. The concentration of calcium in the leaves of *J. secunda* as determined in this study was found to

be significantly higher than the levels reported for other similarly useful medicinal plants such as *Parquetina nigrescens* (Afzel.) Bullock (Kayode & Yakubu 2017; Odukoya *et al.* 2018), *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffrey, *Solanum americanum* Mill. (as *Solanum nigrum* L.) (Odukoya *et al.* 2018) and *Crescentia cujete* L. (Olaniyi *et al.* 2018). However, higher calcium concentration was reported in *Azadirachta indica* A.Juss., *Parkia biglobosa* (Jacq.) G. Don and *Leea guineensis* G. Don (Awotedu *et al.* 2019) compared with the levels determined in this study. Moreover, phosphorus was found to have the lowest value (0.025 %) for macro elements. This concentration is comparable with that reported for *P. nigrescens* (Kayode & Yakubu 2017) a medicinal plant also used to improve blood parameters. Phosphorus is reported to be present in edible vegetables in the region of 0.016-0.437 % and like calcium, phosphorus is interconnected in the maintenance of acid-base balance and the formation of strong bones and teeth (Martínez-Ballesta *et al.* 2010). Phosphorus is also vital component of energy rich substances such as adenosine triphosphate (ATP), nucleic acids and many coenzymes (Soetan *et al.* 2010; Martínez-Ballesta *et al.* 2010). Other macro mineral contents of the leaves of *J. secunda* are consistent with the diverse range generally observed in different plants; for instance, the ratio of sodium to potassium intake has continuously been linked to either higher or lower risk of hypertension and blood pressure, with lower ratio favouring lower risk of blood pressure and hypertension (Perez & Chang 2014; Iwahori *et al.* 2017; Park *et al.* 2016). The ratio of Na:K in *J. secunda* (calculated to be 0.13) is therefore, in conformity with this requirement which suggest that the plant could supply both mineral in the required ratio to minimize the risk of high blood pressure and hypertension. The micro mineral trend in the leaves of *J. secunda* also revealed that, iron had the highest concentrations, a much lower iron and zinc concentration was reported in the aqueous leaf extracts of *Justicia carnea* while copper concentration was found to be comparable in same plants (Orjiakor *et al.* 2019).

In spite of the beneficial effect of herbal plants in traditional medicine, one major drawback is the misidentification and mistaken substitution of the medicinal plant with closely related species which may lead to the problem of adulteration of the

herbal product (Sharaibi & Afolayan 2017). Apart from the key roles of morphological characters' assessment in plant taxonomy, studies on the foliar micromorphology of medicinal plants are also important in establishing standards for identifying adulterated medicinal plants. Consequently, the evaluation of the leaf powdered sample's micromorphology provide a cost effective means in the identification, safety assessment and authentication of original medicinal plant from crude drug materials (Shekhawat & Manokari 2017; Lawal *et al.* 2018). Results from the microscopic evaluation of the powdered sample of *J. secunda* indicated the presence of starch granules which was further confirmed in the chemo-microscopic test while calcium carbonate was also detected during the chemical test. The epidermal features as obtained from the light microscope photomicrographs shows that the leaf of the plant is amphistomatic with stomata type identified as anomocytic, which is more concentrated in the abaxial surface. This implies efficient exchange of gases at both surfaces of the leaves of *J. secunda* which is in consonance with what was earlier reported for *J. carnea* which has been observed to be amphistomatic (Nduche & Offor 2019). This could suggest that both species are closely related.

The shape of the epidermal cells is notably irregular, polygonal, undulating, and the anticlinal walls patterns are sinuate in both upper and lower epidermis as observed by visual comparison with similar sinuate anticlinal walls observed in the study by Cheng (2006). Cuticles which are characterized by cuticular intrusions either identified as 'T' bits or rod/bar located between the ends of each guard cell (Cheng 2006) are present. Trichomes are tiny outgrowths that can be found in plant epidermis. Some trichome types such as the glandular trichomes are known to be specialized secretory structures that perform different functions in different plant species (Robles-Zepeda *et al.* 2009). Non-glandular trichomes are densely distributed in both the upper and lower epidermis of the leaves of *J. secunda*, these trichomes are unicellular possessing fine-curved tips and broad base, characteristics that were also observed and reported in *Cardiospermum halicacabum* L., a medicinal climber (Shekhawat & Manokari 2017), likewise, study by Cassola *et al.* (2019) revealed the presence of both glandular and non-glandular type of trichomes in three species of *Justicia* (*Justicia brandegeana* Wssh. & L.B.Sm., *Justi-*

cia gendarussa Burm.f. and *Justicia pectoralis* Jacq.) from Brazil, where the presence of phytochemicals like flavonoid was observed in the glandular trichome. The high density of this trichome type in both surfaces may be useful in reducing the transpiration rate in the plant. So, the densely packed non-glandular trichomes noted in this study may offer taxonomic information which could be peculiar to this *Justicia* species thus, highlighting its importance as a diagnostic tool in taxonomy (Adeniji and Ariwaodo, 2012; Sonibare *et al.* 2014). The transverse section of the leaves is characterized by different cells arranged in typical manners serving diverse functions such as the parenchyma cells, collenchyma cells, vascular bundles among the other common features. Overall, these features are useful in establishing standards for ensuring the quality and safety of medicinal plants.

It could be deduced from this study that the medicinal attributes of *J. secunda* as evident from ethnobotanical and folkloric uses may be attributed to the presence of the secondary metabolites. Accordingly, *J. secunda* is a rich source of phytochemicals and minerals which in no small measure might help in the treatment, prevention and management of diverse medical complications. The foliar anatomical features of the leaves also revealed some micromorphological characters identified as: amphistomatic leaves, anomocytic stomata, irregularly shaped epidermal cells, sinuate patterned anticlinal walls and dense concentration of non-glandular trichomes in both upper and lower epidermal surfaces; chemo-microscopic test reveals the presence of starch and calcium carbonate. These features could be useful in the systematics, proper identification, standardization and ultimately contribute to the development of African pharmacopoeia.

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