Egypt. J. Bot. **Vol. 64**, No. 2, pp. 567 - 579 (2024)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Foliar Micromorphology and Histo-phytochemistry of Cucumber Trees (*Kigelia africana* (Lam.) Benth)

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KIGELIA AFRICANA belongs to the family Bignoniaceae, and this species has been exploited for many years for its medicinal properties caused by the secondary metabolites found in its fruit. Thus, this study aimed to determine the foliar micromorphology and histo-phytochemistry of *K. africana* leaf extracts using microscopy, histochemistry, and phytochemical screening. Microscopy revealed glandular peltate trichomes on the adaxial and abaxial surfaces of young and mature leaflets. Histochemical analysis showed that the peltate trichomes secreted lipids and alkaloids, and histo-phytochemical analysis indicated that the leaf extracts contained various secondary metabolites, including alkaloids, saponins, tannins, and phenols. The presence of glandular trichomes is a characteristic feature of species belonging to the family Bignoniaceae, and the phytochemicals present in the leaf extracts were similar to those found in fruit extracts. This study revealed the presence of peltate trichomes on the surfaces of young and mature leaflets, and histo-phytochemistry showed that the leaves contained various classes of phytochemical compounds. However, further work must be conducted to investigate the fine ultrastructure of the leaves and peltate trichomes as well as the biological activity of various phytochemicals.

Keywords: Diacytic stomata, Micromorphology, Peltate trichome, Secretory phase.

Introduction

The use of plants as sources of medicinal agents dates back thousands of years (Owolabi et al., 2007; Saini et al., 2009; Shama & Marwa, 2013). Traditional herbal medicinal practitioners have described the use of plants for the treatment of various diseases (Bhandary et al., 2012), and much of this knowledge has been accumulated over many decades by treating human diseases with herbal medicinal products and extracts (Azu et al., 2010a). Plant medicinal properties originate from secondary metabolites that exhibit biological activity, such as alkaloids, phenolic compounds, and tannins (Abdulkadir et al., 2015). Many secondary metabolites extracted from medicinal plants are used for their antimicrobial, antipathogenic, or antivirulence properties (Chenia, 2013; Abdulkadir et al., 2015; Eisawi et al., 2022; Singh et al., 2022; Alphonse et al., 2023). The use and trade of traditional medicines in South Africa is a huge industry, with approximately 60%–80% of South Africans depending on traditional medicine for the treatment of human and animal diseases (Mander et al., 2007; dos Santos et al., 2014). Several members of the family Bignoniaceae have high medicinal value because they contain a variety of secondary metabolites that exhibit biological activity (Castillo & Rossini, 2010; Babu et al., 2015; Mahmoud et al., 2016). Of these, *Kigelia africana* (Lam.) Benth. [synonym: *K. pinnata* (Jacq.) D.C.] has received particular interest because of the high biological

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Received 21/10/2023; Accepted 01/01/2024
DOI: 10.21608/ejbo.2024.243874.2540
Edited by: Prof. Dr. Fawzy M. Salama, Faculty of Science, Assuit University, Assuit, Egypt.

activity in its leaves (Mobark et al., 2016).

K. africana is the sole species in the genus (Saini et al., 2009; Shama & Marwa, 2013) and is commonly known as the "sausage tree" because of the large fruit it produces. It is a tropical species that is widely distributed in South, Central, and West Africa (Kothiyal & Gupta, 2011). It is exploited for its medicinal properties and is used by many traditional healers to treat various ailments, such as fungal infections, snake bites, boils, leprosy, and cancer (Owolabi et al., 2007; Kothiyal & Gupta, 2011; Oyelami et al., 2012). *K. africana* has also been used for its cosmeceutical properties by many women, who use the fruit on their breasts for skin tightening, firming, and enlargement. The fruit is also used as an ointment for women's faces to provide blemish-free skin (Saini et al., 2009; Oyelami et al., 2012). The medicinal and cosmeceutical properties of *K. africana* are due to the secondary metabolites in the fruit, which include flavonoids, iridoids, sterols, and naphthoquinones that have antimicrobial, antifungal, antibacterial, anticarcinogenic, and antioxidant effects (Saini et al., 2009; Kothiyal & Gupta 2011; Chenia, 2013).

Specialized secretory cells produce various valuable organic chemical compounds (Fahn, 2000). Trichomes are small structures or protuberances that range in size from a few microns to centimeters and originate from specialized epidermal cells in many plant species (Marin et al., 2008; Schilmiller et al., 2008; Tissier, 2012). They can be found on the surfaces of aerial parts in almost all plants, including the leaves, stems, fruits, and sepals (Marin et al., 2008; Adebooye et al., 2012). Trichomes have many functions, including mechanical abrasion reduction, water loss reduction by increasing light reflection, temperature regulation, and protection against ultraviolet (UV)-B radiation, pathogens, insects, and herbivores (Wagner 1991; Valkama et al., 2004; Wagner et al., 2004; Marin et al., 2008). The presence of trichomes in the family Bignoniaceae has been well documented, and 21 genera in this family have trichomes. However, a thorough investigation of this family has yet to be conducted (Nogueira et al., 2013; Fróes et al., 2015).

Although considerable work has been conducted on *K. africana* fruit extracts for their medicinal and cosmeceutical properties and uses (Owolabi et al., 2007; Azu et al., 2010b; Kothiyal & Gupta 2011), to the best of our knowledge, few studies have been conducted on the leaf micromorphology and histo-phytochemistry of species. Therefore, this study was conducted to describe the foliar micromorphology of *K. africana* at two developmental stages and to determine its leaf histo-phytochemistry. Electron microscopy was used to investigate the adaxial and abaxial surfaces of young and mature leaflets. Histochemical tests were performed on fresh, hand-cut leaflet sections to identify chemical compounds. Furthermore, preliminary phytochemical screening was conducted to determine the phytochemicals present in the leaves, and statistical analyses were performed to compare the number of trichomes on the adaxial and abaxial leaf surfaces in young and mature leaflets.

Materials and Methods

Plant collection and sampling

Leaves from *K. africana* trees were collected at the University of KwaZulu-Natal, Westville Campus (29°49′04.4″S, 30°56′34.0″E). Leaflets from compound leaves, which were classified as young (5 to 7cm) or mature (>10cm), were sampled and used for microscopic analysis. Leaves were also collected and left to dry for phytochemical extraction, and a fresh material was used for histochemical analysis.

Stereomicroscopy

The abaxial and adaxial surfaces of whole leaflets at each developmental stage were observed under a stereomicroscope (Nikon AZ100) equipped with a digital camera (Nikon DXM 1200C). Then, images were taken at different magnifications using NIS-Elements (NIS-Elements D 3.00) for a general overview of the leaflet surfaces.

Scanning electron microscopy (SEM)

The leaflet material from both developmental stages (young and mature) was cut and placed in a buffered fixative containing 2.5% glutaraldehyde for 24h at 4°C. Then, the fixative was removed, and the leaflet segments were washed three times for 5 min with phosphate buffer (0.1M at pH 7.2). Thereafter, the leaflet material was postfixed by placing the samples in 0.5% osmium tetroxide at room temperature for 1h. Leaflet segments were

then dehydrated in a graded series of alcohol: 25%, 50%, 75% (each twice for 5min), and 100% ethanol (twice for 10min). The dehydrated leaflet segments were critical-point dried using a critical-point dryer mounted on brass stubs secured with a carbon conductive tape and sputter coated with gold using a Polaron SC500 sputter coater. The prepared samples were observed under a Leo 1450 scanning electron microscope at a working distance of 15mm.

Histochemistry

For histochemical analysis, fresh, hand-cut sections of leaflets were stained and observed using a light microscope (Nikon ECLIPSE 80i) equipped with a Nikon DS-Fi1 camera. The following histochemical tests were performed: Wagner's and Dittmar's reagents to detect the presence of alkaloids (Furr & Mahlberg, 1981), NADI reagent to detect essential oils (David & Carde, 1964), ferric trichloride and sodium carbonate to detect phenolic compounds (Johansen, 1940), and Sudan III/IV (saturated alcoholic solution) and Sudan Black (Pearse, 1968) to detect lipids. Images were then captured at different magnifications using NIS-Elements.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed on the number of peltate trichomes in the adaxial and abaxial surfaces of young and mature leaflets using IBM SPSS Statistics 23. This study was conducted to determine a significant difference in the number of peltate trichomes per unit area on the adaxial and abaxial surfaces between the young and mature leaflets. A P value of 0.05 was considered significant. The assumptions of ANOVA had to be fulfilled to validate the results. As the data were not normally distributed, they were transformed (ranked), and ANOVA was performed again. Then, the assumptions were tested again.

Results

Trichomes were present on the abaxial and adaxial surfaces of young and mature leaflets (Figs. 1, 3), but they were more abundant on the abaxial surfaces in young and mature leaflets (Figs. 1, 3). The *K. africana* leaves possessed one type of glandular trichome, namely, peltate (Fig. 2). Peltate trichomes exhibited a scattered distribution along the lamina of leaflets, with rare occurrences of trichomes on the midveins (Fig. 1). A significant difference in the number of peltate trichomes was observed between young and mature leaflets (P= 0.0005). However, no significant difference in peltate trichome density was found between the young and mature leaflets (P= 0.372), and no significant interaction was found between the developmental stage and leaflet surface (P= 0.606). The peltate trichomes were in shallow depressions of the epidermis, with short stalk cells appearing to be embedded in the epidermal layer (Fig. 2). The trichomes had large, broad heads, inside of which were subcuticular cavities (Fig. 2), where secretory products accumulated cavitated hairs. Stereomicroscopy and SEM revealed that the surfaces of young and mature leaflets had anticlinal cell walls, and

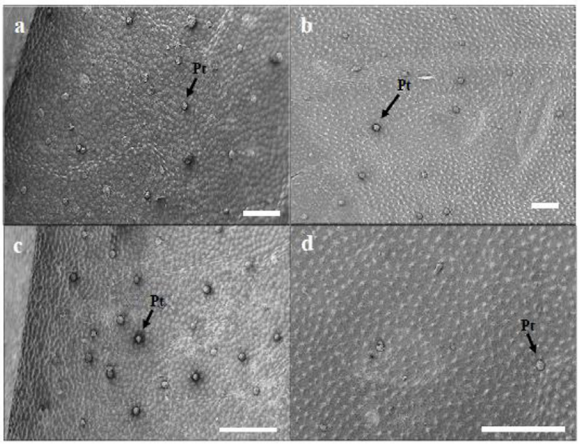

Fig. 1. Scanning electron micrographs showing peltate trichome distribution on the adaxial and abaxial surfaces of young and mature *Kigelia africana* leaflets [a) Distribution of peltate trichomes on the abaxial surface of a young leaflet. b) Adaxial surface of a young leaflet showing the distribution of peltate trichomes. c) Abaxial surface of a mature leaflet showing the distribution of peltate trichomes. d) Distribution of peltate trichomes on the adaxial surface of a mature leaflet. Scale bars: 200 μm in a, c, and d and 100 μm in b. Pt, peltate trichome]

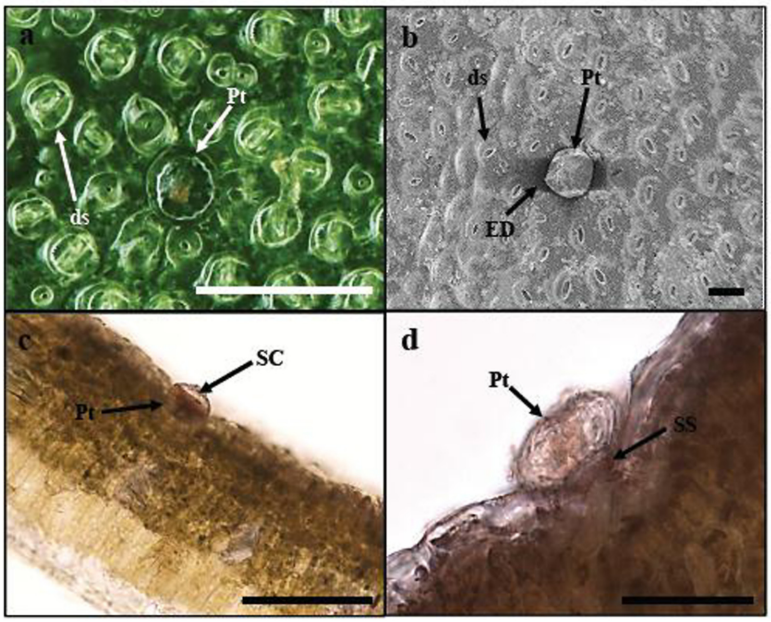

Fig. 2. Morphology of peltate trichomes on the leaves of *Kigelia africana* [a) Stereomicrograph of the abaxial surface of a young leaflet showing a single peltate trichome with secretory products surrounded by raised diacytic stomata. b) Scanning electron micrograph of a single peltate trichome embedded in the epidermal layer forming an epidermal depression on the leaflet surface. c) Light micrograph of a longitudinal section through a leaflet showing a single peltate trichome containing secretory products in a subcuticular cavity embedded in the epidermal layer. d) Light micrograph of a longitudinal section of leaflet showing the short stalk of a single peltate trichome embedded in the epidermal layer. Scale bars: 0.1 mm in a, 20μm in b, 100μm in c, and 50μm in d. Pt, peltate trichome; ds, diacytic stomata; ED, epidermal depression; SC, subcuticular cavity; SS, short stalk]

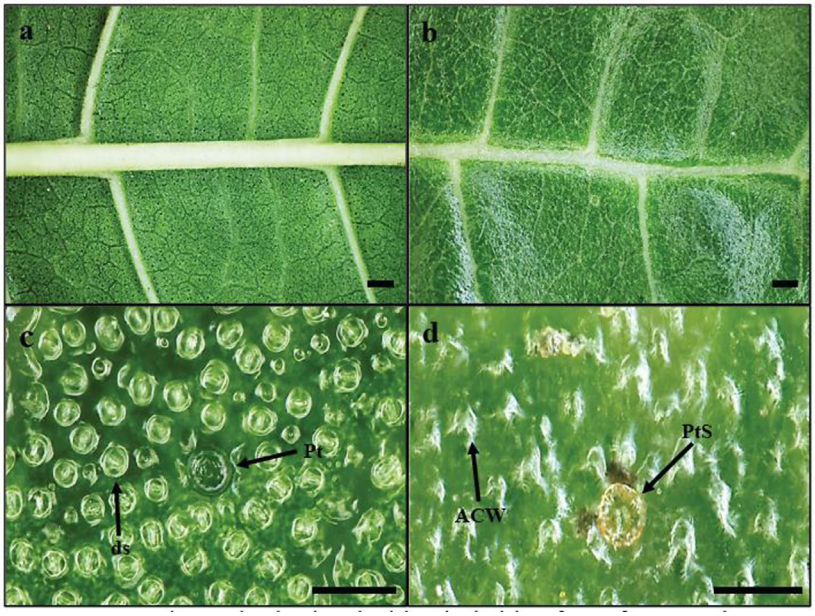

Fig. 3. Stereomicrographs showing abaxial and adaxial surfaces of young and mature leaflets of *Kigelia africana* [a) Abaxial surface of a young leaflet with stomata visible. b) Adaxial surface of a young leaflet appearing shiny due to a waxy cuticle. c) Single peltate trichome surrounded by raised diacytic stomata on the abaxial surface of a young leaflet. d) Adaxial surface of a mature leaflet showing anticlinal cell walls and a single peltate trichome with secretory products. Scale bars: 1mm in a and b and 0.1mm in c and d. *Pt*, peltate trichome; *ds*, diacytic stomata; *PtS*, peltate trichome with secretory products; *ACW*, anticlinal cell walls]

the abaxial surfaces had raised diacytic stomata, which were densely distributed on the surface.

Under a stereomicroscope, the peltate trichomes were only observed at high magnifications. At low magnifications, the surface of the leaflets appeared glabrous, with only stomata visible on the abaxial surfaces of young and mature leaflets. The adaxial surfaces of young and mature leaflets appeared shiny because of a waxy cuticle. However, at high magnifications, peltate trichomes were visible on both surfaces of young and mature leaflets. They were spherical in shape and appeared transparent on the surface of the leaflets. The diacytic stomata also appeared transparent at high magnifications (Figs. 2a and 3c). Several peltate trichomes contained secretory products, as highlighted in yellow on the heads of the trichomes (Figs. 2a, 3d).

Under a scanning electron microscope, only the heads of the peltate trichomes were visible on the surfaces of young and mature leaflets. The trichomes were in depressions of the epidermal layer, as indicated by the shadow surrounding the trichomes. The presecretory, secretory, and postsecretory phases of the peltate trichomes were visible on leaflet surfaces. During the presecretory phase, the heads of the peltate trichomes appeared flattened and "cushioned" between the epidermal depressions. During the secretory phase, the heads of the peltate trichomes appeared round and smooth, and they have a pore in the center. An intermediate phase between the secretory and postsecretory phases was also observed. Ruptured trichomes were found on leaflet surfaces during the secretory phase, and during the postsecretory phase, the heads of the peltate trichomes appeared shriveled (Fig. 4). Secretory products appeared to be released from the center of the heads of the cavitated trichomes, as indicated by what appeared to be a pore and exudates (Figs. 4b and c). Abaxial surfaces had greater abundances of glandular trichomes than adaxial surfaces in both developmental stages. Diacytic stomata were densely distributed on the abaxial surfaces of

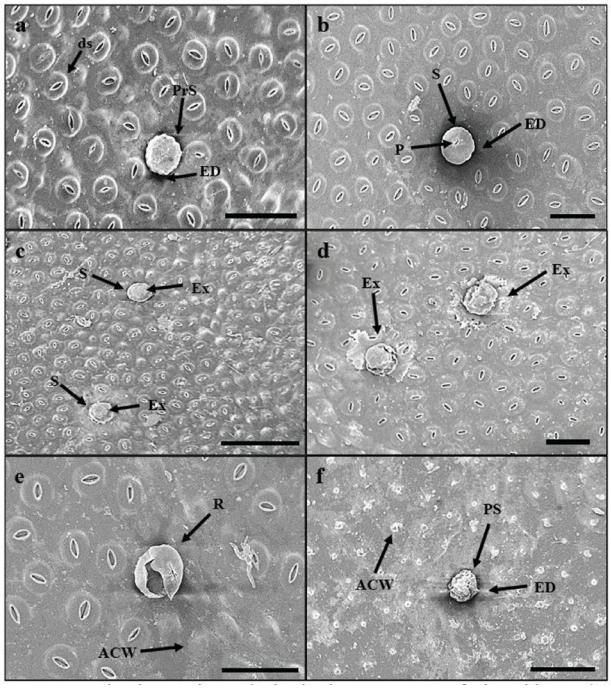

Fig. 4. Scanning electron micrographs showing the secretory stages of peltate trichomes [a) Pre-secretory peltate trichome located in an epidermal depression surrounded by diacytic stomata. b) Secretory peltate trichome with what appears to be a pore at the center of its head, located in an epidermal depression surrounded by diacytic stomata. c) Secretory peltate trichomes showing exudates that appear to be released from the center of their heads. d) Intermediate phase between the secretory and post-secretory phases showing exudates surrounding peltate trichomes. e) Ruptured peltate trichome. f) Post-secretory peltate trichome that appears shriveled, and is located in an epidermal depression. Scale bars: 20μm in a, b, and d-f and 100μm in c. *PrS,* pre-secretory trichome; *ED,* epidermal depression; *ds,* diacytic stomata; *S,* secretory peltate trichome; *P,* pore; *Ex,* exudates; *R,* ruptured peltate trichome; *ACW,* anticlinal cell wall; *PS,* post-secretory]

young and mature leaflets, and anticlinal cell walls were visible on both surfaces (Figs. 1 and 4).

Histochemical analyses of fresh, hand-cut sections revealed the presence of alkaloids, phenol, and lipids (Table 1, Fig. 5). Wagner's and Dittmar's reagents revealed that the leaflets contained alkaloids, as indicated by the orange color of the peltate trichomes and an orange/brown coloration of the mesophyll. Sudan III/IV and Sudan Black revealed lipids. Tissues in the midveins were stained blue, whereas the peltate trichomes were stained black with Sudan Black and orange with Sudan III/IV. The midvein tissue and mesophyll contained phenolic compounds, as indicated by a black color after staining the sections with ferric trichloride. The NADI reagent produced negative results, indicating the absence of essential oils.

The preliminary phytochemical screening of the four crude leaf extracts (hexane, chloroform, methanol, and distilled water) revealed the presence of various classes of compounds, including saponins, oligosaccharides, alkaloids, phenolic compounds, tannins, and sterols. Monosaccharides, fixed oils, and fats tested negative in all four crude leaf extracts, indicating their absence in the leaves of *K. africana* (Table 2). The TLC plate containing samples of three crude leaf extracts (hexane, chloroform, and methanol) indicated the presence of several different compounds in each extract, which varied in size, class, and migration distance. This finding is indicated by the various bands formed on the TLC plate. The compounds were separated after the TLC plate was allowed to develop in the mobile phase of chloroform and methanol (9.9:0.1). Based on the TLC plate, chloroform (B) was the best extraction solvent because of its remarkable extraction efficiency (Fig. 6).

Discussion

This study was conducted to determine the foliar micromorphology and histo-phytochemistry of *K. africana* leaves. Nogueira et al. (2013) reported that plant species belonging to the family Bignoniaceae possess glandular and nonglandular trichomes that are present on the aerial surfaces of the vegetative and reproductive parts. However, the trichome structure, size, position, and abundance vary among species in this family. Fróes et al. (2015) stated that glandular trichomes are the characteristic features of the family Bignoniaceae. Only one type of glandular trichome, namely, peltate, was observed on the surfaces of young and mature leaflets of *K. africana*. This result is consistent with the findings of Ogundipe & Wujek (2004) and Ugbabe et al. (2013), who stated that glandular peltate trichomes

TABLE 1. Results from the histochemical tests on the fresh hand-cut sections on the leaves of *K. africana* to identify the various compounds found in the leaves

Compound group	Test	Peltate	Leaf tissue	Reaction observed
Lipids	Sudan III/IV	++	-	Orange colouration in the peltate trichomes
	Sudan Black	+	+	Black colouration in the peltate trichomes Blue colouration in the midvein of the leaflets
Alkaloids	Wagner & Dittmar's Reagent	++	++	Orange colouration in the peltate trichomes Brown colouration in the mesophyll and orange/brown colouration in the midvein of the leaflets
Phenols	Ferric Trichloride	-	++	Black colouration in the midvein and mesophyll of the leaflets
Essential oils	NADI Reagent	-	-	Sections turned black

+/- indicates presence or absence of compound

++ indicates intense reaction

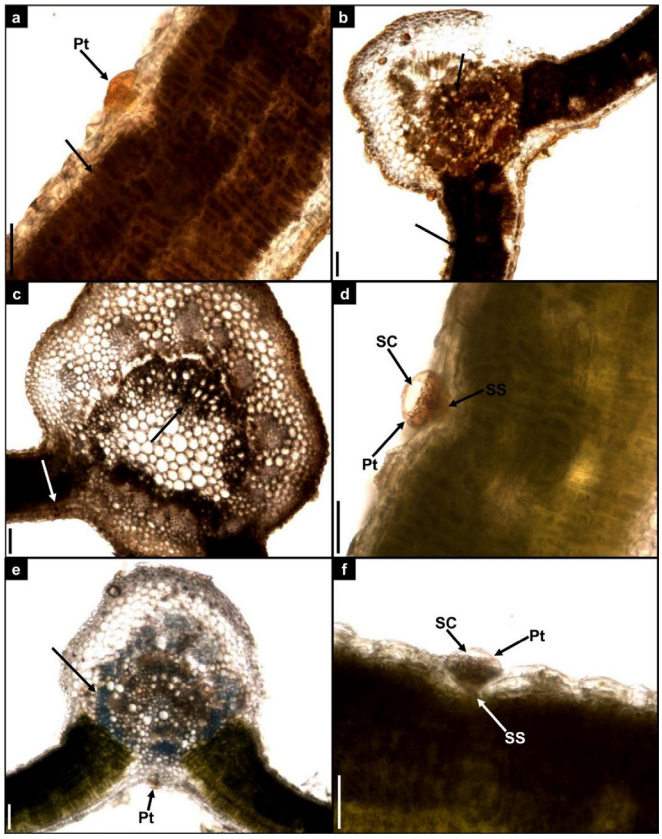

Fig. 5. Light micrographs of transverse sections through *Kigelia africana* leaflets stained with Wagner's and Dittmar's reagents [(a, b), ferric trichloride (c), Sudan III/IV (d), and Sudan Black (e, f). a) Peltate trichome and mesophyll of a leaflet stained positive (brown and orange) for alkaloids. b) Mid-vein tissue stained positive (brown and orange) for alkaloids. c) Tissue in the mid-vein and mesophyll stained positive (black) for phenolic compounds. d) Peltate trichome showing an embedded stalk cell and subcuticular cavity stained positive (orange) for lipids. e) Mid-vein tissue stained positive (blue) for lipids. f) Peltate trichome showing an embedded stalk cell and subcuticular cavity stained positive (black) for lipids. Scale bars: 50 μm in a and d-f and 100 μm in b and c. *Pt,* peltate trichome; *SS* short stalk; *SC,* subcuticular cavity]

TABLE 2. Preliminary phytochemical screening of the crude hexane (H), chloroform (C), methanol (M) and distilled water (DW) extracts from the dried ground leaves of *K. africana* to identify the different compounds found in the leaves

Compound Group	Test	Reaction observed	Present (+)/Absent (-)			
			H	C	M	DW
Fixed oils & fats	Filter paper	No reaction	-	-	-	-
Monosaccharides	Fehling's	No reaction	-	-	-	-
Saponins	Foam	Formation of foam layer after shaking solution for 15min	-	-	-	+
Oligosaccharides	Molisch's	Reddish-brown rings formed	+	+	+	+
Alkaloids	Wagner's & Dittmar's Reagent	Reddish-brown precipitate present	-	-	+	-
Phenolic compounds	Ferric trichloride	A dark green colouration	-	+	-	+
Tannins	Braemer's	Green colouration	-	-	+	+
Sterols	Salkowski's	Reddish-brown interface	+	-	++	++

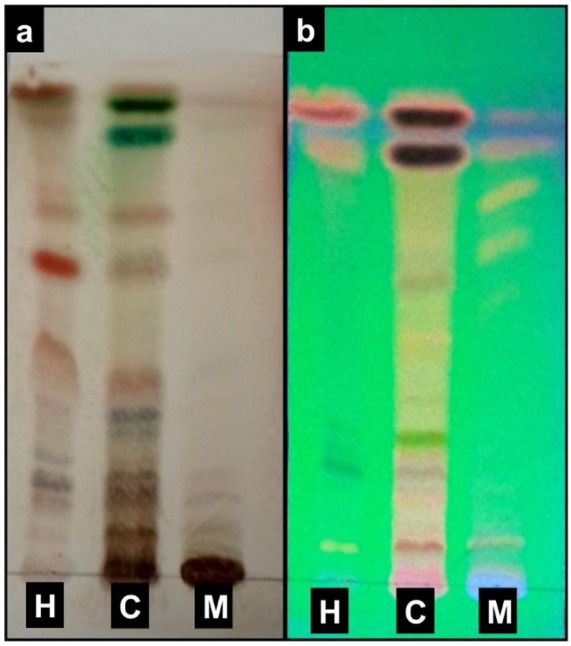

Fig. 6. Thin layer chromatography (TLC) results of crude hexane, chloroform, and methanol extracts (H, C, and M, respectively) from dried, ground leaves of *Kigelia africana* developing in a mobile phase of chloroform and methanol (9.9:0.1) [The TLC plate shows that each crude extract contained different compounds. a) TLC plate after being dipped in anisaldehyde-sulfuric acid reagent. b) TLC plate under ultraviolet light]

are present on the leaves of *K. africana*, but no microscopy results were presented in these studies.

The images captured by stereomicroscopy and SEM revealed glandular cavitated trichomes on the abaxial and adaxial surfaces of young and mature leaflets. Peltate trichomes were also observed in other species belonging to the family Bignoniaceae (Machado et al., 2006; Fróes et al., 2015). The peltate trichomes present on the leaves of *K. africana* were embedded in the epidermal layer, as indicated by a shadow around the trichomes. The peltate trichomes had large, broad, smooth heads with a large subcuticular space and a short stalk (Figs. 2, 5d, and 5f). According to Baran et al. (2010) and Choi & Kim (2013), peltate glandular trichomes consist of short stalks that are either unicellular or bicellular, with a large secretory head comprising four to eight cells. Peltate trichomes in species belonging to the family Bignoniaceae consist of a basal cell and a short stalk that is usually composed of one or two cells. However, certain species possess stalks that consist of three to five cells, whereas the heads of peltate trichomes in the family Bignoniaceae are composed of numerous cells, some of which possess a head consisting of six or eight cells (Machado et al., 2006; Fróes et al., 2015). Glandular trichomes are formed by a sequence of anticlinal and periclinal divisions of epidermal cells (Wagner, 1991; Khokhar et al., 2012). Choi & Kim (2013) stated that secretory cavities are present in peltate trichomes, and the bulb-like shape of peltate trichomes is due to a large subcuticular cavity in the head, which is also the storage site for secretory products (Glas et al., 2012). Figures 8d and 8f show secretory products in the subcuticular spaces of peltate trichomes, and Fig. 7c shows the secretory products of the trichomes released onto the leaflet surface. Zizovic et al. (2005), Kaya et al. (2007), and Choi & Kim (2013) reported that the secretory products of peltate trichomes accumulate and remain in these spaces until, or unless, the cells are physically damaged, causing them to rupture.

Three peltate trichome phases were observed on the surfaces of young and mature leaflets: presecretory, secretory, and postsecretory. The heads of the peltate trichomes appeared flat at the presecretory phase probably because the secretory products had not yet started accumulating in the subcuticular cavity. During the secretory phase, the heads of the peltate trichomes appeared round and spherical and protruded out from the epidermal depression. This phenomenon may be due to the accumulation of secretory products

in the subcuticular cavity during this phase. During the secretory phase, the heads of the peltate trichomes possessed a pore at the center. This pore may be the site from which secretory products are released. Ruptured peltate trichomes were also present on the leaves of *K. africana* probably because of physical damage. The release of secretory products from the subcuticular cavity through a micropore or rupture is relatively common in glandular trichomes and is primarily due to the pressure on the head of the trichome caused by the accumulation of secretory products (Zarinkamar & Sharebian, 2016). In the postsecretory phase, the heads of the peltate trichomes appeared shriveled probably because of the release of secretory products from the subcuticular cavity onto the leaflet surfaces (Fig. 4). Zarinkamar & Sharebian (2016) also reported that the head of the glandular trichome shrinks after secretory products are released from the subcuticular cavity. Peltate trichome phases have been reported by Ascensão et al. (1995), Turner et al. (2000), and Kalicharan et al. (2015).

Under a stereomicroscope, the surfaces of the leaflets appeared shiny because of the cuticular wax present on the leaflet surfaces (Fig. 3). The abaxial surfaces of both developmental stages had high densities of diacytic stomata, and anticlinal cell walls were also present on the surfaces of young and mature leaflets (Figs. 3d and 4e). The presence of cuticular wax, diacytic stomata, and anticlinal cell walls was also reported by Ugbabe et al. (2014). The cuticular wax coating protects the epidermis from a dry atmosphere and UV rays, whereas the dense presence of stomata on the abaxial surface allows for gaseous exchange, in which photosynthesis and respiration are conducted, and allows the plant to "breathe." Anticlinal cell walls are also observed in other species belonging to the family Bignoniaceae (Ugbabe et al., 2014).

Peltate trichomes were primarily found on the lamina of leaflets, with rare occurrences on the midvein. They were more abundant on the abaxial side of both developmental stages. This result is consistent with that of Turner et al. (2000), who reported that abaxial leaf surfaces have two times as many glandular trichomes as adaxial leaf surfaces. In this study, statistical analysis supported this observation because a significant difference in trichome density was found between the adaxial and abaxial surfaces. However, no significant difference in trichome density was found between young and mature leaflets, and no significant interaction was observed between the developmental stage and leaflet surface. Therefore, the trichome density remained constant, although the leaflets expanded and the trichome density on the abaxial surfaces of young and mature leaflets was higher than that on the adaxial surfaces. This result differs from those obtained by Ascensão et al. (1995), who reported that the number of glandular trichomes decreases as leaf size increases. This result is known as the leaf expansion theory, which was also reported by Valkama et al. (2004), who found that the formation and establishment of all trichomes occur at the early developmental stages of leaves; therefore, as the leaf expands, the number of trichomes remains constant, resulting in decreased density. However, Turner et al. (2000) stated that the formation of new peltate trichomes continues to increase through leaf expansion and ceases only when leaf growth ceases.

The results obtained by histochemistry, phytochemistry, and TLC revealed the presence of various compounds in the leaves of *K. africana*, which are presented in Tables 1 and 2 and Figures 5 and 6. Histochemical analysis revealed the presence of lipids, alkaloids, and phenols, indicating that the secretory products present in peltate trichomes consist of lipophilic and hydrophilic compounds. The peltate trichomes of bignoniaceous species also contain alkaloids and lipids (Machado et al., 2006). The NADI reagent was also used to stain sections, but no reactions were observed, and the sections turned black, indicating the absence of essential oils. However, this result may have been due to human error, or the peltate trichomes were at the presecretory phase because most essential oils (the main constituent of which are terpenes) are secreted by peltate trichomes (Huang et al., 2008; Choi & Kim, 2013). Sections stained with Sudan III/IV and Sudan Black produced positive reactions, indicating the presence of lipids. Positive reactions to Sudan III/IV and Sudan Black indicated that the peltate trichomes primarily consisted of lipophilic substances. This finding is consistent with the results of Huang et al. (2008) and Choi & Kim (2013), who reported that lipophilic substances are one of the main products secreted by peltate trichomes.

Peltate trichomes perform various functions,

such as temperature regulation, mechanical abrasion reduction, and water loss reduction by increasing light reflection (Wagner et al., 2004), and their secretory products may be involved in pollination and protection against pathogens and herbivores (Serrato-Valenti et al., 1997; Choi & Kim, 2013). Considering that peltate trichomes were found on the surfaces of *K. africana* leaves and contained secretory products, they could perform these functions.

Preliminary phytochemical screening of the four crude leaf extracts (hexane, chloroform, methanol, and distilled water) revealed the presence of saponins, oligosaccharides, alkaloids, phenolic compounds, tannins, and sterols. This result is consistent with those of Priya et al. (2013), who identified alkaloids, steroids, saponins, tannins, and phenols in crude leaf extracts of *K. africana*. Monosaccharides, fixed fats, and oils tested negative possibly because of human error or because the leaf extracts were too diluted to react with the reagents. Similar phytochemicals have also been found in *K. africana* fruit extracts. Azu et al. (2010a) identified alkaloids, saponins, and tannins in fruit extracts.

Given their medicinal value, species belonging to the family Bignoniaceae are important because of their biologically active constituents and pharmacological activity. The plants in this family have been extensively used by traditional medicinal practitioners to treat various ailments (Ogundipe & Wujek, 2004; Rahmatullah et al., 2010). Medicinal properties are induced by secondary metabolites, such as alkaloids, terpenes, and steroids, which are derived from plants in this family that have antimicrobial and antiparasitic properties (Azu et al., 2010b; Castillo & Rossini, 2010; Choudhury et al., 2011).

Conclusion

This study revealed the presence of glandular cavitated trichomes on the surfaces of young and mature leaflets, which consisted of a short stalk and a large secretory head with a subcuticular cavity. No significant difference in the number of peltate trichomes was observed between young and mature leaflets. However, a significant difference was found in the number of peltate trichomes between the abaxial and adaxial leaflet surfaces. Histo-phytochemistry revealed various phytochemicals, including alkaloids, phenolic compounds, and lipids, indicating that peltate trichomes consist of lipophilic and hydrophilic substances. However, further studies must be conducted on the fine ultrastructure of the leaves and the peltate trichomes to determine their exact composition. Leaf extracts must also be investigated in future work to determine their full range of phytochemicals because TLC displayed various classes of compounds, and only a few were tested in this study. Furthermore, phytochemicals found in the leaves must be explored to determine whether they exhibit any relevant biological activity.

Acknowledgments: The authors acknowledge Researchers Supporting Project number (RSP-2024R375), King Saud University, Riyadh, Saudi Arabia. We would like to thank all of the staff of the Microscopy and Microanalysis Unit, University of KwaZulu-Natal, Westville Campus, and Ashlin Munsamy and Kashmira Raghu for assistance with the histochemistry and phytochemistry.

*Competing interests***:** The authors declare no conflict of interest.

Authors' contributions: Conceptualization, D.S. and Y.N.; methodology, D.S. and Y.N; formal analysis, S.B, Y.N., Y.H.D; investigation and data curation, D.S. and Y.N.; validation, Y.H.D, T.A., WMH and N.M.D.; Visulaization, Y.H.D T.A., WMH and N.M.D.; writing—original draft preparation, D.S. and Y.N.; writing—review and editing, Y.N, Y.H.D., T.A. and N.M.D. All authors approved the final version of the manuscript.

Ethics approval: Not applicable.

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