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MICROMORPHOLOGY, AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *SPIROSTACYS AFRICANA* L. LEAF AND BARK.

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ABSTRACT.

Background:
Spirostachys africana is indigenous to Southern Africa and is often widely used in African traditional medicine. The utilization of this species has been investigated for its medicinal properties. However, the current investigation aimed to analyze the micromorphology of *S. africana* leaves and at the same time screen for the presence of secondary metabolites in the leaf and latex extracts.

Methods:
Stereomicroscopy and scanning electron microscopy were used to study morphological characters of both the abaxial and adaxial surfaces of *S. africana* leaves. Phytochemistry screening was performed using a standard protocol involving chemical reagents and a series of reactions to determine the presence of the phytochemical compound classes.

Results:
It was found that *S. africana* leaves possess both non-glandular and glandular trichomes on the adaxial surface, and no trichomes on the abaxial surface of the leaves. The phytochemical analysis revealed that the latex crude extract contains six phytochemical classes (alkaloids, steroids, flavonoids, coumarins, saponins, terpenoids) while the leaf crude extract contains seven phytochemical classes (alkaloids, phenols, flavonoids, coumarins, saponins, terpenoids, tannins).

Conclusion:
The micromorphological analysis of *Spirostachys Africana* leaf and bark provided valuable insights into their structural characteristics, revealing unique features such as thick cuticles, trichomes, and distinctive epidermal cells that are likely to play crucial roles in the plant's defense mechanisms and ecological adaptations. Preliminary phytochemical screening further identified the presence of key bioactive compounds. These findings not only corroborate the traditional uses of *S. africana* in herbal medicine but also highlight its potential for pharmacological applications.

Recommendations:
The identification of the bioactive compounds lays a foundational framework for future studies aimed at exploring the therapeutic potential and bioactivity of S. africana, thereby contributing to the broader understanding of its medicinal properties and promoting the sustainable use of this Indigenous species.

Keywords: *Trichomes, secondary metabolites, Traditional medicine, latex, Euphorbiaceae*
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INTRODUCTION.

Plants and plant-based medicines are considered the oldest source of pharmacologically active compounds that have been useful to mankind throughout the years (Omwenga et al., 2005). Plants possess a diverse category of phytochemicals that can be utilized in medicine formulation and other applications (e.g. cosmetics, insecticides, food preparation) (Coopoosamy et al., 2023).

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Techniques such as phytochemistry have been used for decades to identify the composition of compounds that may be present in medicinal plants (Ascensao et al., 1997; Kalimuthu and Prabakaran, 2015). Several classes of compounds such as alkaloids, tannins, carbohydrates, terpenoids, proteins, phenolics, flavonoids, naphthoquinones, saponins, glycosides, fixed fats, and oils are phytochemical/ biologically active compounds that are naturally produced by plants (Kennedy and Wigthman, 2011; Pourmorad et al., 2006). These compounds exhibit a broad range of medicinal properties such as antimicrobial, anticancer, antidiabetic, antiulcer, anti-inflammatory, antimalarial, antifertility, and antioxidant (Coopoosamy et al., 2023). Of the compounds present, alkaloids and phenolic compounds are probably the two most important phytochemical compounds that possess medicinal value (Garba and Okeniyi, 2012). These compounds are produced, stored, or excreted in specialized secretory plant structures (Ascensao et al., 1997).

Secretory structures occur in the form of trichomes, salt glands, idioblasts, resin ducts, laticifers, collectors, and nectaries located in various reproductive and vegetative organs of the plant (Singh et al., 2018). Secretory structures are species specific and their function is characteristic of that species. These structures occur in various forms as a result of living conditions, genetic processes, and phylogenetic characteristics. Visible changes in these structures are noted in varying growth conditions due to environmental factors. Conversely, it is not possible to observe such changes through the naked eye (Umah et al., 2017; Singh et al., 2018) Micromorphological studies on plants enable micro-level analyses of secretory structures through the use of light and electron microscopy (Yigit, 2016). Techniques employing microscopy, analyze characteristics such as the presence or absence of trichomes, canals, oil glands, salt-secreting glands, seed or pollen morphology, and particular cell types (Subramanion and Sreenivasan, 2012). The types of electron microscopes used for these micro-structural studies are Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM).

*Spirostachys africana*Sond. (tamboti tree), a member of the Euphorbiaceae family is found throughout southern and central Africa (Hutchings, 1996; Singh et al., 2020). This plant is described as an average-sized, monoecious or dioecious, hardwood, deciduous shrub or small tree that ranges from 10 - 20 m in height, with young green leaves and bright red mature leaves (Lennox et al., 2015; Singh et al., 2020). A toxic white latex is characteristic of the family Euphorbiaceae and is exuded from the trunk of *S. africana* (Lennox and Bamford, 2015). The trunk has a bark that varies from dark brown to grey or black, with a rough surface possessing rectangular scales. The tree is widely used in rural areas for traditional medicine to combat a wide range of diseases (Singh et al., 2020). Decoctions made from tamboti material have been reported to be effective against constipation, gonorrhea, diarrhea, and malaria amongst other diseases (Singh et al., 2020). A variety of phytocompounds have been successfully identified, and have been positively associated with the plant's medicinal properties (Singh et al., 2020). At least 12 000 secondary metabolites have been successfully identified, and tested positive for providing plants with medicinal properties (Singh et al., 2020). However, given the toxic nature of the latex, usage of the species in traditional medicine has to be administered with caution.

The majority of research has focused primarily on the traditional and medicinal properties of *S. africana* with limited focus on the ultrastructure of the species. Therefore, this study aims to investigate the micromorphology of *S. africana* leaves to improve our understanding of the plant's leaf morphology and screen for the presence of phytocompounds that are known to be of medicinal importance in the leaves and latex of *S. africana.*

MATERIALS AND METHODS.

Plant Collection.

Plant material of *Spirostachys africana*leaves and latex were collected around Umlazi, KwaZulu-Natal. Leaves were randomly collected by breaking twigs off and plucking the leaves from the twigs. The leaves were separated into two samples, one sample was used for micromorphological analysis and the other sample was used for qualitative phytochemistry analyses. Latex was obtained by making small incisions (using a surgical blade) on the trunk of the tree and collecting the latex into a test tube as it exuded from the trunk. This process was done in the morning (07:00-09:00) as the latex was less viscous and less evaporative during this period.

Stereomicroscopy.

Stereomicroscopy was used to study morphological characters of the surface of *S. africana* leaves of both the abaxial and the adaxial surfaces. Samples were examined with the Nikon AZ100 stereomicroscope, Japan, equipped with Nikon Fibre Illuminator and images were captured using the Nikon DXM1200C color camera. The images were taken using the NIS-Element Software.

Scanning Electron Microscopy. (SEM)

For scanning electron microscopy, samples were prepared by chemical fixation according to Singh et al. (2019). Fresh

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leaf samples were trimmed into segments and fixed overnight in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) at 4°C. The samples were washed three times (5 min per wash) with the 0.1M phosphate buffer. The samples were post-fixed in 0.5% osmium tetroxide for 1-2 hours. Thereafter, the samples were subjected to three 5-minute washes with phosphate buffer. Dehydration was accomplished with a graded series of 30%, 50%, and 75% alcohol (two changes, each of 5 min) followed by two changes for 10 min in 100% alcohol. The samples were then dried with the use of a Hitachi Critical Point Dryer. Critical point-dried samples were mounted onto brass stubs secured with carbon conductive tape and sputter coated with a Quorum Gold QISORES Sputter Coater, UK. Specimens were then coated with gold for approximately four minutes. They were then viewed with a Leo 1450 SEM, Germany, at 5 kV. Images were captured using the Smart SEM version 5.03.06.

Phytochemistry.

Sample preparation.

The samples were prepared using methods adapted from Mathabe et al. (2008) and Harborne (1998).

Leaves.

Leaves were placed in a brown paper bag and were oven-dried for 48 hours at 50°C. They were then ground to a fine powder using a household food blender. Powdered material (150 g) was placed in a round-bottomed flask, together with 1.5 L ethanol. The mixture was left in a shaker for 24 hours at room temperature. The mixture was then filtered and the filtrate was subjected to a Butchi rotavapor (R-124) to obtain a concentrated crude extract. This process was repeated with dichloromethane (DCM). A variety of phytochemical tests were performed on the ethanol, and DCM extracts to determine the presence of phytochemical compounds.

Latex.

Approximately 15 g of the latex was dissolved in 150 ml of ethanol in a round-bottomed flask. The mixture was left on a shaker for 24 hours and was subsequently filtered using Whatman No. 1 filter paper to obtain the final extract. This process was repeated with dichloromethane(DCM). A variety of phytochemical tests were performed on the ethanol, and DCM extracts to determine the presence of phytochemical compounds.

Phytochemical Screening.

Test for alkaloids.

One ml of Wagner's reagent was added to 1 ml of extract. The formation of a brown precipitate indicated the presence of alkaloids.

Test for coumarins.

Three ml of 10% sodium hydroxide were added to 2 ml of extract. The formation of a yellow precipitate indicated the presence of coumarins.

Test for flavonoids.

Three ml of diluted lead acetate solution was added to two ml of extract. A yellow, dirty white, or bulky white precipitate indicated the presence of flavonoids.

Test for phenols.

One ml of 10% ferric chloride was added to two ml of extract. The formation of a dark green, blue, or bluish-black color indicated the presence of alkaloids.

Test for saponins.

Two ml of extract was placed in a test tube thereafter the extract was diluted with 20 mL of distilled water. The mixture was shaken by hand for 15 min. The formation of a persistent (lasting ≥ 5 minutes) foam layer on the top of the test tube indicated the presence of saponins.

Test for steroids.

One ml of extract was dissolved in 10 ml chloroform and 10 ml of concentrated sulfuric acid was added to the mixture. The formation of a red upper layer and a sulfuric layer showing yellow with green fluorescence was a positive indicator of the presence of steroids.

Test for tannins.

Three ml of 1% ferric chloride solution was added to a 10 ml extract. The formation of a black or blue-green color indicated the presence of tannins.

Test for terpenoids.

Two ml of chloroform was added to 5 ml of extract. 3 ml of concentrated sulphuric acid was carefully added to the

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mixture. The formation of a reddish-brown color indicated the presence. of terpenoids.

RESULTS.

Microscopy.

Stereomicrographs (Figures 1 a and b) and Scanning Electron micrographs (Figures 2 a and b) revealed that both glandular and non-glandular trichomes were present on *S. africana* leaf surfaces. However, they were only observed on the adaxial leaf surface. Scanning electron micrographs (Figure 2) also showed a visual difference in the distribution of stomata between the two surfaces as there were more stomata observed on the abaxial than on the adaxial leaf surface.

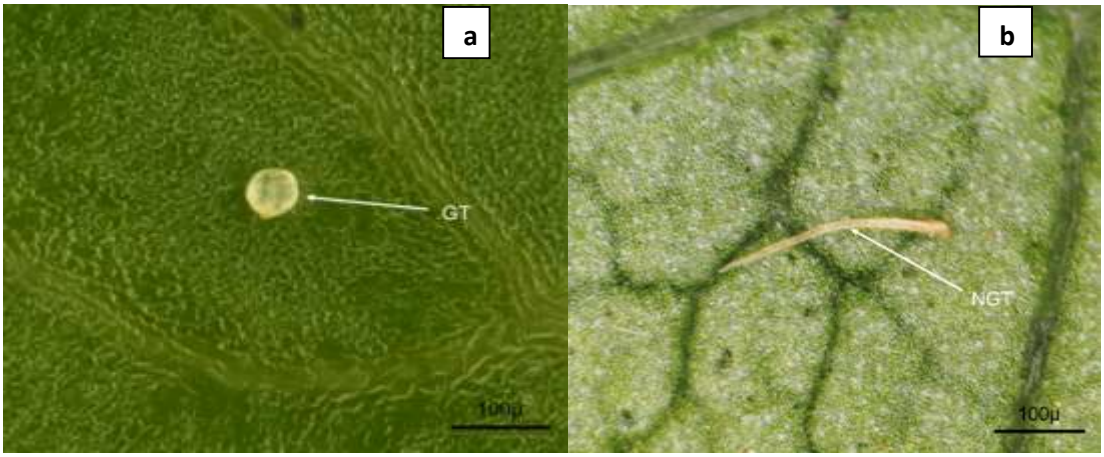

Figure 1: Stereomicrographs of the adaxial surface of S. africana mature leaf a) showing glandular trichome (GT) and b) non-glandular trichome (NGT).

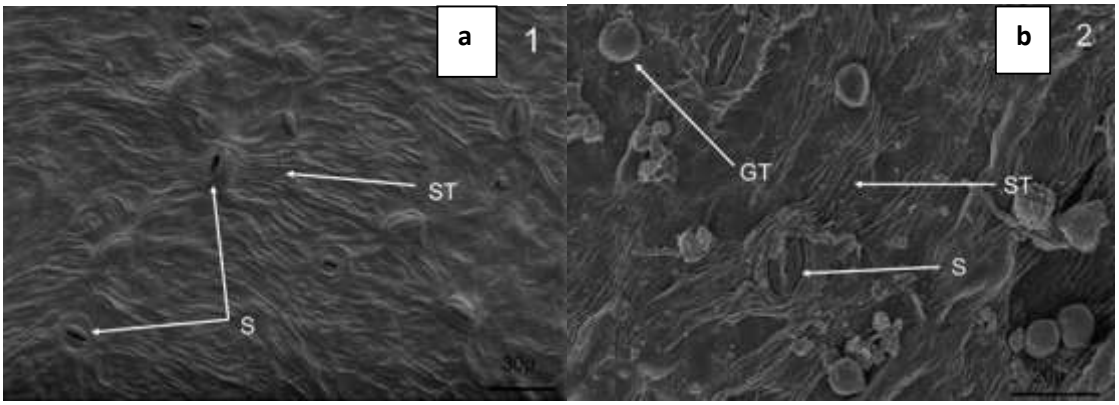

Figure 2: Scanning electron micrographs of both the abaxial (a) and the adaxial (b) surfaces of S. africana mature leaves. GT – glandular trichome, S – stomata, ST – striations.

Phytochemistry.

Phytochemical screening results presented in Table 1 reveal that both the leaf extracts and the latex extracts contain phytochemicals. Eight classes of secondary metabolites were found in the leaf extract except for steroids. However, the latex extracts tested positive for the presence of steroids and the remaining phytochemicals, except tannins and phenols.

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Table 1: Phytochemical screening of *S. africana* leaf and latex extracts.

Compound classes	Plant Part	Plant Part
	Leaves	**Latex**
Alkaloids	+	+
Flavonoids	+	+
Coumarins	+	+
Tepernoids	+	+
Saponins	+	+
Steroids	-	+
Tannins	+	-
Phenols	+	-

+ indicates presence; - indicates absence

DISCUSSION.

Microscopy.

Scanning electron micrographs (Figure 1) indicated that *S. africana* consists of paracytic stomata. Although their distribution was not quantified, visual observations revealed that the stomata were more distributed on the abaxial surface than on the adaxial surface. Such difference in stomatal distribution has been reported as an adaptive strategy to warm and dry places as it reduces water loss through transpiration (Essiet *et al*., 2012). It was mentioned that *S. africana*leaves exhibit such stomatal distribution as the species commonly inhabits warm and dry areas (Aniesua and Silas, 2012; Gandiwa *et al*., 2012). Furthermore, both scanning electron micrographs (Figure 1) and stereomicrographs (Figure 2) indicated that *S. africana* leaves consisted of both glandular and non-glandular trichomes, however, these were only located on the adaxial surface of the leaf. This study was the first to report the presence of trichomes on *S. africana* leaves. The presence of trichomes on *S. africana* leaves could be attributed to numerous ecophysiological adaptations. Wagner (1991) reported that non-glandular trichomes usually assist in mediating photosynthesis and respiration by reflecting light on the surface of the leaves. As such, the appearance of these trichomes on *S. africana*leaves is logical as the species commonly inhabits warm and dry areas, where there is a constant need to regulate the amount of sunlight absorbed and the amount of water lost through transpiration. Singh *et al*. (2018) further explained that non-glandular trichomes could influence a plant's ability to repel pests by acting as a protective physical barrier. This is an unlikely role for non-glandular trichomes of *S. africana* as the microscopic images exhibited a shallow distribution of these trichomes across the leaf surface (Figure 2).

The presence of glandular trichomes on the leaf reduces herbivory towards the plant (Singh et al., 2019). However, this has not been further investigate. Glandular trichomes in general have been reported to reduce herbivory by producing chemical compounds that are toxic or repulsive to pests and herbivorous mammals (Singh et al., 2018). Among the most common exudates from trichomes, terpenoids have received significant attention as it is known to be particularly poisonous to animals (Singh et al., 2019). Glandular trichomes have been extensively researched, particularly as the compounds that they secrete have been found to have several medicinal uses (for example flavonoids have been found to exhibit antioxidant activity)

Phytochemical Screening.

The qualitative phytochemistry results presented in this study suggest that both the leaves and latex of *S. africana* are potentially utilized in the traditional and pharmacological market as they consist of compounds that have numerous biological activities. The presence of alkaloids makes *Spirostachys*one one of the few Euphorbiaceae genera to consist of this compound class (Salatino*et al*., 2007). Alkaloids derived from plant material reportedly contain antimicrobial activities (Augusto *et al*., 2011). Dua *et al*. (2013) explained that alkaloids exhibit antimalarial activity, this justifies the use of *S. africana* material in traditional medicines that aim to treat malarial infections in the eastern parts of Africa (Singh et al., 2020). Additionally, alkaloids are commonly reported as a powerful poison whose presence in *S. africana* plant material accounts for the plant's toxicity to humans (Harborne, 1973; Iqbal *et al*., 2015).

The leaves and latex of the plant have great potential for medical applications due to the presence of coumarins in the extracts. Isolated coumarins have displayed positive results for antimicrobial activities in plant species (including those from the family Euphorbiaceae) (Poumale *et al*., 2013). Moreover, previous research on the biological activity of coumarins uncovered that this class of phytochemicals exhibits anti-leishmanial activity (Iranshahi *et al*., 2007).

This is of medicinal significance as leishmaniasis (a disease caused by parasitic protozoa of the *Leishmania* genus) is responsible for approximately 30,000 deaths every year (Alvar *et al*., 2012). Furthermore, coumarins have been found to possess enzyme-inhibitory activity. This is of importance in the treatment of diseases such as Alzheimer's disease (Poumale *et al*., 2013).

Flavonoids are exceedingly common in vascular plants and Euphorbiaceae is recognized as being particularly rich in this class of phytochemicals (Salatino*et al*., 2007). As such, the presence of flavonoids in the extracts of *S. africana* is less surprising and suggests that similarly to other species in which flavonoids occur, *S. africana*possesses antioxidant activities which can be found in both the leaves and the latex of the species (Saxena *et al*., 2013). The antioxidant activities of the leaves and latex of *S. africana* are further supported by the presence of terpenoids in the tested crude extracts. Terpenoids are a diverse group of secondary metabolites that have antioxidant properties in humans and other animals (Saxena et al., 2013). Although terpenoids exhibit antioxidant activities that are of medicinal significance, they are also toxic to animals, and potentially deadly when ingested (Lennox and Bamford, 2015).
Saponins are one of the phytochemical classes that are known to have antimicrobial activities (Saxena *et al*., 2013). Saponins also play a significant physiological role in plants by protecting them from insects, a property of which has been used in the development of insecticides (Rattan *et al*., 2015). The presence of saponins in *S. africana* extracts suggests that the species could potentially assist insect control, particularly those that act as vectors for pathogens (Pelah *et al*., 2002). This emphasizes the importance of *S. africana* in medicinal practices as when used topically it may circumvent vector-borne infections, eliminating diseases such as malaria.

Steroids present in the latex extract and absent from the leaves extract are also known to exhibit insecticidal activities. However, steroids also have a more direct application in medicine as they have been reported to have cardiotonic effects (Iqbal *et al*., 2015). Like steroids, tannins and phenols were only present in the leaf extract but absent in the latex extract (Table 1). This was unsurprising as numerous studies have revealed that different parts of the same plant may not contain similar chemical compounds (Iqbal *et al*., 2015). Due to the presence of tannins and phenols, it has been investigated that *S. africana* leaves have antiviral and antitumor activities (Hisanori *et al*., 2001). Additionally, tannins work against diarrhea, this is evidenced by the use of *S. africana* stem bark as a cure in traditional medicine for this purpose (Saxena *et al*., 2013; Iqbal *et al*., 2015).

Despite the valuable insights gained from micromorphological and preliminary phytochemical analyses of *Spirostachys africana* L. leaf and bark, several limitations must be acknowledged. The preliminary phytochemical screening was based on qualitative tests, which, while useful for detecting the presence of various bioactive compounds, do not provide quantitative information on their concentration or bioavailability. This limitation prevents definitive conclusions about the relative potency or therapeutic efficacy of the compounds present. Furthermore, the study focused on a narrow range of phytochemical classes, potentially leaving out other important compounds that could contribute to the plant's medicinal properties. Furthermore, the extraction methods used were conventional and may not have fully captured all bioactive constituents, implying that more thorough extraction techniques could provide a more complete phytochemical profile. Finally, the lack of *vivo*or clinical data makes it difficult to directly correlate phytochemical findings with therapeutic outcomes, emphasizing the need for additional research to validate these preliminary findings and investigate their practical applications in medicine.

CONCLUSION.

Preliminary investigations of *S. africana* revealed that leaves possess both non-glandular and glandular trichomes on the adaxial surface. The presence of glandular trichomes can be associated with the secretion of some phytochemicals that the plants use for defense. However, these chemicals have also been found to be of medicinal importance to humans. The leaf and latex extracts were found to possess most of the phytochemicals of interest with a range of therapeutic properties. Further research is needed to validate the medicinal potential of *S. africana* leaf and latex extracts with a focus on isolating and quantifying the content of each compound. Histochemical analysis of plant tissue will be beneficial to better the understanding of the localization and mode of secretion of the secondary metabolites. Additionally, the biological activity of *S. africana* leaf and latex crude extracts should be tested against a broader range of microbial pathogens, to provide evidence that the species can be used in the synthesis of new drug development.

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DECLARATION.

Availability of Data.

The raw data supporting the conclusions of this article will be made available by the principal investigator Karishma Singh without undue reservation.

Competing Interests.

All authors declare no competing interests.

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Authors' contribution.

The conception of the idea, initial manuscript drafting, analysis, result interpretation, and subsequent manuscript revisions were collaborative efforts among all authors who reviewed the initial draft and subsequently contributed to further revisions of the manuscript and granted approval for the final version of the manuscript.

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