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(RESEARCH ARTICLE)

PHARMACOGNOSTIC STANDARDIZATION AND ANTI-INFLAMMATORY ASSESSMENT OF THE METHANOL LEAF EXTRACT OF *KEETIA VENOSA* (OLIV.) BRIDSON (RUBIACEAE)

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ABSTRACT

This study evaluated the pharmacognostic and anti-inflammatory properties of the methanol leaf extract of *Keetia venosa* (Rubiaceae). Pharmacognostic evaluation was carried out using standard macroscopic, microscopic and chemo-microscopic techniques. Phytochemical screening and Thin-Layer Chromatography were performed using standard procedures, while acute toxicity was evaluated using the Organisation for Economic Co-operation and Development (OECD) up-and-down method. Anti-inflammatory activity was assessed using egg albumin-induced paw oedema in albino rats at doses of 250, 500 and 1000 mg/kg, with ibuprofen (40 mg/kg) serving as the standard anti-inflammatory drug. Macroscopic analysis showed opposite leaf arrangement, pubescent surface, elliptic shape and acute apex and base. Microscopic studies revealed polygonal epidermal cells, paracytic stomata, unicellular trichomes and prism-shaped calcium oxalate crystals. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, phenolics and saponins. No mortality or severe toxicity was observed at 5000 mg/kg. Although the extract demonstrated dose-dependent inhibition of paw oedema the anti-inflammatory activity was not statistically significant compared with the control group ($p > 0.05$). The study provides useful pharmacognostic standards for the identification of *Keetia venosa* and establishes baseline information for future phytochemical and pharmacological investigations.

Keywords: *Keetia venosa*, Pharmacognostic, Phytochemicals, Anti-inflammatory activity, Medicinal plants, Rubiaceae

1 INTRODUCTION

Medicinal plants remain an important source of primary healthcare, particularly in developing countries, where approximately 80% of the population relies on traditional and plant-based medicines for healthcare needs [1]. Herbal medicines have gained global interest due to their accessibility, affordability and perceived safety. However, many medicinal plants remain poorly characterized, highlighting the need for systematic evaluation of their quality, safety and therapeutic potential [2].

The family Rubiaceae is one of the most diverse families of angiosperms, comprising approximately 637 genera and 13,000 species. Members of the family are known to produce a wide range of secondary metabolites, including alkaloids, anthraquinones, terpenoids, flavonoids and other phenolic compounds, many of which possess important pharmacological activities [3]. Among the less extensively investigated members of this family is *Keetia venosa* (Oliv.) Bridson. The specie occurs mainly in hilly forests, riverine areas, savannas and woodlands, they are scandent shrubs or climbers distributed across tropical Africa which can attain a height of approximately 2–7 m [4]. Although several species within the genus *Keetia* have demonstrated biological activities, including antimalarial, antitrypanosomal and antimicrobial effects, information on the pharmacognostic characteristics, phytochemical composition and anti-inflammatory potential of *Keetia venosa* remains limited [5,6,7].

Pharmacognostic standardization is an essential step in the scientific evaluation of medicinal plants, as it provides diagnostic, physicochemical and phytochemical parameters necessary for the authentication, quality assessment and reproducibility of crude plant materials. Establishing such parameters is particularly important for medicinal plants that are traditionally used but remain inadequately characterized. Previous work has demonstrated the value of integrating pharmacognostic, physicochemical and phytochemical evaluations in establishing quality-control parameters for medicinal plant materials[8]. Such evaluations are important because the quality and purity of plant-derived materials may vary according to several factors, including species identity, geographical origin, environmental conditions and methods of collection and processing [9].Standardization is essential for ensuring the quality, safety and reproducibility of herbal medicines. Phytochemical evaluation aids plant characterization by identifying bioactive secondary metabolites. Rubiaceae is rich in diverse phytochemicals, including iridoids, anthraquinones, triterpenes and alkaloids.[3]. Several plant species containing phenolic compounds, flavonoids, tannins, saponins and terpenoids have demonstrated anti-inflammatory activity, supporting the importance of phytochemical investigation in the discovery of natural anti-inflammatory agents [10].

Inflammation is a complex protective response to infectious, physical, chemical or endogenous stimuli and is essential for the elimination of harmful agents and the initiation of tissue repair [11,12]. Although acute inflammation is generally protective, persistent inflammation can result in progressive tissue damage and contribute to the development and progression of several chronic diseases, including cardiovascular diseases, diabetes, respiratory disorders, autoimmune diseases and cancer [13,10]. Inflammatory responses are mediated by a complex network of cellular and molecular events involving mediators such as histamine, bradykinin, cytokines, leukotrienes and prostaglandins. Prostaglandin E_2 (PGE_2), in particular, plays a significant role in the development of pain and inflammation and is synthesized from arachidonic acid through the cyclooxygenase (COX) pathway [14]. NSAIDs are widely used to manage inflammatory pain and disease by inhibiting cyclooxygenase enzymes and reducing prostaglandin synthesis. However, conventional NSAIDs may cause gastrointestinal and renal complications, while selective COX-2 inhibitors may increase cardiovascular and cerebrovascular risks.

[15,10]. These limitations have driven the search for safer and more effective anti-inflammatory agents from natural sources. Plant secondary metabolites, including flavonoids, phenolics, terpenoids, tannins and alkaloids, exhibit diverse pharmacological activities and offer promising anti-inflammatory lead compounds. [10].

Despite the increasing interest in plant-derived anti-inflammatory agents, there is limited information on the pharmacognostic characteristics and anti-inflammatory activity of *Keetia venosa*. The limited scientific documentation of the species represents a gap in the systematic evaluation of its potential medicinal value. Establishing its diagnostic pharmacognostic features, phytochemical constituents and biological activity may therefore provide a scientific basis for further investigation and potential development of natural therapeutic agents.

Accordingly, this study evaluated the pharmacognostic characteristics, TLC and phytochemical screening of the methanol leaf extract of *Keetia venosa* (Oliv.) Bridson. It also investigated its anti-inflammatory activity using the egg albumin-induced paw oedema model in rats. The findings are expected to contribute to the scientific documentation

and standardization of *Keetia venosa* and provide preliminary evidence regarding its potential as a source of bioactive compounds with anti-inflammatory properties.

2 MATERIAL AND METHOD

2.1 Plant Collection and Identification

Fresh leaves of *Keetia venosa* (Oliv.) Bridson were collected in Enugu, Nigeria, in October 2021. The plant material was thoroughly washed to remove adhering foreign matter and authenticated by a taxonomist in the Department of Botany, University of Nigeria, Nsukka. The leaves were shade-dried at room temperature, pulverized into a coarse powder and stored in a moisture-free container until further use.

2.2 Preparation of Plant Extract

100 g of the powdered leaves were macerated in 1000 mL of methanol in a covered glass container for 24 hours with intermittent agitation. The mixture was filtered through a clean white cloth, and the filtrate was concentrated using a rotary evaporator. The remaining plant marc was re-macerated with methanol, and the extraction procedure was repeated. The concentrated extract was stored in a refrigerator and used for subsequent pharmacognostic, phytochemical, acute toxicity and anti-inflammatory investigations.

2.3 Pharmacognostic Evaluation

2.3.1 Microscopic Evaluation of the Leaf

The adaxial and abaxial epidermal surfaces of the fresh leaves were prepared using a clearing method. Leaf samples were immersed in commercial bleach containing 3.5% sodium hypochlorite for 18 hours. The epidermal layers were subsequently scraped gently with forceps, mounted on clean glass slides, stained with safranin and covered with coverslips. The prepared slides were examined using a light phase-contrast microscope at ×40, ×100, and ×400 magnifications. Photomicrographs were obtained using a digital camera attached to the microscope.

The epidermal cell characteristics, stomatal type, stomatal size, stomatal density, stomatal index, trichome characteristics, vein-islet number, vein-islet termination number and palisade ratio were evaluated on both leaf surfaces. Stomatal index was calculated using the formula:

$$SI = \frac{S \times 100}{S+E}$$

Where S represents the number of stomata and E represents the number of epidermal cells within the same field of view [16].

Transverse sections of the leaves were prepared using a sledge microtome following standard procedures described by Johansen [17]. Sections approximately 10–15 µm thick were collected and stained with safranin and fast green for the differentiation of lignified and non-lignified tissues.

2.3.2 Chemo-microscopic Evaluation

Powdered leaf samples were subjected to chemo-microscopic examination for the detection of starch, lignified tissues, cellulose, tannins, calcium oxalate crystals, gums and mucilage using standard pharmacognostic procedures. The cleared powdered samples were treated with appropriate reagents and examined microscopically for characteristic colour reactions or structural features indicative of the respective constituents.

2.4 Thin-Layer Chromatography

Thin-layer chromatography (TLC) was performed using pre-coated silica gel plates as the stationary phase. The plates were activated at 105 °C for 30 min before use. The mobile phase consisted of n-hexane and ethyl acetate in a ratio of 9:2 respectively. The methanol extract was applied to the plates using a capillary tube and developed in a pre-saturated chromatographic chamber containing the mobile phase. Following development, the plates were dried and examined under ultraviolet light. The observed spots were recorded and the retention factor (Rf) values calculated according to standard procedures [8].

$$\text{Retardation Factor (Rf)} = \frac{\textit{Distance moved by solute}}{\textit{Distance moved by solvent}}$$

2.5 Phytochemical Screening

Qualitative phytochemical screening of the *Keetia venosa* was performed using standard methods to determine the presence of major secondary metabolites. Tests were conducted for alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, glycosides, saponins and steroids in the leaf extract using different solvents with varying polarity. The solvents include methanol, n-hexane, ethylactate and butanol. The presence of individual phytochemical constituents was determined based on characteristic colour changes, precipitate formation or other specific reactions following the addition of appropriate reagents [8].

2.6 Acute Toxicity and Anti-inflammatory Study Using Experimental Animals

The acute toxicity study was conducted using four albino mice, while 30 albino rats were used for the anti-inflammatory study. The animals were obtained from the animal house of Chukwuemeka Odumegwu Ojukwu University, Igbariam. They were housed under standard laboratory conditions and allowed to acclimatize for 7 days before experimentation. Animals had free access to standard feed and water, except where fasting was required before specific experimental procedures.

All experimental procedures were conducted in accordance with applicable guidelines for the care and use of laboratory animals.

2.6.1 Acute Oral Toxicity Study

Acute oral toxicity was evaluated using the OECD Up-and-Down Procedure [18]. The methanol leaf extract was administered orally at a dose of 5000 mg/kg body weight using 5% Tween 80 as the vehicle. The control animal received the vehicle alone. Animals were observed closely following administration for signs of toxicity, including changes in behaviour, skin, hair, eyes, mucous membranes, respiration, locomotor activity, tremors, convulsions, salivation, diarrhoea, lethargy and sleep. Observations were conducted at regular intervals during the first 24 hours and continued for 7 days to monitor delayed toxic effects or mortality.

2.6.2 Anti-inflammatory Activity

The anti-inflammatory activity of the methanolic leaf extract was evaluated using the egg albumin-induced paw oedema model in albino rats, following the method described by Nile & Park [19]. 30 albino rats were randomly allocated into 5 groups of 6 animals each:

- Control
- Group 1 (Standard)
- Group 2 (Low Dose)
- Group 3 (Medium Dose)
- Group 4 (High Dose)

The initial paw diameter was measured before induction of inflammation. Paw oedema was induced by sub-plantar injection of 0.1 mL of fresh egg albumin into the right hind paw of each rat. Paw diameter was subsequently measured at 1, 2, 3, 4, 5, and 6 hours after induction using cotton thread to determine the circumference of the paw. Ibuprofen (40 mg/kg) was used orally as the standard anti-inflammatory drug. The extract was also used orally for the low, medium and high doses, while the control group received the vehicle.

The degree of paw oedema was determined from the change in paw diameter relative to the baseline measurement. The percentage inhibition of oedema produced by the extract was calculated in comparison with the control group at each time point using the formula.

$$\%\text{Inhibition of oedema} = \frac{CONTROL(Ec) - EXTRACT(Et)}{CONTROL(Ec)} \text{ X } 100$$

Where Ec represents the mean oedema in the control group and Et represents the mean oedema in the treatment group.

2.7 Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation. The difference between groups and statistical analysis were performed using in one-way analysis of variance (ANOVA) followed by an appropriate post hoc test. Values of $p < 0.05$ were considered statistically significant.

3 RESULTS

3.1 Pharmacognostic Evaluation

3.1.1 Extractive Yield

The yield of extract obtained from 100 g of the crude leaves of *Keetia venosa* when macerated in 1000 ml of methanol was 33.45 g

Percentage Yeild (%) = $\frac{Actual\ yield}{Theoretical\ yeild}\ X\ 100\ =\ \frac{33.45g}{100g}\ X\ 100\ =\ 33.45\%$

3.1.2 Macroscopic Characteristics

The macroscopic observations of *Keetia venosa* leaf are presented in Table 1.

Table 1: Macroscopic Characteristics of the Leaf of *Keetia venosa*

Characteristic	Observation
Leaf arrangement	Opposite
Shape	Elliptic
Surface	Pubescent
Colour	Green
Apex	Acute

3.1.3 Organoleptic Properties

The organoleptic observations of *Keetia venosa* leaf are presented in Table 2.

Table 2. Organoleptic Characteristics of the Powdered Leaf of *Keetia venosa*

Taste	Slightly bitter
Colour	Greenish
Texture	Coarse powder
Odour	Uncharacteristic

3.1.4 Microscopic Characteristics of *Keetia venosa* leaf

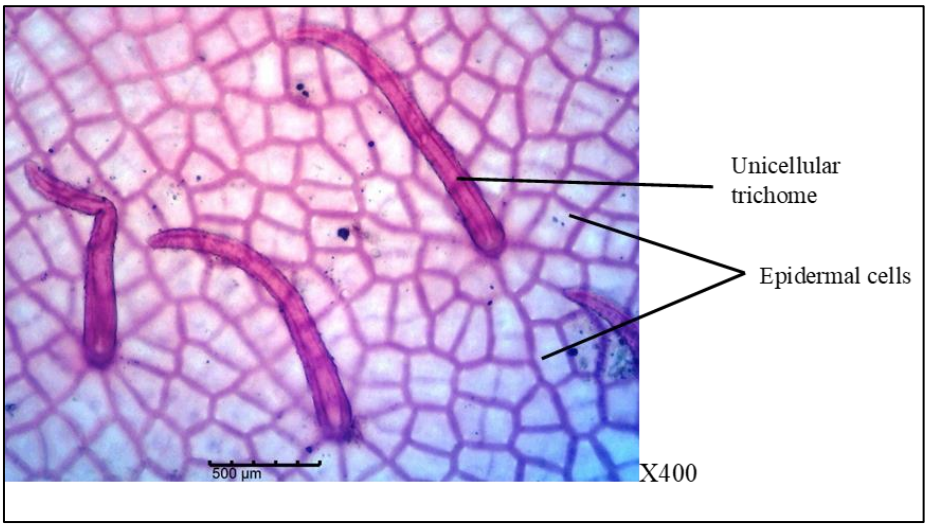


Figure 1: Adaxial surface of *Keetia venosa* leaf showing the uniseriated trichomes and epidermal cells

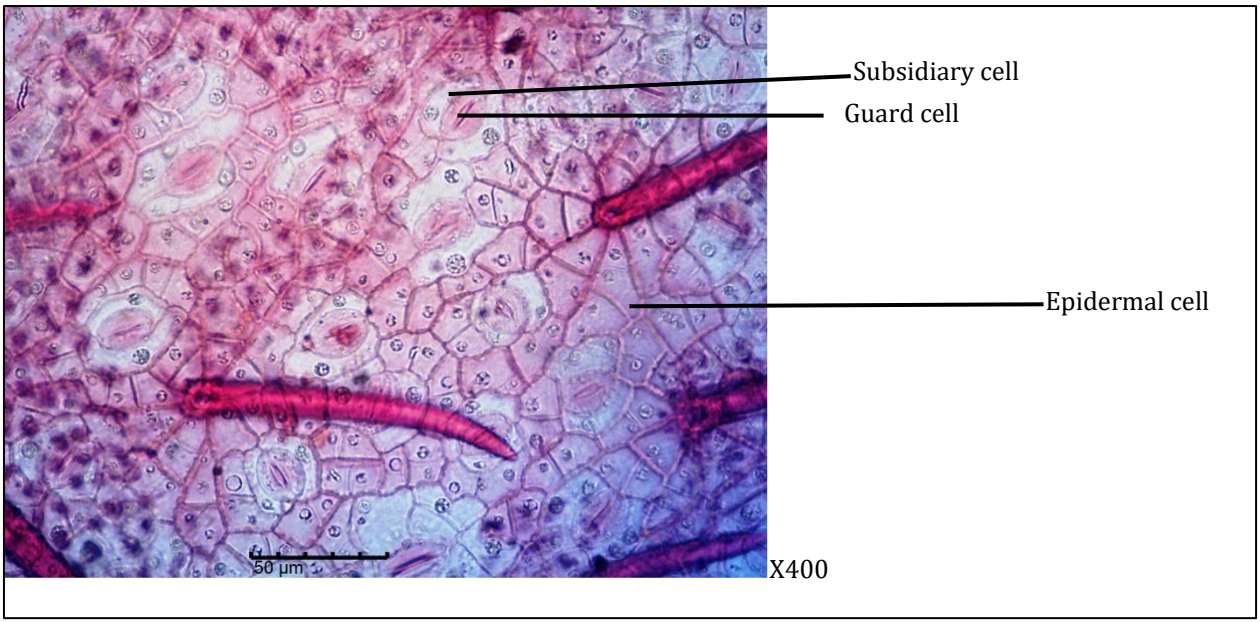


Figure 2: Abaxial surface of *Keetia venosa* leaf showing the subsidiary and guard cells of the stomata

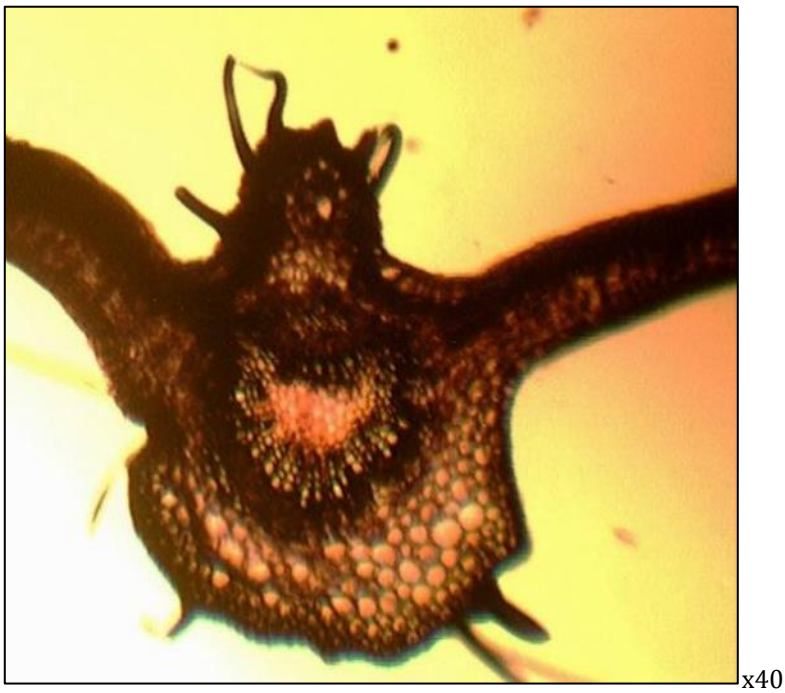


Figure 3. Transverse section of the leaf of *Keetia venosa* showing upper and lower epidermises and a U-shaped vascular bundle

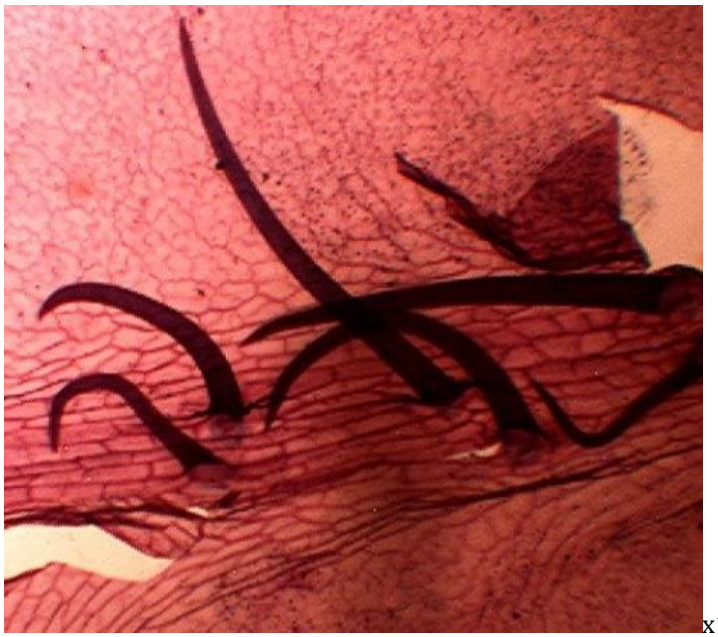
 x100

Figure 4: Photomicrograph of the leaf of *Keetia venosa* abaxial side of the midrib showing covering trichomes

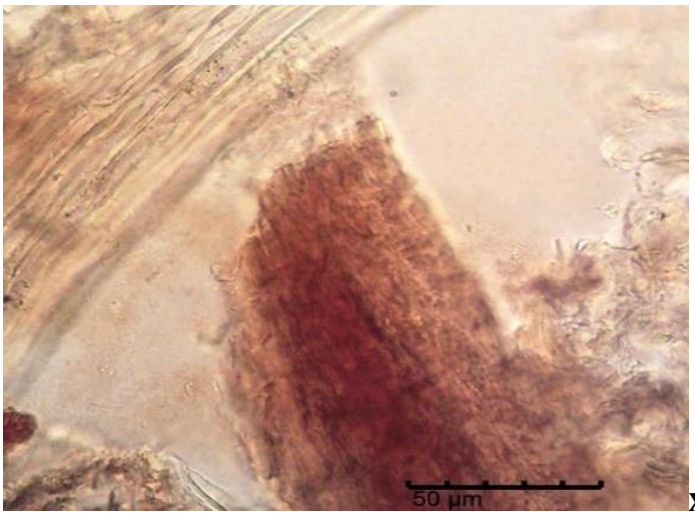
The summary of the microscopic characteristics of *Keetia venosa* leaf is presented in Table 3.

Table 3: The Microscopic Characteristics of *Keetia venosa* Leaf

Epidermal cell	Epidermal cells are polygonally-shaped with straight anticlinal cell walls on both the adaxial and abaxial surfaces
Stomata type	The leaf is hypostomatic (stomata occur only on the lower surface) with paracytic type of stomata (two subsidiary cells occur parallel on both sides of the guard cells)
Trichome	Present; Uniseriate covering trichomes seen
Stomata density	27.32 ± 0.93 mm^{-2}
Stomata length	22.13 ± 1.42 µm
Stomata width	16.84 ± 0.73 µm
Stomata size	372.67 ± 21.06 μm^2
Vein islet number	8.23 ± 0.32 mm^{-2}
Veinlet termination number	11.62 ± 1.82 mm^{-2}
Palisade ratio	7.33 ± 0.14

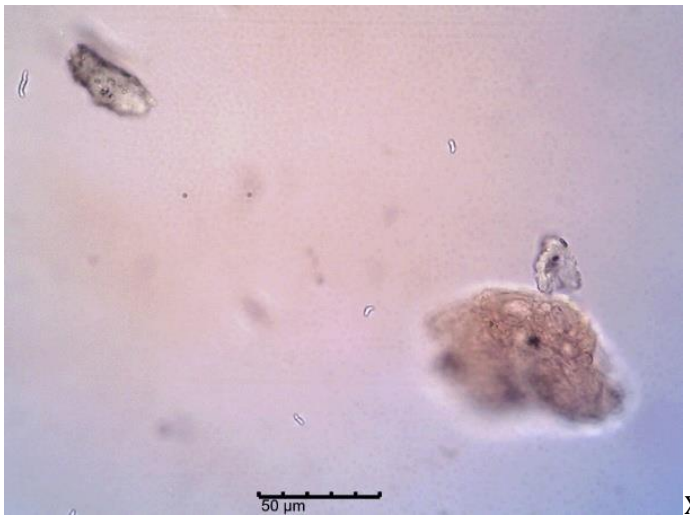
3.2 Chemo-microscopic Characteristics

The chemo-microscopic features of the powdered *Keetia venosa* leaf are shown in Figure 5-7.



X400

Figure 5: Chemo-microscopy of *Keetia venosa* powder showing lignified fibre and parenchyma



X400

Figure 6: Photomicrograph of the powdered drug showing calcium oxalate crystals and palisade tissue

X100

Figure 7: Chemo-microscopic study showing isolated and coiled fibre element of *Keetia venosa* powder

The summary of powdered chemo-microscopic characteristics of *Keetia venosa* leaf is presented in Table 4.

Table 4: The Powdered Chemo-microscopic Characteristics of *Keetia venosa* leaf

Parameter	Reagent(s)	Result
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism shape
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride;Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Ninihydrin	Present
Oil	Sudan III reagent	Present

3.3 Thin-Layer Chromatography

N-Hexane 9: 2 Ethyl acetate Solvent front: 5.80 cm.

The Rf values obtained from the Thin-Layer Chromatography of *Keetia venosa* Leaf extract is presented in Table 5.

Table 5: Rf Values of *Keetia venosa* Leaf Extract

Distance moved by solute (cm)	Rf Value
0.30	0.05
0.60	0.10
1.00	0.17
2.70	0.47
3.70	0.64

3.4 Phytochemical Screening Result

Different phytochemical constituents that can be obtained from *Keetia venosa* leaf using different solvent fractions with varying polarity is presented in Table 6.

Table 6: Phytochemical Constituents Present or Absent in *Keetia venosa* Leaf from Different Solvent Fractions

Phytochemical	Methanol Fraction	n-Hexane fraction	Ethylacetate fraction	Butanol Fraction
Alkaloids	+	-	-	+
Phenolics	+	-	+	+
Tannins	+	-	-	+
Flavonoids	+	-	+	+
Terpenoids	+	+	+	+
Glycosides	+	-	-	+
Saponins	+	-	+	+
Carbohydrates	+	_	_	+
Proteins	+	_	+	+
Fixed oil/fats	+	_	_	+

(-) Absent (+) Present

3.5 Acute Toxicity

Neither mortality nor severe toxic or side effects were observed during the 24 hours of acute toxicity experimental period even when 5000 mg/kg of the extract was administered to the animals.

3.6 Anti-Inflammatory Activity

The anti-inflammatory activity of *Keetia venosa* extract was evaluated using the egg albumin-induced paw oedema mode and the values are presented in Table 7.

Table 7: The Anti-inflammatory Activity of Methanol Leaf Extract of *Keetia venosa*

	Mean oedema ± SD (Percentage inhibition relative to control)					
Treatment	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
Control	0.99± 0.39	0.99± 0.23	0.91± 0.17	0.73± 0.18	0.61± 0.24	0.57± 0.22
Ibuprofen (40 mg/kg)	0.96± 0.33 (3.03%)	0.78± 0.39 (21.21%)	0.70± 0.29 (23.07%)	0.64± 0.25 (12.33%)	0.59± 0.27 (3.27%)	0.50± 0.25 (12.28%)
Extract (250 mg/kg)	0.84± 0.22 (15.15%)	1.02± 0.21 (-3.03%)	0.82± 0.28 (9.89%)	0.66± 0.31 (9.59%)	0.53± 0.29 (13.11%)	0.46± 0.26 (19.30%)
Extract (500 mg/kg)	0.93± 0.36 (6.06%)	0.85± 0.27 (14.14%)	0.77± 0.30 (15.38%)	0.64± 0.40 (12.33%)	0.59± 0.37 (3.28%)	0.56± 0.37 (1.75%)
Extract (1000 mg/kg)	1.11± 0.50 (-12.12%)	0.89± 0.41 (10.10%)	0.76± 0.42 (16.48%)	0.61± 0.35 (16.44%)	0.50± 0.28 (18.03%)	0.43± 0.26 (24.56%)

$$\%\text{Inhibition of oedema} = \frac{CONTROL - EXTRACT}{CONTROL} \text{ X } 100$$

Values in the table are presented as mean ± Standard deviation (n =5), $p > 0.05$: Not statistically significantly different from control.

4 DISCUSSION

Pharmacognostic evaluation is an important component of the standardization of medicinal plants because it provides essential information for the identification, authentication and quality assessment of crude plant materials. Accurate identification and quality control of starting materials are essential for ensuring the reproducibility, safety and efficacy of herbal medicines [20]. In the present study, a combination of macroscopic, organoleptic, microscopic, chemo-microscopic and phytochemical evaluations were used to characterize the leaves of *Keetia venosa*. The macroscopic characteristics observed (Table 1) included opposite leaf arrangement, an elliptic shape, an acute apex and base, pubescence and a green colour. These features are consistent with previously reported morphological descriptions of *Keetia* species, including the presence of hair-like structures on the leaves and similar leaf arrangement patterns [21,22]. The organoleptic evaluation (Table 2) further characterized the powdered leaves as greenish, coarse, slightly bitter and possessing an uncharacteristic odour. These macroscopic and organoleptic characteristics may serve as useful preliminary diagnostic features for the identification and authentication of the crude drug.

Microscopic examination (Table 3) revealed distinct features on leaf surfaces. The adaxial epidermis (Figure 1) consisted of polygonal cells with straight anticlinal walls, while stomata were absent indicating a hypostomatic leaf surface, uniseriate covering trichomes were present. The abaxial epidermis (Figure 2) also consisted of polygonal cells with straight anticlinal walls and contained paracytic stomata, together with uniseriate covering trichomes. These anatomical characteristics provide useful diagnostic features for the identification and authentication of the species. Transverse anatomical examination (Figure 3) revealed single-layered upper and lower epidermises and a U-shaped vascular bundle containing lignified xylem tissues. The ground tissue and pith were non-lignified, while covering trichomes were observed along the abaxial surface of the midrib (Figure 4). These anatomical characteristics, particularly the arrangement of the epidermal tissues, vascular bundle, lignified xylem, stomatal distribution and trichome characteristics, may serve as important microscopic parameters for the standardization of *Keetia venosa* leaves.

Powdered chemo-microscopic examination (Table 4) further revealed reticulate vessel elements associated with fibre and parenchyma cells. The vessel and fibre elements were lignified (Figure 5). Prism-shaped calcium oxalate crystals,

palisade tissue, isolated and coiled fibre elements were also observed (Figure 6 and Figure 7). These features provide additional diagnostic characteristics for the crude drug and may be useful in distinguishing *Keetia venosa* from potential adulterants or substitute materials. The use of microscopic and chemo-microscopic parameters is particularly important in the quality assessment of powdered herbal materials, where macroscopic identification may be limited [23].

Thin-layer chromatography analysis demonstrated the presence of multiple detectable constituents in the methanol leaf extract (Table 5). The observed separation of spots under ultraviolet illumination indicates differences in the physicochemical properties and relative affinities of the extract constituents for the stationary and mobile phases. The resulting chromatography profile provides a preliminary chemical fingerprint of the extract and may be useful for future quality control and standardization studies of *Keetia venosa*.

Phytochemical screening of the methanol leaf extract (Table 6) revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, glycosides and saponins etc. The detection of these secondary metabolites is pharmacologically relevant, as many of these compound classes have been associated with antioxidant, antimicrobial, anti-inflammatory, analgesic and other biological activities. The findings are consistent with previous reports of phytochemical constituents in the genus *Keetia*. Freire [6], using Proton Nuclear Magnetic Resonance (^{1}H-NMR) metabolic fingerprinting, reported the presence of triterpenoids and phenylpropanoid-related metabolites in *Keetia* species, although terpenoids resonances were more pronounced in *Keetia leucantha* than in *Keetia venosa*. The phytochemical profile observed in the present study therefore provides preliminary evidence of the chemical constituents that may contribute to the biological properties of *Keetia venosa*.

The acute toxicity study showed no mortality or observable signs of toxicity following administration of the methanol leaf extract at the tested dose of 5000 mg/kg. The absence of abnormal behavioural or physical symptoms during the observation period suggests that the extract has a relatively wide margin of acute oral safety under the conditions of the present study. Based on the OECD classification framework, the extract may be regarded as practically non-toxic at the tested dose [18]. However, the findings should be interpreted within the context of an acute toxicity assessment and do not exclude the possibility of sub-acute, chronic or organ-specific toxicity following prolonged exposure.

The anti-inflammatory activity of the extract was evaluated using the egg albumin-induced paw oedema model, a commonly used model for assessing acute inflammation. The induction of inflammation resulted in an increase in paw volume in the control animals. Treatment with different doses of the *Keetia venosa* methanol leaf extract appeared to reduce the increase in paw volume relative to the control, suggesting a possible inhibitory effect on the inflammatory response. However, the observed reductions were not statistically significant compared with the control group ($p > 0.05$) (Table 7). The observed anti-inflammatory effect may be attributed to the phytochemicals detected in the extract. Alkaloids, tannins, saponins, glycosides, phenolics, flavonoids and terpenoids have been associated with anti-inflammatory activity through modulation of inflammatory mediators and pathways involved in oedema formation. Similar associations have been reported in other Rubiaceae species, including *Wendlandia heynei*. [24]. Nevertheless, the absence of statistical significance in the present study indicates that the anti-inflammatory activity of *Keetia venosa* under the experimental conditions employed was not sufficiently pronounced to demonstrate a significant difference from the control group.

5 CONCLUSION

Overall, the findings of this study provide useful pharmacognostic and phytochemical information for the identification, authentication and preliminary standardization the methanol extract of *Keetia venosa* (Oliv.) Bridson. The extract contained alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, glycosides, and saponins, In addition, the pharmacognostic characteristics established in this study provide valuable diagnostic parameters for the authentication and quality control of the plant material. Although the extract demonstrated an apparent reduction in egg albumin-induced paw oedema, the anti-inflammatory effect was not statistically significant compared with the control group. The extract did not produce observable signs of acute toxicity at the tested dose of 5000 mg/kg, suggesting relative safety under the conditions of the acute toxicity study. The present study demonstrates that the methanol leaf extract of *Keetia venosa* possesses promising pharmacological activity, as evidenced by its ability to reduce egg albumin-induced paw oedema in rats. The extract produced variable inhibitory effects across the tested doses and observation periods, with the 1000 mg/kg dose producing the highest inhibition of oedema (24.56%) at the sixth hour. However, the observed reductions were not statistically significant compared with the control group ($p > 0.05$). These findings suggest that the methanol leaf extract of *K. venosa* may possess anti-inflammatory potential, although the effect under the present experimental conditions appears modest.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines and were approved by the appropriate Animal Research Ethics Committee. Ethical approval number PHAC/COOU/AREC/2021/004.

Author Contribution

- Dr. E.I. Ezeonyi: Supervision, Validation, Writing - Methodology, review & editing.
- Okeke Chukwuebuka Thankgod: Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft.
- Chukwunonso. C. Onwuzuligbo-Review & editing.
- Madubogwu Ngozi U-Editing
- Immaculeta Chikamnele Umeyor- Review

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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