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Original Research Article

Preliminary Standardization of *Fadogia cienkowskii* Schweinf (Rubiaceae) Leaves and Evaluation of the Anti-inflammatory Activity of the Methanol Extract

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ABSTRACT

Fadogia cienkowskii (family, Rubiaceae) has shown diverse activities in ethnomedicine. However, there are no established pharmacognostic standards for the plant. The study evaluated the phytochemical, proximate, microscopic and macroscopic properties of *F. cienkowskii* leaf and its methanol extract´s anti-inflammatory effects in animal models. The phytochemical and proximate analyses were evaluated by standard methods. The sodium hypochlorite (3.5%)-stained leaf was subjected to macroscopic examination. The leaf was bleached with and stained with safranin solution for light microscopic examination. An acute toxicity test in mice adopted the Lorke method, while an anti-inflammatory study in rats adopted egg albumin- and xylene-induced models. Alkaloids, saponins, tannins and flavonoids were detected in the extract. The proximate composition showed high moisture, total and sulphated ash values of 2.33, 3.85 and 3.0% respectively. The leaf (8 x 2.5 cm size) appeared rough in texture with a pubescent surface and bitter taste. Microscopic examination showed the stomata density, index, length, width, size and shape of 98.82 mm^{-2}, 18.92, 19.70, 14.11 and 227.97 µm, respectively, with the upper epidermis covered by a cuticle to prevent excessive perspiration. With LD_{50} > 5000 mg/kg, a 200 mg/kg dose of methanol extract elicited 75.20 and 56.74% inhibition of egg albumin- and xylene-evoked paw and topical ear oedema, respectively, in rats with no significant difference ($p > 0.05$) compared with diclofenac (68.8 and 69.3 %). The phytochemical, proximate, macroscopic and microscopic characteristics of the leaf provided empirical data necessary for establishing the standards for the crude herbal anti-inflammatory property of *F. cienkowski*.

Keywords: Acute Toxicity, Anti-Inflammatory, *Fadogia Cienkowski*, Macroscopy, Microscopy, Phytochemical, Proximate

Introduction

Herbal medicine has remained an essential component of traditional healing, especially in African and Asian countries.[1] At a time, the use of herbs for therapy was the only available option in African traditional medicine, resulting in total reliance on them. However, the advent of modern medicine has dwarfed the dependency on herbal medicines in some regions due to toxicity, potency and regulatory concerns.[1] The recent resurgence in the therapeutic use of herbs and related herbal products is motivated by the accessibility, availability and sustainability of alternative herbal remedies across the regions. Herbal medicines can be made from different morphological parts of a plant and have been extensively used to alleviate pain and inflammation.[2]

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Inflammatory disorders are complex biological responses of the body to harmful stimuli such as radiation, chemicals, physical, infectious and immunological factors.[3] The response is initiated when the cells in the diseased, injured or infected tissue produce and release chemical agents which cause swelling, redness, pain, heat and loss of function.[2] An inflammatory response is beneficial to health when it targets a specific biological event, has a defined start and endpoint, is not too intense and does not interfere with daily activities. It can lead to significant health challenges when the response becomes chronic, resulting in far-reaching effects such as systemic lupus erythematosus, psoriasis, inflammatory bowel diseases, asthma, rheumatoid arthritis, type 2 diabetes, cardiovascular disease and cancer.[2] Several cellular mechanisms, such as angiogenesis, tissue remodelling, macrophage activation, T cell activation, cytokine production and infiltration of mononuclear cells, have been associated with chronic inflammation.[4] Anti-inflammatory agents, secondary metabolites, omega-3 fatty acids, antioxidants, dietary fibres and herbal remedies from plants play significant roles in alleviating chronic inflammation.[3]

Fadogia cienkowskii Schweinf is an erect underground flowering shrub in the family of Rubiaceae, representing one of the 13,000 species of the commonly referred to bedstraw or coffee family.[5] As a native to tropical Africa, the plant is well distributed across Nigeria´s Savannah regions.[5] It has been used in folkloric remedies for the management of inflammation, male impotency and gastrointestinal tract problems, especially in children. The Igede people of Benue State in Nigeria use

the leaf macerate of the plant to treat pyrexia and convulsions in children.[6] In Cameroon, the powdered root of the plant has been used in the management of male impotency.[6] Several Fadogia species have demonstrated diverse activities including antimalarial, antioxidant, antimicrobial, antihypertensive and anti-inflammatory effects.[5-7] The biological activities and diverse phytochemotypes of the *F. cienkowskii* and other Rubiaceae species are currently at a vantage position in ethnomedicine, thus necessitating their standardization and further development.

Standardization of medicinal plants represents a crucial step in ensuring compliance with quality, safety and efficacy standards of herbal medicines as specified in monographs and official compendia. It provides the basis for quality assurance and compliance with regulatory requirements in the development, production and utilisation.[8] Some of the standardization processes included botanical identification (taxonomic and botanical characteristics), plant part specification (leaves, root, stem bark, flowers), authentication (microscopy, chemical profiling, DNA fingerprinting), quality control (physicochemical properties), chemical standardization (high performance liquid chromatographic, gas chromatography-mass spectrometric analysis), microbial load test, heavy metal analysis, pesticide residue testing, stability testing as well as compliance with pharmacopoeia standards.[9]

Apart from the safety and efficacy of medicinal plants, a lack of basic standards can hinder the development of the plants into an acceptable herbal formulation for therapy.[10] Despite the widespread use of *F. cienkowskii* leaves in ethnomedicine for its medicinal properties, such as anti-inflammatory claims, there are scarce data regarding the quality, safety and efficacy of the various species of Fadogia.[5-7] The lack of standardized protocols for the extraction, identification and quantification of bioactive secondary metabolites poses a significant challenge to their integration into modern medicine.[11] There is also a lack of information on and underexplored exact mechanisms of the anti-inflammatory activity of *F. cienkowskii* leaves. To address this gap, the study evaluated the phytochemical, proximate, microscopic and macroscopic properties of *F. cienkowskii* leaves and its anti-inflammatory effects in rat models.

Materials and Methods

Plant sample

The leaves of *F. cienkowskii* were harvested at Obukpa (GPS location: 6.9003 °N, 7.3935 °E) in Nsukka, Nigeria, on the morning of 10th February 2023. The leaves were authenticated by Mr. Felix Nwafor, a taxonomist with the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. A voucher identification code PCG/UNN/0092 was assigned to the plant sample and the specimen was deposited at the herbarium of the institute for future reference.

Preparation of plant extract

The leaves of *F. cienkowskii* were dried in the shade for 10 days and then ground to a coarse powder using a hammer mill (BSF-8, China). The dried ground leaves (518 g) were placed in a conical flask and cold-macerated in 2.5 L of 95%v/v methanol. The container was sealed and agitated intermittently using a mechanical agitator for 48 h to facilitate extraction. Thereafter, the mixture was filtered using a fine mesh strainer cloth, after which, the filtrate collected was transferred into a rotatory evaporator at 40 ℃ to concentrate the extract.[12] The concentrated crude extract (FCMe) was weighed and stored in a refrigerator at 4 ℃ until needed.

Phytochemical analysis

The phytochemical content of *F. cienkowskii* was tested for the presence of alkaloids, saponins, tannins, flavonoids, resins and steroids following standard phytochemical methods.[13]

Macroscopic analysis

The collected leaves of *F. cienkowskii* were observed for their lamina, apex, texture, base, surface, taste, vein arrangement and general architecture.[14]

Microscopy analysis

The clearing process was used to prepare the foliar epidermis of the adaxial and abaxial surfaces of the leaf sample. The leaf was first cleaned by immersion in commercial bleach solution of sodium hypochlorite (3.5%) for 18 h.[15] The epidermal strips were then scraped gently using forceps, stained with safranin solution, mounted on a dust-free slide and a cover slip was gently placed. The prepared slides were examined with a light microscope (Olympus 271961, Tokyo) at magnifications of x40, x100, and x400, and photomicrographs were obtained with a Motican camera 2.0.

Proximate analysis

Moisture content determination

The moisture content was evaluated using the loss-on-drying method.[16] An empty dish, heated to a constant weight and allowed to cool, was placed in a desiccator. Then, 3.0 g of the pulverised leaf was weighed, placed into the dish and the contents were dry-heated to a constant weight in a muffler at 105 ℃.[16] The constant dry weight was achieved by weighing at 30-minute intervals after an hour of initial drying. The moisture load (%) was obtained relative to the original powdered leaf.

Determination of total ash value

The total ash was determined by weighing 2 g of powdered leaves into an empty nickel crucible, previously heated to 105 ℃, cooled and dried. The crucible and its contents were placed in a furnace set at 450 °C. The crucible was heated until a charred black mass was obtained free of moisture. The heating temperature was slowly increased until a greater proportion of the carbon residue was evaporated and then the heat was applied vigorously until the residue was almost white.[16] After cooling, the crucible and its contents were weighed. The cycle was repeated until the ash weight remained constant. The ash value (%) was then determined relative to the original weight.

Determination of acid-insoluble ash

The acid-insoluble ash was measured by placing the total ash in 25 mL dilute HCl in a pre-weighed beaker, allowing it to simmer for 5 min and then straining through an ashless filter paper. The contents of the beaker were washed with water through filter paper. The filter paper was dried in an oven at 105 ℃, folded into a cone shape and placed in a pre-weighed crucible. Thereafter, the crucible was heated to char the filter paper into a black mass, then intensely heated for a few minutes for total incineration.[16] The crucible's contents were cooled and weighed and the acid insoluble ash value (%) was computed using the total ash value experiment's initial weight of the powdered sample.

Determination of water-soluble ash

A 2 g powder of leaves was dispersed in a crucible previously fired at 450 ℃ to a constant weight. The carbon content was burnt out by firing at low temperatures and gradually increased until all the carbon had been burned away, cooled and reweighed until a steady weight was obtained. The contents were transferred to a 25 mL water in a beaker, heated for 5 min, filtered using ashless filter paper and then the residue was oven-dried and compressed into a tiny cone.[16] This was placed in the crucible, and the heating was continued until the ashless filter paper was removed. The water-soluble ash was obtained from the total ash.

Determination of sulphated ash

A 2.0 g powdered leaves were placed in a nickel crucible previously ignited to a constant weight at a dull red heat. The carbon content was burnt off, the material was wet with dilute H_2SO_4, heated at 500 °C and the crucible was cooled in a desiccator. More dilute H_2SO_4 was added, and the temperature was raised to around 800 ℃, with periodic cooling and reweighing until a steady weight was attained.[16]

Pharmacological evaluation

Experimental rats and mice

Albino rats (60-100 g) and Swiss mice (20-30 g), both male and female, were procured from the Pharmacology and Toxicology Department, University of Nigeria, Nsukka. Ethical approval was obtained from the institutional animal ethics committee on the use of laboratory animals (approval ID: UN/FPS/2023-2003). The animals were provided with water and standard rodent commercial diet whenever necessary, housed in cages with sawdust beddings at 25 ± 2 ℃, 50 ± 10% relative humidity

with a 12 h day/night cycle and acclimatised for 7 days before the experiments.

Acute toxicity test
The Lorke procedure of LD_{50} determination was adopted.[17] A total of 12 mice of either sex were used (n = 3). The extract (1000, 100 and 10 mg/kg) was administered orally to the mice in appropriate groups and monitored for 24 h for signs of tingling, inflammation and mortality. If no death of mice per group was recorded at the end of 24 h, the study proceeded to the next stage. In this stage, separate mouse each received 1600, 2800 and 5000 mg/kg doses of the extract. The mice were observed for death or signs of intoxication for 24 h. The LD_{50} of the extract was obtained from the geometric mean of the least toxic dose and the highest non-lethal dose.[18]

Egg albumin-induced rat paw oedema
The rats, divided into five groups (n = 5), were orally given methanol extract of *F. cienkowskii* (50, 100 and 200 mg/kg) to groups B to D respectively while groups A and E rats received 5 mL/kg of normal saline and 10 mg/kg of diclofenac respectively. After 30 min, inflammation was evoked by injecting fresh hen´s egg albumin (0.10 mL) into the sub-plantar region of the right hind paw.[19] The paw volumes of all the groups were determined by displacement of water at 0, 1, 2, 3, 4, and 5 h after egg albumin injection. The increase in paw volume was noted and compared with the basal volume. The inhibition (%) was obtained using equation 1.

$$Inhibition\ (\%) = \left(1 - \frac{dt}{dc}\right)100 \qquad \text{eq. 1}$$

Where dt = difference in paw volume in the treatment group, and dc = difference in paw volume in the control group.

Xylene-induced ear oedema
The rats were divided into 5 groups (n = 5) of A to E. Doses 0.5 mL/kg of normal saline, graded doses of *F. cienkowskii* (50, 100 and 200 mg/kg) and diclofenac (10 mg/kg) were administered topically to the posterior surface of the right ear of the rats using a micropipette to groups A-E respectively.[20] Xylene (0.03 mL) (Merck KGaA, Darmstadt) was administered to the anterior surface of the right ear thirty minutes after topical administration of the extract and diclofenac. The rats were left for 15 min, after which they were sacrificed under chloroform anaesthesia and both ears examined. Using a 6 mm cork borer, a circular section of the right and left ear was removed and weighed.[20] The ear oedema inhibition (%) was calculated relative to the left ear without xylene using equation 2.

$$Inhibition\ (\%) = 1 - \left(\frac{wR}{wL}\right)100 \qquad \text{eq. 2}$$

Where wR = right ear weight and wL = left ear weight (control)

Data analysis
The results were analysed with one-way ANOVA and post hoc multiple comparisons to check for the least significant difference and represented as the mean ± SEM (standard error mean). Differences between means were accepted to be significant at $p < 0.05$.

Results and Discussion

The World Health Organisation guidelines for herbal drug or formulation standardization stipulated that several parameters, such as botanical properties, foreign organic matter contents, microscopic, histochemical, macroscopic, histological assessment, phytochemical fingerprinting, physicochemical characterisation, biological activities, microbial load and other parameters must be determined as criteria for acceptance.[21] While these properties may not be determined in a single study such as the present study, there is a need to identify and document the most crucial standards (including physical, chemical and biological properties) to complement several other biological activities of *F. cienkowskii*.

Extraction yield
The yield (64 g) of the methanol extraction of *F. cienkowskii* was expressed as a percentage of the 518 g of coarsely powdered sample and was calculated to be 12.35%. One of the basic steps in the standardization of herbal medicines or their formulation involves the extraction of their constituents with suitable solvents. The identification of the optimum solvent systems for the extraction of phytochemicals represents a significant step in herbal medicine standardization and development. Previous studies have identified alcoholic extraction as the optimum solvent for extracting both polar and non-polar secondary metabolites due to their bipolarity.[22] To be considered for inclusion in the pharmacopoeia, the most effective extraction method and the optimum extracting solvent must be established. This study, with a 12.35% yield in methanol following cold maceration, indicated a high yield compared with similar protocols on different plants. Techniques such as the Soxhlet extraction could provide high yields in different plants. However, the challenges associated with this protocol, such as denaturing of thermolabile constituents and batch extraction procedure, could limit their applicability.[23]

Phytochemical content of F. cienkowskii
The phytochemical tests identified the presence of all the secondary metabolites- alkaloids, saponins, tannins, flavonoids, resin, steroids and terpenes/terpenoids- tested. Most of the properties of herbal medicines are controlled by the phytochemical constituents of the plants. This places the secondary metabolites as a major factor in the standardization of herbal drugs and their formulation. Standardization of herbal formulation plays a significant role in maintaining the stability and quality of formulations, considering various factors that affect the content of the secondary metabolites in plants.[22] In this study, qualitative assessment of the phyto marker compounds provided a vital parameter for quality authentication. The presence of alkaloids, saponins, tannins, flavonoids, resin, steroids and terpenes/terpenoids in FCMe serves as preliminary testing for different chemotypes, quantification of the chemotypes and fingerprint profiling [22]

Proximate contents of F. cienkowskii
The proximate contents *of F. cienkowskii* leaves showed high total ash, sulphated ash and moisture content (Table 1). Plants are generally known to provide economic, therapeutic and nutritional values, with a thin line between the nutritional and therapeutic benefits. Several assurances of quality, efficacy and toxicity of herbal medicines or their formulation have been attributed to their proximate composition, such as the ash values, moisture content and extractive values, which are utilised to judge the purity and identity of herbal medicine.

Table 1: Proximate content of *Fadogia cienkowskii*

Parameters	Content (%)
Total ash	3.85±0.59
Acid insoluble ash	1.00±0.06
Water-insoluble ash	0.50±0.14
Sulphated ash	3.00±0.42
Moisture content	2.33±0.84

Moisture is crucial in the storage and quality control of herbal medicines, especially at a crude level, since water held in the matrix of a dried crude drug sample can initiate enzymatic activity, leading to the biodegradation of the drug and consequent deterioration.[16] Equally important is the false weight of the sample that can arise due to the presence of excess moisture. Hence, limits have been set by both the British and the United States Pharmacopoeias establishing the maximum amount of percentage moisture content in a given crude drug sample for quality control, assessment and acceptability.[24] The total ash value is an analytical procedure used for the determination of the presence of impurities and extraneous materials, especially that of silica, in a given crude drug sample.

Macroscopic characterization of F. cienkowskii
The macroscopic characteristics of the leaves of *F. cienkowskii* showed an 8 cm by 2.5 cm size with oblong-elliptic-shaped green upper and

grey lower surfaces (Table 2). The macroscopic characterization of the leaves was carried out to complement the phytochemical and proximate analyses to establish adequate standards for crude *F. cienkowski* leaves. This is essential for maintaining the efficacy, quality, purity, authenticity and consistency of crude drug samples.[14, 15] The study, therefore, described the detailed standards for the colour, texture and shape, odour and taste, surface characteristics, size and venation patterns, as well as the presence of any foreign matter or adulterants.

Table 2: Macroscopic properties of *Fadogia cienkowskii*

Feature	Observation
Shape	Oblong-elliptic
Size	8 cm in length and 2.5 cm in breadth
Colour	Green on the upper surface, Grey on the lower surface
Taste	Bitter
Texture	Rough
Apex	Acute
Base	Acute
Margin	Entire
Surface	Pubescent
Leaf arrangement	Whorl (three leaves at each node)
Vein	Lateral with mixed alternate and opposite

Microscopic properties of F. cienkowskii leaves.

The microscopic properties of *F. cienkowskii* leaves showed stomata parameters such as the density, index, length, width, size and shape of 98.82 ± 1.18 mm^{-2}, 18.92 ± 0.18, 19.70 ± 0.45, 14.11 ± 0.20 and 227.97 ± 7.95, respectively (Table 3). To further complement other established standards for the *F. cienkowski* sample, the microscopic analysis showing the epidermal, stomata and trichome properties of the leaves was determined. The epidermal cell is a vital microscopic component that confers structural integrity to the leaves and can be used to identify and authenticate crude drug leaves using their size, shape and arrangement.[15] Apart from regulating water loss and gas exchange, the stomata properties of leaves are unique to each plant species, thus providing key identifiers in herbal drug standardization. The presence, density and morphology of trichomes also provide identification fingerprints since they house secondary metabolites in some plants.[25]

Transverse sections of the leaf of F. cienkowskii

The presence of the uniseriate upper and lower epidermis, with no chloroplasts, was revealed in a transverse section of the leaf. The upper epidermis was covered by a cuticle to prevent excessive water loss. Tightly packed palisade mesophyll cells were loosely fitted to allow air gaps (Figure 1). The midrib carries the vascular bundle, which includes the phloem (exteriorly positioned) and xylem (interiorly located) - the main conducting organs. A mass of parenchymatous cells created the pith in the middle of the *F. cienkowski* leaf for easy authentication. Some markers present in the sections, such as the vascular bundle arrangement, palisade and spongy parenchyma and the structure of the epidermis, provide strong evidence for the identification of plant species.[15,26] The microscopic structures of the *F. cienkowskii* leaf (Figure 2) showed the characteristic markers such as the calcium oxalate crystal, non-glandular trichome with oil cells, cystoliths, cicatrix and trichome (with its oils and base). These microscopic features are valuable in identifying, authenticating and quality control of crude drugs.[26]

Table 3: Foliar epidermal properties of *Fadogia cienkowskii*

Parameters	Properties
Epidermal cell	The adaxial surface of epidermal cells is polygonal, with straight anticlinal cell walls, but the abaxial surface is irregular.
Stomata type	The leaf is hypostomatic (stomata only appear on the lower surface)
Trichome type	There is an absence of multicellular covering trichomes. Glandular trichomes are absent.
Stomata density	98.82±1.18 mm^{-2}
Stomata index	18.92±0.18
Stomata length	19.70±0.45 µm
Stomata width	14.11±0.20 µm
Stomata size	227.97±7.95 µm
Trichome density	Adaxial surface =1.89 mm^{-2}; abaxial surface = 4.46 mm^{-2}
Trichome index	Adaxial surface = 1.05; abaxial surface 1.03
Trichome length	Adaxial surface = 207.74±33.42 µm; abaxial surface = 267.55±41.16 µm
Vein islet number	5.46 mm^{-2}
Veinlet termination number	4.82 mm^{-2}

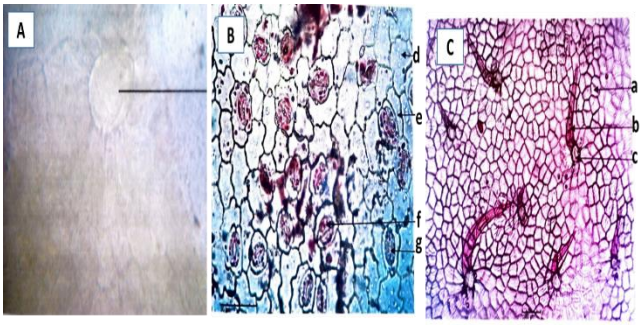

Figure 1: Transverse section of *Fadogia cienkowskii* leaf x40 (A) showing abaxial surface x400 (B) and adaxial surface x100 (B); epidermal cells (a), subsidiary cell (b), guard cell (c), stomatal pore (d), epidermal (e), trichome (f), trichome base (g)

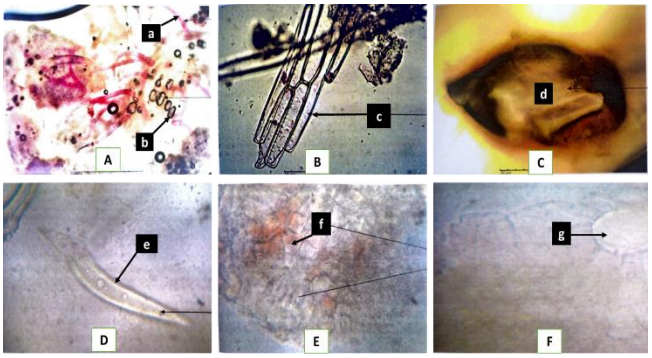

Figure 2: Microscopic properties of *Fadogia cienkowskii* leaf showing the general microscopy of the powdered leaf x400 (A), epidermal cell x400 (B), calcium oxalate crystal x400 (C), non-glandular trichome with oil cells x400 (D), epidermal cell from the midrib (E) and transverse section of the trichome x400 (F); trichome (a), cystoliths (b), cicatrix (c), calcium oxalate crystal (d), trichome (e), trichome with oils (f) and trichome base (g)

Acute toxicity test

The acute toxicity assessment showed no death in the first phase during the administration of 10, 100, and 1000 mg/kg of the methanol extract

of *F. cienkowskii* leaf. In the second phase of the administration of 1600, 2900 and 5000 mg/kg, no deaths were also recorded, indicating no toxicity at 5000 mg/kg. Acute toxicity assessment in the standardization of herbs is crucial in evaluating the immediate harmful effects of crude herbal medicines for safety evaluation, risk assessment and regulatory compliance.[27] In this study, the high safety margin of the extract provides a strong basis for further evaluation of biological activity, such as the anti-inflammatory properties.

Anti-inflammatory activity of F. cienkowskii

Egg albumin-induced anti-inflammatory activity of FCMe

Anti-inflammatory activity showed a dose-dependent effect with the 200 mg/kg dose of FCMe eliciting the highest inhibition of 75.20 ± 0.98% compared with 50.20 ± 0.35% inhibition caused by the 50 mg/kg dose after 5 h (Figure 3). The 200 mg/kg dose elicited significantly higher inhibition ($p < 0.05$) than the standard within the 3rd and 5th hour, with no significant difference ($p > 0.05$) between 0-3 h. All treated groups showed significantly higher inhibition ($p < 0.05$) than the untreated. There was a more significant inhibition from 0-3 h, which later rose gradually from 3-5 h. An unusual trend by the 100 mg/kg dose showed a decline in inhibition between 2-3 h below the 50 mg/kg dose, which suddenly rose with no significant difference from diclofenac at 4 h and achieving 60.18 ± 0.42% inhibition at 5 h.

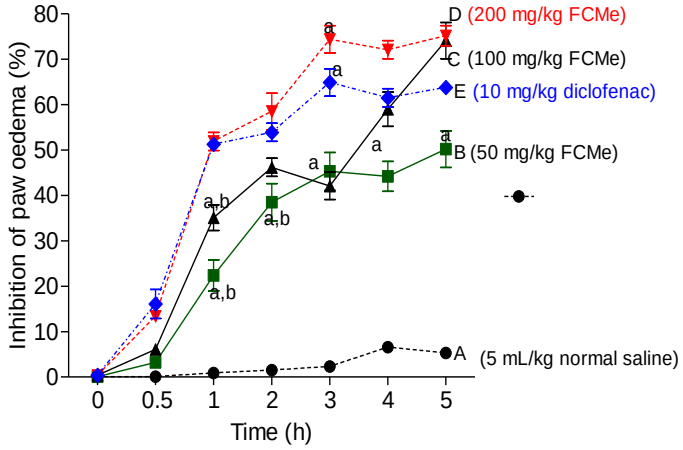

Figure 3: Anti-inflammatory activity of *Fadogia cienkowskii* methanol leaf extract on egg albumin-induced inflammation. Groups A to E represent normal saline, 50, 100, and 200 mg/kg FCMe and standard diclofenac-treated groups, respectively; $^{a}p < 0.05$ and $^{b}p < 0.05$ showed statistically significant differences compared with groups A and E.

Standardization of herbal products plays a significant role in maintaining the quality and potency of the medicine. The inhibition of paw oedema by 50, 100 and 200 mg/kg doses of *F. cienkowskii* peaked at 3-4 h and remained constant thereafter with 50.20 ± 0.35, 60.18 ± 0.42 and 75.20 ± 0.98% respectively, after 5 h compared with diclofenac (68.80 ± 1.12%). The egg albumin-induced paw oedema has been associated with the early release of serotonin, histamine and bradykinin, followed by eicosanoid bump.[28] The sustained reduction of paw oedema by *F. cienkowskii* methanol leaf extract at both early and late stages of inflammatory reactions could be useful in acute and chronic inflammatory disorders [19]

Xylene-induced anti-inflammatory activity of FCMe

The result of the topical anti-inflammatory activity shows that the FCMe showed significant inhibition of inflammation for 50, 100 and 200 mg/kg doses with 16.28 ± 1.87, 50.30 ± 7.02 and 56.74 ± 5.10% inhibition, respectively, compared with 69.30 ± 4.15% of diclofenac. There was a significant increase ($p < 0.05$) between the inhibition of inflammation by the 50 mg/kg dose and the untreated control. The observed activity in the egg albumin-induced model was validated by the xylene-induced ear oedema model, which resulted in a dose-dependent reversal of vasodilation and enhanced vascular permeability caused by substance P and bradykinin release. The overall effects of *F. cienkowskii* extract involved the inhibition of the egg albumin-evoked paw oedema caused by bradykinin, or serotonin, as well as the inhibition of substance P-mediated topical neurogenic inflammation [3,4,20]

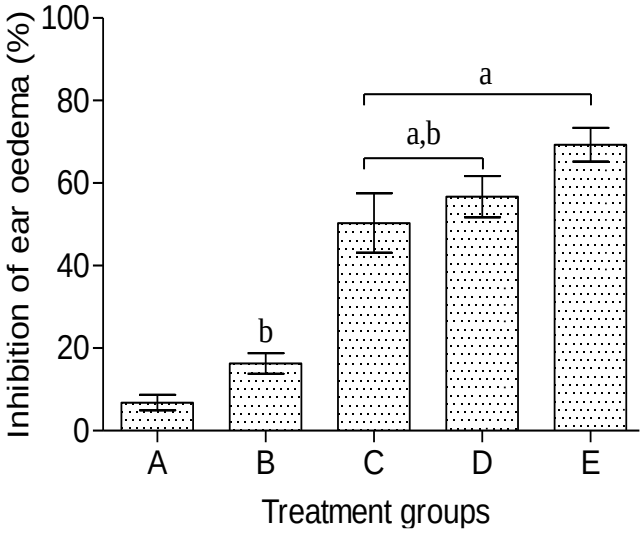

Figure 4: Effect of *Fadogia cienkowskii* methanol leaf extract on xylene-induced inflammation; Groups A to E represent normal saline, 50, 100, and 200 mg/kg FCMe and standard diclofenac-treated groups, respectively; $^{a}p < 0.05$ and $^{b}p < 0.05$ showed statistically significant difference compared with groups A and E

The study showed that *F. cienkowskii* methanol leaf extract has both strong *in vivo* and topical anti-inflammatory activity in both xylene and egg albumin-induced models, which could be attributed to the rich presence of secondary metabolites and proximate content of the leaves. The anti-inflammatory property of some Fadogia species, such as *F. agrestis*, *F. andansonii* and *F. homblei*, has been attributed to their phytochemical and proximate composition in previous studies.[29-31] This study, however, could neither establish the exact concentrations of the phytochemicals in *F. cienkowski* extract nor the mechanisms underlying its anti-inflammatory activity. The anti-inflammatory activity-guided isolation, characterization and quantification of secondary metabolites of importance are currently underway.

Conclusion

The methanol extract of *Fadogia cienkowskii* at all doses, but prominently at the 200 mg/kg dose, showed significant anti-inflammatory properties comparable with diclofenac in egg albumin and xylene-induced models. The high bioactivity of the low dose, LD_{50} > 5000 mg/kg of extract and the established standard parameters provide markers for the quality, potency and authenticity of crude *F. cienkowsk* leaf extract. These standards established for *F.sdogia cienkowski* provide promising starting points for the inclusion of the plant in the herbal pharmacopoeia or other monographs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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