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Original Research Article

PHARMACOGNOSTIC PROFILE AND ANTI-INFLAMMATORY ACTIVITY OF THE METHANOL EXTRACT OF *Sphenocentrum jollyanum* PIERRE (MENISPERMACEAE) STEM

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ABSTRACT

Sphenocentrum jollyanum is traditionally used by the Nsukka people to manage inflammatory conditions. This study established pharmacognostic standards for its stem and scientifically validated its anti-inflammatory efficacy. Fresh, powdered and anatomical sections of the stem were evaluated to establish the macromorphological, micromorphological, chemomicroscopic and some physicochemical and extractive parameters using established methodologies. Phytochemical screening was determined using standard methods. Acute toxicity study was conducted following Lorke's procedure. The anti-inflammatory effect of the methanol crude extract was assessed using egg-albumen-induced and carrageenan-induced paw oedema in rats. Macroscopic features include a thin, erect, sparingly branched stem up to 1.5 m tall; young stems are short-hairy, becoming glabrous when mature; grey bark with yellowish inner stem; woody and firm in older parts, softer in young shoots. Microscopy revealed prism-shaped calcium oxalate crystals, oil cells, lignified trichomes, vascular bundles, cambium, and epidermal cells; transverse sections showed a dorsiventral structure. Chemomicroscopy confirmed starch grains, lignified tissues, calcium oxalate, tannins, cellulose, and oil in the powdered stem. Physicochemical parameters of the powdered stem were: moisture content 11.30 ± 0.98%, total ash 6.53 ± 0.03%, acid-insoluble ash 3.50 ± 0.02%, water-soluble ash 2.91 ± 0.14%; extractive values: n-hexane 1.96 ± 0.02%, ethyl acetate 3.64 ± 0.02%, water 6.24 ± 0.01%, alcohol 7.30 ± 0.04 %. Phytochemical screening detected alkaloids, flavonoids, tannins, saponins, and anthocyanins. Acute toxicity indicated safety up to 5000 mg/kg body weight.The methanol crude extract exhibited significant anti-inflammatory activity ($P<0.05$) in rat paw oedema models. At 400 mg/kg, it achieved 47.02% inhibition in egg albumin-induced oedema and 41.93% in carrageenan-induced oedema, comparable to acetylsalicylic acid (46.26% and 43.57%, respectively).The established pharmacognostic standards serve as diagnostic tools for standardization and identification. The results confirm the stem's anti-inflammatory potential, positioning it as a promising source of safer anti-inflammatory agents.

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INTRODUCTION

A medicinal plant is a remarkable species from the plant kingdom, characterized by its diverse parts be it flowers, leaves, roots, stems, fruits, or seeds that are harnessed, either directly or through elaborate preparations, to ameliorate ailments and diseases. Understanding the therapeutic properties of these plants is not only vital for health but also plays an indispensable role in preserving the rich biological and cultural diversity of various societies[16]. The therapeutic potential of these plants derives largely from their secondary metabolites, which exhibit a myriad of pharmacological effects. An impressive array of medicinal plants serves as invaluable sources for compounds used to develop treatments for conditions ranging from cancer and viral infections to epilepsy, bacterial infections, inflammation, and pain relief, among others[4]. In many regions, especially rural areas grappling with limited communication, persistent poverty, and ignorance, modern health facilities often remain inaccessible, compelling many to revert to traditional medicine for everyday health concerns. It is noteworthy that those most affected often represent the most marginalized in the trade of medicinal plants [5].

Sphenocentrum jollyanum is a robust perennial plant that thrives in the shaded areas of dense forests, preferring regions with a mean annual rainfall of 1800 mm or more and mean temperatures ranging from 20 °C to 29 °C. Typically growing to an average height of 1.5 m with few branches, its smooth, wedge-shaped leaves measure 5–12 cm wide and can reach up to about 20 cm in length with a small-arrowed apex [27]. Different morphological parts of *Sphenocentrum jollyanum* including leaves, roots, stems, fruits, and seeds have been extensively used in the traditional and modern management of various diseases. The stem is used in ethnomedicine to treat various conditions including inflammation. Although the stem has been reported to be portent in ameliorating different disease conditions, its Pharmacognostic profile and anti-inflammatory activity have not been adequately elucidated. Hence, this study was undertaken to establish diagnostic parameters for the standardization of *Sphenocentrum jollyanum* stem and to validate its traditional medicinal use in the management of inflammatory condition scientifically.

Nia et al. [19] reported the anti-angiogenic activities of the methanol stem extract of *Sphenocentrum jollyanum* using in vivo chick chorioallantoic membrane (CAM) angiogenesis assay.

Olorunnisola et al[24] reported the hepatoprotective and antioxidant effect of *Sphenocentrum jollyanum* stem bark extract against CCl4-induced oxidative stress in rats.

Alese et al[3] on the other hand reported the Hypolipidemic and hypoglycemic activities of methanol extract of *Sphenocentrum jollyanum* on stretozocin induced diabetic Wistar rats.

Inflammation is a key and complex biological reaction of body tissues to different types of harmful triggers, like pathogens, damaged cells, or irritants. It is a natural and defensive process triggered by the body's white blood cells and the substancess they release, aimed at protecting against harm or infection caused by infectious agents such as bacteria and viruses[9]. .

Both the carrageenan and egg albumin-induced paw edema models are well-established and highly reproducible tools for inducing acute inflammation, widely employed in the screening of anti-inflammatory compounds. A significant commonality lies in their reliance on rapid release of early-phase mediators. Both models involve the prominent roles of histamine and serotonin in mediating the initial increase in vascular permeability and the onset of edema. Bradykinin is also a key early mediator in both models, contributing to vasodilation and pain. In their later phases, both models involve the sustained production of prostaglandins, contributing to the ongoing edema, pain, and fever [13].

MATERIAL AND METHODS

Collection and preparation of plant material

Sphenocentrum jollyanum stem collected at Iheowere Nsukka, identified and authenticated at the herbarium unit of the Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka by a taxonomist, Mr. Felix Nwafor. A voucher specimen (Number: UNN/11749) was deposited at the herbarium unit of the above-mentioned institution. The collected stem was dried under the shade (to a constant weight) at room temperature over two weeks. It was then pulverized to a coarse powder by a mechanical grinding machine. The stem powder (1.7 kg) was extracted with methanol (3.5 litres) by cold maceration at room temperature for 72 hours, filtered and concentrated and subsequently stored for further use in airtight containers in the refrigerator to prevent microbial attack.

Macroscopical and microscopic examination

The detailed macroscopic study of the stem and the microscopic examination of its powder were carried out using standard methods [11,20] .

Phytochemical Analysis.
Qualitative and quantitative phytochemical analysis of the extract were carried out using the method of Harborne [14]

Determination of some Physicochemical Parameters
The physicochemical parameters; moisture content, extractives and the ash-values are carried out by the methods described in Nwafor et al [21] .

Animal Ethical Committee approval
The research protocol received authorization from the Research Ethics Committee at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka with the Ethics Committee approval code FPSRA/UNN/2500132. The study adhered to the regulations set by the Committee for the Purpose of Control and Supervision on Experiments on Animals.

Acute Toxicity Test
An acute toxicity test was conducted following Lorke's technique [18]. Eighteen healthy adult albino rats of both genders, weighing between 100g and 125g, were sourced from the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The animals were housed in the animal house with free access to standard feed and water, and were allowed to acclimatize for one week prior to experimentation. The rats were randomly assigned to six groups of three animals each and treated as follows:
Group A recieved 10 mg/kg of stem extract.
Group B received 100 mg/kg of stem extract.
Group C received 1000 mg/kg of stem extract.
Group D received 1600 mg/kg of stem extract.
Group E received 2900 mg/kg of stem extract.
Group F received 5000 mg/kg of stem extract.
All the rats in each group were given the extract based on their body weight using oral administration and was watched for twenty-four (24) hours.

Evaluation of Anti-inflammatory Activity
The stem extract of S. jollyanum was tested for its anti-inflammatory effects using egg-albumin and carrageenan-induced oedema models in rats [2,34] . A total of 50 healthy albino rats of both genders were utilized for the study. Half of these rats, weighing between 125g and 130g, were subjected to the egg albumin-induced paw edema model, while the other half, weighing between 153g and 172g, were used for the carrageenan-induced paw edema model. Each group of 25 rats was further divided into five subgroups (A-E).
To induce acute inflammation, a 0.2 mL injection of either fresh egg albumin (for the first five groups of rats) or a freshly prepared 2% aqueous carrageenan suspension (for the remaining five groups) was administered into the sub-plantar region of the right hind paw [8]. 30 minutes post-induction, the animals were treated with the plant extract, aspirin (positive control), and distilled water. The linear diameter of the injected paw was measured using a digital Vernier caliper at 0, 1, 2, 3, 4, 6, and 24 hours following the administration of the phlogistic agent [1]. Oedema formation was determined by calculating the difference in paw diameter between the control group and the treated groups at each corresponding time point. The percentage of inhibition was subsequently calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\textit{Control paw diameter} - \textit{Treated paw diameter}}{\textit{Control paw diameter}} \times 100$$

Statistical Analysis
Analysis of variance (ANOVA) was used to analyze the data obtained which were expressed as mean $\pm$ standard error of the mean and statistical significant differences were determined at $P< 0.05$ using statistical package for service solution (SPSS) version 23.

RESULTS

Macroscopical Evaluation of the Stem of *S. jollyanum*
The stem structure was thin, erect, and branches sparingly. While the younger stems feature short hairs, the mature stems transition to a smooth (glabrous) texture with grey bark. Internally, the stem is yellowish; older sections display a woody, firm texture, whereas younger shoots remain softer. The plant typically reaches a height of up to 1.5 meters.



Figure 1: Picture of *S. jollyanum* in its natural habitat.



Figure 2: Picture of the cut stem of *S. jollyanum*

Microscopical Evaluation of the Stem of *S. jollyanum*

Chemomicroscopy revealed that the powdered stem contains starch granules, wood-aligned cell walls, crystals of calcium oxalate, tannins, plant cellulose, and droplets of oil, which were identified by applying specific staining reagents directly to the tissue under a standard light phase microscope(Motic B3, Motic Carlsbad, CA,USA). Transverse section of the stem revealed that the structure is dorsoventral, consisting of upper and lower epidermis with cells of varying sizes. The vascular bundles, composed of xylem and phloem, are of the conjoint type (Figures 7 and 8).

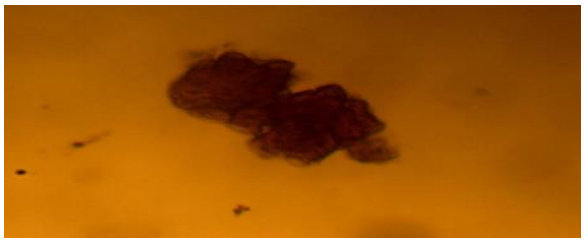


Figure 3: Chemomicrograph of the stem powder showing pack parenchymatous cells.

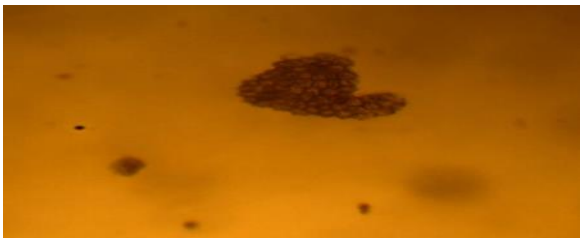


Figure 4: Chemomicrograph of the stem powder showing lignified cork cells.

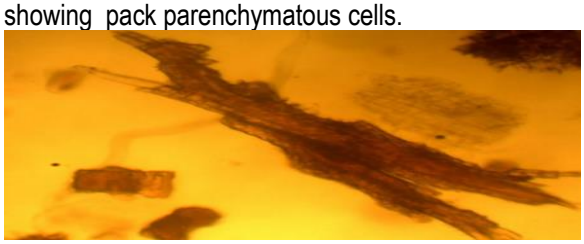


Figure 5: Chemomicrograph of the stem powder showing lignified vessel tissues



Figure 6: Chemomicrograph of the stem powder showing fibre elements (black arrow) and pitted vessel element (white arrow.

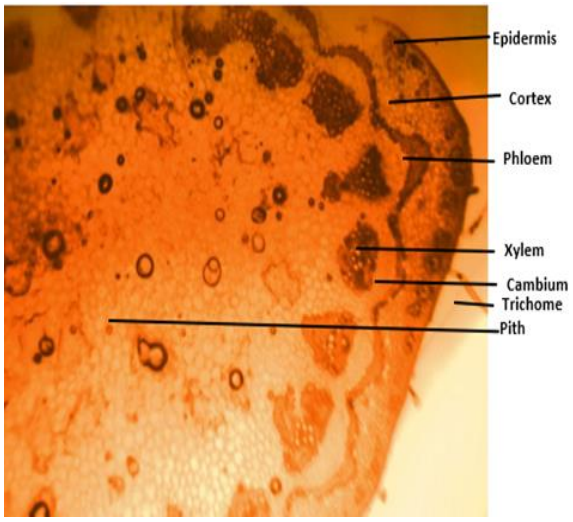


Figure 7: Transverse section of the stem of *S. jollyanum* (×40)

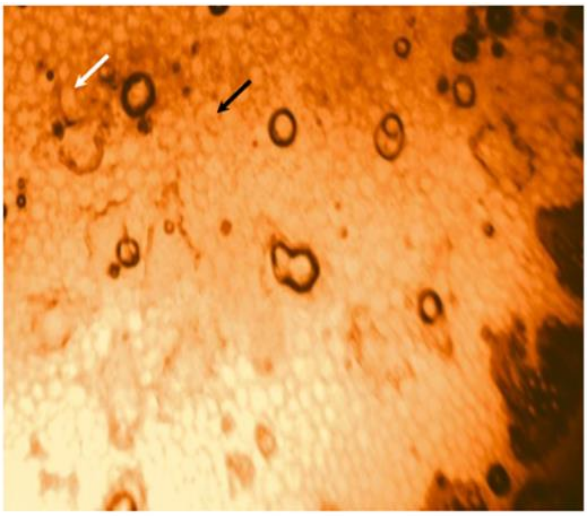


Figure 8: Transverse section of the stem showing oil cells (black arrow) and prism-shaped calcium oxalate crystals (white arrow)

Table 1: Physicochemical Parameters of *S. jollyanum* Stem Powder

Sample	Moisture content(%)	Total ash (%)	AIA (%)	WSA (%)	Percentage yield (%)	n-hexane soluble (%)	Ethylacetate soluble (%)	Water soluble (%)	Alcohol soluble (%)
Mean	11.30±0.98	6.53±0.03	3.50±0.02	2.91±0.14	7.79±0.03	1.96±0.02	3.64±0.02	6.24±0.01	7.39±0.04

Results are presented as mean ± SEM; n=5; $p<0.05$ is considered significant.
Key: AIA=Acid Insoluble ash, WSA=Water Soluble ash

Table 2: Qualitative and quantitative phytochemical analysis of the stem of *Sphenocentrum jollyanum*.

	Steam	
Phytochemicals	**Qualitative**	**Quantitative (mg/g)**
Alkaloids	+	1.65±0.48
Flavonoids	+	1.90±0.02
Tannins	+	2.85±0.02
Saponins	+	1.29±0.04
Terpenoids	+	0.84±0.02
Anthocyanins	+	0.08±0.01

Results presented as mean ± SEM; n=5. +=Present; -=Absent

Table 3: TOXICITY TEST

Phase one	Dose(mg/kg)	Mortality
	10	0/3
	100	0/3
	1000	0/3
Phase Two		
	1600	0/3
	2900	0/3
	5000	0/3

Table 4: Effect of methanol stem extract on egg albumin induced-paw oedema in rat.

Group	Treatment	Dosage	Mean paw diameter (mm) at the indicated time						
			0 h	1 h	2 h	3 h	4 h	6 h	24 h
A	Acetylsalicylic acid (mg/kgbw)	100	3.45±0.07 (2.54%)	6.46± 0.26 (-14.13%)	5.76± 0.24* (26.05%)	5.70± 0.15* (28.75%)	4.67± 0.15* (41.33%)	4.24± 0.20* (46.26%)	3.85± 0.06* (44.20%)
B	Distilled water (ml/kg)	0.5	3.54± 0.17	5.66± 0.23	7.79± 0.26	7.27± 0.22	7.96± 0.30	7.89± 0.27	6.90± 0.33
C	*S. jollyanum* (mg/kgbw)	200	3.27± 0.07 (4.80%)	7.45± 0.30# (21.03%)	5.83± 0.20* (26.19%)	5.41± 0.21* (27.37%)	4.64± 0.18* (44.60%)	4.79± 0.13 (48.04%)	3.89± 0.06 (44.06%)
D	*S. jollyanum* (mg/kgbw)	400	3.39± 0.14 (7.91%)	7.38± 0.21 (-23.14%)	6.12± 0.27* (28.11%)	5.46± 0.13*# (29.44%)	4.74± 0.14* (46.98%)	4.18± 0.05* (46.98%)	3.72± 0.10* (46.09%)
E	*S. jollyanum* (mg/kgbw)	600	3.10± 0.11 (2.26%)	7.06± 0.37 (-28.62%)	5.58± 0.29* (25.03%)	5.43± 0.18* (21.60%)	4.73± 0.21* (38.82%)	4.50± 0.24* (38.82%)	3.95± 0.03* (42.90%)

Results presented as mean ± SEM; n=5; p<0.05 is considered significant. The data in parenthesis showed the percentage inhibition of paw edema. * indicates Significant statistical decrease in mean paw edema diameter as compared to negative Control while # indicates Significant statistical (p< 0.05) decrease in mean paw edema diameter as compared to positive Control.

Table 5: Effect of methanol stem extract on carrageenan induced-paw oedema in rat.

Group	Treatment	Dosage	Mean paw diameter (mm) at the indicated time						
			0 h	1 h	2 h	3 h	4 h	6 h	24 h
A	Acetylsalicylic acid (mg/kgbw)	100	4.50±0.14 (2.18%)	8.70± 0.12* (-4.82%)	8.20± 0.05* (10.38%)	7.61± 0.10* (19.18%)	6.66± 0.11* (33.13%)	6.30± 0.08* (37.99%)	5.84± 0.05* (43.57%)
B	Distilled water (ml/kg)	0.5	4.63± 0.15	8.30± 0.08	9.15± 0.07	9.49± 0.09	9.96± 0.03	10.16± 0.02	10.35± 0.03
C	*S. jollyanum* (mg/kgbw)	200	4.34± 0.07 (6.26%)	8.74± 0.19 (-5.30%)	8.36± 0.18 (8.63%)	7.72± 0.07 (18.86%)	7.06± 0.05* (29.12%)	6.36± 0.10* (37.40%)	6.04± 0.05* (41.46%)
D	*S. jollyanum* (mg/kgbw)	400	4.80± 0.06 (-3.67%)	8.53± 0.07 (-2.77%)	8.12± 0.04 (11.26%)	7.54± 0.10 (20.55%)	7.09± 0.03* (28.8%)	6.41± 0.04* (36.91%)	6.01± 0.05* (41.93%)
E	*S. jollyanum* (mg/kgbw)	600	4.13± 0.04 (10.80%)	8.85± 0.20 (-6.63%)	8.48± 0.23 (7.32%)	8.01± 0.20 (15.60%)	7.57± 0.20*# (23.10%)	6.86± 0.23*# (32.48%)	6.13± 0.06*# (40.77%)

Results presented as mean ± SEM; n=5; p<0.05 is considered significant.The data in parenthesis showed the percentage inhibition of paw edema. * indicates Significant statistical decrease in mean paw edema diameter as compared to negative Control while # indicates Significant statistical (p< 0.05) decrease in mean paw edema diameter as compared to positive Control.

DISCUSSION

The macroscopic and microscopic examination of crude drugs is conducted to identify the appropriate variety and detect any adulterant in plant materials .

This report presents a range of macroscopic, microscopic, and physicochemical standards that have been established to assist in the accurate identification and standardization of S. jollyanum.

Microscopic analysis has played a vital role in setting standards for various herbal remedies like *Warbugia ugandensis* [26] and different species of Ficus [6].

The anatomical characteristics of internal plant structures provide important diagnostic indicators for identifying both whole and powdered crude drugs, as well as for detecting adulterants [20].

The transverse section of the stem revealed some prominent features like the epidermis, oil cells, prism-shaped calcium oxalate crystals, cortex, xylem, phloem, cambium, trichome and pith (Figure 3). The above mentioned diognostic features were displayed among members of the Menispermaceae family [28].

Chemomicroscopical analysis of the stem of *S. jollyanum* revealed the presence of cellulose, lignified tissues, calcium oxalate, tannins, starch, and oil. Identifying powdered drugs relies heavily on their structural characteristics, chemical reactions, and colour changes, which are essential for their recognition. This identification process is significantly influenced by their physical form and the presence or absence of specific cell wall types and inclusions [17].

The physicochemical properties, such as moisture content, total ash content, water-soluble ash content, and acid-insoluble ash content, are essential factors in the standardization and quality control of herbal medicines. These measurements are crucial for determining the authenticity and purity of raw medicinal plants and can help detect contaminants like carbonates, oxalates, and silicates in plant materials . The moisture contents (11.00%) is relatively low, indicating a lower risk of microbial spoilage during storage. The standard requirement for moisture content in raw medicinal plants is typically below 14% [7], a criterion met in this study. Lower moisture values suggest a decreased risk of product deterioration, implying enhanced stability. Moisture is considered an adulterant as it increases weight and promotes mold and bacterial growth.

Ash values serve as indicators of the purity and quality of a crude drug, revealing various impurities such as carbonates, oxalates, and silicates. The water-soluble ash percentages (2.91)± 0.14%) primarily consist of silica, which indicates contamination with earthy substances. The acid-insoluble ash values found in this study was 3.50 ± 0.02%. The total ash value of 6.53 ± 0.03% reflect both physiological and non-physiological ashes present in the plant. Non-physiological ash indicates inorganic residues left after incineration of the plant material. The total ash value is a reliable measure for identifying drug adulteration[29].

Extractive values help estimate the chemical constituents in the crude drug and assess the solubility of phytoconstituents in a specific solvent. Extracting a crude drug with a chosen solvent yields a solution containing various phytoconstituents, whose composition is influenced by both the nature of the drug and the solvent used. This also helps determine whether the crude drug has been fully extracted or not[33]. The extractive values showed that the constituents of *Sphenocentrum jollyanum* are more soluble in polar solvents with ethanol having the highest extractive value. These proofs the traditional use of the local infusion of the plant material in alcohol[25].

Results from phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins and anthocyanins in varying proportions in the crude extract of the stem with tannins being the largest proportion in the extract (Table 2).

Acute toxicity assessments of the extracts from the stems of *S. jollyanum* demonstrated that the plant is safe for oral use at a maximum dosage of 5000 mg/kg body weight, which likely explains the absence of mortality observed in the animals (Table 3).

The plant stem extract significantly ($p<0.05$) inhibited inflammation caused by egg albumin in a time-dependent manner, showing a similar effect to Acetylsalicylic acid, especially after 24 hours of treatment. The rat paw edema induced by egg albumin is a widely accepted method for evaluating anti-inflammatory drugs. This model consists of three stages: The initial phase (0 - 1.5 hours post egg albumin injection) of edema is due to the release of histamine and 5-hydroxytryptamine (serotonin) in the nearby damaged tissue. The second phase (1.5 - 2.5 hours) is controlled by the release of bradykinin. The third phase (after 3 hours) is influenced by prostaglandin-like substances [23] .

The findings of this research indicate that the plant stem extract demonstrates a notable time-dependent anti-inflammatory impact at the 24th hour following the carrageenan injection, with statistical significance ($p<0.05$). This influence might be attributed to the suppression of the cyclooxygenase enzyme, which plays a role in transforming arachidonic acid into signaling molecules like prostaglandins, thromboxane A2, prostacyclin, and resolvins [30]. Carrageenan is known to react to cyclooxygenase inhibitors and is a suitable method for assessing non-steroidal anti-inflammatory drugs (NSAIDs) that hinder cyclooxygenase's role in prostaglandin production [12,31]. The swelling response induced by carrageenan involves three distinct stages characterized by the sequential release of different inflammatory signaling molecules. The initial stage (0 - 1.5 hours after carrageenan injection) triggers the discharge of histamine, 5-HT (serotonin), and neuropeptides from mast cells. The second stage (1.5 - 2.5 hours) is governed by the liberation of kinins. The final phase (after 3 hours) includes the release of prostaglandins, thromboxane A2, prostacyclin, resolvins, and lipoxygenase byproducts [23].

The plant's stem extract contains phytochemical components like tannins, saponins, terpenoids, alkaloids, anthocyanins,

and flavonoids that have anti-inflammatory properties [15]. These compounds in the stem could play a role in its anti-inflammatory effects. By isolating and purifying these bioactive constituents, a more powerful anti-inflammatory agent with reduced side effects and improved therapeutic index could be developed.

CONCLUSION

This study establishes some pharmacognostic standards for the stem of *Sphenocentrum jollyanum* for the first time, to the best of our knowledge. These findings can serve as a diagnostic tool for the standardization and accurate identification of this medicinal plant.

Acute toxicity tests on extracts from the stems indicate that the plant is safe at a maximum dosage of 5000 mg/kg of body weight. The current research confirms that methanol extracts of the stems of *Sphenocentrum jollyanum* effectively reduce paw edema induced by egg albumin and carrageenan. This explains the basis of its use in traditional medicine to manage inflammatory conditions and suggests the plant may be a promising source of anti-inflammatory agents with minimal side effects. It also contains some bioactive constituents worthy of further investigation.

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AUTHORS' CONTRIBUTION

Conceptualization and supervision: COE and MTK; methodology: BNU, FIN and OCE; Experimentation and data analysis: BNU, MTK, FIN and CCA; Manuscript drafting: BNU and OCE; Manuscript proofreading: CCA and SCE.

CONFLICT OF INTEREST

The authors alone are responsible for the content of this research and report no conflict of interest.

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