



Evaluation of Membrane Stabilizing, Proteinase and Lipoxygenase Inhibitory Activities of Ethanol Extract of Root and Stem of *Sphenocentrum jollyanum* Pierre

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOS designed the study and wrote the protocol. Author FOS managed the analyses of the study and the literature searches. Authors AA and OA performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at evaluating the anti-inflammatory potential of ethanol extract of the stem and root of *Sphenocentrum jollyanum*.

Study Design: Red blood cell Membrane stabilization, anti-lipoxygenase and proteinase inhibitory activities of the extracts were assayed *in-vitro* as a measure of anti-inflammatory potential of ethanol extract of root and stem of *Sphenocentrum jollyanum* Pierre.

Place and Duration of Study: All the work was carried out in the Department of Biochemistry, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria between April 2015-February, 2016.

Methodology: Four hundred grams of the stem and root was extracted in batches with 90%

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ethanol in a soxhlet apparatus and concentrated using a rotatory evaporator. Inhibitory effect of the extracts on erythrocytes membrane stabilization, trypsin and lipoxygenase (*in vitro*) were used to assess anti-inflammatory properties according to standard procedures. The reactions were performed in triplicates and changes in optical density of test samples and control were measured using a 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA) and inhibitions were calculated.

Results: The result of this study revealed that the extraction yield of the stem and root was 32.00 g and 26.00 g respectively, while the stem extract exhibited a significantly ($p < 0.05$) higher dose dependent erythrocyte membrane stabilization when compared with the root extract with IC_{50} of $325 \pm 13.81 \mu\text{g/ml}$ and $541 \pm 6.33 \mu\text{g/ml}$ respectively. The Proteinase inhibitory assay showed that the stem extract demonstrated a stronger inhibitory activity to the root with IC_{50} $1000 \pm 3.35 \mu\text{g/ml}$ and $1080 \pm 2.67 \mu\text{g/ml}$. Lipoxygenase inhibition activity assay revealed that the stem and the root extract of *Sphenocentrum jollyanum* inhibited 50% of lipoxygenase at concentration of $426 \pm 6.33 \mu\text{g/ml}$ and $541 \pm 6.67 \mu\text{g/ml}$ respectively. Hence, the results of this study showed that S.J have some bioactive compounds which are effective in management of inflammatory disorders and thus validates the folkloric use of the plant.

Keywords: *Sphenocentrum jollyanum pierre*; stem and root extract; membrane stabilizing; proteinase; lipoxygenase inhibitory activity.

1. INTRODUCTION

Inflammation can be defined as a cascade of reactions in which the organism's immune system reacts to invading foreign agents and simultaneously initiate the healing process of any damaged tissue. These often result in the discomfort of the host [1,2]. The pathogenesis of different inflammatory diseases has been linked to membrane destabilization of the cells of the immune system (mast cells, neutrophils, basophils, eosinophils and leukocytes), which involves the release of their lysosomal constituents (myeloperoxidase, serine proteases, phospholipases, and lipoxygenases) in response to stimuli. These degradative enzymes damage endothelial, epithelial cells and destroy connective tissues thus resulting in different physiological responses which are evident in the classical signs of inflammation (pain, swelling, heat and redness) [3]. Lipoxygenases and cyclooxygenases are well documented and reported in the generation of reactive oxygen species and inflammatory disorders [4]. They mediate the release of prostaglandins, thromboxanes, leukotrienes and other eicosanoids [5,6] which play predominant roles in pathophysiology of many acute and chronic inflammatory diseases such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, dermatitis, and cancer [7,8]. Non steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids have been effective in suppressing inflammatory responses but their prolonged administration can result to drug resistance and significant harmful effects

such as increased risk of infection, cancer, cardiovascular diseases [9] damage to connective tissue and adrenal gland atrophy [10,11]. Hence, there is a dire need for development of natural alternative therapies that do not produce significant side effects. Medicinal plants have been the source of traditional drugs and therapies since time immemorial. According to the report of Gurib-Fakim [12], Mantle et al. [13], Annan and Houghton [14], about 80 percent of people in Africa and Asia relies on medicinal plants as alternative to modern medicine in the management of various health disorders.

Till this present day, medicinal plants continue to play a major role in providing raw materials for modern drug development which can be linked to their diverse bioactive compounds and phytochemical constituents [15]. *Sphenocentrum jollyanum* Pierre is a shrub that belongs to the family of Menispermaceae. It predominantly grows in West-African Countries of Nigeria, Ghana, Sierra-Leone and Cameroon [16]. Interestingly, traditional usage and scientific research on the plant has been richly documented. Various biological and pharmacological activities of its various parts have been reported, they include anti-oxidant, anti-angiogenic [16,17], anti-malaria, anti-diabetes [18,19] hypoglycemic, hypolipidemic [20,21] anti-inflammatory [22] and enhancement of reproductive activity in male albino rats [23]. Although, thorough extensive studies have been carried out on extracts of *S. jollyanum*, but from information available to us, there is paucity of scientific documentation on the membrane stabilization, lipoxygenase and

protease inhibition activity of the root and stem extracts. Hence, evaluation of the above mentioned parameters will be of great importance to knowledge.

2. MATERIALS AND METHODS

2.1 Plant Materials

The root and stem of *Sphenocentrum jollyanum* (SJ) were collected from the Botanical garden of University of Ibadan, Nigeria and were identified by Professor A.J Ogunkunle of the Department of Pure and Applied Biology, Ladoko Akintola University of Technology. Voucher samples (LHO 240 and LHO 241) of the plant were also deposited at the University herbarium. The stem and root were rinsed with clean water to remove sandy particles and air dried for two weeks after which they were chopped into pieces and pounded using a wooden mortar and pestle. This was later blended into powder with electrical blender (Tower brand, 220-240v 50/60HZ 500W) and milling machine (Honda, Japan).

2.2 Preparation of Ethanol Extracts

Blended plant material (400 g) was loaded in a soxhlet extractor in batches for 5 hours each and subjected to extraction with 90% v/v ethanol. The extracts were concentrated at 45°C using a rotary evaporator and the dried extracts were kept in a refrigerator (4°C) till it was needed.

2.3 Membrane Stabilization Assay

Membrane stabilization method was carried out according to the method of [24,25] with slight modification. Briefly, blood was collected from male albino rats and transferred to the centrifuge tubes. The blood was centrifuged at 3000 rpm for 10 min and was washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline. Various concentrations of extracts were prepared (100, 150, 200, 250 and 300 µg/ml) adding distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of red blood cell suspension were added. The mixture was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatants solution was estimated. The reactions were performed in triplicates in 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA) at 560 nm and mean value was

considered. Diclofenac sodium (100 µg /ml) was used as reference standard. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

$$\% \text{ inhibition of hemolysis} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

2.4 Protease Inhibition Activity Assay

The test was performed according to the modified method of [26,25]. The reaction mixture (2 ml) containing 0.03 mg trypsin, 0.5 ml of 10 mM Tri HCl buffer (pH7.4) and different concentrations of the extracts. The reaction mixture was incubated at 37°C for 5 min after which 1 ml of 0.8% (W/V) casein was added. The mixture was incubated for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm using 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA). The experiment was performed in triplicate and the percentage protein inhibitory activity was calculated by;

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5 Anti-lipoxygenase Activity Assay

Anti-lipoxygenase activity was assayed according to the method of [27]. Briefly, linoleic acid was used as substrate and lipoxidase as the enzyme. Test samples were dissolved in 0.25 ml of 2 M borate buffer (pH 9.0), 0.25 ml of lipoxidase enzyme. The solution was incubated for 5 min at 25°C, after which 1.0 ml of linoleic acid solution (0.6 mm) was added and properly agitated. Absorbance was measured at 234 nm, and indomethacin was used as reference standard. The percentage inhibition was calculated from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorption of extract}}{\text{Absorbance of control}} \times 100$$

2.6 Statistical Analysis

All data were presented as mean ± standard error of mean (SEM) of triplicates and IC₅₀ µg/ml was determined. Independent T- Test was used to determine the significant difference between

groups and control using the software Graph Pad prism 5.0 (Graph Pad Software Inc. California, USA).

3. RESULTS AND DISCUSSION

3.1 Extraction of Yield

The ethanol extraction of 400 grams of stem and root of *S. Jollynum* gave a yield of 32.00 g and 26.00 g respectively (Table 1).

Table 1. Yield in (grams) of ethanol extract from 400 grams of *S. jollyanum* stem and root

Plant organ	Yield in grams
Stem	32.00 g
Root	26.00 g

3.2 In-vitro Anti--Inflammatory Assays

3.2.1 Membrane stabilization activity of ethanol extracts of stem and root of *S. jollyanum*

Membrane stabilization assay is a widely used technique in the screening of test compounds and plant extracts for anti-inflammatory potentials. In this study, the ethanol extract of the stem and root of *S. jollyanum* showed considerable dose-dependent membrane stabilization activity. The ethanol extract of the stem showed a significant ($P < 0.05$) higher membrane stabilizing ability when compared with the ethanol extract of the root. The highest percentage erythrocyte stabilization of 42.60% and 30.20% respectively was observed at concentration of 300 $\mu\text{g/ml}$ with IC_{50} ($\mu\text{g/ml}$) values of 355 ± 13.81 $\mu\text{g/ml}$ and 541 ± 6.33 $\mu\text{g/ml}$ respectively. However, the standard drug diclofenac exhibited the highest erythrocyte membrane stabilization activity with IC_{50} of 51 ± 6.12 (Table 2). Previous phytochemical investigations reported that *S. jollaynum* stem extracts constitutes in abundance bioactive compounds such as saponins, tannins, flavonoids, alkaloids and terpenes [16]. Polyphenolic compounds have been reported to inhibit the tissue damaging effect of photolytic, bactericidal enzymes and reactive oxygen species that are released by the polymorpho nuclear cells in response to invading foreign agents [28]. Flavonoids and alkaloids have also been reported to modulate cellular activities of inflammatory related cells by stabilizing their membranes, thus preventing de-granulation [29] [30] and therefore impairing lysosomal enzyme

release of arachidonic acid, elactase and glucuronidase [31]. Also, Fawole et al. [32] and [33] reported that flavonoids and saponin are known to posses anti-inflammatory activity. Hence, the likely mode of action of the extracts observed in (Table 2) may be through the above mentioned biochemical processes. Furthermore, the result obtained in this study is in conformity with earlier submissions of [34-36] that reported significant activities of *Solanum aethiopicum*, *Calliandra surinamensis* extracts and honey respectively against hypotonic and heat induced lysis of red blood cells. In addition, works by Sacchin et al. [37] and [38] reported that *Erythrina indica* and *Lagenaria breviflora* extracts significantly inhibited erythrocytes membrane disruption.

3.2.2 Proteinase inhibition activity of ethanol extracts of stem and root of *S. jollyanum*

Proteinases such as trypsin and other serine proteases are essential enzymes that mediate the hydrolytic breakdown of peptide bonds in proteins [39]. They have been extensively studied with respect to their roles in inflammation, cardiovascular diseases, tissue remodeling, matrix destruction, auto immune arthritis and skin allergies [40]. Therefore, therapeutic inhibition of these enzymes will be of novel contribution. The result from the present study indicates that the ethanol extract of the stem exhibited a significantly ($P < 0.05$) higher percentage inhibition when compared to the root extracts with IC_{50} values of 1000 ± 3.35 $\mu\text{g/ml}$ and 1080 ± 2.67 $\mu\text{g/ml}$ respectively. It was also observed that inhibition was concentration dependent and the standard drug Indomethacin demonstrated the highest Proteinase inhibition (Table 3). Various plants and natural products have been recorded to demonstrate considerable proteinase inhibition. According Gulnaz et al. [41], ethanolic extract of *Randia uliginosa* exhibited significant proteinase inhibition, while [42-44] also have similar observations.

3.2.3 Anti-lipoxygenase activity of ethanol extracts of stem and root of *S. jollyanum*

Lipoxygenases (LOXs) are family of non-heme iron-containing enzymes that have been implicated in the metabolism of arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites which are known mediators of inflammatory and immune responses [45,7]. Hence, the inhibition of these

enzymes becomes imperative, and could pave way for discovery of new anti-inflammatory agents. In this study, it was observed that the stem and root extracts of *S. jollaynum* exhibited a significant ($P<0.05$) concentration dependent lipoxygenase inhibition. It was also observed that the stem extract at concentration of 300 $\mu\text{g/ml}$ demonstrated the stronger percentage LOX inhibition of $40.45\pm 0.55\%$ with IC_{50} of 426 ± 6.33 $\mu\text{g/ml}$ when compared to the root extract. However, Indomethacin demonstrated the highest LOX inhibition (Table 4). This observed LOX inhibitory activity might be due to synergistic effect of the prevalent phytochemicals (flavonoids, alkaloids, tannins and saponins) according to the phytochemistry report of [16]. Secondary metabolites inhibits the expression of

LOX and other related enzymes by suppressing the activation of transcription factors, inhibition of phospholipases A2 [46,46], modulating arachidonic acid [47,48,49]. Csaki et al. [50], reported that isolated compounds such as flavonoids from natural products have been implicated in prevention and regulation of inflammation. According to Kim et al. [40], bioactive principles in plants have the ability to protect against disease such as inflammation and cancer. Bioactive compounds have been reported to inhibit pro oxidants such as LOX of its radical scavenging ability and promote anti-oxidant enzymes activity of super oxide dismutase, catalase and glutathione peroxidase [51,52]. Quite a number of plant

Table 2. IC_{50} ($\mu\text{g/ml}$) and percentage (%) membrane stabilization activity of ethanol extracts of *S. jollaynum* stem and root

Concentration ($\mu\text{g/ml}$)	% Membrane Stabilization		
	Stem	Root	Diclofenac
100	18.59 \pm 0.02	14.60 \pm 0.01	54.60 \pm 0.03
150	21.69 \pm 0.09	18.40 \pm 0.02	55.00 \pm 0.06
200	33.00 \pm 0.01	22.86 \pm 0.06	62.50 \pm 0.05
250	38.61 \pm 0.11	27.20 \pm 0.09	65.67 \pm 0.09
300	42.60 \pm 0.07	30.20 \pm 0.01	68.00 \pm 0.04
IC_{50} ($\mu\text{g/ml}$)	325 \pm 13.81*	541 \pm 6.33*	51 \pm 6.12

Values represent mean \pm SEM ($n=3$) * $p<0.05$ considered as IC_{50} significant when compared to the Standard Drug (Diclofenac)

Table 3. IC_{50} ($\mu\text{g/ml}$) and percentage (%) Proteinase inhibition activity of ethanol extracts of *S. jollaynum* stem and root

Concentration ($\mu\text{g/ml}$)	% Proteinase Inhibition		
	Stem	Root	Indomethacin
100	12.10 \pm 0.07	7.64 \pm 0.08	40.00 \pm 0.05
150	15.31 \pm 0.01	8.65 \pm 0.05	43.20 \pm 0.06
200	17.50 \pm 0.04	10.42 \pm 0.03	47.00 \pm 0.05
250	19.94 \pm 0.01	12.25 \pm 0.04	51.00 \pm 0.06
300	20.05 \pm 0.03	16.90 \pm 0.01	54.00 \pm 0.03
IC_{50} ($\mu\text{g/ml}$)	1000 \pm 3.35*	1080 \pm 2.67*	246 \pm 5.66

Values represent mean \pm SEM ($n=3$) * $p<0.05$ considered as IC_{50} values significant when compared to the Standard Drug (Indomethacin)

Table 4. IC_{50} ($\mu\text{g/ml}$) and percentage (%) lipoxygenase inhibitory activity of ethanol extracts of *S. jollaynum* stem and root

Concentration ($\mu\text{g/ml}$)	% Lipoxygenase Inhibition		
	Stem	Root	Indomethacin
100	24.79 \pm 0.51	14.6 \pm 0.03	45.50 \pm 0.06
150	28.21 \pm 0.60	19.40 \pm 0.35	47.60 \pm 0.05
200	31.12 \pm 0.96	22.86 \pm 0.92	51.60 \pm 0.73
250	36.70 \pm 0.83	27.20 \pm 0.43	56.40 \pm 0.35
300	40.45 \pm 0.55	30.20 \pm 0.19	59.80 \pm 0.77
IC_{50} ($\mu\text{g/ml}$)	426 \pm 6.33*	541 \pm 6.67*	172 \pm 5.97

Values represent mean \pm SEM ($n=3$) * $p<0.05$ considered as IC_{50} significant when compared to the Standard Drug (Indomethacin)

extracts have been able to demonstrate lipoxygenase inhibition. Ali et al. [53] reported the anti-lipoxygenase of some indigeneous medicinal plants, while [54-57] reported the lipoxygenase inhibitory activities of *Caralluma arabica*, *Fraxinus rhynchophylla*, *Thespesia lampas* and *M. malabaricum* with IC₅₀ values of 30.77 µg/ml, 62.6 ug/ml, 586.5 µg/ml and 29.87 µg/ml respectively.

4. CONCLUSION

From the results of this study, it is suggested that *S. jollyanum* could really have anti-inflammatory properties.

ETHICAL APPROVAL

As per international standard or university standard, Ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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