

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2230-480X
JPHYTO 2016; 6(1): 20-26
January- March
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Anti-allergy potential and possible modes of action of *Sphenocentrum jollyanum* pierre fruit extracts

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ABSTRACT

Sphenocentrum jollyanum (SJ) is widely used traditionally in the management of various ailments. Information on its anti-allergy property and possible modes of action is scanty in the literature. Thus, this study was aimed at evaluating the anti-allergic potential of crude and secondary metabolites (Tannins, Saponins, Flavonoids and Alkaloids) of S.J fruit extracts. Aqueous, ethanol extracts and the secondary metabolites were extracted using standard techniques. Inhibitory effect of the extracts on erythrocytes membrane stabilization, trypsin and lipoxygenase (*in vitro*) were used to assess anti-inflammatory properties, while extract with the most potent anti-inflammatory activity was used to assess the anti-allergy property of the fruit in milk induced eosinophilia and leukocytosis mice. Result of the study revealed that the aqueous extract has highest percentage yield (38.00g), while saponins (10.20%), alkaloids (8.51%) and tannins (6.70%) are the predominant phytochemicals. The ethanol extract of the fruit demonstrated significant ($p < 0.05$) high dose dependent erythrocytes membrane stabilization ($IC_{50} = 263 \pm 12.44 \mu\text{g/ml}$), trypsin inhibition ($IC_{50} = 770 \pm 6.33 \mu\text{g/ml}$) and anti-lipoxygenase activities ($IC_{50} = 583 \pm 6.80 \mu\text{g/ml}$) when compared with the secondary metabolites, but significantly ($p < 0.05$) lower than the standard drugs (Diclofenac and Indomethacin). The saponins extract demonstrated highest anti-inflammatory activity when compared with other secondary metabolites. The significant ($p < 0.05$) dose dependent reduction in the eosinophils and lymphocytes counts in the ethanol fruit extract of SJ treated milk induced eosinophilia and leucocytosis Wistar mice suggested anti-allergy property of *Sphenocentrum jollyanum* fruit extract. Although, membrane stabilization effect of the tannin in the fruit may play a dominant role, the anti-allergy effect may involve multiple mechanisms due to phytochemicals interactions.

Keywords: Anti-Allergy, Anti-Inflammation, *Sphenocentrum jollyanum*, Fruit, Phytochemicals.

INTRODUCTION

Allergic disorders are characterized by the hyper-sensitivity of the immune system to non-infectious stimulus in the environment^[1] and this can be detrimental reactions to the host.^[2] In recent years, there is increase in occurrence of allergy and inflammatory diseases world-wide. Allergic disorders such as bronchial asthma, eczema, allergic rhinitis and inflammatory bowel disease affects about 300 million people and they also accounts for 1 out of every 250 deaths worldwide.^[3] Food and drug allergy are reported to affect about 6% of the world population, while about 20% are affected by atopic dermatitis at some point in their life time.^[4] The patho-physiology of the disease is complex and it may involve many inflammatory cells and multiple mediators such as basophils, eosinophils, leukocytes, histamine, tryptases, arachidonic acid metabolites and immunoglobulin E.^[5] Orthodox drugs such as anti-histamines, mast cell stabilizers among others are widely used in treatment of hyper-sensitivity related diseases. Unfortunately these drugs have numerous negative side effects such as life threatening ailments.^[6] In lieu of this, there is an urgent need for the discovery of cheap, effective, readily available drugs with lesser side effects.

Traditional plants usage in folk medicine is not a new thing, it dated back to many years ago.^[7] Not less than 50,000 species of higher plants have been reportedly used to treat various ailments.^[8] In developing countries, folkloric medicine is an integral part of primary and secondary health care system. It is believed that the use of plants in the treatment of diseases is effective, cheap, and relatively safe.^[9] They are also rich source of bioactive compounds such as, phenolics, flavonoids, alkaloids, glycosides, quinine, saponins, steroids, triterpenes, tannin and essential oils.^[10] *Sphenocentrum jollyanum* is a shrub that belongs to family of Menispermaceae, a wide range of plants known for their medicinal activities which include remedy for metabolic disorders and are predominantly found along the west coast of

Africa from Sierra Leone to Cameroun and Nigeria [11]. SJ has a wide range of biological and pharmacological activities such as anti-oxidant^[12], anti-hyperglycemic^[13] and libido enhancing activity^[14]. Also, isolated secondary metabolites from the fruit of *Sphenocentrum jollyanum* has been reported to show significant anti-inflammatory activity in carrageenan-induced hind paw oedema in healthy adult albino rats.^[15] In spite of the wide pharmacological application of *Sphenocentrum jollyanum*, information on anti-allergy property is scanty in literature. The present study was therefore designed to evaluate the anti-allergic activity of the *Sphenocentrum jollyanum* fruit extracts and to predict the possible mode of action.

MATERIALS AND METHODS

Plant materials

The Fresh fruits of *Sphenocentrum jollyanum* (SJ) were bought from a local market in Ogbomosho, Oyo State, Nigeria and were identified by Professor EO Ogunkunle of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology. A voucher (LHO 240) sample of the plant was also deposited at the University herbarium.

Preparation of ethanol fruit extract of SJ

The ethanol extraction was carried out according to the method of^[13] with slight modification. Briefly, blended fruit of *Sphenocentrum jollyanum* (400g) was loaded in a soxhlet extractor in batches for 5 hours each and subjected to extraction with ethanol. After extraction, the solvent was evaporated at 78 °C using a rotary evaporator and the extract were kept in a refrigerator (4 °C) until it was needed.

Preparation of aqueous fruit extract of SJ

The aqueous extract was carried according to the method described by^[13] with slight modification. Briefly, the blended fruit of SJ (400g) was loaded in batches in soxhlet extractor extracted with water in three cycles for about 72 hours. The filtrate obtained was dried in an electric oven between 30-36 °C and in kept in a refrigerator (4 °C) for further analysis.

Extraction of Saponins

Saponins was extracted by the method described by^[16] with slight modification. Briefly, 200 ml solution was prepared in distilled water using 100g of blended fruit of *Sphenocentrum jollyanum*. This was extracted thrice with 100 ml diethyl ether. The diethyl ether layer was discarded and the retained aqueous layer was extracted further with 120 ml n-butan-1-ol (four times). The n-butan-1-ol extracts was pulled together and washed four times using 40 ml of five percent sodium chloride (NaCl). The washed extract was concentrated at < 80°C in an oven and air dried at room temperature and stored in the refrigerator (4 °C) till it was needed. Method described by^[17] was employed for qualitative determination.

Extraction of alkaloids

The extraction of the alkaloid was carried according to method of^[18] using the continuous extraction method and soxhlet apparatus. One hundred grams (100 g) of blended material was weighed and packed in a cheesecloth bag, which served as an extraction thimble. The

thimble was then placed into a suitable jar with cover. The sample was moistened with 4.8 liters 95% ethanol. The sample in the thimble was macerated overnight and then placed in the soxhlet extractor till the next day. The sample was extracted for about 3 – 4 hours. The ethanol extract was filtered and concentrated in a soxhlet distilling apparatus at 60 °C. The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinized with ammonia and placed in a separating funnel. Measured quantities of chloroform was added into the separating funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The combined chloroform extract was concentrated in soxhlet distilling apparatus at 60 °C and evaporated in water bath maintained at that temperature until semi-dry.

Extraction of flavonoids

Flavonoids rich extract was prepared^[19] by immersing 100 gm of blended material in 500 ml ethanol (100%) for 24 hours at room temperature using magnetic stirrer. The mixture was then filtered using Whatman No. 1 filter papers and the process was repeated using the remaining residue with 300 ml ethanol alcohol to ensure the complete extraction in each time. The two filtrates were added and treated with 100 ml lead acetate (1%) for 4 hours for precipitation. The mixture was filtered, and a mixture of 250 ml acetone and 30 ml of concentrated hydrochloric acid was added to the precipitate, and filtered. The extract was again dissolved in ethanol. The extraction process was repeated for 1 hour, filtered to produce red filtrate. Finally, the filtrate was placed in a clean and dry Petri dish away from light at room temperature until deep red brown powdered was obtained and later stored in the refrigerator (4 °C) till it was needed. Qualitative test described by^[19] was done to determine the presence of flavonoids.

Extraction of tannins

Tannins was extracted by method described by^[20] with slight modification. Briefly, powdered materials (100g) were macerated 1000 ml of acetone for 72 hours. The supernatant was then separated from the residue by filtration using Whatman no.1 filter paper, the fraction was concentrated using a rotary evaporator at 45°C and the residues obtained was stored in a (4 °C) before further analysis. Qualitative test for presence of tannins was carried out by method described by^[21].

laboratory animals and ethical protocol

A Total of 10 adult male albino rats and 36 male albino mice with average weight of 160g and 25g were used for the *in-vitro* anti-inflammatory and *in-vivo* anti-allergy experiment respectively. The animals were obtained from the Department of Anatomy Animal House, LADOKE Akintola University of Technology (LAUTECH). The Animals were fed standard food pellets throughout the period of investigation and were allowed access to clean fresh water *ad libitum* in bottles. The experiment was carried out after its approval by the ethics committee of the Ladoke Akintola University of Technology in accordance with the recommendations of the proper care and use of laboratory animals.

In-vitro anti-inflammatory assays

Human red blood cell (hrbc) membrane stabilization

The human red blood cell (HRBC) membrane stabilization method was carried out according to the method of [21,22] with slight modification. The Blood was collected from male rats that has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were 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 was prepared (100 ,150, 200, 250and 300 µg/ml) adding distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension was added. It 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:

$$\% \text{ Membrane stabilization} = \frac{\text{Optical density of Test sample}}{\text{Optical density of Control}} \times 100$$

Protein inhibition activity

The test was performed according to the modified method of [23, 24]. The reaction mixture (2ml) containing 0.03mg trypsin, 0.5ml of 10mM Tris HCl buffer (pH7.4) and 0.5ml test sample of different concentrations of different solvents. The reaction mixture was incubated at 37 °C for 5 min and then 1ml of 0.8% (W/V) casein was added. The mixture was incubated for an additional 20 min, 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm 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;

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

Anti-lipoxygenase activity

Anti-lipoxygenase activity was assayed according to the method of [25]. Briefly, linoleic acid was used as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml of 2m borate buffer pH 9.0 and added 0.25ml of lipoxidase enzyme solution (20,000u/ml) and incubated for 5 min at 25 °C. After which, 1.0ml of lenoleic acid solution (0.6mm) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation,

$$\% \text{ Inhibition} = \frac{A \text{ control} - A \text{ test}}{A \text{ control}} \times 100$$

A control = absorbance of the control

A test = absorbance of sample in presence of extract

Anti-allergy assay

Induction of allergy in mice

This was carried according to the method described by [26] with slight modification. Briefly, mice were randomly divided into six (6) groups (n=6) and were sensitized by subcutaneous administration of 5ml/kg boiled and cooled milk thirty minutes after oral administration of *S.j* fruit extract. Group I served as the normal control group (no sensitization and received only distilled water), group II-IV received 166, 250 and 500 mg/kg of ethanol fruit extract of *S.j* respectively , group V received 5ml/kg of boiled and cooled milk (sensitized and untreated group) while group VI received standard drug Dexamethasone 50mg/kg.

Determination of total white blood cell and absolute eosinophil count

Twenty four hours after subcutaneous administration of milk, mice were slightly anesthetized with chloroform and whole blood was collected through jugular vein into heparinized bottles. Total white blood cell and absolute eosinophil count was carried out at the Hematology laboratory of Ladoko Akintola University of Technology Teaching Hospital, Ogbomoso Oyo State.

Statistical Analysis

All data were presented as mean ± standard error of mean (SEM) of triplicates and IC₅₀µg/ml (defined as the concentration of drugs or extracts necessary for 50% inhibition of the enzyme activity) 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).

RESULTS

Extraction Yields of Crude Extracts SJ fruit

(Table 1) show that the aqueous extract has the higher yield of the crude extracts 38.00g, while the ethanol extract yielded 34.00g.

Table 1: Yield in (grams) of aqueous and ethanol extracts from 400grams of *Sphenocentrum jollyanum* fruit

Extracts	Yield in grams(g)
Aqueous	38.00
Ethanol	34.00

Extraction Yields (%) of Secondary Metabolites SJ fruit

As shown in Table 2, saponins have the highest yield of 19.20%, followed by alkaloids 11.51% and tannin 10.70% respectively.

Table 2: Percentage (%) yield of tannins, saponins, alkaloids and flavonoids extracts from 250 grams of *Sphenocentrum jollyanum* fruit

Secondary metabolites	Percentage (%) yield
Flavonoids	2.00%
Tannin	6.70%
Saponins	10.20%
Alkaloids	8.51%

In-vitro Anti--Inflammatory Assays

Membrane stabilization activity of Sphenocentrum jollyanum fruit extracts

The crude (ethanol and aqueous) and secondary metabolites (tannin, saponins, flavonoids and alkaloids) extracts of *Sphenocentrum jollyanum* fruit showed significant ($p < 0.05$) dose dependent membrane stabilization activity. Table 3 showed that the ethanol extract of the fruit demonstrated the highest membrane stabilization

activity with an IC_{50} of $263 \pm 12.44 \mu\text{g/ml}$ when compared with the aqueous extract or any of the secondary metabolites. Table 3 also showed that tannin extract demonstrated highest membrane stabilization activity ($IC_{50} = 584 \pm 7.14 \mu\text{g/ml}$) when compared with other secondary metabolites. No membrane stabilization activity was demonstrated by the flavonoids and alkaloids extract. However, the ethanol and tannin extracts showed weaker membrane stabilization activity when compared with the standard drug Diclofenac (IC_{50} of $51 \pm 6.12 \mu\text{g/ml}$).

Table 3: IC_{50} ($\mu\text{g/ml}$) values and percentage (%) Membrane Stabilization activity of fruit extracts of SJ at concentrations of (100, 150, 200, 250, 300 $\mu\text{g/ml}$)

Extract/Drug	%Inhibition					$IC_{50} \mu\text{g/ml}$
	100 $\mu\text{g/ml}$	150 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	
Ethanol	27.20 \pm 0.01	30.46 \pm 0.05	38.50 \pm 0.04	48.00 \pm 0.03	57.30 \pm 0.03	263 \pm 12.44*
Aqueous	32.30 \pm 0.02	35.00 \pm 0.04	38.80 \pm 0.03	40.00 \pm 0.09	43.80 \pm 0.08	415 \pm 4.46*
Saponin	N/A	N/A	1.12 \pm 0.02	4.04 \pm 0.03	5.85 \pm 0.04	1740 \pm 2.17*
Tannin	9.20 \pm 0.04	10.50 \pm 0.06	14.60 \pm 0.01	20.20 \pm 0.03	26.40 \pm 0.03	584 \pm 7.14*
Akaloids	N/A	N/A	N/A	N/A	N/A	N/A
Flavonoids	N/A	N/A	N/A	N/A	N/A	N/A
Diclofenac	54.60 \pm 0.03	55.00 \pm 0.06	62.50 \pm 0.05	65.67 \pm 0.09	68.00 \pm 0.04	51 \pm 6.12

Values represent mean \pm SEM (n=3) * $p < 0.05$ considered as IC_{50} values significant when compared to the Standard Drug (Diclofenac). N/A- No activity was observed.

Table 4: IC_{50} ($\mu\text{g/ml}$) values and percentage protein inhibition activity of fruit extracts of S.J at concentrations of (100, 150, 200, 250, 300 $\mu\text{g/ml}$)

Extract/Drug	Inhibition					$IC_{50} \mu\text{g/ml}$
	100 $\mu\text{g/ml}$	150 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	
Ethanol	10.10 \pm 0.01	11.20 \pm 0.01	15.32 \pm 0.01	18.18 \pm 0.05	21.25 \pm 0.06	770 \pm 6.33*
Aqueous	13.78 \pm 0.04	15.00 \pm 0.05	18.88 \pm 0.02	22.94 \pm 0.07	24.22 \pm 0.02	740 \pm 4.63*
Saponin	7.77 \pm 0.062	11.05 \pm 0.08	12.14 \pm 0.05	13.32 \pm 0.06	16.50 \pm 0.07	1160 \pm 3.18*
Tannin	12.00 \pm 0.05	13.64 \pm 0.05	14.32 \pm 0.09	15.82 \pm 0.06	16.50 \pm 0.05	1810 \pm 1.78*
Akaloids	4.36 \pm 0.02	4.77 \pm 0.02	5.45 \pm 0.01	6.41 \pm 0.08	7.09 \pm 0.02	3370 \pm 1.13*
Flavonoids	3.95 \pm 0.08	5.86 \pm 0.02	6.41 \pm 0.02	7.09 \pm 0.03	7.91 \pm 0.024	2630 \pm 1.13*
Indomethacin	40.20 \pm 0.06	43.20 \pm 0.06	47.00 \pm 0.05	51.00 \pm 0.06	54.00 \pm 0.03	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)

Protein inhibition activity of Sphenocentrum jollyanum fruit extracts

All fruit extracts of *Sphenocentrum jollyanum* showed a significant ($p < 0.05$) dose dependent protein inhibition activity. As shown in Table 4, the aqueous extract of the fruit demonstrated insignificant high protein inhibition activity ($IC_{50} = 740 \pm 12.44 \mu\text{g/ml}$) when compared with ethanol extract ($IC_{50} = 770 \pm 6.33 \mu\text{g/ml}$) but significantly ($p < 0.05$) higher when compared with flavonoids, saponins and

alkaloids extracts respectively. The saponins extract demonstrated higher protein inhibitory ($IC_{50} = 1160 \pm 3.18 \mu\text{g/ml}$) effect when compared with other secondary metabolites. However, the aqueous and saponins extracts showed weaker protein inhibition activity than the standard drug Indomethacin ($IC_{50} = 246 \pm 5.66 \mu\text{g/ml}$).

Anti-lipoxygenase activity of *Sphenocentrum jollyanum* fruit extracts

The crude ethanol, aqueous and secondary metabolites (tannin, saponins, flavonoids and alkaloids) extracts of *Sphenocentrum jollyanum* fruit showed significant ($p < 0.05$) dose dependent anti-lipoxygenase activity. Table 5 showed that the ethanol extracts of the fruit demonstrated insignificant high anti-lipoxygenase activity with an IC_{50} of $645 \pm 5.15 \mu\text{g/ml}$ when compared with the aqueous extract (IC_{50} $684 \pm 4.98 \mu\text{g/ml}$). It also showed a significant $p < 0.05$ higher dose dependent anti-lipoxygenase activity when compared with tannin, flavonoids, saponins and alkaloids extracts. Among the secondary metabolites, saponins extract showed the highest anti-lipoxygenase activity ($IC_{50} = 1204 \pm 3.24 \mu\text{g/ml}$). However, the ethanol and saponins extracts showed a weaker anti-lipoxygenase activity than the standard drug indomethacin with an IC_{50} of $172 \pm 5.95 \mu\text{g/ml}$.

In-vivo anti-allergy assay

Effect of ethanol fruit extract of *sphenocentrum jollyanum* in milk induced eosinophilia and leukocytosis

The result as shown in Table 6 revealed that there was a maximum increase in leukocytes and absolute eosinophils count in the untreated group of mice 24 hours after subcutaneous administration of boiled and cooled milk (5ml/kg). Oral administration of ethanol fruit extract of *S.J* caused significant ($p < 0.05$) dose dependent reduction in milk induced leukocytosis and eosinophilia mice to near normal. The activity of the ethanol fruit extracts compared favorably with the standard drug (dexamethasone).

Table 5: IC_{50} ($\mu\text{g/ml}$) values and percentage Lipoxygenase inhibition activity of fruit extracts of S.J at concentration of (100, 150, 200, 250, 300 $\mu\text{g/ml}$)

Extract/Drug	%Inhibition					$IC_{50} \mu\text{g/ml}$
	100 $\mu\text{g/ml}$	150 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	
Ethanol	27.20 \pm 0.11	30.46 \pm 0.66	38.50 \pm 0.69	48.00 \pm 0.95	57.30 \pm 0.49	263 \pm 12.44*
Aqueous	32.30 \pm 0.94	35.00 \pm 0.59	38.80 \pm 0.37	40.00 \pm 0.83	43.89 \pm 0.76	414 \pm 4.46*
Saponin	5.17 \pm 0.51	8.17 \pm 0.03	10.34 \pm 0.63	12.55 \pm 0.7	13.00 \pm 0.54	1204 \pm 3.24*
Tannin	N/A	N/A	0.48 \pm 0.11	0.96 \pm 0.71	1.97 \pm 0.35	5687 \pm 0.82*
Akaloids	5.16 \pm 0.37	6.68 \pm 0.63	7.34 \pm 0.21	8.39 \pm 0.75	8.53 \pm 0.41	2880 \pm 1.38*
Flavonoids	3.62 \pm 0.71	4.53 \pm 0.87	5.54 \pm 0.15	5.67 \pm 0.15	7.67 \pm 0.52	2682 \pm 1.51*
Indomethacin	45.50 \pm 0.67	47.60 \pm 0.56	51.60 \pm 0.73	56.40 \pm 0.35	59.80 \pm 0.77	172 \pm 5.95

Values represent mean \pm SEM (n=3) * $p < 0.05$ considered as IC_{50} significant when compared to the Standard Drug (Indomethacin) N/A- No activity was observed

Table 6: The effect of ethanol fruit extract of *Sphenocentrum jollyanum* on milk induced leukocytosis and eosinophilia mice

Treatment	TWBC($\mu\text{g/ml}$)	Neu (%)	Lym (%)	Eos (%)	AbsEos(c/cmm)
I=(control)	6300 \pm 251	22.0 \pm 1.0	44.0 \pm 1.67	1.0 \pm 0.67	50.0 \pm 0.23
II=Allergen+166mg/kg	14400 \pm 351*	23 \pm 1.1	60.0 \pm 2.50*	3.0 \pm 0.46	100.0 \pm 0.29*
III=Allergen+250mg/kg	10700 \pm 360*	22 \pm 1.0	55.0 \pm 0.31	2.0 \pm 0.55	50.0 \pm 0.34
IV=Allergen+500mg/kg	10000 \pm 251*	22 \pm 1.0	50.0 \pm 2.65	2.0 \pm 0.73	50.0 \pm 0.66
V=(Allergen only)	15700 \pm 200*	52 \pm 1.0*	81.0 \pm 3.38*	4.0 \pm 0.76	150.0 \pm 1.6*
VI=Allergen+50mg/kgSTD	9990 \pm 170*	22 \pm 0.57	47.0 \pm 5.40	2.0 \pm 0.34	50.0 \pm 0.33

DISCUSSION

The disruption of the cell membrane is a well-documented mechanism in the pathogenesis and development of inflammatory and allergic disorders.^[22] The membrane of the erythrocytes is analogous to the lysosomal membranes, thus its stabilization can be extrapolated to lysosomal membranes. It is important to stabilize the lysosomal membrane so as to limit the release of its contents such as enzymes and other inflammatory mediators which cause further tissue damage that are evident in their extracellular release.^[27] Therefore, the human

red blood cell membrane assay is an important technique in studying anti-inflammatory activities of drugs, chemicals, herbal preparations.^[28] The significant membrane stabilization activity of the crude extracts of *S. j* fruit (Table3) may be attributed to the presence of different phytochemicals in the fruit extracts. On the basis of this result, the observed high membrane stabilization activity of the ethanol fruit extract might be due to the high presence and high activity of tannin see in Table 2 and Table 3 respectively.

Tannins are a complex family of naturally occurring polyphenols which have molecular weights ranging from 0.5 to 22 kD produced by plants to provide protection from disease and mammalian herbivores.^[29] They are potent metal ion chelators ^[30] and this is responsible for its ability to stabilize biological system and macromolecules by binding tightly to cations (calcium and iron) and intracellular electrolytes.^[31] Thus, tannins have been targeted as good candidates for therapeutic against inflammatory related disorders and oxidative stress.^[32] Proteinases are enzymes that are ubiquitous in all living organisms, mediating numerous physiological reactions from breaking down of food to blood clotting cascade. They are involved in the breaking down and modification of peptide bonds into their primary structures.^[33] During inflammatory states, neutrophils are activated and they contains in their lysosomal granules serine proteases which attack tissues, causing damages that is evident in swelling and pain.^[34] In respect of the aforementioned, the role of proteinases in inflammation cannot be overemphasized and its inhibition becomes imperative. In this present study, it can be seen that the ethanol fruit extract of *Sphenocentrum jollyanum* demonstrated the highest trypsin inhibition activity, with all the secondary metabolites demonstrating considerable protein inhibition activities (Table 4). This activity of the ethanol fruit extracts might be due to the synergistic activity of the various constituent phytochemicals. Wide range of phytoconstituents such as alkaloids, polyphenols and terpenoids has been reported for their anti-inflammatory activities ^[35] and protein inhibition has been reported as one of the several mechanism of their anti-inflammatory action. Mechanism of protein inhibition can be through modulation of cellular activities of proteins such as protein kinases involved in cell activation process of the T-cell and cytokines production ^[36], inhibition of lysosomal enzymes (elactase and glucorinidase) release from stimulated neutrophil ^[37] and impairment of lysosomal enzyme release from polymorpho nuclear leukocytes^[38].

Lipoxygenases have been reported to play an important role in the pathophysiology of several inflammatory and allergic diseases. Lipoxygenase mediates the incorporation of molecular oxygen into arachidonic acid to generate its metabolites that are known mediators in allergy and inflammatory disorders.^[39] The result of this present study revealed that various extracts of *S.J* fruit demonstrated significant anti-lipoxygenase activity (Table5). Be as it may, Table 5 also revealed that the ethanol extract of the fruit demonstrated the highest anti-lipoxygenase activity. The high anti-lipoxygenase activity showed by the ethanol extracts of the fruit might be due to the synergistic effect of the various bioactive compounds (alkaloids, tannin, flavonoids and saponin).

Bioactive compounds such as polyphenols have been reported to target prostaglandins which are involved in the late phase of acute inflammation and pain perception.^[40,41] Flavonoids are also capable of modulating arachidonic acid related enzymes thereby inhibiting the release of pro inflammatory enzymes such as (COX, LOX and NOS) from different sources.^[42]

Allergic disorders are characterised by the hypersensitivity of the immune system to non-infectious stimulus in the environment.^[1] Induction of allergy by milk may be classified as type I hypersensitivity due to the increase in cells associated i.e. neutrophils, lymphocytes, eosinophils and macrophages.^[43] These cells during allergic state release the inflammatory mediators like cytokines, histamine, and major basic protein, which promote the ongoing inflammation.^[43] The result of this study showed that there was an

increase in total white blood cell and eosinophil count 24 hours after milk administration (Table 6 Group V). An abnormal increase in eosinophil count is termed as eosinophilia and this is seen in allergic conditions. In this study ethanol fruit extract of SJ was tested for its anti-allergic property and result showed that the various concentration (Table 6, Group II, 166mg/kg, III 250mg/kg, IV 500mg/kg) of the extract significantly reduced the leukocytes and oesinophil count, in a dose dependent manner. The highest oesinophil and leukocytes count was observed in the allergen group only (V), while the strongest anti-allergic activity was observed in standard drug treated group (VI). The ability of the extract to reduce the oesinophil and leucocytes counts in the infected mice might be due to the different bioactive constituents present in it. Phytochemicals have been reported to inhibit chemical mediator release and cytokine production by mast cells ^[44] which is hall mark of type 1 hypersensitivity disorder. Saponins have been reported to possess mast cell stabilizing activity ^[45] while flavonoids have shown to possess anti-histamine activities by inhibition of transport ATPase, smooth muscle relaxant and bronchodilator activity.^[46]

CONCLUSION

This study confirmed that the fruit extracts of *Sphenocentrum jollyanum* demonstrated anti-inflammatory and anti-allergy properties. The result of this study validates the ethno-botanical use of *Sphenocentrum jollyanum* fruit in the treatment of inflammation and allergic disorders. Thus, further studies need to be carried out to isolate and purify the bioactive compounds that are responsible for the various anti-inflammatory and anti-allergy activities.

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HOW TO CITE THIS ARTICLE

Olorunnisola OS, Adetutu A, Fadahunsi OS. Anti-allergy potential and possible modes of action of *Sphenocentrum jollyanum* pierre fruit extracts. J Phytopharmacol 2017;6(1):20-26.