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Phytochemical Composition and Anti-Inflammatory Potential of Flavonoid-Rich Fraction of *Erythrina senegalensis* DC (Fabaceae) Leaf

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ABSTRACT

Erythrina senegalensis (leaves, root, and bark) has been used in folkloric medicine in West Africa to manage inflammation for centuries, although this has not been scientifically validated. Herein, we investigated the anti-inflammatory properties of flavonoid-rich fraction of ethanol extract of *Erythrina senegalensis* leaves. In vitro anti-inflammatory parameters were assayed using human red blood cell (HRBC) methods. Also, acute toxicity study was performed in adult mice (between 3 to 4 months), while in vivo anti-inflammatory activity was evaluated using egg albumin paw oedema adult (between 3 to 4 months) rat model. Phytochemical examination revealed high levels of flavonoids, tannins, terpenoids, and phenolics. No mortality in mice was recorded up to 5000 mg/kg. At the various concentrations (0.2-1.0 mg/ml), the extract was potent in stabilizing the HRBC membrane, and significantly ($p < 0.05$) suppressed albumin denaturation, platelet aggregation, phospholipase A2 and protease activity in a concentration-dependent trend in a similar way with the reference drug. Also, the in vivo study showed that the extract significantly ($p < 0.05$) decreased rat paw oedema formation in a time-dependent (0.5-5 hrs) manner. The extract at 100 mg/kg elicited the greatest Inhibition (68.30 %) and competes favourably with the standard group (65.70 %). These, thus, affirm the anti-inflammatory potency of the extract.

***Keywords*:** Albumin Denaturation, Protease Activity, Rat paw oedema, Platelet aggregation.

Introduction

Inflammation refers to one of the diverse defensive mechanisms employed by the immune system against infiltrating pathogens and disease-causing agents.[1] It is usually accompanied with painful episodes due to increased blood flow and vascular permeability at the injured site and recruitment of leucocytes, lysosomal leakages, proteins denaturation, and cellular membrane breakdown, and may progress to tissue damage upon persistent and exaggerated inflammatory condition.[1,2] Non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, indomethacin and aspirin are potent anti-inflammatory agents but are associated with several adverse effects like hypertension, weight gain and ulcer, especially when used for a long period.[3] Thus, agents from plant origin with fewer side effects and better efficacy are being sought for. The plant *Erythrina senegalensis* is an open crowned spiny shrub that usually grows about 3-4.5 m high and may grow at times up to 12-15 m high. The bole consists of stout prickles and a tough and greyish bark. It is commonly used as a vegetable and called 'Minjirya' (Hausa, Nigeria).[4] Studies have shown that *Erythrina senegalensis* stem, bark and root extracts possess antimalarial, antibacterial, anti-inflammatory, and analgesic activities.[5,6,7]

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The leaf of *E. senegalensis* has also been shown to contain some important phytochemicals known to possess several biological activities.[8,9] Traditionally, *Erythrina senegalensis* is used to manage female infertility, arthritis, jaundice, asthma, liver and gastrointestinal diseases, fever, eye infections and renal bilharziasis.[10,11] The Indigenous people of South Eastern Nigeria have used *Erythrina senegalensis* leaves to manage inflammation for decades, although these effects have not been scientifically tested. Therefore, the present study seeks to ascertain the in-vitro and in-vivo anti-inflammatory potential of the flavonoid-rich fraction of ethanol extract of *Erythrina senegalensis* leaves.

Materials and Methods

Equipment
Weighing balance (Vikas Ltd, England), measuring cylinder (Pyrex, England), spectrophotometer (E-312 model, Jenway, UK), centrifuge (Vikas Ltd, England), refrigerator (Thermocool, England), water bath (Gallenkamp, England), rotary evaporator (Hujin, China), beakers, test tubes.

Animals
Forty-two (42) adult (3 to 4 months old) whistar Hannover rats (Whistar HAN) and eighteen (18) adult (3 to 4 months old) mice (C57BL/6 strain) of both sexes, with weight ranges of 80 – 150 g and 20 – 30g, respectively, were used in the study. The animals were gotten from the Department of Zoology and Environment Biology animal holding unit, University of Nigeria, Nsukka. The animals were maintained in an highly hygiene environmental condition (relative humidity of 55 ± 5 %, temperature of 24 ± 1 0C, and a 12 /12 hour light/dark period), in cages and feed with rat chow (Vital feed) and were acclimatized with the environment for 14 days. All experiments involving animals were done in compliance with international ethics and protocols in research as

enshrined in the NIH Guide (revised National Institute of Health publication #85-23; 1985).

Sourcing and Identification of the study plant
The fresh leaves of Erythrina senegalensis were sourced from their natural habitat at Ugwu-Awgbu village (6.1175º N, 7.0896º E) situated in Orumba North of Anambra-Nigeria on 30th April, 2021. The leaves were identified by Mr. Ozioko Alfred, BDCP, and a sample was reserved at the herbarium of the Bioresources Development and Conservation Program Research Centre, Nsukka, Enugu, Nigeria (voucher no: BDCP20210629).

Crude extraction process
The plant leaves were washed in clean water to remove debris, after which they were shade-dried for 10 days to a constant weight. The shade-dried leaves were ground into a powdered form using an electric blender. Weighed mass (402.4g) of the pulverized leaves was macerated in 95 % (1 Litre) ethanol for 72-hours with occasional stirring, and the macerated content was filtered with Whatman No. 1 filter paper. The filtrate was concentrated at 45 ℃ to get the crude ethanol extract in a rotary evaporator.

Preparation of Flavonoid-Rich Fraction
This was performed according to the outlined procedures of Chu *et al*.[12] A weighed amount (3 g) of ethanol extract was introduced into a small flask containing 20 mL of 10 % H_2SO_4. The hydrolysis was facilitated by incubating in a water bath at 100 ℃ for 30 min. The reaction mixture was allowed to stay on ice for 15 minutes. The filtrate of the resulting precipitated solution was filtered, and being the flavonoid aglycone was further dispensed into a warm (50 °C) volume of 95 % ethanol (50 mL). The resulting mixture was then poured into a 100ml graded flask and was made up to 100ml using ethanol (95 %). A rotary evaporator was used to concentrate the filtrate at 45°C in order to obtain the flavonoid-rich fraction. A sufficient quantity of the flavonoid-rich fraction was further obtained by repeating the procedures several times.

Ethical Clearance
The ethics committee of the Department of Biochemistry University of Nigeria Nsukka granted ethical approved the experimental protocols. The ethical approval number for the use of animals in this experimental procedure is UNN/FBS/EC/1094

Acute toxicity (LD_{50}) study
This was performed in mice according to the method of Lorke.[13]
The LD_{50} was determined using the geometric average of the greatest non-fatal dosage and the minimum deadly dosage

Phytochemical analysis
This was performed using standard procedure, as outlined by Trease and Evans.[14] The phytoconstituent concentrations were estimated as follows:

$$Concentration\left(\frac{mg}{100g} or \frac{g}{100g}\right) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Dilution\ factor$$

$$Dilution\ factor = \frac{Total\ volume}{Weight\ of\ extract}$$

Anti-inflammatory study
Inhibition of albumin denaturation
This was performed according to the method of Sakat *et al*.[16] and Mizushima and Kobayashi.[15] with minor modifications. The reaction mixture constitutes the test sample mixed with 1 % bovine albumin aqueous solution with occasional adjustment of the pH by the introduction of a small quantity of 1 N HCl. It was allowed to stay at ambient temperature for 10-minutes and incubated in a water bath at 51 °C for 20 minutes. The absorbance was read at 660 nm after cooling to room temperature. The experiment was repeated three times; with the % prevention of albumin denaturation by the test samples evaluated by the formula thus;

$$\%\ Inhibition = \frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$$

Where Abs = absorbance

Protease inhibition assay
This was performed using the method of Oyedepo and Femurewas.[17] and Sakat *et al*.[16] with minor modifications. The reaction mixture constitutes, trypsin (0.06 mg), 1 ml 20 mM TrisHCl buffer of pH 7.4 mixed with 1 cm^3 of the fraction or reference drug at varying concentrations (200-1000 mg/ml). The reaction mixture was heated for 5-min at ambient temperature, followed by the introduction of 1 cm^3 of 0.8% (w/v) casein into it. The resulting mixture was heated further for 20 min. Thereafter, a graded volume of 70 % perchloric acid (2 ml) was introduced into it to quench the reaction and obtain a hazy suspension. The resulting cloudy solution was spun for 10-min at 3000 rpm, after which the supernatant's absorbance was measured against a blank buffer at 210 nm, spectrophotometrically. The experiment was carried out three times, and the proportion of proteinase inhibitory activity of the various concentrations of the test sample and reference drug were determined, using the formula below;

$$\%\ Inhibition = \frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$$

Where Abs = absorbance

Phospholipase A_2 Activity
The ability of the plant leaf fraction to suppress the activity of phospholipase A_2 was estimated according to the method of Vane.[18] By acting on membrane phospholipids to liberate free fatty acids, phospholipase A2 damages the cell membrane, resulting in cellular components leaking into the media. The activity of phospholipase A_2, which is directly correlated with the quantity of dispersed haemoglobin in the reaction milieu, was evaluated spectrophotometrically at a wavelength of 418 nm as explained in Section 2.9.3.2

Preparation of Enzymes
The enzyme was isolated from a pure fungal culture of the *Aspergillus niger* strain. After homogenizing a weighed quantity of Sabouraud dextrose agar (15 g) diluted with distilled water (1.0 dm^3), and warm on a water bath for 10-minute, it was transferred unto 250-ml marked vessel. The flask holding the resulting nutrient broth was stopped with foil paper and cotton wool before being autoclaved for 14 min at 123 °C. After allowing the broth to cool to ambient temperature, it was used to aseptically inoculate the organisms in Petri plates, which were then stored at room temperature for 72 hours. The purified isolate was dispensed in test tubes that contained 3 ml of phosphate-buffered saline and spun for 10 minutes at 3000 pm. The fungi cells are represented by the pellet, while the crude enzyme constitutes the supernatant solution.

Preparation of the Substrate
The blood samples from human volunteers were centrifuged at 3000 rpm for 10-min to discard the supernatant. The red blood cell pellets were rinsed thrice with normal saline of an equivalent amount, and the packed cell volumes were reconstituted as a 40% suspension (v/v) in 10 mM phosphate-buffered solution (pH 7.4). This was taken as the phospholipase A_2 enzyme's substrate. Varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) of the test samples or concentrations (0.2, 0.4, 0.8 mg/ml) reference drug (Acetylsalicylic acid) were combined with 0.2 cm^3 of 2 mM $CaCl_2$, 0.2 cm^3 of HRBCs, and 0.2 cm^3 of crude enzyme in sets of three test tubes and incubated for one hour. The erythrocyte suspension, $CaCl_2$, and free enzyme are all included in the normal control except the test sample. In each case, 0.2 ml of the heat-denatured enzyme was used as a blank. The mixtures were spun at 3000 rpm for ten minutes after they had been incubated. The solutions' absorbance was read at 418 nm following the introduction of 10 ml of normal saline into 1.5 ml of each supernatant. The standard drug used is Prednisolone (0.2, 0.4, 0.8mg/ml), which suppresses the activity of phospholipase A_2. The % maximal enzyme activity and % inhibition was calculated thus:

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$$\%\ maximum\ activity = \frac{OD\ of\ test/t}{OD\ of\ control/t} \times 100$$

$$\%\ Inhibition = 100\ -\ \%\ maximum\ enzyme\ activity$$

Where;
OD = Optical density
t = Time

Membrane stabilization assay
This was performed based on the outlined method of Oyedepo *et al*.[17] and Ezekwesili and Nwodo.[19] with minor modifications.

Preparation of HRBCs Suspension
Fresh blood samples were obtained from healthy human volunteers and distributed into tubes containing Ethylenediaminetetraacetic acid (EDTA) (1%) solution using a 5 ml syringe. The blood was centrifuged at 3000 rpm in 10 minutes, and the resulting pellets were rinsed with the same volume of normal saline, repeated three times. The measured packed cells were made up of a 40% suspension (v/v) in normal saline.

Test procedure
Low concentrated (0.2mg/ml) (Hypotonic) solution was made separately using a small mass of the test sample or reference drug (Acetylsalicylic acid) diluted with a larger volume of distilled water. Done in triplicates, aspirated volumes (1 ml) of the various concentrations (200-1000 mg/ml) of the extracts or the standard drug (Indomethacin) were dispensed into sets of test tubes. Using distilled water (vehicle), each test-tube volume was made up to 4.9 ml mark. The test used two control test tubes, a hypotonic solution made up of the vehicle (4.9 ml distilled water) and an isotonic solution consisting of 4.9 cm^3 of normal saline. After transferring 0.1 ml of HRBCs suspension into each tube with gentle shaking, the reaction tubes were warmed at 37ºC for an hour, after which they were then spun for 10 minutes at 3000 rpm, and the supernatants' absorbance were read at 418 nm using a spectrophotometer. The experiments were performed thrice. Test solutions consisted of HRBC suspension and graded volume (1 ml) of varying concentrations of the plant fraction of reference drug, which was made up to 5.0 ml mark using normal saline. The blank of the control test tubes were tubes containing normal saline void of HRBC. The degree of Inhibition of hemolysis was estimated by the formula, thus:

$$\%\ Inhibition\ of\ Hemolysis = 1\ -\ \frac{OD2 - OD1}{OD3\ -OD1} \times 100$$

The control I (isotonic solution) absorbance is OD1, the test or reference drug sample absorbance is OD2, while the control II (hypotonic solution) absorbance is OD3.

Platelet Aggregation Inhibition
This was performed according to the method of Born and Cross.[20] with minor modifications. Increased platelet aggregation in the solution medium leads to higher transmittance and lower light absorption. By measuring the reduction in absorbance of the reaction at 520 nm, the degree of $CaCl_2$-induced platelet aggregation may be measured.

Platelet-rich plasma (PRP) preparation
Blood was obtained from voluntary human subjects and distributed into 1% EDTA containing bottles and was spun in 10-minutes at 3000 rpm. Following centrifugation, normal saline was used to rinse the supernatant twice before being adopted as PRP.

Procedure
The tests were performed three times. An aspirated quantity of PRP (0.2 cm^3) was distributed into three test-tubes, each consisting of 1 ml of the plant fraction in normal saline or the reference drug (Acetycylic acid) in normal saline at various concentrations (200-1000 mg/ml). The vehicle (normal saline) was used to make up to the 2.2 cm^3 volume mark. Graded volumes of PRP (0.2 ml) and normal saline (2 ml) were in the normal control test tube. The test tubes were incubated before introducing 1.47% $CaCl_2$ (0.4 ml) to cause platelet clumping. A spectrophotometer was used to record the difference in absorbance (at 520 nm) of the solutions at 2 min intervals for 8-min. In each case, the blank contains PRP-free plants fraction of reference drugs. The degree of Inhibition of platelet clumping (X) was estimated as in % by the formula, thus;

$$\%\ Inhibition\ of\ platelet\ agggregation\ (x) = \frac{A\ -B}{A} \times 100$$

Where A is the control's maximum aggregation rate, and B is the test sample's maximum aggregation rate.

Egg albumin induced paw oedema
This was done following the method of Winter *et al*.[21] with minor modifications. Five (5) groups of rats (n = 5) were administered different treatments as thus; group I was administered with normal saline, group II was treated with 10 mg/kg indomethacin while groups III-IV were administered with 100, 200 and 350 mg/kg extract using normal saline . The right hind paw volumes were estimated using a Plethysmometer (LETICA-7500) on the concept of volume dislocation instantly prior to the zero time of the experiment and in 1 hr interval following egg albumin administration for a total time-space of 5-hours. The changes in the volume displaced after the egg albumin injection compared to the volume displaced at time zero of the rat paw (expressed thus; Vt- Vo) was used to estimate average oedema at each interval. Each treatment group's oedema inhibition percentage was also determined using the equation as thus;

$$\text{Percentage (\%)inhibition of oedema} = \frac{(Vt\ -Vo)\ control\ -\ (Vt\ -Vo)\ treatment}{(Vt\ -Vo)\ control} \times 100$$

Where; rat paw oedema = (Vt – Vo)
Vo = rat paw oedema at time zero
Vt = rat paw oedema at the different time intervals

Statistical Analysis
Data were examined with one-way and two-way ANOVA (analysis of variance) using version 23.0 of SPSS (Statistical Product and Service Solution). The results were presented as the Mean ± SEM, and the difference in mean values was deemed significant at $p < 0.05$.

Results and Discussion

Acute toxicity (LD_{50}) of Erythrina senegalensis leaves extract
The administration of *Erythrina senegalensis* leaves extract caused no death in mice, and there was no sign of weakness or decreased activity/movement upto 5000 mg/kg. Based on acute toxicity test results, the doses for the study was chosen as 100, 200 and 350 mg/kg.

Phytochemical screening of Erythrina senegalensis extract.
The phytochemical examination showed that *Erythrina senegalensis* leaves extract possess flavonoids, phenols, terpenoids and tannins with a little amount of saponins, glycosides, alkaloids, and steroids.

Effect of Erythrina senegalensis leaves extract on *denaturation of albumin*
The percentage inhibition of albumin denaturation exhibited by *Erythrina senegalensis* extract and standard drug (Prednisolone) is presented in Table 2. The result revealed that the extract demonstrated appreciably % inhibition of albumin denaturation in a dose-dependent manner and was greatest (46.0 %) at 1.0 mg/ml. *Erythrina senegalensis* leaves extract exhibited an inhibitory effect 37.0 % on the enzyme activity and the standard drug exhibited 36.0 %, at a similar concentration (0.8 mg/ml), and this was not significantly ($p > 0.05$) different.

Effect of Erythrina senegalensis leaves extract on protease activity
Table 3 shows the reducing effect of protease activity elicited by *Erythrina senegalensis* leaves extract. It was observed that extract effectively suppressed protease activity in a manner that is directly

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proportional to the concentration. *Erythrina senegalensis* leaves extract caused 25 ± 0.51 % at 0.8 mg/ml, which was similar to the % inhibition (22 ± 0.13 %) exhibited by the reference drug (Acetylsalicylic acid) at 0.8 mg/ml.

Effect of Erythrina senegalensis leaves extract on Phospholipase A2 Activity

The inhibitory potential of *Erythrina senegalensis* leaves extract on the activity of phospholipase A2 is presented in Table 4. It was observed *Erythrina senegalensis* leaves extract significantly ($p < 0.05$) decreased the enzyme activity in a dose-dependent fashion which was greatest (35.99 %) at 1.0 mg/ml. However, the standard drug (Prednisolone) exhibited significantly ($p < 0.05$) caused 70.24 % suppression in the enzyme activity, even at 0.8 mg/ml.

Effect of Erythrina senegalensis leaves extract on CaCl2-induced platelet aggregation

The anti-platelet aggregatory effect of *Erythrina senegalensis* leaves extract is shown in Table 5. It was observed that *Erythrina senegalensis* leaves extract significantly ($p < 0.05$) suppressed platelet aggregation induced by the introduction of $CaCl_2$ in the reaction vessel in a manner that is directly proportional to both concentration and time. The maximum anti-platelet aggregatory activity of leaves extract and reference drug were reached at the 8th minute and was coparable to Aspirin.

Effect of Erythrina senegalensis leaves on HRBC membrane stabilization

The stabilization of the HRBC membrane elicited by *Erythrina senegalensis* leaves extract is presented in Table 6. It was observed that leaves extract demonstrated membrane stabilization in a concentration-dependent fashion, with 47.40 ± 0.05 at 1.0 mg/ml. However, the standard drug showed significantly ($p < 0.05$) caused 63.20 ±0.40 % at 0.8 mg/ml.

In vivo anti-inflammatory activity

Effect of Erythrina senegalensis leaves extract on egg albumin-induced paw oedema

The inhibition of egg albumin induced-paw oedema exhibited by *Erythrina senegalensis* leaves extract is presented in Table 7. The Animals pretreated with *Erythrina senegalensis* leaves extract showed a significant ($p < 0.05$) decrease in the volume of paw oedema. The reduction in paw size progressively occurred in a time-related manner. 100 mg/kg of *Erythrina senegalensis* leaves extract had inhibition of 68.30 %. These were not significantly ($p > 0.05$) different relative to the inhibition (65.70%) by the standard control group.

The LD_{50} of *Erythrina senegalensis* leaves extract higher than 5000 mg/kg. Findings from the qualitative and quantitative phytochemical analysis of *Erythrina senegalensis* leaves extract showed the presence of flavonoid, tannins, terpenoids and phenols, a moderate amount of alkaloids and a trace quantity of glycosides, steroids, and saponins. This is in agreement with the findings of Bako and Madu.[8] Plants secondary metabolites constitute biologically potent compounds which are being harnessed as pharmacological agents. Flavonoids and alkaloids, for example, have been related to analgesic, antipyretic, and other effects.[22] Various studies have proposed the anti-inflammatory potential of flavonoids .[7,23,24,25] Terpenoids, steroids, saponins, tannins and alkaloids are reported to elicit anti-inflammation potentials by a cohort of studies.[25,28] Doughari.[9] also submitted that glycosides and steroids are present in the leaf of *E. senegalensis*. The occurrence of steroids in the leaf fraction suggests a steroidal anti-inflammatory mechanism as opposed to that of NSAIDs, which are not without several complications.

Table 1: Phytochemical constituents of *Erythrina senegalensis* leaves extract

Phytochemical Constituents	Relative abundance	Concentration (mg/100 g)
Flavonoids	+++	1277.42 ± 22.581
Phenols	++	588.80 ± 46.123
Terpenoids	+++	737.11 ± 45.020
Tannins	+++	970.00 ± 19.323
Saponins	+	0.21 ± 0.003
Glycosides	+	3.86 ± 0.041
Alkaloids	+	27.05 ± 0.674
Steroids	+	1.02 ± 0.104

Results are presented in Mean ± SE; +, ++, and +++ = mildly, moderately and highly abundant

Table 2: Effect of *Erythrina senegalensis* leaves extract on albumin denaturation

Treatments	Conc. (µg/ml)	Absorbance at 660nm	% Inhibition of albumin denaturation
Control	---	0.320 ± 0.003^{g}	----
leaves Extract	0.2	0.247 ± 0.003^{f}	23.01^{a}
	0.4	0.233 ± 0.004^{e}	27.03^{b}
	0.6	0.221 ± 0.004^{d}	31.02^{c}
	0.8	0.202 ± 0.003^{b}	37.02^{d}
	1.0	0.173 ± 0.013^{a}	46.04^{e}
Acetylsalicylic acid	0.2	0.244 ± 0.011^{ef}	24.00^{a}
	0.4	0.233 ± 0.002^{e}	27.03^{b}
	0.8	0.204 ± 0.004^{c}	36.00^{d}

n=5, the mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.

Table 3: Effect of *Erythrina senegalensis* leaves extract on protease activity

Treatments	Conc. (mg/ml)	Absorbance	% Inhibition of Protease activity
Control	----	0.306 ± 0.001^{d}	----
leaves extract	0.2	0.268 ± 0.008^{c}	12.00^{a}
	0.4	0.263 ± 0.003^{c}	14.01^{b}
	0.6	0.256 ± 0.037^{bc}	16.03^{c}
	0.8	0.230 ± 0.003^{a}	25.05^{e}
	1.0	0.224 ± 0.008^{a}	27.03^{f}
Acetylsalicylic acid	0.2	0.277 ± 0.002^{c}	10.02^{a}
	0.4	0.271 ± 0.002^{c}	11.07^{a}
	0.8	0.240 ± 0.001^{ab}	22.02^{d}

n=5, the mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.

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Table 4: Effect of *Erythrina senegalensis* leaves extract on phospholipase A_2 activity

Treatments	Conc. (mg/ml)	Absorbance	% Max	% Inhibition of Phospholipase A_2 activity
Positive control	---	0.803 ± 0.001^{i}	----	----
leaves extract	0.2	0.656 ± 0.003^{h}	81.69	18.31^{a}
	0.4	0.650 ± 0.001^{g}	80.95	19.05^{a}
	0.6	0.571 ± 0.002^{f}	71.11	28.89^{b}
	0.8	0.522 ± 0.002^{d}	65.01	34.99^{c}
	1.0	0.514 ± 0.001^{c}	64.01	35.99^{c}
Prednisolone	0.2	0.538 ± 0.001^{e}	67.00	33.00^{c}
	0.4	0285 ± 0.002^{b}	35.49	64.51^{d}
	0.8	0.239 ± 0.001^{a}	29.76	70.24^{e}

n=5; mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.

Etkin.[5] demonstrated the anti-plasmodia, anti-inflammation, pain-relieving, and antibacterial actions of the stem and root extracts of *E. senegalensis*, which contained glycosides and steroids. Das *et al*. [25] reported that these phytochemical constituents contribute to the anti-inflammatory activity of medicinal plants. Egharevba *et al*.[29] also established through a scientific investigation that plants with saponins in their substance have anti-inflammatory, antioxidant, anti-cancer, and anti-viral properties. When biologically active proteins are denatured, they lose their activity, a known cause of inflammation.[30] This implies that any substance that can inhibit albumin denaturation could act as an anti-inflammatory agent since albumin is a protein. The result of the present study showed that *Erythrina senegalensis* leaves inhibited albumin denaturation significantly ($p < 0.05$), following a concentration-dependent fashion akin to standard drugs, thus possessing anti-inflammatory activity. Leukocyte proteases play key roles in the progression of tissue damage at the inflammation milieu.[6] Therefore bioactive substances with the ability to inhibit protease activity are deemed, anti-inflammatory agents. The findings of this study showed that *Erythrina senegalensis* leaves inhibited protease activity significantly ($p < 0.05$), following a dose-dependent trend, and its inhibitory activities were similar to the standard drug. These suggest that FREES leaves possess anti-inflammatory properties.

Table 5: Effect of *Erythrina senegalensis* leaves extract on calcium chloride-induced platelet aggregation

Treatment	Conc (mg/ml)	Δ Absorbance 2 min	4 min	6 min	8 min
Control		$0.421 \pm 0.006^{a,G}$	$0.434 \pm 0.002^{b,G}$	$0.439 \pm 0.005^{c,G}$	$0.445 \pm 0.002^{d,D}$
leaves extract	0.2	$0.320 \pm 0.001^{a,E}$	$0.329 \pm 0.001^{b,E}$	$0.332 \pm 0.001^{c,E}$	$0.337 \pm 0.001^{d,C}$
		(24.0 %)	(33.0 %)	(68.0 %)	(69.0 %)
	0.4	$0.245 \pm 0.005^{a,B}$	$0.247 \pm 0.001^{a,D}$	$0.248 \pm 0.006^{a,B}$	$0.255 \pm 0.005^{b,B}$
		(42.0 %)	(43.0 %)	(61.0 %)	(67.0 %)
	0.6	$0.297 \pm 0.001^{c,D}$	$0.295 \pm 0.001^{b,D}$	$0.292 \pm 0.011^{g,D}$	0.295 ± 0.005^{aB}
		(29.0 %)	(32.0 %)	(55.0 %)	(60.0 %)
	0.8	$0.36 \pm 0.001^{a,F}$	$0.365 \pm 0.002^{a,F}$	$0.363 \pm 0.001^{a,F}$	$0.332 \pm 0.059^{a,C}$
		(13.0 %)	(15.0 %)	(17.0 %)	(25.0 %)
Aspirin	0.2	$0.267 \pm 0.001^{d,C}$	$0.263 \pm 0.001^{c,C}$	$0.265 \pm 0.001^{b,C}$	$0.256 \pm 0.001^{a,B}$
		(66.0 %)	(68.0 %)	(71.0 %)	(73.0 %)
	0.4	$0.209 \pm 0.001^{d,A}$	$0.205 \pm 0.001^{c,A}$	$0.202 \pm 0.001^{b,A}$	$0.199 \pm 0.001^{a,A}$
		(63.0 %)	(64.0 %)	(69.0 %)	(70.0 %)

n=4; mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.

The result also showed that *Erythrina senegalensis* leaves extract exerted a significant ($p < 0.05$) decrease in the activity of phospholipase A_2 in a that is concentration-dependent and exhibited maximum inhibitory effect at the higher dose (1.0 mg/ml). However, this was significantly ($p < 0.05$) lesser than the inhibitory activity exerted by the standard drug. Injury to immune cells and the endothelium at the inflammation milieu results in an increased influx of calcium ions into these cells, leading to the activation of phospholipase A_2. The activated enzyme rips out phospholipids on the membrane lipid bilayer. It liberates fatty acids, including arachidonic acid, a precursor for the synthesis of several inflammatory mediators, via key pathways that utilize enzymes such as 5-lipooxygenase and cyclooxygenases I and II. The resulting pro-inflammatory mediators, including prostaglandins, thromboxanes, leukotrienes, and lipoxins, aggravate the inflammatory process.[31,32] The capacity of *Erythrina senegalensis* leaves to decrease phospholipase A_2 activity suggests that it can stop the inflammatory process. This implies that *Erythrina senegalensis* leaves mimic the mechanism of some steroidal anti-inflammatory agents by blocking fatty acid breakdown from membrane lipid bilayers, which is required for the formation of pro-inflammatory mediators.[33] Furthermore, the results revealed that *Erythrina senegalensis* leaves exhibited a time-dependent, significant ($p < 0.05$) reduction in $CaCl_2$-induced platelet aggregation. This was comparable to that obtained for the reference drug. Platelets are component blood cells that are essential in the formation of blood clots and release several inflammatory mediators that play a role in intensifying inflammation cascades such as leukocyte mobilization and endothelial responses to a variety of inflammatory stimuli, regardless of whether they are attached to blood vessels or circulate in the bloodstream.[34] As a result, pharmacological agents' potential to reduce inflammation is reflected in their anti-platelet aggregatory activity. Also, the result showed that *Erythrina senegalensis* leaves fostered appreciably high membrane stabilization on red cells, compared to the reference drug at a similar concentration. This was in consonance with the submission of Obidah *et al*.[4] that demonstrated the ameliorative effect of *Erythrina senegalensis* leaves extract on haematological indices. The erythrocyte membrane correlates to the lysosomal membrane, according to Yogananda *et al*.[34] As a result, pharmacological agents' capacity to suppress erythrocyte hemolysis reflects their lysosomal membrane stabilization potentials. The membrane-stabilizing potential of *Erythrina senegalensis* leaves could be due to their high flavonoids content.

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Table 6: Effect of *Erythrina senegalensis* leaves extract on membrane stabilization

Treatments	Conc. (mg/ml)	Absorbance	% Membrane stabilization
Control	----	0.19 ± 0.002^{h}	
Extract	0.2	0.16 ± 0.003^{g}	15.80^{a}
	0.4	0.14 ± 0.002^{f}	23.30^{b}
	0.6	0.12 ± 0.004^{e}	36.80^{c}
	0.8	0.11 ± 0.002^{cd}	42.10^{d}
	1.0	0.10 ± 0.010^{b}	47.40^{e}
Indomethacin	0.2	0.11 ± 0.001^{d}	42.10^{d}
	0.4	0.10 ± 0.001^{bc}	47.40^{e}
	0.8	0.07 ± 0.004^{a}	63.20^{f}

n=4; the mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.

Based on their chemical structures, the orientation and permeability of flavonoids across the non-polar cell membrane bilayers can give insight into their antioxidant and lipid-peroxidation inhibitory action.[36] The ability of flavonoids to scavenge free radicals reflect their membrane-stabilizing potentials, as elevated levels of free radicals correlate with increased leucocytes activities, protein denaturation and tissue damage. The *Erythrina senegalensis* leaves stabilized the red cells membrane and prevented the release of proteases and other bioactive inflammatory markers, including histamine, 5-hydroxytryptamine, and kinins. This correlates with the submission of Enechi *et al*.[3] which reported that plant extracts' capacity to preserve the integrity of the lysosomal membrane and prevent leakage of protease, inflammatory mediators, and reactive oxygen and nitrogen species (ROS/RNS) reflects their ability to maintain the integrity of the lysosomal membrane. Moreso, the anti-inflammatory activity of *Erythrina senegalensis* leaves was also assessed *in vivo* by its propensity to reduce oedema in rat paw, induced by injecting a phlogistic agent (egg albumin).[37] Carrageenan is also a phlogistic agent commonly used to inflict acute inflammation in an animal model.[21]

Table 7: Effect of *Erythrina senegalensis* leaves extract on egg albumin-induced paw oedema in rats

	Paw circumference (mm) at various times (hrs)					
Group	**0.5 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
I	$3.79 \pm 0.57^{a, C}$	$3.89 \pm 0.63^{a,B}$	$4.03 \pm 0.65^{a,B}$	$4.07 \pm 0.63^{a, B}$	$4.25 \pm 0.62^{a,B}$	4.29 ± 0.67^{aB}
II	$3.03 \pm 0.42^{b, B}$	$2.46 \pm 0.30^{b,A}$	$1.80 \pm 0.50^{a,A}$	$1.61 \pm 0.46^{a,A}$	$1.52 \pm 0.48^{a,A}$	1.36 ± 0.47^{aA}
	(20.05%)	(36.80 %)	(55.30 %)	(60.40 %)	(64.20 %)	(68.30%)
III	$3.42 \pm 0.50^{c, BC}$	$2.93 \pm 0.46^{c,A}$	$2.13 \pm 0.22^{b,A}$	$1.70 \pm 0.25^{ab,A}$	$1.64 \pm 0.28^{ab,A}$	$1.52 \pm 0.30^{a,A}$
	(9.76%)	(24.70 %)	(47.10 %)	(58.20%)	(61.40 %)	(64.60 %)
IV	$3.14 \pm 0.36^{d, ABC}$	$2.6 \pm 0.51^{cd,A}$	$2.45 \pm 0.59^{bc,A}$	$2.00 \pm 0.27^{abc,A}$	$1.89 \pm 0.28^{ab,A}$	$1.68 \pm 0.39^{a,A}$
	(17.15%)	(32.90 %)	(39.20 %)	(50.90 %)	(55.50 %)	(60.80 %)
V	$2.54 \pm 0.40^{e, A}$	2.32 ± 0.26^{deA}	$2.03 \pm 0.10^{cd,A}$	$1.82 \pm 0.16^{bc,A}$	$1.63 \pm 0.70^{ab,A}$	$1.47 \pm 0.74^{a, A}$
	(32.20%)	(40.40 %)	(49.60 %)	(55.30 %)	(61.60 %)	(65.70 %)

n=4, mean ± SEM; Subsets in the same column with distinct superscripted alphabets as are deemed significantly ($p < 0.05$) different.
Group I: 3 mg/kg body weight normal saline; Group II: 10 mg/kg body weight indomethacin; Group III: 100 mg/kg body weight *Erythrina senegalensis* leaves; Group IV: 200 mg/kg body weight *Erythrina senegalensis* leaves; Group V: 350 mg/kg body weight *Erythrina senegalensis* leaves.

The present study used an egg albumin-induced oedema model. The infusion of egg albumin in the rat paw subcutaneously produces oedema due to the exudation of fluid rich in plasma protein, accompanied by the extravasation of neutrophil and increased tissue water.[38] The increased volume of paw oedema of the rats following the infusion of egg albumin suggests egg albumin's ability to be a phlogistic agent. Eodemogenesis induced by egg albumin is biphasic.[39] The first phase commences immediately, following a phlogistic agent's introduction and lasts for about 2 hours. The early phase is proposed to be characterized by the liberation of vasodilators such as histamine and serotonin, which mediates increased blood flow, vasodilation and migration of immune cells at the inflammation milieu. The later phase occurs 3 to 5 hours later and is characterized by the increased activities of prostaglandins, bradykinin, protease, and lysosome.[40,41] The observed time-dependent inhibitory effect of *Erythrina senegalensis* leaves on paw oedema formation in rats implies its ability to block the different phases of inflammation. The reduction in oedema observed was similar to that obtained for the standard drug (indomethacin). This entails the attenuation of the first phase of inflammation, characterized by histamine and serotonin production, and the suppression of kinin and prostaglandin formation, which are evident in the late phase. These mediators induce vasodilatation and increase vascular permeability.[39] Hence, the progressive decrease in the volume of paw oedema observed in the groups administered with varying concentrations of *Erythrina senegalensis* leaves suggests its ability to prevent vascular permeability and inflammatory assaults.

Conclusion

The result of this study suggests that *Erythrina senegalensis* leaves possess anti-inflammatory properties, which follows several mechanisms, including the suppression of protease and phospholipase A_2 activity and inhibiting protein denaturation, platelet aggregation, and fostering lysosomal membrane stabilization. The anti-inflammatory activities of *Erythrina senegalensis* leaves may be due to its biologically active principles. This study gives credence to the folkloric uses of *Erythrina senegalensis* leaves in managing several inflammatory disorders.

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.

References

1. Arulselvan P, Fard MT, Tan WS, Gotham S, Fakurazi S, Norhaizan ME, Kumar SS. Role of antioxidants and natural products in inflammation. Oxid Med Cell Longev. 2016; 1–15,

2. Sarveswaran R, Jayasuriya WJA, Suresh TS. *In vitro* assays to investigate the anti-inflammatory activity of herbal extracts: a review. World J Pharm Res. 2017; 6:131-141.
3. Enechi OC, Okeke ES, Abonyi UC, Nwankwo NE, Ndimele UJ, Benneth ME. Evaluation of *in vitro* anti-inflammatory and antioxidant potentials of methanol leaf extract of *Fagara zanthoxyloides*. Indo Am. J Pharm Sci. 2019; 6:14365-14371.
4. Obidah W, Badung HL, Ajuji J, Peter H, Bello H. Effects of *Erythrina senegalensis* aqueous leaf extract in rats. Am J Res Comm. 2014; 2:179-185.
5. Aliyu M, Abdullahi BN, Abdulkadir IU, Asma'u AK. Evaluation of analgesic and behavioural effects of ethanol root bark extract of Erythrina senegalensis DC (Fabaceae). Afr J Pharmacol Ther. 2016; 5(2):81-86.
6. Parker EJ, Ikechukwu JO, Daniel EE, Innocent UO, Victor NO. Methanol extract of *Erythrina senegalensis* leaves (MEES) ameliorates *Plasmodium berghei*-ANKA 65-parasitised aberrations in mice. All Life. 2020; 13(1):66-77.
7. Shailasree S, Ruma K, Kini KR, Niranjana SR, Prakash HS. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats India. Pharmacogn Comm. 2012; 2:2-12.
8. Osuntokun OT, Ajayi AO, Olorunnipa TA, Thonda OA, Taiwo OV. Phytochemical screening and antimicrobial properties of partially purified ethyl acetate extracts of Erythrina senegalensis leaf and bark against selected clinical isolates. J Med Pl St. 2016; 4(3):259-269.
9. Doughari JH. Evaluation of antimicrobial potentials of stem bark extracts of *Erythrina senegalensis.* Afri J Clin Exp Microbiol. 2010; 4:1836-1841.
10. Gbadamosi IT and Erinoso SM. A review of twenty ethnobotanicals used in the management of breast cancer in Abeokuta, Ogun State, Nigeria. Afr J Pharm Pharmacol. 2016; 10(27):546-564.
11. Togola A, Austarheim I, Theïs A, Diallo D, Paulsen B. Ethnopharmacological uses of *Erythrina senegalensis*: a comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. J Ethnobiol Ethnomed. 2008; 4:6-15.
12. Chu YF, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem. 2002; 50:7449–7454.
13. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 53:275-289.
14. Trease GE and Evans WC. Drugs of biological origin: Textbook of Pharmacognosy (12th edition). London: Ballere Tindall. 1983.
15. Mizushima Y and Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol. 1968; 20:169-173.
16. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Sci Res. 2010; 2:146-56.
17. Oyedepo OO, Akindele VR, Okunfolami OK. Effect of extracts of Parham Peter. The immune system. New York (NY): Garland Publishing. 1997.
18. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971; 231:232-235.
19. Ezekwesili CN and Nwodo OFC. Anti-inflammatory properties of *Vigna unguiculata* seed extract. J. Med. Lab. Sci. 2000; 9:141-145.
20. Born GVR and Cross MJ. The aggregation of blood platelets. J Physiol. 1963; 168:178-195.
21. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine. 1962; 111:544-547.
22. Mustapha AT, Garba TM, Yagana TA, Thiza BA, Fanna IA. Phytochemical analysis, analgesic and antipyretic properties of ethanolic leaf extract of Vernonia amygdalina Del. J Herbmed Pharmacol. 2017; 6(3):95-99.
23. Abdel-Aleem ER, Attia EZ, Farag FF, Samy MN, Desoukey SY. Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Cordia myxa* L. leaves Clin Phytosc. 2019; 5:29-37.
24. Daniel MG, Amos MM, Godfrey MG, Mathew PN, David NM. Phytochemical screening and antipyretic activities of dichloromethane-methanolic leaf and stem bark extracts of *Ximenia americana* in rat models. J Herbmed Pharmacol. 2017; 6(3):107-113.
25. Das S, Haldar PK, Pramanik G, Suresh RB. Evaluation of anti-inflammatory activity of *Clerodendronin fortunatum* Linn extract in rats. Global J Pharm. 2010; 4:48-50.
26. Gepdireman A, Mshvildadze V, Suleyman H, Elias R. Acute anti-inflammatory activity of four saponins isolated from ivy: Alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw oedema. Phytomedicine. 2005; 12:440-444.
27. Neukirch H, D'Ambrosio M, Sosa S, Altinier G, Loggia RD, Guerriero A. Improved anti-inflammatory activity of three new terpenoids derived by systematic chemical modifications from the abundant triterpenes of the flowery plant *Calendula officinalis*. Chem Biodivers. 2005; 2:657-671.
28. Shareen S, Thakur GS, Kriti M, Sonia D. Medicinal plants used against various inflammatory biomarkers for the management of rheumatoid arthritis. J Pharm Pharmacol. 2020; 72:1306–1327.
29. Egharevba HO, Odigwe AC, Abdullahi MS, Okwute SK, Okogun JI. Phytochemical analysis and broad-spectrum antimicrobial activity of *Cassia occidentalis* L. (whole plant). N Y Sci J. 2010; 3:74-81.
30. Yunbo L, Subha M, Bruce AH, Gerald RH. Egg white as a blood coagulation surrogate. J Acoust Soc Am. 2010; 128:480-489.
31. Schimmer BP and Parker KL. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones: (A.G. Gilman, J.G. Hardman, L.E. Limbird, Eds) 10th Edition. Goodman and Gilman's the pharmacological basis of therapeutics. New York (NY): McGraw Hill Co. 2001; 1649-1677.
32. Anoop MV and Bindu AR (2015) *In-vitro* anti-inflammatory activity studies on *Syzygium zeylanicum* (L.) DC Leaves. Int. J. Pharma Res. Rev. 4: 8-27.
33. Coutinho AE and Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 2011; 335:2–13.
34. Hosseinzadegan H and Tafti DK. Mechanisms of platelet activation, adhesion, and aggregation. Res. Pract. Thromb. Haemost. 2017; 1:1008-1015.
35. Yoganandam GP, Mohamad AS, Israf DA. Evaluation of anti-inflammatory and membrane stabilizing properties of various extracts of *Punica granatum* L. (Lythraceae). Int. J. Pharmtech. Res. 2010; 2:1260-1263.
36. Saha S, Ranieri E, Suzen S. The interaction of flavonols with membrane components: potential effect on antioxidant activity. J Membrane Biol. 2020; 253:57–71.
37. Omkar A, Jeeja T, Chhaya G. Evaluation of anti-inflammatory activity of Nyctanthes arbour-tristis and Onosma echioides. Pharmacogn Mag. 2007; 2:258-260.
38. Yankanchi SR and Koli SA. Anti-inflammatory and analgesic activity of mature leaves of methanol extract of *Clerodendrum inerme* L. (Gaertn). Int J Pharm Sci Res. 2010; 11:782-785.
39. Anosike CA, Onyechi O, Ezeanyika LUS. The anti-inflammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin-induced oedema and granuloma tissue formation in rats. Asian Pac J Trop Biomed. 2010; 62-66.
40. Wallace JM. Nutritional and botanical modulation of the inflammatory cascade--eicosanoids, cyclooxygenases, and lipoxygenases--as an adjunct in cancer therapy. Integer. Cancer Ther. 2002; 1:7-37.

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41. Kang HS, Lee JY, Kim CJ. Anti-inflammatory activity of arctigenin from *Forsythiae fructus*. J. Ethnopharmacol. 2008; 116:305-310.