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In-vitro Anti-lipoxygenase and Membrane Stabilization Activity of Crude Extracts of *Euphorbia lateriflora* (Schum and Thonn)

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Authors' contributions

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

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ABSTRACT

Aims: This study was aimed at evaluating the *in-vitro* anti-inflammatory potential of crude extracts of *Euphorbia lateriflora*.
Place of Study: Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
Methodology: The *in-vitro* anti-inflammatory activity of aqueous and ethanol extracts *of E. lateriflora* were evaluated via anti-lipoxygenase and red cell membrane stabilization assays using spectrophotometric technique.
Results: The aqueous and ethanol extracts demonstrated significant anti-lipoxygenase activity with the aqueous extracts (IC_{50}: 0.34 mg/ml) and the ethanol extracts (IC_{50}: 0.25 mg/ml), showing comparable activity with standard indomethacin (0.27 mg/ml). Contrary to what was observed in the

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anti-lipoxygenase study, the aqueous extract demonstrated a better membrane stabilization activity with IC_{50} (1.13 mg/ml) than the ethanol extracts (5.44 mg/ml). The standard diclofenac drug had an IC_{50} of 1.02 mg/kg. Both the aqueous and the ethanol extracts demonstrated significant anti-inflammatory activity.

Conclusion: Observation from this study established the considerable anti-inflammatory potential of this plant by inhibiting lipoxygenase activity and *stabilization* the membrane of the red blood cells.

Keywords: E. lateriflora; crude extracts; anti-lipoxygenase; membrane stabilization; anti-inflammation.

1. INTRODUCTION

Inflammation is an important physiological reaction that occurs in response to a wide variety of injurious agents, and aim to perform the function of promoting tissue repair [1]. This process protects the body from diseases by releasing cells and mediators that resist foreign substances and prevent infection [2,3]. The inflammation process is complex, mediated by variety of signaling molecules released by leucocytes, macrophages and mast cells undergoing various cellular response such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandins E2 (PGE_2), and tumor necrosis factors (TNF-α) [4].

Acute and chronic inflammations are the two phases in which inflammatory process occurs, a complicated process induced by several different classes of chemical mediators; e.g. prostaglandins, leukotrienes, and platelet-activating factor. Anti-inflammatory agents elicit their effect by a range of different mechanism of action [5]. However, diseases such as rheumatoid arthritis, inflammatory bowel diseases, and psoriasis are caused by excessive or inappropriate inflammation [6].

Many drugs both steroidal and non-steroidal such as indomethacin, ibuprofen, and diclofenac reduce inflammation. There are over 50 different non steroidal anti-inflammatory drugs (NSAIDs) available [7] and can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards COX-1 or COX-2 [8]. Prolonged administration of corticosteroids has been implicated in the etiopathogenesis of pathological states such as hyperglycemia, insulin resistance, diabetes mellitus, obesity, osteoporosis, and anxiety disorders [9-11].

Medicinal plants have the ability to synthesize a wide variety of secondary metabolites, which help to defend against attacks from predators such as insects, fungi and herbivorous mammals [12-14]. These active compounds are believed to be responsible for the biological and pharmacological activities of these plants [15-18]. There are extensive scientific literatures and information on the important biological and pharmacological potentials of medicinal plants which include but not limited to anti-radical, anti-allergy, anti-obesity, anti-diabetic, anti-malaria and anti-inflammatory [19-22]. *Euphorbia lateriflora* (Fig. 1) also known among the Yoruba and Hausa speaking people of Nigeria as *Enu Opiri and Fidda sartse*, respectively [23]. It is a shrub with smooth-gracious and erect branches. The latex of the plant is used for the treatment of ringworm, and in a dilute aqueous solution as purgative [24]. Its leaves also serve as treatment for dermatosis [25] and to kill intestinal parasites in children [26]. There are many scientific submissions on the numerous biological activities and potential of this plant. They include anti-viral and chicken pox [27,28], insecticidal [23], and anti-microbial properties [29]. Extensive ethno-botanical studies have reported the plant to be used traditionally in the management of inflammation [30], diabetes mellitus [31], female reproductive health problems [32], and in the management of sexually transmitted diseases [33]. This present study therefore aims to validate the anti-inflammatory potential of aqueous and ethanol extracts of *E. lateriflora* through the red blood cell stabilization and anti-lipoxygenase assay.

2. MATERIALS AND METHODS

2.1 Plants Collection

Whole plant of *E. lateriflora* was obtained within Ogbomosho North Local Government Area, Oyo State, Nigeria through the help of a traditional healer. Ogbomoso North lies between latitude 8.156697 and longitude 4.264409. The plant was identified and authenticated by a taxonomist (Prof. Ogunkunle) at the Department of Pure and

Applied Biology, Ladoke Akintola University of Technology, Oyo State Nigeria. The plant sample was deposited in the University Herbarium. The leaves were washed in clean water, drained, and then air-dried in the laboratory for two weeks. The air-dried sample was thereafter pulverized with electric blender.

Fig. 1. *E. lateriflora*

2.2 Preparation of Plant Extracts

The ethanol extraction of the plant sample used a modified method of Mbaka et al. [34]. Four hundred grams of the pulverized plant sample was loaded in soxhlet extractor in batches for 5 h each and subjected to extraction with ethanol. After extraction, mixtures were concentrated at 78°C using rotary evaporator and the concentrate was kept in a refrigerator (4°C) until use. For the aqueous extraction, 400 g of plant sample was soaked in water for 72 hours with occasional agitation. After 72 hours, the solution was pressed and sieved with a traditional muslin bag. The resulting filtrate was allowed to dry in an electric oven between 25-30°C until dryness. The extraction yield was determined by using electronic balance and extract were kept in a refrigerator (4°C) until further use.

2.3 Experimental Animals

Ten male albino rats (Wistar stock) weighing between 150-200 g were obtained from the Department of Anatomy, Ladoke Akintola University of Technology Ogbomosho Oyo State, Nigeria. They were acclimatized for a period of two weeks and kept under the normal 12 h light/dark cycle. The animals were allowed access to food and water *ad libitum* throughout the study period. The animal feed was specially prepared from chick Grower's mash (Feed Mill Company, Ogbomosho Oyo State, Nigeria). Animals were handled following the standard protocol on animal care and use.

2.4 *In-vitro* Anti-inflammatory Study

2.4.1 Red blood cell (RBC) membrane stabilization assay

The method as described by Sadique et al. [35] and Sakat et al. [36] was adopted with some modifications. Animals which had not taken any NSAIDS for 2 weeks prior to the experiment were sacrificed by cervical dislocation and blood was collected into sterile plane bottles. The blood samples were mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and then 10% suspension was made. 100 and 120 mg/ml concentration of extracts was prepared in distilled water. To each extracts concentrate, 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of RBC suspension was added thereafter incubated at 37^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes. The reactions were performed in triplicates in 96-well micro plate reader Spectra Max 384 plus (Molecular Devices, USA) and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The percentage (%) of RBC membrane stabilization or protection was calculated using the following formula:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

2.4.2 Anti-lipoxygenase assay

The lipoxygenase activity was assayed according to the method of Tappel et al. [37]. A total volume of 200 µl assay mixture contained, 160 µl sodium phosphate buffer (100 mM, PH 8.0), 10 µl test extract (25 to 100 µg extracted material in 100 mM Tris buffer PH 7.4), and 20 µl lipoxygenase enzyme. The contents were pre-incubated for 10 min at 25 ºC. The reaction was initiated by the addition of 10 µl linoleic acid solution as substrate. The change in absorbance was observed after 6 min at 234 nm. All reactions were performed in triplicates in 96-well microplate reader Spectra Max 384 plus

(molecular Devices, USA). The negative and positive controls were included in the assay. The percentage inhibition (%) was calculated by the following formula:

Inhibition (%) = [(Abs of control – Abs of test sample)/ Abs of control] x 100.

*Abs = Absorbance.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield of *E. lateriflora* Leaves Crude Extracts

The extraction yield of the crude extracts from 400 grams of blended plant material is depicted in Table 1. The results revealed that the ethanol extract had a lower yield than the aqueous extract with 27 and 29 grams yield, respectively. The observed difference might be due to the techniques and solvent used in the extraction procedure, which have been reported to have effect on yields and efficacy of plant extracts [38]. These results are in tandem with Kim et al. [39] who also reported a better extraction yield in aqueous extract.

Table 1. Approximate yield (grams and %) of aqueous and ethanol extracts from 400 grams of *E. lateriflora* leaf

Extract	Yield in (g) grams	Percentage (%) yield
Ethanol	27.00	6.75
Aqueous	29.00	7.25

3.2 *In vitro*-anti-inflammatory studies on *E. lateriflora*

The Tables 2 and 3 shows the IC_{50} values of anti-lipoxygenase and membrane stabilization activities of the ethanol and aqueous extracts of *E. lateriflora*, respectively. It can be observed in (Table 2), that the extracts of *E. lateriflora* exhibited anti-lipoxygenase activity in a concentration-dependent manner. Although, the ethanol extract exhibited a better anti-lipoxygenase activity than the aqueous extract, however, indomethacin (standard anti-inflammatory drug) exhibited the strongest anti-lipoxygenase activity.

Table 3 reveals the protective effects of the ethanol and aqueous extracts of *E. lateriflora* on membrane of red blood cells. It can be observed that the extracts of *E. lateriflora* demonstrated a significant membrane stabilization activity. Both the aqueous and ethanol extracts showed a concentration-dependent membrane stabilization activity. However, contrary to what was observed in the anti-lipoxygenase study (Table 2), the aqueous extract demonstrated a better membrane stabilization activity with IC_{50} (1.13 mg/ml) than the ethanol extracts (5.44 mg/ml) as shown in (Table 3). The standard diclofenac drug had an IC_{50} of 1.02 mg/kg, which is comparable to the aqueous extracts.

In-vitro evaluation of compounds provides an initial platform for drug discovery. Therefore, building a consistent and reliable extrapolation procedure from *in vitro* results to *in vivo* is extremely pertinent [40,41]. *In-vitro* anti-lipoxygenase and membrane stabilization assays are few of the many approaches employed by scientists and researchers in the discovery of anti-inflammatory drugs. This is because the mediators of these pathways in-vivo have been widely implicated and involved in the etiopathogenesis of inflammation [42-45]. There are many scientific reports and submission on the *in-vitro*-inflammatory activities of medicinal plants [46-50].

Euphorbiaceae are large family of plants that have about 300 genera and 7,500 species reported [51]. There is no dearth of literature on the anti-inflammatory and other pharmacological

Table 2. Anti-lipoxgenase activity and IC_{50} values of the ethanol and aqueous extracts of *E. lateriflora*

Conc. (mg/ml)	*E. lateriflora* extracts		Standard drug
	Ethanol	Aqueous	Indomethacin
0.2	13.92±0.23	19.22±0.21	18.10±0.21
0.25	20.63±0.34	33.08±0.54	42.23±0.32
0.3	48.78±0.53	58.02±0.62	62.49±0.45
0.35	55.28±0.45	67.02±1.12	82.50±0.21
0.40	61.48±0.43	81.53±0.92	94.0±0.83
IC_{50} (mg/ml)	0.25±0.13	0.34±0.02	0.27±0.04

Table 3. Membrane stabilization activity of the ethanol and aqueous extracts of *E. lateriflora*

Conc. (mg/ml)	*E. lateriflora*		Diclofenac (drug)
	Ethanol	Aqueous	
0.2	1.95±0.12	0.88±0.23	2.78±0.14
0.25	2.00±0.21	2.27±0.11	4.57±0.23
0.3	2.62±0.14	5.29±0.15	7.77±0.22
0.35	3.10±0.15	8.29±0.21	10.97±0.12
0.40	3.70±0.21	11.31±0.13	14.07±0.14
IC50 (mg/ml)	5.44±0.16	1.13±0.03	1.02±0.04

potential of plants belonging to the genus *Euphorbia* [52-56]. The results depicted in (Tables 2 and 3), however revealed the considerable anti-inflammatory potentials of *E. lateriflora* extracts. These anti-inflammatory activities demonstrated can be suggested to be a function of the constituent's phytochemicals previously reported to be present in the plant extracts. Earlier phytochemical screening of *E. lateriflora* extracts revealed it contains active principles such as tannins, lectins, diterpenes, euphorbin, alkaloids, and ingenol mebutate [28,57-59]. Furthermore, compounds such as anhydriobisfarnesol and sesquilavandulol have been isolated from the latex of the plant Faure et al. [60].

According to the submissions of Abrantes [61], Rogerio et al. [62], Leal et al. [63], and Campos et al. [64], lectins which is one of the compounds present in this plant are capable of eliciting anti-inflammatory effect. However, the suggested mechanism is by antagonizing neutrophils from migrating into the tissues in response to inflammatory stimuli [65-66]. This possibly may be due to competitive inhibition of glycosylated selectin binding sites on the membranes of leukocytes and endothelial cells [67-68]. More so, the presence of terpenes and tannins in the extract might also be responsible for the observed anti-inflammatory activities. These compounds have been hitherto reported to inhibit lipoxgenase and suppress other inflammatory mediators by various mechanisms [69-75]. Inhibition of these inflammatory enzymes would help to control the process of inflammation [76-77]. However, from the empirical results it can be hypothesized that the observed anti-inflammatory activities of the crude extracts of *E. lateriflora* might be due to the synergistic activities of constituting phytochemicals and active compounds.

4. CONCLUSION

This research validates the folkloric usage of morphological organs of *E. lateriflora* in management of inflammation and related disorders. Hence, the in-vivo anti-inflammatory studies, identification and isolation of the active compounds should be carried so as to give more scientific credence to pharmacological potential of *E. lateriflora*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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