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Investigation of the anti-inflammatory activity and nutritional value of the leaves of *Calopogonium mucunoides*

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Author contributions

Egele ME Funding acquisition, Methodology, Investigation, Writing of the original draft of the manuscript; Enechi OC Conceptualization, Supervision and Editing of the manuscript; Amah CC Methodology, Supervision, Original manuscript draft writing, manuscript editing, handled peer review, analyzed and interpreted the data; Iyidiegwu FC Editing of the manuscript, Analyzed and Interpreted the Data; Okoro IJ and Amadi EB Editing of the manuscript. All authors critically revised and finalized the manuscript for publication.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

EECML, ethanol extract of *Calopogonium mucunoides* leaves; LD_{50}, median lethal dose; b.w, body weight; DPPH, 2,2-diphenyl-1-picrylhydrazyl; OD, absorbance; NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; PRP, platelet- rich plasmal; TXA_2, thromboxane A_2; PLA_2, phospholipase-A_2; LOX, lipoxygenase.

Citation

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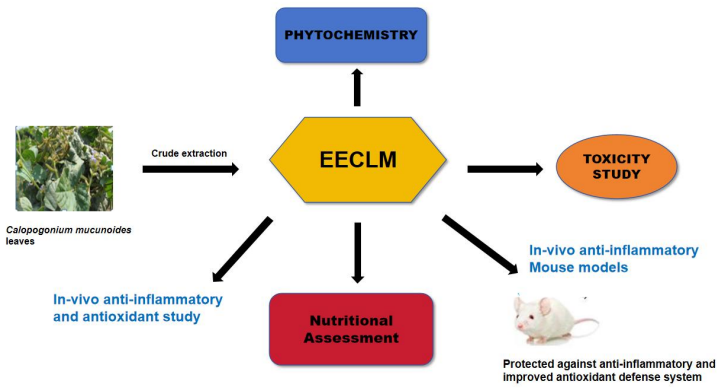
Abstract

Background: *Calopogonium mucunoides* (*C. mucunoides*) is traditionally used in southeastern Nigeria for managing pain and inflammation. The quest for safer options compared to traditional anti-inflammatory medications like ibuprofen, aspirin, diclofenac, indomethacin, and others has increasingly drawn interest. This research assessed the nutritional makeup and anti-inflammatory properties of the ethanol extract from *C. mucunoides* leaves (EECML) through in-vitro and in-vivo models. **Methods:** In vitro tests assessed EECML for its effects on platelet aggregation inhibition, phospholipase-A_2 activity, albumin denaturation, hemolysis induced by hypotonicity, antioxidant capabilities, and nutrient makeup employing established biochemical techniques. The paw edema model was employed to assess in-vivo anti-inflammatory effects. Twenty-five male albino rats weighing 120–160 g each were split into five groups (n = 5). Group 1 was administered normal saline; Group 2 was given 10 mg/kg body weight (b.w) of Indomethacin, whereas Groups 3, 4, and 5 were administered 100, 200, and 400 mg/kg b.w of EECML, respectively. **Results:** The extract of 1,500 g of plant material yielded 28.24 g, accounting for 1.88 % of the sample used. The phytochemical analysis of EECML showed higher concentration of steroids (1.295 ± 0.090 mg/100 g) and flavonoids (1.118 ± 0.121 mg/100 g) compared to other secondary metabolites found. The EECML exhibited an LD_{50} > 5,000 mg/kg b.w, and contained significant antioxidant vitamins and minerals, plus appreciable amounts of carbohydrate (34.14 ± 0.02 %), moisture (32.05 ± 0.02 %) and protein (12.74 ± 0.02 %) contents. The paw sizes of rats administered escalating doses of the EECML and the standard medication, indomethacin, significantly ($P < 0.05$) reduced markedly over time. At the 5-hour mark, the oedema inhibition percentage in the indomethacin group surpassed that of the 400 mg/kg EECML groups; nonetheless, this difference wasn't statistically significant ($P > 0.05$). The EECML demonstrated a strong inhibition of 2,2-diphenyl-1-picrylhydrazyl at 160 µg/mL, showing a markedly ($P < 0.05$) elevated IC_{50} value in comparison to the standard ascorbic acid. The EECML considerably ($P < 0.05$) reduced platelet aggregation, phospholipase-A_2 activity, albumin denaturation, and hypotonicity-induced hemolysis in a concentration-dependent manner, similar to the standard anti-inflammatory medication. **Conclusion:** The results indicate that EECML has substantial anti-inflammatory and antioxidant effects, as well as nutritional advantages, reinforcing its traditional application.

Keywords: anti-inflammatory; *Calopogonium mucunoides*; nutritional composition; antioxidant



Introduction

Inflammation is a complicated physiological response of blood vessels to damaging stimuli and serves as the body's defense mechanism against harmful agents, facilitating tissue repair [1]. This is how the body signals the immune system to recuperate and repair damaged tissue, as well as defend itself from foreign invaders, for example, viruses and bacteria. Without inflammation as a physiological response, the injury would deteriorate and infections could become dangerous. Whenever a part of the human body is harmed, the arterioles in the tissue around it dilate and this presents elevated blood dissemination towards the area (redness) [2]. The beginning of inflammation initiates the release of inflammatory agents such as prostaglandins, histamine, kinin, leukotrienes, and thromboxane, leading to enhanced blood flow and the movement of neutrophils from blood vessels into the damaged tissues [3]. Vasoactive agents also enhance the permeability (larger pore size) of these arterioles, enabling the accumulation of blood cells, chemicals, blood proteins, and fluids in this region. The accumulation of fluid leads to swelling and can put pressure on nearby nerves, resulting in pain [2]. The increasing occurrence of excitation in both developed and developing countries has challenged scientists to further explore various therapeutic agents that can be applied in the treatment of redness and inflammatory disorder. Non-Steroidal anti-inflammatory medications (NSAIDs) belong to a category of drugs that alleviate pain, lower fever, prevent blood clots, and, in larger doses, diminish inflammation. NSAIDs act by restraining cyclooxygenase activity (COX-1 and/or COX-2) and reducing prostaglandin production. Prostaglandins are synthetics (chemicals) that promote inflammation, pain, and fever [4]. The use of traditional anti-inflammatory medications for treating inflammatory diseases is constrained by factors like unavailability, cost, side effects, and drug interactions [5]. Plants have been crucial in supporting human health and enhancing people's quality of life. Numerous native plants contain phytochemical compounds which have anti-inflammatory and antioxidant properties [6]. Consequently, it is important to develop new anti-inflammatory medications, especially from natural sources. *Calopogonium mucunoides* (*C. mucunoides*) is an annual leguminous plant native to tropical regions around the world. The leaves of the plant are widely used in South-East Nigeria for managing bacterial infections, diarrhea, ulcers, gastric pain and skin infections. Studies have demonstrated that the leaf extract of *C. mucunoides* has an anti-ulcerative activity [7], along with anti-diarrhea and anti-bacterial activities [8]. Furthermore, phytochemical analysis of plant leaves revealed a relative abundance of flavonoids among other phytochemicals of pharmacological significance [8]. Phytochemicals, antioxidants and proximate analyses of *C. Mucunoides* extracts were also noted [9]. There is a lack of recorded data on the application of *C. mucunoides* as an anti-inflammatory substance; therefore, this research was aimed at assessing the anti-inflammatory and nutritional benefits of the plant.

Materials and methods

Plant material and extraction process

In July 2022, fresh leaves of *C. mucunoides* were gathered from Opanda-Nimbo in the Uzo-uwani Local Government Area of Enugu State, Nigeria. The specimen was recognized and verified by Mr. Alfred Ozioko, a taxonomist at the Bioresources Development and Conservation Programme Research Centre located in Nsukka, Enugu State. A voucher specimen marked Intercedd/842 was dispatched to the herbarium. The leaves were cleaned and air-dried in the shade for a week, occasionally being turned until they became brittle. The dried leaves were ground into a coarse texture with a mechanical grinder. The powder (1,500 g) was submerged in 5.0 L of ethanol and left to stand for 72 h at room temperature. The blend was filtered through a white muslin cloth. Additional filtration was performed using Whatman No. 1 filter paper manufactured by Cytiva (Marlborough, MA, USA) to remove fine particles, and the resulting filtrate was concentrated with a rotary evaporator produced by IKA (Staufen, Germany) at temperatures between 40–50 ˚C to yield a brownish semi-solid extract.

The equation provided was utilized to determine percentage yield.

$$\% \text{ yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1} \quad (1)$$

Animals

Eighteen (18) clean-grade male Swiss albino mice (12 weeks old, 22–28 g) and twenty-five (25) clean-grade male adult Wistar rats (12 weeks old, 120–160 g) were used for the study. The conventional Wistar mice and rats utilized in this research were sourced from the animal house of the Faculty of Veterinary Medicine at the University of Nigeria, Nsukka. The animals were acclimated in standard laboratory conditions at the animal facility of the Department of Biochemistry, University of Nigeria, Nsukka, for seven days before the commencement of the study, maintained on a half-day light and dark cycle, housed under SPF conditions (20 ± 2 ˚C, 48 ± 12% humidity, 12 h light/dark cycle) with ad libitum access to food (grower's mash pellets: Grand Cereals Ltd., Enugu, Nigeria) and had unrestricted access to water, the animals received attentive care in accordance with the protocols established by the organization, Animal Care and Use Committee of the Faculty of Biological Science's Institutional Board of Research, University of Nigeria Nsukka, with ethical number UNN/FBS/EC/1057 that approved the study and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Chemicals, reagents and drugs

All chemicals employed in this research were of analytical quality and sourced from Sigma Aldrich in St. Louis, MO, USA, Qualikems (Delhi, India), Fluka (Darmstadt, Germany), and May and Baker (London, England). Randox Laboratories Limited. The biochemical assay kits were produced by Randox Laboratories Ltd. (Kearneysville, WV, USA). The conventional anti-inflammatory medications, indomethacin and Prednisolone, utilized in this research were obtained from a trusted pharmacy, Elofex Pharmacy and Drug Stores located in Enugu, Enugu State, South-East Nigeria.

Qualitative and quantitative phytochemical analysis of the ethanol extract from *C. mucunoides* leaves (EECML)

The examination for the qualitative phytochemical components of the plant leaves was conducted following the methods described by Harborne JB [10]. Quantitative analysis was performed as outlined by Ali and Shuab [11]. The levels of the various phytochemical components were determined as outlined below:

$$\text{Concentration mg/100 g} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Dilution factor} \quad (2)$$

Collection of human blood sample

A new sample of human blood (5 mL) was obtained from a donor and placed into heparinized centrifuge tubes. The tubes underwent centrifugation at 3,000 rpm for 10 min and were rinsed three times with an equal volume of normal saline. The blood volume was assessed and prepared as a 10% (v/v) suspension using normal saline.

Acute toxicity

The assessment of the acute toxicity of the EECML, aimed at determining the median lethal dose (LD_{50}), was performed utilizing Lorke's method, incorporating particular modifications to discern both the lethal and safe dosage ranges of the extract [12]. Eighteen albino Swiss male mice were utilized in the research after fasting for 18 h but permitted to drink water. They were divided into six groups, containing three mice each, arranged into two phases. The EECML acquired from maceration was dissolved in distilled water due to its solubility and then administered via oral intubation at different doses (10, 100, 1,000 mg/kg body weight (b.w) for phase I and 1,600, 2,900, and 5,000 mg/kg b.w for phase II). The mice were subsequently monitored for anxiety, death, lethargy, and changes in

behavior over a period of 24 h.

Proximate analysis

The parameters identified for proximate analyses includes: ash, moisture, crude protein, fat, fiber, and carbohydrates; these were conducted utilizing the methods outlined by by AOAC [13].

Determination of vitamin compositions

The levels of vitamins E, C, and A in the sample were assessed using the technique outlined by William and AOAC [14].

Determination of mineral contents

Absorption spectrometry was employed to examine Zn, Fe, Se, and Ca, whereas flame photometry was utilized to assess K and Na. To wet digest the sample, 2 mL of the plant samples was placed in a digesting glass tube. Twelve milliliters of hydrochloric acid were added and kept at room temperature overnight. Perchloric acid (4.0 mL) was incorporated into the mixtures and maintained in the furnace block for digestion. The temperature was raised slowly, beginning at 50 ˚C and reaching as high as 150 ˚C. The digestion ended in approximately 70–85 min, as shown by the emergence of white vapors. The blend was allowed to cool before the contents of the tubes were transferred into 100 mL volumetric flasks. The wet digested solution was moved to plastic bottles and labeled correctly. The digest was preserved and utilized for mineral analyses. All of these actions were conducted following the approach outlined in William and AOAC [14].

Egg albumin-induced oedema

This evaluation was carried out following the procedure outlined by Sakat et al. [15]. Inflammation was triggered in rats by administering egg albumin (0.1 mL) into the subplantar area of the right hind paw. The crude extract (100, 200, and 400 mg/kg b.w) obtained through ethanol maceration was dissolved in distilled water due to its solubility, before being administered intraperitoneally 1 h before the onset of inflammation. The extent of swelling in the injected paw was assessed prior to and at 30 min, 1, 2, 3, 4, and 5 h following the administration of the inflammatory agent. The variation in paw size of the injected paws at the baseline in comparison to different time points following egg albumin injection was utilized to evaluate the advancement of oedema. These values were used to calculate the percentage inhibition of edema for each extract dosage and for indomethacin at various time intervals using the equation given below:

$$(Vc - Vt) \times 100/Vc \quad (3)$$

Where, Vc = Paw volume in control, Vt = Paw volume in test drug.

Experimental design

A total of twenty-five male Wistar rats were divided into five groups, with each group consisting of five rats, and were treated as follows:

Group 1: Egg albumin (0.1 mL) and not treated (positive control).

Group 2: Egg albumin (0.1 mL) and treated 10 mg/kg b.w of Indomethacin (standard control).

Group 3: Egg albumin (0.1 mL) and treated 100 mg/kg b.w of EECML.

Group 4: Egg albumin (0.1 mL) and treated 200 mg/kg b.w of EECML.

Group 5: Egg albumin (0.1 mL) and treated 400 mg/kg b.w of EECML.

Determination of in-vitro antioxidant

DPPH free radical scavenging activity. The potential of the plant extracts to neutralize free radicals in comparison to the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), was assessed using the method outlined by Denaro et al. [16]. In this experiment, 1.5 mL of the methanol extract at various concentrations was combined with 0.5 mL of DPPH methanol solution (0.1 mM). A control was established with the same quantity of methanol and DPPH, excluding any sample. Following a 30-minute reaction at room temperature in the dark, the absorbance was recorded at 517 nm using methanol as the blank on a UV-VIS spectrophotometer (UVmini-1240), product of Shimadzu Corporation (Kyoto, Japan). The percentage of free radical scavenging activity was evaluated using the formula given below:

$$\% \text{ DPPH scavenging activity} = \frac{\text{AbsControl} - \text{AbsSample}}{\text{AbsControl}} \times 100 \quad (4)$$

Evaluation of in-vitro anti-inflammatory activity

Determination of platelet aggregation. The assessment of the anti-platelet effect was conducted utilizing an updated version of the Born and Cross method [17]. Healthy volunteers provided blood samples. New blood samples (5 mL) were collected intravenously in a plastic tube with 0.01 mL of 1% EDTA (Thermo Fisher Scientific, Waltham, MA, USA) as an anticoagulant using a 5 mL plastic syringe. The tube underwent centrifugation at 3,000 rpm for 10 min, following which the supernatant was gathered, diluted twofold with normal saline, and then used as the platelet-rich plasma (PRP). Changes in the absorption of the PRP were evaluated. The PRP 62.5, 125, 250, 500, and 1,000 mL of 2 M $CaCl_2$, combined with various concentrations of normal saline and extract, were allowed to incubate. The solutions' absorbance was measured at 520 nm and recorded every 2 min.

$$\% \text{ Inhibition} = \frac{\text{ODtest}}{\text{ODcontrol}} \times 100 \quad (5)$$

$$\% \text{ Maximum enzyme activity} = \frac{\text{ODtest}}{\text{ODcontrol}} \times 100 \quad (6)$$

$$\% \text{ Inhibition} = 100 - \% \text{ maximum enzyme activity} \quad (7)$$

Phospholipase-A_2 inhibition assay

The technique outlined in was utilized to extract phospholipase-A_2 from Aspergillus niger and evaluate the effect of the extract on its activity [18]. Aliquots (0.5 mL) of resuspended red blood cells were mixed with normal saline containing 2 nM calcium chloride. The mixture was allowed to incubate and subsequently centrifuged at 3,000 rpm for 10 min, after which the absorbance of the supernatant was recorded at 418 nm using a blank as a reference. Prednisolone, known to function as an enzyme inhibitor, served as the control.

$$\% \text{ Maximum enzyme activity} = \frac{\text{OD test}}{\text{OD control}} \times 100 \quad (8)$$

$$\% \text{ Inhibition} = 100 - \% \text{ maximum enzyme activity} \quad (9)$$

Inhibition of albumin denaturation

The impact on inflammation was assessed through the albumin denaturation inhibition method outlined in Sakat et al. [15]; Mizushima and Kobayashi [19]. The mixture of the test extract and a 1% aqueous bovine albumin solution was adjusted to the desired pH with a few drops of 1 N HCl. The sample extract was kept at 37 ˚C for 20 min and then heated to 51 ˚C for an additional 20 min; after cooling, the turbidity was assessed at 660 nm. The experiment was conducted in three repetitions. The calculation of the inhibition percentage of protein denaturation was performed utilizing the formula given below:

$$\% \text{ Inhibition} = \frac{\text{AbsControl} - \text{AbsSample}}{\text{AbsControl}} \times 100 \quad (10)$$

Membrane stabilization

Segments of the extract were dissolved in purified water (a hypotonic solution). The hypotonic solution (5 mL) with varying extract concentrations (20, 40, 80, 160, and 320 µg/mL) was organized into duplicate sets for each concentration. A 5 mL isotonic solution with varying concentrations of the extracts (20–320 µg/mL) was introduced into pairs of centrifuge tubes, duplicated for each dosage. Control tubes held 5 mL of the vehicle (distilled water) and 5 mL of 200 µg/mL indomethacin, respectively. A 0.1 mL suspension of human red blood cells was introduced into each tube and mixed gently. The blend was allowed to sit for 1 hour at room temperature (37 ˚C) and then centrifuged for 3 min at 1,300 rpm. The absorbance (OD) of the hemoglobin concentration in the supernatant was measured at 540 nm with a Spectronic 21D (Milton Roy, Houston, TX, USA). The percentage of haemolysis inhibition for the extract was assessed in the subsequent way:

$$\% \text{ Inhibition of Haemolysis} = 1 - \frac{\text{OD2}-\text{OD1}}{\text{OD3}-\text{OD1}} \times 100 \quad (11)$$

OD1 = absorbance of the sample being tested in an isotonic solution; OD2 = absorbance of the sample being tested in a hypotonic solution; OD3 = absorbance of the reference sample in a hypotonic solution.

Statistical analysis

The collected data were examined using the statistical package for the social sciences version 21. The outcomes were presented as mean ± standard deviation. A notable difference in results was found through one-way and two-way analysis of variance, with the significance threshold set at $P < 0.05$.

Results

Percentage yield and qualitative and quantitative phytochemical screening

The ethanol extract from 1,500 g of plant material produced 28.24 g, representing 1.88% of the fresh leaves of *C. mucunoides* utilized. The qualitative and quantitative phytochemical analysis of the EECML revealed various secondary metabolites in the plant, as displayed in Table 1. It is noteworthy that substantial concentrations of steroids, glycosides, and saponins were discovered, while significant quantities of alkaloids, flavonoids, phenols, tannins, and terpenoids were also identified.

Toxicity study

The findings showed that even with the maximum dosage of 5,000 mg/kg of b.w given, the EECML was non-lethal, and no behavioral changes were noted, as demonstrated in Table 2. Consequently, the median lethal dose (LD_{50}) of the extract is probably more than 5000 mg/kg b.w.

Proximate composition

The results of the proximate analysis of EECML as shown in Table 3 revealed that percentage carbohydrate and moisture contents were relatively high compared to protein, crude fibre, fat and ash.

Anti-oxidant vitamins

The findings regarding the antioxidant vitamins of EECML are presented in Table 4. The findings indicated that vitamin E has a greater concentration of 0.329 ± 0.237 in comparison to vitamin A and C, which have concentrations of 0.290 ± 0.219 and 0.271 ± 0.274, respectively

Mineral compositions

The results of the mineral compositions of EECML as shown in Table 5 were found in varying concentrations of which calcium having the highest concentration while selenium was observed to be low.

Egg albumin-induced

Figure 1 illustrates the effect of EECML on paw swelling induced by egg albumin in rats. It displays the typical swelling of the paw and the percentage reduction in swelling induced by egg albumin in the rat paw, lasting for a period of 5 h. In comparison to the control, all treatment groups exhibited a significant ($P < 0.05$) decrease in the average paw oedema from 30 min to 5 h. At various time intervals and in a dose-dependent manner, no significant ($P > 0.05$) decreases in the average paw swelling of rats were observed in the control group. The size of the paws in animals given larger doses of the EECML and the standard drug, indomethacin, significantly ($P < 0.05$) decreased over time. At the 5-h point, the percentage of oedema inhibition in the indomethacin group exceeded that of the 400 mg/kg EECML groups; nonetheless, this disparity was not statistically significant ($P > 0.05$).

Table 1 Qualitative and quantitative phytochemical composition of the EECML

Constituents	Inference	Concentration (mg/100 g)
Saponins	+ + +	0.816 ± 0.018
Glycosides	+ +	0.102 ± 0.009
Alkaloids	+ + +	0.277 ± 0.017
Flavonoids	+ + +	1.118 ± 0.121
Tannins	+ + +	0.589 ± 0.003
Phenols	+ +	0.652 ± 0.016
Steroids	+ +	1.295 ± 0.090
Terpenoids	+ + +	1.042 ± 0.020

Quantitative results are expressed in means ± standard deviation (n = 3). + + = moderate concentration, + + + = present in large amount. EECML, ethanol extract of *Calopogonium mucunoides* leaves.

Table 2 Results of Phase I and II of the acute toxicity test of the EECML

Groups	Dose of extract (mg/kg b.w)	Mortality	Behavioral changes
Phase I			
1	10	0/3	Nil
2	100	0/3	Nil
3	1,000	0/3	Nil
Phase II			
1	1,600	0/3	Nil
2	2,900	0/3	Nil
3	5,000	0/3	Nil

EECML, ethanol extract of *Calopogonium mucunoides* leaves.

Table 3 Proximate composition of EECML

Component	(%) Composition
Protein	12.740 ± 0.020
Ash	9.750 ± 0.020
Fat	2.353 ± 0.015
Moisture	32.050 ± 0.020
Crude fiber	8.967 ± 0.015
Carbohydrate	34.140 ± 0.020

Results presented as mean ± standard deviation (n = 3). EECML, ethanol extract of *Calopogonium mucunoides* leaves.

Table 4 Concentrations of some anti-oxidant Vitamin in the EECML

Vitamins	Concentration
C (mg/100g)	0.271 ± 0.274
E (mg/100g)	0.329 ± 0.237
A (µg/100g)	0.290 ± 0.219

Results presented as mean ± standard deviation (n = 3). EECML, ethanol extract of *Calopogonium mucunoides* leaves.

Table 5 Determination of mineral composition of EECML

Minerals	Concentration
Calcium (mg/100g)	24.001 ± 0.001
Iron (mg/100g)	10.149 ± 0.001
Potassium (mg/100g)	3.790 ± 0.002
Sodium (mg/100g)	2.087 ± 0.002
Zinc (mg/100g)	0.892 ± 0.003
Selenium (mg/100g)	0.052 ± 0.001

Results presented as mean ± standard deviation (n = 3). EECML, ethanol extract of *Calopogonium mucunoides* leaves.

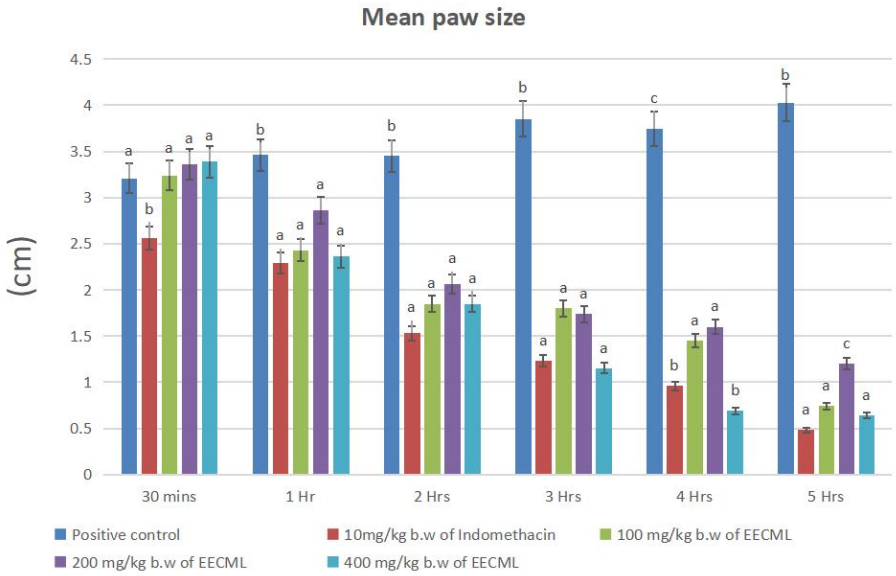


Figure 1 Effect of EECML on egg albumin-induced rat paw oedema.
The findings presented as mean ± standard deviation (n = 5). Mean values with differing superscript alphabets a, b, c show a significant difference at $P < 0.05$; same superscript alphabets a, b, c denote not significant difference at $P > 0.05$. Group 1: positive control; Group 2: 10 mg/kg b.w of indomethacin; Group 3: 100 mg/kg b.w of EECML; Group 4: 200 mg/kg b.w of EECML; Group 5: 400 mg/kg b.w of EECML. EECML, ethanol extract of *Calopogonium mucunoides* leaves; b.w, body weight.

DPPH free radical scavenging activity
According to Figure 2, the EECML at varying concentrations significantly reduced ($P < 0.05$) oxidative stress induced by DPPH scavenging radicals. The DPPH showed a significant percentage of inhibition at a concentration of 160 µg/mL, with IC_{50} displaying a notable ($P < 0.05$) rise compared to standard ascorbic.

Platelet aggregation
The outcome illustrated in Figure 3 demonstrated the influence of the EECML on the platelet aggregatory response induced by $CaCl_2$ from 2 to 8 min. In a manner dependent on concentration, both the extract and the reference drug, diclofenac, notably ($P < 0.05$) diminished the response of platelet aggregation. The various concentrations of the extract suppressed $CaCl_2$-induced platelet aggregation in a dose and time-dependent manner, with peak platelet aggregation seen at a concentration of 1,000 µg/mL after 8 min.

Phospholipase-A_2 inhibition activity
The results shown in Figure 4 indicated that the EECML at varying concentrations significantly ($P < 0.05$) inhibited the phospholipase-A_2 activity in a concentration-dependent fashion. This was shown by a decrease in the percentage inhibition of the enzyme activity. The IC_{50} for the EECML was 10.38 ± 1.06, whereas it was 24.80 ± 1.48 for prednisolone.

Albumin denaturation inhibition assay
The present study demonstrated in-vitro anti-inflammatory effect of EECML by inhibiting protein denaturation illustrated in Table 6. The highest inhibition observed in the extract at 20 and 80 µg/mL showed significant ($P < 0.05$) rises in comparison to other concentrations. Nevertheless, the effect of inhibition on the standard drug Diclofenac was not considerable ($P > 0.05$) at the concentration doses used. The extract's IC_{50} was determined to be 57.82 ± 6.10 compared to 9.50 ± 1.24 for diclofenac.

Assessment of membrane stabilization
Data presented in Table 7 demonstrate that the EECML significantly ($P < 0.05$) inhibited lysis triggered by water. This is demonstrated by the considerable percentage inhibition of haemolysis (4.64 and 11.4) reached at concentrations of 160 and 320 µg/mL, respectively. The extract's ability to inhibit hemolysis was shown to depend on its concentration, increasing with higher amounts of the extract in the medium, and resembled the effect of the standard drug indomethacin.

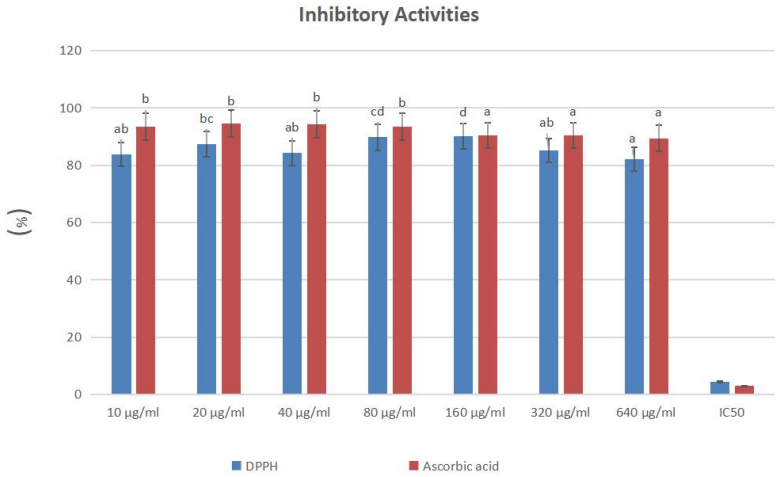


Figure 2 DPPH radical scavenging activity of EECML.
Results shown as Means ± SD (n = 3). Mean values with differing superscript alphabets a, b, c, d show a significant difference at $P < 0.05$; same superscript alphabets a, b, c, d denote not significant difference at $P > 0.05$. IC_{50}, inhibitory concentration 50; EECML, ethanol extract of *Calopogonium mucunoides* leaves; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

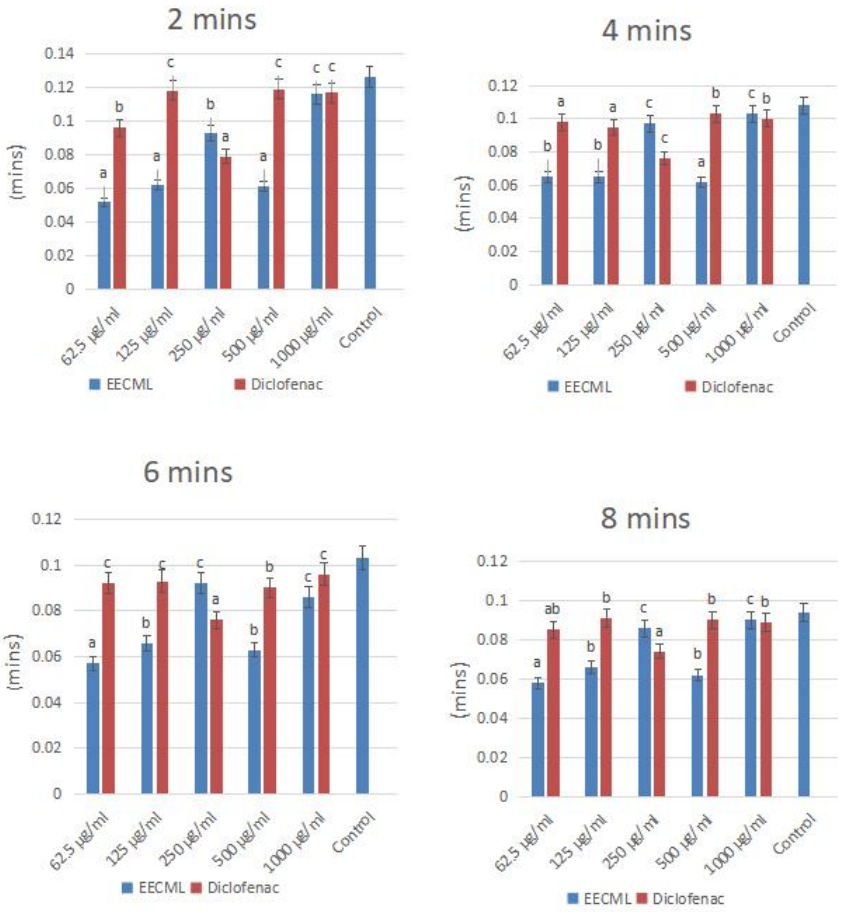


Figure 3 Effect of EECML on platelet aggregatory response.
Results presented as means ± SD (n = 3). Mean values with differing superscript alphabets a, b, c show a significant difference at $P < 0.05$; same superscript alphabets a, b, c denote not significant difference at $P > 0.05$. EECML, ethanol extract of *Calopogonium mucunoides* leaves.

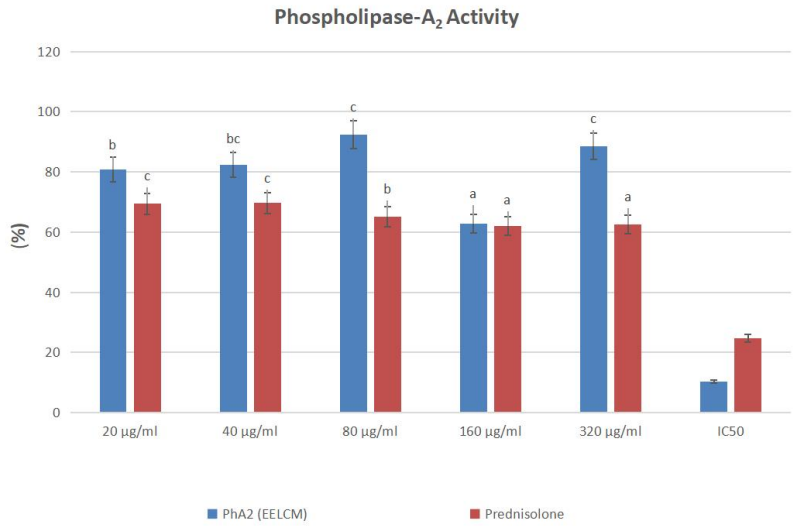


Figure 4 Percentage inhibition of EECML on phospholipase-A_2 activity.
Results presented as Means ± SD (n = 3). Mean values with differing superscript alphabets a, b, c show a significant difference at $P < 0.05$; same superscript alphabets a, b, c denote not significant difference at $P > 0.05$. PhA_2, Phospholipase-A_2; EECML, ethanol extract of *Calopogonium mucunoides* leaves; IC_{50}, inhibitory concentration 50.

Table 6 Percentage inhibition of EECML on albumin denaturation

Concentration (µg/mL)	EECML	Diclofenac
20	50.95 ± 0.83[b]	69.05 ± 10.53[a]
40	42.38 ± 2.1[a]	74.76 ± 4.36[a]
80	51.42± 5.15[b]	63.80 ± 7.33[a]
160	37.62± 2.97[a]	76.42 ± 1.01[a]
IC_{50}	57.82 ± 6.10	9.50 ± 1.24

Results presented as Means ± SD (n = 3). Mean values that have different letters as superscripts in the column are deemed significant at $P < 0.05$. The superscript letters a and b separates the mean values, different superscript alphabets denote significant difference at $P < 0.05$ and same superscript alphabets denotes no significant difference at $P > 0.05$ down the group's concentrations. EECML, ethanol extract of *Calopogonium mucunoides* leaves; IC_{50}, inhibitory concentration 50; SD, standard deviation.

Table 7 Effect of EECML on membrane stabilization

	Concentration (µg/mL)	Hypotonic solution	Isotonic solution	Control
EECML	20	0.26 ± 0.03	0.60 ± 0.04	2.33 ± 0.00
	40	0.27 ± 0.01	0.68 ± 0.01	3.79 ± 0.00
	80	0.27 ± 0.01	0.71 ± 0.01	3.33 ± 0.00
	160	0.28 ± 0.01	0.75 ± 0.02	4.64 ± 0.00
	320	0.31 ± 0.01	0.75 ± 0.03	11.4 ± 0.00
Indomethacin	200	0.29 ± 0.10	0.29 ± 0.10	11.8 ± 0.00

Results expressed as means ± SD (n = 3). EECML, ethanol extract of *Calopogonium mucunoides* leaves; SD, standard deviation.

Discussion

The use of medicinal plants to treat common infections has gained popularity in developing nations because of its lower cost and assertions regarding its effectiveness and reduced side effects compared to synthetic medications. A wide variety of compounds that offer potential for the treatment of chronic and infectious diseases are particularly present in traditional medicinal plants. Numerous minor constituents of foods, like secondary plant metabolites, have been demonstrated to influence biological processes, potentially lowering the risk of certain chronic diseases in humans.

The cold maceration technique was employed to extract the secondary metabolites found in *C. mucunoides* leaves. The maceration technique is regarded as one of the standard methods for extracting medicinal plants [20]. It was used to extract a broad and extensive variety of polar and non-polar phytochemicals found in EECM. Solvents selected for extracting biomolecules from plants are determined by their polarity [20]. Dried *C. mucunoides* powder (1,500 g) processed through cold ethanol extraction produced 28.24 g, representing 1.88 % of the initial material utilized. Several factors, including the type and concentration of solvent for extraction, the ratio of solvent to solid, the size of the plant material particles, the characteristics of the leaves, the timing of the harvest, the level of acidification, the temperature for drying, as well as storage conditions and the temperature and duration of the extraction, may influence the yield of plant extracts [20].

The current research showed that EECML has varying quantities of flavonoids, tannins, saponins, glycosides, alkaloids, phenols, terpenoids, and steroids, as indicated in Table 1. The existence of these phytochemicals indicates that EECML might be utilized to address various health issues. Flavonoids found in considerable quantities in plants have demonstrated the ability to scavenge free radicals and influence the production of COX-1 and COX-2, which are involved in prostaglandin synthesis [21]. Consequently, the existing study indicates that blocking prostaglandin production is a key way in which the plant extract shows anti-inflammatory properties. Alkaloids and flavonoids are demonstrated to exhibit antidiuretic, antispasmodic, anti-inflammatory, and analgesic properties in both humans and animals [22]. The acute toxicity evaluations in this study showed that the oral doses of EECML have a strong safety profile. The animal trials showed that the plant extract was well accepted at doses as high as 5,000 mg/kg b.w, with no fatalities noted as shown in Table 2, indicating that the extract's doses were fairly safe for both human and animal consumption. Therefore, the toxicity test of the plant extract must surpass 5,000 mg/kg b.w.

The proximate composition results presented in Table 3 demonstrate that the plant contains different but notable ($P < 0.05$) quantities of essential nutrients. The proximate composition percentages are as follows: carbohydrate (34.140 ± 0.020), moisture (32.050 ± 0.020), protein (12.740 ± 0.020), ash (9.750 ± 0.020), crude fiber (8.967 ± 0.015), and fat (2.353 ± 0.015). Carbohydrates are essential components of a healthy diet, as they supply energy to cells such as brain, muscle, and blood cells [23]. The moisture level discovered in the plant indicates that it serves as an excellent source of water from vegetables for cellular construction [24]. The low-fat content found is less than the specified range (8.3–27.0%) noted for different leafy vegetables utilized in Nigeria, and research indicates that leafy vegetables are inadequate sources of lipids [25]. The protein content was average and is essential for numerous bodily functions, including growth, fluid balance maintenance, hormone and enzyme production, and supporting a robust immune system [23]. Plants rich in crude fiber can aid in the absorption of trace elements and promote bowel regularity [26]. Incorporating vegetables into our diet can help relieve constipation. Dietary fibers assist in lowering cholesterol and triglycerides, and in addition, they help avert cancer and digestive issues [27]. The ash content indicates the total quantity of mineral substances present in a plant [28].

Table 4 indicates that the plant holds considerable amounts of antioxidant vitamins. The concentrations of Vitamin C, E, and A were 0.271 ± 0.274, 0.329 ± 0.237, and 0.290 ± 0.219, respectively. Vitamins serve as crucial antioxidants that safeguard the cell membrane against oxidative stress and damage inflicted by free radicals [29]. The plant holds significant quantities of ascorbic acid and flavonoids, which are both powerful antioxidants. Vitamin C has antioxidant properties and is essential for maintaining normal connective tissues, promoting wound healing, and aiding the absorption of dietary iron from the intestine [30]. Vitamin A is crucial for sharp eyesight. Insufficient levels of these vitamins make the red cell membranes more susceptible to damage, resulting in haemolysis [31]. Due to the presence of these vitamins in the EECML, it makes an excellent option for antioxidant treatment.

The mineral composition of EECML was examined, revealing that the plant extract has diverse yet significant amounts of minerals, with calcium (24.001 ± 0.001 mg/100 g) present in the highest quantity and selenium (0.052 ± 0.001 mg/100 g) in the lowest (Table 5). Calcium is essential for blood coagulation, the formation of bones and teeth, and serves as a co-factor in the activation of various enzymes. The production of hemoglobin necessitates iron, and a lack of iron is a widespread nutritional issue impacting individuals globally. Primarily, it arises due to chronic bleeding, infections, insufficient consumption of bioavailable iron, and deficiencies in folic acid, vitamin A or B_{12}, as well as during pregnancy. The reasonable level of iron present in EECML suggests that the plant might be an excellent dietary source of iron to address its deficiency when included in the diet. The potassium level relative to sodium produced a significantly low Na/K ratio, which is advantageous from a nutritional standpoint since diets with a low Na/K ratio are associated with a reduced risk of hypertension. Extracellular and intracellular fluids hold potassium and sodium cations, which aid in sustaining the body's electrolyte equilibrium [32]. Potassium is essential for the growth and reproduction of plants and is needed in large quantities. Zinc is essential for protein synthesis, cellular differentiation, immune replication, and sexual functioning [33]. Selenium is a trace element essential for numerous bodily functions, such as cognitive abilities and a strong immune system. It functions as an antioxidant, protecting against oxidative harm, infections, lowering inflammation, and cancer [34].

Figure 1 illustrates the effect of EECML on paw swelling caused by egg albumin in rats. The ability of EECML to prevent the initial stage of edema indicates that it may inhibit the release of histamine and serotonin. Moreover, its capacity to reduce swelling in the second and third stages of inflammation indicates that the extract's anti-inflammatory effects are associated with the inhibition of kinin and prostaglandin production induced by egg albumin in these stages [35]. Since these mediators induce edema by enhancing vascular permeability and promoting vasodilation at the site of injury, the extract reduces vascular permeability and fluid leakage, likely by influencing endothelial cells, thereby reducing edema.

Figure 2 demonstrates that different doses of EECML significantly ($P < 0.05$) decreased oxidative stress caused by DPPH radicals. At a concentration of 160 µg/mL, the DPPH exhibited a notable percentage inhibition with an IC_{50} that was significantly ($P < 0.05$) greater than that of ascorbic acid. Antioxidant substances neutralize DPPH free radicals by donating electrons or hydrogen atoms to DPPH, altering its hue from purple to the stable yellow diamagnetic compound known as diphenylpicryl-hydrazine [16]. The extent of color change reflects the scavenging capacity of the antioxidant substances regarding their hydrogen donating capability [36].

The impact of EECML on platelet aggregation triggered by $CaCl_2$ is illustrated in Figure 3. The extract, similar to diclofenac, notably ($P < 0.05$) reduced the platelet aggregation response in comparison to the control. The highest platelet aggregatory activity was reached at the 6th minute. The extract's effect on platelet inhibition was comparable to that of diclofenac. As the extract concentrations rose from 62.5 to 1,000 µg/mL, it reduced the ability of $CaCl_2$ to induce aggregation of human platelets. The inhibition of the platelet aggregation response induced by $CaCl_2$ may be due to the extract's ability to block phospholipase-A_2 (PLA_2) and COX, which are crucial for producing thromboxane A_2 (TXA_2) [37]. Inhibiting TXA_2 is essential since it initiates platelet aggregation by increasing intracellular calcium ion (Ca^{2+}) concentrations, aiding the fusion of platelet granules with the membrane and releasing substances such as ADP that further promote platelet aggregation. The EECML's capacity to prevent platelet aggregation may suggest reduced vascular permeability and leukocyte extravasation, both processes influenced by histamine released from the granules of platelets, indicating a possible antithrombotic effect [38]. The noted observation may result from the existence of secondary metabolites like phenols, tannins, and flavonoids, which have shown anticoagulant and anti-platelet aggregatory effects [37, 39, 40].

Figure 4 shows that the EECML notably ($P < 0.05$) reduced PLA_2 activity in a dose-dependent fashion. This was demonstrated by a decline in the percentage decrease of enzyme activity. The IC_{50} of the EECML was notably ($P < 0.05$) lower than that of prednisolone, a conventional anti-inflammatory that exhibited a comparable trend where enzyme activity reduced with increased doses of prednisolone. PLA_2 is an enzyme that releases free fatty acids from phospholipids in membranes. Arachidonic acid derived from these phospholipids is processed by COX and lipoxygenase (LOX) enzymes, resulting in the generation of lipid mediators from scratch. COX's action on arachidonic acid results in the formation of mediators like TXA_2, prostaglandin E2, D2, and I2, whereas 5-LOX's influence on arachidonic acid triggers the release of leukotrienes, including leukotriene B4 [41]. The extract's inhibition of PLA_2 activity indicates it could diminish the release of free fatty acids from phospholipids in

red blood cell membranes, consequently limiting the availability of COX and LOX precursors required for generating inflammatory mediators, thereby reducing effects like vasodilation, vascular permeability, chemotaxis, and pain, ultimately assisting in inflammation prevention. The way the extract inhibits PLA_2 might resemble the influence of corticosteroids that promote lipocortin [41, 42].

This study explored the ability of EECML to prevent protein denaturation as part of the investigation into its anti-inflammatory activity mechanism. Table 6 indicates that EECML demonstrated a peak inhibition of 51.4 ± 5.15 percent at 80 µg/mL, showing an IC_{50} of 57.82 ± 6.10, in contrast to diclofenac (9.50 ± 1.24) as the reference drug. Albumin denaturation happens when proteins lose their quaternary, tertiary, and secondary configurations due to the application of external stressors or substances. The majority of biological proteins lose their functional properties when they are denatured [43]. In certain arthritic diseases, auto-antigens may lead to the denaturation of proteins within tissues. Therefore, it can be stated that the denaturation of tissue proteins serves as an indicator for inflammatory and arthritic conditions [43, 44].

Table 7 demonstrates that both EECML and a conventional anti-inflammatory medication safeguarded the human erythrocyte membrane from lysis induced by hypotonic solution at different concentrations. The EECML significantly ($P < 0.05$) reduced lysis triggered by water. This is evidenced by the heightened percentage inhibition of haemolysis (4.64 and 11.4) observed at concentrations of 160 and 320 µg/mL, respectively. In the course of inflammation, lysosomes break down and release their enzymes, leading to various diseases. NSAIDs function by either inhibiting the release of lysosomal enzymes or preserving the integrity of lysosomal membranes [45]. When red blood cells encounter adverse factors like hypotonic conditions or elevated temperatures, the membrane degrades, leading to haemolysis and the oxidation of hemoglobin. Because of the similarities between the membrane components of human red blood cells and those found in lysosomes, the observed prevention of membrane lysis induced by hypotonicity in red blood cells in this study was regarded as evidence of the anti-inflammatory effects of EECML [46]. The haemolytic impact of a hypotonic solution arises from an overabundance of fluid inside the cell, leading to the rupture of the cell membrane. Injury to the red cell membrane heightens the cell's susceptibility to additional damage from lipid peroxidation induced by free radicals [47]. In a phase of increased permeability due to inflammatory mediators, the stabilization of membranes blocks the movement of serum proteins and fluids into tissues [3]. The EECML could have strengthened the red blood cell membrane, thereby preventing the release of lytic enzymes and active inflammatory mediators.

Conclusion

This study indicates that the EECML possesses anti-inflammatory and antioxidant effects. Additionally, the plant was found to be abundant in essential dietary nutrients. Moreover, the results indicate that the plant extract could act as a possible source of anti-inflammatory agents and an economical source of nutritional food elements if used properly. Further study is however required in bioassay fractionation of the EECML with different solvent to get bioactive principle that are more active. Also, to characterized and ascertain the molecular mechanism (s) of anti-inflammatory properties of EECML.

References

1. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med.* 2019;25(12):1822–1832. Available at: https://doi.org/10.1038/s41591-019-0675-0
2. Ouedraogo N, Atchade C, Traore TK, et, al. Anti-inflammatory activity of extracts from Parkiabiglobosa (Jacq.) R.Br. Ex G. Don. (Fabaceae-Mimosoideae) trunk bark. *J Pharmacol Toxicol.* 2021;16:1–8. Available at: https://doi.org/10.3923/jpt.2021.1.8
3. Ikpeazu OV, Ezeja MI, Igwe KK. Anti-Inflammatory Activity of the Stem Bark Methanol Extract of Picralima nitida. *Eur J Med Plants.* 2021;32(1):22–28. Available at: https://doi.org/10.9734/ejmp/2021/v32i130360
4. Chy MU, Adnan M, Rauniyar AK, et al. Evaluation of Anti-nociceptive and Anti-inflammatory activities of Piper sylvaticum (Roxb.) Stem by experimental and computational approaches. *Adv Tradit Med.* 2019;20:327–341. Available at: https://doi.org/10.1007/s13596-019-00395-9
5. Jia YW, Pang C, Zhao KX, et al. Garcinol Suppresses IL-1β-Induced Chondrocyte Inflammation and Osteoarthritis via Inhibition of the NF-κB Signaling Pathway. *Inflammation.* 2020;42(5):1586–1587. Available at: https://doi.org/10.1007/s10753-019-01145-4
6. Kaushik S, Jain P, Satapathy T, Purabiya P, Roy A. Evaluation of anti-arthritic and antiinflammatory activities of Martyniaannua L. Ethanolic extract. *Clin Phytosci.* 2021;7:7. Available at: https://scholar.google.com/scholar?hl=zh-CN&as_sdt=0%2C45&q=Evaluation+of+anti-arthritic+and+antiinflammatory+activities+of+Martyniaannua+L.+Ethanolic+extract&btnG=
7. Enechi OC, Odo CE, Okafor C. Assessment of antiulcer action of *Calopogonium mucunoides* Desv leaf extract in Wistar rats. *J Pharm Res.* 2014;8(1):24–27. Available at: https://doi.org/10.1186/s40816-021-00250-y
8. Enechi OC, Abugu M. Antidiarrheal and Antibacterial activities of Calopogonium mucunoides Desv leaf extract. *Glob Vet.* 2016;16(2):155–164. Available at: https://www.idosi.org/gv/gv16(2)16/4.pdf
9. Fadeyi AE, Akiode SO, Falayi OE, Fatokun AO, Orjiajogun JO. Phytochemical, antioxidant, proximate and FTIR analysis of Calopogonium mucunoides Desv. Extracts using selected solvents. *World J Biol Pharm Health Sci.* 2020;4(1):14–22. Available at: https://doi.org/10.30574/wjbphs.2020.4.1.0069
10. Harborne JB. *Phytochemical Methods: A Guide To Modern Techniques of Plant Analysis.* 3rd Edition. London, UK: Chapman and Hall; 1998. Available at: ISBN: 9780412572708
11. Ali M, Shuab M. Characterization of the chemical constituents of Daturametel Linn. *Indian J Pharm Sci.* 1996;5(6):243–245.
12. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol.* 1983;54(4):275–287. Available at: https://doi.org/10.1007/bf01234480
13. AOAC. *Official Methods of Analysis.* 18th Edition. Washington DC: AOAC International; 2010. Available at: ISBN:978-093558480
14. William H, AOAC International. *Official Methods of Analysis.* 17th Edition. Washington, DC: AOAC International; 2000. Available at: ISBN: 9780935584677
15. Sakat SS, Juvekar AR, Gambhire MN. In vitro Antioxidant and Anti-Inflammatory Activity of Methanol Extract of Oxalis corniculata Linn. *Int J Pharm Pharm Sci.* 2010;2(1):146–155. Available at: https://scholar.google.com/scholar?lr=&q=In+vitro+Antioxidant+and+Anti-Inflammatory+Activity+of+Methanol+Extract+of+Oxalis+corniculata+Linn&hl=zh-CN&as_sdt=0,5
16. Denaro M, Smeriglio A, Trombetta D. Antioxidant and Anti-Inflammatory Activity of Citrus Flavanones Mix and Its Stability after In Vitro Simulated Digestion. *Antioxidants* (*Basel*). 2021;10(2):140. Available at: https://doi.org/10.3390/antiox10020140
17. Born GVR, Cross MJ. The aggregation of blood platelets. *J Physiol.* 1963;168(1):178–195. Available at: https://doi.org/10.1113/jphysiol.1963.sp007185

18. Vane JR. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. *Nat New Biol.* 1971;231(25):232–235. Available at: https://doi.org/10.1038/newbio231232a0
19. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol.* 1968;20(3):169–173. Available at: https://doi.org/10.1111/j.2042-7158.1968.tb09718.x
20. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants.* 2015;4(3):2–6. Available at: https://scholar.google.com/scholar?q=A+Review+on+the+Extraction+Methods+Use+in+Medicinal+Plants,+Principle,+Strength+and+Limitation&hl=zh-CN&as_sdt=0&as_vis=1&oi=scholart
21. George A, Chinnappan S, Chintamaneni M, et al. Anti-inflammatory effects of Polygonum minus (Huds) extract (Lineminus™) in in-vitro enzyme assays and carrageenan induced paw edema. *BMC Complement Altern Med.* 2014;14:355. Available at: https://doi.org/10.1186/1472-6882-14-355
22. Othman ZA, Ghazali WSW, Noordin L, Yusof NAM, Mohamed M. Phenolic compounds and the anti- atherogenic effect of bee bread in high fat induced obese rats. *Antioxidants* (*Basel*). 2019;9(1):33. Available at: https://doi.org/10.3390/antiox9010033
23. Shemishere UB, Taiwo JE, Erhunse N, Omoregie ES. Comparative study on the proximate analysis and nutritional composition of *Musanga cercropioides* and *Maesobotyra barteri* leaves. *J Applied Sci Environ Manage.* 2018;22(2):287. Available at: https://doi.org/10.4314/jasem.v22i2.22
24. Kochhar A, Nagi M, Sachdeva R. Proximate Composition, Available Carbohydrates, Dietary Fibre and Anti Nutritional Factors of Selected Traditional Medicinal Plants. *J Hum Ecol.* 2006;19(3):195–199. Available at: https://scholar.google.com/scholar?hl=zh-CN&as_sdt=0%2C45&as_vis=1&q=Proximate+Composition%2C+Available+Carbohydrates%2C+Dietary+Fibre+and+Anti+Nutritional+Factors+of+Selected+Traditional+Medicinal+Plants.&btnG=
25. Ogunyinka BI, Oyinloye BE, Osunsanmi FO, Kappo AP, Opoku AR. Comparative study on proximate, functional, mineral, and antinutrient composition of fermented, defatted, and protein isolate of Parkia biglobosa seed. *Food Sci Nutr.* 2016;5(1):139–147. Available at: https://doi.org/10.1002/fsn3.373
26. Margier M, Georgé S, Hafnaoui N, et al. Nutritional Composition and Bioactive Content of Legumes: Characterization of Pulses Frequently Consumed in France and Effect of the Cooking Method. *Nutrients.* 2018;10(11):1668. Available at: https://doi.org/10.3390/nu10111668
27. Ayanda IO, Dedeke GA, Ekhator UI, Etiebet MK. Proximate Composition and Heavy Metal Analysis of Three Aquatic Foods in Makoko River, Lagos, Nigeria. *J Food Qual.* 2018;2018(1):1–6. Available at: https://doi.org/10.1155/2018/2362843
28. Agbai CM, Olawuni IA, Azeke AE, Nwokenkwo EC, Alagbaso SO. Evaluation of proximate and functional properties of rubber (Heveabrasiliensis) seed meals. *Afr J Food Sci.* 2020;14(11):407–413. Available at: https://scholar.google.com/scholar?hl=zh-CN&as_sdt=0%2C45&as_vis=1&q=Evaluation+of+proximate+and+functional+properties+of+rubber+%28Heveabrasiliensis%29+seed+meals&btnG=
29. Olatunji TL, Afolayan AJ. Comparison of nutritional, antioxidant vitamins and capsaicin contents in Capsicum annuum and C. frutescens. *Int J Veg Sci.* 2019;26(1):1–18. Available at: https://doi.org/10.1080/19315260.2019.1629519
30. Mohapatra D, Patel AS, Kar A, Deshpande SS, Tripathi MK. Effect of different processing conditions on proximate composition, anti-oxidants, anti-nutrients and amino acid profile of grain sorghum. *Food Chem.* 2019;271:129–135. Available at: https://doi.org/10.1016/j.foodchem.2018.07.196
31. Daniel IE, Mathew KN, John PL. Evaluation of Vitamin Contents, Antioxidant and Antimicrobial Activities of Different Leaf Extracts of Taraxacum officinale (Dandelion). *J Complement Altern Med Res*. 2021;13(2):13–26. Available at: https://doi.org/10.9734/jocamr/2021/v13i230220
32. Jenkins DJA, Spence JD, Giovannucci EL, et al. Supplemental Vitamins and Minerals for CVD Prevention and Treatment. *J Am Coll Cardiol.* 2018;71(22):2570–2584. Available at: https://doi.org/10.1016/j.jacc.2018.04.020
33. Pathak P, Kapil U. Role of trace elements zinc, copper and magnesium during pregnancy and its outcome. *Indian J Pediatr.* 2004;71(11):1003–1005. Available at: https://doi.org/10.1007/BF02828116
34. Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. *J King Saud Univ Sci.* 2015;27(3):224–232. Available at: https://doi.org/10.1016/j.jksus.2015.02.003
35. Enechi OC, Amah CC, Okoro JI, et al. Assessment of the Anti-inflammatory and Anti-oxidant Activities of Flavonoid-rich Fraction of Pleiocarpa mutica Leaves. *Adv Biochem.* 2022;10(1):25. Available at: https://doi.org/10.11648/j.ab.20221001.14
36. Siahbalaei R, Kavoosi G, Shakeri R. In vitro antioxidant and antidiabetic activity of essential oils encapsulated in gelatin-pectin particles against sugar, lipid and protein oxidation and amylase and glucosidase activity. *Food Sci Nutr.* 2020;8(12):6457–6466. Available at: https://doi.org/10.1002/fsn3.1935
37. Reindersa Y, Pohlc F, Ahrensd N, Prantla L, Kuehlmanna B, Haubnere F. Impact of platelet- rich plasma on cell migration processes after external radiation. *Clin Hemorheol Microcirc.* 2019;73:43–51. Available at: https://doi.org/10.3233/CH-199218
38. Kuhnlaa A, Reinthalera M, Braunea S, et al. Spontaneous and induced platelet aggregation in apparently healthy subjects in relation to age. *Clin Hemorheol Microcir.* 2019;71(4):425–435. Available at: https://doi.org/10.3233/CH-199006
39. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. In Vitro Anti-Inflammatory Properties of Selected Green Leafy Vegetables. *Biomedicines.* 2018;6(4):107. Available at: https://doi.org/10.3390/biomedicines6040107
40. Angel Morales León J, González Santisteban A, Peña Fuentes D, Guardia Puebla Y, Torres Rodríguez E. In vitro anti-inflammatory activity of aqueous, ethanolic and ethereal extracts of rhizomes, leaves and stems of Anredera vesicaria. *J Anal Pharm Res.* 2018;7(4):459–461. Available at: https://doi.org/10.15406/japlr.2018.07.00266
41. Forsyth C, Kouvari M, D'Cunha NM, et al. The effects of the Mediterranean diet on rheumatoid arthritis prevention and treatment: a systematic review of human prospective studies. *Rheumatol Int.* 2017;38(5):737–747. Available at: https://doi.org/10.1007/s00296-017-3912-1
42. Tedeschi SK, Frits M, Cui J, et al. Diet and Rheumatoid Arthritis Symptoms: Survey Results From a Rheumatoid Arthritis Registry. *Arthritis Care Res (Hoboken).* 2017;69(12):1920–1925. Available at: https://doi.org/10.1002/acr.23225
43. Thirumalaisamy R, Ameen F, Subramanian A, Selvankumar T, Alwakeel SS, Govarthanan M. In-Vitro and In-Silico Anti-inflammatory Activity of Lupeol Isolated from Crateva adansonii and Its Hidden Molecular Mechanism. *Int J Pept Res*

Ther. 2020;26(4):2179–2189. Available at: https://doi.org/10.1007/s10989-019-10006-5

44. Sangeetha G, Vidhya R. In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). *Int J Herb Med.* 2016;4(3):31–36. Available at: https://scholar.google.com/scholar?hl=zh-CN&as_sdt=0%2C45&as_vis=1&q=In+vitro+anti-inflammatory+activity+of+different+parts+of+Pedalium+murex+%28L.&btnG=
45. Vadivu R, Lakshmi KS. In vitro and In vivo anti-inflammatory activity of leaves of Symplocos cochinchnensis (Lour) Moore ssp laurina. *Bangladesh J Pharmacol.* 2008;3(2):121–124. Available at: https://doi.org/10.3329/bjp.v3i2.956
46. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy K, Shanbhag T. Evaluation of antiinflammatory activity of Tephrosia purpurea in rats. *Asian Pac J Trop Med.* 2010;3(3):193–195. Available at: https://doi.org/10.1016/S1995-7645(10)60007-7
47. Yesmin S, Paul A, Naz T, et al. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*). *Clin Phytosci.* 2020;6:59. Available at: https://doi.org/10.1186/s40816-020-00207-7