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Inhibition of phospholipase A2, platelet aggregation and egg albumin induced rat paw oedema as anti-inflammatory effect of *Peltophorun pterocarpus* stem-bark

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Abstract

Background: Most medicinal plants presently employed in traditional medicine are used without scientific evidence, thereby suggesting a need to explore efficient and reliable investigations of their potential. We, therefore, conducted the present study to ascertain the efficacy of flavonoid-rich extract of *Peltophorum pterocarpum* sterm-bark in the treatment and management of inflammatory disorders as employed in folk medicine.

Materials and methods: Flavonoid-rich extract of *Peltophorum pterocarpum* sterm-bark and a total of fifty-five (55) Wistar rats were used for this study. Eighteen (18) mice were used for toxicity testing, and the phytochemical analysis was done using the Trease and Evans method, while the acute toxicity was done using Lorke's method. In vivo anti-inflammatory study was done using the egg albumin-induced paw oedema method, while the in vitro anti-inflammatory studies were performed for the extract using phospholipase A2 inhibition and calcium chloride-induced platelet aggregation assays.

Results: The phytochemical analysis revealed that the extract of *Peltophorum pterocarpum* sterm-bark contains tannins, terpenoids, steroids, phenols, alkaloids, flavonoids, glycosides, and saponins ranging from 0.307 ± 0.02 to 1279.567 ± 149.868. The acute toxicity test of the extract showed no toxicity up to 5000 mg/kg body weight. In the systemic oedema of the rat paw, scalar doses of the extract significantly ($p < 0.05$) suppressed the development of paw oedema induced by egg albumin, particularly with the Indomethacin (1.77 ± 0.41) when compared with the control (5.50 ± 0.26). However, varying doses of the extract significantly ($p < 0.05$) inhibited phospholipase A_2 activity and CaCl2-Induced platelet aggregation in a concentration, dose, and time-dependent manner, in comparison to prednisolone.

Conclusion: These results indicate that the extract exhibited anti-inflammatory potential, and the mechanism of this activity has a promising ability to inhibit phospholipase A_2 activity and platelet aggregation in rats inflicted with paw oedema.

Keywords: Inflammation, Phospholipase A_2, Platelet aggregation, Prednisolone, *Peltophorum pterocarpum*, Indomethacin

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Introduction

Inflammation is a form of the protective mechanism employed by the biological system to get rid of stimuli that are harmful to the system, such as pathogens, damaged cells, or irritants, thereby initiating the healing process in the body. The isolation and elimination of the injurious agents, repair of tissues damaged at the site of the injury, and restoration of functions are the desired result of inflammatory response. The damage of cell membrane caused by various injurious agents could lead to the activation of phospholipase A_2, which consequently mediates the release of arachidonic acid, which is further processed by cyclo-oxygenase (COX) and lipoxygenase (LOX) to synthesize pro-inflammatory mediators [1], which can either be cell-derived or plasma-derived. The high cost, side effects, and drug reaction associated with some conventional non-steroidal anti-inflammatory drugs (NSAIDs) make their use unattractive despite their efficacy [2], hence the need for the development of novel anti-inflammatory drugs from natural sources as alternatives to these drugs.

Despite the increasing need and use of herbal medicine to treat a number of diseases and ailments, there is still a wide knowledge gap regarding their mode of action. *Peltophorum pterocarpum* (family, Leguminosae) is a tropical tree found in a different part of Nigeria but most dominant in Eastern Nigeria. A literature review showed that different *Peltophorum pterocarpum* trees are used to treat several diseases [3]. In most applications, the traditional healers utilize the leaves in decoction for treating skin disorders, while stem infusion and flowers were used in muscular pain [4]. Different parts of *Peltophorum pterocarpum* have been reported to possess hepatoprotective effect [3], the neuroprotective effect [5], etc. Phytochemical screening carried out on ethanol leaves extract indicated the presence of some secondary metabolites such as flavonoids, alkaloids, saponins, sterols, and cardiac glycosides [6–11]. This study aims to evaluate the efficacy and anti-inflammatory activity of flavonoid-rich extract of *Peltophorum pterocarpum* Stem-Bark on Wistar rats.

Materials and methods

Plant collection

Fresh stem-bark of *Peltophorum pterocarpum* was collected from Nkpeshi, Ugwu-Awgbu, Orumba North LGA, Anambra State, Nigeria. The stem-bark was identified and authenticated by Mr. Alfred Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. The stem-bark were air-dried and pulverized. Voucher specimen of the plant with No. INTERCEED/086 was deposited at the InterCEED Herbarium.

Experimental animals

The animals used for this study were purchased from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized under standard laboratory conditions in the animal farm of the Department of Biochemistry for 1 week prior to the commencement of the experiment with 12-h light and dark cycle and maintained on a regular feed (commercial chicken grower's mash) and water *ad labium*. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85–23, revised 1985) at the Animal house, Department of Biochemistry.

Chemicals and reagents

All chemicals used in this study were of analytical grade and products of Sigma Aldrich, USA, British Drug House (BDH) England, Burgoyne, India, Harkin and Williams, England, Qualikems India, Fluka Germany, May and Baker England. Reagents used for the assays were commercial kits and products of Randox, USA and Teco (TC), USA.

Extraction procedure

The Fresh Stem bark of *Peltophorum pterocarpum*was collected and washed to remove dirt. The plant material was cut into pieces and shade-dried with regular turning to avoid decaying. The dried stem-bark was pulverized into powdered form using a mechanical grinder (Royalstar, Model, RZ-2051). A known weight of the pulverized stem bark(1 kg) was macerated in 3.5 L absolute ethanol using a maceration flask. The mixture was left for 72 h with occasional stirring, after which it was filtered into a flat-bottomed flask using a muslin cloth. Further filtration was achieved with Whatman No 1 filter paper so as to remove fine residues. The filtrate was concentrated using a rotary evaporator at 45 °C to obtain the crude ethanol extract. The concentrated extract was stored in a labelled sterile screw-capped bottle at 2–8 °C.

Phytochemical screening

A number of quantitative chemical tests were performed to establish the phytochemical profile of the crude extract by using the standard procedures [12].

Preparation of flavonoid-rich extract

Extraction of flavonoid-rich extract of *Peltophorum pterocarpum*stem-bark was carried out according to the method described by Chu et al [13]. Exactly 3 g of the crude extract was dissolved in 20 mL of 10% H_2SO_4 in a small flask and was hydrolysed by heating on a water bath for 30 mins at 100 °C. The mixture was placed on ice for 15 min, so as to allow the precipitation of the flavonoids aglycones. The cooled solution was filtered and the filtrate (flavonoid aglycone mixture) was dissolved in 50 mL of warm 95% ethanol (50 °C). The resulting solution was again filtered into 100 mL volumetric flask which was made up to the mark with 95% ethanol. The filtrate collected was concentrated to dryness using a rotary evaporator.

Acute toxicity and lethal dose determination

Investigation on acute toxicity of the extract with estimation of the median lethal dose (LD_{50}) was carried out using the modified method of Lorke [14]. This study was done only in two phases and a total of eighteen (18) mice were used. Six (6) groups of three (3) mice each were administered orally, doses of ethanol extract (10, 100 and 1000 mg/kg body weight) respectively for the first phase and (1900, 2600 and 5000 mg/kg b.w of the extract) for second phase by oral intubation. The mice were then observed for 24 h for lethality, neurological and behavioural change (signs of toxicity). The LD_{50} of the plant was calculated using the formula below:

$$LD_{50} = \sqrt{\text{highest dose that produced no mortality} \times \text{lowest dose that produced mortality}}$$

In vivo anti-inflammatory study

Determination of the effect of flavonoid-rich extract of Peltophorum pterocarpum stem-barkon egg albumin-induced rat paw oedema

This was done according to the method of Winter et al [15]. The increase in the right hind paw size of the rats induced by the sub-plantar injection of freshly prepared egg albumin was used as a measure of acute inflammation.

Principle

Egg albumin just like agar, releases mediators of acute inflammation responsible for causing oedema. The ability of the ethanol extract to inhibit this release of mediators is a measure of the anti-inflammatory effect of the extract.

Experimental design

A total of twenty-five (25) male Wistar albino rats were used for the study. They were divided into five (5) groups of five (5) rats each and treated as follows:

Group 1: Received normal saline.
Group 2: Received 10 mg/kg body weight of Indomethacin (standard drug)
Group 3: Received 100 mg/kg body weight of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark
Group 4: Received 250 mg/kg body weight of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark
Group 5: Received 400 mg/kg body weight of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark

Procedure

Rats were fasted for 18 h before the experiment to ensure uniform hydration and minimize variability in oedematous response, after which the right hind paw size of the rats at time zero (before the induction of oedema) was measured using a vernier calliper. This was followed by intraperitoneal administration of test substances as outlined above. One hour after administration of test substances, acute inflammation was induced by injecting 0.1 ml of freshly prepared egg albumin into the subplantar of the right hind paw of rats. The increase in the right hind paw size of rats was subsequently measured at 0.5, 1, 2, 3, 4, 5 and 24 h after egg albumin injection. The difference between the paw size of the injected paws at time zero and at different times after egg albumin injection was used to assess the formation of oedema. These values were used in the calculation of the percentage inhibition of oedema for each dose of the extract and for Indomethacin at the different time intervals using the relation below:

Paw oedema = (Vt-Vo)
Vo = Paw oedema at time zero
Vt = Paw oedema at time t (0.5, 1, 2, 3, 4, 5, 24 h)

$$\text{Percentage inhibition of oedema} = \frac{(\text{Vt-V}_o)\text{control-}(\text{Vt-V}_o)\text{treated groups}}{(\text{Vt-V}_o)\text{control}} \times \frac{100}{1}$$

Determination of the effect of flavonoid-rich extract of Peltophorum pterocarpum stem barkon phospholipase A_2 activity

The effect of the extract on phospholipase A_2 activity was determined using modifications of the methods of Vane [16].

Principle

Phospholipase A_2 activity was assayed using its action on erythrocyte membrane. It releases free fatty acids from the membrane phospholipids thereby causing leakage, allowing haemoglobin to flow into the medium in the process. The enzyme activity is thus directly related to the amount of haemoglobin in the medium. This was measured at 418 nm since haemoglobin absorbs maximally at this wavelength.

Enzyme preparation
Fungal enzyme preparation was obtained from *Aspergillus niger*strain culture. The nutrient broth was prepared by dissolving 15 g of Sabouraud dextrose agar in 1000 ml of distilled water, homogenized in a water bath for 10 min and dispensed into 250 ml conical flasks. The conical flasks were sealed with cotton wool and foil paper. The broth was then autoclaved at 121 °C for 15 min. The broth was allowed to cool to room temperature and then the organisms in the Petri dishes were aseptically inoculated into the broth and incubated for 72 h at room temperature. The culture was transferred into test tubes containing 3 ml phosphate buffered saline and centrifuged at 3000 rpm for 10 min. The fungal cells settled at the bottom of the test tube while the supernatant was used as the crude enzyme preparation.

Substrate preparation
Fresh human blood samples were centrifuged at 3000 rpm for 10 min and the supernatant (plasma) discarded. The red cells were washed three times with equal volume of normal saline, measured and reconstituted as a 40% (v/v) suspension with phosphate buffered saline. This served as the substrate for phospholipase A_2.

Assay procedure
$CaCl_2$ (2 mM) (0.2 ml), human red blood cell (HRBC) (0.2 ml), 0.2 ml of the crude enzyme preparation and varying concentrations of normal saline, the extract and the reference drug were incubated in test-tubes for 1 h. The control contained the human red blood cell suspension, $CaCl_2$ and free enzyme. The blanks were treated with 0.2 ml of boiled enzyme separately. The incubation reaction mixtures were centrifuged at a speed of 3000 g for 10 min. Samples of the supernatant (1.5 ml) were diluted with 10 ml of normal saline and theabsorbance of the solutions read at 418 nm. Prednisolone, a known inhibitor of phospholipase A_2, was used as the reference drug. The percentage maximum enzyme activity and percentage inhibition was calculated using the following relation:

$$5 \text{ maximum activity} = \frac{OD \text{ of test}}{OD \text{ of Control}} \times \frac{100}{1}$$

$$\% \text{ inhibition} = 100 - \% \text{ maximum activity of enzyme}$$

Determination of the effect of flavonoid-rich extract Ofpeltophorum pterocarpum stem-bark on platelet aggregation
This was achieved following a modification of the method of Born and Cross [17].

Principle
The aggregation of platelets leads to increase transmittance, therefore less absorbance of light. $CaCl_2$-induced platelet aggregation is thus shown by reduced absorbance at 520 nm. Any substance that has anti-aggregatory effect would thus lead to increased absorption by the medium.

Preparation of platelet-rich plasma (PRP)
Blood samples were taken from healthy volunteers. Fresh blood samples (5 ml) were drawn intravenously using 5 ml plastic syringe into plastic tubes containing 1% EDTA as an anticoagulant. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant was collected, diluted twice with normal saline and used as the platelet rich plasma (PRP).

Procedure
An aliquot of PRP (0.2 ml) was put into each of a set of five test tubes containing 1 ml each of varying concentrations of extract (0.1, 0.2, 0.4, 0.6 and 0.8 mg/ml dissolved in normal saline). Also, another set of two test tubes contained an aliquot (0.2 ml) of PRP and 1 ml of 0.6 mg/ml indomethacin in normal saline. The contents of the respective tubes were made up to 2.2 ml with the vehicle. A control tube contained 2 ml of the vehicle and 0.2 ml of PRP. The tubes were allowed to incubate before the induction of aggregation by the addition of 0.4 ml of 1.47% calcium chloride ($CaCl_2$) solution. The tests were performed in triplicates. Changes in the absorbance of the solutions were taken at intervals of 30 s for 2 min at 520 nm. The blanks contained the extract or Indomethacin without PRP.

Statistical analysis
The data obtained were expressed as Mean ± SD. Significant differences of the result were established by one-way and two-way ANOVA and the acceptance level of significance was $p < 0.05$ for all the results. This was done using the Statistical Package for Social Sciences (SPSS) version 22.0.

Results
Quantitative phytochemical evaluation of ethanol extract of *Peltophorum pterocarpum* stem-bark
The analysis of the phytochemical content of the stem-bark extract of *Peltophorum pterocarpum* showed that the plant contains terpenoids, flavonoids, phenols, glycosides, saponins, tannins, alkaloids, and steroids in different proportions as shown in Table 1.

Acute toxicity studies
After the phase one of the acute oral toxicity, mice in the group administered 10, 100 and 1000 mg/kg of

Table 1 Quantitative Phytochemical Composition of Ethanol Extract of *Peltophorum pterocarpum* Stem-Bark

Phytochemical Constituents	Concentrations
Tannins	641.209 ± 4.137
Flavonoids	658.436 ± 7.479
Phenols	1279.567 ± 149.868
Alkaloids	62.574 ± 1.552
Steroids	0.309 ± 0.076
Terpenoids	211.214 ± 3.647
Glycosides	7.414 ± 0.089
Saponins	0.307 ± 0.02

n = 3.

flavonoid-rich extract of *peltophorum pterocarpum* stem-bark showed no sign of mortality, so the extract was non-toxic at this phase as shown in Table 4. Phase two was conducted and mortality did not occur at 1900, 2600 and 5000 mg/kg this is represented in Table 2.

Effect of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark on egg-albumin induced rat paw oedema

Table 3 shows the effect of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark on egg albumin-induced paw oedema in rats. It shows the mean paw oedema and percentage inhibition of egg albumin-induced oedema in the rat paw which was sustained over a period of 24 h. The result showed a decrease in the mean sizes of the paw across the different time intervals for all groups. At the time intervals, 30 mins and 24 h. There were significant ($p < 0.05$) differences between the mean sizes of the paw for the different concentrations of the extracts (100, 250 and 400 mg/kg) when compared to the control and the standard. Also, there was an increase in the percentage inhibition across the time interval for the treated groups.

Table 2 Acute toxicity profile of *Peltophorum pterocarpum*

Group	Dosage of Extract (mg/kg)	Mortality Rate
Phase1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase 2		
Group 1	1900	0/3
Group 2	2600	0/3
Group 3	5000	0/3

Effect of flavonoid-rich extract of *Peltophorum pterocarpum stem bark* on phospholipase A_2 activity

Table 4 shows the effect of ethanol extract of *Peltophorum pterocarpum* stem-bark on phospholipase A_2 activity. There was a decrease in the absorbance of the sample with increasing concentration of the extract hence a decrease in enzyme activity. Prednisolone followed a similar trend with the enzyme activity decreasing with increasing concentration of prednisolone. The absorbance of varying concentrations of the extracts were significantly ($p < 0.05$) lower when compared with control, the same is also observed with increasing concentrations of the standard drug (prednisolone) when compared to the control.

Effect of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark on $CaCl_2$-induced Aggregatory response

The Table 5 shows the effect of flavonoid-rich extract of *Peltophorum pterocarpum*stem-bark on $CaCl_2$-Induced platelet aggregatory response. The different concentrations of the extract as well as Indomethacin (the standard drug) significantly ($p < 0.05$) Inhibited Platelet Aggregatory response. The different concentrations of the extracts inhibited $CaCl_2$induced platelet aggregation in a concentration and time dependent manner; as the concentration of the extract increases, the percentage inhibition degreases. The extract (0.1 mg/ml) at 30 s and 60 s exhibited the equal inhibition of 63%, the same effect was observed for extract (0.2 mg/ml) which exhibited a 62% inhibition at 0 s, 30 s, 60 s and 90 s. The highest percentage inhibition was observed under 120 s at extract concentration of 0.1 mg/ml.

Discussion

The present study was carried out to evaluate the anti-inflammatory effect of flavonoid-rich extract of *Peltophorum pterocarpum* stem-bark using egg albumin-induced paw oedema, calcium chloride-induced platelet aggregatory response and phospholipase A_2 assay. Findings from the quantitative phytochemical analysis of the crude extracts of *Peltophorum pterocarpum* stem-bark revealed the presence of Tannins, Flavonoids, Phenols, Alkaloids, Steroids, Terpenoids, Glycosides and Saponins. This observation is consistent with the observation made by Sukumaram [18–20], which reported the presence of most of these phytochemicals in the stem-bark of *Peltophorum pterocarpum*. Some of these constituents are believed to be responsible for the anti-inflammatory properties of some plants [21]. For example, flavonoids have been shown to exhibit anti-inflammatory effects [22]. Acute toxicity studies of oral doses of flavonoid-rich extract of *Peltophorum pterocarpum* stem-barkin mice revealed that it has a high safety profile, as the

Table 3 Effect of Flavonoid-rich Extract *Peltophorum Pterocarpum* Stem Bark on Egg Albumin-Induced Rat Paw Oedema

Treatments/ Groups	30 Minutes	Mean 1 Hour	(mm) and 2 Hours	Duration 3 Hours	4 Hours	5 Hours	24Hours
Control (Saline Vehicle)	4.36 ± 0.29^{Ca}	4.67 ± 0.31^{Cab}	4.99 ± 0.42^{Cbc}	5.12 ± 0.40^{Dbcd}	5.01 ± 0.26^{Dbc}	5.37 ± 0.29^{Dcd}	5.50 ± 0.26^{Dd}
Indomethacin (10 mg/kgb.w)	3.45 ± 0.16^{Ce} (20.87%)	3.35 ± 0.29^{Be} (28.27%)	3.21 ± 0.42^{Bd} (35.67%)	2.73 ± 0.20^{Cc} (46.68%)	2.45 ± 0.22^{Cbc} (51.10%)	2.31 ± 0.22^{Cb} (56.98%)	1.77 ± 0.41^{Ca} (67.82%)
Extract (100 mg/kgb.w)	3.76 ± 0.10^{Be} (13.76%)	3.60 ± 0.07^{Be} (22.91%)	3.04 ± 0.20^{Bd} (39.08%)	2.64 ± 0.30^{BCc} (48.44%)	2.46 ± 0.27^{Cbc} (50.90%)	2.32 ± 0.27^{Cb} (56.80%)	1.95 ± 0.28^{Ca} (64.55%)
Extract (250 mg/kgb.w)	3.61 ± 0.33^{Abd} (17.20%)	3.27 ± 0.22^{Ad} (29.98%)	2.77 ± 0.26^{ABc} (44.49%)	2.06 ± 0.43^{ABb} (59.77%)	1.82 ± 0.49^{Bb} (63.67%)	1.68 ± 0.44^{Bb} (68.72%)	1.20 ± 0.20^{Ba} (78.18%)
Extract (400 mg/kgb.w)	3.33 ± 0.13^{Ac} (23.62%)	3.03 ± 0.20^{Ac} (35.12%)	1.94 ± 0.56^{Ab} (61.12%)	1.51 ± 0.76^{Ab} (70.51%)	0.75 ± 0.47^{Aa} (85.03%)	0.49 ± 0.41^{Aa} (90.88%)	0.29 ± 0.29^{Aa} (94.73%)

$n = 5$ absorbances.
Results expressed as mean ± SD.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) down the column. Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) across the row.

extract was tolerated by the animals up to 5000 mg/kg. On administration of the extract, no immediate behavioural changes were noted. The mice moved about and fed normally. After 20 min, piloerection was noticed and the animals became restless, some trying to escape through the holes in the cages. The animals did not vomit, neither was there ptosis.

Some of the phytochemicals may exert their anti-inflammatory activities through decrease in the production of pro-inflammatory cytokines, pro-inflammatory lipid mediators such as prostaglandins and decrees in the proteolytic activity of leukocytes; hence, limiting damage of tissues probably through the action of flavonoids. The anti-inflammatory activity of an anti-inflammatory agent is determined by measuring its ability to reduce local oedema induced in the rat paw by injection of an irritant/ phlogistic agent [23]. The flavoinoid-rich extract at 100, 250, 400 mg/kg b.w showed a good anti-inflammatory activity as it significantly ($p < 0.05$) inhibited the increase in paw volume from 1 h to 24 h. Since these mediators cause oedema by increasing vasodilatation and vascular permeability at the site of injury, the extract therefore reduces vascular permeability and fluid exudation, probably by preventing the contraction of endothelial cells, thus, suppressing oedema. This is in an accordanc e with the findings of Nofou [19], which reported significant anti-inflammatory effect using croton oil induced-ear eodema. The anti-inflammatory activity could be due to the presence of flavonoid which is a NSAIDs-like constituents in the extract.

The flavonoid-rich extract of *P. pterocarpum* stem bark was highly effective in inhibiting phospholipase A_2 activity. The inhibition of phospholipase A_2 may be either directly or by an action of extract on the membrane. The activity of the enzyme was enhanced by calcium ion availability in the medium. Enzyme inhibitory activity may be due to interference with calcium utilization. Calcium ion is bound to the catalytic site of the enzyme and directs coordination of substrate

Table 4 Effect of flavonoid-rich extract of *Peltophorum pterocarpum stem back*on phospholipase A_2 activity

Treatment	Concentration (mg/ml)	O. D_{418nm}	Percentage enzyme activity (%)	Percentage inhibition of enzyme activity (%)
Control	–	0.418 ± 0.003^{h}	–	–
Extract	0.1	0.335 ± 0.004^{g}	80.14	19.86
	0.2	0.317 ± 0.002^{f}	75.84	26.16
	0.3	0.300 ± 0.003^{e}	71.53	28.47
	0.4	0.221 ± 0.003^{c}	52.87	47.13
	0.5	0.215 ± 0.005^{b}	51.44	48.56
(Prednisolone)	0.4	0.230 ± 0.003^{d}	55.02	44.98
(Prednisolone)	0.5	0.208 ± 0.003^{a}	49.76	50.24

$n = 3$.
Result expressed as Mean ± S.D.
Mean values with different lowercase letters as superscripts down the groups are considered significant at ($\underline{P} < 0.05$).

Table 5 Inhibition of Calcium Chloride-Induced Platelet Aggregatory Response by Flavonoid-Rich Extract of *Peltophorum pterocarpum*

Group	Concentration (ml)	Absorbance (520 nm)				
		0 s	30 s	60 s	90 s	120 s
Control	–	0.418 ± 0.002^{Fa}	0.420 ± 0.001^{Ga}	0.423 ± 0.002^{Gb}	0.428 ± 0.001^{Fc}	0.430 ± 0.001^{Gc}
Extract 1	0.1	0.261 ± 0.001^{Ea}	0.265 ± 0.002^{Fa}	0.266 ± 0.002^{Fa}	0.290 ± 0.018^{Eb}	0.307 ± 0.002^{Fc}
		(62%)	(63%)	(63%)	(68%)	(71%)
Extract 2	0.2	0.259 ± 0.002^{Ea}	0.261 ± 0.001^{Eb}	0.263 ± 0.001^{Eb}	0.264 ± 0.001^{Dc}	0.271 ± 0.001^{Ed}
		(62%)	(62%)	(62%)	(62%)	(63%)
Extract 3	0.4	0.190 ± 0.002^{Ca}	0.194 ± 0.001^{Cb}	0.197 ± 0.002^{Cb}	0.204 ± 0.003^{Cc}	0.216 ± 0.001^{Cd}
		(45%)	(46%)	(47%)	(48%)	(50%)
Extract 4	0.6	0.145 ± 0.001^{Ba}	0.150 ± 0.001^{Bb}	0.156 ± 0.001^{Bc}	0.161 ± 0.002^{Bd}	0.166 ± 0.001^{Be}
		(35%)	(36%)	(37%)	(38%)	(39%)
Extract 5	0.8	0.127 ± 0.002^{Aa}	0.130 ± 0.002^{Aa}	0.134 ± 0.001^{Ab}	0.139 ± 0.003^{Ac}	0.147 ± 0.002^{Ad}
		(30%)	(31%)	(32%)	(32%)	(34%)
Indomethacin	0.6	0.196 ± 0.003^{Da}	0.203 ± 0.002^{Bb}	0.210 ± 0.001^{Dc}	0.214 ± 0.001^{Cd}	0.220 ± 0.001^{De}
		(47%)	(48%)	(50%)	(50%)	(51%)

$n = 3$ Absorbance; Results expressed as Mean ± Standard Deviation. Mean values having Different uppercase letters as superscripts are considered significant ($P < 0.05$) down the column.
Mean value having different lowercase letters as superscripts are considered significant across the row.
() = % Inhibition of Platelet Aggregation.

carbonyl oxygen atom. Phospholipase A_2 cleaves free fatty acid from erythrocyte phospholipids. The enzyme activity assayed using its action on erythrocyte membrane, creates leakage thus causing haemoglobin to flow out into the medium. Inhibition of phospholipase A_2 by the extract could be the ability of the rich flavonoid constituent to reduce the mobilisation of free fatty acids from membrane phospholipids. It shows that phospholipase A_2 catalyses the hydrolysis of membrane glycerophospholipids to liberate arachidonic acid, a precursor of eicosanoids including prostaglandins and leukotrienes. The same reaction also produces lysophospholipids [24].

This study demonstrated that the *Peltophorum pterocarpum* flavonoid-rich extract produced a concentration and time-dependent inhibition of $CaCl_2$-induced platelet aggregatory response compared to the control. This is in accordance with the findings of Nofou [19], which recorded dose-dependent and time-dependent maximal inhibition due to the biosynthetic activity of the flavonoid-rich extracts. The ability of the extract to inhibit $CaCl_2$-induced platelet aggregatory response could probably be through the inhibition of PLA_2 and COX, which are key enzymes required for the synthesis of Thromboxane A_2. TXA_2 plays a vital role in the induction of platelet aggregation by elevating the intracellular concentration of $Ca^{2+,}$ which promotes the fusion of granules of platelets with the membrane, thereby releasing its rich contents ADP, which is also known to promote platelet aggregation. Flavonoids have been reported to play a vital role in the anticoagulant and anti-platelet aggregatory activity [25]. Additionally, the anti-platelet aggregatory activity of the extract could be through the mechanism of decreased vascular permeability and leukocyte extravasation, a process which is mediated by histamine and P-selectin (released from the platelet granules), respectively [26]. It is well known that blood platelets play a key role in pathological thrombosis with resultant conditions such as stroke, embolism, myocardial infarction, and peripheral vascular thrombosis [6–8, 27, 28], the inhibitory effect of the extract on platelet aggregation indicates its potential role as an anti-thrombotic agent and could be employed in the management of these disorders.

Conclusion

The results indicate that the extract produced significant ($p < 0.05$) anti-inflammatory activity compared to the untreated control, probably due to its high flavonoid content. The results suggest that the mechanisms of this anti-inflammatory effect may be by inhibiting phospholipase A_2 and platelet aggregation. The investigation provides empirical evidence for the use and promotion of *P. pterocarpum* stem-bark in folkloric treatment of inflammatory disorders.

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Authors' contributions
Dr. Enechi and Okeke ES conceptualized, designed and supervised the whole research work and drafting manuscript. Awoh EO and Odo KC conducted all procedures within laboratories, Okeke ES compiled data, analyzed those and wrote manuscript. Finally, Okoye CO critically reviewed manuscript for final

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Declarations

Consent for publication
We assure that this manuscript has not been submitted and published somewhere else. All named authors have approved the final version of manuscript and agreed with the submission.

Competing interests
The authors declare that they have no competing interest.

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