

EVALUATION OF THE PHYTOCHEMICAL CONSTITUENTS AND ANTI-INFLAMMATORY POTENTIAL OF *FAGARA ZANTHOXYLOIDES* ROOT-BARK USING IN VIVO AND IN VITRO MODELS

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Abstract

Fagarazanthoxyloides (Lam.) root-bark is widely used in Nigerian folk medicine to treat inflammatory disorders among other ailments. This study was conducted to evaluate the anti-inflammatory potential of the root-bark extract to confirm its folkloric claims. The phytochemical screening on the root-bark of Fagarazanthoxyloides revealed the presence of tannins, terpenoids, steroids, phenols, alkanoids, flavonoids and saponins. The acute toxicity study 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. This compares well with a standard anti-inflammatory drug indomethacin (10 mg/kg b.w) which at 5 hours inhibited egg albumin induced rat paw oedema (65.31 %). Varying doses of the extract significantly ($p < 0.05$) inhibited phospholipase A2 activity in a concentration-related manner provoking inhibition comparable to that of prednisolone, a standard anti-inflammatory drug. Similarly, the extract significantly ($p < 0.05$) inhibited CaCl₂-Induced platelet aggregation in a dose and time dependent manner. These results indicate that the extract produced good anti-inflammatory activity and the mechanism of this activity may be due to the inhibition of phospholipase A2 activity and platelet aggregation.

Key words: *Inflammation; Phospholipase A2; Platelet aggregation; prednisolone; Indomethacin.*

Introduction

Inflammation is a protective mechanism by which the body removes harmful stimuli such as pathogens, damaged cells or irritants thereby initiating the healing process in the body [1]. No matter the initiating stimulus, the classic inflammatory response is characterised by five clinical signs: calour (warmth), dolour (pain), rubour (redness), tumour (swelling) and functiolaesa (loss of function) [2]. The clinical symptoms such as fever, aches and pains associated with several diseases are directly or indirectly due to inflammatory disorders. The desired result of inflammatory response is the isolation and elimination of the injurious agents, repair of tissues damaged at the site of the injury and restoration of functions. Cell membrane damage caused by various injurious agents could lead to the activation of phospholipase A₂, which mediates the release of arachidonic acid, which is further processed by cyclo-oxygenase (COX) and lipoxygenase (LOX) to synthesize pro-inflammatory mediators [3], and other cellular mediators like histamins, bradykinin, platelet-activating factor and interleukins. 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 [4], hence the need for the development of novel anti-inflammatory drugs from natural sources as alternatives to these drugs.

Natural products such as plant extracts, either as pure compounds or as standardized extracts over the years have provided unlimited opportunities for new drug discoveries because of the unmatched availability of their chemical diversity [5, 6]. These diverse natural products from medicinal plants have been the basis of many traditional medicine systems throughout the world [7], due to their accessibility, efficacy and minimal side effect [4, 8]. The plant derived natural products such as flavonoids, sterols, polyphenols, alkaloids, tannins and terpenes have gained importance in recent years due to their wide range of pharmacological activities. Nowadays researchers are engaged in evaluation of biological activities possessed by these plant derived natural products [9]. The identification of such natural products which possess lesser side effects could

play an important role in treatment of inflammatory disorder.

Fagarazanthoxyloides (Lam.) is widely distributed in Uganda and other West African countries [10]. Fagarazanthoxyloides is well known for its varied uses in traditional medicinal practices which include elephantiasis, toothache, abdominal pain and malaria. Studies have shown that the root-bark extract of the plant possesses antihelminthic and anti-bacterial [11], hypotensive [12] and gastroprotective [13] activities. Also the radical scavenging activity of the stem extract [14] as well as the antidiabetic and hypolipidaemic effects of the leaf extract [15] have been reported. There are little or no documented reports on the use of this plant as an anti-inflammatory agent; hence this study was designed to evaluate the anti-inflammatory potential of Fagarazanthoxyloides.

Materials and Methods

Chemicals

Chemicals and reagents: all chemicals and reagents were obtained from Sigma- Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade. Indomethacin (product of Yangzhou Pharmaceutical Co. Ltd.) used as reference drug for paw oedema and anti-platelet aggregation assay and prednisolone (product of Kunimed Pharmaceutical Ltd.) used as the reference drug for the phospholipase A₂ activity assay.

Collection and Identification of Plant Material

Fresh root-barks of *F. zanthoxyloides* were collected, from Opanda-Nimbo, Uzo-uwani Local Government Area of Enugu State, Nigeria. The root-bark was authenticated by Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme (BDCCP) Research Centre, Nsukka, Enugu State, Nigeria.

Preparation and Extraction of Plant Crude Extract.

Fresh *F. zanthoxyloides* root-bark was collected and washed. The plant was cut into pieces and air

dried with regular turning to avoid decaying, until crispy. The dried root-bark was pulverized into powdered form using a mechanical grinder. A known weight of the pulverized sample (1000 g) was macerated in 3.5 liters of absolute methanol, vigorously shaken and allowed to stand for 72 hours. The mixture was filtered using Whatman No.1 filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator at 45 °C to obtain the crude methanol extract. The concentrated extract was stored in an air-tight container in a refrigerator at 4 °C until required. The percentage yield for the extract was calculated.

Phytochemical screening

The preliminary phytochemical screening was performed for identifying the phyto-constituents present in methanol extracts of root-bark of *F. zanthoxylodes* by the method of [16] to test for the presence of secondary metabolites including alkaloids, flavonoids, phenols, tannins, steroids, glycosides and saponins.

Animals

Adult Wistar albino rats (25 males) weighing between 130 g and 250 g and mice (18 males) purchased from the animal house of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were acclimatized under standard laboratory condition in the animal farm of the Department of Biochemistry for one week prior to the commencement of the experiment with a 12 hour light and dark cycle and maintained on a regular feed (commercial chicken grower's mash) and water ad libitum throughout the period.

Assay of Biological Activity

Acute toxicity and lethality study

Investigation on acute toxicity of the extract with estimation of the median lethal dose (LD₅₀) was carried out using the modified method of [17]. 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 methanol 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).

Effect of the Extract on Egg Albumin-Induced Rat Paw Oedema

This was done according to the method of [18]. 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. Wistar rats were assigned to one of 5 groups consisting of 5 animals each as follows: group 1-control (treated with 5 ml/kg of 3 % (v/v) Tween 80 in distilled water), group 2-standard (treated with Indomethacin 10 mg/kg, orally), while the tested groups (3, 4, 5) were treated with 100, 200 and 400 mg/kg bw of methanolic extracts dissolve in 3 % Tween 80 respectively.

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 and 5 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: Where, V_0 = Paw oedema at time zero

V_c = Mean paw oedema volume of control group

V_t = Paw oedema at time t (0.5, 1, 2, 3, 4, 5 h)

$\% \text{ Inhibition of oedema} = 100 \times (V_c - V_t / V_c)$.

Effect of Extract on Phospholipase A2 Activity

The effect of the extract on phospholipase A2 activity was determined using modifications of the methods of [19]. Phospholipase A2 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.

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 minutes. 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 3,000 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 A2.

Assay Procedure

CaCl₂ (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 hr. The control contained the human red blood cell suspension, CaCl₂ 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 minutes. Samples of the supernatant (1.5 ml) were diluted with 10 ml of normal saline and the absorbance of the solutions read at 418 nm. Prednisolone, a known inhibitor of phospholipase A2, was used as the reference drug. The percentage maximum enzyme activity and percentage inhibition was calculated using the following relationship:

$\% \text{ maximum enzyme activity} = (\text{O.D of test} / \text{O.D of control}) \times 100$

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

Determination of Anti-Platelet Aggregatory Activity

This was achieved following a modification of the method of [20]. The aggregation of platelets leads to increase transmittance, therefore less absorbance of light. CaCl₂-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 free from any form of drug for at least 2 weeks. 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 minutes 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 three test tubes containing 1 ml each of varying concentrations of extract (0.1, 0.2 and 0.4 mg/ml) in 3 % tween 80 (dissolved in normal saline). Also, another set of two test tubes contained an aliquot (0.2 ml) of PRP and 1 ml each of 0.2 and 0.4 mg/ml indomethacin in 3% tween 80. 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 (CaCl₂) solution. The tests were performed in triplicates. Changes in the absorbance of the solutions were taken at intervals of 2 min for 8 min at 520 nm. The blanks contained the extract or indomethacin without PRP. The percentage inhibition was calculated thus;

$$\% \text{ inhibition} = (\text{O.D of test} / \text{O.D of control}) \times 100$$

Statistical Analysis

The data obtained were expressed as Mean \pm 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

When subjected to methanolic extraction, 1000 g root-bark of *Fagarazanthoxyloides* yielded 3.36 % of greenish and slurry-like extract. This extract was used in all biological activity determinations.

In the investigation, there was no lethality or behavioural change in the three groups of mice that received 10, 100 and 1000 mg/kg of the extract. Based on this result, further increased doses of 1900, 2600 and 5000 mg/kg of the extract were administered. There was no change in behaviour or death in animals that received 1900 and 2600 mg/kg of the extract; those that received 5000 mg/kg showed weakness and drowsiness but no death was recorded within 24 h of administration.

Table 1 shows the results of the qualitative phytochemical composition of methanol extract of *Fagarazanthoxyloides* root-bark. The result shows that methanol root-bark extract of *Fagarazanthoxyloides* contain relative amounts of tannins and saponins. Terpenoids, steroids and alkaloid were present at moderate amounts while flavonoids and phenols were present in high quantity.

Table 2 shows the effect of methanol extract of *Fagarazanthoxyloides* root-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 5 hours. A significant ($p < 0.05$) reduction in the mean paw oedema was observed for all the treatment groups from 30 minutes to 5 h when compared to the control. There were no significant ($p > 0.05$) reductions in the mean paw oedema of rats in the control group at the different time intervals. The paw size of animals treated with increasing doses of the extract and indomethacin significantly decreased with time. The percentage oedema inhibition for rats treated with 100mg/kg of extract at 30min, 1h, 2h, 3h, 4h, and 5h were 12.00 %, 27.27 %, 36.95 %, 44.68 %, 50.00 % and 57.14 % respectively. The percentage oedema inhibition for rats treated with 200mg/kg of extract after 30 min, 1 h, 2 h, 3 h, 4 h and 5 h were 22.00 %, 34.09 %, 39.13 %, 46.81%, 52.08 % and 59.18 % respectively. The percentage oedema inhibition of the group treated with 400mg/kg of extract was higher than that of indomethacin at 5 hours period. The inhibitory effect of the extract was dose dependent with the group treated with 400 mg/kg showing the highest oedema inhibition over a 5 hours period.

Table 3 shows the effect of methanol extract of *Fagarazanthoxyloides* root-bark on phospholipase A₂ activity. The methanol extract of *Fagarazanthoxyloides* root-bark significantly ($p < 0.05$) inhibited the activity of PLA₂ in a dose-dependent manner when compared to the control. This is shown by the reduced absorbances of the supernatant solution. There was a decrease in the absorbance of the sample with increasing concentration of the extract hence a decrease in enzyme activity. The percentage inhibition for extracts (1 ml, 1.2 ml, 1.4 ml, 1.6 ml and 1.8 ml) were 58.47 %, 63.71 %, 65.32 %, 72.18 % and 76.20% respectively. Prednisolone, a standard anti-inflammatory drug followed a similar trend with the enzyme activity decreasing with increasing doses of prednisolone. The absorbance of extracts 1ml, 1.2ml, 1.4ml, 1.6ml and 1.8ml were significantly ($p < 0.05$) lower when compared with control, the same was also observed with increasing concentrations of the

standard drug (prednisolone) when compared to the control.

Table 4 shows the effect of methanol extract of *Fagarazanthoxyloides* root-bark on CaCl₂-induced platelet aggregatory response. The extract, like indomethacin, significantly ($p < 0.05$) inhibited platelet aggregatory response. The different concentrations of the extract inhibited CaCl₂-induced platelet aggregation in a concentration-dependent manner. The maximum platelet aggregatory activity was attained at the 6th min. The inhibition of platelet by the extract was similar to that of indomethacin. For example, 0.1 mg/ml of the extract gave a percentage inhibition of 67.0 %, 67.0 %, 68.0 %, 69.0 % at different time interval (2, 4, 6 and 8 min.). As the concentrations of the extract increases, from 0.1 to 0.4 mg/ml, it inhibited the capacity of the CaCl₂ which induce aggregation of human platelets (i.e platelet aggregation decreases).

Discussion

The present study was carried out to investigate the anti-inflammatory effect and mechanisms of action of methanol extract of *Fagarazanthoxyloides* root-bark using in vivo (egg albumin-induced rat paw oedema) and in vitro (inhibition of phospholipase A₂ and platelet aggregation) approaches. The acute toxicity studies of oral doses of methanol extract of *F. zanthoxyloides* root-bark in mice showed revealed that it has a high safety profile, as the extract was tolerated by the animals up to 5000 mg/kg.

A host of traditional drug systems can be a source of a variety of new drugs which can provide relief in inflammation and inflammatory disorders. The possession of anti-inflammatory properties by the extract would depend on its ability to affect patho-physiological changes accompanying inflammatory diseases. The methanol root-bark extract at 100, 200 and 400 mg/kg body weight showed a good anti-inflammatory activity as it significantly ($p < 0.05$) inhibited the increase in paw volume from 0.5 to 5 hour. This shows that the extract inhibited all the phases of the inflammatory response. The inhibition of the early phase of oedema exhibited by the methanol extract in this study suggests that it blocks the release of

histamine and serotonin. The suppression of oedema in the second and third phase of inflammation suggests that the anti-inflammatory activity of the extract may also be due to the suppression of kinin and prostaglandin formation induced by egg albumin within this period. 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. The anti-inflammatory activity could be due to the presence of NSAIDs-like constituents present in the extract of *F. zanthoxyloides*.

Consistent with this finding is the inhibitory effect of the extract on phospholipase A₂ activity an acyl-hydrolase. Phospholipase A₂ an enzyme that cleaves free fatty acids from phospholipids of the biological membrane, releasing arachidonic acid which is acted upon by COX and LOX leading to the de novo synthesis of lipid mediators [21]. The action of COX on arachidonic acid produces mediators such as PGE₂, PGD₂, PGI₂ and TXA₂, while the action of 5-LOX on arachidonic acid releases leukotrienes such as LTB₄. The methanol extract of *F. zanthoxyloides* root-bark from 1.0 to 1.8 ml exhibited a significant ($p < 0.05$) and concentration-dependent inhibition of PLA₂ activity. This inhibition of PLA₂ by the extract implies that it was probably able to suppress the release of free fatty acids from RBC membrane phospholipids and the consequent deprivation of COX and LOX substrates for the synthesis of inflammatory mediators, hence limiting their effects such as vasodilatation, vascular permeability, chemotaxis and pain, thus preventing inflammation. This inhibition of PLA₂ also shows that the extract has potentials for preventing atherosclerosis and cancer as PLA₂ has been implicated in their aetiology [22, 23]. The mechanism of inhibition of PLA₂ by the extract could be like similar to that of corticosteroids which induce lipocortin [24]. This effect could also be attributed to the presence of flavonoids in the extract as many studies have shown that flavonoids inhibit PLA₂ [25-27]. Tannins have also been found to inhibit PLA₂ activity [28].

The result of this study showed that that the methanol extract produced significant

concentration and time-dependent inhibition of CaCl₂-induced platelet aggregatory response compared to control. Inhibition of CaCl₂-induced platelet aggregatory response could probably be due the extract's ability to inhibit PLA₂ and COX which are required for the synthesis of TXA₂. The inhibition of TXA₂ is important because it induces aggregation of platelet by increasing intracellular Ca₂₊ which promotes fusion of platelet granules with the membrane, releasing its contents such as ADP which also promotes platelet aggregation. The inhibition of platelet aggregation by the extract could also imply decreased vascular permeability and leukocyte extravasation which is mediated respectively by histamine and P-selectin which are normally released from the granules of the platelet [29]. Inhibition of platelet aggregation by the extract is thus indicative of its possible role as an antithrombotic agent and could be useful in the management of the above named disorders. This demonstrated effect of the extract could be due to the presence of phenolic compounds and flavonoids since the anticoagulant and anti-platelet aggregatory activity of these substances have been reported [30, 31]. The beneficial effects of antioxidants on the inhibition of platelet activation and aggregation have also been reported [32]. This confirms the involvement of flavonoids in the inhibition of platelet aggregation by the extract, since flavonoids are anti-oxidants [27]. This effect could also be attributed to the presence of tannins, as the anticoagulant or anti-platelet aggregation activity of tannins have also been demonstrated [33-35; 26]. Mosa et al. [36] also demonstrated the reduced anti-platelet aggregatory activity of some Zulu medicinal plants induced with each of thrombin, ADP and epinephrine due to the removal of tannins from the crude extracts, thus, confirming the involvement of tannins in the anti-aggregatory effect of the extract.

Conclusion

The present investigation revealed that the methanol root-bark extract of Fagarazanthoxyloides produced significant anti-inflammatory activity. The results suggest that the mechanisms of this anti-inflammatory effect may be by inhibiting phospholipase A₂ activity and platelet aggregation.

The investigation provided an empirical evidence for the use of Fagarazanthoxyloides root-bark extract in folkloric treatment of inflammatory disorders. These findings suggest that Fagarazanthoxyloides could serve as a potential new source of natural drug with anti-inflammatory activities.

Recommendation

I recommend that further works could be carried out in the following areas:

- Safety use of different parts of the plant
- Whether the extracts may inhibit both isoforms of COX (COX-1 and COX-2) or if it is a COX selective herb.
- The capacity of the extract to inhibit 5-LOX
- Isolation, characterization and structure elucidation of the biologically active principles.

Conflicts of interest

The authors declare no conflicts of interest.

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Table 1: Phytochemical constituents of methanol Extract of *FagaraZanthoxyloides*Root-bark.

Phytochemical Constituents	Qualitative remarks
Flavonoids	+++
Phenols	+++
Terpenoids	++
Steroids	++
Alkaloids	++
Tannins	+
Saponins	+

Key

+ Present

++ Moderately present

+++ Highly present

Table 2: Root-Bark on Egg Albumin-Induced Rat Paw Oedema

TREATMENT GROUP	Mean Oedema (cm) and Duration					
	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Control (saline vehicle)	0.41±0.02 ^{ba}	0.44±0.01 ^{bb}	0.46±0.01 ^{bBC}	0.47±0.01 ^{bCD}	0.48±0.01 ^{Bde}	0.49±0.01 ^{CE}
Indomethacin (10mg/kg b. w)	0.34±0.04 ^{ad} (17.07%)	0.31±0.06 ^{aCD} (29.55%)	0.26±0.03 ^{aBC} (43.48%)	0.23±0.06 ^{aAB} (51.06%)	0.21±0.05 ^{aAB} (56.25%)	0.17±0.02 ^{aA} (65.31%)
Extract (100mg/kg b. w)	0.36±0.03 ^{abd} (12.20%)	0.32±0.04 ^{aCD} (27.27%)	0.29±0.04 ^{aBC} (36.95%)	0.26±0.04 ^{aAB} (44.68%)	0.24±0.04 ^{aAB} (50.00%)	0.21±0.03 ^{ba} (57.14%)
Extract (200mg/kg b. w)	0.32±0.03 ^{ae} (22.00%)	0.29±0.02 ^{aDE} (34.09%)	0.28±0.02 ^{aCD} (39.13%)	0.25±0.03 ^{aBC} (46.81%)	0.23±0.03 ^{aAB} (52.08%)	0.20±0.02 ^{ba} (59.18%)
Extract (400mg/kg b. w)	0.33±0.04 ^{ad} (20.0%)	0.30±0.05 ^{aCD} (31.82%)	0.25±0.06 ^{aBC} (45.65%)	0.22±0.05 ^{aB} (53.19%)	0.19±0.05 ^{aAB} (60.42%)	0.14±0.01 ^{aA} (71.43%)

Results expressed as Mean ± SD n = 5

Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across the row.

() Represents percentage inhibition of paw oedema calculated relative to control.

Table 3: Effect of Methanol Extract of *Fagara zanthoxyloides* Root-Bark on Phospholipase A₂ Activity

Treatment	Extract/Drug volume (ml)	Normal saline (ml)	HRBC (ml)	Crude enzyme (ml)	CaCl ₂ (ml)	Absorbance(418nm)	% Maximum Enzyme Activity	% Inhibition
Control	--	2.0	0.2	0.2	0.2	0.248±0.023 ^f		
Extract	1.0	1.0	0.2	0.2	0.2	0.103±0.013 ^{cd}	41.53	58.47
	1.2	0.8	0.2	0.2	0.2	0.090±0.001 ^{bc}	36.29	63.71
	1.4	0.6	0.2	0.2	0.2	0.086±0.003 ^{bc}	34.68	65.32
	1.6	0.4	0.2	0.2	0.2	0.069±0.004 ^a	27.82	72.18
	1.8	0.2	0.2	0.2	0.2	0.059±0.011 ^a	23.80	76.20
Drug (Prednisolone)	1.0	1.0	0.2	0.2	0.2	0.135±0.005 ^f	54.44	45.56
	1.2	0.8	0.2	0.2	0.2	0.122±0.004 ^{ef}	49.19	50.81
	1.4	0.6	0.2	0.2	0.2	0.110±0.001 ^{bc}	44.35	55.68
	1.6	0.4	0.2	0.2	0.2	0.092±0.004 ^{bc}	37.10	62.90
	1.8	0.2	0.2	0.2	0.2	0.075±0.006 ^{bc}	30.24	69.76

Results expressed as Mean ± SD. n = 3. Mean values having different lowercase letters as superscripts are considered significant (p < 0.05) down the column

Table 4: Effect of Methanol extract of *Fagara zanthoxyloides* Root-Bark on Calcium Chloride-Induced Platelet Aggregation.

Treatments (mg/ml)	Concentrations		Δ Absorbance (520nm)		
	2MINS	4MINS	6MINS	8MINS	
Control	-	0.154±0.001 ^{eA}	0.157±0.001 ^{eA}	0.158±0.003 ^{bE}	0.163±0.003 ^{eC}
Extract	0.1	0.103±0.002 ^{dA} (67.0%)	0.105±0.005 ^{dA} (67.0%)	0.107±0.002 ^{cAB} (68.0%)	0.112±0.002 ^{bB} (69.0%)
		0.089±0.003 ^{bA} (58.0%)	0.093±0.001 ^{bB} (59.0%)	0.097±0.001 ^{bc} (61.0%)	0.109±0.003 ^{bD} (67.0%)
		0.074±0.003 ^{aA} (48.0%)	0.083±0.003 ^{aB} (53.0%)	0.087±0.003 ^{aB} (55.0%)	0.097±0.004 ^{aC} (60.0%)
Indomethacin	0.2	0.102±0.002 ^{dA} (66.0%)	0.107±0.001 ^{dAB} (68.0%)	0.112±0.003 ^{dB} (71.0%)	0.119±0.006 ^{cdC} (73.0%)
		0.097±0.005 ^{cA} (63.0%)	0.100±0.003 ^{cA} (64.0%)	0.109±0.001 ^{cdB} (69.0%)	0.114±0.001 ^{bcB} (70.0%)

Results expressed as Mean ± SD n = 3 absorbances

Mean values having different lowercase letters as superscripts are considered significant (p < 0.05) down the column.

Mean values having different uppercase letters as superscripts are considered significant (p < 0.05) across the row.

() = % Inhibition of Platelet aggregation.