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Therapeutic Evaluation of the Potential Mechanisms of Anti-Inflammatory Activities of *Fagara zanthoxyloides Lam.* Leave Extract in Wistar Rats

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ABSTRACT
Fagara zanthoxyloides is a medicinal plant used in traditional medicine for the treatment of elephantiasis, toothache, sexual impotence, malaria, dysmenorrheal and abdominal pain among rural dwellers. The aim of the present study was to evaluate the *in vivo* and *in vitro* anti-inflammatory effect of the methanol extracts of the leaves of *Fagara zanthoxyloides* to validate its use in folklore medicine. The analysis of the phytochemical content of the leaf extract of *Fagara zanthoxliodes* revealed the presence of different secondary metabolites (tannins, saponin, terpenoids, steroids, flavonoids, alkaloids, phenols and glycoside) in varying proportions. In the systemic oedema of the rat paw, scalar doses (50, 100 and 250 mg/kg b.w) of the extract significantly ($p < 0.05$) suppressed the development of paw oedema induced by egg albumin at different time intervals (0.5 hr- 5 hrs). This compares well with a standard anti–inflammatory drug indomethacin (10 mg/kg b.w). Varying doses of the extract (1.0, 1.2, 1.4, 1.6 and 1.8 ml) significantly ($p< 0.05$) inhibited phospholipase A_2 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 $CaCl_2$-Induced platelet aggregation in a dose and time dependent manner when compared to the control and the standard drug (indomethacin). The extract also significantly ($p< 0.05$) inhibited hypotonicity induced membrane stabilization when compared to that control and standard drug (Indomethacin). These results indicate that the extract produced potential anti-inflammatory activity which could be as a result of the rich phytochemical constituents.

Keywords: Inflammation, Membrane stabilization, Paw oedema, Phospholipase A2, Platelet aggregation.

Introduction

Inflammation is a protective mechanism by which the body eliminates stimuli like pathogens, damage cells or irritants thus initiating the body's healing process.[1] Inflammation has been implicated in several health conditions. The clinical symptoms such as fever, aches and pains associated with several diseases are directly or indirectly due to inflammatory disorders.[2] Non-steroidal anti-inflammatory agents (NSAIDs) and corticosteroids are the major classes of anti-inflammatory agents but their toxic adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome have limited their use.[2,3] Hence, medicinal plants could provide an excellent fountainhead to develop new anti-inflammatory agents, which could be more efficacious, safer, affordable, and accessible for patients.

Fagara zanthoxyloides Lam. Lam. (family Rutaceae) is a West African tree used in traditional medicine. The root-bark extract is used in treating elephantiasis, toothache, sexual impotence, gonorrhea, malaria, dysmenorrheal and abdominal pain.[4] In West Africa, the root of *Fagara zanthoxyloides* Lam. is commonly used indigenously for tooth cleaning as chewing stick and it has been shown that the root extract exhibited a very high antimicrobial activity against oral flora.[5] The anti-pyretic, analgesic[6] and anti-inflammatory activities[2] of the root-bark extract of the plant have been reported. The study evaluated the *in vivo* and *in vitro* anti-inflammatory activity of the methanol extract of *Fagara zanthoxyloide* leaves in Wistar rats.

Materials and Methods

Plant materials

Fresh leaves of *F. zanthoxyloides* were collected from Opanda-Nimbo in Uzo-uwani Local Government Area of Enugu State, Nigeria in June 2018. The plant was authenticated by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug production (InterCEED), Nsukka Nigeria. Voucher specimen of the plant with No. INTERCEED/059 was deposited at the InterCEED Herbarium. The leaves were identified and authenticated by a taxonomist. The fresh leaves were washed to remove dirt. The leaves were then shade-dried The dried leaves were pulverized into powdered form using a mechanical grinder. The pulverized leaves (1.5 kg) were macerated in 3.5 L absolute methanol using a maceration flask. The mixture was left for 72 h with occasional stirring, after which it was filtered into a flat-bottom 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 methanol extract. The concentrated extract was stored in a labelled sterile screw-capped bottle in the refrigerator until use for the study.

Chemicals and reagents

Chemicals were all of analytical grade and products of May and Baker England, British Drug House (BDH) England, Fluka Germany, Burgoyne, India, Harkin and Williams, England and Sigma Aldrich.

Animals

Adult male Wistar rats (120 -130 g) were purchased from the animal house of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The animals were grouped into five groups, each containing five rats. The animals were acclimatized for one week prior to the commencement of experiment with a 12 h light and dark cycle and maintained on a regular feed (commercial chicken grower's mash) and water *ad labium* at the department animal house. The experimental protocols were 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).

Ethical clearance

Clearance and approval (with approval number UN/ZEB/2125) for conducive experimental conditions and humane use and handling of laboratory animals were given by the ethical committee of the Department of Zoology and Environmental Biology, University of Nigeria Nsukka.

Quantitative phytochemical screening

The preliminary phytochemical screening of the methanol leaves extract of *F. zanthoxyloides* was carried out to ascertain the presence of some plant secondary metabolites and thus those detected were further quantified. Both determinations were done by utilizing standard conventional protocols.[7]

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on egg-albumin induced paw oedema

This was done according to the method of Winter *et al.*[8] 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. The animals were fasted for 18 h to prior the experiment. The animals received normal saline, Indomethacin (10 mg/kg, orally), or extract (50, 100 and 250 mg/kg i.p) respectively which are within the lethal dose. Inflammation was induced by subplantar injection of the right hind paw of the animals. The right hind paw size of the rats at time zero (before the induction of oedema) were measured using a vernier calliper. One hour after administration of the test substances, acute inflammation was induced by injecting 0.1 mL of freshly prepared egg albumin into the subplantar area of the right hind paw of the 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:

% Inhibition of oedema = 100 X (Vc-Vt/Vc)

Where; V_c = Mean paw oedema volume of control group

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

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on Phospholipase A_2 activity

The effect of the extract on phospholipase A_2 activity was determined using modifications of the methods of Vane.[9] 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.

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 121ºC 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 (healthy volunteer who has not taken any form of NSAID for at least two weeks- from whom informed consent was obtained) 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 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 (prednisolone) 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 3000x g for 10 min. Samples of the supernatant (1.5 mL) were diluted with 10 mL of normal saline and the absorbance of the solutions was 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 were calculated using the following relationship:

% Maximun enzyme activity = $OD_{Test}/OD_{Control}$ X 100

% Inhibition = 100 - % maximum enzyme activity

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on Platelet aggregation

The modified method of Born and Cross[10] was used to determine the anti-platelet activity. Fresh blood samples (5 mL) from healthy volunteer (from whom informed consent was obtained) who has not taken any form of NSAID for at least two weeks 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). Changes in absorption of the platelet rich plasma (PRP) were determined. PRP (0.2 mL), 0.4 mL of 2M $CaCl_2$, varying concentrations of normal saline (1.5, 1.4, 1.2, 1.0 mL) and extract (0.1, 0.2, 0.4, 0.6 mg/mL) were incubated at room temperature for 2 min. The absorbances of the solutions were measured at 520 nm. Changes in absorption at 520 nm were taken at intervals of 2 min for 8 min. Indomethacin was used as the reference drug.

% Inhibition = $OD_{Test}/OD_{Control}$ X 100

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on membrane stabilization (Hypotonicity-induced Haemolysis of Red Blood Cells)

The effect of extract on hypotonicity was investigated using the method of Shinde *et al.*[11] Fresh blood sample (5 mL) was collected from healthy volunteers into plastic tubes containing 0.01 mL of 1% EDTA to prevent coagulation. These tubes were centrifuged at 3000 rpm for 15 min. The supernatant was collected and discarded. Twice the volume of normal saline equivalent to the volume of the supernatant was used to redissolve the red blood cell pellet for use, sixteen test tubes were used. Eight for the main test and the other eight as the blanks for each test tube. The test tube

1 contained 0.1 mL of blood and 2.4 mL of normal saline. Test tube 2 contained 0.1 mL of blood, 1.9 mL of normal saline and 0.5 mL of distilled water. Test tube 3-6 contained the same volume of blood and distilled water but varying volumes of extract and normal saline. Test tubes 7 and 8 contained the same volume of blood and distilled water but varying volumes of indomethacin and normal saline. The volume of each test tube was 2.5 mL. Subsequently, the test tubes were incubated for 1 h at 37°C in a water bath. After the incubation, the test tubes were centrifuged at 3000 rpm for 20 min to terminate the reaction. The absorbance of the supernatants collected was read at 540 nm. These experiments were done in triplicates and mean absorbance value taken. The percentage inhibition of haemolysis was evaluated using the following relation:

$$\%\ \text{inhibiton of haemolysis} = 100\left(1 - \frac{\text{OD2} - \text{OD1}}{\text{OD3} - \text{OD1}}\right)$$

Where; OD1 = Absorbance of control I (isotonic solution)
OD2 = Absorbance of test sample
OD3 = Absorbance of control II (hypotonic solution)

Statistical analysis
The data obtained were analyzed using both one and two-way analysis of variance (ANOVA) in Statistical product and Service Solution (SPSS) version 22.0 and presented as Mean ± SD. Mean values with $p < 0.05$ of the result was accepted significant.

Results and Discussion

Quantitative phytochemical evaluation of crude extract of Fagara zanthoxyloides
Table 1 shows the results of the quantitative phytochemical constituents of the methanol extract of *Fagara zanthoxyloides*. The results revealed the presence of tannins, saponins, terpenoids, steroids, flavonoids, alkaloids, phenols and glycosides in different proportions. The phytochemical constituents in the methanol extract of *F. zanthoxyloides* leaves could be responsible for its anti-inflammatory properties.

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on egg-albumin induced paw oedema
The result of the effect of methanol extract of *F.zanthoxyloides* leaves on egg-albumin induced paw oedema showed a decrease in the mean sizes of the paw across the different time intervals for all groups (Table 2). At the time intervals, 0.5 - 5 h, there were significant ($p < 0.05$) differences between the mean sizes of the paw for the different concentrations of the extracts (50, 100, 250 mg/kg) when compared to the control. Also, there was an increase in the percentage inhibition across the time interval for the treated groups. The egg-albumin-induced paw oedema model was used to screen the *in vivo* anti-inflammatory activity of this plant. The resultant swelling of the paw after the administration of the egg-albumin is due to oedema formation. The formation of oedema arises from the action of inflammatory mediators such as histamine, serotonin and bradykinin which work in synergy at the site of a local inflammatory insult[12] leading to increased permeability of vessels and blood flow. The early phase of oedema which starts immediately after the administration of the irritant and lasting up to 2 h is probably due to the release of histamine, 5-hydroxyltryptamine, kinins and serotonin, while the later phase which is from 3 h to 5 h after administration of the irritant is induced by bradykinin, protease, prostaglandins and lysosome.[12,13] Sub-plantar injection of egg-albumin into the paw produces oedema resulting from extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation.[14] The extract reduced the mean sizes of the egg-albumin induced paw oedema for all groups at the different time intervals and the percentage inhibition of the different treatment groups increased with increasing time intervals. The reduction of the oedema in the first phase by the extracts at high dose, suggests that it contains active constituents which inhibit the release or action of the early phase mediators, thereby reducing vascular permeability, fluid exudation and thus, suppressing oedema. Suppression of oedema in the second phase of inflammation suggests that the anti-inflammatory activity of the leaf extracts may also be due to the inhibition of phlogistic mediators such as prostaglandins, antagonizing their interaction with their respective receptors or it may be due to general mechanism like increasing the membrane stability of the cell or suppression of kinin formation induced by the egg-albumin within this period.

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on phospholipase A_2 activity

Table 3 shows the effect of methanol extract of *Fagara zanthoxyloides Lam.* leaves on phospholipase A_2 activity. There was a significant decrease ($p < 0.05$) in the absorbance of the sample with increasing concentration of the extract hence a decrease in enzyme activity when compared to the control. Prednisolone followed a similar trend (with the extract) of enzyme activity decreasing with increasing concentration of prednisolone. The absorbances of varying concentrations of the extracts (1.0, 1.2, 1.4, 1.6, 1.8 mg/ml) were significantly ($p< 0.05$) lower when compared to the control, similar effects were observed with increasing concentrations of prednisolone when compared to the control. The methanol leaf extract of *F. zanthoxyloides* was highly effective in inhibiting phospholipase A_2 activity. 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 carbonyl oxygen atom.[15] 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 implies that the methanol extract of *F. zanthoxyloides* leaves may suppress the mobilisation of free fatty acids from membrane phospholipids. It has been reported that anti-inflammatory and immunosuppressive steroids inhibit arachidonic acid and its metabolites.[16] The results were similar to the findings reported by Okeke *et al*[2] on the root-bark extract of the plant.

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on Platelet aggregation

The result of the effect of methanol extract of *Fagara zanthoxyloides Lam.* leaves on platelet aggregation (Table 4) showed an increase in the absorbance values of the different concentrations of the extracts compared to the standard control across the different time intervals. The extract at 0.1 mg/mL gave a percentage inhibition of 75% at the 8th minute when compared with the standard controls (0.4, 0.6 mg/mL) which gave 61% and 57% at the 8th minutes, respectively. The role of platelets in the inflammatory processes is being increasingly recognized, in addition to their function in haemostasis and thrombosis.[17]

Platelets accumulate in inflammatory sites concomitantly with leukocytes[18] and regulate a variety of inflammatory response by secreting or activating adhesion proteins, growth factors, chemokines, cytokine-like factors and coagulation factors. These proteins induce widely differing biological activities, including cell adhesion chemotaxis, cell survival, and proliferation, all of which accelerate inflammatory process.[19] Maximum platelet aggregation was attained at the 6th minute. The percentage inhibition of platelet aggregation increases with increase in time. This shows that the inhibition of platelet aggregation by the extract is concentration-dependent and is consistent with the findings of Okeke *et al*.[2] on the root-bark extract of the plant.

Effect of methanol extract of Fagara zanthoxyloides Lam. leaves on hypotonicity-induced haemolysis of red blood Cells

The extract at different concentrations protected significantly ($P < 0.05$) the erythrocyte membrane against lysis induced by hypotonic solution when compared to the control (Table 5). As the concentration of extract increased the percentage inhibition increased simultaneously. Reduction in the absorbance reading of the different concentrations of the extracts was indicative of the effect of the membrane stabilizing properties of the extracts. The methanol extract of *F. zanthoxyloides* leaves were found to exhibit high membrane stabilization effect against hypotonicity-induced haemolysis of the red cells as is shown by the level of inhibition of haemolysis. Protection against hypotonicity-induced haemolysis is related to membrane stabilization which is an anti-inflammatory index.[20] The inhibition of haemolysis was found to be dose-dependent and was comparable to that of indomethacin. Hypotonicity-induced haemolysis of human red blood cells (HRBC) occurs due to water uptake by the cells and leads to the release of haemoglobin which absorbs maximally at 540 nm. Hence, the reduced optical density at 540 nm obtained for the various *F. zanthoxyloides* test samples was a reflection of the stabilization of the red cell membrane caused by the extract.

Conclusion

It can be concluded that the methanol extract of the leaves of *F. zanthoxyloides* possess anti-inflammatory activity thus validating the ethnopharmacological claims. This anti-inflammatory activity could be due to the inhibition of histamine, serotonin and bradykinin, phlogistic mediators such as prostaglandins, inhibition of phospholipase A_2 activity, the extract's ability to inhibit platelet aggregation and protection against hypotonicity-induced haemolysis which is an anti-inflammatory index. This knowledge could hence be tapped to formulate new agents that will be easily affordable with reduced side effects for the treatment of inflammatory ailments as against chemically synthetic drugs used as anti-inflammatory agents with side effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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Table 1: Quantitative Phytochemical Constituent of *Fagara zanthoxyloides Lam.* Extract

Phytochemical constituent (%)	Amount
Tannins	13.510 ± 1.085
Saponin	0.653 ± 0.110
Terpenoid	905.298 ± 10.144
Steroids	0.713 ± 0.153
Flavonoids	1592.593 ± 8.900
Alkaloids	156.097 ± 4.531
Phenols	699.140 ± 2.612
Glycosides	128.223 ± 0.936

Table 2: Effect of methanol extract of *Fagara zanthoxyloides Lam.* leaves on egg-albumin induced paw oedema

Treatment Group	Mean Paw Size (cm) and Duration					
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control (normal saline)	3.308 ± 0.556^{aA}	3.713 ± 0.707^{aB}	4.355 ± 1.021^{abB}	5.055 ± 0.526^{bB}	5.450 ± 0.933^{bB}	5.580 ± 0.828^{bB}
Indomethacin	3.083 ± 0.611^{dA}	2.550 ± 0.274^{cdA}	2.393 ± 0.313^{bcA}	2.113 ± 0.332^{abcA}	1.845 ± 0.337^{abA}	1.753 ± 0.331^{aA}
(10 mg/kg b. w)	◊6.80	◊31.32	◊45.05	◊58.29	◊66.15	◊68.58
Extract	2.573 ± 0.228^{bA}	2.328 ± 0.517^{abA}	2.270 ± 0.469^{abA}	1.975 ± 0.512^{abA}	1.890 ± 0.464^{abA}	1.798 ± 0.480^{aA}
(50 mg/kg b. w)	◊22.22	◊37.30	◊47.88	◊60.93	◊65.32	◊67.78
Extract	3.105 ± 0.236^{cA}	2.428 ± 0.706^{bA}	1.880 ± 0.545^{abA}	1.568 ± 0.443^{aA}	1.398 ± 0.289^{aA}	1.290 ± 0.327^{aA}
(100 mg/kg b. w)	◊6.14	◊34.61	◊56.83	◊68.98	◊74.35	◊76.88
Extract	2.903 ± 0.651^{bA}	2.390 ± 0.962^{abA}	1.983 ± 0.703^{abA}	1.713 ± 0.608^{aA}	1.523 ± 0.677^{aA}	1.345 ± 0.671^{aA}
(250 mg/kg b. w)	◊12.24	◊35.63	◊54.46	◊66.11	◊72.06	◊75.89

Values are expressed as Mean ± SD, n = 5. ◊Represents percentage inhibition of paw oedema calculated relative to control.
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.

Table 3: Effect of Methanol Extract of *Fagara zanthoxyloides Lam.* Leaf on Phospholipase A_2 Activity

Treatment	Extract/Drug (mg/mL)	Normal saline (mL)	HRBC (mL)	CE (mL)	$CaCl_2$ (mL)	Absorbance (418 nm)	% Maximum Enzyme Activity	% Inhibition
Control	--	2.0	0.2	0.2	0.2	1.788 ± 0.002^{i}		

Extract	1.0	1.0	0.2	0.2	0.2	1.671 ± 0.001^{h}	93.46	6.54
	1.2	0.8	0.2	0.2	0.2	1.602 ± 0.003^{g}	89.59	10.41
	1.4	0.6	0.2	0.2	0.2	1.534 ± 0.002^{f}	85.83	14.17
	1.6	0.4	0.2	0.2	0.2	1.469 ± 0.003^{d}	82.11	17.89
	1.8	0.2	0.2	0.2	0.2	1.300 ± 0.001^{b}	72.71	27.29
Prednisolone	1.0	1.0	0.2	0.2	0.2	1.671 ± 0.001^{h}	93.46	6.54
	1.4	0.6	0.2	0.2	0.2	1.501 ± 0.005^{e}	83.95	16.05
	1.6	0.4	0.2	0.2	0.2	1.380 ± 0.025^{c}	77.19	22.81

Values are expressed as Mean ± SD, n = 3.
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column
CE: Crude enzyme, HRBC: Human red blood cells

Table 4: Effect of Extract of *Fagara Zanthoxyloide* on Platelet Aggregation

Group	Extract (mg/mL)	Drug (mg/mL)	N.S (mL)	PRP (mL)	$CaCl_2$	Absorbance (520 nm)			
						2 min	4 min	6 min	8 min
Control	-	-	1.6	0.2	0.2	0.552 ± 0.001^{Af}	0.555 ± 0.001^{Bf}	0.557 ± 0.001^{Cg}	0.559 ± 0.001^{Cg}
Extract	0.1	-	1.5	0.2	0.2	0.372 ± 0.003^{Ae}	0.377 ± 0.001^{Be}	0.399 ± 0.001^{Cf}	0.421 ± 0.002^{Df}
						(67%)	(68%)	(72%)	(75%)
	0.2	-	1.4	0.2	0.2	0.360 ± 0.001^{Ad}	0.372 ± 0.003^{Bd}	0.393 ± 0.001^{Ce}	0.400 ± 0.001^{De}
						(65%)	(67%)	(71%)	(72%)
	0.4	-	1.2	0.2	0.2	0.340 ± 0.000^{Ac}	0.357 ± 0.003^{Bc}	0.359 ± 0.003^{Bd}	0.367 ± 0.002^{Cd}
						(62%)	(64%)	(64%)	(67%)
	0.6	-	1.0	0.2	0.2	0.321 ± 0.004^{Ab}	0.332 ± 0.001^{Bb}	0.343 ± 0.003^{Cc}	0.355 ± 0.003^{Dc}
						(58%)	(60%)	(62%)	(64%)
Indomethacin	-	0.4	1.4	0.2	0.2	0.327 ± 0.001^{Ab}	0.330 ± 0.000^{Bb}	0.337 ± 0.002^{Cb}	0.341 ± 0.001^{Db}
						(59%)	(60%)	(61%)	(61%)
	-	0.6	1.6	0.2	0.2	0.296 ± 0.008^{Aa}	0.307 ± 0.002^{Ba}	0.316 ± 0.001^{Ca}	0.319 ± 0.002^{Ca}

Values are expressed as Mean ± S.D, n = 3. **( )** = % Inhibition.
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the row
PRP = Platelet-Rich Plasma, N.S = Normal Saline

Table 5: Effect of the extract of *Fagara zanthoxyloide* on hypotonicity-induced haemolysis in RBC

Test tube	HRBC (ml)	Normal Saline (mL)	Distilled water (mL)	Extract (mg/mL)	Indomethacin (mg/mL)	OD at 540 nm	Inhibition (%)
1	0.1	2.4	-	-	-	0.261 ± 0.009^{d}	-
2	0.1	1.9	0.5	-	-	0.460 ± 0.001^{g}	-
3	0.1	1.8	0.5	0.1	-	0.385 ± 0.002^{f}	16
4	0.1	1.7	0.5	0.2	-	0.333 ± 0.003^{e}	28
5	0.1	1.5	0.5	0.4	-	0.225 ± 0.005^{b}	51
6	0.1	1.3	0.5	0.6	-	0.206 ± 0.006^{c}	55
7	0.1	1.3	0.5	-	0.4	0.219 ± 0.002^{b}	52
8	0.1	1.3	0.5	-	0.6	0.200 ± 0.001^{a}	57

9	0.1	2.4	-	-	-	0.261 ± 0.009^{d}	-

Values are expressed as Mean ± S.D. Mean, n = 3. Values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down.

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