CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3382215

Available online at: http://www.iajps.com Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ANTI-INFLAMMATORY EFFECT OF FLAVONOID-RICH SEED EXTRACT OF *BUCHHOLZIA CORIACEA* IN RATS

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Article Received: June 2019 **Accepted:** July 2019 **Published:** August 2019

Abstract:

Background: *The increasing discovery of more medicinal plants have screening of their bioactivity in order to provide data that will help physicians and patients make wise decision before using them.*

Objective: *This study was designed to elucidate comparative phytochemical screening and anti-inflammatory properties flavonoid-rich seed extracts of Buchholzia coriacea.**Materials and Methods:*** *Flavonoid-rich seed extracts of Buchholzia coriacea were used for this study. In vivo anti-inflammatory studies was done using the paw oedema method while the in vitro anti-inflammatory studies were performed for the extract using phospholipase A_2 inhibition andcalcium chloride-induce platelet aggregation assays.*

Results: *The analysis of the phytochemical content of the hydro-ethanol seed extracts of Buchholzia coriacea revealed the presence of different secondary metabolites in varying proportions. 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. Varying doses of the extract 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.*

Conclusion: *These results indicate that the extract produced good anti-inflammatory activity which could be as a result of the rich phytochemical constituents and the mechanisms ofaction of this anti-inflammatory effect could be as a result of th may be due to the inhibition of phospholipase A_2 and platelet aggregation.*

Key words*: Inflammation; Phospholipase A_2; Platelet aggregation; prednisolone; Indomethacin.*

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QR code

Please cite this article in press Okeke, E. S. et al., ***Phytochemical Screening and Evaluation of the Anti-Inflammatory Effect of Flavonoid-Rich Seed Extract of Buchholzia Coriacea In Rats.,*** *Indo Am. J. P. Sci, 2019; 06(08).*

INTRODUCTION:

Natural product is a source for bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [1]. Inflammatory diseases treated currently with steroidal and non-steroidal anti-inflammatory drugs exert their effects by inhibiting the metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways [2]. Both steroidal and non-steroidal anti-inflammatory drugs currently used as anti-inflammatory drugs are known to exhibit some levels of toxicity effects. Because of this effect, compounds with less toxicity potential are needed. Thus, the search for natural products from plant origin having protective properties and possessing minimal side effects is largely becoming the final hope for mankind.

Buchholzia coriacea belongs to the family Capparaceae and is widely distributed in several tropical countries. The seeds gave the plant its common name of "wonderful kola" because of its popular usage in traditional medicine in different ways as an alternative medication to promote people health in Nigeria, Africa and other parts of the world. The plant is widely distributed in most African countries, and its parts have been used to treat a variety of sickness in most rural communities in Nigeria. Some of the diseases locally treated with wonderful kola gonorrhoea and gastrointestinal infections application of wonderful kola leaves and fruits on the body are used to relieve fever in Sierra. The plant is documented to possess diverse medicinal potentials. Okoli *et al*. [3], reported the anti-plasmodial properties of the plant, the ground seeds were therefore routinely mixed with palm oil and taken orally as treatment for malaria [4]. The Cameroonians use the seed as remedy to relieve chest pain [5]. It was also reported to have analgesic effects [6]and anthelminthic potentials [7],anti-malarial [8],antihelmintic [9], antibacterial [10], antimicrobial [11], hypoglycemic [12], abortifacient and cytotoxicity effects [4], anti-fertility potentials [13] and anti-ulcer [14]. Although the beneficial effects of *Buchholzia coriacea* seed extract have been exploited, no work has reported the anti-inflammatory potentials of the flavonoid-rich extract of the seed, this study was therefore undertaken to evaluate the *in vivo* and *in vitro* anti-inflammatory activity of flavonoid-rich seed extract of *B. coriacea* in Wistar albino rats.

MATERIALS:

Plant Materials

Fresh Seeds of *B. coriacea* were collected from Ugwu-Awgbu in Orumba North LGA, Anambra State, Nigeria. The seed was identified and authenticated by Mr. Alfred Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. The seeds were air-dried and pulverized.

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.

METHODS:

Extraction Procedure

The Fresh Seed of *B. coriacea* 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 seed were pulverized into powdered form using a mechanical grinder. A known weight of the pulverized seed (1kg) was macerated in 3.5 L absolute ethanol using a maceration flask. The mixture was left for 72 hours 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 were concentrated using a rotary evaporator at 45°C to obtain the crude ethanol extract. The concentrated extract was stored in a labeled sterile screw-capped bottle at 2-8°C.

Preparation of Flavonoid-Rich Extract of *Buchholzia coriacea* Seeds

Extraction of flavonoid-rich extract of Seed of *B. coriacea* was carried out according to the method described by Chu *et al.* [15]. Exactly 3g of the crude extract was dissolved in 20mL of 10% H_2SO_4 in a small flask and was hydrolyzed by heating on a water bath for 30 minutes at 100^0C. The mixture was placed on ice for 15 minutes, so as to allow the precipitation of the flavonoids aglycones. The cooled solution was filtered and the filtrate (flavonoid aglycone mixture) was dissolved in 50mL of warm 95% ethanol (50^0C).

The resulting solution was again filtered into 100mL volumetric flask which was made up to the mark with 95% ethanol. The filtrate collected was concentrated to dryness using a rotary evaporator.

Phytochemical Analysis of Flavonoid-Rich Seed Extract of *B. coriacea*

The preliminary phytochemical screening of the ethanol extract of seeds of *B. coriacea* was carried out in order to ascertain the presence of some plant secondary metabolites and thus those detected was further quantified. Both determinations were done by utilizing standard conventional protocols as illustrated by Trease and Evans [16].

Acute Toxicity (LD_{50}) Study

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 [17]. This study was done only in two phases and a total of eighten (18) mice were used. six (6) groups of three (3) mice each were administered orally, doses of ethanol flavonoid rich extract (50, 100 and 200 mg/kg body weight) respectively for the first phase and (1900, 2600 and 5000mg/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).

***In vivo* Anti-inflammatory Study**

Determination of the Effect of Flavonoid-rich Seed Extract of *B. coriacea* on Egg Albumin-Induced Rat Paw Oedema

This was done according to the method of Winter *et al*. [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.

Principle

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

Experimental Design

A total of twenty (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 50 mg/kg body weight of *Bulchhozia coriasea* seed flavonoid rich extract.

Group 4: Received 100 mg/kg body weight of *Bulchhozia coriasea* seed flavonoid rich extract.

Group 5: Received 200 mg/kg body weight of *Bulchhozia coriasea* seed flavonoid rich extract.

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)

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

***In vitro* Anti-inflammatory Study**

Determination of the Effect of Flavonoid-rich Seed Extract of *Buchholzia coriacea* on Phospholipase A_2 Activity

The effect of the extract on phospholipase A_2 activity was determined using modifications of the methods of Vane [19].

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 15g of Sabouraud dextrose agar in 1000ml of distilled water, homogenized in a water bath for 10min and dispensed into 250ml conical flasks. The conical flasks were sealed with cotton wool and foil paper. The broth was then autoclaved at 121^{o}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 hours at room temperature. The culture was transferred into test tubes containing 3ml phosphate buffered saline and centrifuged at 3000rpm 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 A_2.

Assay Procedure

$CaCl_2$ (2mM) (0.2ml), human red blood cell (HRBC) (0.2ml), 0.2ml of the crude enzyme preparation and varying concentrations of normal saline, the extract and the reference drug were incubated in test-tubes for 1hr. The control contained the human red blood cell suspension, $CaCl_2$ and free enzyme. The blanks were treated with 0.2ml of boiled enzyme separately. The incubation reaction mixtures were centrifuged at a speed of 3000g for 10 minutes. Samples of the supernatant (1.5ml) were diluted with 10ml of normal saline and the absorbance of the solutions read at 418nm. 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:

% Maximum enzyme activity = $(OD_{Test})/OD_{Control}$

% Inhibition= 100-% Maximum enzyme activity

Determination of the Effect of Flavonoid-Rich Seed Extract of *Buchholzia coriacea* on Platelet Aggregatory response

The method of Born and Cross [20] with little modification was adopted. Human blood samples were obtained from healthy adult male subjects who had not taken any drug for at least one week. Blood samples (10.0ml) were collected by venepuncture into plastic anticoagulant (3.8% trisodium citrate) tubes and centrifuged at 300 rpm for 15 minutes. The supernatants were drawn out and used as platelet-rich plasma (PRP). Reaction medium (2.5ml) containing normal saline (2.0ml) and PRP (0.5ml) served as the control, whereas varying concentrations of the extract (0.1- 0.8 ml final concentrations) were included in the test media and allowed 15 seconds incubation with platelet-rich plasma before the induction of aggregation. Aggregation of platelets was induced by the addition of 4.0 mM CaCl2 (0.1ml) and the absorbance at 600nm monitored for 30 sec intervals using Spectrophotometer. Appropriate blanks containing the extract but without PRP were used. The extent of platelet aggregation is expressed as % Inhibition (X) using the following equation: X = (A-B/A) x 100, where A is the maximal aggregation rate of control, B-maximal aggregation rate of sample.

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 16.0 and presented as Mean ±SD. Mean values with $p<0.05$ of the result was accepted significance.

RESULTS:

Quantitative Phytochemical Composition of Ethnol Seed Extract of *Buchholzia coricea*

The results of the quantitative analysis of the phytochemicals contained in the ethanolic extract of *Buchholzia coriacea* seeds are shown in table 1. The analysis of the phytochemical content of the seeds extract of *Buchholzia coriacea* showed that the plant has a high concentration of Tannins (425.88±8.00), terpenoids (248.43±7.79), Phenols (860.45±25.03), Alkaloids (49.87±1.45) and Flavonoids (95.47±0.75) and low concentration of Cyanide (0.13±0.05), steroids (3.73±0.44), Glycoside (5.65±0.21), and Saponins (0.35±0.14).

Table 1: Quantitative Phytochemical Conposition of Ethanol Extract of *Buchholziacoriacea*seeds

Phytochemical constituent (mg/ml)	Mean ± stardard deviation
Tannins	425.88±8.00
Terpenoids	248.43±7.79
Steroids	3.73±0.44
Phenols	860.45±25.03
Alkaloids	49.87±1.45
Glycoside	5.65±0.21
Flavonoids	95.47±0.75
Cyanide	0.13±0.05
Saponin	0.35±0.14

n= 3

Result of Acute Toxicity Studies.

After the phase one of the acute oral toxicity, mice in the group administered 10, 100, and 1000 mg/kg of flavoniod-rich extract of *Buchholzia coriacea* seed showed no sign of mortality so the extract was non–toxic at this phase as in the table 2. Phase two was conducted and mortality did not occur at 1600, 2900, 5000mg/kg. This is represented in table 2.

Table 2. Acute Toxicity and Lethal Dose Determination

PHASE/GROUP	Dosage of extract (mg/kg b. w)	Mortality rate
PHASE 1		
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

n=3

Effect of Flavonoid-rich Seed Extract of *Buchholzia Coriacea* on Egg Albumin-Induced Rat Paw Oedema

Table 3 shows the effect of flavonoid rich extract of *Buchholzia Coriacea seed* 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 hours. Different concentrations of the flavonoid-rich extract significantly ($p < 0.05$) inhibited oedema formation from 30 minutes to 24 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.

Table 3: Effect of Ethanol Extract of *Bulchholzia coriacea* Seed on Egg albumin-induced Rat Paw Oedema

TREATMENT GROUP	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	24 Hours
Control Saline (vehicle)	2.89 ± 0.02^{Ea}	3.04 ± 0.02^{Eb}	3.04 ± 0.01^{Ec}	3.26 ± 0.015^{Ed}	3.34 ± 0.04^{Ee}	3.41 ± 0.04^{A}	3.44 ± 0.02^{Eg}
Indomethacin (10 mg/kg b. w)	2.15 ± 0.02^{Bg} (25.60%)	1.07 ± 0.02^{Af} (64.80%)	0.84 ± 0.02^{Ac} (72.90%)	0.71 ± 0.0^{Ad} (78.22%)	0.64 ± 0.04^{Ac} (80.54%)	0.41 ± 0.04^{Af} (89.97%)	0.35 ± 0.01^{Ba} (89.82%)
Extract (50 mg/ kg b.w)	2.43 ± 0.03^{Dg} (15.60%)	2.31 ± 0.0^{Df} (24.01%)	1.78 ± 0.02^{De} (42.22%)	1.54 ± 0.05^{Dd} (52.76%)	1.28 ± 0.01^{Dc} (61.67%)	1.17 ± 0.01^{Bb} (65.68%)	1.03 ± 0.02^{Da} (70.05%)
Extract (100 mg/kg bw)	2.23 ± 0.05^{Cg} (22.84%)	21.14 ± 0.03^{Bf} (29.20%)	1.65 ± 0.02^{Ce} (46.77%)	1.42 ± 0.04^{Cd} (56.44%)	1.22 ± 0.02^{Cb} (63.47%)	1.02 ± 0.02^{Cb} (65.68%)	0.83 ± 0.01^{Ea} (75.87%)
Extract (200 mg/kg bw)	1.93 ± 0.08^{Ag} (33.22%)	1.72±o.03^{Bf} (43.42%)	1.51 ± 0.03^{Be} (51.29%)	1.36 ± 0.07^{Bd} (58.28%)	0.94 ± 0.02^{Bc} (71.85%)	0.63 ± 0.02^{Bb} (81.52%)	0.33 ± 0.02^{Ea} (90.41%)

N = 3 absorbance
Result expressed as Mean ±SD
Mean values having different upper case letters as subscripts are considered significant (P<0.05) down the column
Mean values having different lower case letters as subscripts are considered significant (P<0.05) across the column
() = % inhibition of platelete aggregation

Effects of Flavonoid-Rich Extract of *Buchholzia coriacea* Seed on Phospholipase A_2 Activity

Table 4 shows the effect of ethanol extract of *Buchholzia coriacea* seed on phospholipase A_2 activity. The extract showed a significant ($p < 0.05$) inhibition of phospholipase A_2 activity when compared to the control. The maximum enzyme activity was observed at 0.1mg/ml with a corresponding percentage inhibition of 32.44%. As the concentration increases from 0.1mg/ml to 0.4mg/ml, the percentage enzyme activity decreases with a comparatively decrease of percentage inhibition. At concentration 0.5mg/ml, there was a deviation, the standard drug (prednisolone) at concentrations (0.2 and 0.4mg/ml) showed a percentage inhibition of 47.14 and 50% respectively.

Table 4: Effects of Flavonoid-Rich Extract of *Buchholzia coriacea* Seed on Phospholipase A_2 Activity

Treatment	Concentration (mg/ml)	Δ $O.D_{418nm}$	Percentage Enzyme activity(%)	Percentage Inhibition(%)
Control	-	0.524 ± 0.002^{e}	-	-
Extract	**0.1**	0.354 ± 0.003^{d}	67.56	32.44
	0.2	0.294 ± 0.001^{c}	56.11	43.89
	0.3	0.277 ± 0.002^{b}	52.86	47.14
	0.4	0.259 ± 0.123^{a}	49.43	50.57
	0.5	0.264 ± 1.003^{a}	50.38	49.62
Standard Drug (Prednisolone)	**0.2**	0.277 ± 0.003^{b}	52.86	47.14
	0.4	0.262 ± 0.006^{a}	50.00	50.00

n=3

Result expressed as Mean ± S.D
Mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Effect of Flavonoid-Rich Extract of *Buchholzia coriacea* Seeds on Calcium Chloride-Induced Platelet Aggregation

The table 5 below shows the effect of Flavonoid-Rich Extract of the *Buchholzia coriacea* seeds on $CaCl_2$-Induced Platelet Aggregatory response. The different concentrations of the extract, and Indomethacin (the standard drug), significantly ($p < 0.05$) inhibited platelet aggregatory response. The different concentrations of the extract inhibited $CaCl_2$ induced platelet aggregation in a concentration and time dependent manner. As the concentration of the extract imcreases, the percentage inhibition decreases. The extract (0.1mg/ml) at 30secs, 60secs and 90secs exhibited equal inhibition of 62%. The highest percentage inhibition was observed under 120secs at extract concentration of 0.1mg/ml.

Table 5: Effect of the flavonoid-rich extract of *Buchholzia coriacea* seeds on platelet aggregation

Group	Extract/Drug (ml)	Δ Absorbance (520nm)				
		0secs	30secs	60secs	90secs	120secs
Control	-	0.307 ± 0.002^{Ga}	0.309 ± 0.001^{Gb}	0.311 ± 0.001^{Gbc}	0.313 ± 0.001^{Gc}	0.315 ± 0.002^{Gd}
Extract	0.1	0.187 ± 0.002^{Fa} (61%)	0.192 ± 0.001^{Fb} (62%)	0.193 ± 0.003^{Fb} (62%)	0.195 ± 0.000^{Fbc} (62%)	0.197 ± 0.002^{Fc} (63%)
Extract	0.2	0.169 ± 0.001^{Ea} (55%)	0.172 ± 0.003^{Eb} (56%)	0.175 ± 0.002^{Eb} (56%)	0.179 ± 0.001^{Ec} (57%)	0.182 ± 0.002^{Ec} (58%)
Extract	0.4	0.134 ± 0.001^{Ca} (44%)	0.137 ± 0.001^{Cb} (44%)	0.139 ± 0.001^{Cc} (45%)	0.142 ± 0.003^{Cd} (45%)	0.145 ± 0.000^{Ce} (46%)
Extract	0.6	0.120 ± 0.001^{Ba} (39%)	0.127 ± 0.001^{Bb} (41%)	0.129 ± 0.001^{Bc} (41%)	0.131 ± 0.001^{Bd} (42%)	0.135 ± 0.001^{Be} (43%)
Extract	0.8	0.115 ± 0.003^{Aa} (37%)	0.119 ± 0.001^{Ab} (39%)	0.121 ± 0.002^{Ab} (39%)	0.124 ± 0.001^{Ac} (40%)	0.127 ± 0.001^{Ac} (40%)
Indometh	0.6	0.142 ± 0.002^{Da} (46%)	0.146 ± 0.003^{Db} (47%)	0.150 ± 0.001^{Dc} (48%)	0.157 ± 0.000^{Dd} (50%)	0.161 ± 0.001^{De} (51%)

Results expressed as Mean ± Standard Deviation; n=3
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
() = % Inhibition of platelet aggregation.

DISCUSSION:

The present study was carried out to investigate the *in vitro* and *in vivo* anti-inflammatory activity of the flavonoid-rich extract of *Buchholzia coriacea* seeds. It was carried out using paw oedema, phospholipase A_2 and platelet aggregation approaches.

Results from the quantitative phytochemical evaluations of the crude extracts of *Buchholziacoriacea*seeds shows the presence of flavonoids, tannins, terpenoids, steroids, phenols, alkaloids, glycoside, cyanide and saponins.

Acute toxicity studies of oral doses of flavonoid-rich seed *extract of Buchholzia coriacea* in mice revealed that it has a high safety profile, as the 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 twenty minutes, 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 optosis.

The anti-inflammatory activity of the flavonoid-rich extract of *Bulchhoilzia coriacea* seed was confirmed by measuring its ability to reduce local oedema induced in the rat paw by injection of an irritant/phlogistic agent [21]. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation [18]. The use of carrageenan is synonymous to the use of freshly prepared egg-albumin as used in this study. Subcutaneous injection of egg-albumin into the rat paw produces oedema resulting from plasma protein-rich fluid exudation along with neutrophil extravasation [22]. Oedema induced by a phlogistic agent has three distinct phases based on the principle of release of various inflammatory mediators. The first phase (0-1.5 hour) is predominantly nonphagocytic and mainly mediated by histamine and serotonin, the second phase (1.5-2.5 hour) is mediated by kinin and the last phase (2.5-6 hour) is due to the liberation of PGs [23, 24] which produces oedema dependent on neutrophils mobilization. The flavonoid-rich extract at 50, 100 and 200 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 24 hour. This shows that the flavonoid-rich extract inhibited all the phases of the inflammatory response. The inhibition of the early phase of oedema exhibited by the flavoniod-rich 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, thus, suppressing oedema. The suppression of oedema formation by the flavoniod-rich extract shows that it possesses anti-inflammatory activity. Plant flavonoids have been reported to exhibit anti-inflammatory activity [25].

The effect of the ethanol seed extract of *Buchholzia coriacea* on phospholipase A_2 activity was considered. The ethanol seed extract of *Buchholzia coriacea*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. Direct enzyme inhibition is equally probable. 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 carbonyl oxygen atom. Phospholipase A_2 cleaves free fatty acid from erythrocyte phospholipids. The enzyme activity assayed using its actionon erythrocyte membrane, creates leakage thus causing haemoglobin to flow out into the medium. Inhibition of phospholipase A_2 implies that the ethanol flavonoid-rich seed extract of *Buchholzia coriacea* may suppress the mobilization of free fatty acids from membrane phospholipids. It was reported that anti-inflammatory and immunosuppressive steroids inhibit arachidonic acid and its metabolites (prostaglandins) by induction which inhibits phospholipase A_2[26].

Platelets play a vital role in the inflammatory processes, in addition to their function in haemostasis and thrombosis. Platelets accumulate in inflammatory sites concomitantly with leukocytes [27] where they regulate a host 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 adhesionchemotaxis, cell survival, and proliferation, all of which accelerate inflammatory process.Table 3 indicates that the extract inhibited significantly ($P < 0.05$) calcium-chloride-induced platelet aggregation *in vitro.* Maximum platelet aggregatiory inhibition was attained at 120 secs. The percentage inhibition of platelet aggregation is time and concentration dependent, though there are noticeable exceptions. Platelet aggregation is brought about by binding of an agonist to a specific receptor on platelet surface. This leads to a release of lipase which converts arachidonic acid to thromboxane A_2 (TXA_2). TXA_2increases intracellular ionised calcium (Ca^{2+}), which promote fusion of dense and alpha granules with the platelet membrane releasing their contents. This result in activating binding sites for specific peptide sequences found on the fibrinogen and von Willebrand factor, vWF. Multiple platelets bind to the same fibrinogen molecule forming a molecular bridge that result in aggregation.

CONCLUSION:

These results indicate that the extract produced good anti-inflammatory activity which could be as a result

of the rich phytochemical constituents, the mechanism of this activity may be due to the inhibition of phospholipase A_2 activity and platelet aggregation.

ACKNOWLEDGEMENT

The authors are thankful to the Head of department and lecturers of the department of biochemistry, University of Nigeria, Nsukka for their constant help and support in conducting this work to full satisfaction.

Conflict of Interest

None declared.

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