European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 4; Issue 5; May 2016; Page No. 01-08

Comparative effect of aqueous and methanol extract of *Buchholzia coriacea* seeds on carrageenan-induced inflammation in rats

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Abstract
This study was designed to investigate the comparative effect of aqueous and methanol extract of *Buchholzia coriacea* seeds on carrageenan-induced inflammation in rats. Twenty four albino rats of both sexes weighing 162.53±3.55 g were used. For the aqueous extract, 12 animals were divided into 4 groups of 3 rats each. Animals in group 1 (positive control) were administered 0.5 ml of diclofenac sodium (reference anti-inflammatory drug) while those in group 2, 3 and 4 were administered 100, 300 and 500 mg/kg body weight of the aqueous extract of *Buchholzia coriacea* seeds respectively. For the methanol extract, 12 animals were divided into 4 groups of 3 rats each. Animals in group 1 (positive control) were administered 0.5 ml of diclofenac sodium (reference anti-inflammatory drug) while those in group 2, 3 and 4 were administered 100, 300 and 500 mg/kg body weight of the methanol extract of *Buchholzia coriacea* seeds respectively. Thirty minutes after treatment of animals in both the aqueous and methanol groups with the drug and extract, 0.2 ml of 2% carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis. Paw volume of rats were measured before and after carrageenan injection at 30 minutes interval over a period of 3 hours (30, 60, 90, 120, 150 and 180) minutes, to determine the anti-inflammatory response of both extracts, using carrageenan-induced paw odema method. Preliminary phytochemical screening revealed that the aqueous extract contains terpenoids, flavonoids and steroids while the methanol extract contains saponins, flavonoids, tannins, anthraquinones, cardiac glycosides, terpenoids and steroids. Proximate analysis of *Buchholzia coriacea* seeds revealed that crude fibre has the lowest percentage of (1.10±0.05), ash content (5.20±0.01), crude fat (4.10±0.02) moisture content (9.10±0.13), crude protein (10.20±0.20) and carbohydrate with the highest percentage of (70.10±0.04). In terms of percentage inhibition, paw volume was brought to near normal levels by the methanol extract at 100 and 300 mg/kg body weight which showed better anti-inflammatory activity than 500 mg/kg body weight of the same extract as well as all doses of the aqueous extract and even more than the reference drug. Overall, the anti-inflammatory effect may be traced to single or synergistic activity of phytoconstituents present in the methanol extract.

Keywords: *Buchholzia coriacea*, comparative effect, inflammation, carrageenan, anti-inflammatory activity, phytoconstituents, diclofenac sodium

Introduction
Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al*., 2007) [14]. Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair (Cotran, 1998) [7]. Inflammation is a general term for the local accumulation of fluid, plasma proteins, and white blood cells that is initiated by physical injury, infection, or a local immune response. It is initiated by changes in blood vessels that promote leukocyte recruitment. Although inflammation serves a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease (Eming *et al*., 2007) [11]. Inflammation is often characterized by symptoms like redness, swollen joints, warmth, joint stiffness, loss of joint function and sometimes pain and some immobility (Rather, 1971) [39].

There are two types of inflammation: acute and chronic (sometimes called systemic) inflammation. Acute inflammation arises after a cut or scrape in the skin, an infected in grown nail, a sprained ankle, acute bronchitis, a sore throat, tonsillitis or appendicitis. It is short-term and the effects subside after a few days (Kumar *et al*., 1996) [27]. Chronic inflammation is long-term and occurs in "wear and tear" conditions, including osteoarthritis, and autoimmune diseases, such as lupus and rheumatoid arthritis, allergies, asthma, inflammatory bowel disease and Crohn's disease (Xu *et al*., 2003; Ploeger *et al*., 2009) [43, 38]. Habitual or environmental factors, such as excess weight, poor diet, lack of exercise, stress, smoking, pollution, poor oral health and excessive alcohol consumption can also lead to chronic inflammation.

The most common anti-inflammatory drugs are the non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin, naproxen (Aleve), ibuprofen (Advil and Motrin) and corticosteroids are taken to alleviate pain caused by inflammation (Wiedermann *et al*., 1996) [42]. However, these chemical drugs have side effects like gastrointestinal side effects such as indigestion, stomach upset (including nausea or feeling sick) or stomach pain is commonly caused by NSAIDs. Use of NSAIDs can also cause ulcers and bleeding

in the stomach and other parts of the gastrointestinal tract (gut). This is mainly because these medicines also attack the cyclooxygenase-1 enzyme (COX-1) that protects the stomach lining from normal stomach (gastric) acid. However, medicinal plants could mediate in this regard. One of such medicinal plants is *Buchholzia coriacea*.

Buchholzia coriacea Engl. (Capparaceae) is an evergreen under storey tree of the lowland rain forest up to 20 m high occurring from Guinea to west Cameroon (Burkill, 1985). The bark-slash is deep red, and the sap exudes with a violently spicily pungent smell that causes sneezing (Burkill, 1985). The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds. In Nigeria, the plant has various common names including; 'Ovu' (Binin), and 'Aponmu' (Akure). *B. coriacea* is found widely distributed in other African countries such as Ivory Coast and Gabon (Keay *et al*., 1964; Koudogbo *et al*., 1972). The plant's fruit is about 5 inches long and 2 - 3 inches in diameter and resembles avocado pear, yellowish when ripe with a yellow flesh containing a few large, blackish seeds about 1 inch long. They are edible and taste peppery. The bark is an ingredient of herbal traditional remedies for sinusitis, nasal congestion, otitis and ophthalmias in Cote d'Ivoire. Whole leaves are applied to boils in Sierra Leone while the seeds are used on skin eruptions in Liberia (Burkill, 1985) [6]. In Ghana, fresh barks of the plants were used for earache (Irvine, 1961).

Antimicrobial compounds like lupeol and sitosterol have been isolated recently by Ajaiyeoba *et al* (2003) [4], in addition to cytotoxic activity. Obembe *et al* (2012) [33] have reported the antifertility effect of *Buchholzia coriacea* on male reproductive parameters in rats. The analgesic and anti-inflammation effect of *Buchholzia coriacea* have also been reported (Olaleye *et al*., 2012; Ezike *et al*., 2015) [35, 13]. The antimicrobial, antibacterial anti-trypanosomal and larvicidal properties of *Buchholzia coriacea* have been reported by Ezekiel and Onyeoziri (2009) [12], Mbata *et al* (2009) [29], Nweze *et al* (2008) [31] and Adediwura *et al* (2011) [1] respectively. Despite these studies, there has not been any report, in the open scientific literature that has provided information on the comparative effect of aqueous and methanol extract of *Buchholzia coriacea* seeds in rats. Therefore, this study was designed to assess the comparative effect of aqueous and methanol extract of *Buchholzia coriacea* seeds on carrageenan-induced inflammation in rats.

Materials and Methods

Materials

Plant Sample Collection

The seeds of *Buchholzia coriacea* were purchased in Mararaba Market in Abuja. They were identified at the Department of Medicinal Chemistry and Quality Control (MC&QC) of the National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria.

Experimental Animals

Albino rats of both sexes weighing 162.53±3.55 g were obtained from the Animal House of Bingham University, Karu, Nasarawa State, Nigeria.

Drugs and Chemicals

Carrageenan was purchased from Hayslip and Zost Pharmacy Brokers, LLC 6700 Woodlands Parkway, Texas, USA while diclofenac sodium was a product of Jiangxi Xier Kangtai Pharmaceutical Co., Pingxiang, Jiangxi, China.

Other Reagents

All other chemicals and reagents used which were of analytical grade were products of Sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water except otherwise stated.

Methods

Preparation of Aqueous Extract of *Buchholzia coriacea* Seed

The seed of *Buchholzia coriacea* were chopped into small pieces and dried at room temperature; it was then reduced to coarse powder by pounding using mortar and pestle. It was further grinded using a grinding machine (Honda engine with gasoline pump, Model G200, Japan) in order to set the powder. 300g of the powder was soaked in 1500 ml of distilled water for 48 hours. The mixture was filtered with Whatman No. 1 filter paper after which the filtrate was concentrated using the water bath (Model: NL-420S, NEWLIFE® MEDICAL INSTRUMENT, England) at 40 ^{0}C to give the required brownish-black residue.

Preparation of Methanol Extract of *Buchholzia coriacea* Seed

300g of *Buchholzia coriacea* powder was used for the extraction with methanol as the solvent using the Soxlet Apparatus at about 65 ^{0}C. The extract was transferred to a Rotary Evaporator (MODEL: RE-52A, SHANGHAI YA RONG BIOCHEMISTRY INSTRUMENT FACTORY, China) and further evaporated to dryness in a water bath.

Anti-inflammatory Study

The animals were housed in clean aluminium cages placed in well ventilated standard housing conditions (temperature: 28-31^{0}C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets rat pellets (Vital Feed®, Grand Cereals Ltd, Jos, Plateau State, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. Twenty four albino rats of both sexes weighing 162.53±3.55 g were used. For the aqueous extract, 12 animals were divided into 4 groups of 3 rats each. Animals in group 1 (positive control) were administered 0.5 ml of diclofenac sodium (reference anti-inflammatory drug) while those in group 2, 3 and 4 were administered 100, 300 and 500 mg/kg body weight of the aqueous extract of *Buchholzia coriacea* seeds respectively. For the methanol extract, 12 animals were divided into 4 groups of 3 rats each. Animals in group 1 (positive control) were administered 0.5 ml of diclofenac sodium (reference anti-inflammatory drug) while those in group 2, 3 and 4 were administered 100, 300 and 500 mg/kg body weight of the methanol extract of *Buchholzia coriacea* seeds respectively. Thirty minutes after treatment of animals in both the aqueous and methanol groups with the drug and extract, 0.2 ml of 2% carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis. Paw volume of rats were measured before and after carrageenan injection at 30 minutes interval over a period of 3 hours (30, 60, 90, 120, 150 and

180) minutes, to determine the anti-inflammatory response of both extracts, using carrageenan-induced paw odema method.

Phytochemical Screening

Test for Tannins

Test was performed by using the method of (Kumar *et al*., 2007) [26]. About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Saponins

Test was performed by using the method of (Edeoga *et al*., 2005) [10]. About 2 g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled Water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

Test was performed by using the method of (Harborne, 2005) [19]. A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Steroids

Identification of steroids was done by adopting the method described by (Edeoga *et* al., 2005) [10]. 2 ml of acetic anhydride was added to 0.5 g methanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Salkowski test was performed by using the method of (Edeoga *et al*., 2005) [10]. 5 ml of the extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac Glycosides (Keller-Killani test)

Kellar-Kiliani test was performed by using the method of (Parekh and Chanda, 2007) [36]. 5ml of the extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Anthraquinones

0.5g of the extract was boiled with 10ml H_2S0_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change (Trease and Evans, 1983) [41].

Proximate Analysis

The chemical analysis of the proximate composition of powdered plants was determined according to the methods of Association of Official Analytical Chemists A.O.A.C. (1990).

Determination of Moisture Content (MC)

Thermal drying method was used for the determination of moisture content as described by Nwinuka (2005) [32].

Principle

This method is based on measuring the mass of water in known mass of sample. The moisture content is determined by measuring the mass of sample before and after the water is removed by evaporation.

Procedure

A clean dried small beaker was weighed. 10 g of powdered plant was added to the beaker. The beaker was then placed in an oven at a temperature of 100 ^{0}C for 3 hours. The sample was then cooled inside a dessicator and weighed. Again the sample was heated, cooled and weighed until a constant weight was obtained. Two other determinations were done using the procedure above.

Calculation

$$MC\ (\%) = W_0/W_1 \times 100$$

Where,

W_0 = Loss of weight (g) on drying
W_1 = Initial weight (g) of sample
MC (%) = Percentage of moisture content

Determination of Percentage Ash Content (Mineral)

Introduction: Ashing is dry digestion of a sample to determine quantitatively the mineral content. Ash content was determined using ignition method as described by Nwinuka (2005) [32].

Principle

The ash content determines the minerals or organic residue of a biological material. It may not give the absolute content of inorganic matter because there may be volatilization of some material during ashing.

Procedure

A clean and dry crucible was warmed for 2-3 minutes and labeled with china maker. The empty crucible (in triplicate) was weighed. 1.0 g sample was weighed into the crucible and put into a furnace at a temperature of 500 ^{0}C for 3 hours. [Note: temperature was first set at 200 ^{0}C for 1 hour and later increased to 500 ^{0}C for 2 hours]. After 3 hours the furnace was switched off and allowed to cool properly till the following day. The crucible was removed into a dessicator for 3-5 minutes, and then weighed. The weight of ash was recorded.

Calculation

% Ash= weight of Ash x 100/ weight of sample.

Determination of Percentage Crude Protein

Introduction

The determination of crude protein was done by first determining the total nitrogen using the micro kjeldahl method as described by Nwinuka (2005) [32]. Crude protein

determination is carried out in three stages: digestion, distillation and titration. The kjeldahl method of protein determination gives an estimation of the amount of protein in the sample.

Principle
The sample is digested with concentrated sulphuric acid followed by the addition of Nessler reagent. The sulphuric acid converts the nitrogen of the protein and other nitrogen containing compounds into ammonia and ammonium sulphate. The total nitrogen can then be estimated from the digest and subsequently the crude protein by multiplying the total nitrogen by a factor of 6.25.

Procedure
1.0 g of dried powdered plant was weighed into a micro kjeldahl flask (digestion flask). 15 g of anhydrous sodium sulphate and 1 g of weighed copper sulphate were added to the flask. 1 tablet of selenium catalyst was also added into each of the digestion flask of about 0.5%. Digestion commenced by adding 20 ml of concentrated H_2SO_4 and heated strongly until a clear colourless solution was obtained. The digest was filtered into 100ml volumetric flask. The filtrate was diluted carefully with distilled water by making it up to 25 ml mark of the beaker. 0.5 ml was pipette from the diluted digest into clean test tubes and 6.5ml of distilled water were added to each test tube bringing the content to 7.0 ml. Also 1ml of colour developer (Nessler reagent) was pipette into each tube and allowed to stand for 20minutes. Absorbance was read in spectrophotometer at wavelength 520 nm after zeroing the machine. Blank was also titrated the same way as the sample. A series of nitrogen standards of 2, 4, 6, 8, 10ppm were read at the same wavelength. The graph of the absorbance against amount (ppm) was plotted and the slope reciprocal determined.

Calculation

$$N\ (\%) = IR \times SR \times FV \times CD \times 100 / \text{Weight of sample} + \text{aliquot} \times 10^6$$

$$\text{Also, } CP\ (\%) = N\ (\%) \times 6.25$$

Where,

N (%) = Total percentage of organic nitrogen
IR = O.D or instrumental reading
SR = Slope reciprocal
FV = Final volume
CD = Colour developer
Aliquot = Volume of filtrate used
CP (%) = Percentage crude protein
6.25 = Protein nitrogen conversion factor

Determination of Percentage Crude Lipid (Fats and Oils)
Introduction
The determination of percentage lipid (fats and oils) of the sample was done by the method described by A.O.A.C. (1990).

Principle
The fat content in plant tissue is generally extracted using the soxhlet extraction method. Fats are materials soluble in organic solvent such as hexane, diethyl ether and petroleum.

Procedure
25 ml extraction flask was dried in an oven at 105 ^{0}C. The flask was allowed to cool in desiccator and weighed empty. 2 g of powdered plants was weighed into a labeled porous thimble. The porous thimble was tied and then petroleum ether was added up to two-third of the 250 ml extraction flask. The covered porous thimble was placed into the condenser and the apparatus assembled. Extraction was done for about 5-6 hours. The porous thimble was then removed with care and the petroleum ether was collected into the tube for reuse. The extraction flask was removed from the water when it was most free of petroleum ether. The extraction flask containing the oil was oven dried at 105-110 ^{0}C for one hour. The flask was then cooled in the desiccator and the weight taken after cooling.

Calculation

$$\%\ \text{Crude Lipid} = (\text{Weight of flask + oil}) - (\text{Weight of flask}) / \text{Weight of sample used}$$

Determination of Percentage Crude Fibre
Introduction
Crude fibre was estimated using the method of A.O.A.C. (1990).

Principle
Crude fibre (CF) is that portion of the plant materials which does not ash or dissolves in boiling solution of 1.25% H_2SO_4 or 1.25% NaOH or 5% of each. Crude fibre was originally thought to be indigestible portion of any main food. It is known however that fibre consists of cellulose, which can be digested to a considerable extent by ruminants and non-ruminants.

Procedure
2 g of powdered plant was weighed into a 250 ml beaker and 150 ml of 1.25% H_2SO_4 was added and boiled for 30 minutes. Warm water was added continuously into the beaker to maintain constant volume. The solution was stirred while heating, then filtered while hot with Whatman No. 1 filter paper using Buckner suction apparatus. Residue was washed with hot water to neutrality. Test for acid with blue litmus paper to red. The plant sample was washed back into beaker with spatula, using 1.25% NaOH (boiling). Boiling 150 ml 1.25% NaOH was added and boiled for 30 minutes. Few drop of antifoaming agent was added into the solution and volume was kept constant. It was filtered and washed to neutrality with hot water and tested with red litmus paper (i.e red to blue). It was washed with 5% HCl (once in filter paper) and washed with distilled water (4 times) to neutrality. It was rinsed with ethanol and residue scraped into a known weight empty clean and dry crucible. The crucible and content were dried in oven at 105^{o}C for 1 hour and cooled in a desiccator before weighing (weight of crucible + dry sample residue). The crucible and content was placed in a muffle furnace at 500 ^{0}C for 3 hours. The crucible and ashed sample was placed into desiccators and weighed again.

Calculation

% CF = (weight of ash + sample) – (weight of dish + ash) x 100/Weight of sample used

Where,

% CF = percentage of crude fibre

Determination of Carbohydrate Content (CHO)

Carbohydrate content was determined using estimation by "difference" i.e. the Anthrone method as described by Nwinuka (2005) [32].

Principle

The carbohydrate content is also called the Nitrogen Free Extract (NFE). It represents the soluble carbohydrate that is present in a feed. E.g. sugar and starch

Procedure

The sum of percentages of the entire proximate component was subtracted from 100.

Calculation

% CHO = 100 – (%MC + %CF + %CL + %CP + %ASC)

Where,

% CHO is percentage of carbohydrate content.

Statistical Analysis

Results were expressed as the mean ± SD of three determinations. Means were analyzed using Duncan's Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at $p<0.05$. All these analyses were done using SPSS 16.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

Results

Preliminary phytochemical screening revealed that the aqueous extract of *Buchholzia coriacea* seed contains terpenoids, flavonoids and steroids while the methanol extract contains saponins, flavonoids, tannins, anthraquinones, cardiac glycosides, terpenoids and steroids (Table 1).

Table 1: Phytochemical Analysis of *Buchholzia coriacea* seed

Parameters	Aqueous Extract	Methanol Extract
Steroids	+	+
Terpenoids	+	+
Cardiac Glycosides	-	+
Athraquinones	-	+
Flavonoids	+	+
Tannins	-	+
Saponins	-	+

Proximate analysis of *Buchholzia coriacea* seeds revealed that crude fibre has the lowest percentage of (1.10±0.05), ash content (5.20±0.01), crude fat (4.10±0.02) moisture content (9.10±0.13), crude protein (10.20±0.20) and carbohydrate with the highest percentage of (70.10±0.04) (Table 2).

Table 2: Proximate analysis of *Buchholzia coriacea* seed

Parameter	Composition (%)
Ash Content	5.20±0.01
Crude Fibre	1.10 ± 0.05
Carbohydrate	70.10 ±0.04
Crude Protein	10.20±0.20
Crude Fat	4.10±0.02
Moisture Content	9.10±0.13

Results are expressed as mean ± standard deviation for 3 determinations.

The percentage inhibition of aqueous extract of *Buchholzia coriacea* seed indicated that at time 60min, there was significant ($p<0.05$) difference between the positive control (48.28) % and the group treatment (100 mg/kg B.W= -6.90, 300 mg/kg B.W=62.07) % but no significant ($p>0.05$) difference at 500 mg/kg B.W (48.28) %. At time 120min, there was significant ($p<0.05$) difference between the positive control (88.89) % and all other group treatment (100 mg/kg B.W=53.33, 300 mg/kg B.W=66.67 and 500 mg/kg B.W=77.78) %. At time 180min, the percentage inhibition indicated that the control (94.34) % differed significantly ($p<0.05$) from the other extract treated groups (100 mg/kg B.W=84.91, 300 mg/kg B.W=88.68 and 500 mg/kg B.W=90.57) % (Table 3).

Table 3: Percentage Inhibition (%) of Aqueous Extract of *Buchholzia coriacea* seed at Time Interval (t) Minutes

Treatment Group	t=30 mins	t=60 mins	t=90 mins	t=120 mins	t=150 mins	t=180 mins
POS CONTROL (DICLOFENAC + CARRAGEENAN)	-132.76	48.28	68.57	88.89	94.55	94.34
100mg/kg B.W Aq EXTRACT + CARRAGEENAN	-63.79	-6.90	45.71	53.33	87.27	84.91
300mg/kg B.W Aq EXTRACT + CARRAGEENAN	-63.79	62.07	51.43	66.67	80.00	88.68
500mg/kg B.W Aq EXTRACT + CARRAGEENAN	5.17	48.28	62.86	77.78	89.09	90.57

POS. = Positive; **B.W.** = Body weight. **Aq.** = Aqueous
Results are expressed in percentage.

The percentage inhibition of methanol extract of *Buchholzia coriacea* seed indicated that at time 60min, there was significant ($p<0.05$) difference between the positive control (48.28) % and the group treatment (100 mg/kg B.W=10.34, 300 mg/kg B.W=51.72 and 500 mg/kg B.W=10.34) %. At time 120min, there was significant ($p<0.05$) difference between the positive control (88.89) % and all other treatment groups (100 mg/kg B.W=64.44, 300 mg/kg B.W=84.44 and 500 mg/kg B.W=64.44) %. At time 180min, the percentage inhibition indicated that the control (94.34) % differed significantly ($p<0.05$) from the other extract treated groups (100 mg/kg B.W=96.23, 300 mg/kg B.W=96.23 and 500 mg/kg B.W=88.68) %. But no difference ($p>0.05$) between the methanol extract treated group of 100 mg/kg B.W=96.23 %, 300 mg/kg B.W=96.23 % which was greater than that of the positive control (diclofenac) =94.34 % (Table 4).

Table 4: Percentage Inhibition (%) of Methanol Extract of *Buchholzia coriacea* seed at Time Interval (t) Minutes

Treatment Group	t=30 mins	t=60 mins	t=90 mins	t=120 mins	t=150 mins	t=180 mins
POS CONTROL (DICLOFENAC + CARRAGEENAN)	-132.76	48.28	68.57	88.89	94.55	94.34
100mg/kg B.W MeOH EXTRACT + CARRAGEENAN	-158.62	10.34	60.00	64.44	85.45	96.23
300mg/kg B.W MeOH EXTRACT + CARRAGEENAN	-106.90	51.72	65.71	84.44	92.73	96.23
500mg/kg B.W MeOH EXTRACT + CARRAGEENAN	-141.38	10.34	54.29	64.44	89.09	88.68

POS. = Positive; **B.W.** = Body weight. **MeOH** = Methanol
Results are expressed in percentage

Discussion

Phytochemical analysis of *Buchholzia coriacea* seed revealed the presence of different chemical contents/phytocompounds of *Buchholzia coriacea* (both aqueous and methanol extract) that have been documented to have anti-inflammatory activities. Saponins, steroids, flavonoids, terpenoids, tannins and glycosides have all been shown to exhibit good anti-inflammatory activities (Ahmadani *et al*., 2000; Mills and Bone, 2000; Herng-Huey *et al*., 2007; Okere *et al*., 2014). In the present study, the methanol extract exhibited more/higher concentration of chemical components compared to the aqueous extract.

Result of the proximate analysis of *Buchholzia coriacea* shows the various proximate parameters examined with the composition of each. The result shows that the carbohydrate composition is the highest compared to other parameters.

Result of the acute inflammation study revealed that the aqueous extract 500 mg/kg has better anti-inflammatory activity than 100 mg/kg and 300 mg/kg body weight of the same extract after 3hr of induction of inflammation with carrageenan which the anti-inflammatory activity is close or a little lower than that of the standard drug (25 mg/kg of diclofenac sodium drug). In terms of percentage inhibition (%), while that of methanol extract, 300 mg/kg and 100 mg/kg body weight has better anti-inflammatory activity than 500 mg/kg body weight of the same extract and 100 mg/kg, 300 mg/kg and 500 mg/kg body weight of the aqueous extract and even more than the reference drug (diclofenac sodium) after 3hr of induction of inflammation with carrageenan in terms of percentage inhibition.

Anti-inflammatory activity of the methanol extract may be traced to its high content of saponins, steroids, glycosides, flavonoids, terpenoids and tannins. These phytochemical constituents have been shown to exhibit anti-inflammatory effects (Ahmadiani *et al*., 1998; Ahmadiani *et al*., 2000; Mills and Bone, 2000; Herng-Huey *et al*., 2007) [2, 30, 21]. The methanol extract showed significant acute and sub-chronic anti-inflammatory activities. The anti-inflammatory activity of the methanol extract could be attributed to its phytochemical constituents, tannins (Gago and Castelae, 2006) [16], flavonoids (Santosh *et al*., 2008) [40], saponins (Desai *et al*., 2009; Payal *et al*., 2010) [8, 37] and glycosides (Jian-Yu *et al*., 2011) [23].

Terpenoids have been documented to possess antioxidant property (Gulacti *et al*., 2007) [17]. Many studies have shown that flavonoids have good antioxidant activity (Harborne and Williams, 2000) [20]. High flavonoid content of the methanol extract might account for their antioxidant activity. Tannins (Magdalena, 2000) and saponins (Gulcin *et al*., 2004) [18] have equally been shown to possess antioxidant activity. Steroids act as antioxidant through membrane stabilizing effect, depressing peroxidation of membrane lipids (Dirk *et al*., 2002) [9]. Compounds with membrane stabilizing property are well known for their ability to interfere with the early phase of inflammatory response, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators (Aitadafoun *et al*., 1996) [3].

Conclusion

Available evidence from the present study indicated that the methanol extract of *B. coriacea* seed exhibited better anti-inflammatory effect than the aqueous extract. The anti-inflammatory effect may be traced to single or synergistic activity of phytoconstituents present in the methanol extract of *B. coriacea* seed.

Recommendation

Isolation and characterization of the active constituent of *Buchholzia coriacea* seed extract (both aqueous and methanol) should be carried out to elucidate its possible molecular mechanism of pharmacological action.

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