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ANTI- INFLAMMATORY EFFECTS OF *BUCHHOLZIA CORIACEA* ETHANOL LEAF-EXTRACT AND FRACTIONS IN FREUND'S ADJUVANT-INDUCED RHEUMATOID ARTHRITIC ALBINO RATS

Ibiam, Udu Ama, Alum Esther Ugo, Orji, Obasi Uch, Aja, Patrick Maduabuchi, Ezeani Nkiru Nwamaka, Ugwu, Okechukwu Paul-Chima

Department of Biochemistry, Ebonyi State University, PMB 053, Abakaliki, Ebonyi State, Nigeria.

Abstract:

The anti-inflammatory effects of Buchholzia coriacea ethanol leaf-extract, aqueous and ethylacetate fractions in Freund's adjuvant-induced rheumatoid arthritic albino rats were investigated. The phytochemical constituents of the extract and fractions were analyzed using standard methods. A total of 216 albino rats were used for this study. Rats used for acute toxicity using Lorke method were 36, while 180 rats were randomly divided into 12 groups, each containing 15 rats for the sub-acute study. Group 1 served as normal control. Rheumatoid arthritis was induced in groups 2 to 12 by intradermal administration of 0.1 ml complete Freund's adjuvant into the left hind paws of the albino rats. Group 2 (positive control) received normal saline while group 3 (standard control) received 5 mg/Kg indomethacin™ (standard drug). Groups 4 to 12 were given ethanol leaf-extract, aqueous and ethylacetate fractions of Buchholzia coriacea at doses of 200, 400 and 800 mg/Kg body weight via oral intubation for 31 days. The inflammatory parameters were measured by standard laboratory procedures. Phytochemical constituents (mg/100g) in all samples were in the order of:

Terpenoids>phenols>alkaloids>flavonoids>tannins>saponins>steroids>glycosides. Some phytochemicals were significantly ($P<0.05$) higher in the extract than fractions. The acute toxicity study showed that the ethanol leaf-extract caused no death to the rats even at 5000 mg/Kg body weight. Adjuvant administration significantly ($P<0.05$) increased the paw sizes and decreased the body weight of rats. In the arthritic rats, the levels of interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), as well as the activity of adenosine deaminase (AD) were significantly ($P<0.05$) higher than the normal group. Treatment with standard drug and varied doses of the ethanol leaf-extract and fractions significantly ($P<0.05$) reduced these parameters to levels comparable to the normal control in a time and dose-dependent manner. The aqueous fraction had most significant ($P<0.05$) positive effect on most of the parameters studied at a dose of 800mg/Kg. The results show that Buchholzia coriacea possesses some phytochemicals which could be responsible for the anti-inflammatory potentials of the leaf extract and fractions. Therefore, this study provides scientific evidence that Buchholzia coriacea ethanol leaf-extract and fractions may be useful in the management of inflammation which is very common in rheumatoid arthritis.

Key words: *Rheumatoid arthritis, Buchholzia coriacea, inflammation, anti-inflammatory.*

Corresponding author:

Alum Esther Ugo,

Department of Biochemistry,
Ebonyi State University, PMB 053,
Abakaliki, Ebonyi State, Nigeria.
E-mail address alumesther79@gmail.com
Tel: +234-8034789993.

QR code

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INTRODUCTION:

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation due to synovial hyperplasia which further progresses into massive irreversible bone destruction (Mayada *et al*., 2014; Samar *et al*., 2014; Straub *et al*., 2009). Other symptoms include stiffness and loss of physical movement and systemic features including cardiovascular, pulmonary, physiological, and skeletal disorders (Mclnnes and Schett, 2011; Rodan *et al*., 2012). Epidemiological study shows that about 1% of people all over the world are now affected with RA, which exerts significant impact on the quality of life (Rojas-Villaraga *et al.,* 2009; Boeing *et al*., 2012). In all populations, it is more prevalent among women than men (Theis *et al*., 2007).

Inflammation is the basic way in which the body reacts to irritation, infection, or other tissue injuries. The key features are pain, swelling, redness and warmth (Stankov, 2012). Although inflammation sets the stage for repair process (Punchard *et al*., 2004), it causes discomfort to the victims and lowers their productivity (Maina *et al*., 2015). Medications and lifestyle changes are considered as treatment for RA. Treatment includes use of non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antiarthritic drugs (DMARDs) and anti-tumor necrosis factor therapy (Kavanaugh, 2007; Wen and Baker, 2011; Sanmugapriya *et al*., 2010). Though these drugs ease the pain, they are incapable of repairing damaged tissues. They are mainly taken for managing the pain and slowing the progression of RA, hence, there is no known drug for curing RA completely (Okoli *et al*., 2003). RA itself confers an elevated risk of infection, and DMARDs and biologic therapies suppress the immune system through various targets, thereby increasing this risk (Mushtaq *et al*., 2011). A significant risk of reactivation of tuberculosis has also been noted with anti-TNF medication (Dixon *et al.,* 2010). The most commonly prescribed anti-inflammatory drugs are NSAIDs like indomethacin (Warden, 2010). These drugs inhibit the activity of cyclooxygenase-2 (COX-2) enzyme. COX-2 converts arachidonic acid to prostaglandins responsible for inflammation while COX-1 produces prostaglandins responsible for supporting platelets and protecting the stomach (Shukla and Mehta, 2015). However, NSAIDs are expensive and have been reported to have adverse effects like stomach ulcer (Modi *et al*., 2012). As a result of these drawbacks of conventional drugs, there is need to source for therapeutic alternatives (Mwangi *et al*., 2015). One of such alternative treatments is the use of herbal medicine, especially by those who cannot bear the high cost of conventional drugs.

Naturally-occurring medicinal plants are a rich source of anti-inflammatory formulations and are better alternatives due to their less side effects, affordability, and ready availability (Bordgers *et al*., 2013). Some medicinal plants have proven effective anti-inflammatory sources.

Boswellia serrata is widely recommended as an anti-inflammatory herb (Ammon, 2001). The phytochemical which act as key player is boswellic acid (Wang *et al*., 2014). Boswellic acid inhibits the expression of lipoxygenase-5 and eventually lowering leukotriene synthesis and leukotrienes are well known for their role in inflammation (Singh *et al*., 2008). It has also proved potency to block NF-κβ activation and brought down the levels of pro-inflammatory cytokines like TNF-α, IL-1, IL-2, IL-4, IL-6, and IFN-γ (Ammon, 2010).

Withania somnifera is another plant with potent anti-inflammatory effect (Singh *et al*., 2011). It is rich in Withaferin A, a steroidal phytochemical which can prevent proceeding of NF-κβ signaling pathway (Grover *et al*., 2010).

Buchholzia coriacea (B. coriacea) is commonly used to treat arthritis in many communities in Ebonyi State, Nigeria. It belongs to the family of *Capparidaceae*. Common names of *B. coriacea* include: Wonderful kola, Musk tree, Cola pime, and Elephant cola. It is called '*Ewi'* in Edo State, '*Okpolo'* in Igbo, '*Uwuro'* and '*Aponmu'* in Yoruba (Anowi *et al.*, 2012; Koudogbo *et al.,* 1972). It has multiple medicinal values. Studies have shown that the methanol extract has hypoglycemic, hypolipidemic and lipid peroxidation reducing effects (Chinaka *et al*., 2012; Adisa *et al.*, 2011; Olaiya and Omolekan, 2013; Egwu *et al.*, 2017). It also has anti-microbial, antihelmintic and antifungal properties (Ezekiel and Onyeoziri, 2009; Chika *et al.*, 2012; Nweze *et al.*, 2011). The seed extract has anti-ulcer and gastric anti-secretory activities (Enenche *et al.*, 2014). However, there is scarce information on the anti-inflammatory potentials of this plant. This study was aimed at determining the anti- inflammatory potentials of ethanol leaf extract, aqueous and ethylacetate fractions of *B. coriacea* ethanol leaf extract in Freund's adjuvant-induced rheumatoid arthritic albino rats. This is important as this plant is often employed in treating arthritis by rural dwellers, and if validated, it will provide a science-based evidence of the anti-inflammatory potentials of the plant which is often associated with arthritis.

The use of animal models for RA can serve as platforms for research on the underlying pathology, as well as for drug discovery and validation (Kollias *et al*., 2011; Wekerle *et al*., 2012). Collagen type II-induced arthritis (CIA) in mice and rats, and adjuvant arthritis (AA) in rats are the most widely used arthritis models in academia and industry (Wooley *et al*., 2004; Hegen, 2008). Complete Freund′s adjuvant (CFA) is a mixture of paraffin oils, mannide monooleate, and heat-killed mycobacteria.

MATERIALS AND METHODS:

Materials

Equipment and Instrument: All equipment and instruments used were in good working condition.
Chemicals and Reagents: All chemicals and reagents used were of analytical grade.
Biological materials: Biological materials used for this study are *B. coriacea* leaves and albino rats.
Collection of Biological Materials: Leaves of *B. coriacea* were collected from Ngodo Village in Afikpo North Local Government Area of Ebonyi State, South-Eastern Nigeria. The identification of the plant was carried out by a Taxonomist in the Department of Biological Sciences, Ebonyi State University, Abakaliki. Female albino rats weighing 121–146 g were obtained from the Department of Animal Science, University of Nigeria, Nsukka, Enugu State. The rats were acclimatized for a period of two weeks in the Animal House of Divine Analytical Laboratory, Nsukka under standard laboratory conditions and fed with commercial rat feed and were allowed free access to clean water.

Methods

Preparation of the Crude Ethanol leaf Extract and Fractions of *B. coriacea*

The leaves of *B. coriacea* were washed and shade dried and later pulverized in a grinder and sifted using 0.25 mm sieve. The powdered sample was used for the extraction. Eight hundred grammes of the sample were soaked in 2000 ml of ethanol for 48 hours. Thereafter, they were filtered using white clean cloth and the filtrate heated on a water bath at 35 ^{o}C until the solvents were completely removed. The extracts were stored in airtight container.

The dried crude ethanol leaf extract was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel (70-230 mesh). The column was eluted in succession with 500 ml water, 500 ml ethylacetate to obtain aqueous and ethylacetate fractions, respectively. The crude ethanol leaf extract, aqueous and ethylacetate fractions were subsequently used for other analyses.

Acute Toxicity Study

Acute toxicity study was carried out by the modified method described by Lorke (1983).

Determination of Phytochemical Compositins of Ethanol leaf-extract and Fractions of *B. coriacea*.

Saponin and terpenoids concentrations were assessed according to the method of Sofowora (1993), flavonoids, alkaloids and glycosides compositions were assessed according to the method of Harborne (1973) while tannins concentration were determined the method of Trease and Evans (2002).

Induction of arthritis in albino rats.

Induction of arthritis was done by the method of Pearson (1956). Arthritis was induced intradermally by injection of 0.1ml Complete Freund's adjuvant (CFA) heat killed *Mycobacterium tuberculosis* and sterile paraffin oil (10 mg/ml) into the left hind paws of rats in groups 2 to 12, according to their body weights. The paw size of all the rats were measured using calibrated automatic venier caliper twice weekly throughout the duration of the study before and after the administration of the adjuvant. A qualitative scoring system was used in measuring the severity of paw inflammation in the rats. Rats with no visible swelling were scored 0, a score of 1 was given to rats with mild redness and inflammation of individual digits; rats with moderate redness and swelling of the ankle were given a score of 2 while a score of 3 was given to the rats with highly pronounced redness and inflammation of the entire paw including the digits. Rats that had a score of 3 were considered to have arthritis and were used for subsequent experiments. It was observed that by day 10, arthritis had completely set in.

Experimental Groups

A total of 180 female albino rats were used for the sub-acute study. The rats were randomly divided into 12 groups with fifteen (15) rats assigned to each group. The study lasted for 31 days and route of administration of extract and fractions was by oral intubation. The rats were grouped as follows:

Group 1: Normal control received normal saline 1 ml/kg.
Group 2: Positive control (untreated arthritic rats) received 1 ml/kg normal saline.
Group 3: Standard control (arthritic rats treated with 5 mg/Kg) indomethacine^{TM} (standard drug).
Group 4: Arthritic rats treated with 200 mg/Kg body weight of crude ethanol leaf-extract of *B. coriacea.*
Group 5: Arthritic rats treated with 400 mg/kg body weight of crude ethanol leaf-extract of *B. coriacea.*
Group 6: Arthritic rats treated with 800 mg/kg body weight of crude ethanol leaf-extract of *B. coriacea.*

Group 7: Arthritic rats treated with 200 mg/kg body weight of aqueous leaf fraction of *B. coriacea* ethanol leaf-extract.
Group 8: Arthritic rats treated with 400 mg/kg body weight of aqueous leaf fraction of *B. coriacea* ethanol leaf-extract.
Group 9: Arthritic rats treated with 800 mg/kg body weight of aqueous leaf fraction of *B. coriacea* ethanol leaf-extract.
Group 10: Arthritic rats treated with 200 mg/kg body weight of ethylacetate leaf fraction of *B. coriacea* ethanol leaf-extract.
GROUP 11: Arthritic rats treated with 400 mg/kg body weight of ethlyacetate leaf fraction of *B. coriacea* ethanol leaf-extract.
GROUP 12: Arthritic rats treated with 800 mg/kg body weight of ethylacetate leaf fraction of *B. coriacea* ethanol leaf-extract.

Determination of Body Weight and Paw Size:
The changes in body weight and paw volume were measured. The diameter of tibotarsal joint was measured using digital vernier caliper. The body weight and paw diameter were measured daily. Measurements were recorded on 10^{th}, 17^{th}, 24^{th} and 31^{st} day of study.

Collection of Blood Samples for Analysis
Three rats from each group were sacrificed by cardiac puncture on days 10, 17, 24, and 31 and the blood samples were collected for various analyses.

Determination of Inflammatory markers
TNF-α, IL-1β and IL-6 levels were determined according to the method described by Krakauer (1993). The levels of erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C- reactive protein (CRP), as well as the activity of adenosine deaminase activity (AD) were determined by the method described by Westergren (1957), Jonsson and Faulk (1976), Voila (1981) and Bergmeyer (1983), respectively.

Statistical Analysis.
All the results were expressed as Mean ± Standard deviation (SD) and data were subjected to one-way analyses of variance (ANOVA). Data were analyzed using computer software known as statistical package for social sciences (SPSS), version 20. Value of $P < 0.05$ was considered to be statistically significant.

RESULTS:

Phytochemical Composition of *B. coriacea* of Ethanol Leaf-extract and Fractions.
The result of quantitative phytochemical analyses of crude ethanol leaf-extract, aqueous and ethylacetate fractions of *B. coriacea* is shown in Figure 1. The results revealed that the extract and fractions contain phytochemicals in varying amounts and occurred in the order of:
terpenoids>phenols>alkaloids>flavonoids>tannins>saponins>steroids>glycosides, in both the crude and fractions. The concentration of phytochemicals were in the order of: ethanol extract>aqueous fraction>ethylacetate fraction.

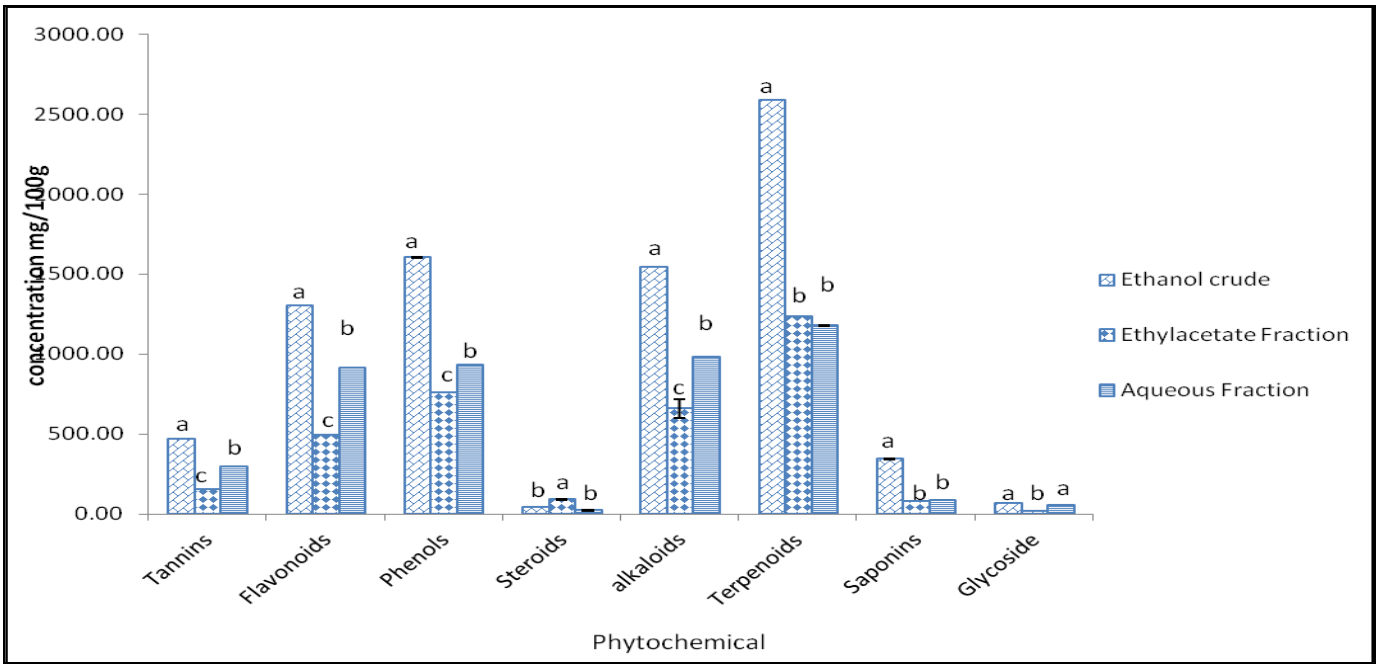

Figure 1: Phytochemical composition of crude ethanol leaf-extract, aqueous and ethylacetate fractions of *B. coriacea* mg/100g.
Bars with different alphabets are significantly different at P<0.05.

Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on Paw Size of Adjuvant-induced Arthritic Rats.

There was increase in paw size in the feet of rats injected with Freund's adjuvant. A significant (P<0.05) reduction in paw size of rats treated with crude ethanol leaf-extract, aqueous and ethylacetate leaf fractions of *B. coriacea* at 200, 400 and 800 mg/kg body weight, relative to normal control was observed. The effect was both dose and time-dependent, as shown in Table 1. There were significant (P<0.05) differences in paw size of rats in all the treated groups. Maximum reduction of paw size with aqueous and ethylacetate fractions at 200 mg/kg and 800mg/kg on day 31, respectively, occurred, relative to normal control. This effect was similar to that of the standard drug.

Table 1: Effect of Ethanol leaf-extract, Aqueous and Ethylacetate fractions of *B. coriacea* on Paw size (mm) of Adjuvant-induced Arthritic Rats.

GROUPS	Before induction	Day 10	Day 17	Day 24	Day 31
1	2.14 ± 0.02^{a}	2.14 ± 0.01^{a}	2.14 ± 0.02^{a}	2.14 ± 0.01^{a}	2.14 ± 0.02^{a}
2	2.20 ± 0.03^{a}	5.39 ± 0.20^{d}	6.59 ± 0.22^{q}	7.33 ± 0.07^{x}	7.78 ± 0.04^{z}
3	2.14 ± 0.07^{a}	4.75 ± 0.55^{e}	3.89 ± 0.59^{g}	2.86 ± 0.32^{gh}	2.16 ± 0.22^{a}
4	2.16 ± 0.02^{a}	5.60 ± 0.20^{cd}	4.53 ± 0.42^{def}	3.48 ± 0.28^{efg}	3.01 ± 0.14^{bc}
5	2.14 ± 0.03^{a}	5.61 ± 0.30^{cd}	4.47 ± 0.32^{def}	4.00 ± 0.22^{bcd}	3.30 ± 0.35^{b}
6	2.21 ± 0.04^{a}	5.57 ± 0.33^{cd}	4.44 ± 0.27^{def}	3.44 ± 0.28^{fg}	3.00 ± 0.45^{bc}
7	2.16 ± 0.02^{a}	5.80 ± 0.20^{bc}	4.48 ± 0.38^{def}	3.36 ± 0.30^{g}	2.16 ± 0.34^{a}
8	2.15 ± 0.02^{a}	5.73 ± 0.24^{bc}	4.64 ± 0.23^{cdef}	4.13 ± 0.10^{b}	3.34 ± 0.28^{b}
9	2.16 ± 0.03^{a}	5.97 ± 0.40^{ab}	5.03 ± 0.67^{d}	3.66 ± 0.48^{efg}	3.25 ± 0.27^{b}
10	2.14 ± 0.03^{a}	5.68 ± 0.32^{c}	4.70 ± 0.26^{bcde}	3.73 ± 0.34^{def}	3.10 ± 0.23^{bc}
11	2.20 ± 0.04^{a}	5.68 ± 0.16^{c}	4.26 ± 0.42^{f}	3.80 ± 0.43^{cde}	3.08 ± 0.41^{bc}
12	2.16 ± 0.03^{a}	5.70 ± 0.40^{ac}	4.33 ± 0.55^{ef}	3.53 ± 0.40^{efg}	2.16 ± 0.44^{a}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.

BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous leaf fraction, BCZ= *B. coriacea* Ethylacetate leaf fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on Body weight of Adjuvant induced Arthritic Rats

Induction of arthritis caused a significant (P<0.05) decrease in body weight of rats. A significant (P<0.05) increase in body weight of rats in the treated groups was observed relative to negative control as shown in Table 2. There were significant (P<0.05) differences in body weight of rats treated with standard drug (indomethacin) relative to those treated with the extract, aqueous and ethylacetate fractions. However, rats treated with ethylacetate fraction had highest increase in body weight.

Table 2: Effect of Ethanol leaf-extracts, Aqueous and Ethylacetate fractions of *B. coriacea* on Body weight (g) of Adjuvant-induced Arthritic Rats.

GROUPS	Day 10	Day 17	Day 24	Day 31
1	145.25 ± 9.53^{a}	146.33 ± 7.57^{a}	147.33 ± 0.58^{a}	148.00 ± 5.29^{a}
2	145.67 ± 8.72^{f}	140.33 ± 4.04^{a}	132.67 ± 5.13^{f}	131.75 ± 3.79^{e}
3	127.17 ± 3.86^{k}	131.00 ± 7.00^{g}	134.00 ± 3.46^{c}	135.00 ± 2.00^{c}
4	125.17 ± 3.33^{k}	127.00 ± 2.65^{k}	132.00 ± 2.65^{e}	136.33 ± 2.08^{b}
5	133.00 ± 3.46^{d}	136.67 ± 4.04^{b}	137.67 ± 1.53^{b}	138.67 ± 2.52^{b}
6	127.50 ± 2.28^{i}	131.67 ± 3.06^{f}	133.00 ± 3.00^{d}	136.00 ± 3.00^{b}
7	126.67 ± 2.57^{k}	130.67 ± 3.06^{h}	136.00 ± 2.65^{b}	132.33 ± 1.53^{e}
8	132.83 ± 4.09^{e}	135.67 ± 1.53^{b}	138.00 ± 2.65^{b}	139.67 ± 3.51^{b}
9	131.92 ± 2.39^{f}	137.00 ± 1.00^{b}	138.33 ± 1.53^{b}	142.00 ± 1.00^{a}
10	121.08 ± 2.94^{m}	122.67 ± 2.52^{l}	131.67 ± 2.08^{f}	134.33 ± 1.53^{c}
11	123.08 ± 2.75^{l}	127.67 ± 1.15^{i}	132.33 ± 4.93^{e}	135.67 ± 5.03^{b}
12	124.50 ± 2.47^{l}	127.33 ± 3.06^{k}	132.33 ± 3.21^{e}	136.00 ± 2.65^{b}

Values are mean ± standard deviation of 3 replicate values.

Values with different superscripts are significantly different at P<0.05.

BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200mg/kg BCE, 5=400mg/kg BCE, 6=800mg/kg BCE, 7=200mg/kg BCA, 8=400mg/kg BCA, 9=800mg/kg BCA, 10=200mg/kg BCZ, 11=400mg/kg BCZ, 12=800mg/kg BCZ.

Effect of Ethanol Leaf-extract, Aqueous and Ethylacetate Leaf Fractions of *B. coriacea* on Inflammatory Markers in Adjuvant-induced Arthritic Rats.

There was significant (P<0.05) increase of TNF- α, IL-1β and IL-6 levels on induction of arthritis, which increased as the days of exposure increased. However, a significant (P<0.05) reduction in TNF-α, IL-1β and IL-6 levels were recorded in the treated groups as shown in Tables 3-5. The cytokine levels in the untreated arthritic rats (group 2) continued to increase significantly (P<0.05) till the end of the 31 days period of study. However, there was no significant (P>0.05) difference in the levels of TNF-α and IL-1β in rats treated with indomethacin relative to those treated with the extract and fractions except on day 31, where a significant reduction was observed in all treated groups. Ethylacetate fraction at dose 200 mg/kg was the most effective in reducing the levels of TNF-α and IL-6 while aqueous fraction at dose 800 mg/kg was the most effective in reducing the level of IL-1β in the treated rats, all on day 31.

ESR, RF, CRP and Adenosine deaminase activity were assayed and the results are presented in Tables 6-9, respectively. The results showed a significant (P<0.05) increase in ESR, RF, and CRP in arthritic rats relative to rats in normal control group. Treatment with standard drug, crude ethanol leaf extract, aqueous and ethylacetate leaf fractions at 200, 400 and 800 mg/Kg body weight significantly (P<0.05) reduced the ESR, CRP and RF levels while

the levels in untreated arthritic rats continued to increase significantly (P<0.05) till the end of the 31 days. The effect of the extract and fractions in reducing the level of ESR was comparable to that of the standard drug. However, the aqueous fraction at 800 mg/Kg on day 31 yielded the most significant (P<0.05) reduction in the ESR level. Treatment with aqueous fraction significantly (P<0.05) reduced the level of RF than the crude ethanol leaf extract and ethylacetate leaf fraction in the arthritic rats in a time and dose-dependent manner. There was no significant (P<0.05) difference on the lowering of CRP level on treatment with standard drug, ethanol extract, aqueous and ethylacetate fractions. However, this lowering effect was time-dependent.

A significant (P<0.05) increase in adenosine deaminase activity was observed in arthritic rats. However, treatment with standard drug, ethanol extract, and fractions at varied doses significantly (P<0.05) reduced the activity of adenosine deaminase in a time and dose-dependent manner as shown in Table 9. There was no significant (P>0.05) difference on the lowering of adenosine deaminase activity on treatment with standard drug, ethanol extract, and fractions.

Table: 3 Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on TNF-α levels of Arthritic Rats.

Groups	Day 10	Day 17	Day 24	Day 31
1	188.24 ± 1.43^{h}	188.65 ± 10.96^{h}	188.76 ± 24.85^{h}	188.67 ± 9.25^{h}
2	274.06 ± 44.53^{a}	277.98 ± 22.28^{a}	280.01 ± 17.45^{a}	290.65 ± 15.27^{a}
3	268.32 ± 54.59^{a}	256.22 ± 11.34^{a}	239..53 ± 37.87^{c}	221.47 ± 39.05^{f}
4	289.13 ± 10.54^{a}	259.61 ± 11.34^{a}	260.01 ± 18.55^{a}	237.81 ± 3.81^{d}
5	274.13 ± 35.00^{a}	270.35 ± 13.07^{a}	259.36 ± 4.38^{a}	251.62 ± 4.66^{a}
6	282.21 ± 25.26^{a}	259.24 ± 13.07^{a}	262.47 ± 2.26^{a}	258.19 ± 6.71^{a}
7	275.01 ± 17.20^{a}	270.36 ± 18.16^{a}	266.00 ± 14.27^{a}	241.05 ± 9.78^{a}
8	272.61 ± 24.98^{a}	262.80 ± 8.83^{a}	271.74 ± 4.33^{a}	249.79 ± 2.11^{a}
9	270.59 ± 27.88^{a}	265.47 ± 5.01^{a}	268.66 ± 14.55^{a}	238.82 ± 18.41^{c}
10	268.70 ± 8.51^{a}	264.68 ± 6.28^{a}	258.84 ± 8.99^{a}	236.80 ± 5.60^{d}
11	264.27 ± 13.31^{a}	255.57 ± 9.86^{a}	250.40 ± 8.62^{b}	244.12 ± 11.46^{b}
12	258.27 ± 7.34^{a}	255.88 ± 8.31^{a}	245.97 ± 12.02^{b}	239.37 ± 7.94^{c}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table: 4 Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on IL-1β levels of Arthritic Rats.

Groups	Day 10	Day 17	Day 24	Day 31
1	240.53 ± 18.65^{m}	242.62 ± 1.22^{k}	240.53 ± 3.30^{m}	243.27 ± 31.83^{k}
2	252.60 ± 11.05^{e}	283.60 ± 18.81^{b}	285.00 ± 19.50^{b}	317.27 ± 9.01^{a}
3	249.27 ± 15.02^{f}	244.73 ± 6.66^{i}	241.93 ± 3.60^{l}	235.60 ± 1.80^{o}
4	272.81 ± 6.43^{b}	271.06 ± 2.96^{b}	268.79 ± 12.41^{b}	245.93 ± 5.67^{h}
5	269.97 ± 9.77^{b}	271.10 ± 10.86^{b}	269.63 ± 5.52^{b}	254.03 ± 8.25^{d}
6	269.70 ± 5.53^{b}	268.80 ± 6.07^{b}	265.03 ± 5.37^{b}	252.64 ± 11.07^{e}
7	273.50 ± 16.49^{b}	270.63 ± 10.60^{b}	261.63 ± 11.12^{b}	240.73 ± 4.27^{m}
8	278.47 ± 4.05^{b}	270.63 ± 1.46^{b}	270.17 ± 7.16^{b}	244.50 ± 22.23^{j}
9	276.40 ± 22.65^{b}	268.27 ± 9.78^{b}	258.93 ± 22.19^{c}	237.83 ± 8.40^{n}
10	272.67 ± 5.11^{b}	267.03 ± 6.11^{b}	264.53 ± 8.58^{b}	237.67 ± 5.35^{n}
11	273.23 ± 6.09^{b}	270.10 ± 4.65^{b}	266.57 ± 16.88^{b}	248.33 ± 4.30^{f}
12	273.63 ± 7.85^{b}	266.16 ± 7.85^{b}	258.37 ± 5.43^{c}	246.91 ± 8.23^{g}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table: 5 Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on IL-6 levels of Arthritic Rats.

Groups	Day 10	Day 17	Day 24	Day 31
1	222.60 ± 18.85^{p}	230.27 ± 35.59^{m}	231.27 ± 14.41	226.20 ± 22.80^{n}
2	268.47 ± 12.93^{b}	282.67 ± 22.11^{a}	285.60 ± 11.48^{a}	296.67 ± 3.93^{a}
3	268.07 ± 17.18^{b}	262.53 ± 5.86^{b}	261.53 ± 18.42^{b}	242.60 ± 15.44^{m}
4	281.31 ± 6.39^{a}	273.00 ± 10.00^{a}	257.32 ± 6.98^{d}	255.02 ± 7.57^{e}
5	272.40 ± 5.24^{b}	269.10 ± 6.84^{b}	265.83 ± 14.53^{b}	258.43 ± 2.20^{c}
6	278.20 ± 2.88^{a}	263.07 ± 3.46^{b}	264.77 ± 6.07^{b}	258.30 ± 6.07^{c}
7	274.67 ± 6.07^{a}	261.83 ± 13.20^{b}	261.03 ± 6.66^{b}	247.37 ± 6.58^{i}
8	273.60 ± 9.96^{a}	264.20 ± 21.55^{b}	258.57 ± 25.72^{c}	253.83 ± 6.87^{e}
9	275.97 ± 8.85^{a}	271.80 ± 6.67^{b}	264.37 ± 20.98^{b}	251.83 ± 8.51^{f}
10	272.08 ± 9.18^{b}	270.80 ± 4.46^{b}	268.00 ± 4.33^{b}	238.37 ± 6.16^{l}
11	271.23 ± 7.21^{b}	266.44 ± 3.76^{b}	258.00 ± 5.76^{c}	248.97 ± 4.25^{g}
12	269.20 ± 8.55^{b}	248.13 ± 2.06^{h}	251.03 ± 1.43^{f}	240.03 ± 9.36^{k}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table 6: Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on Erythrocyte Sedimentation Rate (ESR) (mm/hr) of Adjuvant-induced Arthritic Rats.

Groups	Day 10	Day 17	Day 24	Day 31
1	3.73 ± 0.20^{p}	3.96 ± 0.19^{n}	3.58 ± 0.36^{q}	3.67 ± 0.65^{p}
2	4.67 ± 0.52^{k}	5.26 ± 0.13^{g}	6.48 ± 0.98^{a}	6.98 ± 0.59^{a}
3	5.32 ± 0.39^{g}	4.89 ± 0.21^{j}	4.94 ± 0.15^{i}	3.44 ± 0.75^{r}
4	6.30 ± 0.46^{a}	5.26 ± 0.76	4.97 ± 0.19^{h}	3.73 ± 0.07^{p}
5	6.20 ± 0.08^{b}	5.65 ± 0.49^{e}	4.30 ± 0.03^{m}	3.10 ± 0.88^{n}
6	5.91 ± 0.06^{c}	5.81 ± 0.28^{d}	4.36 ± 0.39^{m}	3.81 ± 0.47^{o}
7	6.34 ± 0.19^{a}	5.45 ± 0.13^{f}	4.29 ± 0.08^{m}	3.75 ± 0.09^{p}
8	6.11 ± 0.53^{b}	5.70 ± 0.24^{e}	4.56 ± 0.18^{l}	3.73 ± 0.12^{p}
9	6.06 ± 0.73^{b}	5.74 ± 0.06^{d}	4.51 ± 0.15^{m}	3.73 ± 0.08^{p}
10	6.64 ± 0.24^{a}	5.41 ± 0.46^{f}	4.40 ± 0.15^{m}	3.74 ± 0.10^{p}
11	6.39 ± 0.10^{a}	6.37 ± 0.23^{a}	4.73 ± 0.37^{j}	3.52 ± 0.45^{r}
12	6.66 ± 0.06^{a}	6.21 ± 0.32^{b}	4.63 ± 0.24^{k}	3.68 ± 0.18^{p}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table 7: Effect of Ethanol leaf-extracts, Aqueous and Ethylacetate leaf fractions of *B. coriacea on* Rheumatoid Factor (RF) (Iu/ml) of Adjuvant-induced Arthritic Rats.

GROUPS	Day 10	Day 17	Day 24	Day 31
1	26.42 ± 0.99^{i}	25.07 ± 0.46^{h}	27.32 ± 0.46^{g}	27.97 ± 0.05^{g}
2	52.64 ± 0.35^{a}	58.41 ± 0.64^{a}	62.23 ± 2.62^{a}	71.29 ± 1.02^{a}
3	38.99 ± 0.88^{h}	32.62 ± 1.81^{i}	29.48 ± 1.29^{g}	27.65 ± 1.41^{g}
4	47.81 ± 0.25^{def}	45.93 ± 0.89^{cde}	44.74 ± 1.04^{cd}	43.86 ± 2.75^{bc}
5	49.26 ± 0.82^{bcd}	46.68 ± 0.95^{cd}	43.02 ± 0.44^{de}	39.18 ± 1.80^{de}
6	49.43 ± 0.51^{bc}	45.93 ± 0.34^{cde}	44.50 ± 1.28^{cd}	41.61 ± 1.19^{cd}
7	46.51 ± 1.27^{fg}	44.30 ± 0.37^{def}	38.41 ± 0.74^{f}	36.82 ± 2.11^{f}
8	46.30 ± 0.60^{fg}	43.89 ± 0.32^{ef}	38.17 ± 0.49^{f}	34.59 ± 0.54^{f}
9	46.89 ± 1.73^{fg}	44.99 ± 0.10^{de}	37.03 ± 0.31^{f}	34.26 ± 0.37^{f}
10	49.31 ± 0.86^{bcd}	47.99 ± 0.82^{bc}	46.61 ± 0.45^{bc}	46.02 ± 0.64^{b}
11	50.69 ± 0.48^{b}	49.36 ± 0.69^{b}	47.81 ± 0.99^{b}	45.61 ± 0.44^{b}
12	49.21 ± 0.67^{bcd}	46.30 ± 0.69^{cde}	41.61 ± 1.76^{e}	40.18 ± 0.96^{d}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table 8: Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea* on C-reactive protein (CRP) (mg/dl) of Adjuvant-induced Arthritic Rats

GROUPS	Day 10	Day 17	Day 24	Day 31
1	4.23±0.15^{i}	4.36±0.86^{d}	4.97±0.01^{e}	4.37±0.01^{c}
2	10.35±0.92^{a}	12.61±0.35^{a}	17.51±0.17^{a}	21.43±1.50^{a}
3	7.53±0.13^{g}	7.41±0.78^{c}	5.37±0.04^{cde}	4.45±0.04^{bc}
4	8.43±0.09^{de}	7.65±0.45^{bc}	5.31±2.60^{de}	5.16±0.39^{bc}
5	9.19±0.13^{c}	7.47±0.14^{bc}	7.15±0.69^{b}	5.13±0.45^{bc}
6	8.32±0.16^{de}	7.50±0.38^{bc}	6.93±0.10^{b}	5.49±0.61^{b}
7	8.57±0.04^{d}	8.03±0.82^{bc}	6.93±0.32^{b}	4.63±0.46^{bc}
8	8.46±0.08^{de}	8.22±0.28^{bc}	6.80±0.18^{b}	5.06±0.30^{bc}
9	8.25±0.20^{de}	7.97±0.19^{bc}	6.48±0.17^{bcd}	4.23±1.04^{bc}
10	9.85±0.06^{b}	8.37±0.25^{b}	6.59±0.17^{bcd}	5.09±0.77^{bc}
11	8.54±0.10^{d}	8.22±0.13^{b}	6.60±0.21^{bcd}	5.46±0.40^{bc}
12	8.45±0.09^{de}	8.35±0.13^{bc}	7.53±0.24^{b}	5.44±0.22^{bc}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ.

Table 9: Effect of Ethanol leaf-extract, Aqueous and Ethylacetate leaf fractions of *B. coriacea on* Adenosine deaminase activity (U/L) of Adjuvant-induced Arthritic Rats.

GROUPS	Day 10	Day 17	Day 24	Day 31
1	0.52 ± 0.00^{d}	0.53 ± 0.01^{e}	0.53±0.01^{bcd}	0.52±0.00^{b}
2	0.79±0.00^{a}	0.79±0.01^{a}	0.85±0.01^{a}	0.89±0.01^{a}
3	0.75±0.01^{ab}	0.63±0.01^{b}	0.53±0.01^{bcd}	0.51±0.01^{b}
4	0.75±0.01^{ab}	0.56±0.05^{de}	0.51±0.07^{bcd}	0.41±0.04^{c}
5	0.74±0.01^{ab}	0.57±0.05^{cde}	0.48±0.04^{d}	0.39±0.01^{c}
6	0.74±0.04^{ab}	0.59±0.02^{bcd}	0.49±0.03^{cd}	0.40±0.04^{c}
7	0.73±0.01^{c}	0.58±0.05^{bcde}	0.49±0.03^{cd}	0.41±0.05^{c}
8	0.74±0.01^{ab}	0.59±0.03^{bcd}	0.53±0.04^{bcd}	0.41±0.02^{c}
9	0.72±0.03^{c}	0.56±0.04^{de}	0.51±0.05^{bcd}	0.39±0.01^{c}
10	0.72±0.01^{c}	0.58±0.03^{bcde}	0.52±0.02^{bcd}	0.44±0.05^{c}
11	0.73±0.01^{c}	0.59±0.03^{bcd}	0.55±0.03^{bc}	0.43±0.07^{c}
12	0.77±0.01^{ab}	0.63±0.04^{bc}	0.56±0.03^{b}	0.41±0.05^{c}

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts are significantly different at P<0.05.
BCE= *B. coriacea* Crude Ethanol extract, BCA= *B. coriacea* Aqueous fraction, BCZ= *B. coriacea* Ethylacetate fraction. 1=normal control, 2=positive control, 3=standard control, 4=200 mg/kg BCE, 5=400 mg/kg BCE, 6=800 mg/kg BCE, 7=200 mg/kg BCA, 8=400 mg/kg BCA, 9=800 mg/kg BCA, 10=200 mg/kg BCZ, 11=400 mg/kg BCZ, 12=800 mg/kg BCZ

DISCUSSION:

Rheumatoid arthritis (RA) is an autoimmune disease in which the body's immune system attacks joints and other tissues. Autoimmune diseases are diseases that occur when the tissues of the body are mistakenly attacked by their own immune system (Kaur *et al.*, 2012).

In this study, result of phytochemical screening of the ethanol leaf-extract and fractions of *B. coriacea* ethanol leaf-extract revealed that the extract and fractions contain the following phytochemicals in varying amounts: terpenoids, phenols, alkaloids, flavonoids, tannins, saponins, steroids and glycosides, in both the crude and fractions. The presence of these phytochemicals in *B. coriacea* have been reported by previous authors (Egwu *et al.,* 2017; Nwachukwu *et al*., 2014; Omolekan and Olaiya, 2013; Lenka *et al*., 2016; Ibrahim and Fagbohun, 2012). Some of these phytochemicals have antioxidant and anti-inflammatory capacity. Flavonoids inhibit the activity of the prostaglandin enzyme synthetase and hence, their anti-inflammatory activity (Chatterjee *et al*., 2015). Steroids reduce inflammation by inhibiting phospholipase A_2 which is responsible for hydrolyzation of arachidonic acid from the membrane phospholipids leading to the formation of prostanoids and leukotrienes (Mencarelli *et al*., 2009). Terpenoids inhibit the production of prostaglandins and also suppresses the function of macrophages and neutrophils, hence their anti-inflammatory activity (Salminen *et al*., 2008).

There was a two-fold increase in paw size in the feet of rats injected with Freund's adjuvant. A significant ($P<0.05$) reduction in paw size of rats treated with standard drug, ethanol leaf-extract, aqueous and ethylacetate leaf fractions of *B. coriacea* at varied, was observed. Our result is in tandem with the work of Chen *et al*. (2015) who reported an average of a 1.5-fold increase in paw volume as indicated by measured paw sizes of arthritic rats (Chen *et al*., 2015). Previous authors have also reported a significant reduction in paw size of rats on treatment with medicinal plants (Das *et al*., 2012; Cong *et al*., 2015). Manifestation of inflammation involves vasodilation which includes increased blood flow causing redness and increased heat. Arthritic paws are subject to increased extra vascular protein infiltration and edema. Increased extra vascular protein infiltration is caused by increased vascular permeability which results in a leakage of plasma proteins and fluid into the tissue resulting to edema and swelling. Some of the released mediators such as bradykinin increase the sensitivity to pain (Cotran *et al*., 1998).

All adjuvant-induced arthritic rats showed a significant ($P<0.05$) decrease in body weight relative to rats in normal control group. A significant ($P<0.05$) increase in the body weight of rats was observed in all the treated groups while progressive weight loss was observed in the untreated-arthritic group till the end of the study. Our present results are in agreement with previous studies that showed that complete Freund's adjuvant (CFA)-injected rats showed decreases in body weight relative to non-arthritic rats (Eissa *et al*., 2016). Suyog *et al*. (2014) also reported a gain in weight of arthritic rats on treatment with *Punica granatum* leaves relative to the untreated rats.

Administration of CFA leads to increase in leptin level, anorexia and weight loss (Mariam *et al*., 2016). Leptin is a hormone secreted by fat cells and is known for suppressing hunger signals, but it also has influences on the immune system. Elevated level of circulating leptin contributes to low-grade chronic inflammation by up regulating inflammatory cytokines (like TNF-α, IL-1β, and IL-6) (Iikuni *et al*., 2008). Leptin levels also increase when there is other inflammatory stimulus, further increasing inflammation (Shen *et al*., 2011). Elevated levels of pro-inflammatory cytokines could exert a strong effect on protein and energy metabolism by promoting muscle breakdown. Increased catabolism leads to resting energy expenditure culminating to weight loss and reduced lean body mass (Rall *et al*., 1996). Increase in body weight of the arthritic rats upon treatment with indomethacin and varied doses of the extract and fractions could be due to the reduction of the inflammatory cytokines and subsequent decrease in protein and muscle breakdown.

Inflammation can also cause a decrease in absorption capacity of the intestine. Elmali *et al.* (2005) reported a restoration of absorption capacity of the intestine upon treatment with anti-inflammatory drugs. Thus, increased body weight of the arthritic rats during the course of treatment with an anti-inflammatory drug (indomethacin) and varied doses of the extract and fractions could be due to the restoration of absorption capacity of the intestine.

There was significant ($P<0.05$) increase of TNF- α, IL-1β and IL-6 levels on induction of arthritis. A significant ($P<0.05$) reduction in TNF-α, IL-1β and IL-6 levels was recorded in the treated groups. Our findings agree with prior studies that reported

elevated levels of these pro-inflammatory cytokines in RA patients (Modi *et al*., 2013; Chen, 2010; Curtis and Singh, 2011).
Cytokines are small proteins which play important roles in cell signaling. Synovial fibroblasts and activated immune cells are responsible for the production of large number of inflammatory cytokines which are believed to play a crucial role in development and progression of RA. Key pro-inflammatory cytokines in RA are IL-1, IL-6 and TNF-α.

The characteristic inflammation of RA occurs due to the abundance of pro-inflammatory cytokines over anti-inflammatory ones (Mateen *et al*., 2016). Among the pro-inflammatory cytokines, TNF-α is the principal cytokine which regulates the formation of other inflammatory mediators in the synovial tissue (Brzustewicz and Bryl, 2015). Production of connective tissue growth factor (CTGF) is mediated by the activation of synovial fibroblasts by TNF-α. This in turn promotes the hyper activation of osteoclasts and thus the destruction of joints (Nozawa *et al*., 2014). It is also involved in the destruction of bone and cartilage via the activation of chondrocytes and osteoclasts (Vasanthi *et al*., 2007). IL-1 and IL-6 are the other key cytokines involved in the pathogenesis of RA. These pro-inflammatory cytokines are also responsible for the formation of chemokines, matrix-metalloproteases (MMPs), inducible nitric oxide synthase, osteoclasts differentiation and the expression of cell adhesion molecules (Mateen *et al*., 2016).

Activation of NADPH oxidase enzyme, which is responsible for the reactive oxygen species generation, is also driven by TNF-α. It is also responsible for the formation of pannus (inflammatory vascular tissue formed over a joint surface of RA patients) by inducing the production of variety of chemokines and endothelial cell activation (Filippin *et al*., 2008). The production of pro-inflammatory cytokines has been found to be decreased by neutralizing the TNF-α in synovial cell cultures.

Erythrocyte sedimentation rate (ESR), Rheumatoid factor (RF), C- reactive protein (CRP) and Adenosine deaminase activity (AD) were also determined in this study. The results are presented in Tables 6-9. The results showed a significant ($P<0.05$) increase in ESR, RF, CRP and AD in arthritic rats relative to rats in normal control group. Treatment with standard drug, and varied doses of ethanol leaf extract and fractions significantly ($P<0.05$) reduced the ESR, CRP, RF and the activity of AD while the levels and activity of AD. For the untreated arthritic rats, the levels of the afore-mentioned parameters and the activity of AD continued to increase significantly ($P<0.05$) till the end of the 31 days. Interestingly, the anti-inflammatory effect of varied doses of ethanol leaf-extract and fractions of *B. coriacea* that was observed in this study was similar to the anti-inflammatory effect of the standard drug indomethacin, a well-known NSAID and a cyclooxygenase (COX-1 and 2) inhibitor. Perhaps this action could be mediated by inhibiting COX-1 or 2.

RF, ESR and CRP are commonly used for diagnosis of RA. ESR and CRP measure the degree of inflammation in the joints. CRP is a protein produced in the liver when there is inflammation anywhere in the body. RF is an auto-antibody (a type of protein made by the immune system that acts against the person's own body tissue) found in the blood of patients with RA. It can also be found in the blood of patients with other inflammatory diseases and also in some other individuals, particularly the elderly. Thus, the presence of RF does not necessarily indicate the presence of RA. However, the possibility of developing RA is high in healthy people with RF (Scott, 2000; Kaltenhauser *et al*., 2001). The key pathogenic markers are IgM and IgA RFs in RA (Scott *et al.,* 2010). RF is an important laboratory parameter because RF positive RA patients have more frequent joint deformity and extra-articular manifestation than RF negative patients. RF, ESR and CRP have associations with RA (Hughes-Austin *et al*., 2013; Goldbach-Mansky *et al*., 2000).

ESR reflects the increase of acute-phase proteins plasmatic concentration, especially of fibrinogen. CRP is an innate immune protein, which helps opsonize pathogens for phagocytosis and activates the complement system. Its levels rise in response to inflammation. CRP production is under the control of IL-1, IL-6, and TNF-α. CRP has a good correlation with therapeutic and radiological progression, better than the ESR (Emery *et al*., 2007). The combination of ESR and CRP may improve sensitivity and specificity of the diagnosis of RA (Barnabe *et al*., 2014). Our results on the effects of *B. coriacea* on ESR, RF, CRP, IL-1, IL-6 and TNF-α validate the anti-inflammatory potentials of this plant.

AD is an enzyme that is involved in purine metabolism. It is ubiquitous in mammalian tissue with the highest concentration in lymphoid tissues (Ibis *et al*., 2007). It is crucial for the differentiation and maturation of the immune cells including lymphocytes and monocyte–macrophage cell lines

(Aldrich *et al*., 2000). It can be used to monitor various diseases in which immunity has been altered (Cakal *et al*., 2010). Serum concentrations of AD have been proposed to be elevated in several inflammatory and autoimmune conditions including RA (Erer *et al*., 2009; Ozturk *et al*., 2008; Cakal *et al*., 2010; Ibis *et al*., 2007). In our study, it was observed that the activity of AD increased significantly ($P<0.05$). This is in accordance with the result of previous studies (Hitogloue *et al*., 2001; Sari *et al*., 2003; Maor *et al*., 2011).

One of the important mediators that provoke inflammatory processes is reactive oxygen species and consequently, their annihilation by antioxidants can alleviate inflammation. Plants which have antioxidant components are thus often found to exhibit anti-inflammatory activity (Warokar *et al*., 2010). Phytochemicals have been reported as potent anti-inflammatory and antioxidant agents in other studies (Samanta and Das, 2015; Tapas *et al*., 2008; Wanja *et al*., 2016). This is true with our plant which contains substantial quantity of antioxidants like flavonoids, alkaloids, etc. Thus, this study suggests that the phytochemicals in the samples, acting either individually or synergistically, could have been responsible for their anti-inflammatory activity. Considering many side effects caused by synthetic drugs in the management of inflammation, *B. coriacea* could be exploited as an anti-inflammatory agent.

CONCLUSION:

Based on the results from this study, *B. coriacea* ethanol leaf-extract and fractions have anti-inflammatory potential. This could be attributed to the phytochemical components, acting either individually or collectively. This study has provided baseline scientific information on the possible anti-inflammatory effects of *B. coriacea.*

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