



Assessing the Analgesic and Anti-inflammatory Effects of *Anthocleista grandiflora* Wood Bark Crude Extract in Albino Rats

Haruna G. Sunday ^{a*}, Halimatu S. Abdullahi ^a,
Ejiogu I. Chibueze ^b and Umar I. Daba ^a

^a Department of Biochemistry, Nasarawa State University, Keffi, Nigeria.

^b Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pain is an unpleasant sensation induced on the body in response to inflammation of tissues. To reduce or stop a painful sensation, a group of drugs broadly classified as pain relievers are administered. The current study aimed at assessing the analgesic and anti-inflammatory effects of the methanol extract of *Anthocleista grandiflora* wood bark crude extract using albino rats as the animal models. Samples of the wood bark were collected from a forest in Keana LGA of Nasarawa State, Nigeria, processed and extracted in methanol to obtain the crude extract. The phytochemical composition of the extract as well as the analgesic and anti-inflammatory properties were analyzed following standard procedures. A total of 25 adult albino rats were randomly grouped into five groups of 5 rats each for both the analgesic and anti-inflammatory studies. Results of the phytochemical analysis showed the presence of tannins, flavonoids, phenols, terpenoids and

*Corresponding author: E-mail: sundayharuna@nsuk.edu.ng;

cardiac glycosides in large amounts. While saponins and steroids in small amounts and alkaloids, glycosides, anthraquinones, phlobatamins and anthracyanine were absent. The results showed a significantly ($p < 0.05$) high analgesic potential of different doses of the extract (50mg/kg body weight) and 100mg/kg bw at 0, 1, 2, 3, 4 and 5 hours compared to the control group. Analysis of anti-inflammatory potential showed a significantly ($p < 0.05$) higher anti-inflammatory properties by the extracts (50mg/kg and 100mg/kg bw) compared to the control. The outcomes revealed that the plant extract may possess good analgesic and anti-inflammatory properties, which may be attributed to the phytochemical compounds present in the extract.

Keywords: Inflammation; pain; phytochemicals; drugs; immune response.

1. INTRODUCTION

The word “pain” maybe defined as a disturbing and unpleasant sensation and emotional physical encounter exacerbated by harsh physical impulse and associated with actual or potential tissue damages or inflammation. It may also be described in terms of such damages [1] and could also be said to be the result or response to a direct tissue injury.

“Pain is usually often initiated by noxious stimuli where the information is transmitted through nerve cells to the CNS where the sensation is interpreted and confirmed as pain. Technically, pain is a response mechanism in which the body exhibits by releasing chemicals to protect the tissues from possible injury” [2].

“A painful sensation that is not curtailed may cause suffering and inability to perform daily activities hence imposing high health costs and economic losses to the victim and society” [3]. Due to its unpleasant, unbearable and disturbing nature, efforts are always made to stop the continuation of painful sensation by administration of drugs known as pain relievers which act through several mechanisms depending on the inducing factor of the pain.

“In a similar way, inflammation, which is employed by both the innate and adaptive immune systems, is a protective response of the body to various obnoxious stimuli such as infections and tissue injury. Inflammation is a characteristic of non-specific immune response that occurs as a result of pathogen entry, injury, allergic reaction or chemical irritation” [4].

“The process of inflammation may persist due to the body's inability to eradicate the irritant or deregulation of mechanisms of the resolution phase. This leads to chronic inflammation which is associated with metabolic diseases such as atherosclerosis, asthma and rheumatoid arthritis” [5,6].

“A category of drugs, known as anti-inflammatory drugs which are broadly classified as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are used to treat and manage inflammation and inflammatory diseases. However, they are not devoid of adverse side effects, such as gastric ulceration” [5]. The inflammatory mediators released during the inflammation might induce pain, redness, swelling and loss of function [7], which are the copious characteristics of inflammation.

“A situation where inflammation is not curtailed may lead to loss of function in terms of missing out on productive and valuable activities and it results in the progression of serious inflammatory diseases, including asthma, autoimmune disease, chronic inflammation, glomerulonephritis, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, hypersensitivities, hay fever, atherosclerosis, and rheumatoid arthritis. These devastating conditions are the major causes of disabilities and may result to death depending on the affected tissues, unless they are properly managed and controlled” [8], which is why there is need for regulation and moderation of drug use.

The substance, Carrageenan is a common food additive that is considered to have no nutritional value. It is usually extracted from a red seaweed, *Chondrus crispus*, popularly known as Irish moss, and is used as a thickener and emulsifier to improve the texture of products like ice cream, yogurt, cottage cheese, soy milk, and other processed foods. Some animal studies have linked “degraded” forms of carrageenan (the type not used in food) to ulcerations and cancers of the gastrointestinal tract. “However, toxic carrageenan such as the type that is widely used in food products have now also been associated with malignancies and stomach complications. It has been found through several research that all forms of carrageenan are harmful and the researchers have used carrageenan to induce

inflammation in tissues in order to test the anti-inflammatory properties of new drugs in experimental models” [9].

“It is a strong chemical that functions in stimulating the release of inflammatory and pro inflammatory mediators, including bradykinin, histamine, tachykinins, reactive oxygen, and nitrogen species. Typical signs of inflammation include edema, hyperalgesia, and erythema, which develop immediately following the administration of carrageenan” [10].

“Alternatively, herbs are globally used to ameliorate pain, fever and inflammation. Herbal medicine entails the use of plants and plant extracts to treat diseases. Herbs exist in many local plant varieties depending on the regional flora and are gradually becoming worldwide” [11]. Many conventional drugs were originally extracted from plant sources and are currently being produced synthetically by pharmaceutical companies [12].

Currently, researchers are now focusing on natural products as alternative and complementary therapy to many diseases. They accomplish these by documenting traditional knowledge relating to medicinal plants, scientifically authenticating them as well as isolating active principles from them [13].

Various plant parts such as leaves, bark, roots, stem, flowers, seeds, fruits among others are associated with many desirable characteristics including suspected *in vivo* safety, affordability and easy accessibility among others, and hence it is often easier to explore their use in the treatment of diseases.

The plant, *A. grandiflora* is commonly used traditionally as herbal medicine and has been documented to have a broad range of therapeutic effects. It belongs to the Gentianaceae family. The Tiv people of Benue State, Nigeria calls it “Kokoso”. The tree has a number of documented medicinal uses. The bark is used traditionally to treat malaria, as an anti-helminthic (specifically for roundworm) agent and in treating diarrhoea, ulcer, diabetes, high blood pressure and venereal diseases [14]. The bark and roots of this plant are also used to treat stomach cramps.

Traditionally, different parts of *Anthocleista* species are used in the treatment of stomachache, fever, constipation, inflammatory

diseases, diabetes, wounds, among others [15,16]. The use of medical plants is still popular today because they are considered safe, less expensive, easily available and effective. This study aims at elucidating the phytochemical composition, analgesic and anti-inflammatory effects of the hydro-ethanol extract of *A. grandiflora* in albino rat models, with the view to its possible scientific validation for use in traditional medicine practice and further exploration for pharmaceutical potentials.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

Fresh barks of *A. Grandiflora* were collected from its natural habitat at a forest in Keana Local Government Area, in Nasarawa State, Nigeria. The local traditional medicinal practitioners assisted in identifying the plant and was confirmed in the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi, Nigeria.

2.2 Chemicals and Reagents

All the chemicals and reagents used were of analytical grade and products of Sigma Aldrich. They include: Carbon tetra chloride (CCL₄), Potassium ferricyanide, Hydrogen chloride, Distilled water, Ammonia (NH₃), Concentrated tetraoxosulphate vi acid (conc. H₂SO₄), Acetic Anhydride, Wagner's Reagent/ Mayer's reagent, Sodium Hydroxide (NaOH), Chloroform, Glacial acetic acid, Ammonium hydroxide (NH₄OH), Acetic acid, Ethanol, Sodium chloride (NaCl).

2.3 Methods

2.3.1 Preparation of plant materials

The collected barks were chopped into small pieces and air dried in the shade for two weeks until they were thoroughly dried. They were then ground into fine powder using an electric blender.

2.3.2 Extraction

The powdered sample material weighing 400g was soaked in one liter of methanol (which have

been known to give a high yield of bioactive compounds due to its high polarity and ability to dissolve a wide range of compounds, at the same time giving rise to extract that could be concentrated at a lower temperature range) for 72 hours with occasional swirling to facilitate the extraction process. The mixture was then filtered using muslin cloth to remove the coarse particles followed by filter paper. The filtrate was concentrated using a rotary evaporator at about 55°C and stored in stoppered containers at 4°C until use.

2.4 Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening of the ethanol bark extract of *A. grandiflora* was carried out to determine the class of secondary metabolites present using standard procedure according to Harbone [17]. Active principles tested included tannins, saponins, alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, anthraquinones, steroids, phlobatannins and anthracyanine, as many of them have been known to be associated with analgesic and anti-inflammatory properties.

2.4.1 Test for Tannins

1ml of extract was added to 2ml of 5% ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.

2.4.2 Test for Saponins

2ml of extract was added to 2ml of distilled water with continuous shaking in a graduated cylinder for 15mins length wise. The formation of 1cm layer of foam indicated the presence of saponins.

2.4.3 Test for Alkaloids

2ml of concentrated hydrochloric acid was added to 2ml of extract. Few drops of Mayer's reagent were added. Presence of green or white colour precipitate indicates the presence of alkaloids.

2.4.4 Test for Flavonoids

1ml of 2N sodium hydroxide will be added to 2ml of extract. Presence of yellow colour indicates the presence of flavonoids.

2.4.5 Test for Glycosides

3 ml of chloroform and 10% ammonia solution was added to 2 ml of extract.

Formation of pink colour indicates presence of glycosides.

2.4.6 Test for Phenols

2ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green colour indicates the presence of phenols.

2.4.7 Test for Terpenoids

0.5ml of the extract was treated with 2 ml of chloroform and concentrated sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

2.4.8 Test for Cardiac Glycosides

2ml of glacial acetic acid and few drops of ferric chloride was added to 0.5ml of the extract. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

2.4.9 Test for Anthraquinones

Few drops of 10% ammonia solution was added to 1ml of extract. Appearance of pink colour precipitate indicates the presence of anthraquinones.

2.4.10 Test for Steroids

To 1ml of extract equal volume of chloroform was added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish ring will indicate the presence of phytosteroids.

2.4.11 Test for Phlobatannins

Few drops of 2% hydrochloric acid was added to 1ml of the extract. Appearance of red colour precipitates indicates the presence of phlobatannins.

2.4.12 Test for Anthracyanine

1ml of the extract was added to 1ml 2N sodium hydroxide and heated for 5mins at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

2.5 Experimental Animals

Albino rats (weighing 116 – 359 g) of both sexes were used for both analgesic and anti-inflammatory tests. They were housed in the animal house, Department of Biochemistry,

Nasarawa State University, Keffi. Ethical guidelines and procedures for handling experimental animals were adhered to according to the guidelines of Vogel, (2002). The animals were kept in cages at room temperature under standard Environmental conditions. They were fed on the standard pellet diet and water *ad libitum*.

2.5.1 Experimental design for evaluation of analgesic activity of methanol bark extract of *A. grandiflora* in albino rat

A total of twenty (20) albino rats were divided into four (4) groups of five rats in each group and administered different substances thus; group 1; normal control, group 2; 15 mg/kg body weight Diclofenac, group group 4; 50mg/kg bw of *A. grandiflora* extract, group 5; 100 mg/kg bw of *A. grandiflora* methanol extract.

After the administrations, individual rats from each group was placed on a hot plate preheated at 50°C and carefully observed for paw licking or jumping off the plate. The average time taken (in seconds) to start licking the paws or jumping off the plate in response to the heat was recorded. The percentage edema inhibition was then calculated using the formula;

$$\% \text{ protection against thermal stimulus} = \frac{(\text{Mean of Control} - \text{Mean of Test})}{\text{Mean of Control}} \times 100\%$$

2.5.2 Experimental design for evaluation of anti-inflammatory activity of methanol bark extract of *A. grandiflora* in albino rat

A total of twenty five (25) adult albino rats were divided into five group of five rats in each group and administered different substances orally thus; group 1; normal control, group 2; 50mg/kg b.w of Diclofenac, group 3; 15 mg/kg bw of Carrageenan (0.5ml), group 4; 50 mg/kg bw of *A. grandiflora* extract + 15 mg/kg bw of Carrageenan (0.5 ml), group 5; 100 mg/kg bw of *A. grandiflora* extract + 15 mg/kg bw of Carrageenan (0.5 ml). The paw diameter of the rats paw was measured in mm using Vernier calipers and recorded before induction of inflammation and at 0,1, 2, 3 and 4 hours after the administrations. Percentage paw edema inhibitory activity was calculated according to the formula below:

$$\% \text{ Edema inhibition} = (\text{Control group} - \text{Test group}) / \text{Control group} \times 100\%$$

2.6 Statistical Analysis

The data obtained were analyzed by one-way ANOVA in SPSS version 23.0 and expressed as mean ± standard deviations. Statistical significance was determined Duncan's post hoc test to compare the levels of analgesic and anti-inflammation between the normal control and test groups. The levels of statistical significance was set at p<0.05.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative phytochemical compositions of *A. Grandiflora* methanol extract

Table 1 is a presentation of the results of the qualitative phytochemical compositions of *A. grandiflora* methanol extract. Results showed the presence of tannins, flavonoids, phenols, terpenoids and cardiac glycosides in large amounts. While saponins and steroids in small amounts and alkaloids, glycosides, anthraquinones, phlobatamins and anthracyanine were absent.

Table 1. Qualitative phytochemical compositions of *A. Grandiflora* methanol extract

S/N	Phytochemical	Inference
1.	Tannins	+++
2.	Saponins	+
3.	Alkaloids	-
4.	Flavonoids	+++
5.	Glycosides	-
6.	Phenols	+++
7.	Terpenoids	+++
8.	Cardiac Glycosides	+++
9.	Anthraquinones	-
10.	Steroids	+
11.	Phlobatamins	-
12.	Anthracyanine	-

Key: (+++) = present in high amount, (++) = present in moderate amount, (+) = present in little amount, (-) = absent

3.1.2 Percentage protection of *A. grandiflora* methanol extract against thermal stimulus in albino rats

Table 2 is a presentation of the results of the analgesic properties of *A. grandiflora* methanol extract, measured as percentage protection against thermal heat stimulus. The results

showed a significantly ($p < 0.05$) high effect of the extract (50 mg/kg bw) and 100mg/kg bw at 0-hour compared to the normal control and standard control. At 1 hour, there was also a significant ($p > 0.05$) increase in the mean percentage analgesic effect in the extract compared to the controls. At 2 hours of the test, the group administered 50mg/kg bw of the extract showed significant increase in analgesic effect while the group administered 100 mg/kg bw of the extract showed a significant ($p < 0.05$) decreases in the mean analgesic property compared to the controls. At 3 hours, there was no significant ($p > 0.05$) change in the percentage analgesic property of the extracts compared to the control groups. At 4 hours, the group administered 50 mg/kg bw of the extract showed significantly ($p < 0.05$) high percentage protection against thermal stimulus compared to the control groups. At 5 hours, there was also non-significant ($p > 0.05$) change in the mean percentage protection against thermal stimulus in the extract groups compared to the control groups.

Results are presented as Mean \pm SD ($n = 5$), mean values with different letters as superscripts are considered to be statistically significant at $p < 0.05$ compared to control group. AGE = *Anthocleista grandiflora* extract.

3.1.3 Percentage paw edema inhibition of *A. grandiflora* methanol extract in albino rats

Table 3 is a presentation of the results of the anti-inflammatory properties of *A. grandiflora* methanol extract, measured as percentage edema inhibition. The results showed a significantly ($p < 0.05$) higher percentage edema inhibition by the extract at (100 mg/kg bw and 50 mg/kg bw) compared to the controls at 0 hours. Similarly, at 1 hour, the extracts at (100 mg/kg bw and 50 mg/kg bw) showed significantly ($p < 0.05$) higher percentage edema inhibition compared to the controls. At 2 hours, the extract (100 mg/kg bw) showed significantly higher

percentage edema inhibition compared to the normal and standard controls. At 3 hours, the extract (100 mg/kg bw) also showed significantly ($p < 0.05$) higher percentage edema inhibition compared to the normal and standard controls. Likewise at 4 hours, the extract (100 mg/kg bw) showed significantly ($p < 0.05$) higher percentage edema inhibition compared to the normal and standard controls.

Results are presented as Mean \pm SD ($n = 5$), mean values with different letters as superscripts are considered to be statistically significant at $p < 0.05$ compared to control group. AGE = *Anthocleista grandiflora* extract.

3.2 Discussion

In this study, the phytochemical composition of *A. grandiflora* methanol extract was determined, its analgesic and anti-inflammatory effects were also evaluated using albino rats as the animal models. The results showed that the extract is rich in phytochemical compounds such as the presence of tannins, flavonoids, phenols, terpenoids and cardiac glycosides in large amounts. While saponins and steroids in small amounts and alkaloids, glycosides, anthraquinones, phlobatamins and anthracyanine were absent. This contradicts (Basu et al., [18]) who stated that, *A. grandiflora* stem bark is highly rich in alkaloids, moderately rich in saponins and flavonoids with little content of glycosides, protease inhibitor and terpenes. These compounds have been reported to have health promoting effects. Also, *A. grandiflora* stem barks are rich in alkaloids. Sunday et al., [19] earlier reported that the wood bark of *A. grandiflora* contains many important phytochemical components such as flavonoids, saponins cardiac glycosides, tannins, phenols, terpenoids and steroids. Therefore, the presence of some of the identified phytochemicals confers medicinal properties on the plant. The presence of these phytochemically-active components in the plant sample might be responsible for their therapeutic activity [20].

Table 2. Percentage protection of *A. grandiflora* methanol extract against thermal stimulus in albino rats

Group	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Control	3.77 \pm 0.60 ^a	3.46 \pm 0.57 ^a	4.07 \pm 0.33 ^a	3.86 \pm 0.84 ^a	3.85 \pm 0.68 ^a	3.31 \pm 0.30 ^a
Diclofenac	2.43 \pm 0.51 ^a	3.39 \pm 0.85 ^a	3.97 \pm 1.10 ^a	3.34 \pm 0.68 ^a	3.91 \pm 0.91 ^a	3.04 \pm 0.31 ^a
50 mg/kg bw Age	4.34 \pm 1.36 ^b	3.90 \pm 1.04 ^c	4.75 \pm 0.64 ^b	3.86 \pm 1.07 ^a	5.62 \pm 0.99 ^b	2.99 \pm 0.29 ^a
100 mg/kg bw Age	3.90 \pm 1.12 ^b	3.61 \pm 0.60 ^c	3.11 \pm 0.79 ^c	3.66 \pm 0.42 ^a	3.66 \pm 0.38 ^a	3.72 \pm 0.78 ^b

Table 3. Percentage paw edema inhibition of *A. grandiflora* methanol extract in albino rats

Group	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Control	0.43±0.05 ^a	0.50±0.00 ^a	0.53±0.05 ^a	0.53±0.05 ^a	0.50±0.00 ^a
Neg control	0.50±0.00 ^a	0.73±0.11 ^a	0.86±0.05 ^b	0.80±0.00 ^b	0.93±0.05 ^b
Diclofenac	0.40±0.00 ^a	0.63±0.11 ^b	0.63±0.11 ^a	0.70±0.00 ^b	0.70±0.00 ^c
50 mg/kg bw Age	0.56±0.05 ^b	0.73±0.11 ^c	0.63±0.05 ^b	0.66±0.05 ^c	0.70±0.00 ^a
100 mg/kg bw Age	0.70±0.10 ^c	0.70±0.10 ^c	0.80±0.00 ^d	0.80±0.00 ^b	0.83±0.05 ^c

“The extract was observed to show significant protection against thermal/heat stimulus at 100mg/kg bw when administered at 2 hours of the analgesic test. At 4 hours, the extract administered 50mg/kg bw showed significantly high percentage protection against thermal stimulus. This phenomenon could be attributed to inhibition of either the synthesis and/or release of pain mediators or through nociceptor blockage by analgesic principles. Plants containing organic acids, terpenoids, alkaloids, saponins and flavonoids are known to show significant analgesic activities. Flavonoids, saponins and alkaloids inhibit prostaglandins involved in pain perception” [21].

The extract exhibited significant edema inhibition which showed a significantly higher percentage edema inhibition by the extract at 100 mg/kg bw and 50 mg/kg bw compared to the controls. The active principles with good anti-inflammatory potential include flavonoids, terpenoids, saponins, phenolics and tannins. Flavonoids and saponins have been found to act in synergy to reduce the rate of inflammation by inhibiting the activities of some major enzymes such as cyclooxygenase, lipoxygenase and nitric oxide synthase. These group of enzymes have been found to play major metabolic roles in the production of inflammatory molecules which serve as mediators and metabolism of arachidonic acid.

As of today, drugs that are in use for the management of pain and inflammation are effective but possess many known side and toxic effects, hence, efforts are ongoing to find alternative and complementary medicine for managing these conditions. Herbal medicines have been extensively used to treat and manage various diseases globally due to its natural origin, availability, safety, cultural acceptability and stronger therapeutic activities with lesser side effects. This study was principally designed to scientifically support the traditional use of the bark of *A. grandiflora* in the treatment of pain and inflammation.

“The therapeutic benefits of the traditional medicine are believed to be due to a combination of active principles. The qualitative phytochemical screening of *A. grandiflora* of methanol extract indicated the presence of flavonoids, steroids, saponins, cardiac glycosides, phenolics, terpenoids and tannins. These phytochemicals are associated with good analgesic anti-inflammatory potential” [21]; (Kumar et al., 2015).

“Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception” [22]. The ability of the extract to prolong the reaction latency to pain thermally induced in rats suggests that the extract has some central analgesic activity, this is in accordance with Williamson et al., [23] and Koster et al., [24]. “In the anti-inflammatory studies, the percentage inhibition obtained showed that the extract exhibited significantly reduction in the edema in the hind paw of the rats. This probably may be due to cyclooxygenase (cox) pathway of arachidonate metabolism produces prostaglandins, which have a variety of effects on blood vessels, on nerve endings and on cells involved in inflammation” [25]. The extracts probably produce its anti-inflammatory effect by inhibiting the release synthesis of inflammatory mediators including polypeptide kinins and prostaglandins.

Therefore, the analgesic and anti-inflammatory activities of the *A. grandiflora* of methanol bark extract could be due to the overall effect of the plant constituents.

4. CONCLUSION

This research shows that *A. grandiflora* methanol extract is rich in phytochemical compounds which include; tannins, flavonoids, phenols, terpenoids and cardiac glycosides in large amounts, and saponins and steroids in small amounts. The results also depicts that the bark extract of *A. grandiflora* showed analgesic and anti-inflammatory activities in animal models, thereby establishing a pharmacological basis for

its use in the treatment of pain and inflammation in folk medicine. It may also serve as a good and effective alternative in the management of painful sensation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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