

Research Article

## Anti-Oxidant, Anti-Inflammatory and Antinociceptive Properties of the Acetone Leaf Extract of *Vernonia Amygdalina* in Some Laboratory Animals

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### Abstract

**Purpose:** *Vernonia amygdalina* is a medicinal plant of great importance that has its fresh leaves rich in vitamins and salt hence, it is valuable in human diet. The anti-oxidant, anti-inflammatory and analgesic activities of its acetone leaf extract were evaluated in this study to validate its folkloric use.

**Methods:** The acetone extract is prepared by dissolving ground plant materials (200g) in 1 L of acetone for 48 h, filtered, and then dried using rotary evaporator before it is used for the pharmacological investigations. Standard phytochemical methods were used to test for the presence of phytoactive compounds in the plant. Acute toxicity was carried out in mice to determine safe doses for use. The anti-inflammatory activities were conducted using carrageenan and histamine to induce oedema in rats while analgesic activities were embarked upon using acetic acid-induced writhing test and formalin-induced paw lick test. The anti-oxidant activities were assessed in vitro using ABTS, DPPH, FRAP and total polyphenolics.

**Results:** The results from this study showed that the 100 and 200 mg/kg doses of the acetone extract caused significant reduction in oedema induced by both carrageenan and histamine. Similar effect was observed in analgesic tests which were comparable to that of indomethacin, the reference drug used in the study.

**Conclusion:** The anti-oxidant effects were also good and the pharmacological activities may be due to the presence of polyphenols and other phytochemicals contained in the plant. The study may have thus validated the folkloric use of this plant as a medicinal and nutritional agent.

### Introduction

*Vernonia amygdalina* Del is commonly called bitter leaf in English because of its bitter taste. African common names include *grawa* (Amharic), *ewuro* (Yoruba), *etidot* (Ibibio), *onugbu* (Igbo), *ityuna* (Tiv), *oriwo* (Edo), *chusar-doki* (Hausa), *muluuza* (Luganda), *labwori* (Acholi), and *olusia* (Luo). It is a member of the Asteraceae family and is a small shrub that grows in the tropical Africa. *V. amygdalina* typically grows to a height of 2--5 m. The leaves are elliptical and can be up to 20 cm long. Its bark is rough.<sup>1</sup> Leaves of this plant are used in Nigeria as a green vegetable or as a spice in soups, especially in the popular "bitter-leaf soup". Such preparation includes freshly harvested leaves which are macerated with either cold or hot water to reduce the bitterness of the leaves to a desirable level. The leaves are then added to other condiments for the soup while the water extract may be taken as a tonic to prevent certain illnesses. The leaves can be taken as an appetizer and the water extract as a digestive tonic.<sup>2</sup> These are largely consumed by the female Hausas in their belief that it makes them more sexually attractive. In Northern Nigeria, it is added to horse feed to provide a strengthening or fattening tonic called 'Chusar Doki' in Hausa.<sup>3</sup> The leaves have also been used in Ethiopia

as hops in preparing 'tela' beer.<sup>4</sup> The leaves are widely used for fevers and also as a quinine-substitute in Nigeria and some other African countries.<sup>5</sup> The young leaves are used in folk medicine as anthelmintic, antimalarial, laxative/purgative, enema, expectorant, worm expeller and fertility inducer in subfertile women. Some wild chimpanzees in Tanzania had been observed to use this plant for the treatment of parasite related diseases.<sup>6-8</sup> Many herbalists and naturopathic doctors recommend the aqueous extracts for the treatment of emesis, nausea, diabetes, loss of appetite-induced abrosia, dysentery and other gastrointestinal tract problems in their patients. *V. amygdalina* is well known as a medicinal plant with several uses including for the treatment of diabetes, fever reduction, and recently for a non-pharmaceutical solution to persistent fever, headache, and joints pain associated with AIDS (an infusion of the plant is taken as needed).<sup>9</sup> The leaves are exported from several African countries and can be purchased in grocery stores aiming to serve African clients for about \$1.50/225gm pkg. frozen. The root of *V. amygdalina* is also used for treating gingivitis and toothache due to its proven antimicrobial activity.<sup>6</sup>

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Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.<sup>10</sup> It is a complex multistep process comprising a dynamic cascade of biological phenomena, which can be subdivided into several stages and phases.<sup>11</sup> Pro-inflammatory molecules like tumour necrotic factor  $\alpha$  (TNF $\alpha$ ), certain interleukins, prostaglandins and even pathogenic concentration of nitric oxide are instrumental in raising inflammatory response.<sup>12</sup> Many current anti-inflammatory drugs target these mediators at different levels, yet they lack specificity and their untoward effects restrict their long-term use.<sup>13</sup> Hence, there is a constant demand for better therapeutic alternatives. An anti-oxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. Anti-oxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.<sup>14</sup> Anti-oxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that anti-oxidant supplements might promote health, later large clinical trials with a limited number of anti-oxidants detected no benefit and even suggested that excess supplementation with certain putative anti-oxidants may be harmful.<sup>15</sup> In this study, we evaluated the anti-oxidant, anti-inflammatory and antinociceptive properties of the acetone leaf extract of *Vernonia amygdalina* to validate its medicinal and nutritional value.

## Materials and Methods

### *Plant collection and identification*

Fresh leaves of *Vernonia amygdalina* Del were collected from the campus of the University of Ibadan, Nigeria (7° 23' 16" North, 3° 53' 47" East) in March 2012. The leaves were identified by Professor Abiodun Ayodele (a botanist) and a voucher specimen (UIH ADE/001/2013) deposited at the herbarium of the Department of Botany, University of Ibadan. The leaves were dried under shade and ground into powder. The powdered plant material (200g) was subjected to maceration in acetone. It was later filtered and the filtrate was concentrated using rotary evaporator. This was also further concentrated using vacuum bath. The final crude extract was placed in the refrigerator until it was ready for use.

### *Animals*

Eighty healthy white Wister strain albino rats (150–230g) and eighty mice (17–30g) of either sex, bred in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria were used for

the study. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. The animals were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

### *Chemicals and drugs*

The chemicals and drugs used were purchased from sigma Aldrich GmbH (Steinheim, Germany) and they include: carrageenan, acetic acid, and formalin. Other chemicals used were acetate buffer, hydrochloric acid, gallic acid, ascorbic acid, ABTS (2, 2'-azino-bis-(3-ethylthiazolone)-6-sulphonic acid), DPPH (1, 1-Diphenyl-2-picrylhydrazyl), TPTZ (2, 4, 6-tri [2-pyridyl]-s-triazine) and ferric acid. The standard drugs used were indomethacin and histamine which were also purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All the chemicals and drugs used were of analytical grade.

### *Acute toxicity of Vernonia amygdalina in mice*

The acute toxicity study of *Vernonia amygdalina* was determined according to the method of Sawadogo *et al.*<sup>16</sup> Thirty five mice fasted for 16 hours were randomly divided into 7 groups of 5 animals each. Graded doses of the extract (100, 200, 400, 800, 1600 and, 3200mg/kg) corresponding to groups B,C,D,E,F and G respectively were separately administered to the mice in each test group by means of oral cannula. The control group representing group A was administered with normal saline (3ml/kg) only. All animals were then allowed free access to feed and water and observed for a period of 48hrs for signs of acute toxicity, morbidity and mortality.

### *Phytochemical screening*

The phytochemical analysis was performed on the ground (powered) leaf of *V. amygdalina* for identification of the constituents. The constituents tested for were alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and flavonoids as described by Sawadogo *et al.*<sup>16</sup> Shale *et al.*<sup>17</sup> and Moody *et al.*<sup>18</sup>

### *Anti-inflammatory studies*

#### *Histamine induced paw oedema in rats*

Using the method of Perianayagam *et al.*<sup>19</sup> the paw oedema was produced by subplantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw oedema was measured at 0, 1, 2, and 3hrs after administration of histamine using thread and ruler. Increase in the linear diameter of the hind paw was taken as indication of paw oedema. Four groups of rats A, B, C and D of five rats per group (i.e. n=5) were pre-treated with 2ml/kg control, 10mg/kg indomethacin and different doses of the plant extract (100 and 200mg/kg) respectively. The drug and extract were administered orally 1 hour

before the induction of paw oedema. The percentage inhibition of inflammation was calculated using the formula:

$$\% \text{ inhibition} = \frac{(D_o - D_t)}{D_o} \times 100$$

Where  $D_o$  is the average inflammation (hind paw oedema) of the control group of rats at a given time and  $D_t$  is the average inflammation of the drug treated (i.e. extract or reference drug, indomethacin) rats at the same time.<sup>16,18,20</sup>

#### *Carrageenan induced paw oedema in rats*

Four groups of rats A, B, C and D of five rats per group (i.e.  $n=5$ ) were pretreated with 2ml/kg vehicle control, 10mg/kg indomethacin and different doses of the plant extracts (100 and 200mg/kg) respectively. These were administered orally. Acute inflammation was then induced after 60minutes by the sub-planter administration of 1% carrageenan in normal saline that contains tween-80 in the right hind paw of the rats. The paw volume was measured at 0, 1, 2, 3 and 4hours after carrageenan injection using thread and ruler. Increases in the linear diameter of the right hind paws was taken as an indication of oedema which was assessed in terms of the difference in the zero-time linear diameter of the injected hind paw and its linear diameter at time  $t$  (i.e. 1, 2, and 3 hr) following carrageenan administration. The percentage inhibition of the inflammation (hind paw oedema) was calculated from the formula:

$$\% \text{ inhibition} = \frac{(D_o - D_t)}{D_o} \times 100$$

Where  $D_o$  is the average inflammation (hind paw oedema) of the control group of rats at a given time and  $D_t$  is the average inflammation of the drug treated (i.e. extract or reference indomethacin) rats at the same time.<sup>16,18,20</sup>

#### *Analgesic studies*

##### *Acetic acid induced writhing response in mice*

To evaluate the analgesic effect of this plant extract, the method described by Sawadogo et al<sup>16</sup> was used though with slight modification. Four groups of mice A, B, C and D ( $n=5$ ) each received orally- administered vehicle control (distilled water or normal saline 2ml/kg) (i.e. control), indomethacin (10mg/kg) and the plant extracts (100 and 200mg/kg) respectively. Sixty minutes later, 0.6% acetic acid (10ml/kg) solution was injected intraperitoneally to all animals in the different groups. The number of writhes occurring between 5 and 20 mins after acetic acid injection was counted. The percentage inhibition of the writhing response was calculated from the formula:

$$\% \text{ inhibition} = \frac{(D_o - D_t)}{D_o} \times 100$$

Where  $D_o$  is the average writhing response of the control group and  $D_t$  is the average writhing response of the treated group. A significant reduction of the writhes in

the tested animals compared to those in the control group was considered as an antinociceptive response.

##### *Formalin paw lick test in mice*

In this experiment, pain was induced by formalin. Following an overnight fast, four groups of mice A, B, C and D ( $N=5$ ) each received orally-administered vehicle control (distilled water or normal saline 2ml/kg) (i.e. control), indomethacin (10mg/kg) and plant extracts (100 and 200 mg/kg) respectively. Thirty minutes after treatment, 0.05ml of 2.5% formalin was injected subcutaneously into the sub-plantar surface of the mice left hind paw, then the number of paw licks by the mice were recorded both at the early (0-5mins) and the late phase (15-30mins), the time interval between the paw licks was also noticed.<sup>21,22</sup> The percentage inhibition of the paw licks for both phases was calculated from formula:

$$\% \text{ inhibition} = \frac{(D_o - D_t)}{D_o} \times 100$$

Where  $D_o$  is the average number of paw licks of the control group and  $D_t$  is the number of paw licks of the treated group.

#### *Anti-oxidant activities of acetone leaf extract of V. amygdalina*

##### *ABTS radical scavenging assay*

The ABTS radical scavenging activity of the hydrophilic extract was determined according to the method described by Re et al.<sup>23</sup> ABTS<sup>+</sup> solution was prepared 24 hours before use by mixing ABTS salt (7 mM) with potassium persulfate (140 mM) and the solution was then stored in the dark until the time of analysis. The ABTS<sup>+</sup> solution was diluted with ethanol (EtOH). Each hydrophilic extract (25  $\mu$ L), 25  $\mu$ L blank, 25  $\mu$ L Standard, was mixed with 300  $\mu$ L ABTS<sup>+</sup> solution in a 96-well clear plate. The plate was incubated for 30 minutes at room temperature and then read in a Multiskan Spektrum plate reader (Thermo Fisher Scientific) at 734 nm. Gallic acid was used as standard. All samples were prepared and read in triplicates.

##### *DPPH radical scavenging assay*

The effect of this extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi.<sup>24</sup> A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard and the results expressed as  $\mu$ mol AAE/L. All samples were prepared and read in triplicates.

##### *Ferric reducing antioxidant power (FRAP) assay*

The ferric reducing ability of the hydrophilic fraction was determined using the method described by Benzie and Strain.<sup>25</sup> Each hydrophilic extract (10  $\mu$ L) was mixed with 300  $\mu$ L FRAP reagent in 96-well clear plate. The FRAP

reagent was a mixture (10:1:1, v/v/v) of acetate buffer (300 mM, pH 3.6), 10 mM TPTZ in 40 mM HCl and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (20 mM). After incubation for 30 minutes, the plate was read at a wavelength of 593 nm in a Multiskan Spektrum plate reader (Thermo Fisher Scientific). Ascorbic acid (AA) was used as the standard and the results expressed as  $\mu\text{mol AAE/L}$ . All samples were prepared and read in triplicates.

#### Determination of total phenolics

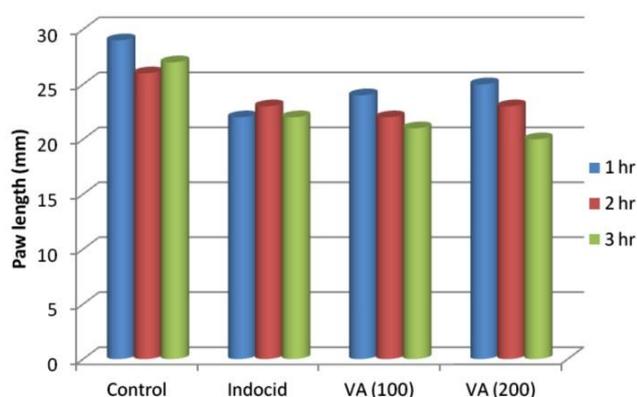
Total phenol contents in the extract were determined by the modified Folin-Ciocalteu method.<sup>26</sup> An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Multiskan Spektrum plate reader. Total phenolic contents were expressed as mg/g gallic acid equivalent (GAE/L).

#### Statistical analysis

Data obtained were expressed as means  $\pm$ SD. Student's *t*-test was used to compare test and control group values. Differences in the test versus control values were considered to be statistically significant at  $P \leq 0.05$ .

#### Results

Phytochemical screening of the powdered leaves of *V. amygdalina* in this study showed the presence of alkaloids, tannin, flavonoids, saponin, anthraquinones and cardiac glycosides. The acute toxicity study also showed that no mortality was recorded in any of the groups including the 1600 mg/kg dose group. In Figure 1, the extract (100 and 200 mg/kg) and indomethacin (10 mg/kg) significantly ( $P < 0.05$ ) reduced the paw oedema at 1, 2 and 3 hr after histamine injection when compared to the control. Anti-inflammatory effect of 200mg is most pronounced after 3hr.



**Figure 1.** Anti-inflammatory activity of the acetone leaf extract of *Vernonia amygdalina* (VA) on histamine-induced oedema in the right hind-limb of rats

Table 1 showed the effect of the acetone extract (100 and 200 mg/kg) and indomethacin on the carrageenan-induced paw oedema in rats and this indicated that both 100mg and 200mg of the extract reduced inflammation at 1hr, 2hr and 3hr compare to control. The effect of 200mg is more pronounced than 100mg at 1hr, 2hr and 3hr. 200mg is the most pronounced at 3hr and this is more pronounced than the standard drug.

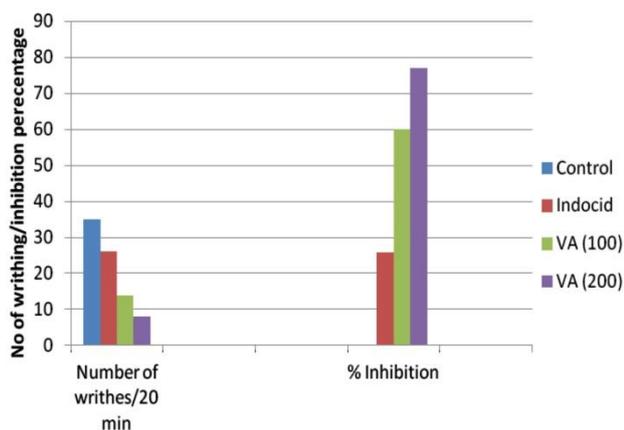
**Table 1.** Anti-inflammatory activity of the acetone leaf extract of *Vernonia amygdalina* on carrageenan-induced oedema in the right hind-limb of rats (in mm); Data is presented as mean $\pm$ S.D., n=5

Time (hr)	Control 3 ml/kg	Indomethacin 10 mg/kg	Extract (acetone)	
			100 mg/kg	200 mg/kg
0	21.8 $\pm$ 1.1	24.6 $\pm$ 1.7	25.2 $\pm$ 2.3	23.4 $\pm$ 1.1
1	23.8 $\pm$ 0.8	23.2 $\pm$ 1.3 (5.7)	22.0 $\pm$ 1.1 (12.7)	22.2 $\pm$ 0.4 (5.1)
2	23.8 $\pm$ 0.8	21.8 $\pm$ 0.8 (11.4)	22.6 $\pm$ 1.6 (10.3)	21.4 $\pm$ 1.7 (8.6)
3	23.4 $\pm$ 0.9	22.0 $\pm$ 0.7 (10.6)	22.0 $\pm$ 1.2 (12.7)	20.8 $\pm$ 2.3 (11.1)

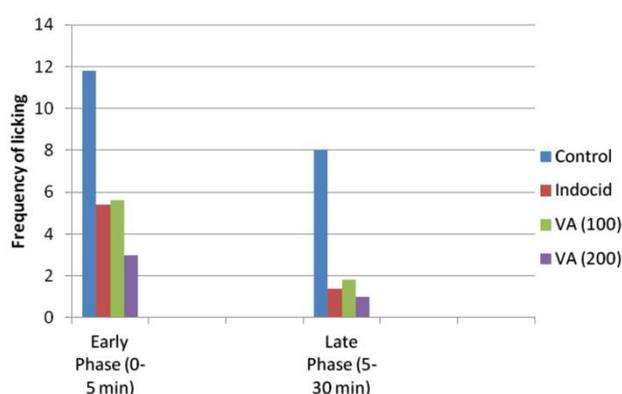
Percentage inhibitions of the carrageenan-induced inflammation (oedema) are in parenthesis.

With respect to analgesic effect, as it relates to acetic acid writhing test, acetone leaf extract of *Vernonia amygdalina* caused a significant decrease in the number of writhes at doses 100 and 200mg/kg when compared to the control. The extract (100 and 200 mg/kg) and indomethacin exhibited a significant antinociceptive power. The 100 and 200mg/kg doses of the extract showed a significant analgesic effect relative to the standard drug. The standard drug indomethacin (10 mg/kg) also caused a significant reduction ( $P=0.05$ ) in the number of writhes when compared to the control (Figure 2). In the paw licking test, the acetone leaf extract of *V. amygdalina* (100 and 200 mg/kg) caused a significant decrease in the number of paw licks induced by formalin when compared to the control. The analgesic effect of both 100 and 200 mg/kg doses of the extract was greater relative to the standard drug (indomethacin). Indomethacin at 10 mg/kg caused a significant decrease ( $P < 0.05$ ) in the number of paw licks induced by formalin when compared to the control. This result has the same trend for both the early phase and late phase. The analgesic effect is more pronounced at the late phase than at the early phase (Figure 3).

The anti-oxidant activities of the acetone extract were shown in Table 2. The ABTS activity was higher than that of DPPH and this was comparable to that of gallic acid. Though the FRAP activity was low; however, the plant is rich in total phenol.



**Figure 2.** Effect of acetone leaf extract of *Vernonia amygdalina* and indomethacin on mice writhing reflex induced by acetic acid (n=5), mean  $\pm$  S.D.



**Figure 3.** Analgesic effect of acetone leaf extract of *Vernonia amygdalina* and indomethacin using formalin test on mice Mean  $\pm$  S.D., (n = 5).

**Table 2.** Antioxidant properties of the acetone leaf extract of *Vernonia amygdalina*

Test	Results
Total Phenol (GAE)	16.1 $\pm$ 0.01
ABTS (GAE)	206.3 $\pm$ 0.15
DPPH (AAE)	118.7 $\pm$ 0.01
FRAP (AAE)	4.3 $\pm$ 0.004

## Discussion

This study showed that the *Vernonia amygdalina* is safe for use medicinally and nutritionally as attributed to by the acute toxicity experiment. Since the acute toxicity describes the adverse effects of a substance that result either from a single exposure as it was done in this study and yet there was no adverse effect at the 3200 mg/kg dose, one may easily assume that this plant is safe. The plant extract caused a reduction in paw oedema induced by carrageenan at 1hr, 2hr and 3hr when compare to the control. The effect of 200mg is more pronounced than 100mg. 200mg at 3 hr is more

pronounced than the standard drug. Carrageenan-induced rat paw oedema is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products and is believed to be biphasic.<sup>27</sup> The first phase (1 hr) involves the release of serotonin and histamine while the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins.<sup>18</sup> This oedema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors, including prostaglandins.<sup>28</sup> It has been shown that species of *Vernonia amygdalina* demonstrated inhibitory effects on the biosynthesis of prostaglandins E<sub>2</sub> (PGE<sub>2</sub>) and prostaglandins D<sub>2</sub> (PGD<sub>2</sub>).<sup>29</sup> It is also known that *Vernonia amygdalina* contains tannins<sup>30</sup> and these compounds are known to be potent cyclooxygenase-1 inhibitors. Because the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation,<sup>18</sup> these results are an indication that *Vernonia amygdalina* can be effective in acute inflammatory disorders. Based on the results of this study, we can suggest that the anti-inflammatory effect of this extract may be attributed to inhibition of prostaglandin release and other mediators.<sup>31-33</sup> Since the plant is rich in flavonoids, steroids, essential oil and tannin, this may also be partly responsible for the analgesic effects exerted in animal models of nociception.<sup>34-37</sup> This result is similar to that of histamine-induced paw oedema.

In the paw licking test, the acetone leaf extract of *V. amygdalina* (100 and 200 mg/kg) caused a significant decrease in the number of paw licks induced by formalin when compared to the control. The analgesic effect of both 100 and 200 mg/kg doses of the extract was greater relative to the standard drug (indomethacin). The paw licking (formalin) test is believed to represent a significant model of clinical pain. This test also produced a distinct biphasic response to pain stimulus and different analgesic compounds may act differently in the early and late phases of this test. The early phase is the result of direct chemical activation of nociceptive primary afferent fibers, but the factors that contribute to the late phase are not well defined.<sup>38,39</sup> Therefore, this test can be used to clarify the possible mechanisms of anti-nociceptive effect of a test compound.<sup>38</sup> However, some studies also believed that the nociception produced in phase 2 of the formalin test is a result of chemical insult resulting in tissue damage. Tissue destruction produces mediators of inflammation such as histamine, bradykinins, prostaglandins and serotonin. NSAIDs block the production of prostaglandins therefore; sensitization of the peripheral nervous tissue is reduced, resulting in less nerve stimulation and ultimately less pain.<sup>40</sup> Centrally-acting drugs such as opioids inhibit both phases equally<sup>41</sup> but, peripherally-acting drugs, such as cyclooxygenase inhibitors (aspirin and indomethacin) and corticosteroids only inhibit the late phase.<sup>27,42,43</sup> This

study also confirmed the fact that indomethacin was more effective at the late phase than the early phase.

In the acetic acid writhing test, acetone leaf extract of *Vernonia amygdalina* caused a significant decrease in the number of writhes at doses 100 and 200mg/kg when compared to the control. The extract (100 and 200mg/kg) and indomethacin exhibited a significant antinociceptive power. The 100 and 200mg/kg doses of the extract showed a significant analgesic effect relative to the standard drug. The writhing test was first described by Koster et al<sup>44</sup> and has since received wide acceptance as an experimental model in the screening of drugs as analgesic and anti-inflammatory agents. It is considered as a typical model for evaluating visceral inflammatory pain. The most important pathway for transmission of inflammatory pain especially by acetic acid is sensitive to bradykinins, prostaglandins, and cytokines including interleukins 1 $\beta$  and 8 as well as TNF- $\alpha$ . Signals are then sent to the central nervous system through the sensory afferent C-fibre entering the dorsal horn. As a result of this, the model allows for evaluation of both central- and peripheral-acting analgesic agents.<sup>45,46</sup> When put together therefore, the extract exhibited both anti-inflammatory and antinociceptive properties.

The anti-oxidant properties of this plant were also evaluated in this study. In recent years, more and more people are exploring how to supplement their diet with antioxidants, especially those derived from natural sources. Several studies have been making evaluation of anti-oxidant properties in natural botanicals and herbs, and making research of their potential uses as nutraceutical ingredients because they are rich in natural ascorbic acid (vitamin C) and dietary fibres.<sup>28,47,48</sup> Some studies on *Vernonia amygdalina* reported that the plant is rich in flavonoids, tannins and saponins; and these may play some roles in the anti-oxidative effect observed.<sup>49-52</sup> The plant is rich in flavonoids and flavonoids are known to be good antioxidants, and luteolin (a flavonoid found in *Vernonia amygdalina*) has been reported to be a strong antioxidant.<sup>53</sup> Igile et al<sup>54</sup> confirmed that luteolin is more potent as an antioxidant than BHT, and reported that its glucosides – luteolin 7-*O*- $\beta$ -glucuroniside and luteolin 7-*O*- $\beta$ -glucoside also have anti-oxidant activities. A study of oxidative stress in diabetic rats showed that the aqueous extracts of VA decreased the levels of serum malondialdehyde, indicative of anti-oxidant property.<sup>50</sup> The findings of Iwalokun et al<sup>55</sup> and Adaramoye et al<sup>56</sup> corroborate the antioxidant properties of this plant. A study by Owolabi et al<sup>57</sup> further showed that both the ethanolic and aqueous extracts of VA have potent anti-oxidant abilities. Antioxidants have the capability to prevent oxidative stress caused by diseases like cancer, inflammation, cardiovascular diseases as well as aging, because the anti-oxidants can eliminate the free radicals, which make these chronic diseases more debilitating.<sup>58</sup> The acetone extract of this plant also exhibited antioxidant properties hence its continuous use as vegetable for human consumption is encouraged.

## Conclusion

It could then be concluded that the acetone extract of this plant exhibited anti-inflammatory, antinociceptive as well as anti-oxidant properties. The results from this study may have thus justified the use of this plant for medicinal and nutritional purposes.

## Acknowledgments

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## Conflict of Interest

The authors declare no conflict of interest.

## References

1. Ijeh II, Ejike CECC. Current perspectives on the medicinal potential of *Vernonia amygdalina* Del. *J Med Plant Res* 2011;5(7):1051-61.
2. Singha SC. Medicinal Plants of Nigeria. Lagos, Nigeria: Nigerian National Press; 1965.
3. Dalziel JM. The Useful Plants of West Tropical Africa. London, UK: Crown Overseas Agents for the Colonies; 1937.
4. Getahun A. Some Common Medicinal and Poisonous Plants Used in Ethiopian Folk Medicine. Addis Ababa, Ethiopia: Faculty of Science, Addis Ababa University; 1976.
5. Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del (Compositae). *Trans R Soc Trop Med Hyg* 2000;94(6):694-5.
6. Huffman MA. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proc Nutr Soc* 2003;62(2):371-81.
7. Adedapo AA, Otesile AT, Soetan KO. Assessment of the anthelmintic efficacy of the aqueous crude extract of *Vernonia amygdalina*. *Pharm Biol* 2007;45(7):564-8.
8. Challand S, Willcox M. A clinical trial of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria. *J Altern Complement Med* 2009;15(11):1231-7.
9. Egedigwe CA. Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats (Thesis). Nigeria: Department of Biochemistry, MOUAU; 2010.
10. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin Exp Immunol* 2007;147(2):227-35.
11. Drozdova IL, Bubenchikov RA. Composition and anti-inflammatory activity of polysaccharide complexes extracted from sweet violet and low mallow. *Pharm Chem J* 2005;39(4):197-200.
12. Van der Vliet A, Eiserich JP, Cross CE. Nitric oxide: a proinflammatory mediator in lung disease? *Resp Res* 2000;1(2):67-72.

13. Dhikav V, Singh S, Anand KS. Newer non-steroidal anti-inflammatory drugs—a review of their therapeutic potential and adverse drug reactions. *J Indian Acad Chem Med* 2002;3(4):332-8.
14. Sies H. Strategies of antioxidant defense. *Eur J Biochem* 1993;215(2):213-9.
15. Baillie JK, Thompson AA, Irving JB, Bates MG, Sutherland AI, Macnee W, et al. Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial. *QJM* 2009;102(5):341-8.
16. Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP, et al. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. *Int J Pharmacol* 2006;2(4):435-8.
17. Shale TL, Stirr WA, Van Staden J. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. *J Ethnopharmacol* 1999;67(3):347-54.
18. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). *J Ethnopharmacol* 2006;104(1-2):87-91.
19. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. *J Ethnopharmacol* 2006;104(3):410-4.
20. Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB, et al. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J Ethnopharmacol* 2005;98(3):267-73.
21. Wheeler-Aceto H, Porreca F, Cowan A. The rat paw formalin test: comparison of noxious agents. *Pain* 1990;40(2):229-38.
22. Vincler M, Maixner W, Vierck CJ, Light AR. Estrous cycle modulation of nociceptive behaviors elicited by electrical stimulation and formalin. *Pharmacol Biochem Behav* 2001;69(3-4):315-24.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999;26(9-10):1231-7.
24. Liyana-Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J Agric Food Chem* 2005;53(7):2433-40.
25. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996;239(1):70-6.
26. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agric Food Chem* 2003;51(3):609-14.
27. Chen YF, Tsai HY, Wu TS. Anti-inflammatory and analgesic activities from roots of *Angelica pubescens*. *Planta Med* 1995;61(1):2-8.
28. Adedapo AA, Sofidiya MO, Masika PJ, Afolayan AJ. Anti-inflammatory and analgesic activities of the aqueous extract of *Acacia karroo* stem bark in experimental animals. *Basic Clin Pharmacol Toxicol* 2008;103(5):397-400.
29. Li RW, Myers SP, Leach DN, Lin GD, Leach G. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. *J Ethnopharmacol* 2003;85(1):25-32.
30. Xu GJ. The Chinese Materia Medica. Beijing, China: Chinese medicine and technology press; 1996.
31. Spector WG, Willoughby DA. The activation by irritants of slow-contracting substances, and their relation to the vascular changes of inflammation in the rat. *J Pathol Bacteriol* 1962;84:391-403.
32. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J Pathol* 1971;104(1):15-29.
33. Ferreira SH, Moncada S, Vane JR. Some effects of inhibiting endogenous prostaglandin formation on the responses of the cat spleen. *Br J Pharmacol* 1973;47(1):48-58.
34. Peres MTL, Delle Monache F, Pizollatti MG, Santos ARS, Beirith A, Calixto JB, et al. Analgesic compounds of *Croton urucurana* Baillon. Pharmacological criteria used in their isolation. *Phytother Res* 1998;12(3):209-11.
35. Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel Filho V, Yunes RA. Naturally occurring antinociceptive substances from plants. *Phytother Res* 2000;14(6):401-18.
36. Amanlou M, Dadkhah F, Salehnia A, Farsam H, Dehpour AR. An anti-inflammatory and antinociceptive effects of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract. *J Pharm Pharm Sci* 2005;8(1):102-6.
37. Hejazian S. Analgesic effect of essential oil (EO) from *Carum copticum* in mice. *World J Med Sci* 2006;1(2):95-9.
38. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain* 1992;51(1):5-17.
39. Abbadie C, Taylor BK, Peterson MA, Basbaum AI. Differential contribution of the two phases of the formalin test to the pattern of c-fos expression in the rat spinal cord: studies with remifentanyl and lidocaine. *Pain* 1997;69(1-2):101-10.
40. Sateesh S, Mathiazhagan S, Anand S, Parthiban R, Suresh S, Sankaranarayanan B, et al. A study on analgesic effect of *Caryophyllus aromaticus* by formalin test in albino rats. *Int J Pharm Sci Invent* 2013;2(1):28-35.
41. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. *Pain* 1989;38(3):347-52.
42. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 1987;30(1):103-14.
43. Rosland JH, Tjolsen A, Maehle B, Hole K. The formalin test in mice: effect of formalin concentration. *Pain* 1990;42(2):235-42.

44. Koster R, Anderson M, Beer EJ. Acetic acid analgesic screening. *Fed Proc* 1959;18:412-6.
45. Deraedt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur J Pharmacol* 1980;61(1):17-24.
46. Risso WE, Scarminio IS, Moreira EG. Antinociceptive and acute toxicity evaluation of *Vernonia condensata* Baker leaves extracted with different solvents and their mixtures. *Indian J Exp Biol* 2010;48(8):811-6.
47. Adedapo AA, Sofidiya MO, Afolayan AJ. Anti-inflammatory and analgesic activities of the aqueous extracts of *Margaritaria discoidea* (Euphorbiaceae) stem bark in experimental animal models. *Rev Biol Trop* 2009;57(4):193-200.
48. Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ. Polyphenolic and biological activities of the acetone, methanol and water extracts of *Argemone subfusiformis* (Order: Ranunculales, Family: Papaveraceae) and *Urtica urens* (Order: Rosales, Family: Urticaceae). *Rev Biol Trop* 2010;58(4):1517-31.
49. Ezekwe CI, Obidoa O. Biochemical effect of *Vernonia amygdalina* on rats liver microsomes. *Nig J Biochem Mol Biol* 2001;16(3):1745-98.
50. Nwanjo HU. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma protein and oxidative status in diabetic rat models. *Nig J Physiol Sci* 2005;20(1-2):39-42.
51. Adesanoye OA, Farombi EO. Hepatoprotective effects of *Vernonia amygdalina* (astereaceae) in rats treated with carbon tetrachloride. *Exp Toxicol Pathol* 2010;62(2):197-206.
52. Lolodi O, Eriyamremu GE. Effect of methanolic extract of *Vernonia amygdalina* (common bitter leaf) on lipid peroxidation and antioxidant enzymes in rats exposed to cycasin. *Pak J Biol Sci* 2013;16(13):642-6.
53. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochem* 1986;25(2):383-5.
54. Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *J Agric Food Chem* 1994;42(11):2445-8.
55. Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA, Akinwande AI. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *J Med Food* 2006;9(4):524-30.
56. Adaramoye OA, Akintayo O, Achem J, Fafunso MA. Lipidlowering effects of methanolic extracts of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vasc Health Risk Manag* 2008;4(1):235-41.
57. Owolabi MA, Jaja SI, Oyekanmi OO, Olatunji OJ. Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *Vernonia amygdalina*. *J Complement Integr Med* 2008;5(1):doi:10.2202/1550-3840.1152.
58. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem* 2006;97(1):109-14.