Trop J Nat Prod Res, January 2019; 3(1):22-25

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of the analgesic potential of *Basella alba* (L.) leaves (Basellaceae)

Adeola V. Abioye*, Zainab Mohammed, Abubakar Ahmed, Ayodeji E. Ayeni

Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria - Nigeria

ARTICLE INFO

Article history:
Received 19 December 2018
Revised 01 February 2019
Accepted 02 February 2019
Published online 04 February 2019

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ABSTRACT

Basella alba is used in traditional medicine as an analgesic, androgenic, anticonvulsant, anti-inflammatory, antifungal, and in the treatment of anaemia, infertility in men, constipation, gonorrhea and hypertension. This study aimed at evaluation of the analgesic potential of *B. alba* leaves towards its further utilization in drug development. The powdered leave of *B. alba* was successively extracted using n-hexane, ethyl acetate and methanol. Acute toxicity studies of the extracts were also conducted using the Lorke's method. The *B. alba* leave extracts were evaluated using the acetic acid-induced writhing test and thermally-induced pain in Albino mice. The results from the study showed that methanol and ethyl acetate extracts showed no sign of toxicity but the highest dose of n-hexane extract (1000 mg/kg) showed a sign of toxicity. The leave extracts of *B. alba* produced a significant ($p < 0.05$) and dose-dependent anti-nociceptive effect against acetic acid-induced peritoneal pain when compared with the standard drug (piroxicam). The latency of the extract increased significantly *($p< 0.05$)* in a dose-dependent manner and the findings showed that the n-hexane extract of *B. alba* has higher analgesic activity when compared to the methanol and ethyl acetate extracts. This provides a rationale for its traditional use in the management of pain.

Keywords: *Basella alba*, Analgesic, Toxicity, Pain.

Introduction

Basella alba (L.) commonly referred to as Indian spinach is one of the traditional leafy vegetables popularly cultivated and used as soup among the Yoruba people of South Western Nigeria. It is commonly referred to as 'Amunututu' among the Yorubas 'Alayyahun zomo' in Hausa, and 'Logi' (Igbo) and 'Alandi' in Fulfulde Language, all in Nigeria languages.

Ever since, medicinal plant is any substance with one or more of its organs containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs.[1] They have been found to be useful in the treatment and management of various health problems.

Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easily accessible and efficacious. A large proportion of the world's population depends on traditional medicine because of the scarcity, high cost of orthodox medicine,[2] and unpleasant side effects,[3] It is estimated that about 75% of the populace by choice solve their health problems consulting traditional healers in Nigeria.[4] Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents.[5] The majority of rural dwellers does not have access to modern health care. In many developed countries, complementary and alternative medicine (CAM) is becoming more popular and the percentage of the population which has used CAM at least once is 48% in Australia, 70% in Canada, 42% in the United States of America, 38% in Belgium and 75% in France, meanwhile In Africa, up to 80% of the population use traditional medicine to help meet their health care needs.[6] Many rural communities have great faith in traditional medicine especially the explicable aspect which also recognize their socio-cultural and religious background which orthodox medicine seems to neglect.

In recent years there has been an increasing interest by researchers in the use of naturally occurring biologically active compounds of medicinal value.[7] Artemesinin, atropine, aspirin, camptothecin, codeine, digoxin, morphine and pilocarpine, are a few examples of useful plant drugs.[8]

The only true medicines ever used initially are plants.[9] The use of plants as medicinal agents have been over thousands of years with records as far back to Mesopotamia,[10] natural products of plant origin have got its own importance and have remained the most important source of new drugs, one of such medicinal plant is *B. alba.*

The International Association for the study of pain clearly defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.[11] This definition emphasizes both the physical and emotional nature of pain.[12] Pain triggers various responses in the spinal cord and the brain, reflexes, conscious perception, cognitive learning and memory processes, emotional reactions such as drug addiction and depression.[13] Some plants species which have been used traditionally as analgesics or have yielded compounds which are used in pain relief include *Cannabis sativa, Papaver somniferum, Conium maculatum*, and *Mandragora officinanum*.

Hence, medicinal plants research with alleged folkloric use as analgesic agents should, therefore, be viewed as a fruitful and logical strategy in the search for new pain relief drugs and *Basella alba* is one of such plant, with little or no scientific evaluation regarding its analgesic efficacy and safety.

Materials and Methods

Experimental animal

Adult Swiss Albino Mice (20 – 30 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard environmental conditions (light/dark cycle) ± 25ºC and fed with standard rodent pellets from PZ Feed Mill,

*Corresponding author. E mail: vabioye9@gmail.com
Tel: +2347039818694

Citation: Abioye VA, Mohammed Z, Ahmed A, Ayeni EA. Evaluation of the analgesic potential of *Basella alba* (L.) leaves (Basellaceae). Trop J Nat Prod Res. 2019; 3(1):22-25. doi.org/10.26538/tjnpr/v3i1.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Zaria-Kaduna State, Nigeria and water was provided *ad libitum*. Experiments were carried out in the Main Laboratory of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. Ethical clearance and consideration on the use and care of laboratory animals were obtained from the Ahmadu Bello University Zaria Animal Ethical Committee.

Plant materials

The leaves of *B. alba* were collected from Ita-Elepa Village, Ilorin West Local Government Area, Ilorin, Kwara State, Nigeria between the month of April - May 2016. Fresh samples of the whole plant were taken to the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification by Mallam Namadi Sanusi and with a voucher no:7519. The leaves were air dried and powdered using mortar and pestle and stored in an airtight container for further use.

Preparation of extracts

Leaf powder (500 g) of *B. alba* was extracted successively in 2 L each of analytical graded n-hexane, ethyl acetate and methanol solvents using cold maceration process. It was evaporated to dryness on exposure to air and stored in a desiccator for further experiment.

Chromatographic procedure

The TLC profile of all the extracts was studied on pre-coated silica gel plates. Developed plates were sprayed with specific detecting reagents such as ferric chloride for phenolic compounds, aluminum chloride for flavonoids, Liberman-Burchard for steroids/triterpene and Dragendorff's for alkaloids.[14] The Thin layer chromatographic plates developed were visualized with different detecting reagents and some were viewed under UV light in order to detect the presence of fluorescent compounds.

Acute toxicity study

The acute toxicity study was carried out using mice of both sexes according to the method previously described.[15] The procedure was divided into two phases, at the first phase, nine mice were divided into three groups of three mice each and treated with the extract at doses of 10, 100 and 1000 mg/kg body via the oral route and observed for signs and symptoms of toxicity for 24 hours.

The second phase was proceeded with the stated doses according to the method [15] i.e. 600 mg/kg, 1000 mg/kg, 1600 mg/kg and 2900 mg/kg for n-hexane, and 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg for methanol and ethyl acetate extracts. This involved 4 groups of one animal each. The volume to be administered to each animal based on their weight was calculated and administered orally. Signs of toxicity were observed for 24 hours. The number of deaths which occurred was recorded.

The lethal dose (LD_{50}) was calculated as the geometric mean of the lowest lethal dose that caused death and the highest non-lethal dose that did not cause death.

$$LD_{50} = \sqrt{\boldsymbol{minimumlethaldose} \times \boldsymbol{maximumnon} - \boldsymbol{lethaldose}}$$

Acetic acid-induced writhing in mice

The analgesic activity of the leaf extracts of *B. alba* was carried out according to a previously described method.[16] Five groups of 6 mice per group (n = 6) were used for this experiment. The first group served as the negative control and was treated with 10 mL/kg of distilled water. The second, third and fourth groups were treated with graded doses of the methanol and ethyl-acetate extract (200, 400 and 800 mg/kg, respectively), and n-hexane extract (100, 200, and 400 mg/kg, respectively), while the fifth group was treated with piroxicam (10 mg/kg) as positive control. After 60 minutes, all five groups were administered 0.1 mL of 0.6% acetic acid solution via intraperitoneal injection, and the number of abdominal writhes produced in the mice were observed and counted 5 times repeatedly after injection for 10 minutes. The percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Mean No. of Writhes(Control)} - \textit{Mean No. ofWrithes(test)}}{\textit{Mean No. ofWrithes (Control)}} \text{ X } 100$$

Hot plate method

This method was carried out using a standard procedure.[17] The paws of mice are very sensitive to heat at temperatures which are not damaging to the skin. The responses such as jumping, withdrawal of the paws and licking of the paws were observed. The temperature of the hot plate was set at $45^{\circ}\pm1^{C}$. Thirty (30) Swiss albino mice of both sexes were used; the mice were fasted 12 hours prior to the experiment and divided into five groups of six mice each and the extracts were administered orally. Group 1 served as negative control (distilled water 10 mL/kg), group 5 received positive control (Morphine 10 mg/kg) while group 2, 3, and 4 received methanol and ethyl-acetate extract 200, 400 and 800 mg/kg, respectively, and n-hexane extract 100, 200, and 400 mg/kg, respectively. The animals were placed on the hot plate individually, and the time, until either lick or jumping occurred (reaction time, RT), was recorded using stop-watch, at time intervals of 0, 60, 90, 120, and 150 minutes after treatment. The percentage protection against thermal pain stimulus was calculated applying the formula:

$$\% \text{ protection against thermal stimulus} = \frac{\text{Treatment Mean (Ta)} - \text{negative Control Mean (Tb)}}{\textit{negativeControlMean (Tb)}} \text{ X } 100$$

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). For data presentation, graphs, tables, plates and figures were used where appropriate. Single point data were analyzed using one-way analysis of variance (ANOVA); while time-dependent point data were analyzed using repeated measure ANOVA. This was followed by *Dunnet* and *Bonferroni* posthoc tests where appropriate, with result considered significant at $p \leq 0.05$.

Results and Discussion

The median lethal dose LD_{50} of n-hexane extract of *B. alba* was found to be 1319.09 mg/kg. This suggests that the extract is slightly toxic.[15] The acute toxicity of ethyl acetate and methanol extracts was greater than 5000 mg/kg. The LD_{50} results for ethyl acetate and methanol extracts could be assumed to be relatively safe and non-toxic. LD_{50} which is the index of acute toxicity is a useful index in evaluating safety margin but not to be regarded as a biological constant as different results are obtained on repetitions or when determinations are carried out in different laboratories.[15]

The oral LD_{50} value of the methanol and ethyl-acetate extract of *B. alba* in mice were greater than 5,000 mg/kg body weight, and the LD_{50} of n-hexane extract was estimated to be greater than 2900 mg/kg. This suggests that the methanol and ethyl-acetate extracts are practically non-toxic orally, at acute dose levels according to the lethal dose classification of toxic levels of chemicals.[15]

The chromatographic profile of the leaf extracts of *B. alba* revealed the presence of cardiac glycosides, saponins, steroids/terpenoids, flavonoids and alkaloids. These constituents are known to be responsible for several pharmacological activities including the observed analgesic effect. Analgesic activities have been observed with flavonoids as well as tannins.[18]

Flavonoids have been reported to inhibit prostaglandin synthesis through inhibition of enzymes involved in prostaglandin synthesis such as prostaglandin synthase.[19] Extracts of the plant containing flavonoids are known to modify the production of cyclooxygenase and lipoxygenase which are essential in prostaglandin synthesis.[20] Flavonoids also possess anti-oxidant activities which are responsible for the inhibitory effect on several enzymes. There are also reports of alkaloids and saponins analgesic effects.[18] However, all the constituents present in the extract might have attributed to the analgesic effect of the methanol leaf extracts of *Basella alba*.

The acetic acid-induced writhing test is a sensitive method used to evaluate potential analgesic drugs or compounds that act peripherally. The injection of acetic acid intraperitoneally produces an abdominal writhing response due to sensitization of chemoreceptors by prostaglandins.[21] This model has been associated with an increased level of prostaglandins particularly PGE_2 and PGEα in peritoneal fluids as well as lipoxygenase products. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesic like NSAIDs.[22] Piroxicam reduced the number of writhes induced by acetic acid by inhibiting cyclooxygenase (COX) in peripheral tissues thereby blocking the release and/or synthesis of inflammatory mediators.[23] Oral administration of the extracts demonstrated significant and dose-dependent attenuation of acetic acid-

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Effect of Methanol leaf Extract of *Basella alba* on Acetic Acid-Induced Writhes in Mice.

Treatment (mg/kg)	Mean No. of writhes	Percentage Inhibition
10 mL/kg Distilled water	29.80 ± 0.74	
200	22.20 ± 1.59	27.52
400	20.60 ± 1.21*	30.87
800	12.40 ±1.21*	58.39
10 mL/kg Piroxicam	5.80 ± 1.24*	80.52

Data are expressed as mean ± SEM (n = 6 per group) and analyzed using one-way ANOVA followed by Dunnett *post hoc* test* $p < 0.001$ significant difference from negative control (Distilled Water 10 mL/kg).

Table 2: Effect of Ethyl acetate leaf Extract of *Basella alba* on Acetic Acid-Induced Writhes in Mice.

Treatment (mg/kg)	Mean No. of writhes	Percentage Inhibition
10 mL/kg Distilled water	29.80 ± 0.74	
200	19.80 ± 1.35*	33.56
400	12.00 ± 1.18*	59.73
800	10.60 ±1.08*	64.43
10 mL/kg Piroxicam	5.80 ± 1.24*	80.52

Data are expressed as mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Dunnett *post hoc* test* $p < 0.001$ significant difference from negative control (Distilled Water 10 mL/kg)

Table 3: Effect of n-Hexane leaf Extract of *B. alba* on Acetic Acid-Induced Writhes in Mice.

Treatment (mg/kg)	Mean No. of writes	Percentage Inhibition
10 mL/kg Distilled water	29.80 ± 0.74	
200	16.40 ± 1.29	13.42
400	12.00 ± 1.18*	44.97
800	8.60 ± 1.17*	71.14
10 ml/kg Piroxicam	5.80 ± 1.24*	80.52

Data are expressed as mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Dunnett *post hoc* test* $p < 0.001$ significant difference from negative control (Distilled Water, 10 mL/kg).

induced writhes in mice (Table 1-3). Similarly, positive control piroxicam (10 mg/kg p.o) also exhibited significant writhes inhibition in mice, eliciting a peripheral acting analgesic effect. The reduction of acetic acid-induced writhes in mice by the extracts indicates that the extract may be acting peripherally and/or centrally via the nociceptors. Thus, the analgesic effect exhibited by the extracts of the leaf of *Basella alba* may be due to inhibition of the synthesis and release of prostaglandins and other endogenous substances.

The hot plate method is the most commonly used thermal nociception model in the evaluation of central analgesic efficacy of drugs or compounds. The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of higher intensity.[24] Pain induced by a thermal stimulus of the hot plate is specific for centrally mediated nociception.[25]

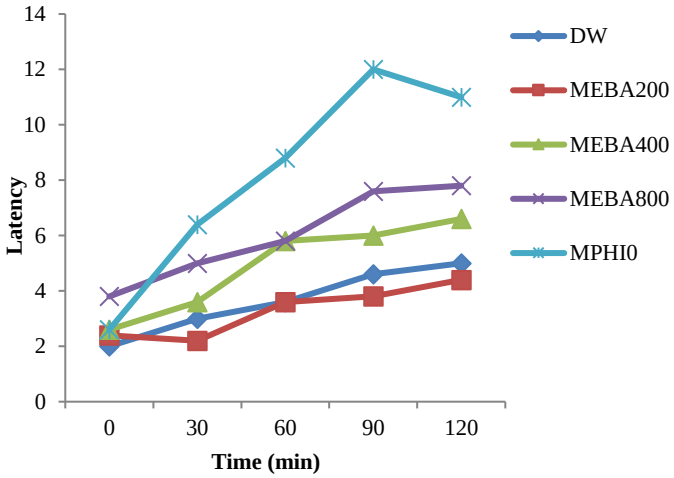

Figure 1: Effect of methanol leaf extract of *Basella alba* on hot plate test in mice.

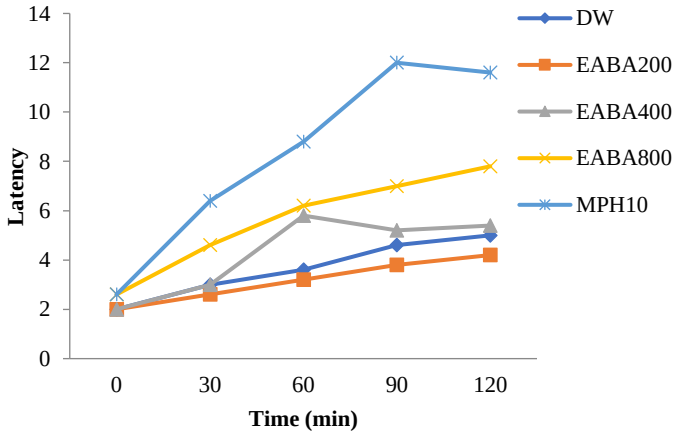

Figure 2: Effect of ethylacetate leaf extract of *Basella alba* on hot plate test in mice.

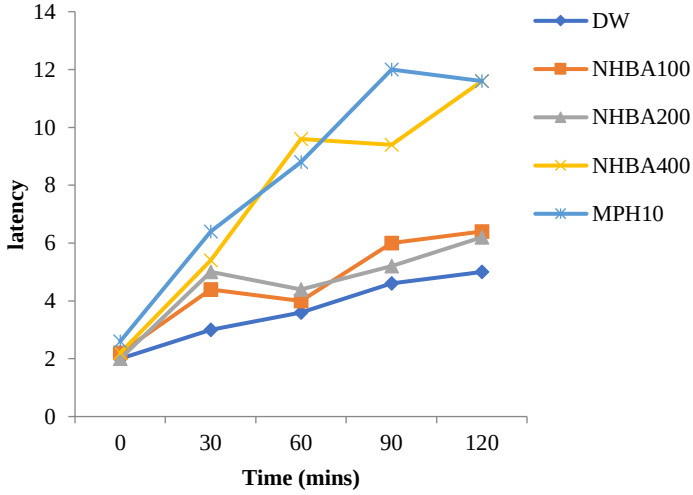

Figure 3: Effect of n-hexane leaf extract of *Basella alba* on hot plate test in mice.

Therefore, the prolongation of reaction latency to pain induced thermally in mice using this model suggests centrally acting anti-nociceptive activity.[26]The methanol leaf extract of *B. alba*, at different doses, significantly increased reaction time of mice in a dose-dependent pattern in hot plate test (Figure 1). The extract at the dose of 800 mg/kg gave the highest percentage of protection against thermally induced pain which was comparable to that of the control morphine (10 mg/kg). This demonstrates that the extracts possess anti-nociceptive activity mediated via a central mechanism.

Conclusion

The n-Hexane extract of *B. alba* showed higher analgesic activity compared to the methanol and ethyl-acetate extracts, which could be attributed to the presence of some non-polar secondary metabolites that could be responsible for the management of pain. The significant analgesic activity of the extract observed might be associated to the secondary metabolites such as flavonoids and tannins present in the plant and this may proffer scientific basis for its use, also the plant has a relatively safe acute toxicity level.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors sincerely appreciate the contributions of all the technical staff in the Department of Pharmacognosy and Drug Development, Faculty of Pharmacy, Ahmadu Bello University Zaria, Nigeria.

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