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Original Research Article

The Antinociceptive Property of *Brachystegia eurycoma* (Fabaceae) Partly Involves Stimulation of Opioid Pathways and Mitigation of the Action of Reactive Radicals.

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Abstract

Introduction: *Brachystegia eurycoma* (Fabaceae) has been reported to mitigate peripheral and centrally mediated nociception. However, the probable mechanisms involved in this antinociceptive effect have not been investigated. This study evaluated the possible mechanisms involved in the antinociceptive effect of *Brachystegia eurycoma* (BE).
Methods: Mice were selected according to standard protocols. They were treated with 100, 200 and 400 mg/kg, per oral of BE. One (1) hour post-treatment, 0.6 %v/v acetic acid (10 ml/kg, ip) was administered, and the number of writhes was recorded every 5 mins for 30 mins. To investigate the possible mechanisms involved in the antinociceptive effect of BE, mice (n = 5) were pretreated with atropine (1.0 mg/kg, ip), naloxone (1.0 mg/kg, ip), haloperidol (0.1 mg/kg, ip), and ondansetron (1.0 mg/kg, ip), followed by treatment with BE (100 mg/kg, po) after 15 mins. 1-hour post-treatments, 0.6 %v/v acetic acid (10 ml/kg, ip) was administered, and the number of writhes was recorded for 30 mins. *In vitro* radical activity, total phenol and flavonoid contents were also determined.
Results: BE reduced writhing at all doses, exerting peak reduction at 100 mg/kg compared to control (130.3 ± 3.1 vs 64.8 ± 3.5, $p < 0.05$). Naloxone caused a 24 % reversal in the antinociceptive effect of BE ($p < 0.05$). BE exerted a concentration-dependent radical scavenging activity with an IC_{50} (2.4 µg/kg) higher than ascorbic acid. The total phenol content of the BE extract was estimated to be 156.1 ± 1.4 µg/ml GAE/g extract, while the total flavonoid content was determined to be -11.9 ± 3.2 mg QE/g extract.
Conclusion: The antinociceptive effect of BE partly involves the stimulation of opioid receptors and reduction in radical-induced nociception. However, further investigation would be needed to elucidate the mechanism involved with its antinociceptive activity.

Keywords: *Brachystegia eurycoma*, antinociceptive, acetic acid, opioid, radical scavenging

Indexing: Index Copernicus, African Index Medicus

Introduction

Brachystegia eurycoma (Fabaceae) is a medicinal plant of interest that is found growing in the forests of Nigeria and Cameroun [1]. It is particularly popular in the eastern part of Nigeria where it is used for culinary purposes. It is a large tree with diffused branches that bear fruits. The fruits, which occur as pods, contain seeds covered in a hard husk. In local communities, the plant is called "Achi" in Igbo, "Ekalado" in Yoruba, "Okweri" in Edo, and "Taura" in Hausa [2]. Aside from its nutritional value, the plant has been used for managing several ailments in ethnomedicine [3]. For instance, the crude extracts have reportedly been used as an analgesic, antihelminth, anti-asthmatic and anti-tubercule. Similarly, the seeds have been used as an anti-pyretic and laxative [1]. These effects have now been related to the presence of secondary metabolites present in these extracts. The stem bark, seed and leaf extracts have been documented to contain alkaloids, flavonoids, saponins, tannins and oil [4].

Several reports have documented the pharmacological and biological activities of this plant [5]. Moreover, the plant has been reported

to possess cytotoxic, analgesic, anti-inflammatory, antioxidant, and hypolipidemic activities, amongst others [1]. The plant has also been documented to be safe at employed doses [4,6].

The search for novel antinociceptive agents from medicinal plants is well documented and has included *Brachystegia eurycoma* (BE) [5]. Hence, the antinociceptive property of the plant has been reported [2]. However, the possible mechanistic pathway/targets involved in this antinociceptive effect have not been evaluated. Considering the role of various endogenous pathways and free radicals in the control of nociception [7,8], this present study evaluated the possible targets in the descending tract involved in the antinociceptive effect of *Brachystegia eurycoma*.

Materials and Methods

Animals

Male Swiss albino mice (18-26 g) were housed in the animal facility of the Department of Pharmacology & Toxicology, University of Benin, Nigeria. The animals had free access to drinking water and pelletized feed. They were maintained under standard lightening and environmental conditions. All experimental procedures were approved by the Ethics Committee, Faculty of Pharmacy, University of Benin (EC/FP/023/03).

Plant material

The stem bark of *Brachystegia eurycoma* (BE) was collected from the Basawa area of Sabongari, Kaduna State. The plant was identified and authenticated by Mr. Muazu of the National Institute for Pharmaceutical Research (NIPRID). A voucher specimen was kept in the Herbarium for future reference (NIPRID H.7096).

Plant extraction

The stem bark was sundried and pulverized to powder using a mechanical mill. The Powdered stem bark (300 g) was suspended in 1.5 L of methanol and extracted using repetitive Soxhlet extractions. The extract was concentrated to dryness using a hot-air oven (50 ºC) and was stored at 4 ºC until when needed for the experiment.

Phytochemical screening

Previous phytochemical screening has shown that the plant contains flavonoids, alkaloids, phenolic compounds, saponin and tannins in stem bark [1].

Acute toxicity study

An acute toxicity study revealed that the methanol extract of the stem bark did not induce any observable signs of toxicity or mortality after twenty-four (24) hours. [2].

Antinociceptive assay

Acetic acid-induced mouse writhing assay

A total of Twenty-five (25) mice were randomly allotted into five (5) groups containing five (5) mice each. Group I was treated with 0.9 % normal saline (10 ml/kg, po), and groups II, III and IV received 100, 200 and 400 mg/kg, po of the BE extract respectively. Group IV was treated with acetylsalicylic acid (ASA) (100 mg/kg, po). After one (1) hour post-treatment, 0.6 % v/v acetic acid was administered intraperitoneally to all treated groups at 10 ml/kg. The animals were immediately placed in individual cages to record the abdominal writhes for a period of 30 min, at intervals of five (5) min [9]. The percentage inhibition of writhes was calculated as stated.

$$\% \text{ inhibition} = \frac{Mean\ number\ of\ writhes\ (control) - Mean\ number\ of\ writhes\ (BE\ extract)}{Mean\ number\ of\ writhes\ (control} \ x\ 100 \ldots (1)$$

Assessment of the possible mechanisms involved in the antinociceptive effect of BE.

A total of twenty (20) mice were randomly selected for this assay. Mice (n = 5) were grouped and pretreated with atropine (1 mg/kg, ip) (muscarinic antagonist) ondansetron (1 mg/kg, ip) ($5HT_3$ antagonist), naloxone (1 mg/kg, ip) (opioid antagonist) and haloperidol (0.1 mg/kg, ip) (dopaminergic antagonist). Fifteen (15) mins after pretreatment with these antagonists, the animals were treated with the BE extract (100 mg/kg, po). One (1) hour after all treatments, 0.6 %v/v acetic acid was administered at 10 ml/kg, ip to all treated groups and animals were placed in separate cages to monitor abdominal writhes for thirty (30) min, at intervals of five (5) mins as in standard protocols [10,11].

In vitro antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (*DPPH) radical scavenging assay*

The DPPH free radical scavenging activity of BE extract was estimated using the modified method described [12]. Briefly, 0.2 mM of DPPH in methanol was prepared, and 1.0 ml of this solution was mixed with 3.0 ml of the extract in methanol (1, 2, 5, 10, 25, 50, 100 and 150 µg/ml). The reaction mixture was vortexed thoroughly and left in the darkroom for 30 min at 37ºC. The absorbance of the resulting mixtures was measured spectrophotometrically at 517 nm. Ascorbic acid was used as a reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A0-A1}{A0} \; x\; 100 \ldots (2)$$

Where, A_0 = DPPH radical + methanol, A_1 = DPPH radical + sample/standard.

The 50 % inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50 % of the DPPH free radical.

Phytochemical quantification assays

Total phenol

The total phenol content of the BE extract was determined by the addition of 5 ml of the BE extract in methanol (1 mg/ml) to 4.5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent. After mixing, the mixtures were maintained at room temperature for 5 minutes followed by the addition of 5 ml of 7 % sodium carbonate and 2 ml of distilled water. After mixing, the samples were incubated for 90 minutes at room temperature. The absorbance was measured by a UV spectrophotometer at a wavelength of 750 nm. The standard curve was prepared by gallic acid in different concentrations (12.5, 25, 50, 75, 100 and 150 mg/l) and was subjected to the same procedure. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract) as previously reported [13].

Total flavonoid

The total flavonoid content of the BE extract was estimated. Briefly, 0.5 ml of the BE extract in methanol (1 mg/ml) was mixed with 1.5 ml of methanol and 0.1 ml of 10 % aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 minutes. A standard curve was prepared with quercetin (12.5, 25, 50, 75, 100 and 150 mg/l). The absorbance of the samples was measured using a UV spectrophotometer at 415 nm. The results were expressed as milligram quercetin equivalent (QE) per gram of extract (mg QE/g extract) [11].

Statistical analysis

All analyses were done using GraphPad® Prism software (6.01). Data are presented as mean ± SEM. Analysis of variance (ANOVA) was used for statistical analysis and the significant differences between control and treatment groups were tested with Dunnett's test. $p < 0.05$ was considered significant.

Results

BE mitigated against acetic acid-induced writhing in mice.

All doses of BE (100, 200 and 400 mg/kg) significantly reduced the number of writhes induced by acetic acid compared to the control ($p < 0.05$) (Figure 1). The highest reduction in writhes (64.8 ± 3.5) occurred at 100 mg/kg. Similarly, the percentage inhibition was highest at 100 mg/kg (50.3 %, $p < 0.001$) compared to the control (Table 1). The reduction in the number of writhes by BE was not dose-dependent.

Pretreatment with naloxone reversed the antinociceptive effect of BE.

As shown in Figure 1, BE exerted its antinociceptive effect at 100 mg/kg compared to the control ($p < 0.001$). Pretreatment with atropine (Figure 2) and ondansetron (Figure 3) had no significant effect on the antinociceptive effect of BE ($p > 0.05$) (Figures 2 & 3), while haloperidol (Figure 4) amplified the antinociceptive effect of BE ($p < 0.05$), compared to BE alone. However, naloxone significantly reversed the antinociceptive effect of BE ($p < 0.05$), compared to BE alone (Fig. 5).

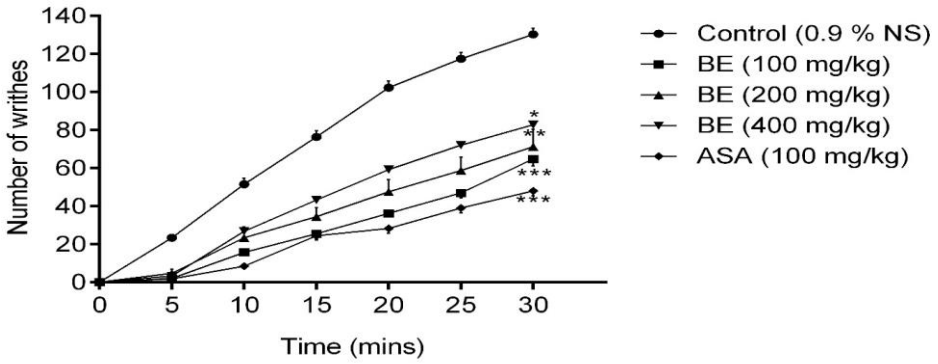

Figure 1: Effect of BE (100, 200 and 400 mg/kg, po) on acetic acid-induced writhing in mice
**p* <0.05, ***p* < 0.01, ****p* < 0.001, compared to control. NS = normal saline, BE = *Brachystegia eurycoma. ASA = Acetylsalicylic acid.*

Table 1: Percentage inhibition of writhes by BE in mouse writhing assay

Treatment	Dose (mg/kg)	% inhibition
Control	10	Nil
BE	100	50.2 ± 3.6***
BE	200	45.2 ± 9.2**
BE	400	36.8 ± 1.0*
ASA	100	63.2 ± 2.5***

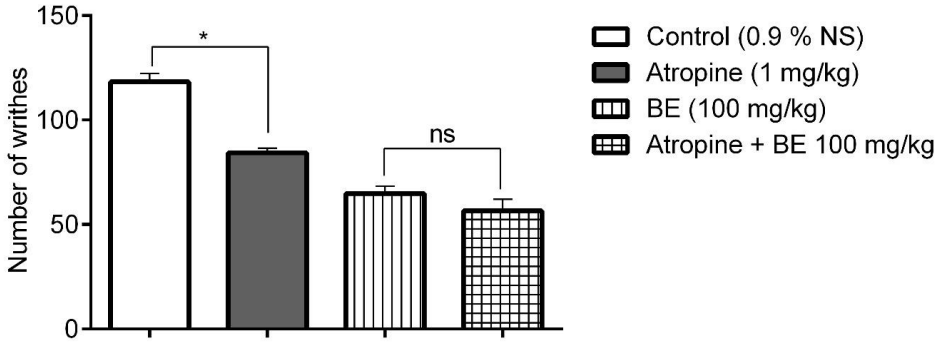

Figure 2: The effect of the muscarinic antagonist, atropine (1 mg/kg, ip) on the antinociceptive effect of BE (100 mg/kg, po) in the mouse writhing assay
**p* < 0.05 vs control. NS = normal saline, BE=*Brachystegia eurycoma*, ns = not significant.

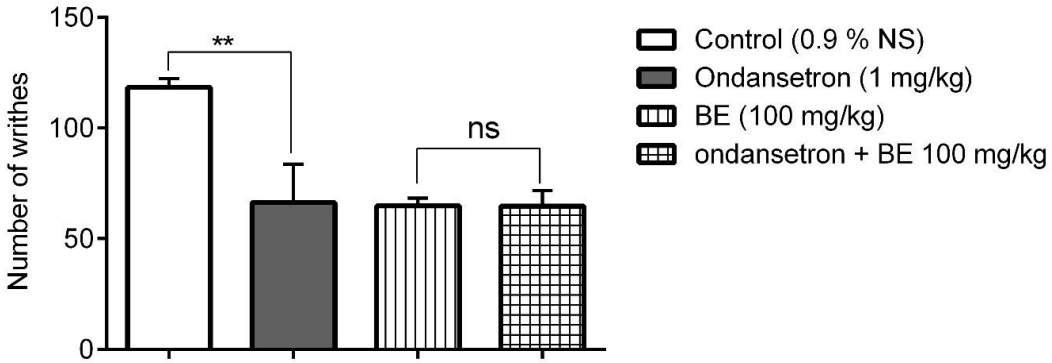

Figure 3: The effect of the $5HT_3$ antagonist, ondansetron (1 mg/kg, ip) on BE-induced antinociception in the mouse writhing assay
***p* < 0.05 vs control. NS = normal saline, BE=*Brachystegia eurycoma*, ns = not significant.

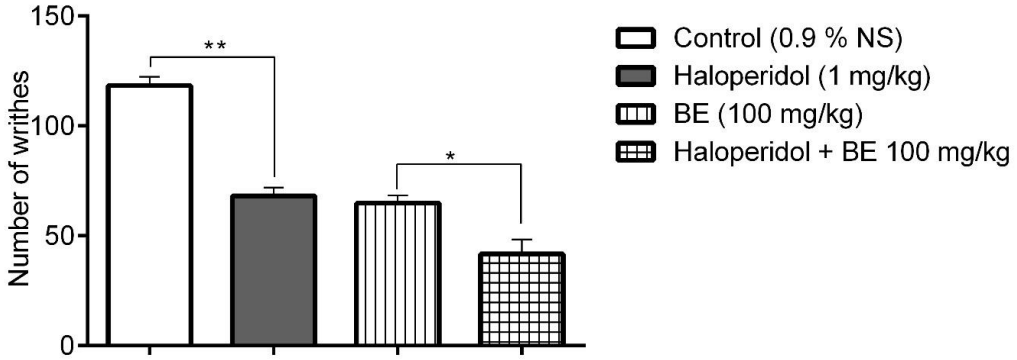

Figure 4: The effect of the dopamine antagonist, haloperidol (0.1 mg/kg, ip) on BE-induced nociception in the mouse writhing assay. *$p < 0.05$ vs BE alone, **$p < 0.05$ vs control. BE=*Brachystegia eurycoma.*

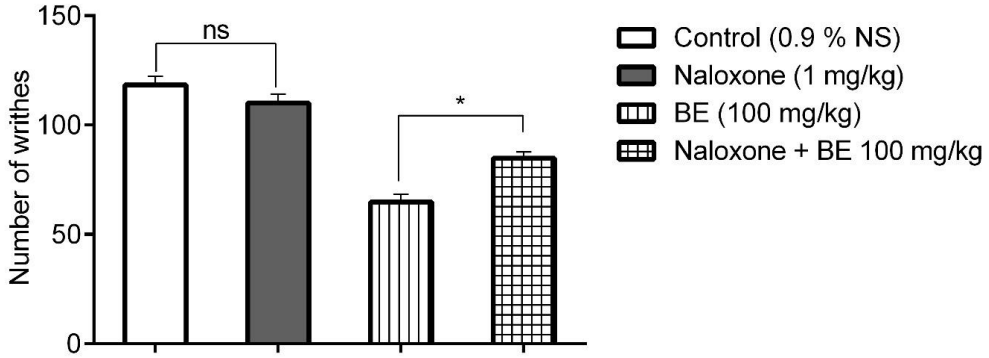

Figure 5: The reversal of BE-induced antinociceptive effect by the opioid antagonist, naloxone (1 mg/kg, ip) in the mouse writhing assay
*$p < 0.05$ vs BE alone, ns = not significant. BE = *Brachystegia eurycoma*.

BE exerted concentration-dependent scavenging activity.

As presented in Fig 6, the BE extract elicited an early onset reduction in DPPH activity. This effect increased with higher concentrations. There was a somewhat equipotent action with ascorbic acid at 25 µg/ml, IC50 values for ascorbic acid and BE were 5.9 µg/mL and 2.4 µg/ml.

Total phenol and flavonoid content.

As shown in Table 2, the total phenol content of the BE was 156.1 ± 1.4 µg/mL. $_{GAE/gextract}$. The total flavonoid content was determined to be - 11.9 ± 3.2 mg $_{QE/gextract}$

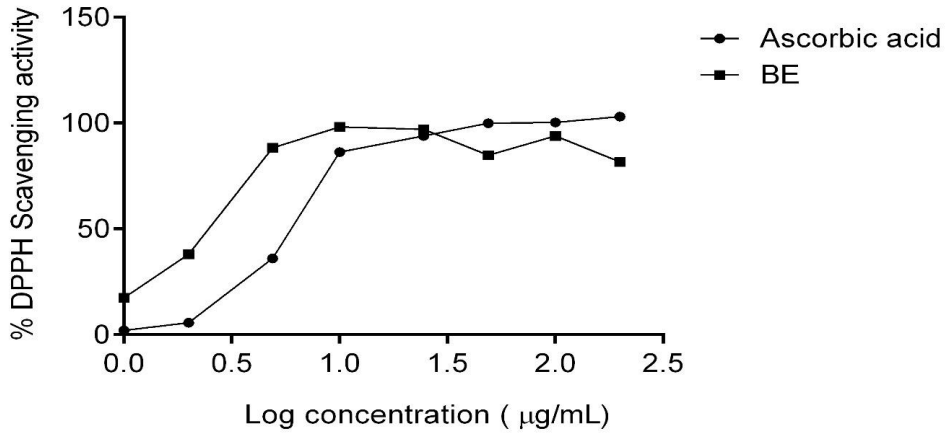

Figure 6: The *in vitro* radical scavenging activity (%) of BE and ascorbic acid (1.0 mg/ml) on 2,2-diphenyl-1-picrylhydrazyl (DPPH). Each data point is presented as triplicates (n = 3). BE = *Brachystegia eurycoma.*

Table 2: Total phenol and flavonoid contents of BE (1 mg/ml)

Assay	BE
Total phenols	156.1 ± 1.4 µg/mL
Total flavonoids	-11.9 ± 3.2 µg/mL

BE = *Brachystegia eurycoma.*

Discussion

The transmission of nociceptive stimuli is a process that involves local mediators, nociceptors, and a myriad of signal transduction mechanisms [13]. Offensive stimuli activate nociceptors, and the resulting signal is interpreted in the central nervous system. The CNS regulates nociception via signal transduction mechanisms involving the descending pathway [14]. This descending pathway (usually called the inhibitory pathway) is documented to express several ligands including opioids, serotonin, acetylcholine, and dopamine and their receptors. There is evidence to show that exogenous agents that suppress nociception act via interaction with these receptors. Similarly, medicinal plants that exert anti-nociceptive effects have been reported to act via the stimulation of some of these receptors in the descending tract [11,15,16].

In this present study, the mouse writhing assay was used to assess the mechanisms involved in the antinociceptive effect of BE because central and peripheral mechanisms are involved in this model [10]. The mouse writhing assay involves the injection of an irritant, acetic acid, into the peritoneum. The presence of acetic acid stimulates the release of local mediators such as bradykinin, prostaglandins, histamines, and substance P which stimulates nociceptors and triggers the nociceptive stimuli. The animal thus constricts its abdomen (writhes) in response to nociception [17].

BE has been reported to significantly reduce the number of writhes in the mouse writhing assay used to assess compounds for peripheral antinociceptive effect [2]. There was a similar observation in our present study where BE reduced the number of writhes at all doses and exerted peak activity at the lowest dose (100 mg/kg). As stated earlier, anti-nociceptive agents can enhance the activity of the descending tract via binding to receptors in this pathway. Hence, it was postulated that BE may also be exerting its antinociceptive effect in the mouse writhing assay via this mechanism and in a bid to elucidate the possible signaling pathway involved, BE was assessed for any likely interaction with the receptors in the descending pathway. Agents such as atropine (muscarinic antagonist), naloxone (opioid antagonist), haloperidol (dopamine antagonist) and ondansetron (serotonin antagonist) were administered to the mice before treating with BE.

The principle was based on the fact that antagonists that block the receptor via which BE exerts its antinociceptive effect would reverse the action of BE (seen as an increase in writhes) [11]. It was observed that only naloxone, the non-selective opioid antagonist, reversed the antinociceptive effect of BE. This insinuates that the antinociceptive effect of BE partly involves the stimulation of opioidergic receptors in the descending tract. This may partly underline the reported effect of BE in both peripheral and central nociception [2]. Opioid-like compounds alter the sensory perception of algesia, and they suppress the nociceptive stimuli irrespective of the cause or origin [18].

Redox species are intimately involved in the pathogenesis of nociception [19]. Local mediators and inflammatory cells release these redox species which assist in the propagating of the nociceptive stimuli. Hence, it is possible that agents that neutralize the production of reactive radicals may suppress nociception [20]. This is in congruence with the observation in our present study where BE neutralized the activity of the reactive radical, DPPH. BE exerted this anti-radical/oxidant effect at an IC_{50} that was 59.3 % higher than the standard antioxidant agent (ascorbic acid).

This observation has also been reported in previous studies that showed that the ethanol stem bark extract possessed anti-radical activity. In this study by Sofidiya and Familoni [3], the ethanol stem bark extract of BE possessed an IC_{50} that was higher than quercetin as it relates to the scavenging of hydroxyl radicals. Similarly, the total phenol content of BE was high and due to their rich electron density, phenols are known scavengers of reactive radicals. This also corroborates with the high reducing power of BE, which was higher than catechin. Hence, it is possible the anti-radical effect of BE may also partly contribute to its antinociceptive effect [21].

Conclusion

The present study has shown the BE may have exerted its antinociceptive effect via part interactions with opioid receptors in the descending tract and a reduction in the stimulation of nociceptors by the scavenging of reactive radicals. The promising expositions from this study have laid the foreground for future studies on the need for further mechanistic evaluations as it relates to the antinociceptive actions of BE.

Abbreviations

BE: *Brachystegia eurycoma*. IP: intraperitoneal. NIPRID: National Institute for Pharmaceutical Research. IC_{50}: 50 % inhibitory concentration. DPPH: 2,2-diphenyl-1-picrylhydrazyl. GAE: gallic acid equivalent.

Declarations

Ethical approval

All experimental protocols for this study were approved by the Ethics Committee of the Faculty of Pharmacy (EC/FP/023/03), University of Benin, Nigeria.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. OE conceptualized and designed the study. OE and SE carried out the pharmacological and biochemical assays. OE analyzed and wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Igbe I., and Okhuarobo A. Expanding the insights into the usefulness of *Brachystegia eurycoma* Harms: a review of its nutritional and medicinal values. J. Intercult. Ethnopharmacol. 2018; 7:82-82.
2. Igbe I., Ayinde B.A., and Izuchukwu A. Anti-inflammatory and analgesic effects of the methanol stem bark extract of *Brachystegia eurycoma* Harms (Fabaceae) Eur. J. Med. Plants. 2012; 2:356-365.
3. Sofidiya M.O., and Familoni O.B. *In vitro* antioxidant activities of ethanolic stem bark extract of *Brachystegia eurycoma*. J. Basic Med. Sci. 2016; 1:1-16.
4. Igbe I., Ayinde B.A., Innih S., Ezeadiugwu N.A., and Okhuarobo A. Sub-acute toxicological evaluation of the aqueous stem bark extract of *Brachystegia eurycoma* (harms). Bayero J. Pure Appl. Sci. 2006; 9:107–113.
5. Karrat L., Abajy M.Y., Nayal R. Investigating the anti-inflammatory and analgesic properties of leaves ethanolic extracts of *Cedrus libani* and *Pinus brutia*. Heliyon. 2022; 8:1-10.
6. Igbe I., Edosuyi O., Okhuarobo A., Maduako N., and Ehimen I. Preliminary assessment of the anti-inflammatory and analgesic effects of methanol leaf extract of *Cussonia barteri* (Araliaceae) in rodents. Herb. Pol. 2019; 65:22–31.
7. Chung J.M. The role of reactive oxygen species (ROS) in persistent pain. Mol. Interv. 2004; 4:248–250.
8. Ren K., and Dubner R. Descending modulation in persistent pain: An update. Pain. 2002; 1:1-16.
9. Koster R., Anderson M., and De Beer E. Acetic acid for analgesic screening. Fed Proc. 1959; 18:412–430.
10. Islam S., Shajib M.S., Ahmed T. Antinociceptive effect of methanol extract of *Celosia cristata* Linn. in mice. BMC Complement. Altern. Med. 2016; 16:1–14.
11. Edosuyi O., Igbe I., and Iniaghe L.O. Antinociceptive and antioxidant activities of *Hunteria umbellata* stem bark: Possible role of the serotonergic, opioidergic and dopaminergic pathways. J. Complement. Integr. Med. 2018; 15:1-17.
12. Jain A., Soni M., Deb L., Jain A., Rout S.P., Gupta V.B., and Krishna K.L. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. J. Ethnopharmacol. 2008; 115:61–66.
13. Lima D.F, Brandã M.S., Moura J.B., Leitão J.M.R.S., Carvalho F.A.A., Miúra L.M.C.V, Leite J.R.S.A., Sousa D.P., and Almeida F.R.C. Antinociceptive activity of the monoterpene α-phellandrene in rodents: Possible mechanisms of action. J. Pharm. Pharmacol. 2012; 64:283–292.

14. Zendehdel M., Torabi Z., and Hassanpour S., Antinociceptive mechanisms of *Bunium persicum* essential oil in the mouse writhing test: Role of opioidergic and histaminergic systems. Vet. Med. (Praha). 2015; 60:63–70.
15. Abubakar A., Nazifi A.B., Odoma S., Shehu S., and Danjuma N.M., Antinociceptive activity of methanol extract of *Chlorophytum alismifolium* tubers in murine model of pain: Possible involvement of α_2-adrenergic receptor and KATP channels. J Tradit. Complement. Med. 2020; 10:1–6.
16. Cavalcante-silva L.H.A., Alice M., Falcão P., Carolina A., Vieira S., Denisson M., Viana M., Araújo-júnior J.X. De., Celestino J., Sousa F., et al. Assessment of Mechanisms Involved in Antinociception Produced by the Alkaloid Caulerpine. Mol. 2014; 7361:14699–14709.
17. Gawade S.P., Acetic acid induced painful endogenous infliction in writhing test on mice. J. Pharmacol. Pharmacother. 2012; 3:348.
18. Rosenblum A., Marsch L.A., Joseph H., and Portenoy R.K. Opioids and the treatment of chronic pain: controversies, status, and future directions. Exp. Clin. Psychopharmacol. 2008; 16:405–416.
19. Sen S., Chakraborty R., Sridhar C., Reddy Y.S.R. and De B. Free radicals, antioxidants, diseases and phytomedicines: Status and future prospect. Int. J. Pharm. Sci. Rev. Res. 2010; 3:91–100.
20. Kayani W.K., Dilshad E., Ahmed T., Ismail H., Mirza B. Evaluation of *Ajuga bracteosa* for antioxidant, anti-inflammatory, analgesic, antidepressant, and anticoagulant activities. BMC Complement. Altern. Med. 2016; 16:2-11.
21. Miller A.L. Antioxidant flavonoids: Structure, function, and clinical usage. Altern. Med. Rev. 1996; 1:103–111.