**ANALGESIC ACTIVITY OF *Alchornea laxiflora* STEMBARK EXTRACT IN MICE**

**ABSTRACT**

*Alchornea laxiflora* is used in Ibibio ethnomedicine for the treatment of various diseases such as pains. This work was designed to validate and confirm the ethnomedical use of this plant in the treatment of pain. The ethanol stembark extract of *Alchornea laxiflora* (141-424 mg/kg) was evaluated for analgesic activity against experimentally-induced pains in mice using acetic acid-induced writhing, formalin-induced hind paw licking and thermally-induced pain models. The stembark extract significantly (p<0.005-0.001) inhibited pains in all the models tested in a dose-dependent manner. The extract caused reduction in acetic acid-induced writhing, inhibited the two phases of formalin-induced pains with a more considerable inhibition of the second phase and prolonged the reaction time of mice dose - dependently in thermally-induced pain. The findings of this study show that the stembark extract possess analgesic activity which confirms its use in traditional medicine in the treatment of pains.

**KEYWORDS:**  *Alchornea laxiflora, acetic acid,*analgesic, pain, stembark

1. **INTRODUCTION**

*“Alchornea laxiflora* [Benth.] Pax and K. Hoffm (Euphorbiaceae) is a deciduous shrub, ~6–10 m tall, that grows in most areas of Africa including Nigeria, Congo, Ethiopia, and throughout East Africa to Zimbabwe” (Burkill, 1994). “*A. laxiflora* is called “Opoto” and “Nwariwa,” respectively, among the Yoruba and Ibibio tribes of Nigeria. Stem bark and branches have also been used in traditional medicine for various purposes, notably for malaria, anemia, emmenagogue, ringworm, venereal disease, typhoid fever, antioxidant, infertility in females, infectious diseases, tumor, inflammation, teething problems, and toothache in South Africa, Ghana, and Nigeria” (Jain *et al.*, 2022).

“Biological activities reported of the stembark include antioxidant” (Farombi *et al.,* 2003; Oloyede *et al.,* 2010), “anti-HIV, antibacterial and cytotoxic activities” (Siwe-Noundou *et al.*, 2019), “larvicidal effect (Oyedeji *et al.,* 2024), anticholinesterase activity” (Elufioye, 2017), “antibacterial activity against multi-drug resistant (MDR) bacteria, including strains of *E. coli* (ATCC 8739, AG102, AG100 Atet), *Enterobacter aerogenes (E. aerogenes)* (ATCC 13048, CM64, EA27), *K. pneumoniae* (ATCC 11296, KP55), *Providencia stuartii* (*P. stuartii*) (ATCC29916, PS299645), *Enterobacter cloacae* (*E. cloacae*) (BM47, BM67), and *P. aeruginosa”* (Mbaveng *et al.,* 2015). The stem bark of *Alchornea laxiflora* contains various phytochemicals, including fatty acid derivatives, ellagic acid and its derivatives, and triterpenoids. “The phytochemical investigation of methanolic extract from the stem bark of *A. laxiflora* resulted in the isolation of some compounds, including ellagic acid, 3-O-methylellagic acid, and 3-O-methylellagic acid-3-O-α- rhamnopyranoside” (Sandjo *et al.,* 2011). “A novel ellagic acid derivative, namely, 3,4,3′-tri-O-methylellagic acid, was isolated from the methanolic extract obtained from the *A. laxiflora* stem bark” (Mbaveng *et al.*, 2015). Sandjo *et al.* (2011) and Tapondjou *et al*. (2016) isolated and established the structure of a known steroidal glycoside, β-sitosterol-3-O-β-D-glucopyranoside from the methanol extract of stem bark, ellagic acid; 3-O-methyl-ellagic acid, 3-O-β-D-glucopyranosyl-β-sitosterol, 3-O-acetyloleanolic acid and 3-O-acetyl-ursolic acid. Several pentacyclic triterpenoids, such as 3-acetyloleanolic acid, 3-acetoxyursolic acid, adipedatol, and betulin, have been found in the stembark. “Squalene and 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl heptadeca-3,7,11,15-tetraenyl) -cyclohexanol have also been identified as triterpenoids in the stembark extract” (Sanjo *et al.,* 2011). This study investigated the analgesic activity of the ethanol stembark extract of *A. laxiflora.*

1. **MATERIALS AND METHODS**

**2.1 Plants collection**

The plant material *Alchornea laxiflora* (stembark) were collected in bushes in Uyo area, Akwa Ibom State, Nigeria in April 2024. The plant was identified and authenticated by a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

**2.2 Extraction**

The leaves were washed and shade dried for two weeks. The dried leaves were cut into smaller pieces and pulverized to powder. The powdered leaves were macerated in ethanol for 72 hours and filtered. The liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator. The extract was stored in a refrigerator at -4°C, until used for the proposed experiments.

**2.3 Phytochemical Screening**

“Phytochemical screening of the crude extract was carried out employing standard procedures and tests” (Trease and Evans, 1996, Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, and cardiac glycosides and others.

**2.4 Experimental animals**

 Swiss albino mice (male and female) that were used in the study were obtained from the University of Uyo’s Animal house. They were kept in standard plastic cages in a well-ventilated room and left to acclimatized for a period of 10 days before the experiments. The mice were fed on standard pelleted diet and water *ad libitum*. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication, 1996). Approval for the study was obtained from the University of Uyo’s Animal Ethics Committee.

**2.5 Determination of median lethal dose (LD50)**

The determination of median lethal dose (LD50) of the extract was carried out in mice using intraperitoneal route by modified method of Lorke ([1983).](#_bookmark34) The animals in groups of three mice each were administered different doses of the extract (10– 5000 mg/kg) in two phases. In the first phase, the animals were administered three doses of the stembark extract (10, 100 and 1000 mg/kg). Higher doses of the stembark extract (2000, 3000 and 5000 mg/kg) were administered when no mortality was recorded in the first phase. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, and decreased mobility and death. The mortality in each group within 24 h was recorded. The LD50 value was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b). LD50 =√ ab .

**2.6 Evaluation of analgesic potential of the extract**

**2.6.1 Acetic acid induced writhing in mice**

Writings (abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hind limbs) resulting from intraperitoneal (i.p) injection of 2% acetic acid, was induced according to the procedure described by Okokon *et al.* (2020). The animals were divided into 5 groups of 6 mice per group. Group 1 served as negative control and received 10 mL/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 141, 282 and 424 mg/kg doses of *A. laxilora* stembark extract intraperitoneally, and group 5 received 100 mg/kg of acetyl salicylic acid. After 30 minutes, 0.2 mL of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing movements were counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

**2.6.2 Formalin – induced hind paw licking in mice**

The procedure similar to that described by Okokon *et al.*, (2021) was adopted for the study. The animals were injected with 20 μL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as the indication of pain. Adult albino mice of either sex were randomized into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before being used but allowed access to water. The animals in group 1 (negative control) received 10 mL/kg of normal saline, groups 2-4 received 141, 282 and 424 mg/kg doses of the extract, while group 5 received 100 mg/kg of acetyl salicylic acid (ASA) 30 minutes before being induced with buffered formalin. The responses were measured for 30 mins after formalin injection.

**2.6.3 Thermally induced pain in mice**

The effect of stem extract on hot plate induced pain was investigated in adult mice. The hot plate was used to measure the response latencies according to the method of Okokon *et al*. (2020) and Okokon and Nwafor, (2010). In these experiments, the hot plate was maintained at 45.0±1˚C, each animal was placed into a glass beaker of 50 cm diameter on the heated surface, and the time(s) between placement and shaking or licking of the paws or jumping were recorded as the index of response latency. An automatic 30-second cut off was used to prevent tissue damage. The animals were randomly divided into 5 groups of 6 mice each and fasted for 24 hours but allowed access to water. Group 1 animal served as negative control and received 10 mL/kg of normal saline. Groups 2, 3 and 4 were pretreated intraperitoneally with 141, 282 and 424 mg/kg doses of *A. laxiflora* stembark extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 minutes prior to the placement on the hot plate.

**2.7 Statistical analysis**

Data collected were analyzed using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were considered at p˂0.001 and p˂0.05.

1. **RESULTS**

 **3.1 Phytochemical screening**

The results of phytochemical screening of the stembark extract of *Alchornea laxiflora* revealed that the extract contains alkaloids, flavonoids, phenol, tannins, terpenes, saponins, cardiac glycosides and reducing sugars.

**3.2 Determination of Median lethal dose (LD50)**

 The median lethal dose determined by administration of different does of the ethanol stem bark extract of *Alchornea laxiflora* to Swiss albino mice in groups of six mice each was 1414.21mg/kg. The minimum dose that produced 100% mortality was observed in the group receiving 2000 mg/kg while the maximum dose that produced 0% mortality was observed in the group receiving 1000 mg/kg. The LD50 was calculated to be 1414.21mg/kg.

**3.3 Effect of ethanol stem extract of *A. laxiflora* on acetic acid-induced writhing in mice**

The administration of *A. laxiflora* extract (141-424 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing in mice. The mice pretreated with the stembark extract exhibited a dose-independent reduction in the number abdominal constrictions and stretching of hind limbs. The reductions were statistically significant (p<0.05- 0.001) relative to control but not comparable to that of the standard drug, ASA. However, the analgesic effect of the stembark extract was more prominent in the middle dose (282 mg/kg) treated than the higher dose (424 mg/kg) pretreated group (Table 1).

**3.4 Effect of ethanol stem extract of *Alchornea laxiflora* on formalin-induced hind paw licking in mice**

The stembark extract exhibited a dose-independent analgesic effect on formalin-induced hind paw licking in mice. The extract prominently inhibited the two phases of formalin-induced pains with a more considerable inhibition of the second phase. These inhibitions were significant relative to the control (p< 0.05-0.001) but not comparable to that of the standard drug, ASA. The middle dose (242 mg/kg) was observed to exert the most prominent activity (Table 2).

**3.5 Effect of ethanol stembark extract of *Alchornea laxiflora* on thermally-induced pain in mice**

The mice pretreated with the stembark extract (141- 424 mg/kg) exhibited a dose-dependent effect on thermally-induced pain in mice. The treated mice demonstrated a dose-dependent increase in latency of response in the hot plate test. The increase in the latency of response (analgesic effect) was statistically significant (p<0.001) relative to the control and were comparable to that exerted by the standard drug, ASA (Table 3)**.**

Table 1: Effect of *Alchornea laxiflora* stem extract on acetic acid induced writhing in mice.

|  |  |
| --- | --- |
| TREATMENT/DOSE (mg/kg) | TIME INTERVALS (hr) |
| 5 | 10 | 15 | 20 | 25 | 30 | TOTAL |
| CONTROL | 3.66±0.59 | 21.66±3.43 | 24.00±2.09 | 24.00±0.84 | 18.00±1.69 | 16.66± 1.53 | 107.98±10.17 |
| EXTRACT141 | .2.33±0.75 | 25.66±1.73 | 15.33±1.20 | 10.66±1.18a  | 6.66±0.48a | 9.66±0.48 | 70.30±5.82c |
| 282 | 0.00± 0.00 | 19.00±2.45 | 15.00±1.92 | 8.33±1.20a | 7.00±0.93a | 5.66±0.937a | 54.99±7.47c |
| 424 | 0.66±0.26 | 14.00±2.26b | 16.66±2.41 | 9.66±1.65a | 8.66±0.71b | 6.33±0.54a | 55.97±7.83c |
| ASA 100 | 0.33±0.13c | 8.33±0.35 c | 9.00± 0.62c | 6.00±0.62c | 3.33±0.35c | 4.66±0.35c | 31.65±2.42c |

Data are expressed as mean ± SEM. significant at ap< 0.05, bp <0.01, cp < 0.001 when compared to control n = 6.

Table 2: Effect of *Alchornea laxiflora* stem extract on formalin-induced hind paw licking in mice.

|  |  |
| --- | --- |
| TREATMENT/DOSE (mg/kg) | TIME INTERVALS (hr) |
| 5 | 10 | 15 | 20 | 25 | 30 | TOTAL |
| CONTROL | 24.0±0.84 | 13.00±0.40 | 6.33±0.26 | 8.00±0.40 | 14.00± 0.62 | 11.00±1.41 | 76.33±3.93 |
| EXTRACT141 | 19.00± 1.54c | 7.66±1.53a | 5.66±1.42 | 4.33±0.13 | 5.66±0.84a | 1.33± 0.26a | 43.64±5.72c |
| 282 | 21.33± 2.12 | 2.00± 0.46 | 2.00±0.62 | 2.33± 0.59 | 5.00±0.62a | 4.33±0.35 | 36.99±4.76c |
| 424 | 22.66± 0.88 | 6.33±0.35 | 3.66±0.95 | 4.00± 1.24 | 3.33±0.82b | 4.33± 0.26 | 44.31±4.50c |
| ASA 100 | 15.00±0.23 | 4.00±0.23a | 5.33± 1.71 | 5.00± 1.24 | 1.00±0.23b | 2.00± 0.46a | 32.33±4.20c |

Data are expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control n = 6.

Table 3: Effect of *Alchornea laxiflora* stem extract on hot plate test

|  |  |  |  |
| --- | --- | --- | --- |
| Group | DoseMg/kg | Reaction time (sec)(mean  SEM) | % inhibition |
| Control | - | 1.33± 1.60 |  |
| *Alchornea laxiflora* | 141 | 32.00± 1.54 | 74.57 |
|  | 282 | 81.00±3.47c | 341.89 |
|  | 424 | 90.66± 3.95c | 390.99 |
| ASA | 100 | 74.00±0.93c | 303.70 |

 Data are expressed as mean ± SEM. Significant at ap< 0.05, b p<0.001 when compared to control. n = 6.

1. **DISCUSSION**

“In this study, evaluation of analgesic activities of ethanol stembark of *Alchornea laxiflora* was carried out using different experimental models. Acetic acid induced writhing reflex model in mice is a widely accepted and effective pain model for evaluating peripherally acting analgesics” (Zulfiker *et al.,* 2010). “Acetic acid induces pain by producing a localized inflammatory response via the cyclooxygenase pathway which results in the release of arachidonic acid and subsequently prostaglandins” (Zihad *et al.*, 2018). “Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting in the release of arachidonic acid from the tissue phospholipids” (Ahmed *et al.,* 2006) “via cyclooxygenase (COX) and prostaglandins biosynthesis” (Okokon *et al.,* 2017). “Elevated levels of PGE2 and PGF2 in peritoneal fluids as well as lipoxygenase products have been linked to acetic acid-induced writhing” (Derardt *et al.,* 1980) “by increasing capillary permeability, an increase in prostaglandins levels within the peritoneal cavity may potentially aggravate inflammatory discomfort and pain” (Zuliker *et al.,* 2010). “It is used to distinguish between central and peripheral pain though insufficient to provide enough evidence to differentiation between the two types of pains. The organic acid may potentially indirectly excite the nociceptive neurons by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics. The inhibition of acetic acid induced writhing by the extract at all the doses when compared to control suggests antinociceptive effect which may have been due to the inhibition of the synthesis of arachidonic acid metabolites and prostaglandins biosynthetic pathway.Formalin exhibits neurogenic and inflammatory pain and exhibits both centrally and peripherally mediated pain activities that are characteristics of biphasic pain response” (Nwafor and Okokon, 2010; Okokon *et al.,* 2013). “The first phase (0 - 5 minutes) known as the neurogenic phase is caused by chemical stimulation, which causes the release of bradykinins and substance P, whereas the second phase which occurs after 15-20 minutes of formalin injection, causes the release of inflammatory mediators like histamine and prostaglandin” (Okokon *et al.*, 2013). “The injection of formalin causes an immediate and intense increase in the spontaneous activity of C fiber afferents as well as a distinct quantifiable pain behaviour in the form of paw licking by the animals, with the first phase being more selective for centrally acting analgesics and the second phase being selective for peripherally acting analgesics” (Okokon *et al.,* 2013). “The study showed that the extract inhibited both phases of formalin-induced paw licking with more inhibition observed in the second phase. This suggests that the extract has both central and peripheral activities which is made possible by its ability to inhibit bradykinins, substance P, histamine and prostaglandins which are mediators in these pains. The hot plate test is one of the most common models for studying central nociceptive activity. This model evaluates the complex response to a non-inflammatory, acute nociceptive input, which is thought to be selective for medicines that act centrally such as opioids” (Zuliker *et al.,* 2010). “Studies have shown that agents that increase latency in hot plate response time are likely acting centrally. Preliminary phytochemical screening of the stembark extract revealed the presence of alkaloid, phenols, terpenes, tannins, flavonoids and saponins. Also, flavonoids such as ellagic acid, 3-Omethylellagic acid, 3-O-methylellagic acid-3-O-α- rhamnopyranoside, 3,4,3′-tri-O-methylellagic acid, 3-O-β-D-glucopyranosyl-β-sitosterol, 3-O-acetyloleanolic acid, 3-O-acetyl-ursolic acid, and β-sitosterol-3-O-β-D-glucopyranoside have been isolated from the methanolic extract obtained from the *A. laxiflora* stem bark” (Mbaveng *et al.*, 2015; Sandjo *et al.* 2011; Tapondjou *et al*. 2016).

“Flavonoids are known to act through inhibition of the cyclooxygenase and lipoxygenase pathways” (Liang *et al.,* 1999; Carlo *et al.,* 1999), phospholipase A2 and phospholipase C (Middleton *et al*., 2000). “Some flavonoids exert their antinociception via opioid receptor activation activity” (Rajendran *et al.,* 2000; Otuki *et al.,* 2005). “The presence of these compounds (polyphenolics and flavonoids) in this plant might account for the activities and may in part explain the mechanisms of its actions in this study. The results of this investigation corroborate previously reported antinociceptive activity of the root extract of *A. laxiflora* whereby significant central and peripheral analgesic activities were reported” (Okokon *et al.*, 2017).

1. **CONCLUSION**

 From the results of this study, the stembark extract of *A. laxiflora* possesses analgesic activity which is due to the activities of its phytochemical constituents. This study also showed that the extract demonstrated a significant dose-dependent increase in latency to thermally induced pain response time, thereby demonstrating central antinociceptive activity.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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