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Antinociceptive Activities and Phytoconstituents of Aqueous Leaf Extract of *Polyalthia longifolia* (Sonn.) Thwaites

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Abstract
The study was aimed at evaluating the antinociceptive activities and phytochemical constituents of aqueous leaf extract of Polyalthia longifolia (Sonn.) Thwaites. Both hot plate nociception and acetic acid-induced writhing reflex models were used to measure the ability of the extract to inhibit painful impulses, while secondary metabolites contained in the extract were determined by qualitative screening tests. The extract, at all doses, provided significant antinociceptive activity (P< 0.05) against thermally-induced pain stimulus at a temperature of 55±1 ^{0}C. The number of writhes, 56.5±0.90, 55.0±0.58 and 32.75±0.48 due to 100, 200 and 800 mg/kg treatments respectively, were significantly reduced (P< 0.05) compared to the value, 71.20±1.04, recorded in the control group. The activity of the extract appears to be mediated by both central and peripheral routes. Alkaloids, flavonoids, saponins and phenols confirmed in the extract may be responsible for the activities of the extract.

Keywords: Antinociceptive; Phytochemistry; Leaf; Extract; *Polyalthia longifolia*

Introduction
Polyalthia longifolia (Sonn.) Thwaites (Annonaceae) is a tall beautiful evergreen tree (1). It is commonly referred to as 'masquerade tree' in Nigeria, maintaining a tall straight main trunk with drooping pendulous branches. The young leaves are slightly brownish, soft and delicate to touch, while the older leaves are dark green, glossy and lanceolate. Flowers are star-like pale green, while its fruits are ovoid and occur in clusters of about 10-20. Usually, the species is cultivated as an ornamental and to alleviate noise pollution (2). It is locally used to treat fever, diabetes, skin diseases, helminthiasis, hypertension and constipation (2). The bark is employed in the treatment of digestive, circulatory and urinary systems, including pyrexia, rheumatism, scorpion sting, menorrhagia and diabetes (2, 3).
Medicinal plants are endowed with bioactive compounds that can be used in the treatment of diseases, and drug production (4, 5, 6). Herbal preparations are prescribed widely for the treatment of pain and inflammatory conditions (6). *Polyalthia longifolia* var. *pendula* is a very popular herb in Bangladesh due to its traditional uses in treatment of rheumatism, bone fracture and gastric ulcer (7). This study therefore evaluates the antinociceptive and phytochemical constituents of aqueous leaf extract of *P. longifolia*.

Materials and Methods
Collection and Preparation of Plant Material
Fresh leaves of *P. longifolia* were collected from matured stands around the Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. The plant was authenticated by Dr. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. A prepared specimen, voucher number UBHp346, was deposited in the herbarium unit of the department. The leaves were air-dried and reduced to coarse powder. A weighed portion (860 g) of the powder was extracted with distilled water by hot infusion. After 24 hours, it was filtered through a clean, colourless mesh. The filtrate was concentrated over a water bath to obtain a semi-solid paste of *Polyalthia longifolia* leaf extract (PLLE). It was collected in a clean container, sealed and stored in a refrigerator until further use.
Experimental Animals
Sixty albino mice of both sexes, with average weight of 24 g, were obtained from the Animal Unit of the Department of Biochemistry, University of Benin, Benin City, Nigeria. They were randomly allotted into twelve groups, containing five mice each, and kept in plastic cages within the animal house of the Department of Animal and Environmental Biology. The mice were allowed two weeks of acclimatization and maintained under standard nutritional and environmental conditions of normal relative humidity, room temperature, and 12 hour light and 12 hour dark cycle, with unrestricted access to standard food pellets and tap water. The feed was withdrawn only during the experimental hours. Procedures were in accordance with the ethical guidelines on the handling and use of experimental animals of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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Hot Plate Nociceptive Test

The method of Eddy and Leimbach (8) was adapted. Six groups from the set of acclimatized mice were used for the experiment. The negative and positive control groups received oral administration of 10 ml/kg distilled water and 100 mg/kg isobutylphenylpropionic (ibuprofen) respectively, whereas the treatment groups received 100, 200, 400 and 800 mg/kg respectively of the plant extract. After one hour, the mice were individually placed (for not more than 15 seconds, cut off time) on a hot plate maintained at a temperature of 55±1 ^{0}C. The time taken to flick the hind paw or lick or attempt jump from the hot plate was considered as the reaction time of a particular animal. The reaction time was recorded at 0, 30, 60, 90 and 120 minutes.

Acetic Acid-Induced Writhing Test

Antinociceptive activity of the extract was evaluated by acetic acid-induced mouse writhing test (9). The extract (100, 200, 400 and 800 mg/kg), acetyl salicylic acid (aspirin 100 mg/kg) and distilled water (10 ml/kg) respectively were administered orally to the other six groups of mice 30 minutes prior to intraperitoneal injection of acetic acid (10 ml/kg of 0.7% v/v). Immediately, each mouse was closely monitored for writhes within a 30 minute period. The numbers of writhes were counted and expressed as a percentage inhibition of abdominal constrictions between the control and treated groups.

$$\text{Inhibition (\%)} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean number of writhes (control)}} \text{ X } 100$$

Phytochemical Screening

The extract was subjected to phytochemical screening for the identification of alkaloids (Dragendorff's, Mayer's, and Hager's tests), flavonoids (lead acetate, and alkaline reagent tests), saponins (frothing test) and phenols (ferric chloride test) according to Evans (10).

Statistical Analysis

Data obtained from the study were subjected to statistical analysis using SPSS version 16.0 software. One way analysis of variance (ANOVA) was performed and mean separation was by Duncan multiple range post hoc tests for five replicates. A value of P< 0.05 was considered to be significant.

Results

Hot Plate Nociceptive Response

As shown in Figure 1, PLLE significantly (P< 0.05) extended the latency period of mice in response to thermal stimulus when compared with control. The activities of the 200, 400 and 800 mg/kg dose were comparable to the standard at 30 and 60 minutes post-treatment phases.

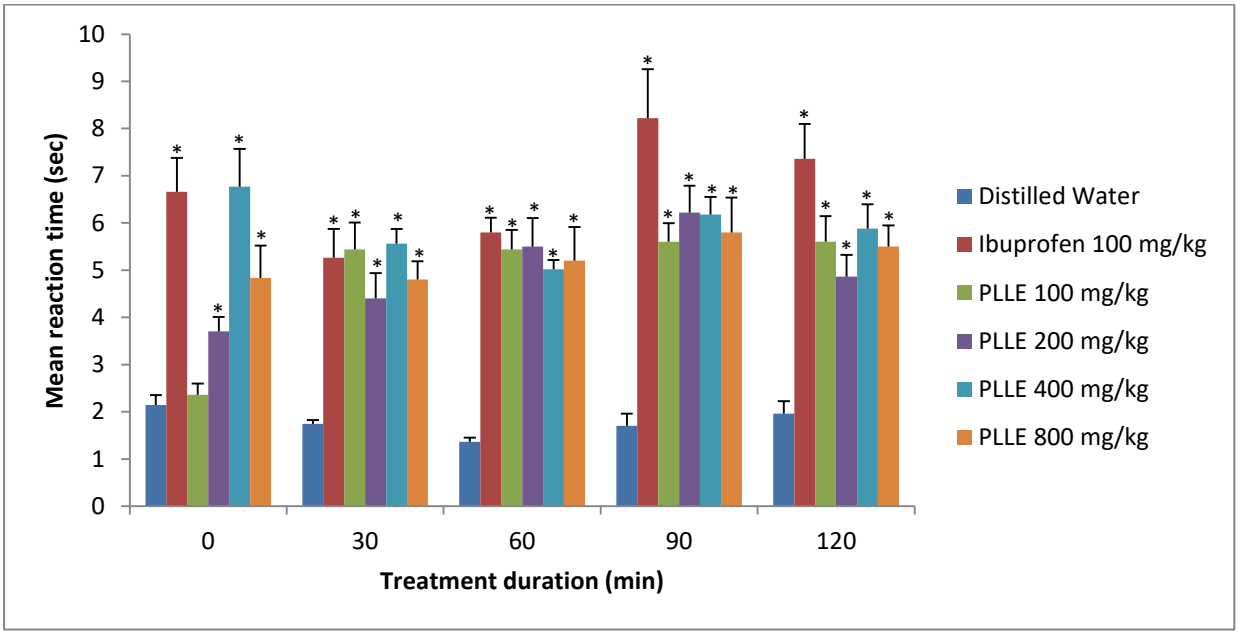

Figure 1: Effect of aqueous leaf extract of *Polyalthia longifolia* (PLLE) on thermal pain latency in mice.
*P< 0.05 as compared with negative control; n =5

Acetic Acid-Induced Writhing Reflex

The 100, 200 and 800 mg/kg aqueous leaf extract of *P. longifolia* (PLLE) reduced the mean number of writhes significantly (P< 0.05) compared to the negative control (Figure 2). However, the effect of the 400 mg/kg was not significant (P> 0.05). The activity of the 800 mg/kg dose (32.75±0.48) was comparable with aspirin (31.50±0.65).

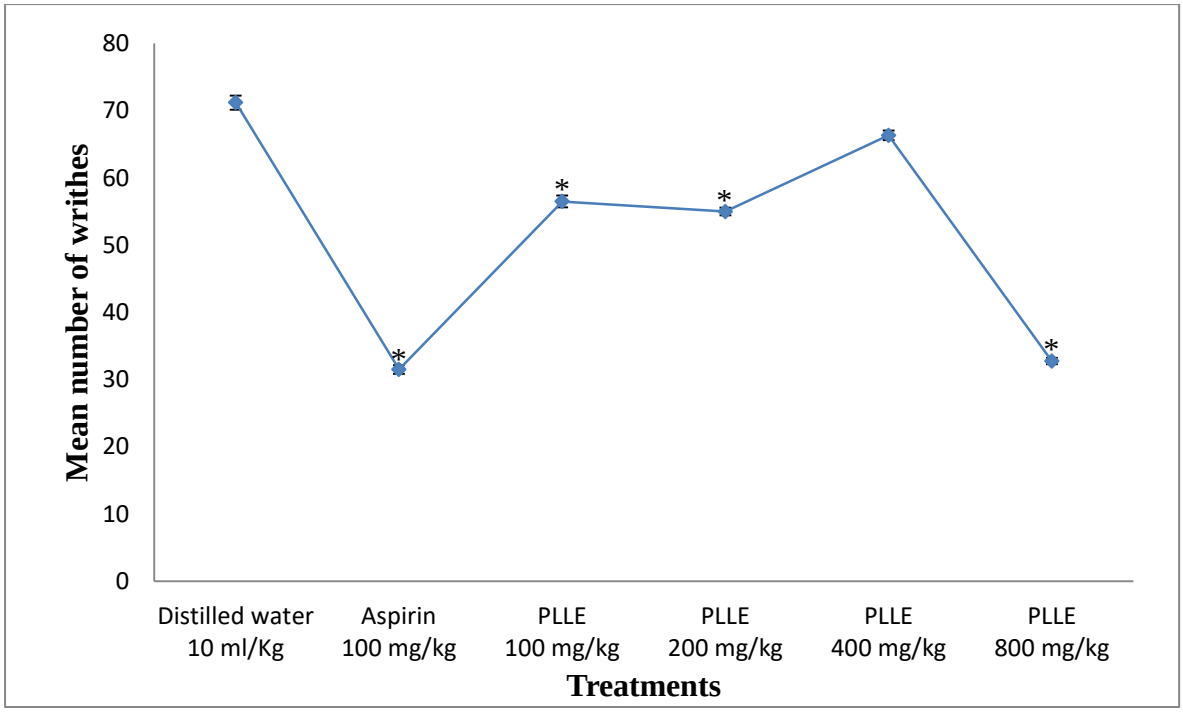

Figure 2: Effect of aqueous leaf extract of *Polyalthia longifolia* on acetic acid-induced writhing reflex in mice. *P< 0.05 as compared to the negative control; n =5

The aqueous leaf extract of *P. longifolia* inhibited acetic acid-induced writhing in mice dose-dependently. The highest percentage inhibition index (54%) was obtained at 800 mg/kg. However, the lower doses (100 and 200 mg/kg) performed better (20.65% and 22.75% respectively) compared to the effect of the 400 mg/kg treatment. No inhibition (0%) was conferred by the control, distilled water (Figure 3).

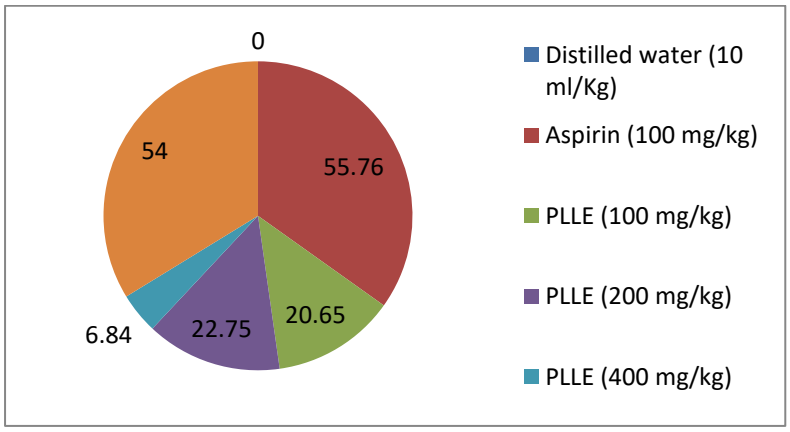

Figure 2: Percentage inhibition of acetic acid-induced writhing reflex in mice due to treatment with aqueous leaf extract of *Polyalthia longifolia*.

Preliminary Phytochemistry

Alkaloids, flavonoids, saponins and phenols are secondary matabolites found to be present in the aqueous leaf extract of *P. longifolia* (Table 1).

Table 1: Phytochemical constituents of *Polyalthia longifolia* aqueous leaf extract.

Test	Observation
Alkaloids	+
Flavonoids	+
Saponins	+
Phenols	+

+ = Present

Discussion

Hot plate latency stimulus and acetic acid-induced writhing models are suitable for screening analgesic potentials of a substance (11). The former is one of the most common tests of nociception that is based on a phasic stimulus of high intensity (12). Thermal-induced pain model is suitable for centrally mediated nociception (13). The present study reveals that the extract significantly ($P< 0.05$) increased the latency period of response to thermal pain stimulus in the experimental mice (Figure 1). With exception to the 1st phase (0 minute), there was no significant difference between the activities of the varying doses of plant extract administered during this study. However, the antinociceptive activities of the extract were significant at all doses throughout the experimental phases (0 - 120 minutes) of the test. Similarly, the activities of the extract were comparable to that of ibuprofen, particularly during the 2nd and 3rd phases (30 and 60 minutes) respectively.

Administration of acetic acid (i.p.) produces abdominal writhing response due to stimulation of chemosensitive nociceptors by prostaglandins (14) as well as lipoxygenase products (15) in the peritoneal fluid. In the present study, aqueous extract of *P. longifolia* leaf significantly ($P< 0.05$) and dose-dependently inhibited the number of writhes when compared with control (Figure 2). Whereas the number of writhes in the control group was as much as 71.20±1.04 the values were significantly lower (56.5±0.90, 55.0±0.58 and 32.75±0.48) in the 100, 200 and 800 mg/kg treatments respectively. However, the 400 mg/kg treatment did not produce any significant difference ($P> 0.05$) compared with control. The highest inhibitory effect (54%) was conferred by the 800 mg/kg dose (Figure 3), which was comparable to the effect of aspirin (55.76%). However, higher inhibitions of 65.63%, 62.43% and 59.66% respectively were obtained at a lower dose (120 mg/kg) of methanol, ethyl acetate and benzene extracts of *P. longifolia* leaf compared to 67.32% recorded in 100 mg/kg of aspirin (16). Although, writhing response test is particularly sensitive for peripherally acting analgesics (17), it is also indicative for both central and peripheral acting analgesics (18). In acetic acid-induced writhing test, the abdominal constriction is sensitive to drugs with analgesic activity similar to aspirin, antagonists of kinin receptors as well as central and peripherally acting opioid analgesics (19). The potent antinociceptive outcomes recorded in both models in the present study suggest that the extract may be acting via both central and peripherally mediated mechanisms. This is similar to the significant antinociceptive activity of ethanol extract of bark of *P. longifolia* (7) and methanol extract of *Dalbergia sissoo* leaves (20) in both heat and chemical-induced nociception. However, methanolic extract and alkaloid fractions of *Berberis integerrima* root were only active in chemical-induced test but inactive in hot plate nociception model (21).

Pharmacological effects of medicinal plants are basically dependent on their phytochemical constituents. This study reveals the presence of alkaloids, flavonoids, saponins and phenols in the aqueous leaf extract of *P. longifolia* (Table 1). The observation is consistent with earlier reports that steroids, alkaloids, terpenoids, phenols and flavonoids are the major phytochemicals in the plant (22, 23, 24). Alkaloids, saponins, flavonoids and phenols have analgesic properties (25, 26). The analgesic activity of *Capsicum* spp. is due to the presence of capsaicinoids, which are simple phenolic compounds (27). Flavonoids (28), saponins (29), and triterpenes (30) are widely reported to exert antinociceptive activity. Flavonoid-based bioactive compounds and their synergistic action with non-volatile bioactive compounds have also been suspected in the antinociceptive activity of *Clinacanthus nutans* (31). It is likely that similar phytochemicals present in the aqueous leaf extract of *P. longifolia* may be responsible for the antinociceptive activity recorded in this study. Further studies are required to isolate the specific bioactive involved in the antinociceptive mechanism of the plant extract.

Conclusion

Aqueous leaf extract of *P. longifolia* possesses significant antinociceptive activities, which may explain its use in the traditional management of pain. The extract contains a number of secondary metabolites, which are known to be responsible for antinociceptive activity.

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