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Full Length Research Paper

Antinociceptive activity of methanol extract and aqueous fraction of the stem bark of *Pentaclethra macrophylla* Benth (Mimosaceae)

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The antinociceptive activity of the methanol extract and the aqueous fraction of the bark of *Pentaclethra macrophylla*, family: (mimosaceae) was evaluated using the acetic acid induced mouse writhing assay and tail immersion assay in mice. The methanol extract and the aqueous fraction at a dose of 50 and 100 mg/kg body weight administered orally exhibited significant inhibition of the acetic acid induced writhing assay in a dose dependent manner ($p < 0.001$) with 100 mg/kg dose giving a higher inhibition. The methanol extract and the aqueous fraction also showed significant increase in the heat tolerance capacity in mice in a dose dependent manner ($p < 0.001$) in the tail immersion assay. These findings therefore justify the ethnomedicinal use of *P. macrophylla* stem bark as an antinociceptive agent.

Key words: Antinociceptive activity, *Pentaclethra macrophylla*, tail immersion assay, mouse writhing assay, stem bark.

INTRODUCTION

Screening of herbal plants has become a potential source of compounds of high therapeutic value. Ethno pharmacological studies have become increasingly invaluable in the development of health care. The green pharmaceuticals have now received extraordinary attention and popularity. The drugs labelled safe and efficacious some years ago had to be recalled and relabelled because of unanticipated side effects (Hussain and Sheikh, 2008).

Pentaclethra macrophylla is planted on the fringes of compound farms mainly for its edible seed. The seed are eaten boiled or roasted. They are also fermented to yield a snack or condiment with a meaty taste, very popular in south-western Nigeria where it is called 'Ugba' (Gugnami and Ezenwanze, 1985).

It is used in African traditional human and veterinary medicine. The ripe fruits are applied externally to heal wounds. Extracts of the leaf, stem bark, seed and fruit pulp have anti-inflammatory and anthelmintic activity, and are used to treat gonorrhea and convulsions, and also used as analgesic (Bouquet et al., 1971; Cousin and Huffman, 2002).

The root bark is used as a laxative, as an enema against dysentery and as a liniment against itch. In Cameroon an infusion of the bark is used as an abortifacient. The aqueous leaf, bark, seed and fruit pericarp of *P. macrophylla* have been examined for their cytotoxicity, while only the leaves and seeds were tested for analgesic and anti-inflamatory activities. Using *in vivo* and *in vitro* experimental models (Githens, 1948; Iwu, 1993).

Pain is not a unitary sensation, as illustrated by its vast range of descriptors. Although analgesics may alleviate different types of pain, their potency may vary from one to another. Thus, evaluating analgesics in a single nociceptive assay may not provide a full understanding of the actions of the drugs or its utility (Okunrobo et al., 2007).

The present study aimed to extract the stem bark of *P. macrophylla* in methanol, partitioned it into aqueous and chloroform fractions and screen the crude extract and the aqueous fraction for antinociceptive property using different experimental animals models.

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MATERIALS AND METHODS

Plant collection and identification

The stem barks were collected based on ethnopharmacologic information. The barks were harvested in Odi-okpe, Ogun State Nigeria, in March 2006. The botanical identification of the plant, its bark and its authentication were done by Mr. Usang Felix Luah (Plant taxonomist) of the Forest Research Institute of Nigeria, Ibadan, where a voucher specimen No. 107278 was deposited for future reference. The barks were then cut into smaller pieces, sun-dried, pulverised in a mixer-grinder, filtered, and the coarse powder was stored in a non-toxic polyethylene bag.

Extraction and fractionation of plant

The coarse powder (600 g) was mixed with 2.0 L of methanol and kept closed in a dark area for 48 h. It was then pressed, filtered and concentrated to a syrupy mass using a rotary evaporator at reduced pressure. The methanol extract (40 g) was dissolved in methanol and distilled water (1:2) and successively and exhaustively partitioned between water and chloroform using a separating funnel. The fractions obtained were concentrated in a rotary evaporator under reduced pressure and weighed. The percentage yields of the aqueous and chloroform fractions were 45.8 and 0.25% respectively. These were stored in universal bottles and refrigerated at -4°C prior to use.

Phytochemical screening

Qualitative assay, for the presence of secondary plant metabolites such as carbohydrate, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the powdered stem bark following standard procedures (Sofowora, 1984; Trease and Evans, 1985).

Drugs and chemicals

Acetic acid (BDH chemical), n-hexane (sigma-Aldrich-Germany), chloroform (Sigma-Aldrich-Germany), methanol (Sigma- Aldrich-Germany), ethylacetate (BDH-chemicals), morphine (BDH chemical), Acetylsalicylic acid (BDH chemicals), and normal saline (Pfizer pharmaceuticals-Nigeria)

Pharmacological evaluation

Animals

Swiss mice (20 – 25 g) of both sexes were kept at the laboratory animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City. Nigeria. The animals were maintained under standard environmental conditions and were allowed free access to feed (Bendel Feeds and Floor mill, Ewu, Edo State, Nigeria) and water *ad libitum*. All the procedures were conducted in accordance with the guide line for Care and Use of Laboratory Animals published by the National Institutes of Health and the ethical guidelines of the International Association for the study of pain.

Mouse writhing assay

The method of Koster et al. (1959) was used. The methanol extract (50 and 100 mg/kg, oral), aqueous fraction (50 and 100 mg/kg, oral), or acetylsalicylic acid (100 mg/kg, s.c) was administered to mice 60 and 30 min respectively before intraperitoneal injection of acetic acid (0.6% v/v in normal saline 10 ml/kg). Normal saline was used as control. The number of writhes was counted for 30 min. A significant reduction in the number of acetic acid induced abdominal constrictions of the treated mice, compared to that in the untreated (control group of mice), was taken as an indication of analgesic effect.

Tail immersion assay

The prescreened animals (reaction time 3 – 4 s) were divided into four (4) groups of five mice each. The lower 5 cm portion of the tail was immersed in a 1 litre beaker of water maintained at 50 ± 2°C. In both the control and test animals the reaction time (in seconds) was chosen as the time when the animals completely withdrew their tails from the hot water in the bath (Parimaladevi et al., 2003). A cut-off time of 15 s was allowed. The reaction time was measured 1hour after oral administration of methanol extract (50 and 100 mg/kg, oral), aqueous fraction (50 and 100 mg/kg, oral), or normal saline (10 ml/kg). Morphine (10 mg/kg) was administered subcuta-neously (s.c), 30 min before the test. The reaction time (in sec-onds), were recorded and the test mean reaction time was calcu-lated for each of the groups.

Statistical analysis

All data were expressed as mean ± SEM and where applicable were analyzed for statistical significance using one way ANOVA followed by Dunnet's test. A P- value < 0.05 was considered significant.

RESULTS

The phytochemical analysis on the stem bark of *P. macrophylla* was carried out using the standard procedures and the result revealed the presence of glycosides, saponins, tannins and alkaloids as shown in Table 1.

Table 1. Phytochemical screenings of *Pentaclethra macrophylla* (PM) stem bark powder.

Powdered stem Bark of pm	Result
Cardiacglyoside	+
Cyanogenetic glycoside	+
Anthraquinone	-
Carbohydrate	+
Saponin	+
Tropane alkaloid	+
Isoquinoline alkaloid	-
Pyrogallo tannin	+
Phlobatannin	+
Steroidal nucleus	+

The antinociceptive action of the methanol extract and the aqueous fraction of the stem bark of *P. macrophylla*, family: (mimosaceae) was evaluated using the acetic acid induced mouse writhing assay and tail immersion assay in mice. Table 2 shown the effect of the methanol extract

Table 2. Effect of the Methanol Extract of *Pentaclethra macrophylla* (PM) stem Bark on Acetic acid Induced Writhing Test.

Treatment	Writhes per 30 min	Percentage inhibition of pain
Normal saline 10 ml/kg (oral)	82.80 ± 0.66	-
PM Extract 50 mg/kg (oral)	50.20 ± 0.77*	39.37
PM Extract 100 mg/kg (oral)	40.80 ± 0.72**	50.72
Acetylsalicylic acid 100 mg/kg (S.C)	36. 60 ± 0.92**	55.80

Values are mean ± S.E.M * p< 0.05, ** p < 0.001, significantly different from control, Paired t- test (n = 5), p.o. = per oral.

Table 3. Effect of the Aqueous partitioned Fraction of the Methanol Extract of *Pentaclethra macrophylla* (PM) stem Bark on Acetic Acid Induced Writhing Test.

Treatment	Writhes per 30 min	Percentage inhibition of pain
Normal saline 10 ml/kg (oral)	74 ±1.67	-
PM Extract 50 mg/kg (oral)	46.60 ±1.66**	37.02
PM Extract 100 mg/kg (oral)	39.60 ± 0.67**	46.49
Acetylsalicylic acid 100 mg/kg (S.C)	36. 60 ± 0.36**	50.54

Values are mean ± S.E.M * p < 0.05, ** p< 0.001, significantly different from control, Paired t- test (n = 5), p.o. = per oral.

Table 4. Effect of the methanol extract of *Pentaclethra macrophylla* (PM) *stem bark* on tail flick latency.

Treatment	Tail flick latency (seconds)
Normal saline 10 ml/kg (oral)	3.60 ± 0.22
PM Extract 50 mg/kg (oral)	6.40 ± 0.45**
PM Extract 100 mg/kg (oral)	9.20 ± 0.77**
Morphine 10 mg/kg (I.P)	12. 60 ± 0.61**

Values are mean ± S.E.M * p < 0.05, ** p < 0.001, significantly different from control, Paired t- test (n = 5), p.o. = per oral.

Table 5. Effect of the aqueous partitioned fraction of the methanol extract of *Pentaclethra macrophylla* (PM) *stem bark* on tail flick latency.

Treatment	Tail flick latency (seconds)
Normal saline 10 ml/kg (oral)	3.40 ± 0.22
PM Extract 50 mg/kg (oral)	5.0 ± 0.28**
PM Extract 100 mg/kg (oral)	7.0 ± 0.28**
Morphine 10 mg/kg (I.P)	9.8 ± 0.59**

Values are mean ± S.E.M * p < 0.05, ** p < 0.001, significantly different from control, Paired t- test (n = 5), p.o. = per oral.

on acetic acid –induced writhing test which revealed that at 50 mg/kg it had 39 percentage inhibition on pain, which was significant ($p < 0.05$) while at 100 mg/kg it shown 51 percentage inhibition on pain and was highly significant ($p < 0.001$) however, Table 3 revealed the effect of the aqueous fraction on acetic acid –induced writhing test which shown that at 50 mg/kg it had 37 percentage inhibition on pain which was significant ($p < 0.05$) while at 100 mg/kg it shows 47 percentage inhibition on pain and was highly significant ($p < 0.001$). Both effects were dose dependent and the 100 mg/kg doses were comparable with that of the standard acetylsalicylic acid used. Table 4 shows the effect of the methanol extract on tail flick latency while Table 5 revealed the effect of the aqueous fraction on tail flick latency both results shown a significant ($p < 0.001$) increase in a dose dependent-manner in the tail flick latency and the activity at 100 mg/kg was comparable to that of morphine. From the results of this study it shown that the activity in the methanol extract is slightly higher than in the aqueous fraction from the experimental models used.

DISCUSSION

Pain is a subjective symptom that is affected by psychological factors. A wide variety of chemical agents could be used to alleviate or kill pain. These agents mediate their effect through central or peripheral mechanisms. The complex nature of the chain of central and peripheral mechanisms that underline pain sensation and the fact that no simple test is good enough to predict the efficiency of a test agent in humans makes the use of various experimental models imperative when screening a drug for pharmacologic activity.

Two models of noxious stimuli were used to investigate the potential analgesic activity of the methanol extract

and the aqueous fraction of *P*. *macrophylla*. The results obtained show a dose dependent antinociceptive activity on the various models used.

It is known that centrally acting analgesic drug elevate the pain tolerance capacity of mice towards heat and pressure (Adeyemi et al., 2004). The effects of the methanol extract and the aqueous fraction at a dose of 100 mg/kg (P.O) were comparable to morphine 10 mg/kg (IP) a standard analgesic drug. Studies have shown that these central actions occur in the spinal cord, midbrain, thalamus and the cortical sites. This may involve direct inhibition of nociceptive neurons, inhibition of excitatory relay inter neurons, as well as excitation of an inhibitory interneuron in the midbrain (Smith and Reynard, 2000). The analgesic activity of the methanol extract and the aqueous fraction of *P*. *macrophylla* was found to be significant on both the acetic acid – induced model ($P < 0.001$) and the tail immersion assay ($P < 0.001$). It appears that the methanol extract the aqueous fraction inhibit both the peripheral and the central pain mechanism.

The preliminary Phytochemical screening of the powdered stem bark of *P*. *macrophylla* revealed that it contains tannins, alkaloids and glycosides. Some of these compounds may be interfering with the mechanism involved in pain perception.

Conclusion

The finding of the present study has justified the traditional use of *P*. *macrophylla* (PM) stem bark extract for the treatment of various types of pain conditions. The methanol extract and the aqueous fraction of the methanol extract possess analgesic activity. The Phytochemical constituent might be contributory to the analgesic effect of *P*. *macrophylla* extract. However, further investigations are required to identify the active constituent(s), to verify the therapeutic merits of the active constituent(s) and to reveal the exact mechanisms of action responsible for the analgesic activity of *P*. *macrophylla*.

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