



**PHYTOCHEMICAL AND SOME BIOLOGICAL ACTIVITIES OF THE STEM BARK  
EXTRACTS OF *Pentaclethra macrophylla* BENTH.**

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### ABSTRACT

*Pentaclethra macrophylla* Benth (fabaceae) also called African oil bean is a tropical tree crop found mostly in the southern rain forest zone of west Africa.. In Nigeria, the stem bark of *Pentaclethra macrophylla* is used traditionally to treat headache, pains, fever, boils and swellings . This study intends to evaluate the analgesic, anti-pyretic and anti-inflammatory, properties of the methanol extract of the stem bark of the plant in 150 albino mice. The result of the phytochemical screening revealed that the extract contained alkaloids, tannins, terpenes, flavonoids, cardiac glycosides, saponins carbohydrates and polyphenols. The lethal dose (LD<sub>50</sub>) of the extract was 346.41 mg/kg in mice. A dose of 103.92mg/kg of the extract reduced the writhing movement (nociceptive response) in mice significantly (P<0.05) from 71.77±5.20 to 22.70 ±1.55. It reduced the rectal temperature (pyrexia response) of the mice significantly (P<0.05) from 38.20± 0.06 to 35.60± 0.19 It also reduced the hind paw licking (inflammatory response) in mice significantly (P<0.05) from 6.15±0.89 to 4.69±0.72 compared to the negative control.

**KEY WORDS:** Phytochemical constituents, Biological activities, *Pentaclethra macrophylla*.

### INTRODUCTION

Pain (nociception) is unpleasant and emotional experience associated with or without actual tissue damage.<sup>[1]</sup> An analgesic is any member of the group of drugs used to achieve analgesia, relief from pain.<sup>[2]</sup>

Fever is a complex physiologic response to disease characterized by a rise in core temperature.<sup>[3, 4]</sup> Inflammation is a patho-physiological response of mammalian tissues to a variety of harmful stimuli.<sup>[5]</sup>

Medicinal plant part like fruit, leaves, stem, bark and roots or even the whole plants are used in the treatment of illness.<sup>[6]</sup> *Pentaclethra macrophylla* Benth (fabaceae) also called African oil bean is a tropical tree crop found mostly in the southern rain forest zone of west Africa. In Nigeria the bark of the plant is used as worm expeller and pain reliever. It is also to treat gonorrhoea, convulsion, swellings and fever.<sup>[7, 8, 9]</sup>

Despite these various uses, there has been insufficient scientific data on the anti-nociceptive, anti-pyretic and anti-inflammatory properties of the stem bark of the plant.

The result of this study may generate enough data to bridge the information gap.

The objectives of the Study shall be to extract the dried pulverized bark of *Pentaclethra macrophylla* with n-hexane, ethylacetate and 70% methanol; evaluate the presence of phytochemicals and also evaluate the antinociceptive, anti-pyretic, anti-inflammatory and effects of the extract in mice after establishing the toxicity profile.

### MATERIALS AND METHODS

#### Collection of plant material

The stem bark was harvested in Obot Akara Local Government Area of Akwa Ibom State, Nigeria in April, 2015 and authenticated by Dr. (Mrs.) Margeret Basse of the department of Botany and Ecological Studies, University of Uyo, Nigeria and a voucher specimen number UUH 42 (d) was assigned to it and deposited in the university of Uyo Herbarium, Department of Pharmacognosy and Natural Medicine, faculty of pharmacy, University of Uyo, Uyo, Akwa Ibom State. The bark was then cut into pieces, sun-dried, pulverized.

**Extraction:** The pulverized plant material (1000g) was extracted with n-hexane for 72 hours. The extract was concentrated the water bath at 40°C and later stored in the refrigerator at -4°C until required.

After extracting the plant with n-hexane, the dried mass was extracted with ethylacetate and 70% methanol respectively, using the same procedure.

#### Phytochemical screening

The above extracts were subjected to preliminary phytochemical screening to determine the presence of bioactive constituents. This was carried out using the standard methods of analysis.<sup>[10]</sup>

#### Acute Toxicity Studies

Acute toxicity was carried out in mice.<sup>[11, 12]</sup>

#### Evaluation of Analgesic Effect of Extract on Formalin-Induced Hind Paw Licking In Mice

Standard procedure was adopted.<sup>[13]</sup>

The animals were injected with 20mL of 2.5% formalin solution (0.9%) made up in phosphate buffer solution (PBS concentration: NaCl 137mM, KCl 2.7mM and phosphate buffer, 10mM) subcutaneously under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as indication of pain. Adult albino mice (20g-26g) of either sex randomized into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours but allowed access to water. The animals in group 1 (negative control) received 10ml/kg of normal saline, groups 2-4 received 34.64mg/kg, 69.28mg/kg and 103.92mg/kg dose of the extract, while group 5 received 100mg/kg of acetyl salicylic acid 30 minutes before being challenged with buffered formalin.

## RESULTS

**Table 1 : Analgesic Effect Of Extract On The Formalin-Induced Hind Paw Licking In Mice**

Treatment/ Dose (mg/kg)	TIME INTERVALS (HR)						
	5	10	15	20	25	30	Total
Control	29.6±1.76	15.66±1.20	12.6±1.76	10.33±0.88	7.66±1.20	7.66±0.33	73.51±7.13
Extract 34.64	28.66±2.02	10.66±1.20	8.33±0.66	6.33±0.88	6.33±0.88	4.66±0.88	64.97±6.52
69.28	23.0±0.57	14.66±0.88	13.0±2.84	12.33±0.88	9.00±1.15	4.00±0.57	76.32±6.89 <sup>a</sup>
103.92	18.33±0.88	14.33±1.20	9.00±0.00	5.66±0.33	7.66±0.88	4.33±0.88	59.31±4.17 <sup>b</sup>
ASA 100	4.00±0.57	6.66±0.88	8.00±0.57	9.00±1.00	7.33±0.88	4.00±0.57	38.99±4.47

Data are expressed as mean ± SEM. Significant at <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, when compared to control. n=6.

**Table 2 : Anti-Pyretic Effect Of Extract On 2, 4-Dinitrophenol-Induced Pyrexia In Mice**

Treatment	Dose (mg/kg)	Time interval (hr)							
		Basal temp	0	0.5	1	2	3	4	5
Control		36.10±0.05	37.33±0.08	38.70±0.11	38.83±0.08	38.80±0.05	38.80±0.05	38.66±0.08	38.20±0.05
Extract	34.64	35.96±0.06	37.16±0.31	38.26±0.23	38.90±0.15	38.56±0.08	37.73±0.03	37.40±0.11	37.46±0.18
	69.28	36.26±0.03	37.53±0.08	38.80±0.05	38.76±0.08	38.10±0.23	37.10±0.23	36.50±0.27	36.50±0.17 <sup>a</sup>
	103.92	36.13±0.17	37.30±0.05	38.66±0.12	38.60±0.15	36.40±0.15	36.40±0.15	35.70±0.17	35.63±0.18 <sup>b</sup>
ASA	100	36.13±0.17	37.36±0.14	37.63±0.38	38.40±0.23	36.06±0.14	36.06±0.14	35.43±0.08	35.43±0.08

Data are expressed as mean ± SEM. Significant at <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, when compared to control. n=6.

#### Evaluation of Anti-pyretic Effect of Extract on 2,4-Dinitrophenol (DNP) induced Pyrexia in mice

Thirty (30) mice of both sexes were fasted for 24 hours allowed water ad libitum and randomized into groups of six mice each for the experiment. DNP (10mg/kg, ip) was administered to the mice after obtaining the basal rectal temperatures. Hyperthermia developed within 30min of DNP administration. Different doses of the extract were administered respectively to the treated and control animals. Rectal temperatures of the animals were obtained at an hour interval for 5 hours.<sup>[14]</sup>

#### Evaluation of Anti-inflammatory Effect of Extract on egg – albumin induced inflammation in mice.

The overnight fasted mice weighing 100-128g of either sex were divided into 5 groups of 6 each. The first group served as control and received distilled water (10mg/kg Per Oral), the second group received Aspirin (100mg/kg P.O), as the standard drug. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups received various doses of methanol extracts (34.64, 69.28 and 103.92mg/kg).

Inflammation was induced in the mice by injection of 0.1mL freshly prepared egg albumin into the plantar-Apo neurosis of the right hind paw. The linear circumference of the injected paw was measured using vernier caliper (that is at 0 hour) and 0.5, 1, 2, 3, and 5 hours after the egg albumin administration.<sup>[15, 16]</sup>

#### Statistical Analysis

Data obtained from this work will be analyzed statistically using student t-test and ANOVA (one – or two way) followed by a post test (Turkey-Kramer multiple comparison test). Difference between means was considered significant at P ≤ 0.05 and 0.01.

**Table 3 : Anti-Inflammatory Effect Of Extract On Egg Albumin-Induced Oedema In Mice**

Treatment/ Dose (mg/kg)	TIME INTERVALS (HR)						
	0	0.5	1	2	3	4	5
Control	4.46±0.17	8.05±0.28	8.38±0.16	8.88±0.16	8.16±0.01	7.32±0.15	6.15±0.15
Extract 34.64	4.53±0.16	7.92±0.24	8.25±0.16	8.00±0.16	7.50±0.16	6.75±0.16	5.71±0.17
69.28	4.56±0.14	7.83±0.39	8.40±0.17	7.89±0.17	6.57±0.45	5.81±0.45	4.84±0.45a
103.92	4.30±0.08	7.56±0.60	8.49±0.52	7.40±0.19	6.23±0.52	5.14±0.43	4.19±0.43b
ASA 100	4.18±0.06	7.14±0.50	7.92±0.17	7.92±0.17	5.21±0.17	4.72±0.17	3.95±0.18

Data are expressed as mean  $\pm$  SEM. Significant at <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, when compared to control. n=6.

## DISCUSSION

The dry pulverized plant material weighed 1000g, the N-Hexane extract 9.55g (1.73% w/w), Ethylacetate extract 27.60g (4.89% w/w) and the Methanol Extract 140.42g (26.41% w/w).

The median lethal dose (LD<sub>50</sub>) was calculated to be 346.4 mg/kg.

The result of phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenes, steroidal glycoside, saponins, phenols, carbohydrate and cardenolides in the extract of the plant. One or a combination of these phytochemicals may be responsible for the antipyretic, anti-inflammatory and antinociceptive activities observed.<sup>[17]</sup>

**Analgesic Effect of Extract on Formalin-induced Hind paw licking in mice:** The injection of formalin caused Hind paw licking (pain) in mice.

The paw licking intensified 5 min following formalin injection. This is called the first phase. It corresponds to the period the animal experiences an acute pain. The second phase occurred 15-30 minutes after injection. This is the more persistent inflammatory state. The methanol extract of the *Pentaclethra macrophylla* reduced the hind paw licking significantly (p<0.05-0.01) compared to the negative control (Table 1). This result corroborates a previous study in which the aqueous extract of the plant also inhibited pain significantly.<sup>[18]</sup>

### Anti-pyretic effect--- DNP- induced pyrexia in mice

Dinitrophenol (DNP) was used to induce pyrexia in mice. DNP causes increased level of intracellular calcium, muscle contraction and hyperthermia.<sup>[19]</sup>

In this study, the extract reduced pyrexia in a dose dependent manner. The reduction was however not significant (P>0.05) compared to positive control (Table 2). Some Phenols including flavonoids have been shown to possess antipyretic properties.<sup>[17]</sup>

### Anti-Inflammatory Activity of the extract on Egg Albumin-Induced Oedema

The egg albumin administered induced oedema (inflammation) in the mice. The extract reduced the oedema significantly (P<0.05 – 0.01) in a dose-

dependent manner compared to the negative control (Table 3). Some Tannins are believed to have anti-inflammatory activities.<sup>[20]</sup>

## CONCLUSION

Based on the findings of this study, it can be concluded as follows

- That the stem bark extract of *Pentaclethra macrophylla* contain flavonoids, saponins, tannins, alkaloids, cardiac glycosides, carbohydrates and polyphenols.
- That the LD<sub>50</sub> of the extract was 346.41mg/kg
- That the extract demonstrated significant anti-inflammatory, antipyretic and anti-nociceptive activities.

## CONTRIBUTION TO KNOWLEDGE

At the end of this study, it was discovered that

- The methanol, n-Hexane and ethylacetate extract of *Pentaclethra macrophylla* Benth has abundant phytochemicals present.
- The methanol bark extract of the plant possesses anti-inflammatory, anti-pyretic, and anti-nociceptive activities.

## RECOMMENDATION FOR FURTHER STUDIES

It is hereby recommended as follows, that

- Anti-inflammatory, anti-pyretic, anti-nociceptive studies should be carried out on the n-Hexane and ethylacetate bark extracts of this plant.
- Isolation should be carried out on the n-hexane and ethylacetate extracts to further elucidate chemical constituents of the plant.

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