Phytochemical,Analgesic and Anti-inflammatory Analysis of the Ethylacetate Fraction of *Paullinia pinnata* Leaf L.(Sapindaceae)

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Abstract

Paullinia pinnata(Sapindaceae) is commonly called sweet gum (English). Traditionally various parts of *P. pinnata* is used in the management of various diseases including chronic arthritis rheumatic pain. Phytochemical investigation of the ethyl acetate fraction of *P. Pinnata* leaf showed the presence of flavonoids, saponins, anthraquinone, steroidal terpenoids and carbohydrates only and it was also observed to be non-toxic with LD_{50} of 1264.9 mg/kg. The fraction displayed significant analgesic activity (21.45, 35.62 and 92.70 % inhibition at 75, 150 and 300 mg/kg body weight respectively) in the formalin induced pain model, whereas ketoprofen had a 28.32 % inhibition of pain. In acetic acid induced writhing model the fraction also displayed significant analgesic activity between 70.03 to 100 % inhibition of the contraction of the abdominal muscle and stretching of the hind limbs at a dose of 75 to 300 mg/kg body weight respectively, where ketoprofen had a 76.60 % inhibition. The fraction had significant anti-inflammatory activity in carrageenan induced paw oedema model with maximum activity up to three hours (60.0 % at first hour, 47.47 % at second hour, 65.38 % at third hour and 63 % at fourth hour) at a dose of 150 mg/kg body weight. The ethyl acetate fraction of *P. Pinnata* leaf poses significant analgesic and anti-inflammatory activities.

Keywords: *Paullinia pinnata*, Acute Toxicity, Analgesic, Anti-inflammatory

Introduction

Paullinia pinnata is an evergreen climbing shrub that climbers up to a height of 2.5 to 8.0 m. The leaves are compound with five leaflets, the terminal leaflet being the largest, inflorescence stand axillary on long stalks[1].*Paullinia pinnata* belong to the Sapindaceae family. It is commonly called sweet gum (English) but locally called *Furenamaryaa, Hannubiyar* (hausa), *Egwubiomekpa (Igala*) and *Kakasela* (*Yoruba)*[2]. Traditionally various parts of *P. pinnata* is used in the management of various diseases. The leaf juice of *P. pinnata* is used for the treatment of sore throat, fever while the root is used in the management of leprosy, jaundice, snake bites, nausea and vomiting[3,4].The roots are also chewed for coughs and pulmonary diseases, gonorrhoea, fractures or abscesses or used on open sores. It is also used as aphrodisiac[4]. The methanol extract of *P. pinnata* had also had observed to have some anti-malarial property against Chloroquine-sensitive *P. berghei* NK65[5].
Paullinia pinnata had also been reported to be used in ethnomedicine for the management of chronic rheumatism

[6].Despite the progress in the development of safe and effective analgesic drugs, pain and inflammation is the most common reason for physician consultation in most developed countries[7]. Hence there is need to develop potent and safe analgesics from plants, since medicinal plants are important source of new chemical substances with potential therapeutic effects[8]. Previous studies on *P. pinnata* indicated that the ethanolic extract possess some analgesic and anti-inflammatory activity and phytochemical investigation of the ethanolic extract revealed the presence of saponins, tannins, glycosides and flavonoids[6]. While the methanol extract revealed the presence of steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids[1].
This study was aimed at investigating the safety, analgesic and anti-inflammatory activities of the ethylacetate fraction of *P.pinnata* crude methanol extract.

Materials and Methods

Plant collection

The aerial part of *Paullinia pinnata* (Linn) was collected from Samaru, Zaria, Kaduna State, Nigeria. The plant was authenticated at the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher number of No 427 was issued.

Plant preparation

The leaves of *P. pinnata*were washed and air-dried under the shade and extracted into distilled methanol. The extract was concentrated using a rotary evaporator (40-50°C), weighed and refrigerated until needed.Crude methanol extract of *P. pinnata*was dissolved in methanol and water (3:1). This was then exhaustively partitioned 5 times using 200 mL ethylacetate. The ethyl acetate fraction obtained was evaporated to dryness *in vacuo* (40-50°C) and refrigerated until needed for analysis.
A portion of the fraction was weighed and dissolved in 50 µL of ethylacetate prior to the addition of distilled water for use on each day of the experiment.

Phytochemical Analysis

Phytochemical constituent of the ethylacetate fraction of *Paullinia pinnata* was performed to test for the presence of alkaloids, saponins, tannins, glycosides, flavonoids resins and carbohydrate [9,10].

Acute Toxicity Testing

The median lethal (LD_{50}) dose of the *P. pinnata* ethyl acetate fraction was determined by the method described by Lorke [11] using intraperitoneal administration. A total of thirteen (13) mice of both sexes were used.
This evaluation was done in two phases. In phase I, three groups of three mice each were treated with 10, 100, and 1000mg/kg of the fraction by intraperitonially administration (*i.p*) respectively. The mice were observed for clinical signs of toxicity and death for 24 hours after treatment. In phase II four (4) untreated mice were each treated with four more specific doses of the fraction obtained from the results of the previous treated mice in first phase. The LD_{50} was then calculated as the geometric mean of the highest non-lethal and the lowest lethal dose.
$LD_{50}= \sqrt{D_0 \times D_{100}}$
D_0 = Highest non-lethal dose
D_{100} = Lowest lethal dose

Animals

Adult Swiss Albino mice and rats of either sex were obtained and maintained in cages in the animal house of the Pharmacology and Therapeutic Department of the University of Ilorin, under standard laboratory conditions in the presence of light

and at room temperature, fed on standard feeds and provided with drinking water *ad libitum*.Animals used for this study were fasted for 12 hours and deprived of water only during the experiment.

Tests for Analgesic Activity

Formalin test in rats: Thirty (30) Wister rats were divided into five groups of six rats. Group 1 rats received normal saline. Groups 2, 3 and 4 rats were pre-treated with 75, 150 and 300 mg/kg body weight of the ethylacetate fraction of the extract intraperitonially respectively, while Group 5 were pre-treated with standard drug/ ketoprofen (10mg/kg).Thirty minutes later each rat was injected with a 2.5% solution of formalin subcutaneously under the plantar surface of the left hind paw. The rats were placed individually in an observation chamber and monitored. The severity of pain for each rat was recorded using the scale described by Dubuisson and Dennis [12]. Where: scale 0 - if rat walked or stood only on the injected paw; scale 1-if the injected paw was slightly elevated; scale2-if the paw was clearly lifted off the floor; scale 3-if the rat licked the injected paw.

Acetic acid-induced writhing test: The ability of the ethyl acetate fraction to inhibit/suppress acetic acid induced contraction of the abdominal muscle and stretching of the hind limbs was estimated by the method described by Winter and co-workers [13]. Thirty (30) mice were divided into five groups of six mice. Group 1 mice received normal saline (*i.p*). Groups 2, 3 and 4 were pre-treated with varying doses of the ethylacetate fraction (75, 150 and 300 mg/kg body weight *i.p*. respectively), while Groups 5 was treated with ketoprofen (10mg/kg). After 30 minutes, the mice were treated with acetic acid (0.6%v/v) at a dose of 10mg/kg (*i.p*). Five minutes later, the mice were then placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 minutes and the percentage inhibition of writhing was calculated.

$$\% \text{ inhibition of Writhing} = \frac{\text{No. of writhes (control) - Mean No of writhes (test)}}{\text{Mean No of writhes (control)}} \times 100$$

Anti-inflammatory Activity

Carrageenan induced paw oedema: The ability of the ethylacetate fraction to inhibit Carrageenan induced oedema was estimated using the method described by Posadas and co-workers[14]. Thirty (30) Wister rats were divided into five groups of six rats. Group1 rats received normal saline (*i.p*). Groups 2, 3 and 4 were pre-treated with the ethylacetatefraction of the extract (75, 150 and 300 mg/kg body weight *i.p*. respectively), Groups 5 was treated with ketoprofen (10mg/kg).After 30 minutes, 0.1mL carrageenan (1%) was injected into the plantar surface of the left hind paw of each rat. The paw volume was measured at 0, 1, 2, 3 and 4 hours using a Venier caliper to determine the diameter of the oedema. The difference between the radii at time 0 hr and different time intervals was taken as diameter of oedema.

Statistical analysis

The results obtained were expressed as mean ± SEM and analyzed using the Student's t-test. A P value ≤ 0.05 was considered to be statistically significant.

Results

Table 1: Phytochemical Analysis of Ethyl acetate Fraction of *P. pinnata* Leaf

Metabolite	*Paullinia pinnata* Leaf Extract
Alkaloid	-
Flavonoid	++
Tannins	-
Saponins	++
Anthraquinone	+
Cardiac glycoside	-
Steriodterpenoid	+
Carbohydrate	+

KEY: -, Absence of component; + Presence of component, ++ Copious amount of component

Table 2: Acute Toxicity of Ethyl acetate Fraction of *P. pinnata*

	Phase I		Phase II	
Group	**Dosage (mg/kg)**	**Mortality**	**Dosage (mg/kg)**	**Mortality**
1	10	0/3	600	0/1
2	100	0/3	1000	0/1
3	1000	0/3	1600	1/1
4	-	-	2900	1/1

Table 3: Analgesic Activity of the Ethylacetate Fraction of *P.pinnata* on Formalin Induced Pain.

Treatment	Dose (mg/kg)	Mean pain severity score	Percentage Inhibition (%)	P Value
Normal Saline		2.33±0.21	-	-
Ethyl acetate Fraction	75	1.83±0.17	21.45	0.496
	150	1.50±0.34	35.62	0.5680
	300	0.17±0.17	92.70*	0.0022
Ketoprofen	10	1.67±0.33	28.32	-

* = Significant difference (P< 0.05)

Table 4: Analgesic Activity of the Ethylacetate Fraction of *P. pinnata* in Acetic acid Induced Writhing in Mice

Treatment	Dose (mg/kg)	Mean No. of Writhes	Percentage Inhibition (%)	P Value
Normal Saline		23.5±3.10	-	
Ethyl acetate Fraction	75	6.83±2.83	70.03	0.5356
	150	6.33±2.46	73.06	0.6672
	300	0.00±0.00	100.00*	0.0073
Ketoprofen	10	5.50±1.89	76.60	-

n=6 * = Significant difference (P< 0.05)

Table 5: Anti-inflammatory Activity of the Ethylacetate Fraction of *P.pinnata* on Carrageenan Induced Paw Oedema.

Treatment	Dose (mg/kg)	1 h	2h	3h	4h
		% inhibition			
Normal Saline	-				
Ethyl acetate Fraction	75	40.00	47.37	57.69	52.63
	150	60.0*	47.37	65.38	63.89*
	300	20.00	42.10	61.54	57.89
Ketoprofen	10	50.00	52.63	73.07	63.16

n=6 * = Significant difference (P< 0.05)

Results and Discussion

Phytochemical investigation of the ethylacetate fraction of *P. pinnata* is shown in Table 1. The fraction was observed to contain flavonoids, saponins, anthraquinone, steroidal terpenoids and carbohydrates. However alkaloids, tannins and cardiac glycosides were absent. In previous studies, the crude methanol extract of *P. pinnata* gave a positive test for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids[1], while the ethanolic extract of *P. pinnata* was positive for the presence of saponins, tannins, glycosides and flavonoids [6]. This is the first report of the

phytochemical constituents of the ethyl aceate fraction of *P. pinnata*.

The acute toxicity study as shown in Table 2 indicated that the ethylacetate fraction of *P. pinnata* caused no death at the doses of 10 – 1000 mg/kg body weight (Phase I). In phase II the fraction was safe up to a dose of 1000 mg/kg body weight, while death was recorded from 1600 up to 2900 mg/kg body weight. Hence, the highest non-lethal dose (D_0) is 1000 mg/kg body weight while the lowest lethal dose (D_{100}) is 1600 mg/kg body weight. The dose of the *P. pinnata* ethylacetate fraction that will kill 50 % of the animal population (LD_{50}) was estimated to be 1264.9 mg/kg. In a previous study, ethanol extract of *P. pinnata* leaf was also observed to be non-toxic with a LD_{50} of 1131 mg/kg.

The ethylacetate fraction of *P. pinnata* was observed to show a concentration dependent analgesic activity in formalin induced pain model as shown in Table 3. The fraction displayed 21.45, 35.62 and 92.70 % inhibition at 75, 150 and 300 mg/kg body weight respectively, while the reference drug / ketoprofen had a 28.32 % inhibition of pain. The ethyl acetate fraction of *P. pinnata* (75 and 150 mg/kg body weight) displayed similar analgesic activity to ketoprofen (10 mg/kg body weight) with $P > 0.05$. However, at 100 mg/kg the ethylacetate fraction displayed over three times inhibition compared to ketoprofen. In acetic acid induced writhing test, the ethylacetate fraction of *P. pinnata* was also observed to show a concentration dependent analgesic activity as shown in Table 4. Acetic acid Induced writhing model is a popular and highly sensitive model for the evaluation of peripheral anti-nociceptive activity of plant extracts and compounds [15,16]. Local peritoneal receptors are postulated to be partly involved in abdominal constriction response. The method has been associated with prostanoids in general, e.g. increase level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products [8]. Hence, it may be postulated that the mechanism of action by which the ethyl acetate fraction inhibit the acetic acid -induced writhing may be by inhibition of prostanoids with inhibition of either lipoxygenase and/or cyclo-oxygenases.

The ethyl acetate fraction of displayed significant anti-inflammatory activity in the carrageenan induced paw oedema as shown in Table 5. The anti-inflammatory activity of ethyl acetate fraction (75 and 150 mg/kg body weight) had significant activity at the fourth hour (52.63 and 63.89 % inhibition respectively). But at a higher dose of 300 mg/kg body a lower anti-inflammatory activity (20 % at first hour, 42 % at second hour, 61.54 % at the third hour and 57.89 % at the fourth hour) was observed compared to a two other lower doses. The standard drug display 50, 52.63, 73.07 and 63.16 % inhibition of the paw oedema at the first, second, third and fourth hour respectively. The fraction displayed significant anti-inflammatory activity. In another study using egg albumin induced paw oedema in rats, the ethanol extract of *P. pinnata* displayed significant anti-inflammatory activity[6]. The observed effect did not appear to be dose-dependent, because maximum inhibitory effects (60.0 % at first hour, 47.47 % at second hour, 65.38 % at third hour and 63 % at fourth hour) were observed at a dose of 150 mg/kg body weight.

Conclusion

Phytochemical investigation of the ethyl acetate fraction of *P. pinnata* showedthe presence of flavonoids, saponins, anthraquinone, steroidal terpenoids and carbohydrates. The ethyl acetate fraction was also observed to be non-toxic and displayed significant analgesic and anti-inflammatory activities in the formalin in

formalin induced pain, acetic acidinducedwrithing and carrageenan induced paw oedema. This study validates the traditional use of *P. pinnata* in ethno medicine for the management of pain and inflammation.

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