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ANALGESIC, PHYTOCHEMICAL AND ACUTE TOXICITY EVALUATION OF THE METHANOL EXTRACT OF THE LEAVES OF *PTEROCARPUS SANTALINOIDES*- FAMILY FABACEA

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ABSTRACT

Pterocarpus santalinoides, family- Fabaceae was claimed to have analgesic properties. The people of Ogidi in Idemili North Local Government Area of Anambra State, Nigeria used it in the management of aches and pains. This study is therefore aimed at determining this claim of the activities of *Pterocarpus santalinoides* using the leaves which will serve as a criterion to recommend the ethno pharmacological use of the plant. The leaves of *Pterocarpus santalinoides* family Fabaceae were dried, powdered and extracted by cold maceration with methanol for 48hrs, it was concentrated using rotary evaporator. The analgesic activity was investigated in rats using hot plate method at a temperature of 40^{o}C. Phytochemical evaluation revealed the presence of tannins, flavonoids, terpenoids, steroids, alkaloids, glycosides, saponins and resins. *Pterocarpus santalinoides* extract (300 mg/kg) induced analgesia in rats ($p< 0.05$) and this effect was comparable to that of Aspirin (100 mg/kg). Acute toxicity test also revealed that the drug is safe. The claimed benefits of *Pterocarpus santalinoides* in traditional medical management of aches and pains could be supported by the results of this investigation.

INTRODUCTION:

Plant Description [1, 2]:

Name: *Pterocarpus santalinoides*

Taxonomy: Domain: *Eukaryot;,* Kingdom: *Plantae;* Subkingdom: *viridaeplanntae;* Phylum: *Magnoliophyta;* Subphylum: *Euphyllophytina;* Infraphylum: *Radiatopses;* Class: *Magnoliopsida;* Subclass: *Rosidae;* Superorder: *Fabanae;* Order: *Fabales;* Family: *Fabaceae* Subfamily: *Faboideae*; Tribe: *Dalbergieae*; Genus: *Pterocarpus*; Specie: *Pterocarpus santalinoides*

Synonym(s): *Pterocarpus amazonicus* Huber; *Pterocarpus esculentus* Schum. & Thonn.; *Pterocarpus grandis* Cowan; *Pterocarpus michelii* Britton.

Common names: (Hausa): gunduru, gyadar kurmi; (Igbo): uturukpa; (Yoruba): gbengbe.

Botanic description: *Pterocarpus santalinoides* is a tree 9-12 m tall, 1 m DBH, with low straggling branches. Bark thin and flaking in small patches, slash yellowish-

white exuding drops of red gum. Leaves compound, 5-9 leaflets ovate-elliptic, abruptly acuminate, rounded at the base or slightly cineaste, glabrous, glossy, rather coriaceous with about 8 pairs of prominent main lateral nerves looping away from the margin, leaf stalk slender, glabrous stalk 10-20 cm long, leaflet stalk stout 2-5 mm long.

Flowers orange-yellow, fragrant in axillary racemes and panicles, inflorescence branches finely hairy, individual flowers with short stalks. Calyx rather narrowly cup-shaped, petals densely hairy outside, about 7 mm long including the prominent triangular teeth, standard petal about 12 mm long and broad. Fruit a light brown glabrous pod, 3.5-6 cm across including the soft, fleshy narrow wing which extends about three quarters way round the body.

Pterocarpus is based on the Greek words 'pteran' meaning a wing and, 'karpos' meaning' fruit. The specific epithet 'santalinoides' refers to its likeness to *P. santalinus* found in Asia.

Ecology and distribution:

- **Natural Habitat**: *P. santalinoides* is a shade tolerant tree commonly found along riverine forests in Africa and tropical South America.
- **Geographic distribution**: Brazil, Cameroon, Ghana, Nigeria, Senegal.
- **Biophysical limits**: Altitude: 200-500 m Mean annual temperature: 26 deg C Mean annual rainfall: 1 600 mm.
- **Soil type:** Prefers well drained soils.
- **Reproductive Biology**: *P. santalinoides* is monoecious, flowering from December-March, fruits ripening between March-April.

Propagation and management:

- **Propagation methods:** Direct seeding, cuttings and rootstocks can be used to propagate *P. santalinoides*.
- **Tree Management**: Pollarding, coppicing and lopping are recommended management practices for *P. santalinoides*.

Functional uses:

Products:

- **Food:** The leaves are eaten as a vegetable [3].
- **Fodder:** Livestock browse its young shoots and leaves.
- **Timber:** Wood white or yellow, not hard but termite- resistant.
- **Gum or resin:** Cuttings on the stem exude a red gum.
- **Tannin or dyestuff:** The bark contains tannins and dyes used for dyeing.
- **Medicine:** The tree bark is used as a stomach ache remedy.

Services:

- **Erosion control:** An important species for soil conservation in water catchment areas.
- **Shade or shelter:** A good windbreak around settled areas and farms.
- **Nitrogen fixation:** *P. santalinoides* forms nodules with nitrogenase activity. The nodules are generally spherical but occasionally elongate.
- **Soil improver:** Leaf litter from *P. santalinoides* on decomposition slowly releases N and significantly increases soil exchangeable Ca and Mg in the soil.
- **Ornamental:** A beautiful tree with good gardening attributes its; showy flowers, beautiful foliage and form make it a suitable ornamental tree.
- **Boundary or barrier or support**: Poles from *P. santalinoides* are used for fencing.

FIGURE 1: *P. SANTALINOIDES*

MATERIALS AND METHOD:

Drugs and Chemicals: Aspirin, distilled water and methanol.

Materials: Miller (Thomas Laboratory Mill, U.K), Mechanical Weighing Balance (Ohaus, Poland), Electronic Weighing Balance (Gulfes Mediqal and Scientific,England), Filter Paper (No. 1 Wattman), White Clean Handkerchief (as porcelain cloth), Rotary Evaporator (Fulton, china), Oven (Harris, England), Mechanical shaker (Surgifrend, England), Beaker (10ml, 25ml, 50ml, 500ml capacities), Cotton wool, Hand gloves, Syringes and Needle (1ml, 2ml, 5ml), Hot plate.

Animal: Albino rats (57 – 220g) and albino mice (18 – 29g) of both sexes.

Collection and Identification of Plant Material: Young fresh leaves of Pterocarpus santalinoides were collected in Ogidi, Idemili North local government area of Anambra State in July, 2011, during the rainy season and was identified by Dr. Ezugwu, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Methanolic Extraction: Fresh leaves of Pterocarpus santalinoides family Fabaceae were dried at ambient temperature until their weight which was measured at intervals was about the same. The dried leaves were pulverized using laboratory miller, 250g of the powder was macerated in 500ml of methanol and were placed on a mechanical shaker for 48 hours, the extract was filtered using clean white handkerchief, then the filtrate was further filtered using No.1 Wattman filter paper. The filtrate was concentrated using rotary evaporator. The extract was stored in the refrigerator for future use.

Phytochemical Screening: Phytochemical tests were carried out on the methanolic extract of *Pterocarpus santalinoides* using the procedure outlined by Harbourne [2]. In general, test for the presence or absence of phytochemical compounds using the above method involves the addition of an appropriate chemical agent to the methanolic extract of the leaves in a test tube and shake (**Table 1**).

Test for Carbohydrate:

- **Molisch's test:** About 0.1g of the extract was boiled with 2ml of water, and filtered. To the filtrate, two drops of naphthol solution in ethanol (molisch reagent) was added. Concentrated sulphuric acid was gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

Test for Reducing Sugar: 0.1g of the leave extract was shaken vigorously with 5ml of distilled water and filtered. The filtrate was divided and used for the following test.

- **Fehling's Test:** To a 1ml, portion of the filtrate was added equal volumes of Fehling's solution 1 and 2 and boiled on a water bath for a few minutes. A brick red precipitate indicates the presence of reducing sugar.

- **Benedict's Test:** To another 1ml portion of the filtrate, 2ml of Benedict's reagents was added. The mixture was shaken, heated on a water bath for five minutes. A rusty precipitate indicates the presence of reducing sugar.

Test for Alkaloids: 20mls of 5% sulphuric acid in 50% ethanol was added to about 2g of the methanolic extract and heated on a boiling water bath for 10minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of Mayer's, Dragendroff's, Wagner's reagent and 1% picric acid. The remaining filtrate was placed in 100ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portion of dilute sulphuric acid. The Mayer's, Dragendroff's, Wagner's and picric acid respectively. The extract gave milky, brick red, reddish brown and yellow precipitate with one drop each of the reagents and therefore showing the presence of alkaloid.

Test For Glycosides: About 5ml of a mixture of equal part of Fehling's solution of the extract, dissolved in water and then heated or a water bath for few five minutes. A brick red precipitate shows the presence of glycosides.

- **Hydrolysis Test:** About 5ml dilute sulphuric acid were added to about 0.1g of leave extract in a test tube and boiled for 15 minutes in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10ml of a mixture of equal parts of Fehling's solution 1 and 2 were added and boiled for 15minutes. A brick red precipitate indicates the presence of glycosides.

Test for Saponin: About 20ml of water was added to 0.25g of the methanolic extract of the leave in 100ml beaker and boiled gently on a water bath for two minutes. The mixture was filtered hot and allowed to cool and the filtrates used for the following tests.

- **Frothing Test:** About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth upon standing indicates the presence of saponins.
- **Emulsion Test:** To the frothing solution was added two drops of olive oil and the content shaken vigorously. The formation of emulsion indicates the presence of saponins.
- **Fehling's Test:** To 5ml of the filtrate was added 5ml of Fehling's solution (equal parts of 1 and 2) and the mixture heated. A reddish precipitate indicated the presence of saponins further heating with sulphuric acid produce a brick red precipitate.

Test for Tannins: About 0.5g of the extract was boiled with 25ml of water, filtered and used for the following test.

- **Ferric Chloride Test:** To 3ml of the filtrate was added few drops of ferric chloride solution. A greenish black precipitate indicates the presence of tannins
- **Lead Sub Acetate Test:** Few drops of lead sub acetate were added to 3mls of the filtrate. A clean precipitate appearing would interfere with the presence of tannins.

Test for Flavonoids: 5ml of ethyl acetate were added to 0.1g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for the following test.

- **Ammonium Test:** About 2ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonia cal layer indicates the presence of flavonoids.

Test for Resins: The plant extract was dissolved in 3ml acetone and 3ml concentrated hydrochloric acid was added. This mixture was heated in a water bath for 30 minutes. A pink color which changes to red indicates the presence of resins.

Test for steroids and triterpenoids: About 9 ml of ethanol was added to 1 g of the extract it was refluxed for a few minutes and filtered. The filtrate was concentrated on a boiling water bath. 5 ml of hot distilled water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of conc. H_2SO_4 to form a lower layer. A reddish brown interface shows the presence of steroids. 0.5 ml of the chloroform was evaporated to dryness on a water bath and heated with 3 ml of the concentrated sulphuric acid for 10 minute on a water bath. A grey color indicates the presence of terpenoids.

Test for Carotenoids: A measured weight of each sample was homogenized in methanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtered to obtain the initial crude extract.20ml of ether were added to the filtrate to take up the carotenoid mixed well and then treated with 20ml of distilled water in a separating funnel. The other layer was recovered and evaporated to dryness at low temperature (35-500C) in vacuum desiccators. The dry extract was then saponified with 20ml of ethanolic potassium hydroxide and left over night in a dark cupboard.

The next day, the carotenoid were taken up in 20ml of ether and then washed with two portions of 20ml distilled water. The carotenoid extract (ether layer) was dried in a dessicator and then treated with a light petroleum (petroleum spurt) and allowed to stand overnight in a freezer (-100C). The next day, the precipitated steroid was removed by centrifugation and the carotenoid extract was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator and weighed. The weight of carotenoid was determined and expressed as a percentage of the sample weight.

Test for Anthocyanins: This was done gravimetrically by the method of Harborne [4].

Pharmacological Tests:

1. **Acute Toxicity Test:** *The* acute toxicity study of *Pterocarpus santalinoides* was assessed by oral administration in albino mice [5, 6]. Briefly, the tests involved two phases. The first phase involved the determination of the toxic range. The mice were placed in three groups (n = 3) and the extract (10, 100 and 1000 mg/kg) suspended in distilled water was administered orally. The treated mice were constantly observed for the next 4hrs, then intermittently for the next 6hrs, then over a period of 24hrs. Then the number of death in each group was recorded. The death pattern in the first phase determined the doses used for the second phase. In this phase, four groups (n = 1) of mice were used for each dose. Each group received different doses of the extract (p. o.) 1500 mg/kg, 2500 mg/kg, 3500 mg/kg and 5000 mg/kg respectively. The animals were observed for lethality or signs of acute intoxication for the next 24hrs. The LD50 was calculated using the relation (**Table 2**).

$$\sqrt{a \times b}$$

Where 'a' is the lowest dose that brought death and 'b' is the highest dose that did not bring death.

2. **Analgesic Activity:** The analgesic activities of *Pterocarpus santalinoides* was carried out using in vivo method of test. A total of 15 adult albino rats were employed. They were grouped into five groups of three rats each in a group. Each of them was placed individually on a hot plate at the temperature of 40˚C. The time the animal started licking its paw, or showing signs of discomfort was noted and it was taken as their normal duration, then the animals were given treatment as follows; group 1 received 0.5ml of distilled water, group 2 received 100 mg/kg of aspirin, group 3 received 100 mg/kg of extract, group 4 received 200 mg/kg of extract, while group 5 received 300 mg/kg of extract. 30mins, 60mins, 90mins and 120mins after oral administration of the drugs, the duration of time each animal in each group can stay comfortably on the hot plate was taken, then the negative control group (group 1) was compared with other groups for significance in analgesic activity using T- test (dinette comparison method) (**Table 3**).

RESULTS AND DISCUSSION

TABLE 1: RESULT OF THE PHYTOCHEMICAL CONSTITUENTS

Phytochemical components	Relative presence
Tannins	+++
Flavonoids	+++
Terpenoids	++
Steroids	+++
Glycosides	++
Resins	++
Alkaloids	+++
Anthocyanins	+
Carotenoids	+
Saponins	++
Carbohydrates	-
Reducing sugars	-

KEY: +; slight presence, ++; medium presence, +++; heavy presence, -; absent

TABLE 2: ACUTE TOXICITY TEST

Phase	Dose (mg/kg)	No. of death
I	10	0/3
	100	0/3
	1000	0/3
II	1500	0/1
	2500	0/1
	3500	0/1
	5000	0/1

*Pterocapus santatinoide*s extract is non-toxic and caused no death or signs of acute-intoxication in mice at a dose up to 5000 mg/kg administered orally.

TABLE 3: ANALGESIC ACTIVITY OF THE METHANOLIC EXTRACT OF THE LEAVES OF *PTEROCARPUS SANTALINOIDES* Mean ± S.E.M

GROUP	Agent & Dose	Initial time (secs)	Treatment (30mins)	Time (secs)		
				60mins	90mins	120mins
1	0.5ml Distilled water	2.5±0.01	3.0±0.00	3.0±0.01	3.5±0.50	0.20±0.02
2	100mg/kg Aspirin	2.0±0.05	ns 2.5±0.01	ns 4.0±1.00	**5.5±0.50	**6.0±0.03
3	100mg/kg Extract	2.0±0.00	ns 2.0±0.03	ns 2.5±0.25	*4.0±2.00	*4.5±0.02
4	200mg/kg Extract	3.0±0.02	ns 3.0±0.05	*5.5±0.05	**7.5±1.50	**5.0±0.56
5	300mg/kg Extract	3.0±0.05	*4.5±0.48	*7.0±2.00	**6.0±0.00	**6.0±0.33

ns = Not significant (p > 0.05), *= slightly significant (p< 0.05), **= extremely significant (p< 0.05)

The extract of *Pterocarpus santalinoides* significantly increased the time the albino rats can stay comfortably on the hot plate in a dose dependent manner [7].

At a dose of 300mg/kg, the effect of extract was comparable to that of the standard drug, Aspirin (100mg/kg). [8] Analgesic activity of extract may be associated with the presence of steroids; a phytochemical constituent which it contains in abundant. Steroids are significant in the treatment of inflammation and pain is a sign associated with inflammation [9- 11].

CONCLUSION: This study shows that the methanolic extract of leaves of P*terocarpus santalinoides* possesses analgesic activity which may be as a result of the presence of steroids. Also, the methanolic extracts of the leaves of P*terocarpus santalinoides* showed no toxicity. This justifies the folkloric use of *Pterocarpus santalinoides* in Ogidi community of Anambra State. However, further studies are recommended to isolate and characterize the structure of the active constituents.

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