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(Research Article)

Preliminary Phytochemical Screening , Evaluation of Acute Toxicity and Antipyretic Activity of Methanolic Extract of *Pterocarpus santalinoides* (Fabaceae)

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ABSTRACT

Pterocarpus santalinoides (Family – Fabaceae) was claimed to have antipyretic properties. The people of Ogidi in Idemili North Local Government Area of Anambra State,Nigeria used it in the management of fever. This study is therefore aimed at determining this claim of the activities of Pterocarpus santalinoides using the leaves which will serve as a criteria to recommend the ethnopharmacological 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 anti-pyretic activity was investigated using brewer's yeast to induce pyrexia in rats. Phytochemical evaluation revealed the presence of tannins, flavonoids, terpenoids, steroids, alkaloids, glycosides, saponins and resins. Pterocarpus santalinoides extract (300 mg/kg) relieved pyrexia in rats ($p<0.01$) and this effect was comparable to that of aspirin (100 mg/kg). Acute toxicity also revealed that the drug is safe. The claimed benefits of Pterocarpus santalinoides in traditional medical management of pyrexia, could be supported by the results of this investigation.

Key Words: *Pterocarpus santalinoides*, Anti pyretic, Brewers yeast, Aspirin, Phytochemicals.

INTRODUCTION

Pterocarpus santalinoides L'Herit ex DC (Family: *Fabaceae-papilnoideae*) has been described[1] as a shade-tolerant tree 9-12m tall, with low straggling branches, commonly found along riverine forests in Africa and tropical South America. The plant is a commonly referred to as Red Sandal wood in English, Gunduru gyadar Kurmi in Hausa, Uturukpa in Igbo and Gbenghe in Yoruba. Various morphological parts of *P. santalinoides* are used in ethnomedicine in many African countries, to treat an array of human ailments. The ethnomedical use of leaves of *P. santalinoides* in the treatment of diarrhoea and other gastrointestinal disorders has been scientifically proved. [1,2] The triglyceride and glucose lowering properties of *P. santalinoides* has been ascertained, as such lending credence to its folkloric use in management of diabetic syndrome. It has been documented that the bark and leaves of the plant possess anti malarial, anti infective and anti abortive properties.[3] Ethnomedically, leaf extract of *P. santalinoides* combined with leaves of *Solanum macrocarpum* is used in the management of high blood pressure among the Igede tribe in central Nigeria.[4] Among Ogidi people, south east Nigeria, *P. santalinoides* is claimed to have anti pyretic property and is used as such. There is paucity of studies on the anti pyretic activities of *P. santalinoides*. Therefore, the aim of the present study was to investigate the plant for the presence of various phytoconstituent, evaluate its toxicity and verify the scientific basis of the use of the leaves of *P. santalinoides* as anti pyretic agent.

MATERIALS AND METHODS

Drugs and Chemicals

Aspirin, Tween 80, Distilled water, Brewers yeast 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 Whatman), White Clean Handkarchief (as porcelin cloth), Rotary Evaporator (Fulton,china), Oven (Harris,England), Mechanical shaker (Surgifrend,England), Beaker (10ml,25ml and 50ml and 500ml capacities), Cotton wool, Hand gloves,Syringes and Needle (1ml,2ml and 5ml), Hot plate.

Animal

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

Plant Material

Collection and identification of plants

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 in the Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.

Extraction of Plant Material

Fresh leaves of *Pterocarpus santalinoides* 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, 200g 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 Whattman 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.[5] 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 shaken .

Test for Carbohydrates

Molisch 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 filterate 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

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.

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 filterate was shaken with 1ml of dilute ammonia solution. The layer were allowed to separate and the yellow colour in the ammoniacal 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 munites. A pink colour 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 a vacuum desiccators. The dry extract was then saponified with 20ml of ethanoic 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 dessicator 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 [5] (1973). 5g of each test sample was hydrolyzed by boiling in 100ml or 2Mhcl solution for 30 min. The hydrolysate was filtered using Whatman No.42 filter paper. The filtrate was transferred into a separation funnel and equal volume of ethyl acetate was added to it, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) was recorded while the aqueous layer was discarded. The extract was separated to dryness in the crucible over a steam bath. The dried extract was then treated with concentrated amyl alcohol to extract the anthocyanins. After filteration, the alcohol extract and the filterate was transferred to a weighed evaporating dish and evaporated to dryness. It was then dried in the oven at 300C for 30min and cooled in a desiccator. The weight of anthocynanin was determined and expressed as percentage of the original sample.

PHARMACOLOGICAL EVALUATION

Ethical Clearance

Ethical clearance was obtained from the dash committee of the Anambra State University Teaching Hospital ,Awka, south east Nigeria.

Acute Toxicity Test

The acute toxicity study of *Pterocarpus santalinoides* was assessed by giving oral administration of the drug to albino mice using the method described by of Lorke and Carvalho et. al.[6,7]. 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 deaths 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

$\sqrt{a \times b}$. Where '**a'** is the lowest dose that brought death and '**b'** is the highest dose that did not bring death.

Evaluation of Anti-pyretic Activity

The anti-pyretic study was carried out using animal model using total of 15 rats were involved. They were divided into 5 groups of 3 animals each. The normal body temperature of each animal were taken using rectal thermometer, then pyrexia was induced by injecting 10 mg/kg of 15% brewer's yeast in normal saline subcutaneously on the neck of each animal. 18hrs after the injection, their body temperature were taken to confirm pyrexia. Group 1 received 0.5ml of distilled water orally. Group 2 received 100 mg/kg of aspirin, while group 3, group 4 and group 5 received 100 mg/kg, 200 mg/kg, 300 mg/kg of extract respectively. 30mins, 60mins, 90mins and 120mins of post – treatment, their body temperature were taken.

Statistical analysis

The procedures were repeated three times and results expressed as mean ±standard error of mean (SEM). Differences in observation were determined by Analysis of Variance (ANOVA) using Dunnette comparison method and regarded as slightly significant at $p \leq 0.05$ and extremely significant at $p \leq 0.01$.

RESULTS AND DISCUSSION

Phytochemical Screening

On preliminary phytochemical anlaysis of methanolic extract of Pterocarpus santalinoides showed the presence of alkaloids, anthocyanins, carotenoids, flavonoids, resins, saponins, steroids, terpenoids and tannins. The result of phytochemical screening are summarised in Table-1.

Table-1: Results of phytochemical screening

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

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

Acute toxicity Test

This test showed that at a dose of up to 5000mg/kg of the test extract administered orally, no mortality or was recorded in the mice (Table 2) .

Table-2: Results of 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	$^0/_1$

Antipyretic Activity

The leave extract of *Pterocarpus santalinoides* relieved pyrexia that was induced in the rats using brewer's yeast in a dose dependent manner. At a dose of 300mg/kg, the effect of extract was comparable to 100mg of the standard drug, Aspirin (100 mg/kg) [8,9].

Anti-pyretic activity of leave extract of *Pterocarpus santalinoides* may be attributed to the presence of steroid[10]. Steroid is significant in amelioration of inflammation and fever is a sign associated with the condition[11-13]. The results of Antipyretic activity are summarised in Table-3.

Table-3: Anti-pyretic activity of the methanolic extract of the leaves of *Pterocarpus santalinoides*

Group	Dose and Agent	Initial Body Temp. (˚C)	Pyrexia Temp. (˚C)	Treatment Body Temperature (°C)			
				30mins	60mins	90mins	120mins
1	0.5ml Distilled water	36.7±0.35	37.6±0.30	37.6±0.30	37.6±0.30	37.6±0.30	37.6±0.30
2	100mg/kg Aspirin	36.4±0.35	38.3±0.10	ns 38.1±0.15	** 35.8±0.15	** 35.2±0.15	** 35.1±0.10
3	100mg/kg Extract	36.1±0.35	37.9±0.20	ns 37.8±0.00	ns 37.7±0.15	Ns 37.0±0.00	* 36.4±0.35
4	200mg/kg Extract	36.9±0.35	38.0±0.05	ns 36.8±0.55	* 36.1±0.30	** 35.7±0.40	** 35.5±0.25
5	300mg/kg Extract	36.0±0.30	37.9±0.20	ns 36.5±0.15	** 35.7±0.15	** 35.3±0.30	** 35.1±0.10

All the values are expressed as Mean ± S.E.M, ns = not significant (p> 0.05), *= slightly significant (p< 0.05)
**= extremely significant (p< 0.01)

CONCLUSION

This study showed that the methanolic leave extract of *Pterocarpus santalinoides* possesses antipyretic.activity which may be as a result of the presence of steroids. This justifies the folkloric use of *Pterocarpus santalinoides* in alleviating pyrexia among the people of Ogidi, southeast Nigeria. However, further studies are recommended to isolate and characterize the structure of the active constituents.

REFERENCES

1) Okpo SO, Ching FP, Ekeleme IC. Evaluation of the Anti-Diarrhoeal Activity of the Aqueous Extract from Leaves of *Pterocarpus santalinoides*, Research Journal of Pharmaceutical, Biological and Chemical Sciences,2011,2(3):590-597
2) Nworu C S, Akah P A, Nwachukwu, J O and Asogwa C, Antidiarrhoeal Activity of *Pterocarpus santalinoides* L'Hérit ex DC Leaf Extract, Journal of Complementary and Integrative Medicine, 2009,6(1) : 1553
3) Okwuosa C.N, Unekwe PC, Achukwu PU, Udeani TKC and U. H. Ogidi UH, Glucose and triglyceride lowering activity of *Pterocarpus santaniloides* leaf extracts against dexamethasone induced hyperlipidemia and insulin resistance in rats, African Journal of Biotechnology, 2011, 10(46) : 9415-9420
4) Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Traditional Medicine Practice amongst the Igede People of Nigeria, Part II. Afr J Trad CAM 2005; 2(2): 134-152.
5) Harbourne JB, Phytochemical methods: A guide to modern technique of plant analysis, 3rd ed., London, Chapman and Hall, 1984, p. 1-302.
6) Lorke D, A new approach to practical acute toxicity testing, Arch. Toxicol.,1983, 53: 275-289.
7) Carvalho, V., Melo V.M., Aguiar A., Matos F.S., Toxicity evaluation of medicinal plant extracts by the brine shrump (Arthenus salina Leah) biossay. Ciência e Cultura, 1988, 40: 1109-1111.
8) Jane J.R. and Bobbling RM; Mechanism of action of non steroidal anti-inflammatory drugs; Am. J. med; 1998,104:25-85.
9) Waldmar RJ. et. al. Aspirin as a risk factor in Reye's syndrome, JAMA, 1982,247:3089 - 3094
10) Palombo EA , Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function, Phytother Res,2006, 20 (9): 717-724
11) Appidi JR, Grierson DS, Afolayan AJ, Ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape, South Africa, Pakistan J Biol Sci, *2088,* 11(15): 1961-1963.
12) Hart F.D. and Huskisson EC. Non steroidal antiimflammatory drugs; Current status and rational therapeutic use, Drugs 1984, 27:232-255.
13) Onunkwo AU, Nwankwo CH, Umolu DN, Stochastic Appraisal of the Routine Serodiagnostic method for Enteric Fever in Nigeria. J. Sci. Eng. Technol.,2001, 8(1): 2964-2973

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