ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 6, Issue – 2, April – 2021 , Page No. : 239 - 249

Chemical characterization, phytochemical evaluation and in vivo antinflammatory activities of pterocarpus osun l.

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Citation this Article: Johnson – Ajinwo, O.R., Udofia Cynthia Emmanuel, "Chemical characterization, phytochemical evaluation and in vivo antinflammatory activities of pterocarpus osun l.", IJMSIR- April - 2021, Vol – 6, Issue - 2, P. No. 239 – 249.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Most chronic diseases are characterized by inflammations, which could be devastating and result in dire consequences. The search for novel anti-inflammatory agents is on the increase as the long-term use of NSAID could result in potentially life-threatening conditions. Pterocarpus osun (Fabaceaea) referred to as camwood is widespread in the tropics such as Africa and parts of Asia. There is abundant data on the ethnopharmacological uses of this plant, which requires scientific validation. Hence this research work is aimed at investigating the anti-inflammatory activities of this plant. Organic and aqueous extracts were obtained from the pulverized heartwood by use of the America national cancer institute protocol (NCI). Phytochemical screening was done using standard procedures. GC-MS analysis was carried out to determine the bioactive compounds in P. osun. Results from the phytochemical screening indicated the presence of alkaloids, anthraquinones, flavanoids, saponins, tannins,triterpenoids/Steriods, glycosides, carbohydrates and phenolic compounds. The GC-MS results reported the presence of five highly bioactive compounds: Hexadecanoic acid, methyl ester (9.56%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (12.12%), 6-Octadecenoic acid, methyl ester, (Z)- (19.23%), Methyl stearate(5.05%) and Homopterocarpin(39.57%). Homopterocarpin;an isoflavonoid with antiulcergenic and cytotoxic activities. Anti-inflammatory investigations of the extracts were carried out by the induction of inflammation. The animals were grouped into 2 test groups and 2 control groups with 6 rats per group. Egg albumin (0.1ml) was administered sub-plantarly followed by treatment. Group A received a dose of 200mg/kg of the plant extracts and Group B received a dose of 400mg/kg of the plant extracts. Group C (positive control) received indomethacin (10mg/kg), while Group D (negative control) received 1 ml of normal saline. Statistical analysis of the percentage inhibition of inflammation demonstrated that the organic extracts at doses of 200 and 400 mg/kg were

significantly (P<0.05) more potent than indomethacin indicating that the plant possess significant antiinflammatory activities and warrants further antiinflammatory studies. Homopterocarpin is the most bioactive compound identified in this plant. There is some evidence to suggest that P. osun may possess anticancer activities, as inflammations are a characteristic feature of cancers. This is the first time report of the antiinflammatory activities of P. osun.

Keywords: Pterocarpus osun, GC-MS, phytochemical, antiinflammatory activity

Introduction

Pterocarpus osun (Fabaceaea) commonly called camwood is widespread in tropical areas such as Africa and Asia. The plant has numerous traditional uses [1]. P.osun is an important specie in the Pterocarpus genus which comprises 35 species, found exclusively in the tropics. A number of the Pterocarpus species are documented as treatments for diabetes, fever, diarrhea, skin infections, UTI's, and toothache [2]. The heartwoods have been reported to exhibit antiulcer, anthelmintic, anti-inflammatory activities. Similarly, the stembark and resins are used traditionally in the treatment of ringworm, chronic ulcers, gland tumors and urethral discharges, [2,3,4,5].

P. osun leaves are employed as an ingredient in local soaps in Nigeria, the pulverized stem, is used as an antiseptic for umbilical cords of newborns, treatment of eczema, acne, rheumatism, gonorrhea, and candidiasis [6]. The stem bark is used in combination with other plants for the treatment of sickle-cell and amenorrhea [7,8]. The heartwood, stembark and roots are powdered and used as skin dye during ceremonies [8]. The antioxidant activity and antimicrobial activities have been reported [8,9,10,11]. Earlier investigations, have documented the analgesic, anti-inflammatory [12,13,14], antifungal[15,16], antioxidant[17,18,19,20], cardiotonic[21], hepatoprotective[22, 23], and cytotoxic activities [24,25,26,27] have been also reported. The red pigments; santarubin and santalin were isolated and recommended for use as dyes in histological staining [8,28]. The proximate analysis and quantification of the vitamin content have been carried out [29]. The phytochemical screening of Pterocarpus species identified the presence of alkaloids, flavonoids, phenols, tannins, saponnins [30] , aurones, pterocarpans, lignans, sesquiterpenes, stilbenes, sterols, triterpenes and isoflavonoids. The isoflavonoids are reported as the most potent class of compounds in P. osun, followed by pterocarpans. The isoflavonoids are biologically active compounds with antiinflammatory, chemopreventive, antioxidant, antiproliferative, antihemolytic, xenobiotic metabolism modulator including activation of estrogen receptors [31,32,33]. Pterocarpans possess antiviral, cytotoxic, antimitotic activities [34,35,36,37,38]. This study aims to investigate the chemical constituents and the anti-inflammatory activities of P. osun.

Material and methods

Plant materials and reagents: The heartwood of P. osun was obtained from a bioreserve. The plant was authenticated by a botanist, Dr. Suleiman Mikailu of the Department of Pharmacognosy & Phytotherapy, University of Port Harcourt. The heartwood was dried, dusted, cleaned and cut into pieces before it was milled to coarse powder. Reagents: Methanol, Dichloromethane, Chloroform, Diethyl Ether, Acetic Anhydride, Glacial acetic acid, Sodium picrate, 2% 3,5-Dinitrobenzoic acid, Picric acid, Iodine solution, Dimethylsulfoxide, (JHD company, Guangdong. Guanghua Sci-Tech. Co. Ltd. China), Hydrochloric acid, Million's reagent, Benedict's solution, Wagner's reagent, Sodium Hydroxide, Ferric chloride solution,

Saturated lead acetate solution, Dragendorff's reagent, Kedde reagent, Ammonia solution, 7.5% Potassium Hydroxide, Fehling's solution A and B (Sigma Aldrich Chemicals, St Louis, USA), Distilled water, Deionized water (Pharmaceutical Chemistry Lab, University of Port Harcourt).

Assay materials

Egg albumin, indomethacin 10mg/kg (oral), normal saline and digital vernier caliper.

Extraction of plant materials

The 1.2 Kg of the pulverized heartwood were extracted according to the American National Cancer Institute (NCI) method of extraction [39]. The pulverized plant material was macerated in a 1:1 mixture of dichloromethane and methanol for 24 h to obtain the extracts. The procedure was repeated thrice. The ratio of plant material to solvent used was 1:5. The residue was further marcerated in methanol for another 24 h to yield the methanol extract, which was combined with the dichloromethane/methanol extract to yield the total organic extract. Deionised water was added to the residue, to obtain the aqueous extract after freeze drying. The obtained dry extracts were further dried in a desiccator to remove any trace of solvent.

Phytochemical screening

Phytochemical tests were carried out on the plants' extract by the method described by Trease and Evans [40].

GC-MS analysis of the crude extracts

The gas chromatography mass spectrometry (GC-MS) analysis of the DSE was quantitatively determined using an Agilent 7890B GC system coupled with an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025μm) (5%-phenylmethylpolysiloxane). The GC-grade helium served as the carrier gas at a constant flow rate of 2 mL/min. The DSE was dissolved with ethanol and filtered before use. The column temperature was maintained at 60°C and gradually increased at 10°C per minute until a final temperature of 300°C was reached. The time taken for the GC-MS analysis was 30 min. The compounds were identified based on computer matching of the mass spectra with the NIST 11 MS library (National Institute of Standards and Technology library).

Experimental design

This study was designed in line with the ethically approved experimental protocols adopted by the department of Experimental Pharmacology and Toxicology, of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. Adult mixed-gender wistar rats weighing between 170-200g, bred by the animal house unit of the department of Experimental Pharmacology and Toxicology were used for the study. The rats were housed in spacious cages, to allow for free movement at room temperature, sufficient humidity and 12 hourly cycles of light and darkness. The animals, had access to standard laboratory animal feed and water. The animals were divided into 2 test groups and 2 control groups with 6 rats per group. Group A received a dose of 200mg/kg P.O of the plant extracts and Group B received a dose of 400mg/kg P.O of the plant extracts. Group C and D received indomethacin (10mg/kg P.O) and normal saline (1ml/rat P.O) respectively.

EGG albumin induced rat paw edema

The initial paw thickness of each rat was measured using a digital vernier caliper and recorded as To. Edema was induced in the paws of the animals by administering 0.1ml egg albumin sub-plantarly. After 30 minutes of inducing edema, treatments were given to the rats orally according to their body weights after

which their paw thickness were determined at time intervals (T) of 1 hour, 2 hours, 3 hours and 4 hours using a digital vernier caliper.

The percentage inhibition was calculated using the formula

$$\frac{(T_t - T_0)Control - (T_t - T_0)treated}{(T_t - T_0)Control} \times 100$$

Statistical analysis

The analytical tool used was Graph Pad Prism version 8 for calculation of the mean values ± standard error of mean (SEM). The one-way ANOVA was used to determine statistical difference between means. A P< 0.05 was considered statistically significant.

Results

Results from the phytochemical screening

The results of the phytochemical screening are contained in Table 1 below:

Table 1: Results of Phytochemical Screening of P. osun

Test	P. OSUN
1. Carbohydrate	
a. Molisch test	+
b. Fehlings test	_
2. Anthraquinone	
a. Free (Bomtrager's test)	+
3. Triterpenoids/Steriods	
a. Liebermann-Buchard Test	+
b. Salkwoski's Test	+
4. Phenolics test	
a. $FeCl_3$ test	+
5. Tannin test	
a. Phlobatannins test	+
6. Flavonoids	
a. Shinoda Reduction Test	+
b. $AlCl_3$ test	_
7. Alkaloids	
a. Dragendorff's (orange colour)	+
b. Mayer's test (cream)	_
c. Hager's test (yellow ppt)	_
8. Saponin	
a. Frothing Test	+
b. Emulsion Test	_

Key: + = present, - = absent

The results presented in Table 1 showed that the organic extract of P. osun contained alkaloids, flavonoids, phenolic compounds, tannins, saponins, triterpenoids/steriods, anthraquinone. This study is in consonance with the work of other researchers who have investigated the phytochemical constituents of P. osun. These earlier researchers identified anthraquinones, flavonoids, alkaloids, saponins, glycosides, and tannins as secondary metabolites in P. osun [30,31,32,33].

Results of the GC-MS analysis

The GC-MS chromatogram of the organic extract of P. osun is presented in Figure 1. A total of 15 peaks were recorded.

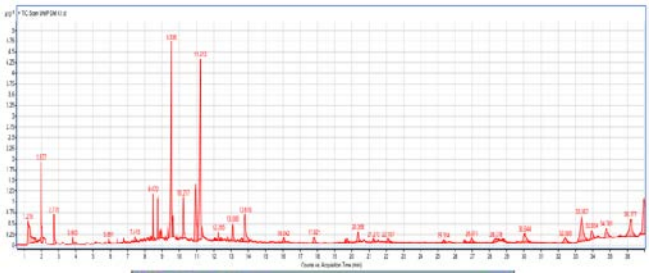

Figure 1: GC-MS Chromatogram of the organic extract of P. osun.

The GCMS chemical characterization of the organic extract of P. osun was carried out and the results

presented in Table 2. The interpretation of GC-MS mass-spectra was based on the NIST library of the equipment. The individual spectrum were matched with that of the library and the following parameter; molecular weight, structure, retention time and fragmentation patterns compared. Five bioactive compounds were ascertained from the spectral match.

Table 2: GC-MS analysis of P. osun stem-bark

Peak	RT	Area %	MW	MF	Name of Compound	Documented Bioactivity
1	8.043	9.56	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Antifungal and Antioxidant, Antimicrobial, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic flavour, haemolytic, 5-Alpha reductase inhibitor [41]
2	9.233	12.12	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Antioxidant and Antimicrobial [42]
3	9.279	19.23	296	$C_{19}H_{36}O_2$	6-Octadecenoic acid, methyl ester, (Z)-	Antipyretic [43]
4	9.450	5.05	298	$C_{19}H_{38}O_2$	Methyl stearate	Antifungal and Antioxidant [44]
5	16.065	39.57	284	$C_{17}H_{16}O_4$	Homopterocarpin	Cytotoxic to human cancer cells, Antiulcerogenic [45,46]

The results of the anti inflammatory studies

The results of the antiinflammatory studies of the extracts of P. osun are shown in figure 2 below. The Mean±SEM of the paw readings versus time intervals were plotted.

Figure 2: Plot showing the mean thickness of egg albumin-induced paw edema at time intervals, after administration of the extracts

Figure 2 shows the mean thickness of egg albumin-induced paw edema. At time intervals, each treatment group recorded a decrease in mean thickness of the induced paw edema at different rates based on their respective inhibitory effects. From the results, the organic extract (400mg/kg) had significant anti-inflammatory activity against the negative control from the first hour ($P<0.05$).The organic extracts (200mg/kg and 400mg/kg), demonstrated better anti-inflammatory activities compared to the aqueous extracts.

The percentage inhibition of Edema of the different treatments at the respective time Intervals were calculated to obtain the significant values. The data is shown in figure 3.

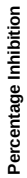

Figure 3: Percentage Inhibition of Edema of Different Treatments At Time Intervals

The percentage inhibition of the 400 mg/kg organic extracts showed significant inhibition from the 1st, 2nd, 3rd and 4th hour as 10.33%, 12.60%, 27.08% and 31.61% respectively against indomethacin which yielded values of 1.16%, 11.37%, 13.3% and 15.03% for the 1st, 2nd, 3rd and the 4th hour respectively. Similarly the percentage inhibition for the organic extract (200mg/kg) was significant against indomethacin at the 1st and 3rd hour with values of 4.1% and 15.9% respectively.

Discussion

According to the ethnopharmacological data on the Pterocarpus genus, the heartwoods of some species are used as antiinflammatory, anthelmintic, antiulcer and in jaundice. Also, reports on uses in cough therapy, treatment of elephantiasis, leucoderma, rectalgia and minimization hair greyness has been documented [2,3,4,5]. P. osun leaves displayed antioxidants properties, induced drug-detoxifing enzymes, minimized lipid peroxidation and diminished acetaminophen-induced redox imbalance in previous studies [47]. Similarly, the antimicrobial activities of P. osun has been reported by several researchers [11,48].

A comparison of the chemical composition of the leaf extracts of P. osun from different geographical regions of Nigeria showed the presence of tannins, cardiac glycosides, phenol, saponins, sterols and terpenes [30], their findings align with the results of this research. Additionally, alkaloids, anthraquinones and flavonoids were recorded in the phytochemical screening of the heartwood used in this study. A recent review of the Pterocarpus genus documented aurones, pterocarpans, lignans, sesquiterpenes, stilbenes, sterols, triterpenes and isoflavonoids as the classes of compounds found in the species. Isoflavonoids were considered the most important class of compounds. Several compounds have been isolated from the genus from species such as P. marsupium, P. soyauxii, P. santalinus, and P. angolensis [49,50,51] while only a six compounds; Santal, Santalins A, Santalins B, per-O-methylsantalin, Santarubins A and Santarubins B from P. osun [52].

The GC-MS analysis of P.osun documented five bioactive compound with significant abundance; Hexadecanoic acid, methyl ester (9.56%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (12.12%), 6-Octadecenoic acid, methyl ester, (Z)- (19.23%), Methyl stearate(5.05%) and Homopterocarpin(39.57%), with established bioactivities. Homopterocarpin, is a derivative of isoflavonoid classed as pterocarpans, found in the Fabaceae family [53]. These compounds are known as benzo-pyrano-furano-benzenes. This compound has been found in P. soyauxii, (wood) P. erinaceus (wood) and P. macrocarpus (stembark) [53]. This is the first time report of this compound in P. osun. The observed anti-inflammatory effect is could be attributed to the presence of phytochemical constituents in the plant. Flavonoids have significant activity against both phases of inflammation; the proliferative and exudative phases. The activities of saponins with anti-inflammatory properties such as Frutice saponin B has been reported [54]. Homopterocarpin has demonstrated antiulcerogenic activities against indomethacin-induced ulcer. The compound induced antioxidative mechanism and modulated gastric homeostasis [55]. Other

researchers have documented the cytotoxicity of Pterocarpus macarocarpus Kurz, with this agent listed as one of the bioactive compounds in the specie. Again the anticancer activities of Pterocarpus santalinoides on six human cancer cell lines: human breast adenocarcinoma MCF7 (ATCC No. HTB-22), BT-20 (ATCC No. HTB-19) prostate adenocarcinoma PC-3, acute T cell leukemia Jurkat and colon adenocarcinoma SW-480 has been documented [56].

Conclusion

In conclusion, the result presented in this study demonstrated that the organic extracts of P. osun possess significant anti-inflammatory activity. From the results, the anti-inflammatory activity of P.osun had a quick onset of action and exhibited very potent activities than Indomethacin (10mg/kg). The results from this work showed that Homopterocarpin, may be considered the most bioactive compound responsible for the anti-inflammatory activities of P. osun. There is strong evidence to suggest that P. osun may possess anticancer activities given that mutated cells and inflammatory mediators are characteristic of most tumour inflammations, given the possibility that inflammation and cancer could be linked by the intrinsic and extrinsic pathways [57,58].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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