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BIOLOGICAL AND PHYTOCHEMICAL INVESTIGATIONS OF EXTRACTS FROM *PTEROCARPUS ERINACEUS* POIR (FABACEAE) ROOT BARKS

Ouédraogo Noufou[1,2,3*], Hay Anne-Emmanuelle[3], Ouédraogo Jean Claude W[1], Sawadogo W Richard[1], Tibiri André[1], Lompo Marius[1], Nikiema Jean-baptiste[2], Koudou Jean[2], Dijoux-Franca Marie-Geneviève[3] and Guissou Innocent Pierre[1,2]

[1]Département de médecine-pharmacopée traditionnelle/pharmacie (IRSS/CNRST) 03 BP 7192 Ouagadougou 03, Burkina Faso.
[2]Laboratoire de développement du médicament, UFR/SDS, Université de Ouagadougou 03 BP 7021 Ouagadougou 03, Burkina Faso.[3]UMR 5557 CNRS-Université Lyon 1, Ecologie Microbienne, Villeurbanne, F-69622, France

Corresponding author E-mail: arnoufou2@yahoo.fr

Abstract

Background: *Pterocarpus erinaceus* Poir. belonging to Fabacae familly is used as medicinal plant in Burkina Faso's folk medicine. Roots of *P. erinaceus* are used to treat ulcer, stomach ache and inflammatory diseases. The objective of the present study was to carry out phytochemical composition of methanol (MeOH) and dichloromethane (DCM) extracts from *Pterocarpus erinaceus* roots, to isolate pure compounds, and to evaluate their pharmacological activities.

Methods: Chromatographic fractionation led to the isolation of active components of the extracts. The structures were established by NMR analysis and comparison with data from literature. The anti-inflammatory activity was evaluated using croton oil-induced edema of mice ear as well as the effect of extracts against lipoxygenase and lipid peroxidation was evaluated. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Cupric-reducing antioxidant capacity (CUPRAC) methods were used to evaluate the antioxidant activity of the extracts.

Results: Friedelin (1), 3α-hydroxyfriedelan-2-one (2), α-sophoradiol (3) and stigmasterol (4) were isolated from DCM extract and maltol-6-*O*-apiofuranoside-glucopyranoside (5) isolated from MeOH. DCM extract and friedelin, 3α-hydroxyfriedelan-2-one, α-sophoradiol showed a significant anti-inflammatory effect against ear edema. Friedelin (1), α-sophoradiol (3) and maltol-6-*O*-apiofuranoside-glucopyranoside (5) exhibited lipoxygenase inhibition. MeOH extract (100 µg/mL) inhibited lipoxygenase and lipid peroxidation activities at 45.1 ± 3% and 30.7 ± 0.5% respectively. MeOH extract, ethyl acetate fraction and butanol fraction exhibited antioxidant property with both two methods used.

Conclusion: The results suggested that the extracts and compounds from roots of *Pterocarpus erinaceus* possessed local anti-inflammatory effect, antioxidant properties and inhibitor effect against lipoxygenase and lipid peroxidation activities.

Keywords: *Pterocarpus erinaceus*, triterpenes, anti-inflammatory, antioxidant, lipoxygenase

Introduction

Natural compounds from plants present an increasing interest for scientific research because of their beneficial effect on human and animal health. Medicinal plants continue to provide new remedies through new active isolated molecules which are used as drugs (Gurib-Fakim, 2006). Their therapeutic effect as antioxidant is considered with great interest worldwide to prevent chronic diseases such as cancer, diabetes, cardiovascular troubles, liver injury (Loganayaki et al., 2013). Antioxidant compounds (flavonoids, tannins, phenolic acids) from plant with redox properties, act against reactive oxygen species (ROS) production. ROS which are well known for causing membranes and proteins damages, and oxidative stress (Sreeramulu et al., 2013). This oxidative stress is involved in chronic diseases that are by far the leading cause of mortality in the world (WHO, 2014).

Other natural compounds such as terpenoids (triterpenes, sterols) from plants possess anti-inflammatory properties. Inflammation is a complex defensive system against any aggression. Lipoxygenases and cyclo-oxygenases enzymes are respectively involved in biosynthesis of leukotrienes and prostaglandins that are mainly mediators of inflammation. In addition, lipoxygenase plays a role in ROS production during inflammation (Holm et al., 2008).

The *Pterocarpus* genus belonging to Fabaceae family includes several species like *P. indicus Willd, P. marsupium Roxb, P. dalbergioides Roxb.* and *P. soyauxii Taubert* distributed in South Asia. In Africa, encountered species are *P. tinctorius Welw., P. osun Craib, P. mildbraedii Harms., P. santalinus L. F.* and *P. erinaceus* Poir. In ethnomedicine reports, Saslis-Lagoudakis et al. (2011) showed that *Pterocarpus* genus is used to treat inflammation, pain, infectious, cardiovascular, gastrointestinal and skin diseases in Africa, Asia and Latin America. Leaves, stem barks and roots of *Pterocarpus erinaceus* Poir are used in Burkina Faso folk medicine to treat inflammatory diseases such as inflammation, ulcer, rheumatism, and fever, bacterial infections or malaria. Roots of *P. erinaceus* are used to treat ulcer, stomach ache and inflammatory diseases (Nadembega et al., 2011). The use of medicinal plants for millennia in diseases treatment, shows efficacy and safety of used plants. The world health organization (WHO) has estimated that 80% of Africa's population practice traditional medicine. Involvement of scientists is necessary to improve traditional medicine contributing with scientific data through pharmacological, toxicological and phytochemical investigations.

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Previous studies reported the pharmacological properties including antimalaria, antibacterial, analgesic, antioxidant and anti-inflammatory of *Pterocarpus erinaceus* stem bark (Karou et al., 2005; Nuhu et al., 2000; Ouédraogo et al., 2011). Muningin, prunetin, pseudobaptigenin, afromorsin and tectorigenin were isolated from heartwood of *Pterocarpus erinaceus* (Bevan et al., 1966). The objective of the present study was to carry out phytochemical investigation, to isolate compounds from methanol and dichloromethane (DCM) extracts from *P. erinaceus* roots, and to evaluate local anti-inflammatory, lipoxygenase and lipid peroxidation inhibition and antioxidant activities of DCM and methanol extracts.

Material and methods

General experimental procedure

1D-NMR (^{1}H-NMR, ^{13}C-NMR) and 2D-MNR (COSY, HMQC and HMBC) spectra were recorded in MeOD and in CDCl3 on a Bruker spectrometer operating respectively at 300 and 100 MHz. Column chromatography were carried out using silica gel 60 (40 – 63 µm, Merck®), Sephadex LH-20 (Pharmacia®) and Lichroprep RP18 (Merck®). Silica gel 60 F254 plates were used for analytical thin-layer chromatography (TLC). Silica gel 60 GF254 used for preparative TLC.TLC were visualized under UV light (254 and 366 nm) and by spraying with sulphuric vanillin.

Plant material

Roots bark of *Pterocarpus erinaceus* were collected from Gourcy (150 km from Ouagadougou, Burkina Faso), identified by Pr Adjirma Thiombiano and a voucher specimen has been deposited at Herbarium of Ecology Laboratory of University of Ouagadougou and referenced with the number ON-01.

Animals and ethical approval

Local anti-inflammatory activity was carried out in accordance with guidelines for care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983). NMRI mice (25 – 30 g) were housed in temperate rooms (22 ± 2 °C) with a 12/12 dark-light cycle. Mice were allowed free access to standard dry pellet diet and given water *ad libitum*. Animals were fasted for 16 hr and weighed before the experiments.

Reagents and Chemicals

DPPH (2,2-diphényl-1-picrylhydrazyl), croton oil, copper II chloride (CuCl2), neocuproin and Lipoxygenase (type I-B) enzyme was purchased from Sigma® (St Louis, USA). Ammonium acetate buffer (Prolabo®, France), trolox (Fluka®, France), ketamine hydrochloride (Rotexmedica®, Germany) were used in this work.

Extraction and isolation

At room temperature, 750 g of dried root barks *of P. erinaceus* were successively extracted by maceration with dichloromethane (DCM) and methanol (MeOH) during 48 h for each extraction. After concentration, 5.6 g of DCM extract and 54.4 g of MeOH extract were obtained. DCM extract (4.2 g) was subjected to silica gel CC (40-63 µM, Merck®) using eluent system n-hexan/DCM (from100/0 to 0/100) then DCM/ethyl acetate (EtOAc) (from 100/0 to 0/100). 23 fractions (E.1- E.23) were obtained.

The fraction E.8 was purified on Sephadex LH-20 column to yield **compound 1** (42.5 mg), using 100% DCM only as eluent system. The fraction E.16 was subjected to Lichroprep RP-18 (40-63 µm, Merck®) and the column was eluted with MeOH/H2O (70/30 to 0/100) then MeOH/DCM (100/0 to 70/30), affording 15 sub-fractions (E.16.1 – E.16.15). The sub-fraction E.16.10 was purified over silica gel chromatography column (40-63 µM, Merck®), with a gradient of n-hexan/EtOAc (100/0 to 60/40). The **compound 2** (2.6 mg) was obtained.

The fraction E.19 was fractionated by SPE over Lichroprep RP-18 (40-63 µm, Merck®) and afforded 18 fractions (E.19.1 to E.19.18) and the **compound 3** (17.7 mg). MeOH/H2O (70/30 to 0/100) and then MeOH/DCM (100/0 to 0/100) gradient system were used as eluent. The fraction E.19.18 from fraction E.19, was purified by Sephadex LH-20 (Pharmacia®) column using DCM as eluent and afforded the **compound** 4 (14 mg).

MeOH extract (54.4 g) was suspended in water (1.2 L) and partitioned with EtOAc (3 x 300 mL) and n-butanol (BuOH) (3 x 300 mL) to yield EtOAc fraction (3 g) and BuOH fraction (11.2 g).

The whole BuOH fraction was chromatographed on VLC over silica gel (Merck) using CHCl3/MeOH (from 100/0 to 0/100) and four fractions (H.1–H.4) were obtained. The fractionation of H.3 (3 g) on silica gel column afforded 11 fractions (H.3.1 – H.3.11)

0.91 (m, H-22), 1.22 (m, H-21), 1.58 (m, H-10), 2.23 (m, H-4), 2.36 - 2.40 (m, H-2). (Fig1)

13C NMR (75 MHz, CDCl3) öC (ppm) 22.26 (C1), 41.50 (C2), 213.23 (C3), 58.19 (C4), 42.12 (C5), 41.26 (C6), 18.21 (C7), 52.65 (C8), 37.41 (C9), 59.44 (C10), 35.59 (C11), 30.48 (C12), 39.66 (C13), 38.26 (C14), 32.38 (C15), 35.98 (C16), 29.96 (C17), 42.75 (C18), 35.31 (C19), 28.14 (C20), 32.73 (C21), 39.23 (C22), 6.80 (C23), 14.63 (C24), 17.92 (C25), 20.24 (C26), 18.64 (C27), 31.06 (C28), 35.01 (C29), 31.76 (C30).

using CHCl3/MeOH (from 100/0 to 0/100). H.3.7 (200 mg) was subjected to Lichroprep RP-18 (40- 63 JLm, Merck®) column, affording 8 fractions (H.3.7.1 - H.3.7.8) with a mixture of H2O/MeOH (from 100/0 to 40/60). Sub-fraction H.3.7.3 (72 mg) was subjected to silica gel preparative TLC to afford the **compound 5** (12.6 mg), in a mixture of MeOH/ EtOAc (50/50). **Compound 1**: Friedelin (42.5 mg)TLC Silica: Rf = 0.3 with eluent: Hexan/ethyl acetate (90/10), Rf = 0.42. Spot: yellow with Sulfuric Vanillin,

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physical aspect: white powder. ^{1}H NMR: (300 MHz, CDCl3) ö(ppm) 0.88, 0.72, 0.86, 1.01, 1.06, 1.19, 0.96, 1.01 (respectively H-23, 24, 25, 25, 26, 27, 28, 29 and 30),

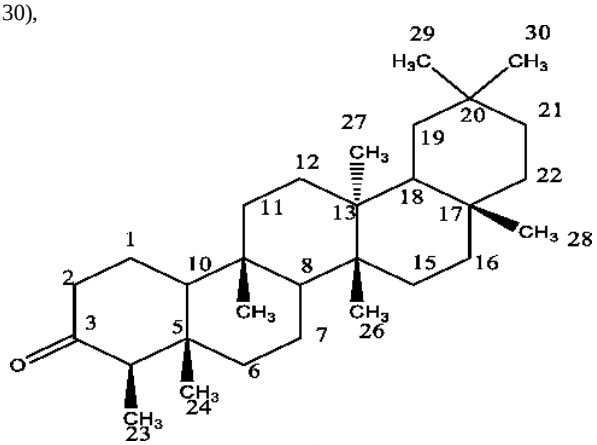

Figure 1: friedelin

Compound 2: 3x-hydroxyfriedelan-2-one (2.6 mg)
TLC Silica: Rf = 0.3 with eluent: hexan/ethyl acetate (90/10, v/v). Spot: orange with sulfuric vanillin. Physical aspect : white powder.^{1}H NMR (400 MHz, CDCl3) öH (ppm) : 2.5 (dd, 1x-H), 2.4 (t, 1f3-H), 3.8 (m, 3f3-H), 1.8 (m, 6f3-H), 1.04 (CH3-23), 1.03 (CH3-24), 0.89 (CH3-25), 1.01 (CH3-26), 0.99 (CH3-27), 1.17 (CH3-28), 0.97 (CH3-29), 0.94 (CH3-30).^{13}C NMR (100 MHz, CDCl3) öC (ppm) : 36.1 (C1), 211.9 (C2), 77.2 (C3), 54.5 (C4), 38.1 (C5), 40.6 (C6),

17.6 (C7), 53.1 (C8), 37.6 (C9), 60.4 (C10), 35 (C11), 30.3 (C12), 39.6 (C13), 38.3 (C14),

32.3 (C15), 35.9 (C16), 30 (C17), 42.7 (C18), 35.3 (C19), 28.2 (C20), 32.7 (C21), 39.2 (C22),

10.8 (C23), 14.2 (C24), 17.4 (C25), 20.2 (C26), 18.6 (C27), 32.1 (C28), 31.7 (C29), 35 (C30).

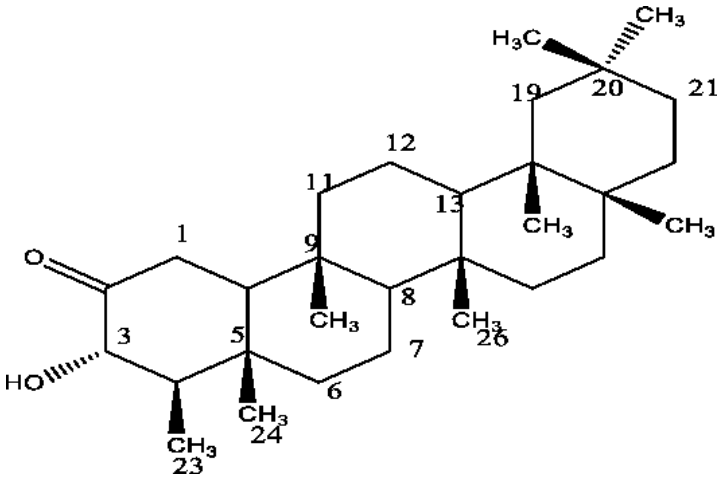

Figure 2: 3α-hydroxyfriedelan-2-one

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Compound 3: α-sophoradiol (17.7 mg)

TLC RP-18: Rf = 0.66 with eluent: methanol/DCM (60/40, v/v). Spot: blue with sulfuric vanillin. Physical aspect: white needles.[1]H NMR (400 MHz, CDCl3) δH: (ppm); 5.25 (t, J= 3.6 Hz, H-12), 3.22 (dd, J=11.0, 4.9 Hz, H-3), 3.44 (t, H-22), 2.1 (d, 11.2), 0.87 (CH3-23), 0.79 (CH3-24), 1.00 (CH3-25), 1.04 (CH3-26), 1.12 (CH3-27), 0.91 (CH3-28), 0.98 (CH3-29), 0.95 (CH3-30).

13C NMR (100 MHz, CDCl3) δC (ppm): 37.0 (C1), 27.2 (C2), 79.0 (C3), 37.5 (C4), 55.2 (C5), 18.4 (C6), 32.9 (C7), 39.7 (C8), 47.6 (C9), 37.0 (C10), 23.5 (C11), 122.5 (C12), 143.9 (C13), 42.1 (C14), 25.9 (C15), 30.5 (C16), 37.4 (C17), 44.7 (C18), 46.1 (C19), 30.5 (C20), 41.5 (C21), 76.7 (C22), 28.0 (C23), 16.8 (C24), 15.5 (C25), 16.8 (C26), 19.9 (C27), 28.1 (C28), 32.7 (C29), 25.3 (C30).

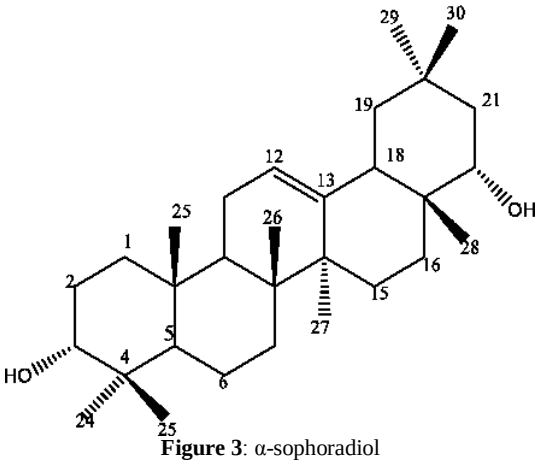

Figure 3: α-sophoradiol

Compound 4 (14 mg): Stigmasterol

TLC Silica: Rf = 0.4 with eluent: hexan/ethyl acetate (80/20, v/v). Spot: purple with sulfuric vanillin. Physical aspect: white powder.[1]H NMR (300 MHz, CDCl3) δH (ppm) : 5.35 (t, J = 11.7 Hz, H-6), 5.16 (dd, J = 15.2, 8.6 Hz, H-22), 5.02 (dd, J = 15.2, 8.6 Hz, H-23), 3.51 (m, H-3), 0.68 (s, H-18), 1.01 (s, H-19), 0.85 (CH3-26), 0.0.78 (CH3-27), 0.82 (CH3-29). 13C NMR (75 MHz, CDCl3) δC (ppm) : 36.5 (C1), 28.3 (C2), 129.3 (C3), 37.3 (C4), 140.7 (C5), 121.7 (C6), 31.6 (C7), 31.9 (C8), 50.6 (C9), 36.7 (C10), 21.2 (C11), 39.8 (C12), 42.3 (C13), 56.9 (C14), 24.4 (C15), 29.1 (C16), 56.1 (C17), 12.1 (C18), 19.0 (C19), 40.5 (C20), 21.2 (C21), 138.3 (C22), 71.8 (C23), 51.2 (C24), 45.8 (C25), 19.4 (C26), 19.8 (C27), 25.4 (C28), 12.3 (C29).

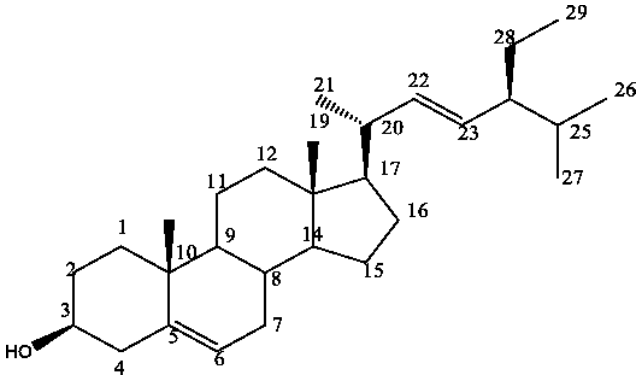

Figure 4: Stigmasterol

Compound 5: maltol 6'-O-α-apiofuranoside-α-glucopyranoside (12.6 mg)

TLC Silica: Rf = 0.36 with eluent: methanol/ethyl acetate (50/50, v/v). Spot: yellow brown with sulfuric vanillin. Physical aspect: white powder. [1]H NMR (300 MHz, CD3OD) δH (ppm) : 4.80 (d, J= 7.2 Hz), 4.9 (d, J= 2.3 Hz), 8.00 (d, H-6), 6.5 (d, H-5). [13]C NMR (75 MHz, in CD3OD): δC (ppm) :164.5 (C 2), 143.5 (C 3), 177.1 (C 4), 118.0 (C 5), 157.3 (C 6), 15.4 (C 7), 105.0 (C 1'), 74.9 (C 2'), 77.9 (C 3'), 71.4 (C 4'), 77.5 (C 5'), 68.6 (C 6'), 110.0 (C 1"), 77.8 (C 2"), 80.5 (C 3"), 75.4 (C 4"), 65.6 (C 5").

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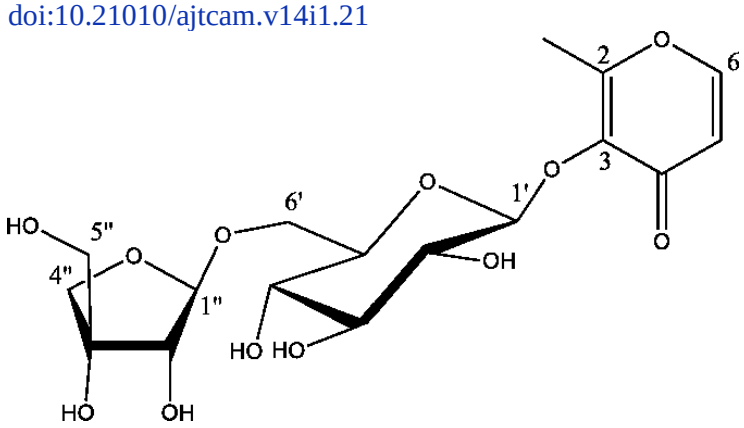

Figure 5: maltol 3-*O*-[f3-apiofuranoside-f3-(1-6)-glucopyranoside]

Anti-inflammatory activity: Ear œdema induced by croton oil

Local anti-inflammatory activity was carried out according to Sawadogo et al. (2008) with slight modification. DCM extract and compounds (**1**, **2** and **3**) were dissolved in a DCM solution of croton oil. NMRI Mice were divided into five groups (n = 6 per group). NMRI mice were anaesthetized with ketamine hydrochloride (150 mg/kg, i.p.) before croton solution application. Ear edema was induced by application of 5 µL of croton oil solution (at 25 mg/mL in DCM) containing extract or compounds (at different concentrations) on the inner surface of the right ear. Control group received only irritation solution. Six hours later, the animals were sacrificed by cervical dislocation and a plug (6 mm Ø) was removed from both the treated (right) ear and the untreated (left) ear at each animal. Œdematous response was measured to be the weight difference between the two plugs. The anti-inflammatory activity was expressed as percent of edema reduction in treated mice compared with the control mice.

Lipoxygenase inhibition assay

Lipoxygenase inhibitor effect of MeOH and DCM extracts was determined using colorimetric method described by Malterud and Rydland (2000) with slight modifications. Extracts were dissolved in borate buffer (pH 9.0). 3.75 µL of lipoxygenase solution (400 U/mL) were mixed with methanol extract (100 µg/mL, final concentration) or compounds (1, 3, 5; several concentrations) or positive control (quercetin and zileuton) and then incubated during 5 min at 25°C. Negative control was the mixture without extract or reference. Reaction was started by adding 150 µL of linoleic acid solution (250 µM) and absorbance variation was recorded at ?234 nm during 90 s using a microplate reader (BioTeck instruments, USA). Experiments were performed in triplicate. The percentage of lipoxygenase inhibition was determined by comparison with negative control.

%inhibition = [(E-S)/E]x100

where E = absorbance of solution at 234 nm without a test sample, and S = absorbance of solution at 234 nm with a test sample.

Lipid peroxidation (LPO) inhibition assay

The LPO inhibition activity of MeOH extract was determined according to the method described by Su et al. (2009) using thiobarbituric acid. Briefly 40 µL of extract (1.65 mg/mL) was mixed with 200 µL of rat liver homogenate (1%), 10 µL of FeCl2 (0.5 mM) and 10 µL of H2O2 (0.5 mM). After 60 min incubation at 37°C, 200 µL of trichloroacetic acid (15%) and 200 µL of 2-thiobarbituric acid (0.67%) were added to the mixture. Then the final mixture was heated up in boiled water during 15 min. The absorbance was measured at ?532 nm using a spectrophotometer (Agilent® 8453) equipped with UV-vis ChemStation software.

DPPH• free radical scavenging method

DPPH• free radical scavenging of methanol extract, ethyl acetate fraction and butanol fraction from *P. erinaceus* roots was evaluated using method of Brand et al. (1995). Twenty (20) µL of different concentrations of methanol extract or fractions were mixed with 200 µL of a methanolic DPPH solution (0.08 mg/mL) in a 96-well microtiter plate. The absorbance was recorded at ?515 nm, after 30 min incubation at room temperature. Standard controls were Trolox and chlorogenic acid. Each determination was carried out in triplicate. The percentage of residual DPPH was evaluated onto the graph in function of quantity of antioxidant: %DPPHres = f(antioxidant quantity/DPPH quantity)

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Antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 % and expressed as antiradical power (ARP = 1/EC50).

Cupric-reducing antioxidant capacity (CUPRAC) assay

CUPRAC method described by Apak et al. (2004) was used to determine the antioxidant capacity of methanol extract, ethyl acetate fraction and butanol fraction from *P. erinaceus* roots. In each well of 96-well microplate 50 µL of extract, fraction or standard control were mixed with 50 µL of distilled water and copper (II) chloride (CuCl2, 17.05 mg/mL, 50 µL), ammonium acetate buffer (NH4Ac, 77.08 mg/mL, 50 µL), neocuproine (1.56 mg/mL, 50 µL). After 1 hour of incubation, the absorbance was recorded at □450 nm using Multiskan EX (Thermo Electron Corporation®) spectrophotometer equipped with Ascent Software 2.6. The cupric ion reducing capacity of extract or fractions was expressed as trolox equivalent.

Statistical analysis

Data obtained were expressed as mean ± standard deviation (SD). The data were analyzed using GraphPad Prism version 6.0. Statistical comparison of data was performed by one-way ANOVA using Prism version 5.0. P-value < 0.05 were considered.

Results and Discussions

Friedelin (1), 3α-hydroxyfriedelan-2-one (2), α-sophoradiol (3) and stigmasterol (4) have been isolated from DCM extract of *Pterocarpus erinaceus* roots. Their structures were confirmed by their ^{1}H NMR and ^{13}C NMR data using Bruker DRX 300 by comparison with published literature data (Ciangherotti et al., 2004; Lingbo et al., 2005 ; Mahato et al., 1994 ; Lagnika et al., 2008). Maltol-6-*O*-apiofuranoside-glucopyranoside isolated from MeOH extract was identified by ^{1}H NMR and ^{13}C NMR data by comparison with those published by Haizhou et al. (2008). According to our knowledge, this is the first time that maltol-6-*O*-apiofuranoside-glucopyranoside was isolated and identified in *Pterocarpus* genus.Sophoradiol exhibited cytotoxicity against cell line HepG2 (Kinjo et al., 2003). Lagnika et al. (2008) reported anti-inflammatory, antipyretic and anti-mutagenic properties of stigmasterol and sitosterol. These results enrich the repertory of secondary metabolites like triterpenoids of the *Pterocarpus* genus, and they can be used to supplement further study of chemotaxonomy of *Pterocarpus* genus. Ear edema induced by application of croton oil was used to investigate local anti-inflammatory property of substances. Results are presented in figure 6. DCM extract reduced ear œdema in dose-dependent manner and at 300 µg/ear DCM extract had the same effect with phenylbutazon (100 µg/ear). This effect of DCM extract indicated the presence of active substances endowed with anti-inflammatory activity. This has been showed by isolated compounds. The effect of 3α-hydroxyfriedelan-2-one was higher than other compounds. Inflammation response induced by croton oil application was characterized by neutrophil infiltration, increased vascular permeability and protein kinase C activation with the subsequent prostaglandins and leukotrienes production. Croton oil application promotes tissue inflammatory response by the release of inflammatory mediators such as cytokines (TNFα, IL 1β, IL6), prostaglandins, (Luo et al., 2014 ; Sanchez-Mateo et al., 2006). It suggests that local anti-inflammatory effect of DCM extract and isolated compounds might interfered with mediator's action or synthesis and cellular activation. Ear edema could also be inhibited by serotonin and histamine antagonist effect of extract and compounds (Santos et al., 2015).

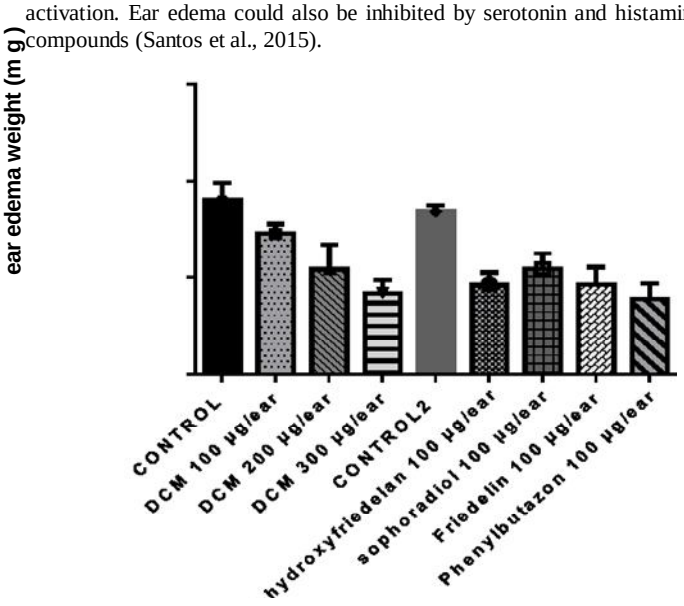

Figure 6: Anti-inflammatory effect of DCM extract and compounds from *Pterocarpus erinaceus* on mice ear œdema induced by application of croton oil solution. Values are mean ±

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S.E.M; n = 6; P < 0.05 significant from control (one-way ANOVA analysis followed by Dunnett's test).

The activity of MeOH and DCM extracts from *P. erinaceus* Poir. against lipoxygenase (LOX) was evaluated and the results are shown in Table 1. DCM extract possesses strong activity compared to MeOH extract and Hydrocortisone. Friedelin (1), α-sophoradiol (3) and Maltol-6-*O*-apiofuranoside-glucopyranoside (5) significantly exhibited inhibitor effect against LOX and compounds (1, 3, 5) were more active than extracts (DCM, MeOH); but their inhibitor effects were less than zileuton (Table 1). However, there's no significant difference between the effect of these compounds on LOX. LOX inhibitor reduce inflammatory response in mouse ear edema (Otuki et al., 2005). Lipoxygenase, a pro-inflammatory enzyme, is involved in leukotrienes biosynthesis which stimulate neutrophil chemotaxis and the release of pro-inflammatory mediators, enzymes and superoxides (Busse, 1998).

Table 1: Lipid peroxydation and lipoxygenase inhibition of methanol, dichloromethane and isolated compounds from *Pterocarpus erinaceus* roots

Samples	**Lipid Peroxydation Inhibition (%) at 100 µg/mL**	**Lipoxygenase Inhibition**	
		100 µg/mL (%)	**IC50 (µg/mL)**
MeOH Extract	30.7 ± 0.5	45.1 ± 3	---
DCM Extract	nd	54.2 ± 0.7	---
Quercetin	37.4 ±1	nd	---
Hydrocortisone	Nd	42.1 ± 1	---
friedelin	---	---	24.44 ± 2.8
sopharadiol	---	---	23.91 ± 1.7
Maltol-6-O-apiofuranoside-glucopyranoside	---	---	23.11 ±1.6
Zileuton	---	---	1.3 ± 0.2

Values are mean ± S.E.M. for triplicate; P < 0.05 significant from control (one-way ANOVA analysis followed by Dunnett's test)

DPPH and CUPRAC methods were used to determine the antioxidant capacity of MeOH extract, ethyl acetate and butanol fractions from *Pterocarpus erinaceus* roots. The results are presented in Table 2. With both methods, MeOH extract and fractions had the same antioxidant activity, however the reference compounds (trolox, rutin, chlorogenic acid) had higher activity. Free radical production is necessary during body aggression by pathogens, because free radicals are involved in defensive system against pathogens aggression; but their excessive production can cause cell damages and oxidative stress. Free radical-mediated oxidative stress in inflammatory diseases including cancer, diabetes, arthritis, infections, alzheimer and atherosclerosis, has been well documented (Oliveira et al., 2014; Sreeramulu et Raghunath, 2010). Antioxidant power of extract shows that extract and fractions may inhibit free radical production.

Table 2: Antioxidant activity of methanol extract and fractions from *Pterocarpus erinaceus* roots.

Samples	**DPPH assay**		**CUPRAC assay**
	IC50 (µg/mL)	**ARP**	**TEAC**
Extract MeOH	0.5 ± 0.05	2.0	0.8
Ethyl acetate fraction	0.5 ± 0.04	2	0.9
Butanol fraction	0.5 ± 0.01	2.0	1
Rutin	---	---	2.9
Trolox	0.20 ± 0.01	4.8	---
Chlorogenic acid	0.30 ± 0.03	3.7	---

Values are mean ± S.E.M. for triplicate; P < 0.05 significant from control (one way ANOVA analysis followed by Dunnett's test)

The lipid peroxidation inhibition activity of MeOH extract and quercetin was evaluated at 100 µg/ml. Quercetin had higher activity than MeOH extract (Table 1). In addition of anti-radicalar activity, MeOH extract possessed a lipid peroxidation inhibition effect. Cellular membrane damage by peroxidising lipid is induced by free radical such as ROS (Khan et al., 2013). Sung-Jin et al. (2013) reported that antioxidant substances can prevent and retard lipid oxidation by acting on ROS and others free radicals productions. It suggests that inhibition of lipid peroxidation contributes to cellular membrane protection against free radicals.

Conclusion

The present study reported that friedelin, 3α-hydroxyfriedelan-2-one, α-sophoradiol, stigmasterol, and maltol-6-*O*-apiofuranoside-glucopyranoside have been isolated from the roots of *Pterocarpus erinaceus*. The results highlighted cytotoxic, anti-inflammatory and antioxidant activities of compounds and extracts of *P. erinaceus*. This study provided supplementary data to promote the traditional medicine and confirmed the use of this plant by the healers to treat inflammatory diseases.

Conflict of interests

The authors have not declared any conflict of interests

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