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Pharmacological properties and related constituents of stem bark of *Pterocarpus erinaceus* Poir. (Fabaceae)

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ABSTRACT

Objective: To screen methanol and dichloromethane extracts of stem bark of *Pterocarpus erinaceus* for anti-inflammatory, analgesic, *in vitro* antioxidant activities and phytochemical analysis. **Methods:** Anti-inflammatory activity was determined by using carrageenan induced-edema of mice paw and croton oil-induced edema of mice ear; analgesic effect was evaluated using acetic acid-induced writhing. Phytochemical screening of extracts was performed by thin layer chromatography. The chromatographic fractionation led to the isolation of main active components as friedelin, lupeol and epicathechin. The structures were established by TLC and nuclear magnetic resonance studies. **Results:** Both methanol and dichloromethane extracts, friedelin, lupeol and epicatechin showed a significant anti-inflammatory effect using croton oil induced-ear edema. Furthermore, the action of dichloromethane extract was more important. At the doses of 100 and 200 mg/kg, the methanol extract was able to reduce the carrageenan induced-hind paw edema, while at the doses of 100, 200 and 400 mg/kg, it showed an important analgesic effect against writhing induced by acetic acid injection of 38.8%, 68.0% and 74.3%, respectively. Antioxidative properties of methanol extract and its dichloromethane and ethyl acetate fractions were assessed by using the 1,1-diphenyl-2-picrylhydrazyl method. The methanol extract showed the stronger radical scavenging activity than dichloromethane and ethyl acetate fractions, with an antiradical power of 5, 3.5 and 2 respectively. The main components isolated from these extracts as friedelin, lupeol and epicathechin were responsible of these activities. **Conclusions:** The results suggest that the stem bark extracts of *Pterocarpus erinaceus* possessed important anti-inflammatory, analgesic activities and strong antioxidant properties, therefore, they could be used as natural potential ingredients for pharma ceutical industry.

1. Introduction

Pterocarpus erinaceus (P. erinaceus) Poir. which belongs to the family Fabaceae, is a tree usually up to 8 and 15 m in height. This plant grows in savannah and is endemic from West Africa to Central Africa[1]. In savannah, *P. erinaceus* is a medicinal plant used to heal various diseases. In central region of Burkina Faso, its stem bark is used to treat inflammatory affections such as ulcer, rheumatism, dermatitis and infections. Recently, several studies demonstrated the antimalarial activity, antimicrobial activity and antiprogestative activity of *P. erinaceus*[2-4]. Earlier, phytochemical investigations revealed the presence of some isoflavonoids as prunetin, muningin, afromosin, tectorigenin, pseudobaptigenin isolated from heartwood of *P. erinaceus*[5]. Research on relationship between antioxidants and prevention of some diseases, such as cancer, cardiovascular and other inflammatory affections has been increasingly sharply in recent years[6]. The role of free radicals and reactive oxygen species is becoming recognized in the pathogenesis of the human diseases including cancer, aging and atherosclerosis[7].

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However, synthetic chemicals such as non-steroidal anti-inflammatory drugs (NSAID) were widely used against inflammatory disorders. Unfortunately, the digestive iatrogenic pathology caused by the use of these non-steroidal anti-inflammatory drugs constitutes a serious problem of public health because of its frequency and its gravity. Therefore, the use of natural antioxidants, plant phytochemicals, fruits, vegetables and medicinal plants is important and less damaging to the human health and environment [8]. To the best of our knowledge, the anti-inflammatory, analgesic and antioxidant effects of *P. erinaceus* have not been reported before and therefore the main objectives of this study are to assess the anti-inflammatory, analgesic and antioxidant activities of the stem bark extracts of this plant and to determine the main active components.

2. Materials and methods

2.1. Plant material

The stem barks of *P. erinaceus* Poir. were collected in March 2007 from Gourcy, Burkina Faso. The plant was authenticated by Dr. Adjirma Thombiano from Section of Botany, UFR SVT of Ouagadougou University. The voucher specimens (N° ON 01) have been deposited in the herbarium of Ouagadougou University.

2.2. Animals

The NMRI mice (24-37 g) were obtained from the animal house of Department of "Médecine-pharmacopée/Pharmacie" of the Institute of Health Sciences Research. The room temperature was maintained at (22±2)℃ with the 12 h light/12h dark cycle. The mice were starved 17 hours before the experiments. The tests, in this work, were performed according to the protocols already approved by the Institute of Health Sciences Research (Burkina Faso) and met the international standards of animal study[9].

2.3. Chemicals

Acetic acid, carrageenan, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), neocuproïn (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma (Sigma, St Louis,USA). Ammonium acetate buffer (Prolabo, France), trolox (Fluka, France) and chlorogenic acid (Acros, France) were used in this work. Paracetamol, hydrocortisone and ketamine hydrochloride (Rotexmedica, Trittau, Germany) were provided by Hospital "Yalgado Ouédraogo" of Ouagadougou. All solvents were analytical grade.

2.4. Extraction and isolation

The dried and committed stem barks of *P. erinaceus* Poir. (661 g) were successively extracted by maceration with dichloromethane (8 L) and methanol (10 L) at room temperature for 48 h. The extracts were concentrated to dryness under reduced pressure at 40℃ to yield dichloromethane (DCM) crude (3.5 g) and a methanol (MeOH) residue (100 g). Both the extracts were dried and stored at 4℃ until use.

The DCM crude (1.5 g) was fractionated by column chromatography over silica gel (40-63 μm, Merck), eluted with *n*-hexane/CH_2Cl_2 gradient (from 5/5 to 0/10, v/v), with CH_2Cl_2/ ethyl acetate gradient (from 5/5 to 0/10, v/v) and afforded thirteen fractions (A to M). The fraction B was purified by column chromatography over silica gel (40-63 μm, Merck), eluting with *n*-hexane/ethyl acetate gradient (10/0 to 7/3, v/v) and yielded the compound 1 (25 mg) and three other fractions. The fraction D was subjected to solid phase extraction (SPE) over silica gel (40-63 μm, Merck) to yield compound 2 (6 mg) by using *n*-hexane/ethyl acetate (0/10 to 7/3, v/v) as gradient.

The MeOH residue (36 g) was suspended in water (600 mL) during 20 hours, and partitioned with CH_2Cl_2 (3×200 mL) and ethyl acetate (3×200 mL) to yield a CH_2Cl_2 fraction (0.10 g) and an ethyl acetate fraction (1.03 g). Ethyl acetate fraction was fractionated by column chromatography over Sephadex LH-20 (Pharmacia) as stationary phase and MeOH as eluent and afforded eight fractions (A.1 to A.8). The fraction A.3 was re-chromatographied on silica gel (40-63 μm, Merck) with *n*-hexane/ethyl acetate (6/4 to 0/10, v/v) as gradient and yielded the compound 3 (27 mg).

On the other hand, the screening of the chemical constituents was carried out with the extracts of the *P. erinaceus* stem barks, using chemical reagents and thin layer chromatography (TLC) methods according to the methodology suggested by Wagner and Bladt[10].

2.5. Anti-inflammatory activity

2.5.1. Carrageenan induced paw edema test

the test was carried out according to Winter[11] with the MeOH extract. One hour prior to injection of carrageenan, the mice were orally treated with MeOH extract (100 and 200 mg/kg bw) and distilled water, and then four other groups received MeOH extract at doses of 50, 100 and 200 mg/kg and hydrocortisone (10 mg/kg) at intraperitoneal. The edema volume was recorded at 1 h, 3 h and 5 h after carrageenan injection using plethysmometer (model Ugo Basil, n° 7141, Italy). The average volume of the right hind paw of each mouse was calculated from three reading. The inhibitory activity was calculated according to the following formula:

$$\%\text{Inhibitian} = \frac{(C_t - C_0)\ \text{control} - (C_t - C_0)\ \text{treated}}{(C_t - C_0)\ \text{control}} \times 100$$

C_t is the paw circumference at time t, C_0 is the paw circumference before carrageenan injection, $C_t - C_0$ is edema, $(C_t - C_0)$ control is edema or paw size after carrageenan injection to control mice at time t.

2.5.2. Ear edema induced by oil croton

Topical inflammation was carried out according to Sawadogo[12] with slight modification. NMRI mice were anaesthetized with ketamine hydrochloride (150 mg/kg, *i.p.*).

Coetaneous inflammation was induced by applying 5 μL of solution of croton oil dissolved in 42% aqueous ethanol and the sample (MeOH extract, compound 3 and hydrocortisone) or in DCM (DCM extract, compound 1 or 2) on the inner surface of the right ear. Control mice received only irritation solution. Six hours later, mice were sacrificed and a plug (7 mm Ø) was removed from both the treated (right) and the untreated (left) ears. Edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percent reduction of edema in treated mice compared with the control mice.

2.6. Analgesic activity

Acetic acid-induced writhing test: the intraperitoneal injection of 0.6% acetic acid solution (10 mL/kg bw) provoked abdominal writhing[13]. Five groups of six mice each have formed. The mice orally received the MeOH extract (100, 200 and 400 mg/kg), paracetamol (100 mg/kg) and distilled water 1 h before acetic acid injection. Five min after acetic acid injection, the number of writhing was recorded during 15 min. The analgesic effect was evaluated by a percentage reduction of writhes in treated mice compared to those in the control group.

2.7. DPPH free radical scavenging method

The method of Brand[14] was used to evaluate the antioxidant power of the extracts. 20 μL of each extract solution at different concentrations was mixed with 200 μL DPPH methanolic solution (0.08 mg/mL) in a 96-well microtitre plate. After 30 min incubation at room temperature, the absorbance was measured at 515 nm. Trolox and chlorogenic acid were used as standard control. Each determination was carried out in triplicate. The percentage of residual DPPH was evaluated onto the graph in function of quantity of antioxidant:

$\%DPPH_{res}$= f(antioxidant quantity/DPPH quantity)

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

2.8. Statistical analysis

The results were expressed as mean±SEM. The data were analyzed using GraphPad Prism version 5.0. The statistical analysis was performed by one way ANOVA followed by Dunnett's test. At $P<0.05$ the differences were considered significant compared to control group.

3. Results

3.1. Chemical composition

The phytochemical screening on TLC revealed the presence of tannins, flavonoids and phenolic compounds in the MeOH and ethyl acetate fraction. Thus in the dichloromethane extract triterpenoids were detected.

The screening indicated that the non-polar compounds such as triterpenoids were extracted from the plant material during maceration into the DCM extract. Both, the MeOH extract and ethyl acetate fraction contained compounds as flavonoids, tannins and phenolic compounds. In generally, TLC analysis of the DCM fraction revealed the presence of friedelin and lupeol as main active constituents while the analysis of the MeOH extract and ethyl acetate fraction revealed the presence of epicatechin as their main active constituent. Friedelin 1 and lupeol 2 were isolated from dichloromethane extract. These triterpenes were identified by their ^{1}H NMR and ^{13}C NMR data using Bruker DRX 300 by comparison with those of the literature data[15,16]. The compound 3 from ethyl acetate fraction was (-) epicatechin. This flavanol was identified with its ^{1}H NMR and ^{13}C NMR spectra compared with those of literature data[17].

Friedelin (1): TLC: Silica (Hexane/ethyl acetate, 90/10), R_f= 0.42. Spot: yellow with sulfuric vanillin, physical aspect: white powder.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.27 (s, 1H), 5.31 (s, 1H), 2.40-2.36 (m, 2H), 2.30-2.23 (m, H), 2.18 (s, 1H), 1.98 (ddd, *J*=9.8, 5.8, 3.4 Hz, 3H), 1.75 (s, 7H), 1.62 (dd, *J*=7.9, 4.8 Hz, 4H), 1.58-1.48 (m, 17H), 1.41-1.22 (m, 36H), 1.19 (s, 9H), 1.06 (s, 9H), 1.01 (d, *J*=1.9 Hz, 17H), 0.96 (s, 9H), 0.89 (d, *J*=5.8 Hz, 18H), 0.73 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 22.26 (C 1), 41.50 (C 2), 213.23 (C 3), 58.19 (C 4), 42.12 (C 5), 41.26 (C 6), 18.21 (C 7), 52.65 (C 8), 37.41 (C 9), 59.44 (C 10), 35.59 (C 11), 30.48 (C 12), 39.66 (C 13), 38.26 (C 14), 32.38 (C 15), 35.98 (C 16), 29.96 (C 17), 42.75 (C 18), 35.31 (C19), 28.14 (C 20), 32.73 (C 21), 39.23 (C 22), 6.80 (C 23), 14.63 (C 24), 17.92 (C 25), 20.24 (C 26), 18.64 (C 27), 31.06 (C 28), 35.01 (C 29), 31.76 (C 30)(Figure 1).

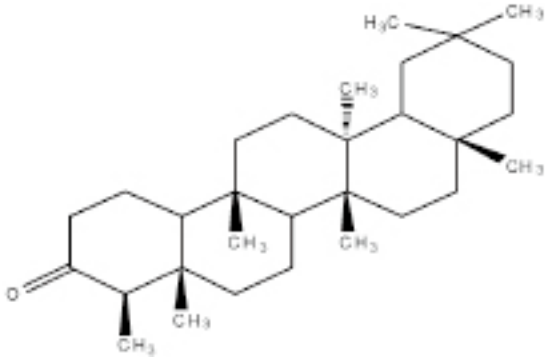

Figure 1. Friedelin.

Lupeol (2): TLC: Silica (CH_2CL_2 100%), R_f =0.57. Spot: purple with sulfuric/vanillin, white powder.

^{1}H NMR: (300 MHz, $CDCl_3$) (300 MHz, $CDCl_3$) δ 7.26 (s, 1H), 4.80-4.48 (m, 2H), 3.21 (s, 2H), 2.48-2.35 (m, 1H), 2.00-1.87 (m, 3H), 1.74-1.53 (m, 16H), 1.46-1.23 (m, 18H), 1.08 (dd, *J*=16.7, 11.1 Hz, 5H), 1.03 (s, 3H), 1.00 (s, 2H), 0.97 (d, *J*=3.1 Hz, 5H), 0.94 (s, 5H), 0.91 (s, 1H), 0.87 (s, 2H), 0.83 (s, 3H), 0.81-0.78 (m, 5H), 0.76 (s, 3H) .

^{13}C NMR: (75 MHz, $CDCl_3$) δ 38.73 (C 1), 27.46 (C 2), 79.01 (C 3), 38.87 (C 4), 55.32 (C 5), 18.33 (C 6), 34.31 (C 7), 40.85 (C 8), 50.46 (C 9), 37.19 (C 10), 20.95 (C 11), 25.17 (C 12), 38.08 (C 13), 42.85 (14), 27.44 (C 15), 35.60 (C 16), 43.01 (C 17), 48.33 (C 18), 48.00 (C 19), 150.96 (C 20), 29.87 (C 21), 40.02 (C 22), 28.00 (C 23), 15.37(C 24),

16.12 (C 25), 15.99 (C 26), 14.56 (C 27), 18.01 (C 28), 109.33 (C 29), 19.32 (C 30) (Figure 2).

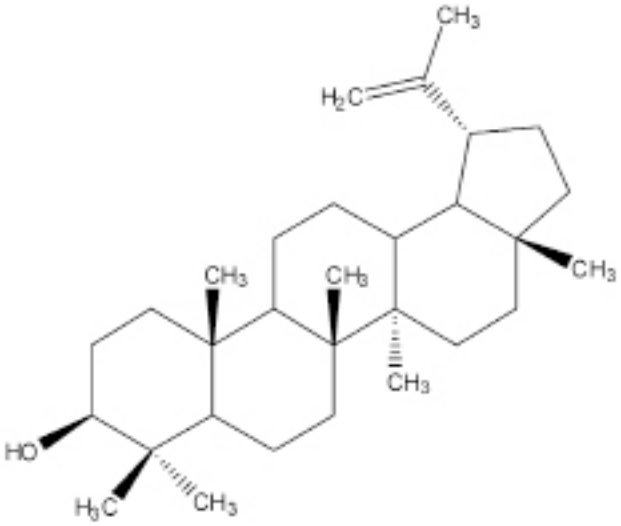

Figure 2. Lupeol.

(−)-epicatechin (3): TLC: silica RP-18 (MeOH/H_2O, 50/50), R_f=0.34. Spot: yellow with NEU (DBA), physical aspect: yellow powder. [α_D] = − 46° (C=0.19, MeOH).

^{1}H NMR (300 MHz, MeOD) δ 6.96 (s, 1H), 6.77 (d, *J*=4.9 Hz, 2H), 5.92 (dd, *J*=7.9, 2.0 Hz, 1H), 4.81 (s, 1H), 4.17 (s, 1H), 3.45-3.13 (m, 3H), 2.79 (ddd, *J*=19.5, 16.8, 3.7 Hz, 2H), 2.15 (s, 1H), 1.28 (s, 1H).

^{13}CNMR: (75MHz,$CDCl_3$) δ 79.59 (C 2), 67.21 (C 3), 28.99 (C 4), 157.6 (C 5), 96.09(C 6), 158.1 (C 7), 95.78 (C 8), 157.0 (C 9), 99.76 (C 10), 132.01 (C 1′), 115.59 (C 2′), 145.50 (C3′), 145.66 (C 4′), 115.03 (C 5′), 119.10 (C 6′) (Figure 3).

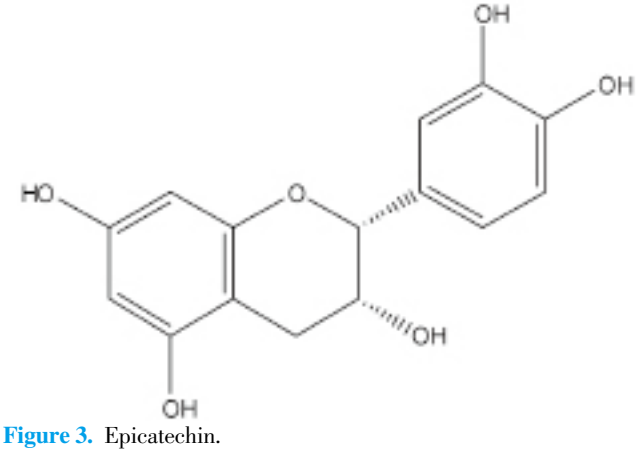

Figure 3. Epicatechin.

3.2. Anti-inflammatory activity

3.2.1.Carrageenan induced paw edema test

The effects of MeOH extract on carrageenan induced-paw edema in mice have been shown in Table 1. At different doses, the oral and the intraperitoneal administration of MeOH extract inhibited significantly the edema induced by carrageenan injection. At doses 50, 100 and 200 mg/kg its intraperitoneal administration reduced dose-dependently the paw edema (Table 1).

3.2.2. Ear edema induced by oil croton

At the doses of 50, 100 and 200 μg/ear, DCM extract caused inhibition with 32.3%, 69.7% and 91.7%, respectively. The MeOH extract reduced the edema by 42.0% and 85.4% at the doses of 300 and 500 μg/ear. Friedelin 1 (100 μg/ear), lupeol 2 (100 μg/ear), epicatechin 3 (75 μg/ear) and hydrocortisone (100 μg/ear) caused inhibition percentage of 61.1%, 48.2%, 16.9% and 62.0%, respectively (Figure 4).

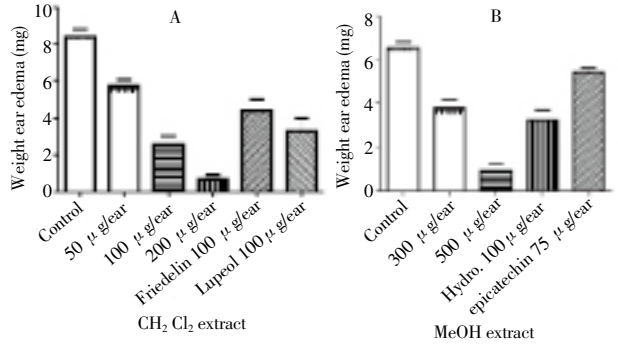

Figure 4. Effect of MeOH extract (A)and CH_2Cl_2 extract (B) on ear edema induced by oil croton.

3.3. Acetic acid-induced writhing test

The writhing induced by acetic acid injection was reduced by the MeOH extract. At doses of 100, 200 and 400 mg/kg oral administration of this extract exhibited an inhibition of 38.8% 68.0% and 74.3%, respectively (Table 2). The reference product (paracetamol) caused a more important inhibition than the MeOH extract at same dose (47.36%).

3.4. DPPH free radical scavenging method

Table 3 showed the free radical-scavenging capacity of MeOH extract and its dichloromethane and ethyl acetate

Table 1

Effect of administration of MeOH extract on carrageenan induced hind paw edema.

	Samples	Doses (mg/kg bw)	Increase in paw volume (mL)			Edema inhibition (%)		
			1 h	3 h	5 h	1 h	3 h	5 h
Oral administration	Control	–	0.21±0.02	0.24±0.03	0.28±0.03	–	–	–
	MeOH extract	100	0.15±0.04*	0.18±0.04*	0.16±0.05**	28.57	25.00	42.86
		200	0.10±0.03**	0.13±0.04**	0.10±0.02**	52.38	45.83	64.28
i.p. administration	Control	–	0.24±0.03	0.34±0.01	0.37±0.06	–	–	–
	MeOH extract	50	0.14±0.02**	0.29±0.04	0.24±0.04*	41.67	14.70	35.13
		100	0.10±0.02**	0.26±0.04**	0.20±0.03**	58.33	23.53	45.94
		200	0.08±0.03**	0.23±0.04**	0.17±0.03**	66.67	32.35	54.05
	Hydro cortisone	10	0.19±0.02	0.28±0.04**	0.25±0.06**	20.83	17.65	32.43

*P<0.05, **P<0.001 significant from control (one way ANOVA analysis followed by Dunnett's test).

fractions. The MeOH extract had the most important antiradical power compared to its fractions and the standard antioxidants (trolox and chlorogenic acid).

Table 2
Effect of oral administration of MeOH extract on writhing induced by acetic acid (mean±SEM, n=6).

Samples	Doses (mg/kg bw)	Number of writhing
Control	–	66.17±2.00
MeOH extract	100	40.50±1.80**
	200	20.17±2.00**
	400	17.00±3.00**
Paracetamo	100	34.83±1.47**

** P <0.001 significant from control (one way ANOVA analysis followed by Dunnett's test).

Table 3
DPPH radical scavenging capacity of MeOH extract, DCM fraction and ethyl acetate fraction.

Samples	IC_{50} (mg/mg DPPH)	ARP
MeOH extract	0.20±0.02	5
DCM fraction	0.50±0.04	2
Ethyl acetate fraction	0.28±0.05	3.57
Chlorogenic acid	0.27±0.03	3.70
Trolox	0.22±0.01	4.55

ARP(Antiradical power)=1/ IC_{50}.

4. Discussion

The paw edema induced by carrageenan is an experiment animal model used to evaluate the acute anti-inflammatory activity of natural substances and drugs. The carrageenan injection provokes the activation of mast cells and the release of chemical mediators. The release of mediator involves three phases, first phase lasted for 1 hour that mediated by histamine, serotonin and the second phase was due to release of bradykinin during 2-3 hours; prostaglandins were produced during the last phase (4-6 h)[18]. The MeOH extract inhibited the inflammatory response during the three phases of edema. At doses of 100 and 200 mg/kg, the oral administration of MeOH extract caused an important inhibition of paw edema induced by carrageenan injection. The maximal inhibition was noted at fifth hour oral administration (64.28% at 200 mg/kg). It suggests that the oral administration could more inhibit the prostaglandins biosynthesis. However, the intraperitoneal administration could reduce more the production of histamine and serotonin because it showed the maximal inhibition during the first phase (65.21% at 200 mg/kg).

The inflammatory response produced by application of croton oil was reduced by the MeOH and DCM extracts, friedelin 1, lupeol 2 and epicatechin 3. The croton oil application in inner of ear of mice is the model tested to essay the topical anti-inflammatory effect of some drugs[19]. The ear edema induced by croton oil application is due to the production of some cytokines such as TNF α, IL1 β and others pro-inflammatory products[20,21]. The DCM extract presented the highest inhibition compared to hydrocortisone, friedelin, lupeol and epicatechin. The high inhibition of DCM extract was due to the apolarity of extract and the products it contains. The reduction of ear edema induced by croton oil application could be due to the action of extracts and compounds on the release of cytokines.

Friedelin and lupeol contributed to the anti-inflammatory activity of DCM extract. Lupeol is known to possess the anti-inflammatory activity[22-24], the ability to inhibit cytokines such as TNF α, IFN γ, IL2, IL4[25] and analgesic effect against glutamate-induced nociception[26], while friedelin is able to delay the release of nitric oxide[27]. Epicatechin was able to inhibit the production of nitric oxide[28]. These three compounds that were responsible of biological effect had not been isolated before from *P. erinaceus*.

The writhing induced by injection of acetic acid is the experimental animal model used to test the peripheral analgesic effect of some substances and NSAIDs. Acetic acid injection provokes the release of substance P, histamine, serotonin and prostaglandins which stimulated the nociceptives neurons[29,30]. Ours results show that the oral administration of MeOH extract was able to inhibit significantly the writhing induced by acetic acid injection. The treatment with MeOH extract has been observed to indicate dose-dependent inhibition. The analgesic effect of MeOH extract may be the inhibition of release of cytokines and pro-inflammatory mediators such as prostaglandins.

The free radicals such as reactive oxygen species (ROS) are produced during the process of inflammation by the mast cells, neutrophils and macrophages. This production of ROS contributes to expand the inflammatory response[31,32]. The present results indicate that methanol extract and its fractions inhibited significantly DPPH free radical. It suggests that these extracts might inhibit the release of free radicals during the inflammation. This antioxidant ability of the extracts contributes to reinforce the anti-inflammatory and analgesic effects.

The present study is the first report to provide data about the anti-inflammatory, analgesic and antioxidant activities of the MeOH and DCM extracts obtained from the stem barks of *P. erinaceus* commonly used in Burkina Faso as folk medicine. In summary, the anti inflammatory, analgesic and antioxidant activities of the MeOH extract were better and stronger than those of DCM extract. But both the extracts showed the same anti inflammatory potency to inhibit the ear edema of mouse induced by croton oil application. Interestingly, the main active compounds isolated from these extracts as friedelin, lupeol and epicatechin contribute to these activities. Possible synergetic actions of other components in the extracts could be taken in consideration. *P. erinaceus* can also represent a useful source of anti-inflammatory, analgesic and antioxidative constituents, which could be easily isolated by the non-polar solvent dichloromethane and the polar solvent methanol. Therefore, it is suggested that further works should be performed on isolation and identification of the active constituents of the MeOH extract of the plant. These results may provide a starting point for the investigations to explore new natural anti inflammatory, analgesic and antioxidant products. *P. erinaceus* can be considered as a promising natural source of ingredients that could be used in food and in pharmaceutical industry. This study has provided the

scientific data to promote the traditional medicine and confirmed the use of this plant by the healers to treat inflammatory diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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