International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.549.555

Research Article
Anti-inflammatory and Anti-plasmodial Activities of Methanol Extract of *Pterocarpus erinaceus* Poir. (Fabaceae) Leaves

[1,2,3]Ouédraogo Noufou, [1,5]Tibiri André, [1]Sawadogo W. Richard, [1]Serge Yerbanga, [1]Traoré Maminata, [1]Sylvin Ouédraogo, [3]E. Hay Anne, [3]Garcia Irène and [1,2]Guissou Innocent Pierre

[1]Département de médecine-pharmacopée traditionnelle/pharmacie, (IRSS/CNRST), 03 BP 7192 Ouagadougou 03, Burkina Faso
[2]Laboratoire de pharmacologie et toxicologie, UFR/SDS, Université de Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso
[3]Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne, CNRS, UMR 5557, Ecologie Microbienne, ISPB, 69373 Lyon, France
[4]Département de Pathologie et d'immunologie, CMU, Université de Genève, 1 rue Michel Servet-CH-1211 Genève 4, Suisse
[5]Unité Mixte Internationale-Environment, Santé, Sociétés (UMI 3189, ESS) CNRS/UCAD/USTTB/CNRST/UGB

Abstract

Background and Objective: *Pterocarpus erinaceus* Poir. (Fabaceae) is used in folk medicine in Burkina Faso to treat inflammatory diseases including malaria, ulcer, rheumatism and infectious disease. The aim of this study was to carry out the evaluation of the safety and efficacy of methanol extract of *Pterocarpus erinaceus* leaves studying anti-inflammatory, analgesic and anti-plasmodial activities. **Methodology:** The anti-inflammatory and analgesic effects of methanol extract of leaves were studied by carrageenan-induced paw edema test and acetic acid-induced writhing test in mice Naval Medical Research Institute (NMRI). Methanol extract effect on Tumor Necrosis Factor alpha (TNF α) and Nitric Oxide (NO) production was evaluated by lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The 2,2-diphenyl-2-picryl-hydrayl, Sigma (DPPH) and cupric-reducing antioxidant capacity (CUPRAC) methods were used to evaluate the antioxidant activity of the extract. Test against *Plasmodium berghei* infected mice was used to evaluate the anti-plasmodial activity of methanol. **Results:** At doses of 100, 200 and 400 mg kg^{-1}, oral administration of methanol extract significantly produced anti-inflammatory and analgesic effects in dose-dependent manner. Methanol extract inhibited release of NO and TNF α in LPS-stimulated RAW 264.7 cells. The capacity of extract to scavenge free radical was lower than trolox and rutin. Plasmodium inhibition of extract was expressed at 50 and 100 mg kg^{-1}. **Conclusion:** These results indicate that the leaves of *Pterocarpus erinaceus* have anti-inflammatory and analgesic properties, which support its traditional medicine use.

Key words: Anti-inflammatory, analgesic, antioxidant, anti-plasmodial, *Pterocarpus erinaceus*

Received: February 05, 2016 Accepted: March 26, 2016 Published: June 15, 2016

Citation: Ouédraogo Noufou, Tibiri André, Sawadogo W. Richard, Serge Yerbanga, Traoré Maminata, Sylvin Ouédraogo, E. Hay Anne, Garcia Irène and Guissou Innocent Pierre, 2016. Anti-inflammatory and anti-plasmodial activities of methanol extract of *Pterocarpus erinaceus* Poir. (Fabaceae) leaves. Int. J. Pharmacol., 12: 549-555.

Corresponding Author: Ouédraogo Noufou, Département de médecine-pharmacopée traditionnelle/pharmacie, (IRSS/CNRST), 03 BP 7192 Ouagadougou 03, Burkina Faso Tel: +226 25363215

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammation is defensive system that protects organism against pathogens (bacteria, fungi, virus) and injury. Several pro-inflammatory endogen mediators including cytokines, such as Tumor Necrosis Factor alpha (TNF α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), Reactive Oxygen Species (ROS) and Nitric Oxide (NO) are produced during inflammatory process[1]. The ROS are a heterogeneous group of oxygen radicals, which play an important role in physiological process but uncontrolled ROS production can lead to oxidative stress, tissue and ADN damage. Excessive production of ROS and cytokines can maintain or amplify the inflammatory response leading to chronic inflammation. The ROS induce the release of cytokines and Nitric Oxide (NO) released by inducible Nitric Oxide Synthase (iNOS). The NO induce biosynthesis of inflammatory prostaglandins (PGE, PGF) via activation of cyclooxygenase 2[2].

Plasmodium infection causes an inflammatory response involving inflammatory mediators production, such as cytokines (TNF α, IL 1β, IL 6) and Nitrite Oxide (NO)[3]. The parasite major toxin glycosylphosphatidylinositol (GPI) of Plasmodium is responsible of TNF α and NO production[4]. Punsawad *et al*.[5] reported that increasing serum TNF level were associated with increased mortality in children with severe malaria.

In Africa, malaria patients use local medicinal plants to treat malaria with eliminating of clinical symptoms such as fever, pain and general malaise. However, there are few scientific data as well as pre-clinical studies about efficacy and safety of traditional herbals.

Pterocarpus erinaceus leaves were used folk medicine in Burkina Faso to treat malaria, parasitosis, fever and anemia. Previous biological investigations on *Pterocarpus erinaceus* revealed *in vitro* anti-malaria and anti-microbial activities[6,7]. Previous study had shown anti-inflammatory and analgesic proprieties of *P. erinaceus* stem bark[8].

The aim of the present study was to investigate the anti-inflammatory activity of methanol extract from leaves of *P. erinaceus* Poir. (Fabaceae) using *in vivo* and *in vitro* assays, then to carry out analgesic and anti-plasmodial activities *in vivo*.

MATERIALS AND METHODS

Plant material: The leaves of *P. erinaceus* were collected in March, 2009 at Gourcy (150 km from Ouagadougou, Burkina Faso). The *P. erinaceus* was identified by Pr. A. Thiombiano and a voucher specimen number ON-01 has been deposited at herbarium of ecology laboratory of University of Ouagadougou.

Animals and ethical approval: *In vivo* studies were carried out in accordance with guidelines for care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals[9]. The NMRI mice (25-32 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 17 h and weighed before the experiments.

Chemicals: The 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproin (2,9-dimethyl1,10-phenanthrolin) and copper II chloride, carrageenan (sigma), acetic acid (sigma) and paracetamol (sigma) were purchased from Sigma (St. Louis, USA). Trolox (Fluka, France), rutin (Merck, France) and ammonium acetate aqueous buffer (Prolabo, France) were also used in this study.

Biological tests

Acute toxicity: Acute toxicity was carried out to determinate Lethal Dose (LD_{50}). Mice fasted overnight were randomly divided into five groups (six per group). Groups were treated with methanol extract at doses of 500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt. Mice were observed from 2-72 h and two weeks period for morbidity or mortality and changes in behavior were recorded compared to control group treated with water.

Anti-edematous activity: Carrageenan induced paw edema test was carried out according to the method described by Winter *et al*.[10]. Mice were divided into five groups (n = 6 per group). One hour prior to injection of carrageenan, control group received distilled water, group II, III and IV were orally treated with MeOH extract (100, 200 and 400 mg kg^{-1} b.wt.) and group V was treated with acid acetylsalicylic (AAS). The edema volume was recorded at 1, 3 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 at each hour. The inhibitory activity was calculated according to the following formula:

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

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

Analgesic activity: The intraperitoneal injection of 0.6% acetic acid solution (10 mL kg^{-1} b.wt.) provokes abdominal writhing according to Sawadogo *et al.*[11]. Five groups (n = 6 mice per group) were formed. Control group (group I) received per os distilled water, group II, III and IV orally received the MeOH extract (100, 200 and 400 mg kg^{-1}) and group V treated with paracetamol (100 mg kg^{-1}) 1 h before acetic acid injection. Five minutes 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 group compared to the control group.

TNF α and nitrite assay: According to Fotio *et al.*[12] extracts were dissolved in a solution of Dulbecco's Modified Eagle's Medium (DMEM). Murine cells of the bone marrow were differentiated into macrophages after culture of 1.5×10^6 cells/well in a 24 well plate. Each well contained DMEM supplemented with 10% fetal calf serum and 20% conditioned RAW 264.7 cells as a source of colony-stimulating factor of granulocytes and macrophages.

After six days of culture, the cell preparation contained 99% of adherent macrophages, which accounted for approximately 10% of total cells initially placed in culture. Macrophages derived from bone marrow bone marrow-derived macrophage (BMDM) were untreated or treated with different concentrations (25, 50 μg mL^{-1}) extracts for 30 min. The BMDM were stimulated by *Escherichia coli* (serotype 055:B5), LPS (1 μg mL^{-1}) or LPS/IFN-γ for 24 h. The supernatant was collected for determination of the amount of nitrite and the cytokine Tumor Necrosis Factor alpha (TNF α). The amount of TNF α in the cell culture supernatant was measured using the enzyme-linked immunosorbent assay (ELISA) test. Accumulation of nitrite in the culture medium was determined as BMDM indicator of the production of nitric oxide by the Griess reagent (1% sulfanilamid and 0.1% in naphthyethylenediamid 2.5% phosphoric acid). About 25 μL of the supernatant collected were mixed with 50 μL of the Griess reagent for 5 min. The absorbance was measured at 570 nm and the concentration of nitrite was determined by comparison with a reference to a standard curve of sodium nitrite.

Antioxidant activity

DPPH radical scavenging assay: Free radical scavenging activity of methanol extract and flavonoids fraction were evaluated by DPPH (2,2-diphenyl-2-picryl-hydrayl, Sigma)[13]. Trolox and ascorbic acid were used as positive control. The IC_{50} was considered as the concentration of the substrate that causes 50% loss in DPPH activity and the Anti-Radical Power (ARP = $1/IC_{50}$) was the antioxidant power.

CUPRAC assay: Cupric-reducing antioxidant capacity (CUPRAC) assay was used to determine the antioxidant capacity of methanol extract and flavonoids fraction[14]. Trolox Equivalent Antioxidant Capacity (TEAC) was used to determine the antioxidant capacity of the substrate.

***In vivo* anti-plasmodial assay:** *In vivo* anti-malaria activity of methanol extract of *P. erinaceus* leaves was performed in mice based on the 4 day suppressive test[15]. *Plasmodium berghei* ANKA strain infected blood was obtained by ear, mixed in Phosphate Buffered Saline (PBS). The test mice were been infected by i.p., injection with 200 μL (10^7 parasitized erythrocytes) on day 0. Infected animals were randomly divided in three groups of six mice. Control group and treated groups received orally respectively distilled water and extract (50 and 100 mg kg^{-1}, b.wt.), 2 h post infection. The groups 50, 100 and 250 mg kg^{-1} were treated once per day from day 0-3. On day 4 of the test, thin blood smears from the tail of treated mice were performed and fixed in methanol. Blood from animals were strained with Giemsa 10% and microscopically examined.

Statistical analysis: Results were expressed as Mean±SEM. GraphPad Prism version 5.0 was used to analyzed study data. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Differences were considered significant compared to control group if $p<0.05$.

RESULTS

Acute toxicity: Oral administration of methanol extract at doses of 500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt. did not provoke any changes in behavior. No mortality of mice NMRI was obtained during two week of observation.

Anti-edematous activity: Carrageenan induced-paw edema was used to evaluate the anti-inflammatory effect of the methanol extract. It showed a significant inhibitory effect on paw edema formation from first to 5th h after carrageenan injection. The highest inhibition was observed during the 5th h, which at 200 and 400 mg kg^{-1} and the percentage of inhibition obtained were, respectively 58.1 and 77.4% (Table 1).

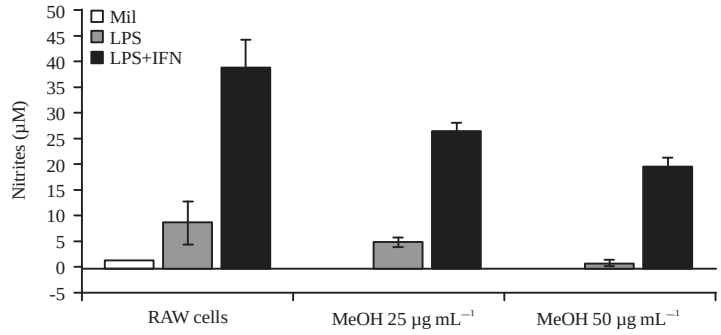

Fig. 1: Effect of methanol extract of *Pterocarpus erinaceus* leaves on nitrites (NO) level in IFN/LPS or LPS-stimulated RAW 264.7 cells. Values are Mean±SEM (n = 8), p<0.05, significant from control (one way ANOVA analysis followed by Dunnett's test)

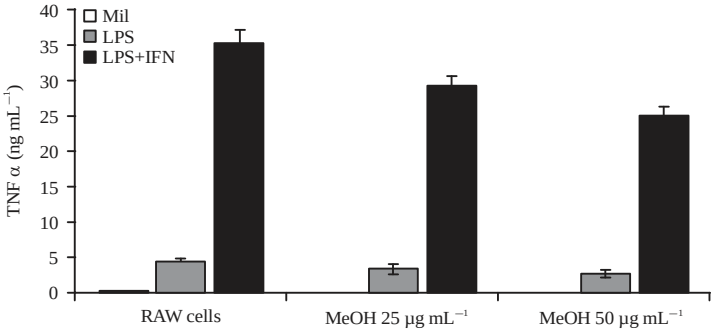

Fig. 2: Effect of methanol extract of *Pterocarpus erinaceus* leaves on TNF α level in IFN/LPS or LPS-stimulated RAW 264.7 cells. Values are Mean±S.E.M, (n = 8), p<0.05, significant from control (one way ANOVA analysis followed by Dunnett's test)

Table 1: Anti-inflammatory effect of methanol extract of *Pterocarpus erinaceus* leaves on carrageenan induced hind paw edema

		Increase in paw volume (ΔV mL)			Edema inhibition (%)		
Extract	Doses (mg kg^{-1} b.wt.)	1 h	3 h	5 h	1 h	3 h	5 h
Control	-	0.21±0.03	0.29±0.03	0.31±0.02	-	-	-
MeOH extract	100	0.18±0.03*	0.25±0.03	0.26±0.05*	14.3	13.8	16.1
	200	0.13±0.02***	0.14±0.04***	0.13±0.04***	38.1	51.7	58.1
	400	0.11±0.04**	0.13±0.04***	0.07±0.02***	47.6	55.2	77.4
Acid acetylsalicylic	100	0.14±0.04**	0.18±0.02**	0.17±0.06**	33.3	25.0	39.3

Values are Mean±SEM (n = 6), *p<0.05, **p<0.01, ***p<0.001 significant from control (one way ANOVA analysis followed by Dunnett's test)

Table 2: Analgesic effect of methanol extract of *Pterocarpus erinaceus* leaves on acetic acid-induced writhing

Extract	Doses (mg kg^{-1} b.wt.)	Number of writhing	Inhibition (%)
Control	-	66.2±2	-
MeOH extract	100	45.5±1.8***	31.20
	200	26.2±2.0***	60.50
	400	21.0±3.0***	68.30
Paracetamol	100	34.83±1.47**	47.36

Values are Mean±SEM (n = 6), **p<0.01, ***p<0.001 significant from

Analgesic activity: Acetic acid injection-induced writhing was significantly reduced by the oral administration of 100, 200 and 400 mg kg^{-1} doses of methanol extract by 31.2, 60.5 and 68.3%, respectively (Table 2). Paracetamol used as reference, inhibited the writhing by 73.6 at 200 mg kg^{-1}.

TNF α and NO determination: The effect of methanol extract on TNF α and NO productions in RAW 264.7 cells simulated by LPS or LPS/IFN was investigated. The TNF α and NO productions were more in the response to LPS/IFN than LPS only in cells. In Fig. 1 and 2, at 25 and 50 µg mL^{-1}, the methanol extract inhibited the level of nitrite (equivalent to the NO level) and the TNF α level in culture supernatants. The highest inhibition (95.14%) of methanol extract was obtained at 50 µg mL^{-1} on NO production in LPS-stimulated RAW 264.7 cells.

Antioxidant activity: The free radical DPPH•-scavenging capacity of methanol extract and flavonoids fraction was less

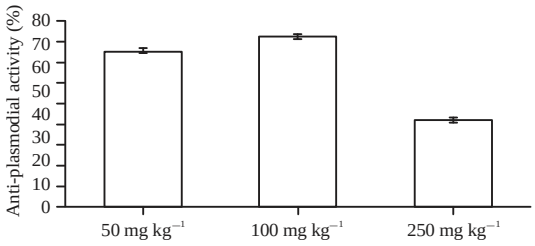

Fig. 3: Anti-plasmodial activity of methanol of *Pterocarpus erinaceus* leaves against *Plasmodium berghei* infected mice. Values are Mean±SEM (n = 6), p<0.05, significant from control (one way ANOVA analysis followed by Dunnett's test)

Table 3: Antioxidant capacity of methanol extract of *Pterocarpus erinaceus* leaves with DPPH method and CUPRAC method

	DPPH		CUPRAC
Extract	IC_{50} (mg mg^{-1} DPPH)	ARP	TEAC
MeOH extract	1.1±0.03	0.9	0.6
Trolox	0.22±0.01	4.55	
Rutin	nd	nd	2.9

ARP: Anti-radical power, TEAC: Trolox equivalent antioxidant capacity, values are Mean±SEM for triplicate, p<0.05 significant from control (one-way ANOVA analysis followed by Dunnett's test)

than trolox (ARP = 4.55) used as reference substance. The antioxidant power using CUPRAC method of methanol extract and flavonoids fraction was less important than rutin (TEAC = 2.9) (Table 3).

***In vivo* antiplasmodial assay:** Methanol extract of *P. erinaceus* leaves exhibiting *in vivo* anti-inflammatory activity was tested for *in vivo* anti-plasmodial activity using three doses (50, 100 and 250 mg kg^{-1} b.wt.). Results presented by Fig. 3 showed suppressive capacity of extract against *Plasmodium berghei* ANKA strain. The anti-plasmodial effect of extract was significant (p<0.05) and observed in a dose dependent in manner.

DISCUSSIONS

The leaves of *Pterocarpus erinaceus* are used to treat inflammatory diseases (malaria, anemia, ulcer, rheumatism and dermatitis) in traditional medicine in Burkina Faso. In the present study, the pharmacological properties of methanol extract were investigated in paw edema induced by carrageenan, writhing induced by acetic acid, antioxidant capacity, on TNF α and NO production in IFN/LPS or LPS-stimulated RAW 264.7 cells and against *Plasmodium berghei* infected mice.

Before pharmacological studies, the acute oral toxicity of methanol extract was initially investigated in NMRI mice. Up to 2,500 mg kg^{-1} b.wt., no mortality and no changes in behavior in treated mice were observed. This suggests that methanol extract can be classified to be safe and low toxic drug according to Hodge and Sterner[16].

Carrageenan-induced paw edema is wildly used to evaluate the anti-inflammatory effect of natural substances or new drugs. Carrageenan injection induce the inflammatory responses, which is biphasic events: Histamine, serotonin, bradykinins are released in the initial phase (1 h) and the second phase (over 1 h) is due the prostaglandins and cytokines (TNF α, IL 1β, Il 6) production[17,18]. Paw edema was reduced by methanol extract at 1, 3 and 5 h after carrageenan injection. The edema inhibition suggests that the methanol extract acts on the release of different pro-inflammatory mediator. Edema reduction was important at 5 h after carrageenan injection, suggesting that methanol extract inhibited the release of prostaglandins and cytokines.

Writhing induced by acetic acid injection on animal is a method used to investigate the peripheral analgesic effect of natural drugs. Acetic acid injection releases endogenous mediators including histamine, serotonin, bradykinin, substance P and prostaglandins ($PGE_{2\alpha}$, $PGF_{2\alpha}$), which are responsible of abdominal writhing[19,20]. Oral administration of methanol extract significantly reduced abdominal writhing in dose-dependent manner. This result could be suggesting that the analgesic effect was related to inhibition of endogenous mediators, such as substance P and prostaglandins ($PGE_{2\alpha}$, $PGF_{2\alpha}$).

In vitro anti-inflammatory activity was evaluated on TNF α and NO production in IFN/LPS or LPS-stimulated RAW 264.7 cells. Pro-inflammatory cytokines as TNF α and Nitric Oxide (NO) play an important role during inflammatory diseases, in addition, TNF α and NO stimulate the production of prostaglandins ($PGE_{2\alpha}$, $PGF_{2\alpha}$), which are involved in inflammation and pain[21,22]. Methanol extract inhibited the release of TNF α and NO in RAW 264.7 cells. According to results (Fig. 1 and 2), the inhibition of methanol extract of *P. erinaceus* on NO production was superior when compared to TNF α-production. The release of NO and TNF α are due to NF-κB activation[23]. It suggests that blocking of NO and TNF α production by extract could be linked to the lock of NF-κB activation. In addition, the anti-inflammatory effect of substance could be the fact that it decreases the COX 2 expression and the nuclear translocation of NF-κB[24].

Large amount of Reactive Oxygen Species (ROS) generated by activated macrophages and granulocytes, contribute to expand the inflammatory response. During inflammation ROS induce the release of pro-inflammatory cytokines (TNFα, IL 1β, IL 6), which in turn increase ROS[25].

The ROS inhibition contributes to the therapeutic anti-inflammatory effect of natural substance[8]. Two methods (DPPH and CUPRAC) were used to evaluate the capacity of methanol extract of *Pterocarpus erinaceus* to inhibit ROS. According to the results obtained through both methods, methanol extract exhibited antioxidant activity but moderated than trolox and rutin used as reference compounds. It could be this antioxidant effect, which contributes to anti-inflammatory and analgesic effect of extract reducing ROS production and pro-inflammatory cytokines release.

Several studies reported the anti-malaria properties of medicinal plants leading to isolate anti-malaria molecules including quinine, artemisinin actually used against malaria[26]. Methanol extract of *P. erinaceus* leaves exhibited anti-plasmodial effect against *Plasmodium berghei* in mice NMRI. Karou *et al.*[6] demonstrating that the *in vitro* anti-plasmodial activity of ethanol extract of leaves of *P. erinaceus* was performed against fresh clinical isolated *Plasmodium falciparum* with $IC_{50} = 14.63$ µg mL^{-1}. According to the classification about anti-plasmodial activity *in vivo*, methanol extract of leaves of *P. erinaceus* exhibited very good activity[27].

The pathogenesis of malaria is associated with an inflammatory response as a defensive reaction of body leading pro-inflammatory cytokines production. Kassa *et al.*[28] reported that during Plasmodium infection, hemozoin formed induces the pro-inflammatory cytokines productions. So the anti-inflammatory activity of methanol extract contributes to antiplasmodial activity *in vivo*.

CONCLUSION

The present study demonstrated the anti-inflammatory properties, analgesic effect and the capacity to inhibit free radicals of *Pterocarpus erinaceus* Poir. (Fabaceae) leaves. The anti-inflammatory and analgesic effects of extract support the traditional use of this plant in malaria and associated symptoms treatment. These results constitute some scientific data to promote the use of *P. erinaceus* in traditional medicine in Burkina Faso.

ACKNOWLEDGMENTS

This research was supported by traditional medicine department of IRSS/CNRST-Burkina Faso and department of Pathology and immunology, CMU, University of Geneva. The authors want to thank Pr Thiombiano Adjourma, at Botany section of University of Ouagadougou, to identify the plant.

REFERENCES

1. Owona, B.A., N.F. Njayou, S. Laufer, P.F. Moundipa and H.J. Schluesener, 2013. A fraction of stem bark extract of *Entada africana* suppresses lipopolysaccharide-induced inflammation in RAW 264.7 cells. J. Ethnopharmacol., 149: 162-168.
2. Shih, M.F., Y.D. Cheng, C.R. Shen and J.Y. Cherng, 2010. A molecular pharmacology study into the anti-inflammatory actions of *Euphorbia hirta* L. on the LPS-induced RAW 264.7 cells through selective iNOS protein inhibition. J. Nat. Med., 64: 330-335.
3. Brunet, L.R., 2001. Nitric oxide in parasitic infections. Int. Immunopharmacol., 1: 1457-1467.
4. Clark, I.A. and L. Schofield, 2000. Pathogenesis of malaria. Parasitol. Today, 16: 451-454.
5. Punsawad, C., S. Krudsood, Y. Maneerat, U. Chaisri and N. Tangpukdee *et al.*, 2012. Activation of nuclear factor kappa B in peripheral blood mononuclear cells from malaria patients. Malar J., Vol. 11. 10.1186/1475-2875-11-191
6. Karou, D., M.H. Dicko, S. Sanon, J. Simpore and S.A. Traore, 2003. Antimalarial activity of *Sida acuta* Burm. f. (Malvaceae) and *Pterocarpus erinaceus* Poir. (Fabaceae). J. Ethnopharmacol., 89: 291-294.
7. Nuhu, A.M., M.S. Mshelia and Y. Yakubu, 2000. Antimicrobial screening of the bark extract of *Pterocarpus erinaceus* tree. J. Chem. Soc. Nig., 25: 85-88.
8. Noufou, O., S.R. Wamtinga, T. Andre, B. Christine and L. Marius *et al.*, 2012. Pharmacological Properties and Related Constituents of Stem Bark of *Pterocarpus erinaceus* Poir. (Fabaceae). Asian Pac. J. Trop. Med., 5: 46-51.
9. Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109-110.
10. Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Exp. Biol. Med., 111: 544-547.
11. Sawadogo, W.R., R. Boly, M. Lompo, N. Some, C.E. Lamien, I.P. Guissou and O.G. Nacoulma, 2006. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. Int. J. Pharmacol., 2: 435-438.
12. Fotio, A.L., M.L. Olleros, D. Vesin, S. Tauzin and R. Bisig *et al.*, 2010. *In vitro* inhibition of lipopolysaccharide and *Mycobacterium bovis* bacillus Calmette Guerin-induced inflammatory cytokines and *in vivo* protection from D-galactosamine/Lps-mediated liver injury by the medicinal plant *Sclerocarya birrea*. Int. J. Immunopathol. Pharmacol., 23: 61-72.
13. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

14. Apak, R., K. Guclu, M. Ozyurek and S.E. Karademir, 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agric. Food Chem., 52: 7970-7981.
15. Peters, W. and B.L. Robinson, 1992. The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials. Ann. Trop. Med. Parasitol., 86: 455-465.
16. Hodge, H.C. and J.H. Sterner, 1943. Determination of substances acute toxicity by LD_{50}. Am. Ind. Hyg. Assoc., 10: 93-99.
17. Jia, N., Y. Li, Y. Wu, M. Xi and G. Hur *et al*., 2012. Comparison of the anti-inflammatory and analgesic effects of *Gentiana macrophylla* Pall. and *Gentiana straminea* Maxim. and identification of their active constituents. J. Ethnopharmacol., 144: 638-645.
18. Mothana, R.A.A., M.S. Al-Said, A.J. Al-Rehaily, T.M. Thabet, N.A. Awad, M. Lalk and U. Lindequist, 2012. Anti-inflammatory, antinociceptive, antipyretic and antioxidant activities and phenolic constituents from *Loranthus regularis* Steud. ex Sprague. Food Chem., 130: 344-349.
19. Reanmongkol, W., T. Noppapan and S. Subhadhirasakul, 2009. Antinociceptive, antipyretic and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. J. Nat. Med., 63: 290-296.
20. Sawadogo, W.R., M. Lompo, N. Some, I.P. Guissou and O.G. Nacoulma-Ouedraogo, 2011. Anti-inflammatory, analgesic and antipyretic effects of *Lepidagathis anobrya* nees (Acanthaceae). Afr. J. Tradit. Complement. Altern. Med., 8: 420-424.
21. De Oliveira, C.M., F.R. Nonato, F.O. de Lima, R.D. Couto and J.P. David *et al*., 2011. Antinociceptive properties of bergenin. J. Nat. Prod., 74: 2062-2068.
22. Kohno, S., T. Murata, A. Sugiura, C. Ito, M. Iranshahi, K. Hikita and N. Kaneda, 2011. Methyl galbanate, a novel inhibitor of nitric oxide production in mouse macrophage RAW264.7 cells. J. Nat. Med., 65: 353-359.
23. Sheeba, M.S. and V.V. Asha, 2009. *Cardiospermum halicacabum* ethanol extract inhibits LPS induced COX-2, TNF-α and iNOS expression, which is mediated by NF-κB regulation, in RAW 264.7 cells. J. Ethnopharmacol., 124: 39-44.
24. Choudhari, A.S., P. Raina, M.M. Deshpande, A.G. Wali, A.A. Zanwar, S.L. Bodhankar and R. Kaul-Ghanekar, 2013. Evaluating the anti-inflammatory potential of *Tectaria cicutaria* L. rhizome extract *in vitro* as well as *in vivo*. J. Ethnopharmacol., 150: 215-222.
25. Xie, W., M. Li, N. Xu, Q. Lv, N. Huang, J. He and Y. Zhang, 2013. MiR-181a regulates inflammation responses in monocytes and macrophages. PloS One, Vol. 8. 10.1371/journal.pone.0058639.
26. Lima, R.B., L.F.R. e Silva, M.R. Melo, J.S. Costa, N.S. Picanco *et al*., 2015. *In vitro* and *in vivo* anti-malarial activity of plants from the Brazilian Amazon. Malaria J., Vol. 14. 10.1186/s12936-015-0999-2.
27. Mustofa, E.N. Sholikhah and S. Wahyuono, 2007. *In vitro* and *in vivo* anti-plasmodial activity and cytotoxicity of extracts of *Phyllanthus niruri* L. herbs traditionally used to treat malaria in Indonesia. Southeast Asian J. Trop. Med. Public Health, 38: 609-615.
28. Kassa, F.A., M.T. Shio, M.J. Bellemare, B. Faye, M. Ndao and M. Olivier, 2011. New inflammation-related biomarkers during malaria infection. PLoS One, Vol. 6. 10.1371/journal.pone.0026495.