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Research Article

Anti-Inflammatory Activity of Extracts from *Parkia biglobosa* (Jacq.) R.Br. Ex G.Don. (Fabaceae-Mimosoideae) Trunk Bark

¹⁻³Noufou Ouedraogo, ^{1,2}Constantin Atchade, ^{1,2}Tata Kadiatou Traore, ¹Benjamin Ouedraogo, ^{1,2}Boly A.G. Laurent, ^{1,3}W.L.M. Esther Kabre, ¹Jules Yoda, ¹Felix B. Kini, ¹Marius Lompo, ²Moussa Ouedraogo and ¹Sylvin Ouedraogo

¹Department of Traditional Medicine-Pharmacopeia/Pharmacy, Research Institute of Health Sciences (IRSS/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso

²Laboratory of Drug Development, Doctoral School of Health, University Joseph KI ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso

³Laboratory of Biochemistry and Applied Chemistry, University Joseph KI ZERBO, 03 BP 848, Ouagadougou 03, Burkina Faso

Abstract

Background and Objective: *Parkia biglobosa* is used in the treatment of several inflammatory pathologies. The work aimed to study the toxicity, anti-inflammatory, analgesic and antioxidant effects of aqueous and hydroethanolic extracts from the bark of the trunk of *Parkia biglobosa*. **Materials and Methods:** A phytochemical study was carried out using the TLC method and the toxicity of the extracts was evaluated. The anti-inflammatory effect of extracts was evaluated using mice paw edema-induced by carrageenan injection and ear edema by croton oil application and these inhibitor effects against LOX and COX. The analgesic effect of the extracts was evaluated according to the writhing test. DPPH, FRAP, ABTS and LPO methods were used to evaluate antioxidant capacity. **Results:** Aqueous and hydroethanolic extracts are considered as slightly toxic substances. Extracts significantly reduced edema caused by carrageenan. At a dose of 500 µg/ear, the extracts used reduced (more than 50%) the edema caused by the application of croton oil to the inner surface of the ear of mice. Both extracts inhibited pro-inflammatory enzymes (LOX, COX 1 and 2) activities. Extracts exhibited antioxidant power using four methods (DPPH, FRAP, ABTS and LPO). **Conclusion:** These results are scientific data that could therefore justify the use of *Parkia biglobosa* trunk bark in the treatment of inflammatory diseases in traditional medicine.

Key words: *Parkia biglobosa*, anti-inflammatory, analgesic, antioxidant, lipoxygenase, cyclooxygenase, traditional medicine

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Corresponding Author: Noufou Ouedraogo, Department of Traditional Medicine-Pharmacopeia/Pharmacy, Research Institute of Health Sciences (IRSS/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso

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

INTRODUCTION

Inflammation is a biological defense process of the orchestrated organism, induced by stimuli that can be of physical, chemical, or biological origin¹. Inflammation is associated with fever, pain, tissue damage, edema, cells (monocytes, macrophages and lymphocytes) infiltration and the formation of granulomas.

The inflammatory process is characterized by the release of several inflammatory mediators, including cytokines (TNF α , IL1 β , IL6), prostaglandins, leukotrienes and Reactive Oxygen Species (ROS). Persistence excessive production of these mediators provokes chronic inflammation which constitutes a component of chronic diseases (cancer, diabetes, hypertension, obesity, etc.)².

There are various components of inflammatory reaction that can contribute to symptoms and associated tissue damage. Edema, leukocyte infiltration and the formation of granulomas represent such components of inflammation. Although it is a defense reaction of the body when it takes on large proportions, it can be detrimental².

To avoid the transformation of the acute phase of inflammation into a chronic phase, modern medicine uses two groups of drugs which are: Steroidal anti-inflammatory drugs (AIS) and non-steroidal anti-inflammatory drugs (NSAIDs). Modern medicine uses two groups of drugs which are steroidal anti-inflammatory drugs (AIS) and non-steroidal anti-inflammatory drugs (NSAIDs) to treat inflammation³. NSAIDs which are more used to treat inflammation, have inhibitor effects against cyclo-oxygenases 1 and 2 that are enzymes intervening prostaglandins synthesis⁴.

These drugs are responsible for undesirable effects which are the subject of therapeutic attention. These include metabolic disorders with AIS, gastric, renal and cardiovascular troubles with NSAIDs in long-term treatment⁴ and renal⁵ with NSAIDs. The advent of Coxib was hope for the reduction of gastrointestinal effects, but they have been shown to have cardiovascular toxicity⁶. In this context, the use of natural resources and more particularly medicinal plants to discover new effective and better-tolerated molecules is necessary.

Several studies in Africa have identified a large number of medicinal plants used in the management of inflammation and pathologies with inflammatory components⁷. All the parts of *Parkia biglobosa* (Jacq.) R.Br. Ex G.Don. is used for therapeutic purposes or nutritional belongs to the family of Fabaceae-Mimosoideae⁸. Commonly called Nere in West Africa, it is known to occur in a variety of agro-ecological zones ranging from tropical rainforest to arid zones⁹. It is a deciduous perennial that typically grows to a height of 7-20 m but can sometimes reach 30 m in exceptional conditions.

Parkia biglobosa is used in the treatment of several pathologies. It has been reported to have vascular properties⁹ and anti-diabetic, anti-hyperlipidemic¹⁰, antibacterial¹¹.

The aim therefore of this study is to carry out a phytochemical screening, to assess the acute toxicity, the anti-edematous, analgesic and antioxidant properties of bark extracts from the trunk of *Parkia biglobosa* (Jacq.) and then inhibitor effect of extracts on cyclo-oxygenase and lipoxygenase.

MATERIALS AND METHODS

Study area: This research project was conducted from July, 2018-August, 2019 in the Traditional medicine department of the Research Institute of Health Sciences (Ouagadougou, Burkina Faso).

Plant material: The trunk bark of *Parkia biglobosa* was collected in July, 2018 in Gonse, about fifteen kilometers from Ouagadougou. The plant was identified by Dr. GANABA Souleymane, Head of the Ecosystem Management and Monitoring program of the Environment and Forests where a herbarium was deposited under the identification number 8749. The bark was dried, at room temperature (20-25°C), in an airy room protected from the sun and dust, then reduced to powder.

Animal: The animals were used under the Directive 86/609/EEC and compliance with animal welfare assurance guidelines for foreign institutions (OLAW A5926-01). The NMRI mice (8-12 weeks) were used with average weight was 21 ± 5 g. These animals were provided by the lab of animal production of the Research Institute of Health Sciences. The temperature of the pet store was between 20 and 25°C with a humidity rate of 75% and a photoperiod of 12-24 hrs.

Chemicals: All solvents and reagents used were of analytical grade. COX-1 and human COX-2, Screening Kit (Item No. 560131) and sPLA2 (Item No. 765001) from Cayman Chemical Co. (MI, USA).

Extractions: Aqueous and hydroethanolic extracts: A sample test of 100 g of powder of *Parkia biglobosa* was dispersed in 1000 mL of distilled water. The whole is macerated for 15 min then brought to the boil for 30 min. The same process was carried out, but this time 80% ethanol was used to obtain the hydroethanolic extract.

Phytochemical study

Phytochemical screening: Phytochemical screening was to characterize the phytochemical groups present in the extracts using TLC (60 F250, 20×20 glass support, Merck-Silica gel). Several specific reagents were used to reveal these groups of compounds, sulfuric vanillin reagent for triterpenes/steroids, sulfuric anisaldehyde for saponins, Neu's reagent (5% ethanolic solution of polyethylene glycol) for flavonoids and trichloro ferric (FeCl₃) reagent.

Determination of total flavonoids content: The dosage of flavonoids was carried out according to the method of Kumaran¹², adapted by Abdel-Hameed¹³ using aluminum trichloride (2%). The number of flavonoids in the plant extract was determined in mg-equivalent quercetin (EQ) per gram dry matter (MS) (mg EQ/100 mg extract).

Determination of total phenolic content: The total phenolic compounds were assayed according to the Singleton *et al.*¹⁴, method using Folin Ciocalteu (FCR) reagent. Total phenol content was expressed in mg of tannic acid equivalents (mg ETA/100 mg extract).

Acute general toxicity: Acute toxicity was conducted using the OECD line 423 "dose adjustment" method¹⁵.

Anti-inflammatory activity *in vivo*

Carrageenan-induced paw edema test: The anti-inflammatory activity was carried out according to the method described by Winter *et al.*¹⁶ slightly modified by Ouedraogo *et al.*⁷. Inflammation is induced by the injection of carrageenin at the level of the plantar arch of the paw right of the rat. The edema caused by this carrageenin was translated into volume and measured by the Plethysmometer (UGO BASILE 7141, Italy).

Croton oil-induced ear edema test: The anti-edematous test with croton oil was carried out according to the method of Marius *et al.*¹⁷. Inflammation was induced on the internal face of the pavilion of the right ear of the mouse, by application of 5 µL of croton oil dissolved in DMSO (for extracts and reference substance).

Analgesic activity: Acetic acid-induced writhing test: The analgesic effect of the extracts was evaluated according to the number of abdominal contortions induced by the intraperitoneal injection of acetic acid (0.6%) according to the method described by Ouedraogo *et al.*⁷.

Anti-inflammatory activity *in vitro*

Inhibition lipoxygenase test: The spectrophotometric method developed by Malterud and Rydland¹⁸ was used to assess the inhibitory activity of the extracts on lipoxygenase.

Inhibition cyclooxygenase's 1 et 2 (COX-1 et COX-2) test: The ability of the extracts to inhibit COX-1 (ovine) and COX-2 (human recombinant) was determined using kits (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Antioxidant activity

Radical DPPH (2,2-diphényl-1-picrylhydrazyl) scavenging test: The capacity of the extracts to reduce free radicals of DPPH was determined by the method of Kim *et al.*¹⁹.

ABTS (2, 2'-azino bis-(3 éthylbenzothiazoline 6-sulfonique)

test: The methodology was followed as described by Art *et al.*²⁰.

Ferric reducing antioxidant power (FRAP): The reducing power of the samples was evaluated according to the spectrophotometric method described by Hinneburg *et al.*²¹.

Lipid peroxidation inhibition (LPO): The inhibitory activity of rat liver lipid peroxidation was determined using 2-thiobarbituric acid. FeCl₂-H₂O₂ was used to induce peroxidation of the liver homogenate according to the method of Ohkawa *et al.*²² modified by Sombié *et al.*²³

Statistical analysis: The analysis of the results of the *in vivo* tests was carried out based on the statistical processing using the software Graph Prism version 5. One-way ANOVA, followed by Dunnett's tests were used as statistical processing. The differences were considered significant if p (p-value) is less than 0.05 compared to the control or the reference.

RESULTS

Phytochemical screening: Phytochemical screening by thin-layer chromatography of the aqueous and hydroethanolic extracts of the bark of the trunk of *Parkia biglobosa* Jacq made it possible to highlight the presence of the following secondary metabolites (Table 1).

Flavonoids and total phenolics contents: The data in Table 2 presents the results of total flavonoids and phenolics content. These results are expressed in quercetin

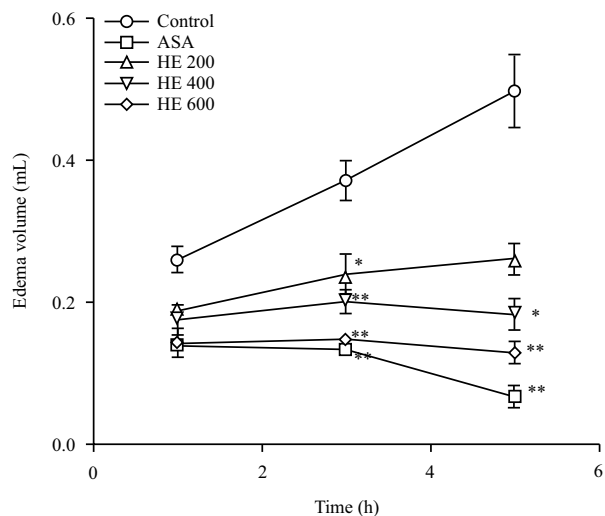


Fig. 1: Effect of Acetyl Salicylic Acid (ASA) and Hydroethanolic Extract (HE)

**p<0.01 vs. control, n = 7

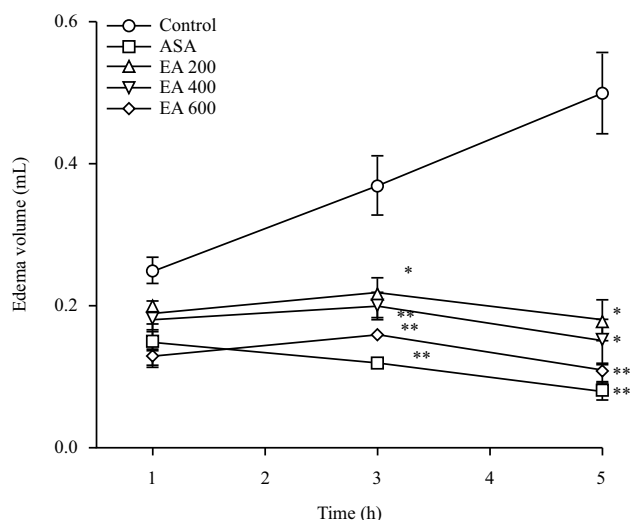


Fig. 2: Effect of Acetyl Salicylic Acid (ASA) and aqueous extract (EA)

**p<0.01 vs. control, n = 7

Table 1: Secondary metabolites sought and their presence in the extracts studied

Secondary metabolites	Extracts	
	Aqueous	Hydroethanolic
Triterpenes et Stérols	+	+
Saponosides	+	+
Flavonoids	+	+
Tannins	+	+

+: Present, -: Absent

equivalent/100 g of dry weight (dw) of plant material (QE/100 g dw) for flavonoids and Tannic Acid Equivalent for total phenolics/100 g of dry weight (TAE/100 g dw).

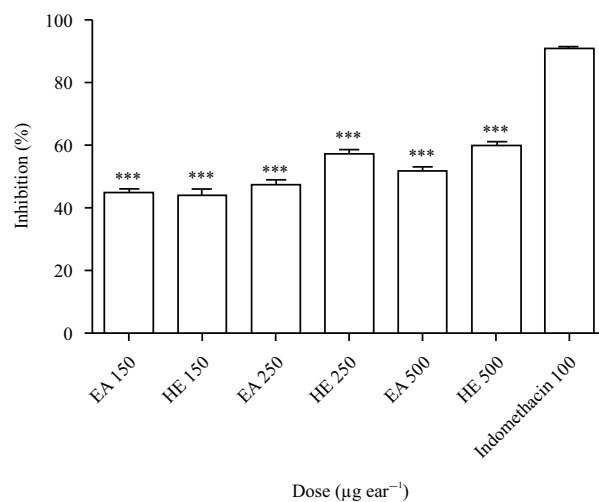


Fig. 3: Percent inhibition of edema induced by croton oil by hydroethanolic extract (HE), aqueous extract (EA) and indomethacin

***p<0.001 vs. indomethacin

The hydroethanolic extract has a higher content of flavonoids and phenolic compounds than the aqueous extract.

Acute toxicity: No signs of toxicity and no deaths were recorded after the administration of the 2000 mg kg⁻¹ b.wt. doses of the extracts. All animals survived after 14 days of observation. According to the Global Classification and Harmonization System (GHS), the LD50 of the extracts used is greater than 5000 mg kg⁻¹.

Anti-inflammatory activity *in vivo*

Paw anti-edema effect: The anti-edema effect of the aqueous and hydroethanolic extracts was evaluated at the doses of 200, 400 and 600 mg kg⁻¹. The edema volumes evolution are shown in Fig. 1-2.

The plots in Fig. 1-2 show the effect of both extracts on the oedema of the mouse paw. It should be noted that from the first hour after the administration of carrageenan, there is a decrease in the volume of oedema compared to the distant white control which received the distilled water. This inhibition is more accentuated from the third hour and better at 5 hrs when we had a significant drop in the volume of oedema.

Local anti-edema effect: The aqueous, hydroethanolic and indomethacin extracts had a local anti-inflammatory effect. The results are reported in Fig. 3.

Table 2: Content of extracts in flavonoids and total phenolics

Concentration of extracts	Total phenolic content (mg TAE/100 mg extract)	Flavonoid content (mg QE/100 mg extract)
Aqueous extract	486.80±25.01	62.55±1.18
Hydroethanolic extract	515.02±21.2	74.50±1.25

Table 3: Inhibitor effect of hydroethanolic and aqueous extracts on pro-inflammatory enzymes

Enzyme	LOX IC ₅₀ (µg mL ⁻¹)	COX 1 (% inhibition at 100 µg mL ⁻¹)	COX 2 (% inhibition at 100 µg mL ⁻¹)	Rio Cox 1/Cox 2
Hydroethanolic extract	4.98±0.31	35.79±0.55	51.18±2.42	0.70
Aqueous extract	10.8±0.17	41.05±2.74	51.69±2.68	0.79
Zileuton	3.11±0.45	Nd	Nd	nd
Indomethacin	Nd	44.93±1.15	53.94±2.05	0.83

LOX: Lipoxigenase, COX: Cyclooxygenase, Rio: Ratio

Table 4: Antioxidant effect of extracts from the different methods used

	DPPH IC ₅₀ (µg mL ⁻¹)	ABTS IC ₅₀ (µg mL ⁻¹)	FRAP TEAC (mol EAA g ⁻¹)	LPO Inhibition (%) (at 100 µg mL ⁻¹)
Hydroethanolic extract	6.21±0.001	2.75±0.0018	1340.1±0.69	51.46±1.98
Aqueous extract	6.40±0.0003	4.89±0.0007	1312.6±1.51	49.18±1.44
Trolox	4.37±0.0005	4.37±0.0005		75.82±0.9

DPPH: 2,2-diphényl-1-picrylhydrazyl, ABTS: 2, 2'-azino bis-(3 éthylbenzothiazoline 6-sulfonique, FRAP: Ferric reducing antioxidant power, LPO: Lipid peroxidation inhibition

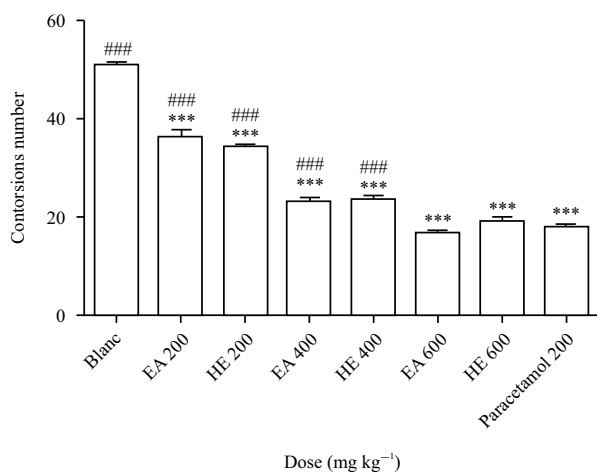


Fig. 4: Analgesic effect of aqueous (EA) and hydroethanolic decoctions (HE) of the bark of the trunk of *P. biglobosa* and paracetamol

***p<0.001 vs. white, ###p<0.001 vs. paracetamol

The extracts produced a dose-dependent edema reduction in mice. The edema inhibition was 44.81, 47.25 and 51.84% for the aqueous extract and 44.17, 57.29 and 60.05% for the hydroethanolic extract respectively at doses of 150, 250 and 500 µg/ear and indomethacin caused a significant inhibition of 90.61% at the dose of 100 µg/ear.

Analgesic effect: The results of the analgesic effect of the aqueous and hydroethanolic extracts are illustrated in Fig. 4.

Treatment of mice with extracts at doses of 200, 400 and 600 mg kg⁻¹ reduced contorsions induced by acetic acid in dose-dependent. However, there is

not a significant difference between the percentage of inhibition of the aqueous extract at the dose of 600 mg kg⁻¹ and paracetamol reference substance at the dose of 200 mg kg⁻¹.

Anti-inflammatory activity *in vitro*

Enzyme inhibitory effect: The data of Table 3 shows the inhibitor capacity of hydroethanolic and aqueous extracts on LOX, COX 1 and COX 2. The hydroethanolic extract gave a better inhibition on LOX (IC₅₀ = 4.98 µg mL⁻¹) but less than zileuton (reference substance) (IC₅₀ = 3.11 µg mL⁻¹). The inhibitory effect on COX 1 and COX 2 of the extracts was evaluated at a single concentration (100 µg mL⁻¹). Both extracts inhibited more COX 2 than COX 1.

Antioxidant effect: The hydroethanolic extract showed the highest capacity to scavenge ABTS and DPPH radicals (Table 4). Its action is better than Trolox using the ABTS method. Both extracts exhibited similar antioxidant activity with FRAP and LPO methods.

DISCUSSION

The plant kingdom represents a huge reservoir of biologically active compounds with various chemical structures and protective/preventive properties of (phytochemical) diseases. These phytochemicals, often secondary metabolites found in smaller amounts in plants, include alkaloids, steroids, flavonoids, terpenoids and tannins²⁴.

The phytochemical screening on TLC highlighted the presence of sterols, triterpenes, flavonoids, tannins, saponins in the extracts. These results are similar to those obtained literature. Phenolic compounds are very common in the plant kingdom. The *Parkia biglobosa* extracts contain significant amounts (Table 2). Tannins and flavonoids are the majority polyphenols present in the bark of the trunks of *P. biglobosa*²⁴.

The aqueous and hydroethanolic extracts of the bark of the trunk of *Parkia biglobosa* (jacq) Benth administered orally at a dose of 2000 mg kg⁻¹ did not cause any mortality throughout the study. The LD50 of the extracts used would therefore be greater than 5000 mg kg⁻¹ the extracts used are considered to be slightly toxic by the oral route in mice, according to the Globally Harmonized Classification System of the guideline 423 OECD and can be classified in category 5. Previous work had the same results with the aqueous extract of the trunk bark of *P. biglobosa*²⁵.

Oral administration of aqueous and hydroethanolic extracts from the barks of *Parkia biglobosa* (jacq) Benth is effective in reducing inflammatory edema induced by carrageenan in a dose-dependent manner. However, this anti-inflammatory effect was weak in the initial phase (1 hr) of edema but important in the late phase (5 hrs). Carrageenan-induced mouse paw edema involves many mediators that induce the inflammatory response in two different phases²⁶. An initial phase, which lasts approximately 1 hr 30 min after the injection of the carrageenan agent attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability. A late phase, which is the result of the overproduction of prostaglandins in the tissues, mediated by cyclo-oxygenase (COX) and which can continue beyond 5 hrs after injection of the carrageenan⁶. The important inhibition of edema was observed at the 5th hour after carrageenan injection. This suggests that the inhibitory action of the extracts would be exerted more on the action of prostaglandins or cyclooxygenases (COX 1 and COX 2) responsible for the biosynthesis of prostaglandins. Carrageenan-induced edema is sensitive to cyclo-oxygenase (COX) inhibitors and lipoxygenase inhibitors²⁷. Several studies reported the anti-inflammatory properties of phenolic compounds (flavonoids, tannins) and triterpenes through their inhibitor actions against prostaglandins release and natural factor-kappa B (NF-κB) activation^{28,29}.

The evaluation of the local anti-edematous effect of the extracts was determined by the induction of the edema by the application of croton oil on the internal surface of the ear of the mouse. Croton oil application induces the release of histamine and serotonin which are responsible for vasodilation and infiltration of leukocytes and cytokines production (I.L. 1β, IL6, TNFα)¹⁸. TPA (12-o-

tetradecanoylphorbol-13-acetate) major component of croton oil activates protein kinase C (PKC) with the stimulation of phospholipase A2 leading to the release of arachidonic acid and the biosynthesis of prostaglandins and leukotrienes (LT4) as well as the release of TNF α and nuclear factor kappa B (NF-κB)²⁹. The aqueous and hydroethanolic extracts from the bark of the trunk significantly inhibited edema induced by croton oil. This inhibition could be due to the blocking of the release of prostaglandins, leukotrienes (LT4) as well as cytokine production (I.L. 1β, IL6, TNFα) and the nuclear factor kappa B (NF-κB) activation. Triterpene and sterol presence in extracts were responsible for topical anti-inflammatory properties¹⁷.

Writhing induced by acetic acid injection on an animal is a method used to evaluate the peripheral analgesic effect of the pharmacological substance. The acetic acid injection causes the release of chemical mediators such as histamine, substance P, kinins such as bradykinin, serotonin, prostaglandins, as a result of COX-2 stimulation, which is responsible for abdominal contortions³⁰. Aqueous and hydroethanolic extracts of the barks of *Parkia biglobosa* significantly reduced the number of abdominal contortions, compared to the control batch. Indeed, the mechanism of the analgesic effect of these extracts may be the reduction of the release of histamine, serotonin and prostaglandins, or the direct blocking of the receptors of these endogenous mediators. Moreover, the synergistic action of tannins, saponins, flavonoids and triterpenes/steroids could be responsible for the analgesic and anti-inflammatory properties of the extracts³¹.

LOX and COX (1 and 2) respectively synthesize leukotrienes and prostaglandins, which play an important role in inflammation processes. The best inhibitor activity against LOX and COX activity was observed with the hydroethanolic extract compared to the aqueous extract. The difference in effectiveness could be due to chemical composition. The high amount of total phenolics in the hydroethanolic extract could explain this strong inhibition. Holsapple and Yim³² reported that injection of carrageenan in animals induces leukotriene biosynthesis. This suggests that the anti-edematous effect of decocts may be associated with inhibition of LOX and COX.

The importance of the dual inhibition of LOX and COX lies in the effective reduction of chronic inflammatory components. Gastrointestinal integrity remains protected with the double inhibition of enzymes, as is clinically demonstrated in other studies³³. The incidence of cardiovascular disorders in the selective inhibition of COX-2 is also reduced in such situations of double inhibition³⁴. Reactive Oxygen Species (ROS) are involved in the inflammation pathophysiology. ROS induce cytokine release (TNF α, IL, 1β, IL 6) and activation of pro-inflammatory enzymes

(cyclooxygenases, lipoxygenase, monoxide of inducible nitrogen synthase) involved in the process inflammatory. Antioxidant substances can inhibit ROS. Thus, four (04) methods (DPPH, ABTS, FRAP and LPO) were used to evaluate the antioxidant power of the hydroethanolic and aqueous extract of the bark of the trunk of *Parkia biglobosa*.

There is a certain complementarity between these different methods of assessing the anti-free radical activity of plants (DPPH, ABTS, FRAP LPO), without one of them being able to be stated as a reference³⁵. The hydroethanolic extract had a better antioxidant capacity than the aqueous extract. This could be explained by the high concentration of our extracts in phenolic compounds. Indeed, phenolic compounds are known for antioxidant capacity. ROS oxidizes unsaturated lipids in the cell membrane leading to the formation of lipid peroxides, which are cytotoxic and involved in the process inflammatory. Injection of carrageenan in rats has caused the activation of lipoxygenase and the formation of lipid peroxides³⁶. This suggests that the inhibition of lipoxygenase and lipid peroxidation by the aqueous extract of *P. biglobosa* could contribute to the anti-edema effect of the extract. It shows that the hydroethanolic extract, as well as the aqueous extract, have a real power of chelating metal ions, including ferric ion (Fe^{3+}). The results of the four methods used show that the different extracts have an antioxidant activity linked to the content of antioxidant compounds, which could act synergistically.

CONCLUSION

In conclusion, the aqueous and hydroethanolic extracts of the trunk bark of *Parkia biglobosa* contain tannins, flavonoids, saponosides and sterols and triterpenes. Both extracts possess anti-inflammatory, analgesic and antioxidant properties and are no toxic substances. The present study brings scientific evidence that could confirm the traditional uses of *Parkia biglobosa* against inflammatory pathologies.

SIGNIFICANCE STATEMENT

This study discovers the proprieties anti-inflammatory, analgesic and antioxidant of extracts from *P. biglobosa* trunk bark. This study showed that extracts inhibited lipoxygenase (LOX) and cyclooxygenases (COX 1 and 2) that are key pro-inflammatory enzymes in the inflammation process. Extracts inhibited more COX 2 than COX 1. Inhibitor action of extracts on LOX and COX is a benefit in the treatment of inflammation. This study shows that extracts

from *P. biglobosa* trunk bark can be used to treat inflammatory diseases safely. These study data are prerequisites to developing an anti-inflammatory herbal drug. This study will help the researcher to uncover the possible mechanism of action of the inflammatory effect through the inhibition of both LOX and COX.

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