Comparative study of extracts of *Parkia biglobosa* (JACQ.) R.BR. EX G. Don (FABACEAE) trunk barks : A plant traditionally used against hemorrhoidal diseases in Burkina Faso

Mathieu NITIEMA[1*], Elisabeth YABRE[2], Wendkouni Leila Marie Esther BELEM-KABRE[1], Souleymane COMPAORE[1], Tata Kadiatou TRAORE[1], Boladé Constantin ATCHADE[1,2], Windingoudi Rimwagna Christian OUEDRAOGO[1], René Dofini MAGNINI[1], Salfo OUEDRAOGO[1], Lazare BELEMNABA[1], Estelle Noëla Hoho YOUL [2], Noufou OUEDRAOGO[1] and Sylvin OUEDRAOGO[1]

Abstract

Parkia biglobosa (Jacq.) R.Br. ex G. Don (Fabaceae) is a West African medicinal plant used for many therapeutic purposes such as anti-inflammatory, anti-hemorrhoids, antidiarrhea, etc. The present study focused on the standardization of the raw material for the formulation of an anti-hemorrhoidal phytomedicine based on extracts of *Parkia biglobosa* trunk bark harvested in three localities of Burkina Faso in the dry and rainy seasons. A microbiological quality and physicochemical study were conducted respectively on powder, and extracts. In addition to these parameters, secondary metabolites were determined on extracts from the three localities. For antioxidant potential, tests using DPPH-radical reduction, $ABTS^{+}$ cation-reducing power, and lipoxygenase inhibition were used to determine which locality provided the plant with the best antioxidant and anti-inflammatory activity over both seasons. The study revealed that the microbiological quality of the powders was acceptable. A residual moisture content was less than 10%, and the extraction yield was between 10.47% and 20.38%. The determination of phenolics compounds gave a high content of total phenolics (minimum = 199.39±4.63 mg/g and maximum = 292.46±0.35 mg/g) with a predominance of tannins (minimum =139.32±0.26 mg/g and maximum = 218.24±0.15 mg/g), especially in Yako locality. Flavonoids were at low levels. ABTS, DPPH, and LOX inhibition tests confirmed the antioxidant activity.

[1] Laboratoire de Recherche-Développement des Phytomédicaments et Médicaments (LR-D/PM), Institut de Recherche en Sciences de la Santé (IRSS), CNRST, Ouagadougou, Burkina Faso, 03 BP 7047 Ouagadougou 03, Burkina Faso.

[2] Laboratoire du Développement du Médicament (LADME), École Doctorale Sciences et Santé, Université Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.

***Corresponding author:** E-mail: nitmat01@yahoo.fr ; Phone number: +22670996924

These data could therefore constitute a scientific basis for the choice of the raw material in the formulation of a phytomedicine based on the decocted of *Parkia biglobosa*, in particular the decoction extract plant from Yako in the dry season.

Keywords: *Parkia biglobosa* - Antioxidant - Harvest season - Extraction

Introduction

The African population relies on traditional medicine for primary healthcare (1). This recourse is mainly largely due to the inaccessibility of modern pharmaceutical products and the weakness of the economic resources of the populations, especially in developing countries like Burkina Faso.

Many plants of the African Pharmacopeia, including *Parkia biglobosa* (Jacq.) R. Br. ex G. Don (Fabaceae), have therapeutic properties in treating heamorrhoidical disease, a prevalent disease in developing countries (1). Heamorrhoidical disease represented 38.5% in Africa, and 60% in Burkina Faso (2). Besides, the different parts of *Parkia biglobosa*, are used not only in nutrition but also as constituents of antihypertensive, antiparasitic, antibacterial, and anti-hemorrhoidal recipes (3-6). Studies on *Parkia biglobosa* trunk bark have shown that it contains several secondary metabolites, including tannins, flavonoids, coumarins, and saponosides (4). These metabolites vary depending on several factors, such as locality, climate, soil composition, harvesting and drying conditions, and the extraction process (7, 8). Also, one of the main safety risks of herbal medicines is contamination by microorganisms of various kinds that can adhere to roots, stems, leaves, flowers, and seeds. Microorganisms are often introduced during harvesting, transportation, air-drying, storage, and manufacturing (9). In order to produce effective and safe anti-hemorrhoidal phytomedicines, a study of the choice of raw materials was initiated. In this sense, the trunk bark of *Parkia biglobosa* was collected in different localities (Baré, Nobéré, and Yako) of Burkina Faso in relation to the 3 climate zones for a comparative study of their microbiological quality, antioxidant properties, and anti-inflammatory activity. This work aimed to determine the extraction conditions, and the most favorable harvesting period to standardize the raw material.

I. Material and methods

1.1. Material

1.1.1. Plant material

Plant material consisted of *Parkia biglobosa* (Jacq.) R. Br. ex G. Don (Fabaceae) trunk bark collected with GPS coordinates in Baré (X=0378375; Y=1223767), Nobéré (X=0687209; Y=1295737), and Yako (X=0564356; Y=1430667) in May 2020 and August 2020. A reference specimen No. 8757 was identified by the botanist Dr GANABA Souleymane and deposited in the Herbier National du Burkina Faso of the Centre National de la Recherche Scientifique et Technologique (CNRST). The trunk bark was dried on racks in a ventilated room protected from sunlight and dust. After drying, the trunk bark was made powdery using a grinder and stored in freezer bags. Figure 1 shows a map of Burkina Faso with the different sites where plant material was collected.

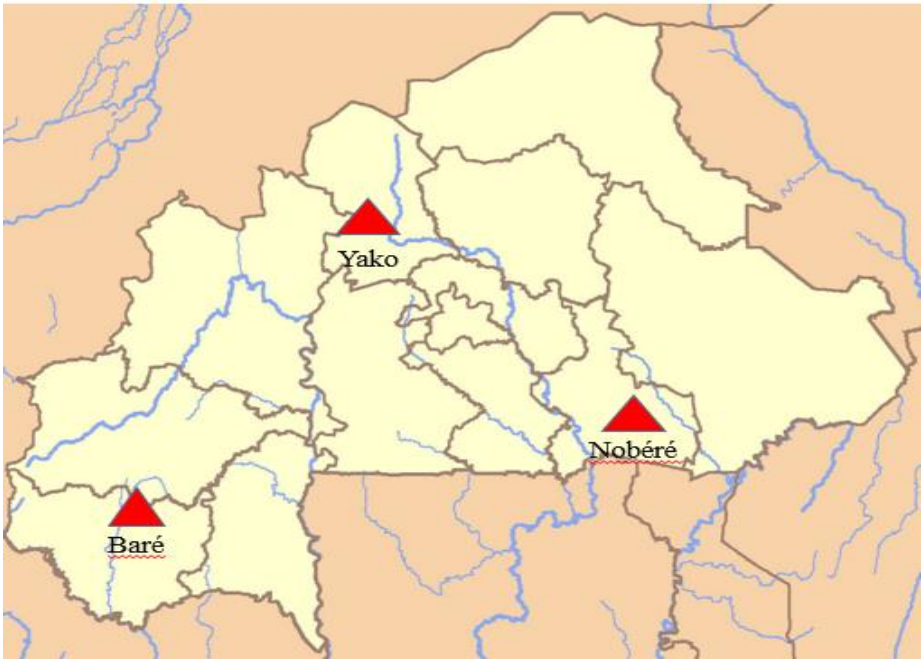

Figure 1: Map of Burkina Faso with the different collection sites of *Parkia Boglobosa* trunk bark

1.1.2. Chemicals and Reagents

Folin Ciocalteu reagent (FCR), Sodium carbonate, Polyvinyle polypyrrolidone (PVPP), Aluminium trichloride, 2,2-diphenyl-β-picrylhydrazyl (DPPH), 2,2'-azino bis-[3-éthylbenzothiazoline-6-sulfonique] (ABTS), potassium persulfate, boric acid, sodium tetraborate, linoleic acid, lipoxidase (type I-B), tannic acid, quercetin, trolox, zileuton were purchased from Sigma-Aldrich. Absolute ethanol and methanol were also used. All chemicals and reagents were of analytical grade.

1.2. Methods

1.2.1. Analysis of the microbiological quality of *Parkia biglobosa* trunk bark powders

The number of microorganisms per mass unit was calculated for each flora studied based on the samples analyzed from each harvest site and compared to the normative reference of the microbiological criteria for herbal medicines in the European Pharmacopoeia (10).

1.2.1.1. Enumeration of the total aerobic mesophilic flora (FMAT)

A stock solution was prepared for each sample. Thus, 10 g of powder to be analyzed was dissolved in 90 mL sterile physiological water by homogenizing for 2 min. The resulting solution was made cascading dilutions down to 10^{-4} dilution. Standard agar or Plate Count Agar (PCA) was used for enumeration of total aerobic mesophilic flora. The spread plating technique was used. For this purpose, 0.1 mL of the different dilutions were aseptically collected in an agar medium and then spread over the entire surface using a sterile Pasteur pipette. Incubation was done at 30 °C for 72±3 h. After the 72 h period of incubation, all colonies were counted on two successive dilution plates (less than 300 colonies and more than 10 colonies on one plate). The standard ISO 4833: 2003 was used for the enumeration (11).

1.2.1.2. Research and enumeration of yeasts and molds

Sabouraud agar with Chloramphenicol added was used for the detection of yeasts and molds according to the standard AFNOR ISO 7954: 1988. Chloramphenicol is an antibiotic that inhibits the growth of bacteria in the sample. A quantity (0.1 mL) of the previously prepared decimal dilutions were inoculated into sterile Petri dishes. The plates were incubated at 30 °C for 48 to 72 h under aerobic conditions. On the agar,

colonies in the form of small circular sizes of milky white color were counted.

1.2.1.3. Research and enumeration of total and thermotolerant coliforms

Total and thermotolerant coliforms were enumerated on EMB medium. The plating was done in double layers. The plates were incubated for 24 h at 37 °C for total coliforms and 44 °C for thermotolerant coliforms. The coliforms showed purplish colonies with a diameter equal to or greater than 0.5 mm (ISO V08-017: 1980).

1.2.1.4. Research and enumeration of *Staphylococcus aureus*, and *Salmonella*

The surface plating technique was used. In each plate, 0.1 mL of the previously prepared decimal dilutions were plated and spread on the entire surface of Mannitol Salt Agar. Petri dishes were incubated aerobically at 37 °C for 24 to 48 h according to the standard ISO 6888-1: 1999. Colonies of *Staphylococcus aureus* appearing yellow surrounded by a yellow halo were counted on Mannitol Salt Agar (11). The SS medium was used for *Salmonella* research. *Salmonella* testing was performed in three successive steps: pre-enrichment, enrichment, and isolation. Buffered peptone water (BPW) broth was used for pre-enrichment. In 225 mL of sterile EPT broth contained in a vial were added 25 g powder sample. Incubation was done at 37 °C for 18 to 24 h in aerobic conditions. Then, to 9 mL of Rappaport Vassiliadis Soy (RVS) broth contained in sterile glass tubes, 0.1 mL of pre-enriched broth was added using a sterile pipette. The broths were then incubated in an oven at 37 °C for 18-24 h. SS agar was used for isolation of *Salmonella*. After solidification of the medium, 0.1 mL of the suspension was plated with a sterile syringe and streaked with a sterile Pasteur pipette. The seeded Petri dishes were incubated at 37 °C for a 24 h period of incubation. Suspect colonies will appear bluish with or without a black center.

The number N of microorganisms expressed in Colony Forming Units (CFU)/mL was calculated by the following formula (ISO 6579:2002).

$$N = \frac{\sum c}{(n_1 + 0{,}1n_2) \times v_i \times d}$$

N: Number of germs ; $\sum C$: sums of the colonies

Vi : µL volume of inoculum; nL : number of Petri dishes counted at 1st dilution
n2 : number of Petri dishes counted at 2nd dilution; d : dilution rate at first dilution retained

1.2.2. Macroscopic and organoleptic characteristics

Macroscopic characteristics such as color and texture were observed (6). The taste was appreciated by the gustatory sensation of tasting and the smell by sniffing powders.

1.2.3. Physicochemical analysis

Residual moisture content (RCM): the RCM of *Parkia biglobosa* trunk bark powder was determined by the thermogravimetric method, the principle of which is based on loss on drying at 103±2 °C for 3 h period (6).

Decoction: ten (10) of plant powder test sample was mixed in 100 mL of distilled water. The mixture was boiled under reflux for 30 min from the boiling point. After cooling, the extract was filtered through a fine mesh nylon cloth.

Infusion: ten (10) g plant powder was dispersed in 100 mL of distilled water previously brought to boil. After 30 min the mixture was filtered.

Extraction yield: both filtrate extracts (decoction and infusion) were centrifuged at 2000 rpm for 15 min. The supernatants were recovered and dried at 45 °C in the oven. The dry extracts obtained were collected in amber vials and stored in a refrigerator for further testing. The extraction yield was determined at the end of the extractions.

1.2.4. Determination of total phenolics, tannins and flavonoids

Determination of total phenolic extracts

Total phenolic compounds were determined using Folin Ciocalteu Reagent described by Singleton et al. (12). The reaction mixture consists of 1 mL of extract, 1 mL of FCR 2N and 3 mL of a 20% sodium carbonate solution. After 40 min incubation at room temperature, the absorbances were measured at 760 nm with a spectrophotometer (Agilent 8453 with UV-visible ChemStation software). A standard curve was drawn with tannic acid (1-5 µg/mL). The tests were performed in triplicate.

The total phenolic concentration of the extract is given by the formula:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

T_{PT}: total phenolic content of the extract expressed as tannic acid equivalent (TEA)/g; $C_{Tube:}$ concentration in mg EAT/mL in the assay tube; D: dilution factor; C_i: concentration in mg/mL in the stock solution.

Determination of tannins

Tannins content were determined according to the method of Singleton et al. (12) and adapted in our laboratory (13). An amount of PVPP required to complex the tannins, determined as previously described, was added to 2 mL of 1 mg/mL concentration extract. The mixture was vortexed, kept at 4 °C for 15 min and then centrifuged at 3000 g for 10 min. The supernatant contained phenolic compounds including tannins, the latter having been precipitated by PVPP. The assay was performed as for total phenolics. After 40 min, it was centrifuged and the absorbance of the supernatant was measured at 760 nm with an Agilent 8453 spectrophotometer with UV-visible ChemStation software. The tannins content was determined as the difference between the first value of total phenolics (which contained tannins) and the second value of total phenolics (in the absence of tannins).

Determination of flavonoids

Flavonoid content was performed according to the method of Kumaran et al. (14) adapted (13). Two mL of extract at a concentration of 1 mg/mL in methanol was mixed with 2 mL of 2% aluminum trichloride in methanol. After 40 min, the absorbance was measured at 415 nm using the spectrophotometer (Agilent 8453 with UV-visible ChemStation software). The absorbance of quercetin (0.10 mg/mL) used as a reference compound was measured under the same conditions. The quantity of flavonoids in the plant extract in Quercetin Equivalent (QE) was determined according to the following formula:

$$T_{Flav} = \frac{A \cdot m_0}{A_0 \cdot m}$$

T_{Flav}: flavonoid content of the extract expressed in mg EQ/mg dry extract; A: extract absorbance; A_0: quercetin absorbance; m: extract mass in mg; m_0: quercetin mass in mg.

1.2.5. Evaluation of the antioxidant activity of the extracts

DPPH Assay

The method of measuring the antioxidant power by 2,2-diphenyl-1-picrylhydrazyl (DPPH) is based on the ability of a compound to reduce the DPPH• radical (5, 13). A series of 10 successive dilutions of sample was carried out, starting from a stock concentration of 1 mg/mL. Twenty (20) mL of sample (extracts or Trolox as reference compound) were mixed with 200 mL of DPPH (4 mg / 100 mL methanol). The blank consisted of a mixture of DPPH and methanol. The tests were performed three times. The reaction was quantified by measuring the absorbance of the solution spectrophotometrically at 490 nm (Agilent 8453 with UV-visible ChemStation software) after a 30-min incubation in dark. The percentage of inhibition (IC_{50}) was calculated according to the formula: Inhibition % = [(A blank - A Sample) /A blank] x 100.

ABTS Assay

This test is based on the redox mechanism of ABTS (ammonium salt of 2,2'-azino bis-(3-ethylbenzothiazoline-6-sulfonic acid)) (5, 13). In the presence of the antioxidant agent, the radical thus formed is reduced to give the $ABTS^{\bullet+}$ cation, which causes the solution to discolor. Indeed, the ABTS cation radical was generated by mixing a solution of 19.2 mg of ABTS in 5 mL of distilled water to which was added a solution of 3.312 mg of potassium persulfate and a solution of ABTS at 3.84 mg/mL, all of which was kept in the dark and at room temperature at a time interval of 12 h to 16 h before use. For dilution, several concentrations of 1 mg/mL were used and this dilution was made up to 10 dilutions of the solution that was prepared of 1 mg of dry extract and 1 mL of solvent. Thus, for each extract evaluation 200 mL of the freshly prepared solution was added to 20 mL of extract. These mixtures were made in 96-well plates and the blanks for this assay consisted of a mixture of ABTS and ethanol. For assay reproducibility, three trials were performed for each concentration of test material. Finally, the reading was taken at 415 nm (Spectrophotometer Agilent 8453 with UV-visible ChemStation software) after 30 min in the dark for each run. The absorption inhibition curve versus the extract or Trolox concentration was drawn for the determination of the inhibitory concentration 50% (IC_{50}).

The percentage of inhibition was calculated according to the formula:

$$\%\text{Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample or standard. .

1.2.6. Anti-inflammatory activity *in vitro* by 15-Lipoxygenase inhibition test

The principle consists in inhibiting the activity of lipoxygenase to produce leukotrienes and lipoxin in the presence of an inhibiting substance. A spectrophotometric method was used to evaluate the inhibitory activity of the extracts on lipoxygenase (13, 15, 16). For this, 146.25 µL of lipoxygenase solution (820.51 U/mL) prepared in boric acid buffer (pH 9.0), 3.75 µL of sample/reference solution (at different concentration starting from a stock concentration of 8 mg/mL) were mixed and incubated for 3 min at room temperature. To initiate the reaction, 150 µL of linoleic acid were added and the absorbances was followed at 234 nm using spectrophotometer (Epoch Biotek Instruments, USA). All assays were performed in triplicate. Zileuton was used as a reference product. Lipoxygenase inhibitory activity expressed as percent inhibition was determined using the following formula: Percent inhibition = (E - S) /E×100.
E is the activity of the enzyme without inhibitor and S is its activity in the presence of the tested extract/reference. The IC_{50} was also determined as previously described.

1.2.7. Statistical Analysis

The results of the different tests were expressed as mean±SEM (standard deviation from the mean) for three independent trials. The observed differences between the variables in this study were evaluated using the analysis of variance (ANOVA) test using GraphPad Prism 7.01 software. The difference was considered statistically significant at the 5% significance level ($p < 0.05$).

II. Results

2.1. Microbiological analysis of *Parkia biglobosa* trunk bark powders

The results in Table II show that all samples were compliant regarding thermotolerant coliforms and total coliforms. The results of this study also indicate compliance with *S. aureus* and a total absence of *salmonella.*

Table II: Microbiological analysis of *Parkia biglobosa* trunk bark powders from 3 harvesting areas in two seasons

SAMPLES	Total aerobic mesophilic flora	Total coliforms	Thermotolerant coliforms	Yeasts and molds	*S. aureus*	*Salmonella* (25 g)
Baré R1	$5.1\ 10^3$	< 10	< 10	$2.5\ 10^2$	< 10	Compliant
Baré R2	$4.2\ 10^5$	< 10	< 10	$4.4\ 10^3$	< 10	Compliant
Nobéré R1	$1.3\ 10^6$	< 10	< 10	$7.6\ 10^4$	< 10	Compliant
Nobéré R2	$8.6\ 10^4$	< 10	< 10	$2.6\ 10^3$	< 10	Compliant
Yako R1	$8.5\ 10^5$	< 10	< 10	$2.2\ 10^2$	< 10	Compliant
Yako R2	$7.2\ 10^6$	< 10	< 10	$1.4\ 10^5$	< 10	Compliant
Required standards	**$\leq 10^5$**	**< 10**	**< 10**	**$\leq 10^5$**	**< 10**	**Absent**

2.2. Macroscopic and organoleptic characteristics

The raw plant powder, regardless of harvest localities and seasons, had the same characteristics: brown color, pungent smell, bitter and astringent taste and sandy texture.

2.3.Residual moisture content (RMC)

All residual moisture content were less than 10% (values ranged from 3.18 to 6.46%). The RCM of trunk bark powders from the first harvest (R1) from Baré, Nobéré and Yako (RCM< 5) were lower than those of the second harvest (R2), under the same treatment conditions (Table III).

Table III: Residual moisture content of *Parkia biglobosa* trunk bark powders, (n=3)

Residual moisture content (%)	Baré	Nobéré	Yako
Harvest 1 (dry season)	3.45±0,46	3.18±0,03	4.19±0,30
Harvest 2 (rainy season)	5.40±0,48	6.46±0,70	6.35±0,75

R1: 1st harvest, May 2020: dry season; R2:2nd harvest, August 2020: rainy season

2.4. Yield of extraction

Extraction yields of *Parkia biglobosa* trunk bark powders of are recorded in table IV. The yields of the first harvest were better with 20.38% in infusion for the locality of Yako and 18.62% for the locality of Baré.

Table IV: Yields of extracts of *Parkia biglobosa* trunk bark powders by decoction and infusion

Locality / Extract	Baré	Nobéré	Yako
Decoction R1	15.71	15.88	14.04
Decoction R2	10.47	15.66	14.83
Infusion R1	18.62	17.62	20.38
Infusion R2	10.92	14.58	13.66

R1: 1st harvest, May 2020: dry season; R2:2nd harvest, August 2020: rainy season

2.5. Total phenolics compounds, tannins and flavonoids content

Table V shows the contents of total phenolics, tannins and flavonoids in the extracts of *Parkia biglobosa*. In the decoction, the total phenolics compound contents were highest in Yako with a content of 292.46±0.23 mg/g in R1. The decoction extract had higher tannin contents of 218.24±5.39 mg/g in Yako in harvest 2.

Table V: Total phenolic, tannins and flavonoids contents of aqueous decocted and infused of *Parkia biglobosa* trunk bark powders according to harvests (n=3)

Harvesting sites	Harvest (R)	Total Phenolics (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)
		Decoction		
Baré	R1	256.06±5.95**	177.52±3.61**	6.89±0.05
	R2	290.30±1.33	207.76±5.39	7.25±0.05
Nobéré	R1	284.35±3.99	208.67±4.35	7.15±0.06
	R2	265.56±1.76**	200.29±0.64	7.50±0.05
Yako	R1	292.46±0.35	205.92±6.37	6.20±0.09
	R2	286.86±3.62	218.24±0.15	7.18±0.02
		Infusion		
Baré	R1	251.69±7.97***	177.52±3.61**	6.73±0.04
	R2	199.39±4.63***	118.46±0.38***	7.78±0.04
Nobéré	R1	271.80±3.99*	199.95±3.16	6.94±0.06
	R2	207.03±1.71***	139.32±0.26***	7.45±0.01
Yako	R1	268.03±11.83*	144.83±1.93***	7.17±0.01
	R2	269.98±3.99**	189.98±0.97*	7.27±0.11

Total Phenolics (mg/g): *p < 0.5 *vs* R1, Yako (decoction, highest content); Tannins (mg/g) : *p < 0.5 *vs* R1, Yako (same extract)

2.6. Antioxidant properties of *Parkia biglobosa* trunk bark extracts

Anti-radical activity by ABTS method

The IC_{50} of the extracts are presented in Figure 2. For the decoction, the antioxidant activity of the powdered trunk bark extracts harvested at Nobéré (harvest 2) had a higher activity (IC_{50} = 1.58 µg/mL) than the

trolox (4.85 µg/mL) but also than the other extracts. For infusion, the antioxidant activity of the extract from harvest 1 in Yako had a higher activity (IC_{50} = 2.6 µg/mL). The extracts from the different harvest sites had better activity compared to trolox except for the infused extract from harvest 2 in Yako which had similar activity to trolox ($p < 0.05$).

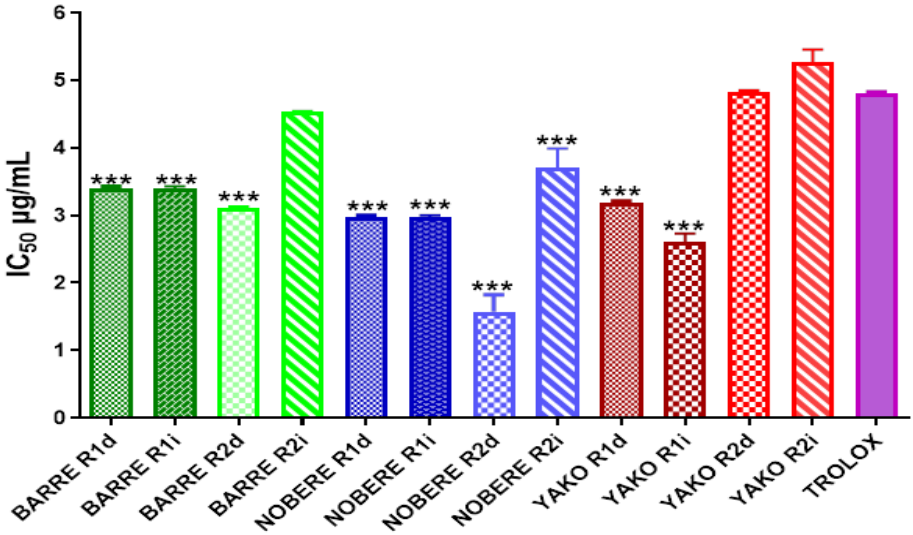

Figure 2: Free radical scavenging activity by ABTS method of decocted and infused as a function of sites and harvest period ***$p < 0.001$ *vs* Trolox (R1d = decocted from first harvest; R1i = infused from first harvest; R2d = decocted from second harvest; R2i = infused from second harvest)

2.7.Anti-radical activity by DPPH method

Figure 3 presents the results of the DPPH free radical scavenging test of the different extracts. The decocted from harvest 2 in Yako (IC_{50} = 2.65 µg/mL) and the infused from harvest 1 in Baré (IC_{50} = 2.88 µg/mL) showed the best antioxidant activities than the trolox (4.85 µg/mL). In contrast, infusions from Harvest 2 in Baré (10.53 µg/mL) and Harvest 1 in Yako (7.10 µg/mL) had lower antioxidant activity than trolox. The other extracts in the study showed statistically similar antioxidant activities to trolox.

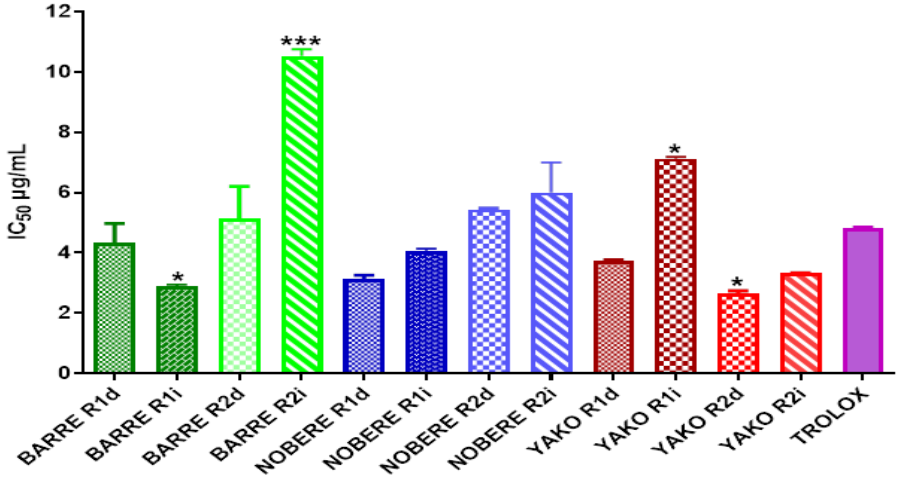

Figure 3: Anti-free radical activity by DPPH method of decocted and infused as a function of sites and harvest period *p < 0.05 *vs* Trolox (R1d = decocted from first harvest; R1i = infused from first harvest; R2d = decocted from second harvest; R2i = infused from second harvest)

2.8. Anti-inflammatory activity by lipoxygenase inhibition

The results of lipoxygenase inhibition of different extracts of *Parkia biglobosa* trunk bark are presented in figure IV. The Zileuton (9.76±0.25 µg/mL) used as a reference substance had a significantly higher activity than extracts from different harvest sites.

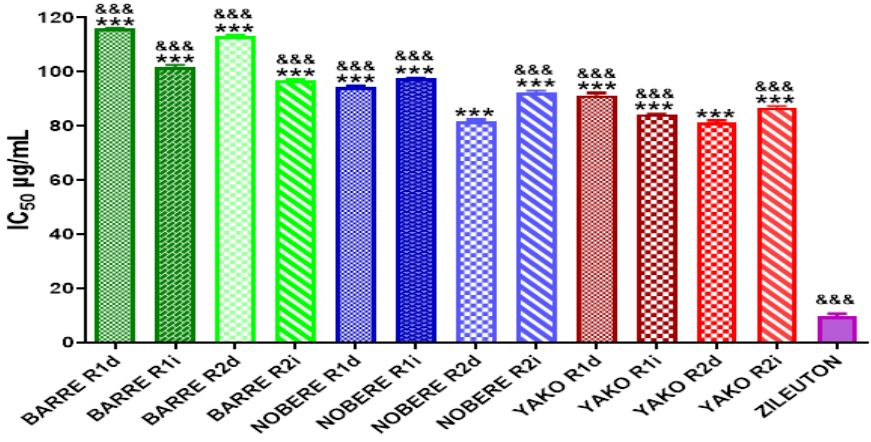

Figure 4: Lipoxygenase inhibition of decocted and infused as a function of site and harvest period ***p < 0.001 *vs* Zileuton; &&&p < 0.001 *vs* Yako R2d (R1d = decocted from first harvest; R1i = infused from first harvest; R2d = decocted from second harvest; R2i = infused from second harvest)

III. Discussion

The presence of total flora, total coliforms, thermotolerant coliforms, yeasts and molds, *Staphylococcus aureus* and *Salmonella* was sought in the powders of the trunk bark of *Parkia biglobosa*. Total flora provides information on preparation conditions and the effectiveness of treatment and preservation processes. According to European Pharmacopoeia standards, compliant samples had a total aerobic mesophilic flora rate of less than 5.10^5 CFU/mL being considered inappropriate (10). The results of our study showed that, a part from the Nobéré sample collected in May and the Yako one collected in August, the other 4 samples comply with the requirements. In accordance with WHO standards, the samples were compliant in terms of biological contamination, excepted the samples from the May harvest in Nobéré and the August harvest in Yako, which had high levels of mold and fungi (greater than 10^5 CFU/g) (9, 17, 18). These results suggest greater caution in the production of raw materials for the manufacture of phytomedicines. Thus, any contamination beyond the standards would cause rejection or treatment of the raw material with the consequence of risks of degradation of certain compounds and an increase in the raw material cost if the process had not foreseen it. In addition, for better sample quality, the residual moisture content of the plant powders was checked and was all below 10%. Lower levels were determined for samples harvested during the dry season than those harvested during the rainy season. These results are in line with other studies which found acceptable levels of contamination on powders harvested during the dry period (6). The presence of moisture, microorganisms, and enzymes are causes of degradation of plant material with negative consequences on the appearance of drugs, their organoleptic characteristics, and their therapeutic properties (6, 19). For the realization of the tests, extractions were carried out in the form of decoction and infusion from the vegetable powders. The yields of these extractions varied between 10.47 and 20.38. Previous work had found a yield of 12.76% with the decoction of the powder of the plant's trunk barks (20). In addition, the content of total phenolics was higher with 292.46±0.23 mg/g dry extract (decocted) from the Yako sample and lower (256.06±5.95 mg/g dry extract) for the decocted of the sample harvested in May at Baré.

The tannins content was very high, unlike the flavonoids content which varied between 6.20±0.09 mg/g and 7.78±0.04 mg/g. Moreover, there was a positive correlation between the phenolics content of the extracts

and their antioxidant activity. Indeed, the results of antioxidant (ABTS, DPPH) showed that the extracts of *Parkia biglobosa* trunk bark were endowed with antioxidant properties, those in decoction bringing out better antioxidant activity than those in infusion, especially during the May harvest in Yako. These antioxidant results corroborate those of Ouedraogo et al. (6) who found almost similar IC_{50} values for both methods with aqueous extract (the mixture is macerated for 15 min then boiled for 30 min). In fact, the secondary metabolites (total phenolics, flavonoids, tannins) known for numerous pharmacological properties (anti-edematous, analgesic, antipyretic, antioxidant...) which could justify the anti-hemorrhoidal effects of the plant (4, 21, 22). However, a better synergy and a diversity of compounds in the extracts could also contribute to this antioxidant activity (13, 23, 24). Previous research has shown that the aqueous extract (macerated followed by decoction) contained at least twice as many total phenolics and flavonoids as both types of extract in the current study (5). In addition to the antioxidant tests, the inhibition of lipoxygenase activity by the extracts were verified, as anti-inflammatory agents are used primarily to relieve the suffering of patients, especially hemorrhoidal patients. Lipoxygenase (LOX) is an enzyme that limits the metabolizing of arachidonic acid into leukotriene, which is involved in the development of inflammation. Inhibition of LOX can reduce leukotriene, thus producing an anti-inflammatory effect (13, 15, 25). All extracts were weakly effective compared to zileuton (IC_{50}=9.76±0.25 µg/mL). Also, our study showed a weak anti-inflammatory effect, using lipoxygenase inhibition, in comparison with previous work (5). Thus, a probable anti-inflammatory activity of the extracts of this plant could be mediated by mechanisms other than LOX inhibition.

Finally, among the extracts from the three localities, those from Yako were more active over the two periods with decoction the best, followed by those from Nobéré and finally those from Barré. The Yako locality could therefore be used for further studies.

Conclusion

The present study has shown that the raw materials harvested are acceptable in terms of microbiological qualities and that the harvesting period did not influence the effectiveness of the extracts. However, the extraction and harvesting areas, which are variable according to the climatic zones influence the pharmacological effects of the extracts.

Moreover, these results proved that the trunk bark of *Parkia biglobosa* is a potential source of secondary metabolites rich in polyphenols with antioxidant properties that would justify its traditional use in treating diseases with hemorrhoidal components. After comparison, trunk bark of *Parkia biglobosa* harvested in dry season of Yako (decoction) is better for formulation of anti-hemorrhoidal phytomedicines.

Declaration of competing interests

We have no conflicts of interest to declare. The manuscript was submitted with the approval of the co-authors who are responsible for writing the article.

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