

# Preclinical evaluation of phytoconstituents, antioxidant activity and acute oral toxicity of the ethyl acetate fraction of the trunk bark of *Sclerocarya birrea* (a. Rich.) Hochst (Anacardiaceae), a plant used in the treatment of cardiovascular diseases

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Lazare BELEMNABA<sup>1\*</sup>, Bibata SAWADOGO<sup>2</sup>, Mathieu NITIEMA<sup>1</sup>,  
Windingoudi Rimwagna Christian OUEDRAOGO<sup>1</sup>,  
Bertrand KAFANDO<sup>2</sup>, Tata Kadiatou TRAORE<sup>1</sup>,  
Boukaré KABORE<sup>1</sup>, Moumouni KOALA<sup>1</sup>,  
Moussa OUEDRAOGO<sup>2</sup>, Sylvin OUEDRAOGO<sup>1</sup>

## Abstract

Cardiovascular diseases, including hypertension, is a public health problem worldwide, with significant proportions in recent years.. Their pathophysiology involves an exacerbation of oxidative stress. The incidence of cardiovascular disease (CVD) is rising sharply worldwide, with around two-thirds of these people living in middle- and low-income countries. Moreover, for their therapeutic use, *Sclerocarya birrea* (A. rich.) Hochst (Anacardiaceae) is one of the candidates for phytotherapy. Thus, this study was undertaken to determine the phytochemical profile, antioxidant activity and safety of the ethyl acetate fraction of this plant's trunk bark (FAE). To achieve this, FAE was obtained following liquid-liquid fractionation from the aqueous decoctate of trunk bark powder. Chemical characterization was performed by HPTLC. The antioxidant activity of FAE was determined using ABTS<sup>•+</sup>, DPPH<sup>•</sup>, FRAP and LPO methods. Acute oral toxicity was also assessed in NMRI mice. In the various tests, FAE revealed the presence of flavonoids, saponosides, tannins and steroids/triterpenes. In addition, FAE was rich in flavonoids and phenolic compounds with interesting antioxidant activity compared with Trolox (p>0.05). The acute oral toxicity test of FAE (2000 mg/kg bw) revealed no mortality or adverse effects, indicating good safety of use. Thus, this study provides a scientific basis for the use of *Sclerocarya birrea* trunk bark in the management of pathologies involving oxidative stress, including hypertension.

**Keywords:** *Sclerocarya birrea*, phytoconstituents, antioxidant, safety use, ethyl acetate fraction

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<sup>1</sup> Laboratoire de Recherche-Développement de Phytomédicaments et Médicaments (LR-D/PM), IRSS/CNRST, 03 BP 7047 Ouagadougou 03, Burkina Faso

<sup>2</sup> Laboratoire de Développement du Médicament (LADME)/CEA-CFOREM, UFR-SDS, Université Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso

\* **Corresponding author** : BELEMNABA Lazare, [belemnabalaz@yahoo.fr](mailto:belemnabalaz@yahoo.fr) ; <https://orcid.org/0000-0002-4519-2603>

## Introduction

Hypertension, often referred to as the “silent killer,” is one of the leading causes of death and a public health problem worldwide, with significant proportions in recent years.. According to the World Health Organization (WHO), hypertension is characterized by a chronic, abnormal increase in blood pressure, with readings equal to or greater than 140/90 mmHg. Its incidence is increasing significantly worldwide, and it is considered the most common and modifiable cause of cardiovascular disease (CVD) (1-3). The danger of hypertension lies in its silent symptoms, which lead to a whole range of complications and morbidities. It is expected to account for 29% of deaths by 2025 (3). Thus, by 2025, an estimated 1.56 billion people worldwide will be affected (4). Currently, 1.28 billion adults worldwide between the ages of 30 and 79 suffer from this condition, representing three out of every ten adults with hypertension. Around two-thirds of these people live in middle- or low-income countries (5). In Africa, cardiovascular disease (CVD) has reached almost epidemic proportions (6). In Burkina Faso, the prevalence of hypertension has reached a worrying 18.2%, according to the Ministry of Health. Alarmingly, if not correctly managed, hypertension can lead to stroke, kidney disease, heart failure, and even death (7, 8). The pathophysiological mechanism behind this disorder is multifactorial (6, 9). Many antihypertensive drugs control and treat hypertension, including vasodilators, diuretics, calcium channel blockers, renin, and angiotensin-converting enzyme inhibitors (3, 9). However, despite improved management with conventional antihypertensives, 20% to 30% of hypertensive patients are resistant to at least three antihypertensive drugs (10, 11). As a result, interest in exploring natural bioactive substances as alternative approaches to hypertension management has grown. For example, 80% of the African population rely on traditional medicine and herbal pharmacopeia to treat several diseases, including hypertension {Nitiéma, 2023 #801}. Natural medicines are also an alternative to synthetic drugs (8, 12, 13). Moreover, plants contain thousands of therapeutic molecules that can improve disease control indicators (12, 14). In Burkina Faso, several studies have been carried out on medicinal plants with antihypertensive properties (9, 15, 16). These plants contain several compounds, including polyphenols (16), which promote endothelium-dependent and endothelium-independent vasorelaxation and improve lipid profile, antioxidant defenses and mitochondrial function (13, 17). Among the multitude of medicinal plants, traditional remedies prepared from the

trunk bark of *Sclerocarya birrea* (A. rich.) Hochst (Anacardiaceae), also known as “African plum” or “marula”, are used for the management of arterial hypertension (14). From a pharmacological point of view, patients frequently use the aqueous decoction of the trunk bark of this plant to treat arterial hypertension. Several studies have demonstrated the antioxidant, antidiabetic, vasorelaxant and antihypertensive properties and safety of extracts from this plant species (14, 18). However, in view of the therapeutic interest of this plant, the lack of scientific data in the literature calls for further research with a view to developing antihypertensive phytomedicines. The biological properties of plant extracts vary according to the polarity of the extraction solvent. Thus, following work on the aqueous extract, this study was undertaken to elucidate the phytoconstituents, antioxidant properties and safety of the ethyl acetate fraction of *Sclerocarya birrea* trunk bark in the treatment of cardiovascular disease.

## **I. Material and methods**

### **I.1. Material**

#### **I.1.1. Plant material**

The trunk barks of *Sclerocarya birrea* (A. rich.) Hochst (Anacardiaceae) were collected in August 2021 in Loagha (13°19'15.2"N 1°38'34.7"W), a village about 118 km from the city of Ouagadougou (Burkina Faso). A specimen (N° 360) of the plant was identified and deposited at the Département Environnement et Forêts / Centre National de la Recherche Scientifique et Technologique (DEF/CNRST), Ouagadougou, Burkina Faso. The collected sample was dried in the open air, protected from sunlight and dust, and then ground to a powder. The powder obtained was used to prepare an extract for biological and chemical investigation purposes.

#### **I.1.2. Experimental animals**

Female NMRI (Naval Medicinal Research Institute) mice, aged 3 months and weighing between 21-25 g, from the animal house of the “Institut de Recherche en Sciences de la Santé/Centre National de Recherche Scientifique et Technologique (IRSS/CNRST), Burkina Faso” were used. The animals were placed in an enclosure at a temperature of 21-23 °C with a relative humidity of 50-60% and subjected to the light/dark cycle of 12 h/12 h according to the rearing conditions of these species. Water and standard laboratory pellets enriched with proteins (29%) were provided for satiation and

experiments were carried out following the procedures of the Guide of Good Practices in Animal Experimentation under the Declaration of Helsinki. Furthermore, all experimental animal procedures have been performed by the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments (13, 14). They were used for toxicological and pharmacological testing of the prepared extract.

## **I.2. Methods**

### **I.2.1. Phytochemical screening**

#### **I.2.1.1. Preparation of the plant extracts**

Liquid-liquid fractionation was performed using the exhaustion technique (12, 19). The aqueous extract was prepared from twenty grams (20 g) of *S. birrea* trunk bark powder in 150 ml of distilled water boiled for 30 min. After cooling, the aqueous solution was filtered and centrifuged at 4000 rpm for 5 min. One hundred (100) mL of the aqueous decoctate was placed in a separatory funnel and 100 mL of ethyl acetate was added, giving rise to two phases: an ethyl acetate phase and a residual aqueous phase. The ethyl acetate organic phase was recovered and concentrated under reduced pressure using the Rotavapor RII. The concentrated ethyl acetate fraction was recovered and oven-dried at 55 °C. Ethyl acetate fraction (FAE) with a 0.37% yield was then recovered and stored in a hermetically sealed plastic bottle protected from light and humidity for the various tests.

#### **I.2.1.2. Analytical chromatography by HPTLC of the fraction**

High-performance thin-layer chromatography (HPTLC) was used to detect flavonoids, sterol-triterpenes, saponosides and tannins in the extracts. It was carried out on chromatoplates (60 F<sub>254</sub>, 10x5 cm, glass support 10x20 cm, Merck) following the literature (13, 14). Approximately 20 µL of FAE was streaked with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 8 mm from the bottom edge of the plate. After deposition and drying, the plates were placed in a tank containing eluent previously saturated (20x10 cm, saturation time: 30 min). The solvent system used depended on the metabolite to be identified: ethyl acetate/formic acid/H<sub>2</sub>O, (8/2/1 v/v/v/) for flavonoids; ethyl acetate/formic acid/H<sub>2</sub>O (18/2/4/2/1 v/v/v/v) for tannins; ethyl acetate/hexane (8/2 v/v) for sterol-triterpenes and hexane/ethyl acetate/methanol (10/5/5 v/v/v) for saponosides. After migration over 8 cm in length, the plates were dried.

The chromatographic profile was determined using specific enhancers for the compounds, including Neu's reagent for flavonoids, sulfuric anisaldehyde reagent for saponins, Liebermann and Burchard's reagent for sterol-triterpenes and 5% FeCl<sub>3</sub> for tannins. The profiles were then observed in visible light for tannins and at the UV wavelength of 366 nm for the others.

### **I.2.1.3. Determination of total phenolic compounds**

Total phenolic compounds was determined using the Folin-Ciocalteu Reagent (FCR) (13, 14). Gallic acid was used as the reference compound to produce the standard curve. Twenty-five(25) µL of sample at a concentration of 1 mg/mL was mixed with 125 µL of FCR. One hundred (100) µL of 7.5% w/v sodium carbonate solution was added to the mixture. After one hour, absorbance at 760 nm was measured using a microplate reader (Spectro UV, Epoch Biotek, USA). Results were expressed as mg gallic acid equivalent (GAE)/g dry fraction.

### **I.2.1.4. Determination of flavonoid compound**

Flavonoid content was assessed using an aluminum chloride reagent (13, 14). A standard calibration curve was plotted with quercetin as the reference. One hundred (100) µL of FAE (1 mg/mL) was mixed with 100 µL of a 2% w/v aluminium trichloride solution. After 10 min, absorbance at 415 nm was measured using a spectrometer (Epoch Biotek, USA). Results were expressed as mg quercetin equivalent (QE)/g dry fraction.

## **I.2.2. Assessment of antioxidant properties**

### **I.2.2.1. DPPH• assay**

Free radical scavenging activity by FAE and Trolox was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich as previously described (13, 14). Briefly, the absorbance of 10 µL of samples and Trolox (Sigma-Aldrich) added to 200 µL of DPPH (0.04 mg/mL) was measured at 490 nm after 30 min of incubation in the dark at room temperature using a Bio-Rad spectrometer (model 680, Japan). The result was expressed as antioxidant capacity equivalent to Trolox. As a function of sample concentration, a DPPH•-inhibition percentage curve was plotted. The percentage inhibition of the DPPH radical was calculated using the following formula:

Inhibition (%) = [(Ac - Ae)/Ac] x 100; Ae and Ac represent the absorbances of the extract/Trolox and the control (DPPH solution without sample). The concentration required to inhibit 50% of DPPH (IC<sub>50</sub>) was determined on the curve.

Anti-radical power (ARP) was determined by the formula: ARP= 1/IC<sub>50</sub>; ARP: Anti Radical Power; IC<sub>50</sub>: 50% inhibitory concentration expressed in µg/mL

### **I.2.2.2. Ferric reducing antioxidant power (FRAP) assay**

The FRAP assay was performed on FAE and Trolox as previously described (13, 14). The mixture of 0.5 mL samples with 1.25 mL phosphate buffer and 1.25 mL potassium hexacyanoferrate aqueous solution (1%) was incubated for 30 min at 50 °C. Next, 1.25 mL of trichloroacetic acid (10%) was added and centrifuged at 3000×g for 10 min. Distilled water (0.625 mL) and FeCl<sub>3</sub> solution (0.125 mL, 0.1%) were added to the supernatant (0.625 mL), and absorbance was measured at 700 nm using a spectrometer (Agilent, Santa Clara, CA) equipped with ChemStation UV-visible software. Trolox was used to plot the calibration curve. The FRAP activity of the samples was expressed as mmol Trolox equivalent/gram dry fraction.

### **I.2.2.3. ABTS•<sup>+</sup> assay**

The ABTS free radical scavenging activity of FAE and Trolox was assessed using the procedure described previously (13, 14). In a volume of 5 mL of distilled water, 19.2 mg of ABTS were dissolved. A mass of 3.312 mg potassium persulphate (Sigma-Aldrich) was added to the ABTS solution at 3.84 mg/mL. After adding the potassium persulphate, the solution was left for 16 h in the dark at room temperature before use. On the day of the experiment, 4.5 mL of the mixture was diluted in 220 mL of absolute ethanol. The range of 8 dilutions to be tested was prepared from the parent concentration of the samples (1 mg/mL). On a 96-well microplate, 200 µL of ABTS solution mixed with 20 µL of the extract or reference was added to each well. After incubation for 30 min at 25 °C, absorbances were read against a blank at 415 nm using an Agilent 8453 spectrometer with ChemStation UV-visible software. Measurements were performed in triplicate. The percentage inhibition of absorbance at 415 nm was calculated according to the formula:

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

A<sub>0</sub> is the absorbance of the control; A is the absorbance of the sample.

The absorbance inhibition curve as a function of the concentration of the FAE or reference substance (Trolox) was constructed to determine the 50% inhibitory concentration (IC<sub>50</sub>). Anti-radical power (ARP) was determined using the formula:  $ARP = (1/IC_{50})$ ; ARP: Anti-radical power; IC<sub>50</sub>: 50% inhibitory concentration expressed in µg/mL.

#### **I.2.2.4. Lipid peroxidation inhibition (LPO) assay**

The lipid peroxidation activity of rat liver was determined using 2-thiobarbituric acid (Sigma-Aldrich) (13, 14). FeCl<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> was used to induce peroxidation of the liver homogenate. A 0.2 mL volume of FAE at a concentration of 1.5 mg/mL was mixed with 1 mL of 1% rat liver homogenate, then 50 µL FeCl<sub>2</sub> (0.5 mM) and 50 µL H<sub>2</sub>O<sub>2</sub> (0.5 mM) were added. The mixture was incubated at 37 °C for 60 min, then 1 mL trichloroacetic acid (15%) and 1 mL 2-thiobarbituric acid (0.67%) were added. The mixture was heated in boiling water for 15 min. The experiment was performed in triplicate and the absorbance was read at 532 nm. Trolox was used as the reference product. The percentage inhibition was calculated using the following formula: Percentage inhibition (%) =  $[1-(A1 - A2)/A0] \times 100$

A1 is the absorbance of the control (without sample); A2 is the absorbance with the sample; A0 is the absorbance without liver homogenate.

#### **I.2.3. Acute oral toxicity**

The acute oral toxicity test for FAE was performed on female NMRI (Naval Medicinal Research Institute) mice in accordance with OECD guideline 423 (13, 14). Two (02) batches, each consisting of three mice, were made up and placed separately in polypropylene cages: a control batch of three mice and a test batch of three mice. After fasting for 3 h, FAE was administered by gavage using an esophageal tube in a single dose to the test mice. A dose of 2000 mg/kg body weight (bw) of FAE was chosen as the starting dose. The control batch received the solvent for dissolving FAE (10 mL/kg of 0.2% Tween 80). The mice were observed individually for 2 h after administration, at the end of which food was restored. They were then observed twice daily for 14 days to monitor for mortality and toxidrome (signs of toxicity) such as changes in skin and fur, eyes, mucous membranes, convulsions, salivation, diarrhea, sleep, and coma. The weight of each mouse and the quantities of water and food consumed were measured every 2 days during the 2 weeks of experimentation. On day 15, the mice were sacrificed after

anesthesia with ketamine (150 mg/kg) and a necropsy was performed on the organs (liver, kidneys, lungs, spleen, and heart) and then weighed. The relative weight of each organ was calculated [(Organ weight (g) / Fasting mouse weight on the day of sacrifice (g)) ×100]. This test was repeated under the same conditions as the 2 other batches (test batch and control batch).

### I.3. Statistical analysis

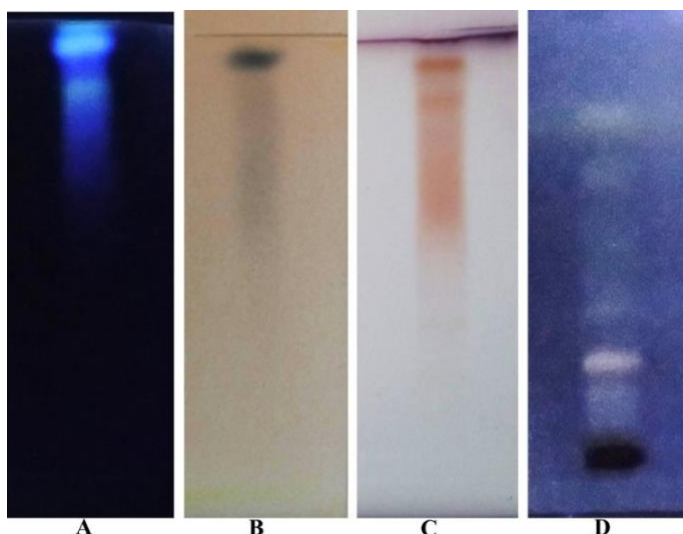
Statistical analysis applies to toxicity and antioxidant test. Values are given as arithmetic means ± SD. The graphics were constructed using GraphPad Prism 8.4.3 Software, San Diego, CA. Statistical comparisons were performed using Student's T-test and one- and two-way ANOVA. A post hoc test was performed using Bonferroni's test analysis to compare all the groups. A p-value < 0.05 was considered statistically significant.

## II. Results

### II.1. Phytochemical screening

#### II.1.1. Compounds revealed in the extract by HPTLC

The phytochemical analysis of FAE highlighted the presence of saponins, flavonoids, steroids and triterpenes, and tannins (figure 1).



**Figure 1:** Chromatogram showing the detection of flavonoids (A), tannins (B), saponosides (C), sterols, and triterpenes (D) from the ethyl acetate fraction of *S. birrea* trunk bark.



### **II.1.2. Total phenolic and flavonoid contents of ethyl acetate fraction of *S. birrea* trunk bark**

The results of the determination of flavonoid and total phenolic contents in the ethyl acetate fraction of *S. birrea* trunk bark were  $27.35 \pm 0.18$  mgquercetin equivalent (QE E/g of dry extract and  $74.63 \pm 7.76$  mggallic acid equivalent (GAE)/g dry of extract respectively.

### **II.2. Antioxidant activity of ethyl acetate fraction of *S. birrea* trunk bark**

The antioxidant activity of FAE is shown in Table I. The antioxidant activity of FAE with ABTS and DPPH assays was lower than with Trolox ( $p < 0.001$ ). Also, the lipid peroxidation inhibitory power (LPO) of FAE was  $40.24 \pm 4.04\%$ , comparable to that of Trolox ( $48.11 \pm 3.88\%$ ) ( $p > 0.05$ ).

**Table I:** Antioxidant test results for the ethyl acetate fraction of *S. birrea trunk bark*

| Extract | DPPH                     |      | ABTS                     |      |      | FRAP         | LPO              |             |
|---------|--------------------------|------|--------------------------|------|------|--------------|------------------|-------------|
|         | IC <sub>50</sub> (μg/mL) | ARP  | IC <sub>50</sub> (μg/mL) | ARP  | TEAC | (mmol EAA/g) | %INH (100 μg/mL) | (100 μg/mL) |
| FAE     | 29.37±7.96**             | 0.03 | 5.27±0.22***             | 0.18 | 0.69 | 1194.5±3.15  | 40.24±4.04       |             |
| Trolox  | 6.34±0.04                | 0.15 | 3.78±0.21                | 0.26 | 1    | -----        | 48.11±3.88       |             |

\*p<0.05 versus Trolox ; \*\*\*p<0.001 versus Trolox; FAE = ethyl acetate fraction of *S. birrea trunk bark*; TEAC = Trolox Equivalent Antioxidant Capacity.

## II.3. Acute oral toxicity

### II.3.1. Mortality and toxidrome

The dose of 2000 mg/kg body weight (bw) showed no remarkable behavioral changes or mortality in female mice at the first and second stages of administration of FAE (Table II)

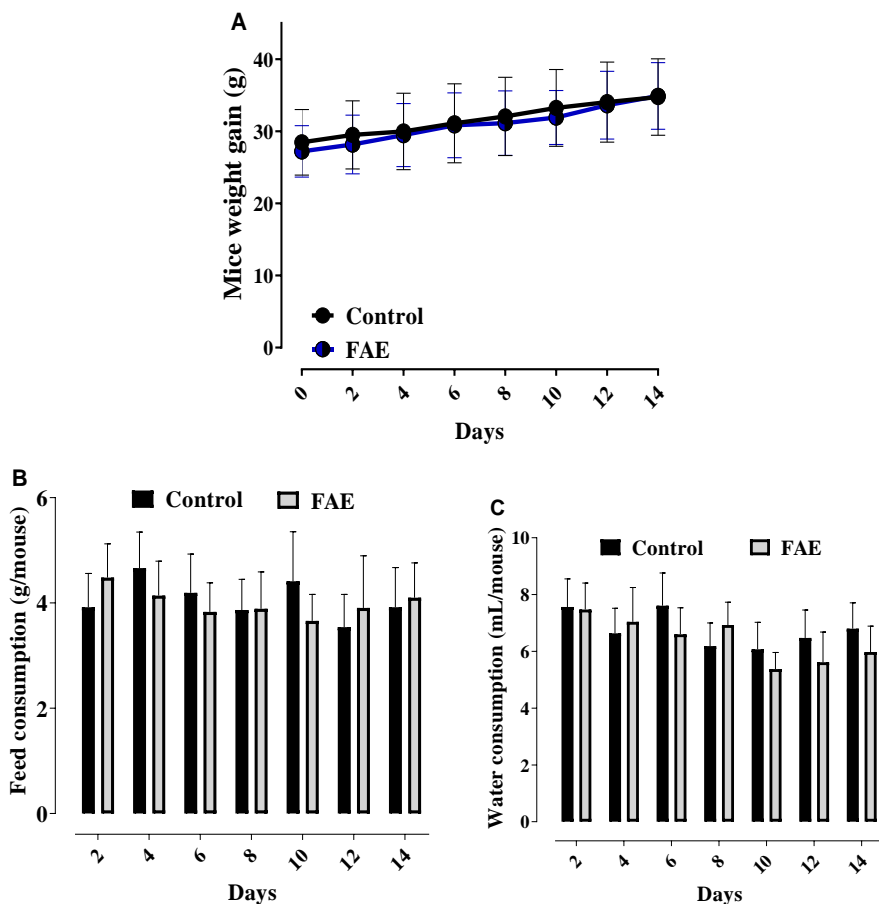
**Table II:** Mortality of female mice administered a single dose of ethyl acetate fraction of *S. birrea* trunk bark

| Samples administered<br>/ Behaviour of mice | Mortality            |                      |
|---|----------------------|----------------------|
|   | 1 <sup>st</sup> test | 2 <sup>nd</sup> test |
| Control (0.2% Tween 80 in distilled water)  | 0/3                  | 0/3                  |
| FAE (2000 mg/kg/bw)                         | 0/3                  | 0/3                  |
| Hair standing up                            | No                   | No                   |
| Lack of appetite                            | No                   | No                   |
| Convulsions                                 | No                   | No                   |
| Sleepiness                                  | No                   | No                   |
| Hyperventilation                            | No                   | No                   |
| Diarrhea                                    | No                   | No                   |

FAE: ethyl acetate fraction of *S. birrea* trunk bark (FAE); n = 6

### II.3.2. Changes in body weight, food, and water consumption of mice

Average weight gain, food and water consumption over two weeks in female mice given a vehicle (0.2% Tween 80) and a single dose of FAE (2000 mg/kg) are shown in Figure 2. No statistically significant differences were observed between the FAE batch and the control for the three parameters evaluated ( $p > 0.05$ ).



**Figure 2:** Changes in weight (A), feed consumption (B) and water consumption (C) of female mice from test (FAE, 2000 mg/kg bw) and control batches during 14 days of follow-up; FAE: ethyl acetate fraction of *S. birrea* trunk bark (FAE); n = 6.

### II.3.3. Macroscopic observation and relative organ weights

Table III shows the relative organ weights of control and FAE-treated mice. The relative organ weights of the 2 batches of mice showed no statistically significant variation. In addition, fresh macroscopic examination of the vital organs (liver, heart, lungs, kidneys, and spleen) of control and FAE-treated mice (2000 mg/kg bw) showed no lesions or changes in the appearance or color of the individual organs.

**Table III:** Average relative organ weights of control mice and mice treated with the ethyl acetate fraction of *S. birrea* trunk bark (FAE, 2000 mg/kg bw); n = 6.

| Sample       | Mice organ (%) |           |           |           |           |
|--------------|----------------|-----------|-----------|-----------|-----------|
|              | Heart          | Liver     | Kidneys   | Spleen    | Lungs     |
| Control      | 0.51±0.03      | 5.23±0.08 | 1.17±0.02 | 0.69±1.20 | 0.80±0.03 |
| FAE (2 g/kg) | 0.46±0.03      | 4.53±0.2  | 1.21±0.11 | 0.34±0.07 | 0.65±0.02 |

### III. Discussion

In the current context of developing new or alternative therapeutic agents from natural sources, the only data available on the potential applications of plant species in phytotherapy are sometimes those provided by ethnopharmacology, given their use by indigenous populations (14, 20). Through its aqueous extract, *Sclerrocaya birrea* is one of the plants used to treat chronic and cardiovascular pathologies linked to oxidative stress, including arterial hypertension (14). Furthermore, to verify the therapeutic potential of this plant, we were interested in identifying its phytoconstituents and their pharmacological capacity in the context of management of hypertension from the medium-polar extract, the ethyl acetate fraction. Chemical analysis of FAE of *S. birrea* trunk bark revealed the presence of flavonoids, saponosides, tannins, sterols and triterpenes. However, it was unlike some authors who noted the absence of sterols and triterpenes in the freeze-dried aqueous extract of this part of the plant (14, 21). Also, previous work has shown the presence of alkaloids in methanolic and acetic extracts of *S. birrea* trunk bark (22). In addition, anthocyanins, coumarins and leuco-anthocyanins were found in aqueous and methanolic extracts of this part of the plant (23, 24). Indeed, this difference in constituents could be explained by the nature of the extraction solvents on the one hand, and by edaphic conditions, and the harvesting period of the raw material on the other. We also collected raw material in Burkina Faso and for other authors in Nigeria. Thus, the localities and rainfall are different. However, not all parts of a plant contain the same levels of phytochemicals (25). Thus, saponosides, tannins, flavonoids, sterols and triterpenic glycosides, coumarin derivatives and anthocyanosides were also found in the aqueous ethanolic extract of *S. birrea* leaves (18, 26). This multitude of

phytoconstituents present in the plant could justify its traditional use to meet the health needs of populations. According to the literature, saponosides (27), tannins (9, 12), sterols and triterpenes (12, 28, 29) have diuretic, antihypertensive, and vasodilator properties on the arteries of normotensive and hypertensive rats. Moreover, FAE, rich in total phenolic and flavonoids, can induce endothelium-dependent relaxation due to their ability to promote endothelial NO• formation and EDHF-mediated vasodilation (12, 30). Furthermore, the mechanism of cardiovascular pathologies is multifactorial and includes inflammation, oxidative stress, the renin-angiotensin system and vascular dysfunction (31-33). To this end, we have formulated research hypotheses that FAE possesses antioxidant properties. During the metabolic process, oxygen undergoes a series of reduction steps leading to the production of free radicals represented by the superoxide ( $O_2^{\cdot-}$ ), oxygen radical ( $O^{\cdot-}$ ), hydroxyl ( $OH\cdot$ ), alkoxyradical ( $RO\cdot$ ) and peroxy radical ( $ROO\cdot$ ). To combat the latter, natural antioxidants exert their protective effect by activating the Nrf2 (NF-E2-related factor 2) signaling pathway, which regulates the endogenous antioxidant defense system by stimulating the expression of antioxidants and detoxification enzymes (34). Interestingly, FAE has proven antioxidant properties following our investigation by four methods (DPPH•, ABTS•+ and FRAP and LPO tests). The antioxidant properties obtained corroborate previous work demonstrating that triterpenes and phenolic compounds, in particular flavonoids and tannins, capture free radicals and inhibit pro-inflammatory enzymes (15, 35). Moreover, there is a correlation between the presence of phenolic compounds and flavonoids and antioxidant activity (36, 37). Also, these phytoconstituents chelate heavy metals involved in the production of free radicals and regulate the protection of the body's antioxidant defense system (38, 39). In addition, triterpenoids and their derivatives inhibit the action of ROS on the expression of protein kinases activated by mitogens ERK1 and ERK2, protein kinase 1c and nuclear factor kappa B (NF- $\kappa$ B) (35). FAE could therefore help combat chronic and cardiovascular diseases. Indeed, these findings are in line with the literature, which has documented that the freeze-dried aqueous extract of trunk barks (14) from the leaves and fruits of *S. birrea* (21) has properties against oxidative stress. However, a health remedy can only be used if it is both effective and non-toxic. As a result, the use of plant extracts in alternative medicine requires vigilance in terms of safety. With this in mind, the acute oral toxicity of FAE was evaluated. The results showed that acute oral administration of FAE (2000 mg/kg bw) produced no

mortality or visible behavioral changes in female NMRI mice. The lethal dose 50% (LD<sub>50</sub>) of FAE is therefore estimated to be greater than 5000 mg/kg bw according to the United Nations Globally Harmonized System for a dose of 2000 mg/kg administered to animals and causing no mortality (40). These findings show that FAE can be safe and is practically non-toxic. These scientific data corroborate earlier work which demonstrated that freeze-dried aqueous extracts from the same part of the plant had an LD<sub>50</sub> in excess of 5000 mg/kg bw (14). Thus, *S. birrea* extracts are free from deleterious effects at high doses.

## Conclusion

This study showed that the ethyl acetate fraction of *Sclerrocarya birrea* trunk bark has good antioxidant activity. Phytoconstituents such as flavonoids, tannins, saponosides, sterols and triterpenes present in the fraction could explain its beneficial properties against oxidative stress and in the treatment of chronic and cardiovascular pathologies. From a toxicological point of view, the use of the fraction is safe in the acute mining. These results scientifically validate the traditional therapeutic uses of this plant in the treatment of cardiovascular disease, including hypertension. However, research into long-term toxicity and antihypertensive effects ex-vivo and in vivo will be of undeniable help in scientifically supporting the use of this fraction in the management of hypertension.

## Conflict of interests

The authors declare that there is no conflict of interests to disclose regarding the publication of this paper.

## Author contributions

Conceptualization, LB and BS; Methodology and Software: SB and MN, WRCO and TKT; Validation: MN, LB, and TKT; Formal Analysis, MN and WRCO; Investigation, BS and BK; Writing - Original Draft Preparation: LB and MN; Writing - Review & Editing: LB and MN; Visualization and Supervision: MO and SO.

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