

ANTIOXIDANT AND VASODILATORY PROPERTIES OF SCLEROCARYA BIRREA (A. Rich.) Hochst TRUNK'S EXTRACTS (ANACARDIACEAE)

ABSTRACT

Sclerocarya birrea (*S. birrea*) is a medicinal plant of the Anacardiaceae family used to treat various diseases, including high blood pressure. The plant has many biological activities. This study aimed to determine the acute toxicity, antioxidant, and vasodilator activities of *S. birrea* trunk bark extracts. The phytochemical testing of the lyophilized aqueous decoction of d of the bark of *S. birrea* trunk was carried out by HPTLC. The vasodilation effect of the lyophilized water extracts of the trunk of *S. birrea* was studied in vivo with DMT 620M myography. The phytochemical study revealed the presence of flavonoids, tannins, saponins, steroids, and triterpenes in the various extracted substances studied. Evaluation of the vascular effect of frozen aquatic decarbonate showed that this extract caused vascular vasodilation of the thoracic artery in NMRI mice, both in the presence of endothelium alone, in the presence of L-NAME, and the absence of endothelium. The effective concentrations of 50% (EC₅₀) were (266.36±65.12 µg/mL, 365.57±97.54 µg/mL, and 592.43±78.05 µg/mL, respectively). As a preventive measure, this extract reduced the vasoconstriction of the mouse aorta ring by 58.04±0.6% compared to U46619 alone. This study reported the presence of botanical compounds of interest, such as phenols, flavonoids, tannins, saponins, steroids, and triterpenes, and the vasodilation effect of the bark decoction of *S. birrea*. These results provide a scientific basis for its use in traditional medicine.

Keywords: *Sclerocarya birrea*; Phytochemical screening; Myography; Vasodilators; Thoracic aorta.

1. INTRODUCTION

Arterial hypertension (AH) is an increase in arterial pressure (>140 mmHg) and diastolic blood pressure (>90 mmHg) that is pathologically continuous and associated with the risk of damage to several noble organs [1]. According to the World Health Organization (WHO), hypertension remains the leading cause of cardiovascular death [2]. High blood pressure is the main risk factor for stroke and has complex interactions with other risk factors [3]. A closer look at the figures shows a clear shift in blood pressure values between high-income countries and low-income regions [3]. In low- and middle-income countries, it is estimated that more than 1,04 billion people suffer from hypertension [3,4]. These countries are, therefore, the most burdened by hypertension, accompanied by low levels of treatment and control of the disease.

Despite several global associations' initiatives to fight this disease, its incidence is increasing, affecting cardiovascular disease and mortality [5–7]. In Africa, about 30% of countries have a HTA management system, most of which are high-income countries and incompatible with the economic and social realities [8,9]. Faced with the failure of the treatment, people turn to traditional doctors to seek help in the form of medical recipes. The main component of these recipes is pharmaceutical plants, which are a complementary therapeutic option to treat this disease [10]. In fact, these plants contain thousands of molecules, and by demonstrating their effectiveness and safety, they can contribute to improving the indicators for control. In Burkina Faso,

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traditional medicine is integral tonational health policy. For this purpose, the Institute for Health Research (IRSS) supports scientific validation of traditional recipes and highly recommended traditional medicines. This is the case for several studies on cardiovascular disease [11–13],infectious and inflammatorydiseases[14–16]. With this in mind, this study aims to scientifically validate the efficacy and safety of extracts from *Sclerocaryabirrea*, a tree with a good reputation in traditional medicine. Indeed, several studies on parts of this tree have demonstrated antioxidant, antidiabetic, and antihypertensive properties[17–20]. Despite the existence of some literature data, the scientific interest of this plant species leads us to deepen and confirm some of this information entering the research and development of medicinal plant-based drugs. This study aims to assess the acute oral toxicity, antioxidant, and vasodilatory efficacy of extracts from the trunk bark of *Sclerocaryabirrea*(Anacardiaceae), also known as "African plum" or "marula."

2. MATERIALS AND METHODS

2.1. Plantmaterial

S. birrea(Anacardiaceae) trunk bark was harvested in August 2021 in Loagha(13°19'15.2"N 1°38'34.7"W), a village located around 118 km from the city of Ouagadougou (Burkina Faso) (Figure 1). The harvested raw material was dried at room temperature, under ventilation, protected from the sun, and finely ground using a GLADIATOR brand mill, type BNI 10161 1083 X 84. The powder obtained was kept at room temperature until extraction (figure 1).

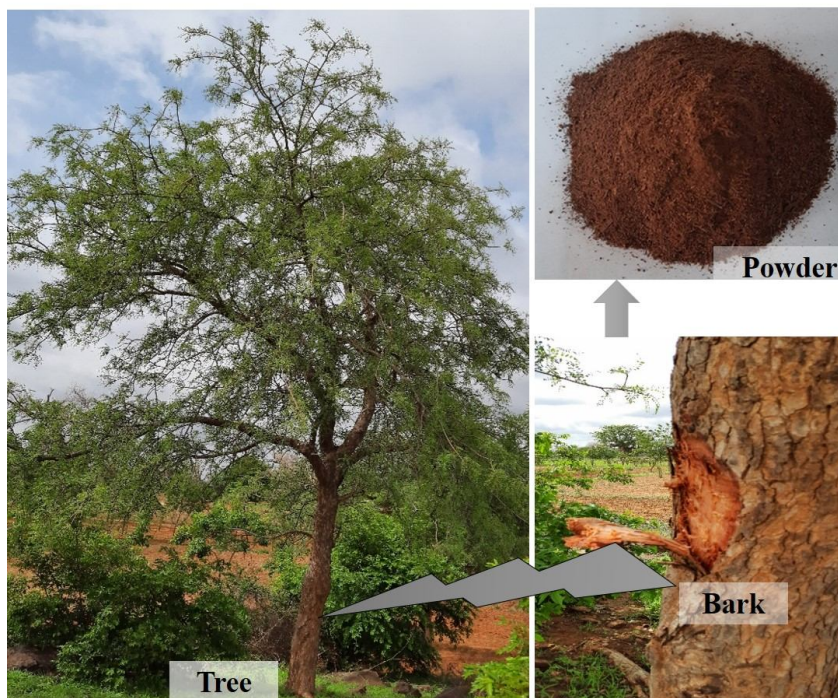


Figure 1: *Sclerocaryabirrea*(Anacardiaceae) (A. Rich.) Hochst at Loagha (13°19'15.2"N 1°38'34.7"W, Kongoussi, Burkina Faso)

2.2. Laboratory animals

Male and female 3-month-old NMRI mice weighing 21.12 ± 2.39 g were used. They were supplied by the IRSS animal facility, fed wheat cake (29%), with free access to running water and an ambient temperature of 23 ± 2 (60% humidity). They were used for toxicological and pharmacological studies of the study extract. All experiments were carried out using the procedures of the Guide to Good Practice in Animal Experimentation under the Declaration of Helsinki [21]. It is in phase with the terms of the Local Ethics Committee of Joseph KI-ZERBO University (Protocol number: CE-UOI/2019-04) relating to using vessels for myography.

2.3. Physico-chemical assay

2.3.1. Residual moisture content (THR) of vegetable matter

The residual moisture content of *S. birreaplant* powder was determined using the method based on water loss on drying. A test sample (P_0) of one gram (1g) of the plant powder was weighed in triplicate and placed in dry watch glasses. The whole set was placed in a TITANOX ELECTRONIC MICROPROCESSOR CONTROLLER CPS oven ventilated at 105°C for three (3) hours. After cooling, the watch glasses containing the steamed powders were weighed (P). The residual moisture content of the plant drug was determined according to [22].

2.3.2. Preparation of freeze-dried aqueous decoction

Twenty grams (20 g) of *S. birreatrunk* bark powder was mixed with 150 mL distilled water and decocted under reflux for 30 min from the boiling point. After cooling, the aqueous solution was filtered and centrifuged at 4000 rpm for 5 min. The supernatant obtained (aqueous decoction) was collected and frozen, then freeze-dried using a CHRIST ALPHA 1-3 LO freeze-dryer. This freeze-dried aqueous decoction of *S. birreabark* (DAL) was stored in a hermetically sealed plastic bottle, protected from light and humidity until it was used for the various tests in the study.

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2.4. Phytochemical screening

Phytochemical screening was performed on HPTLC (10 cm × 10 cm) silica gel plates 60 F₂₅₄ plates (Merck). Ten (10) μL of each extract were deposited in 1 cm strips with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 8 mm from the bottom edge of the plate. The distance between deposit spots was 3.4 mm. The distance between the plate's first spot and left edge and between the last spot and the right edge was 20 mm. A constant application rate of 100 nL/s was used. Upward linear development with a 10 mL mobile phase was performed in a CAMAG double-trough glass chamber lined with filter paper and previously saturated with mobile phase vapor for 20 minutes. The development distance was 70 mm. Plates were dried after development using a hair dryer. In the double-trough chamber, the mobile phases were:

- flavonoids, tannins, and saponins: ethyl acetate-formic acid-acetic acid-water 100: 11: 11: 26, v/v/v. Neu's reagent revealed flavonoids, tannins by FeCl_3 (2%), and saponins by sulfuric anisaldehyde;
- terpenoids: n-hexane-ethyl acetate 20: 4, v/v. Terpenoids were revealed by Liebermann Burchard reagent.

2.4.1. Determination of total phenolic compounds

The total phenolic compounds of DAL were determined by Singleton *et al.* [23]. These compounds react with FolinCiocalteu Reagent (FCR) in an alkaline medium. The loss of a phenolic proton in an alkaline medium lead to a phenolate anion, which can reduce the FCR in which molybdate is reduced, forming a colored blue molybdenum oxide complex with an absorption maximum at 760 nm. The intensity of the blue coloration is proportional to the amount of total phenolics present in the test sample. The reaction mixture consisted of 1 mL extract, 1 mL 2N FCR, and 3 mL 20% sodium carbonate solution. It was left to stand at room temperature for 40 min; then, the absorbance was measured at 760 nm using a spectrophotometer (Agilent 8453). In the white control tube, the extract was replaced by distilled water. A standard curve was plotted with tannic acid (1-5 µg/mL). Trials were performed in triplicate.

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2.4.2. Determination of total flavonoids

Determination of flavonoids in the extract was carried out using the method of Kumaran and Karunakaran adapted by Kaboré *et al.* [24,25]. Two (2) mL of 1 mg/mL extract in methanol were mixed with 2 mL of 2% aluminum trichloride in methanol. After 40 min, absorbance was measured at 415 nm using a spectrophotometer (Agilent 8453). The white control tube consisted of 2 mL methanol. The absorbance of quercetin (0.10 mg/mL) used as a reference compound was measured under the same conditions. Assays were performed in triplicate.

2.5. Antioxidant activity of extracts

Antioxidant potential was measured by determining the resulting products of oxidation or by assessing the radical scavenging ability of reaction models. Four methods (DPPH, FRAP, ABTS, and LPO) were used to assess the antioxidant activity of extracts.

2.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction Assay

The free radical scavenging assay DPPH• was performed according to the method used by Kim *et al.* with slight modification [26]. For this, a series of concentrations were prepared from DAL extract (9×10^{-2} ; 45×10^{-3} ; 22×10^{-3} ; 11×10^{-3} ; 5×10^{-3} ; 28×10^{-4} ; 14×10^{-4} ; 7×10^{-4} ; 3×10^{-4} mg/mL) and Trolox. Twenty (20) µL of these solutions (extracts and Trolox) were placed in wells of a 96-well microplate, containing in advance 200 µL of DPPH solution (0.04 mg/mL). After 30 min incubation, absorbance was read using an Agilent 8453 UV-vis spectrophotometer at 490 nm. The blank was prepared with 200 µL of DPPH and 20 µL of 99.9% pure methanol. A curve of percent DPPH inhibition was then plotted against sample concentration to determine the concentration required to degrade 50% DPPH (IC_{50}).

2.5.2. ABTS (2,2'-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid]) radical assay

The DPPH scavenging activity was performed according to the method described in the literature with minor modifications [25]. A mass of 19.2 mg ABTS plus 3.312 mg potassium persulfate was dissolved in 5 mL distilled water. The mixture was kept at room temperature in the dark for 12 to 16 hours. Different concentration series (9×10^{-2} ; 45×10^{-3} ; 22×10^{-3} ; 11×10^{-3} ; 5×10^{-3} ; 28×10^{-4} ; 14×10^{-4} ; 7×10^{-4} and 3×10^{-4} mg/mL) were then prepared from a stock concentration of each extract (1 mg/mL). Trolox was used as a reference substance. A 96-well microplate was used. The wells were filled with 200 µL of ABTS solution mixed with 20 µL of each extract at different concentrations or with 20 µL of trolox. The whole set was then incubated for 30 minutes at 25°C, and

absorbances were read using an Agilent 8453 UV-vis spectrophotometer at 415 nm. The white control was a mixture of 20 μL of 96° ethanol and 200 μL ABTS. All measurements were performed in triplicate.

2.5.3. Ferric Reducing Antioxidant power (FRAP) assay

The spectrophotometric method described by Hinneburg[27] was used to assess the reducing power of the study sample. To a test tube containing 0.5 mL DAL solution of *S. birreatrunk* bark (1 mg/mL) were added 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$, 1%]. The combination was heated to 50°C in a water bath for 30 minutes. A 1.25 mL trichloroacetic acid solution (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Three 0.625 mL aliquots were prepared in 3 test tubes, adding 0.625 mL distilled water and 0.125 mL freshly prepared FeCl_3 (1%) in water. A blank without any extract was prepared under the same conditions. Readings were taken at 700 nm against an ascorbic acid standard curve ($R^2 = 0.99996$).

2.5.4. Lipid peroxidation inhibition test (LPO)

The inhibitory activity of rat liver lipid peroxidation was determined using 2-thiobarbituric acid. $\text{FeCl}_2 - \text{H}_2\text{O}_2$ was used to induce peroxidation of liver homogenate according to the method of [28,29] with a few modifications. 0.2 mL of DAL extract at a concentration of 1.5 mg/mL was mixed with 1 mL of 1% Wistar rat liver homogenate, then 50 μL of FeCl_2 (0.5 mM) and 50 μL of H_2O_2 (0.5 mM) were added. The mixture was incubated at 37°C for 60 minutes, then 1 mL trichloroacetic acid (15%) and 1 mL 2-thiobarbituric acid (0.67%) was added, and the mixture was heated in boiling water for 15 minutes. Absorbance was read at 532 nm with a BioRad 680 spectrophotometer. Ascorbic acid was used as a reference product. The ability of the extracts to inhibit liver lipid peroxidation is expressed as a percentage of inhibition.

2.6. Determination of acute oral toxicity of freeze-dried aqueous decoction

The test was performed on homogeneous-weight NMRI mice by the Organization for Economic Cooperation and Development (OECD) guideline 423 with slight modifications [30][30]. Healthy fasting NMRI mice were divided into two (02) groups of 3 mice (test and control). The first phase of the test involved oral administration of a single 2000 mg/kg bw dose of lyophilized aqueous decoction (LAD) to the test group, while the control group received distilled water only. Control and test animals were observed every 30 min for the first two hours after treatment. After two hours, the animals were given access to food and observed daily until day 14^{ème}. Observations focused on mortality, behavior, and symptoms of intoxication. After 14 days, the animals were anesthetized with ketamine (150 mg/kg) and then sacrificed. Vital organs such as the heart, liver, lungs, kidneys, and spleen were removed, observed fresh and weighed. The second phase of the test (confirmation test) consisted of repeating the test with the same dose and under the same experimental conditions.

2.7. Pharmacological study

The experiment on *ex-vivo* vasorelaxant effects was performed with isolated mouse thoracic aorta using the method described by Nitiémaet *al.*[11].

2.7.1. Preparing the animal and harvesting the aorta

Male or female NMRI strain mice weighing 21.12 ± 2.39 g from the IRSS animal facility were used for experimentation. The mice underwent cervical dislocation, and the thoracic aorta was harvested. The aorta was placed in a petri dish containing Krebs solution and freed of adherent tissue. Aorta segments (2 mm long) were mounted in isolated organ vessels of a myograph (Danish Myo Technology, Aarhus, Denmark) filled with physiological Krebs solution (in mM): 130 NaCl; 14.9 NaHCO₃; 3.7 KCl; 1.2 MgSO₄ 7H₂ O; 1.6 CaCl₂ H₂ O; 1.2 KH₂ PO₄ and 11 D-glucose. The Krebs solution was maintained at 37°C and aerated with a mixture from a pneumatic pump. Two tungsten wires were inserted through the vessel lumen to hold each aortic ring between the vessel hooks. The mechanical activity was recorded isometrically by a force transducer connected to one of the two tungsten wires; the other wire was attached to a support carried by a micromanipulator screw enabling the voltage to be varied in mN.

2.7.2. Vascular reactivity

The aortic rings were stretched with a passive wall tension of 5 mN for 60 min, during which the rings were washed every 15 min with Krebs solution. After stabilization, the aortic rings were contracted by adding KCl (80 mM). Once the contraction plateau was reached, cumulative concentrations (10^{-9} - 3×10^{-7} M) of U46619, a vasoconstrictor, were added, and the maximum tension was recorded and considered 100% ring contraction. At the contraction plateau, the rings were rinsed thrice with Krebs solution until they returned to baseline contraction. The rings are again contracted U46619 (80% of maximum contractile response) followed by cumulative ACh (10^{-9} - 10^{-5} M) to check their functionality. Rings are considered functional and have no endothelium when relaxation at ACh is greater than or equal to 80% and less than or equal to 10%, respectively. After this check, rings are rinsed as before and contracted again to 80% of maximal contractile response with U46619 followed by cumulative at successively increasing concentrations of DAL extract (1, 3, 10, 30, 100; 300, 1000, and 2000 µg/mL). Rings with endothelium-intact were also pre-incubated with L-NAME (10^{-4} M) for twenty (20) minutes, then contracted with U46619, followed by cumulative treatment with the extract. Other rings were pre-incubated for 15 min with 1 mg/mL of the extract, followed by cumulative U46619 (10^{-9} M - 3×10^{-7} M).

2.8. Statistical analysis

Results were expressed as mean \pm standard deviation of the mean and plotted using GraphPad Prism 7.0 software (GraphPad Software, San Diego, California, USA). They were expressed as mean \pm standard error of the mean (SEM). An analysis of variance (One-way or Two-way ANOVA) followed by a Bonferroni post-test was used, and a p-value < 0.05 was considered statistically significant.

2.9. Ethical considerations

All *in vivo* studies and experiments were carried out by protocols already validated by the Institut de Recherche en Sciences de la Santé (IRSS, Burkina Faso) and which comply with international standards (guidelines established by the European Union on the protection of animals CCE Council 86/609).

3. RESULTS

3.1. Physico-chemical parameters

3.1.1. Residual Humidity Rate (RHR)

The residual moisture content of the crude *S. birrea* bark powder was $7.84 \pm 0.47\%$.

3.1.2. Extraction efficiency

Yields of freeze-dried aqueous extract of *S. birrea* bark were 7.77% .

3.2. Phytochemical analysis

3.2.1. High Performance Thin Layer Chromatography

Figures 2A, 2B, 2C, and 2D show chromatograms of the chemical compounds present in the freeze-dried aqueous decoction (DAL) of *S. birrea* bark.

Examination of the chromatogram in Figure 2A confirms the presence of flavonoids in the DAL extract through four (4) frontal reference spots (R_f) equal to 0.2, 0.24, 0.72, and 0.91. Figure 2B also reports the presence of tannins through a blue-black spot ($R_f=0.9$). In addition, the DAL extract also shows two (2) saponin spots, one observable at 366 nm (UV) (figure 2C: $R_f = 0.87$) and the other in the visible range (figure 2D: $R_f = 0.87$). Sterols and triterpenes were absent from this extract (2E).

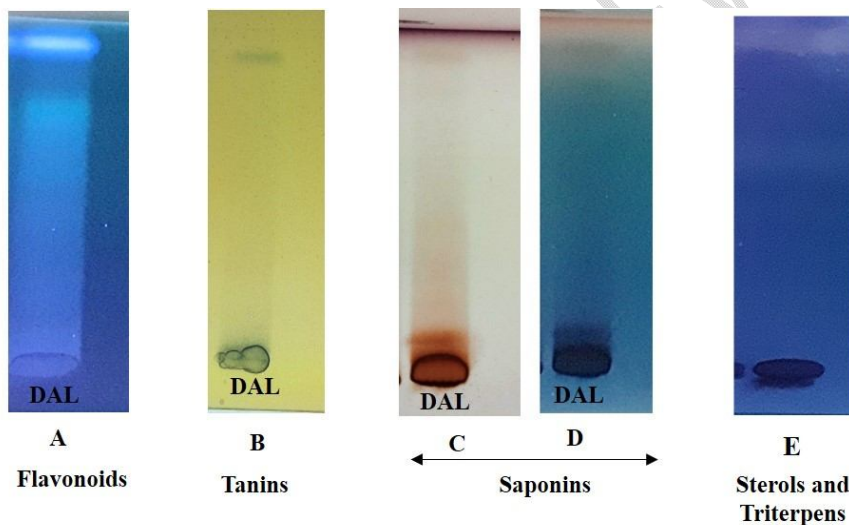


Figure 2: Chromatogram showing the detection of flavonoids (A), tannins (B), saponins (C-D), and sterols and triterpenes (E) in the freeze-dried aqueous decoction (DAL) of *S. birrea* bark.

3.2.2. Determination of total flavonoids and total polyphenols in DAL from *S. birrea*

The results of the flavonoid and total polyphenol assays were 23.75 ± 0.30 mg/g dry extract and 109.82 ± 3.71 mg/g dry extract, respectively, i.e., four times more abundant in total polyphenols than in flavonoids.

3.3. Antioxidant capacity of DAL

Table I shows the results of the antioxidant power assessment of *S. birrea* DAL using the DPPH, ABTS, FRAP, and LPO methods.

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Table I: Antioxidant test results for freeze-dried aqueous decoction (DAL) *S. birrea*.

Extract	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	FRAP (mol EAA/g)	LPO (100 µg/mL)
DAL	5,06±0,06	2,71±0,03**	841,27±2,24	35,78±7,30
Trolox	6,34±0,04	3,78±0,21	-----	48,11±3,88

Abbreviation: DAL = Freeze-dried aqueous decoction **Note:** ** $p < 0.05$ versus Trolox; *** $p < 0.001$ versus Trolox

Analysis of these results shows that antiradical activity by the DPPH and ABTS methods has IC₅₀ of 5.06±0.06 µg/mL and 2.71±0.03 µg/mL, respectively, with no significant difference. Also, the FRAP iron-reducing power of the extracts in the study, expressed in millimole ascorbic acid equivalent per g (mmol EAA/g) dry matter, showed an antiradical activity of 841.27±2.24 mmol EAA/g dry extract. The lipid peroxidation inhibitory power (LPO) of the freeze-dried aqueous decoction was expressed as a percentage (%) and was 35.78±7.30%, comparable to that of Trolox (48.11±3.88%) but with no significant difference ($p > 0.05$).

3.4. Acute oral toxicity of *S. birrea*DAL

3.4.1. Mortality

Freeze-dried aqueous decoction of *S. birrea* trunk bark (DAL) administered orally at a single dose of 2000 mg/kg caused no mortality during the fourteen (14)-day observation period. No signs of toxicity (reduced sensitivity to stimuli and decreased mobility) were observed for two hours after administration of the extract. The LD₅₀ of this extract is estimated at 5000 mg/kg. According to the Globally Harmonized System of Classification (GHS) and OECD guideline 423, the DAL extract of *S. birrea* can be classified in category five and considered slightly toxic.

3.4.2. Effect of DAL on weight growing

During the investigation, changes in the body weight of treated and control animals were recorded during the two (2) weeks of observation (figure 3). Analysis of the results showed that the weight gain of the test groups remained superimposable on that of the control group throughout the 14-day study period ($p > 0.05$).

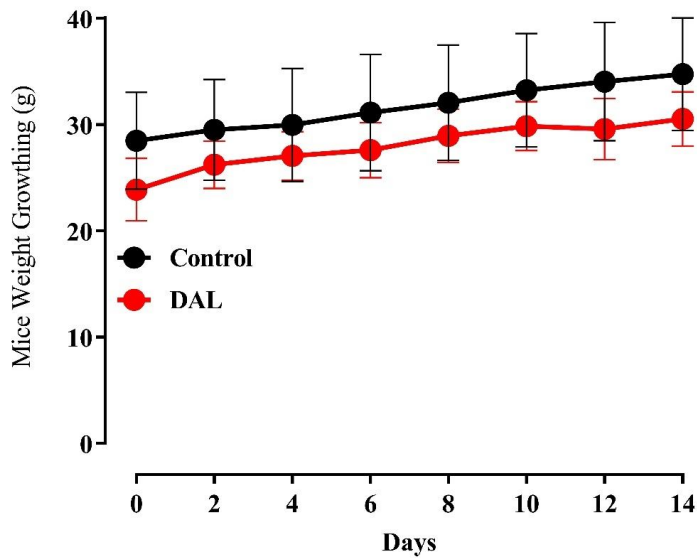


Figure 3: Changes in **daily** weight gain of control and test mice on freeze-dried aqueous decoction (DAL) of *S. birrea*.

3.4.3. Effect of DAL on mice food consumption

Figure 4 shows variations in feed consumption by study animals over the 14 days of inclusion. The feed consumption curves of the animals tested with the extracts are superimposable to those of the control animals. The analysis shows no difference in feed consumption from day 1 of administration to the end of the experiment.

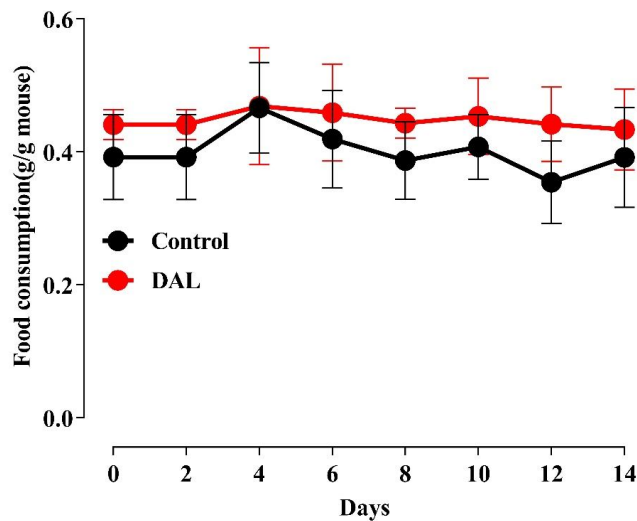


Figure 4: Changes in daily food consumption of mice treated with freeze-dried aqueous decoction of *S. birrebark* (DAL).

3.4.4. Effect of DAL on mice Water consumption

The results of the animals' daily water consumption are shown in Figure 5. These results showed that the average water consumption of mice fed the freeze-dried aqueous decoction was unaffected compared with that of the control group. A slight but insignificant reduction in consumption was observed between days 10^{eme} and 14^{eme} in all groups, compared with their initial consumption.

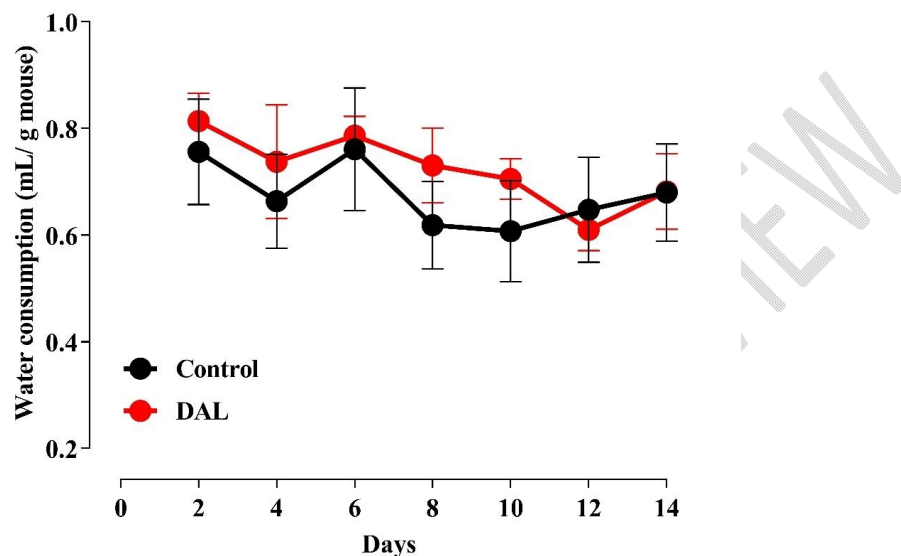


Figure 5: Evolution of average daily water consumption in mice treated with freeze-dried aqueous *S. birrea* bark (DAL) decoction.

3.4.5. Effects of DAL on average relative organ weight

Fresh macroscopic observation of treated animals' hearts, liver, kidneys, spleen, and lungs showed no change in color or appearance of these vital organs compared with those of control animals. In addition, there was no significant difference between the average relative weight of these organs from test mice compared with controls ($p > 0.05$) (Table II).

Table II: Average relative organ weights of control mice and those tested with dried aqueous decoction of *S. birreatrunk* bark (DAL) at 2000 mg/kg.

Organ	Heart	Liver	Kidneys	Rate	Lungs
Control	0,51±0,03	5,23±0,08	1,17±0,02	0,69±0,20	0,80±0,03
DAL	0,49±0,00	4,57±0,11	1,02±0,01	1,76±0,32	0,81±0,00

Abbreviation: DAL: dried aqueous decoction of *S. birrea* bark

3.5. Pharmacology study of dried aqueous decoction (DAL) of *S. birrea*

3.5.1. Ex-vivo vasodilatory effect of DAL on aorta isolated from NMRI mice.

The plots in Figure 6A and 6B illustrate, respectively, the vasodilatory effect of DAL in the presence and absence of endothelium on U46619-contracted mouse thoracic

aortic rings. Figure 6C shows the vasodilatory effect of DAL extract on aortic rings pre-incubated with L-Nitro Arginine Methyl Ester (L-NAME) and contracted with U46619.

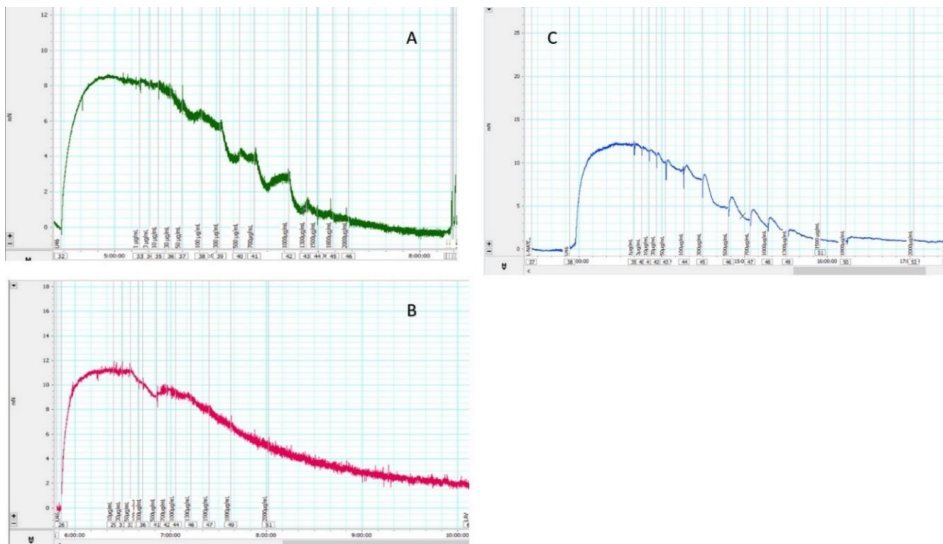


Figure 6: Illustrations of the effect of freeze-dried aqueous decoction of *S. birreatrunk* bark on vasodilation of thoracic aorta rings pre-contracted at U46619. (A) in the presence of endothelium, (B) in the absence of endothelium, and (C) in the presence of endothelium+L-NAME.

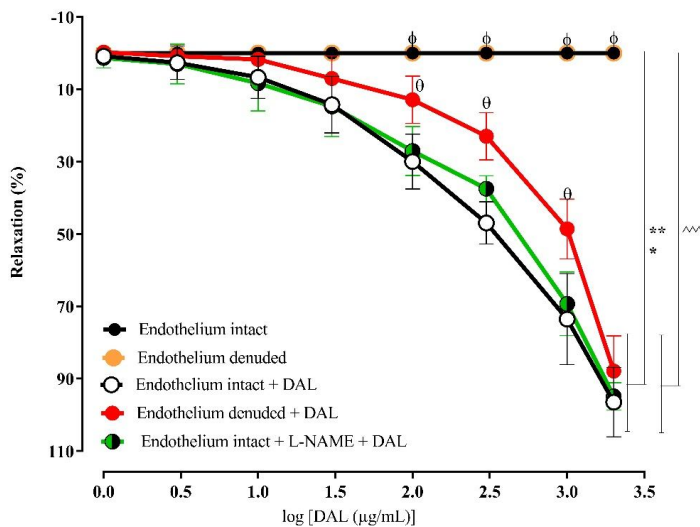


Figure 7: Effect of freeze-dried aqueous decoction of *S. birreatrunk* barks on mouse aorta rings in the presence of endothelium, in the absence of endothelium, and the presence of endothelium+L-NAME+Freeze-dried aqueous decoction pre-contracted at U46619.

Note: $***p < 0.001$ Vs control (+E); $***p < 0.001$ Vs (+E) +DAL; $***p < 0.001$ Vs control (-E); [n=5-7; (+E) = Presence of endothelium; (-E) = Absence of endothelium].

Analysis of these results in Figure 7 shows that DAL induces vessel relaxation in the presence of endothelium that is superimposable on its effect in the presence of L-NAME in a concentration-dependent manner. In the absence of endothelium, this effect is slightly shifted to the right but significantly different from its effect in the presence of endothelium. The 50% effective concentrations (EC_{50}) of DAL were $266.36 \pm 65.12 \mu\text{g/mL}$, $592.43 \pm 78.05 \mu\text{g/mL}$, and $365.57 \pm 97.54 \mu\text{g/mL}$, respectively, for rings in the presence of endothelium, in the absence of endothelium and the presence of endothelium + L-NAME. Maximum effects (E_{max}) were $96.47 \pm 9.54\%$, $87.92 \pm 9.68\%$, and $94.89 \pm 3.72\%$ in the presence of endothelium, in the absence of endothelium and the presence of endothelium +L-NAME.

3.5.2. Effect of DAL (1 mg/mL) on U46619-induced vasoconstriction

The vasodilatation of a substance involves several relaxation pathways, including inhibition of intracellular calcium release into the cytoplasm and calcium ion reuptake into the rough endoplasmic reticulum. Other signaling pathways, such as the prostaglandin I pathway₂ and endothelium-derived hyperpolarizing factors, are also involved. Figure 8 illustrates the contracting effect of cumulative U46619 on mouse aortic rings in the presence of endothelium (A) alone and (B) pre-incubated with DAL (2 mg/mL, 30 min).

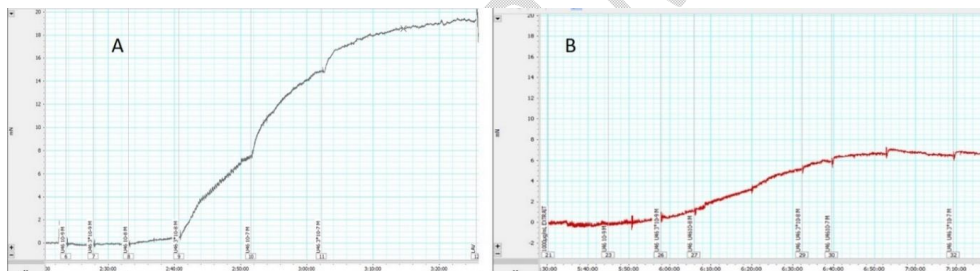


Figure 8: Graphic illustration of cumulative U46619 on mouse aortic rings in the presence of endothelium (A) alone and (B) pre-incubated with DAL (1 mg/mL, 30 min).

Analysis of these results in Figure 9 shows that pre-incubation of the rings with DAL at a concentration of 1 mg/mL significantly ($58.04 \pm 0.6\%$) reduces U46619-induced vasoconstriction compared with control. Maximum contraction intensities were $6.42 \pm 0.30 \text{ mN/mm}$ and $3.23 \pm 0.72 \text{ mN/mm}$ in the absence and presence of DAL pre-incubation, respectively.

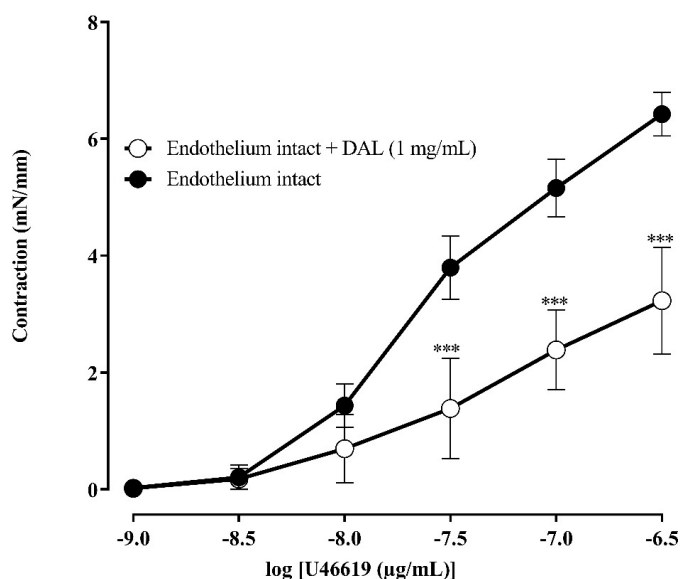


Figure 9: Curves showing the effect of DAL preincubation (1 mg/mL) on U46619-induced vasoconstriction of mouse aorta rings. *** $p < 0.001$ versus U46619 control, $n=6-7$.

4. DISCUSSION

THR is used not only as an indicator to help reduce errors in estimating the actual weight of plant materials but also as a significant component of ensuring their quality during the preservation phase. During this study, vegetable powder's residual moisture content (THR) was 7.840.47 %. This value is less than 10%, and according to the European Pharmacopoeia 9.0 standard, it is sufficiently dry and can be stored for a long time without the development of molds and yeast[22,31,32]. In fact, a high water content (over 10%) can cause enzyme reactions and adversely affect the appearance, organoleptic characteristics, and therapeutic properties of plant medicines during preservation. High residual humidity also promotes the spread of microorganisms such as bacteria and yeast.

The DAL extraction yield was 7.77%. Other authors reported similar results with acetone (70%) and methanol (70%) extracts from *S. birrea* trunk bark, with corresponding yields of 9.71 percent, 16.43 percent, and 14.78 percent (D. ; M. R. ; C. M. ; B. F. ; A. P. B. ; V. P. ; M. L. ; A. M. F. Russo, 2018). This variation in extraction yield may be explained by the difference between extraction methodology and climatic and erosion factors[22].

The chromatography profile showed that DAL extracts contain flavonoids, saponins, and tannins. These results confirm that other authors reported saponin, tannins, flavonoids, sterols, and triterpene glycosides in the hydrocarbon extract of *S. birrea* leaves. In addition to the phytochemicals reported in this study. Lahat Niang *et al.* and Dongocket *et al.*[34,35] also showed the presence of alkaloids in methanol extracts and acetone from the bark of *S. birrea*. Other authors have reported the presence of leuco-anthocyanins, anthocyanins, and coumarins in water and methanol extracted from the trunk bark of *S. birrea* (Kallo *et al.*, 2018; LAOULA *et al.*, 2021). The

extraction solvents and the geographical origin and harvesting period of *S. birrebarks* help explain these different findings.

Statistical analysis of total flavonoid content in DAL (23.75 ± 0.30 mg EQ/g) showed no significant difference ($p > 0.05$). Lower contents were reported by Lahat Niang *et al.*, while other authors found higher flavonoid contents. A high content of total phenolics in DAL was found with a value of 109.82 ± 3.71 mg EAT/g. However, the content obtained in this study was significantly higher than that of Lahat Niang *et al.* and lower than that reported by Russo *et al.* (Niang *et al.*, 2022; D. Russo *et al.*, 2018). All these differences could be justified by variations related to environmental conditions, harvesting sites, harvesting periods, reference compounds used, etc.

Among the extracts studied, DAL extract showed potent ABTS ($IC_{50} = 2.71 \pm 0.03$ μ g/mL) and DPPH ($IC_{50} = 5.06 \pm 0.06$ μ g/mL) free radical scavenging activity. This antioxidant activity of DAL extract was superior to those of *S. birrearoot* extracts reported by Akoto *et al.* and Russo *et al.* [38,39]. Analysis of the FRAP antiradical activity results reported that the DAL extract (841.27 ± 2.24 mol EAA/g) possesses FRAP antiradical activity. This ferric ion-reducing activity of DAL was more effective than that of the ethanolic extract of *S. birrealeaves*, as reported by Paré *et al.* in 2021. This potent free radical scavenging activity of DAL extract could be explained by its high phenolic and flavonoid content. As for antioxidant activity by inhibiting lipid peroxidation (LPO), the analysis reported an efficacy of DAL extract ($35.78 \pm 7.30\%$). The activity of phenolic compounds, which no longer needs to be demonstrated, could help explain this oxidation-reduction property [40]. Indeed, these compounds present in our extracts could act directly as free radical scavengers or chelators of transition metals (Fe, Cu) on the one hand and as inhibitors of lipid peroxidation or stabilizers of reactive oxygen species (ROS) on the other. These differences could be linked to the plant parts and the extraction methods used in these various studies.

A single oral dose of 2000 mg/kg bw of freeze-dried aqueous decoction (FDAD) of *S. birreatrunk* bark caused no mortality during the 14-day acute oral toxicity test. Moreover, no clinical signs of toxicity were observed during the study. Thus, the LD_{50} of each of its extracts was estimated at 5000 mg/kg bw in mice by OECD guideline 423 (OECD, 2001). The DAL extract could be classified in category 5 of the United Nations Globally Harmonized Classification System (United Nations, 2017). Under normal conditions of use, DAL extract from *S. birreawould* be practically non-toxic. These results differ from those of Belemnaba, who found an LD_{50} of 1437.23 mg/kg intraperitoneally with the freeze-dried aqueous decocted of the plant [42]. The administration route of the extracts could justify this difference in LD_{50} . These results corroborate those of other authors who found similar results with the aqueous extract of *S. birreastem* bark (Belemtougri *et al.*, 2006). However, behavioral differences such as slower mobilities and suspicious postures were reported after administration of the extract to rats by Ojewole *et al.* [44]. Body weight analysis of the mice in the study showed no statistically significant difference between the control group and the group tested with DAL (2000 mg/kg). This would be a good indicator of the safe use of the plant extract. [45]. In addition, water and food consumption did not vary significantly despite a slight drop in all 03 groups studied. Furthermore, no significant variations in relative weight nor changes in the color and shape of organs such as the heart, liver, kidneys, spleen, and lungs were observed during animal necropsies. Indeed, changes in the relative weight of noble organs are a sensitive indicator of toxic effects [46].

Furthermore, this study showed that the cumulative effect of successively increasing concentrations of DAL extract generated vasodilation in a concentration-dependent

manner in the aortic rings of NMRI mice pre-contracted with U46619. This finding is comparable to that of Belemnaba et al., who also showed that freeze-dried aqueous decocted of *S. birrea* relaxes endothelium-contracted Wistar rat aorta rings by phenylephrine [42]. Other authors have shown that aqueous extracts of *S. birrea* stem bark have a vasorelaxant effect on intact Wistar rat aortas via nitric oxide [47]. Moreover, this DAL extract could release endothelium-denuded aorta rings in a concentration-dependent manner. Nevertheless, DAL extract's efficacy on aortic rings was statistically significant in the presence of endothelium compared with its effect in the absence of endothelium. This verifies the endothelium-dependent and independent nature of the impact of DAL extract. This suggests that, in the presence of endothelium, the extract may have the capacity to induce the release of vasodilatory chemical mediators, including nitric oxide (NO) in particular, which is responsible for vasodilation (Belemnaba et al., 2019; Nitiema, Soleti, et al., 2019b; W. R. C. Ouedraogo, Belemnaba, Nitiema, Kabore, et al., 2023). Indeed, numerous studies have demonstrated the involvement of NO in the relaxation of vessels with endothelium of natural substances. The second messenger release involves the upstream triggering of chemical mediators such as Src and PI3-Kinase/Akt, the origins of which remain determined [50]. In addition to this endothelium-dependent effect, the results showed that *S. birrea* DAL extract relaxes vessels when the endothelium is degraded. This suggests that the extract has the potential to induce the release of endothelium-independent vasodilators via activation of prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factors. Results also showed that the DAL extract of *S. birrea* significantly reduced the vasoconstrictor effect of U46619. This thromboxane A₂ analog is well known for its ability to induce increased intracellular calcium mobilization (Jiang et al., 2021). Its high affinity for specific G protein-coupled receptors (TP receptors) makes this action possible. Indeed, activation of these TP receptors causes an increase in cytosolic free Ca²⁺ [(Ca²⁺)_i]²⁺ either from the sarcoplasmic reticulum (SR) or by inducing extracellular calcium entry via activation of plasma membrane channels, just like thromboxane A₂ [51,52]. It should be noted that the preliminary results of this work did not confirm all these implications. However, studies have already shown that the vasorelaxant effect of many pharmacological substances is linked to this mechanism of action at the level of vascular smooth muscle. Nevertheless, the present study's results align with other authors, showing the endothelium-dependent and independent character of aqueous extracts of *S. birrea* leaves. Similarly, the endothelium-dependent vasodilator effect was demonstrated by Belemnaba (2007) using phenylephrine as a contracting agonist [42].

This study pre-incubated mouse aorta rings with L-NAME (a competitive inhibitor of endothelial NO synthase) to refine our understanding of this extract's mechanism of action further. The results showed that the presence of L-NAME did not influence the vasorelaxant effect of DAL extract, as evidenced by the superimposable curves. Thus, the endothelium does not play a fundamental role in regulating vasorelaxation linked to *S. birrea* DAL extract. This finding supports the ability of DAL extract to induce an endothelium-dependent and independent effect in the aorta rings of NMRI mice. DAL extract induces total relaxation of U46619-induced aortic contraction. However, compared with EC₅₀, DAL extract was more potent in the presence of endothelium than in its absence and the presence of endothelium + L-NAME. Given these results, the endothelium-dependent relaxation of DAL extract could be linked, at least in part, to its high polyphenol and flavonoid content [53]. Indeed, phytochemical screening has identified chemical compounds such as

flavonoids, tannins, and saponins with various pharmacological properties. Several authors have shown that polyphenols can induce endothelium-dependent NO [49,54,55]. These findings corroborate those of previous studies that reported that plant extracts, rich in polyphenols, could induce endothelium-dependent vasorelaxation due to their ability to stimulate the biosynthesis or activate the release of endothelial NO in vessels [12,56].

5. CONCLUSION

This study showed the presence of phytochemical compounds such as flavonoids, tannins, and saponins in the DAL extract of *S. birrea*, which has a high polyphenol and flavonoid content. This extract is anti-free radical (ABTS and DPPH), iron-reducing (FRAP), and lipid peroxidation inhibiting (LPO). Better still, this DAL extract is an endothelium-dependent and independent vasodilator in the aorta of NMRI mice pre-contracted with U46619. As for safety, DAL extract is reported to have low toxicity, according to the United Nations Globally Harmonized System. Further work will be undertaken to elucidate this extract's pharmacological mechanisms of action. All these findings will undoubtedly strengthen the scientific database of plant species used to treat cardiovascular disease.

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