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Phytochemistry and antioxidant activity of *Sclerocarya birrea* extracts, A plant used in the traditional management of hypertension in Mali

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Abstract

Sclerocarya birrea (A. Rich) Hoscht is a popular African wild tree whose leaves, bark, roots and fruits are used in the food and the traditional medicine. The aim of the current study was to determine the phytochemical composition and the antioxidant potential of extracts (aqueous, ethanolic and ethyl acetate macerates) of *S. birrea* leaves, bark and roots. Thus, a screening based on qualitative techniques was carried out. The total polyphenols were quantified using the Folin-Ciocalteu method, while the flavonoids were quantified using aluminum trichloride. The *in vitro* antioxidant activity was determined by the DPPH and Phosphomolybdate tests. The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, coumarins and terpenoids in all extracts. The ethanolic root extracts showed the highest levels of polyphenols (822.6±36.9 mg GAE/g) and flavonoids (112.59±12.29 mg QE/g). As for the antioxidant potential, the ethanolic extracts of bark showed the best DPPH radical reduction capacity, with an IC_{50} of 65.57±2.32 µg/mL and a total antioxidant capacity of 882.50±25 mg EAA/g. This study showed that *S. birrea* organs are a potential source of natural antioxidants that could play a crucial role in the management of hypertension.

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Introduction

The betterment of human health is now a major concern for many countries and international organizations, and one of the priorities of sustainable development. However, despite the efforts, the cardiovascular diseases remain a major cause of human mortality (Bio *et al.*, 2015; Samaké *et al.*, 2024). These diseases are a group of disorders affecting the heart and blood circulation. They include coronary heart disease, heart failure, high blood pressure and diabetes (Houehanou *et al.*, 2018). The arterial hypertension is a chronic metabolic disease and one of the main risk factors leading to cardiovascular disease in Africa and worldwide.

The statistics show that it affected 5-10% of pregnant women, and is now routinely investigated during prenatal consultations (Traoré *et al.*, 2021). It is the leading cause of maternal and fetal morbidity and mortality, and the second most common reason for admission of pregnant women to intensive care. Associated with pregnancy, the arterial hypertension still remains a major cause of maternal-fetal morbidity and mortality in Malian context where diagnosis is often late (Samaké *et al.*, 2024). The literature reported that 16.5% of annual deaths worldwide are attributable to hypertension. By 2030, the annual deaths are expected to reach 23.5 million people through the world. The main risk factors are linked to age, primigravida, sedentary lifestyle, heredity, obesity, use of estrogen-progestogens, alcohol and tobacco abuse, and stress (Samaké *et al.*, 2024; Traoré *et al.*, 2021). While this pathology used to mainly affect the elderly, nowadays its prevalence is worryingly high among young people. Some studies have reported a prevalence ranging from 16% to 40% in adults aged 18 and over in sub-Saharan Africa (Houehanou *et al.*, 2018). Recent studies in Mali revealed an in-hospital incidence of hypertension of 55.80% in the 20-34 age group and 24% in young people under 20 (Traoré *et al.*, 2021).

The management of arterial hypertension remains difficult, especially in a context of under-equipment associated with a poorly range of antihypertensives. Furthermore, the lack or the inadequacy of paraclinical monitoring exacerbates that situation. The management of this pathology is generally a lifelong treatment. The cost of this treatment is not always within the reach of the majority of the local population in Africa (Traore *et al.*, 2022). As a result, these populations resort to their own culture and tradition of using medicinal plants to treat this disease. Importantly, the use of medicinal plants to manage hypertension is well-documented in several African countries (Traore *et al.*, 2022).

Medicinal plants are widely used by the Malian population to cure high blood pressure (Dembélé *et al.*, 2020; Sanogo *et al.*, 2009). Illiteracy, the limited income of that population, and sociocultural factors, in general, has resulted in a relatively large demand for treatment using plants. Since 2009, a traditional recipe composed on two plant species (*Sclerocarya birrea* and *Vitex doniana*) has been developed in Mali by the Traditional Medicine Department (DMT) under the local name “Nitrokoudang”. This Malian recipe is an improved traditional medicine, which is registered in the National Therapeutic form (Sanogo *et al.*, 2009).

S. birrea is an African wild tree found in many countries across the continent (Sène *et al.*, 2018). Its fruit, as an excellent source of nutrients, are used to fill certain nutritional deficiencies of indigenous peoples in Africa. However, the species is mostly used in Africa for its medicinal properties by about 79% of local population (Sène *et al.*, 2018). Its medicinal properties were highly reported in the literature. It is known as a regulator of the level of cholesterol in the human body, which improves the health of the heart and blood vessels (Abdelwahab *et al.*, 2024; Ngueguim *et al.*, 2015). It was also reputed to help pregnant women with skin issues (Komane *et al.*, 2015). Despite its valuable medicinal properties, very few works have been carried out on the biochemical aspects of *Sclerocarya birrea* in Mali. This study aimed to contribute to the valorization of this species by investigating its phytochemical composition and antioxidant potential.

Materials and methods

The plant material was consisted of *S. birrea* leaves, roots and trunk bark, which were harvested in Niéna, located in Sikasso Region, Mali. After being carefully washed and dried at room temperature, these organs were reduced into powder and then kept cold for future analysis.

Extract preparation

The extracts were prepared at 10% (w/v) in three different types of solvent (distilled water, 70% ethanol and ethyl acetate). The mixture was placed under magnetic stirring for 24 h, and then filtered under vacuum. This operation was repeated twice to extract the maximum amount of the bioactive compounds.

Phytochemical screening

The characterization of phytochemical compounds was performed based on qualitative techniques according to the standard methods reported by Bruneton (2009) and Shaikh and Patil (2020). The test of Dragendorff and Mayer were used to detect the alkaloids while the flavonoids were detected by the alkaline reagent test; the tannins by Braymer test; the sterols and terpenoids by Salkowski test; and the saponins by the foam test. The coumarins were detected by UV fluorescence at 365 nm.

Determination of total polyphenols and flavonoids

The quantification of polyphenols and flavonoids was carried out according to the spectrophotometric method reported by Konaré *et al.* (2023), with slight modifications. The Folin-Ciocalteu reagent was used to quantify the polyphenols and the results are expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g). For the flavonoids, the aluminum chloride ($AlCl_3$) test was used, and the results were expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g).

Calculations

The content of both total phenolic and flavonoids compounds was calculated using the following formula:

$$m = \frac{C \times Vf}{Ti} \times Fd$$

m: Total polyphenol content (mg GAE/g)

C: Concentration of the sample deduced from the standard curve (mg/mL)

Vf: Final volume of the extract (mL)

Fd: Dilution factor

Ti: Test intake (g)

Assessment of in vitro antioxidant potential

The antioxidant potential was assessed by colorimetric assay using the DPPH (2.2-diphenyl-1-picryl-hydrazyl) and phosphomolybdate tests according to the protocols described by Hamata *et al.* (2020) and Konaré *et al.* (2020), respectively.

Phosphomolybdate or total antioxidant capacity (TAC) assay

A working solution consisting of a mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate has been prepared first. The total antioxidant capacity (TAC) was carried out by mixing a volume of 100 µL of the extract with 900 µL of working solution. After sealing, the mixture was incubated at 95°C for 90 min, and then cooled. The absorbance was measured at 695 nm with a spectrophotometer (Thermo Scientific, Biomate 3S) against the blank which contained 100 µL of methanol instead of the extract. The total antioxidant capacity value was expressed in milligrams of quercetin equivalent per gram (Konaré *et al.*, 2020).

DPPH assay

A volume 50 µL of each extract at different concentrations or 50 µL of methanol for negative control were added to 1.95 mL of a methanolic solution of DPPH previously prepared (0.024 g/L). After 30 min of incubation in the dark at room temperature, the absorbance was read using a spectrophotometer (Thermo Scientific, Biomate 3S) against a blank (composed of 2 mL of simple methanol) at 515 nm. The positive control was represented by a standard antioxidant, ascorbic acid whose absorbance was measured under the same conditions as the samples.

The antioxidant potential linked to the trapping effect of the DPPH radical was expressed as a percentage of inhibition calculated from the absorbances obtained according to the formula below. Lastly, the inhibitory concentrations of 50% free radical were deduced (Hamata *et al.*, 2020).

$$\text{Inhibition (\%)} = [1- \frac{Asborbance\ of\ sample}{Asborbance\ of\ negative\ control}] \times 100$$

Statistical analysis of data

The data obtained were processed using Minitab 18.1 software. A single-factor ANOVA using Fisher's test was chosen to compare the means at the α = 0.05 significance level.

Results and discussion

Phytochemical composition

The analysis of this Table 1 reveals the presence of all the chemical groups sought in all the organ extracts investigated, with the exception of terpenoids in the aqueous and ethyl acetate extracts of leaves, and saponins in the ethyl acetate extracts of roots. On the other hand, terpenoids were absent in the aqueous and ethyl acetate leaf extracts, while the saponins were absent in the ethyl acetate root extract. These data obtained confirm those reported by several previous works relating to the presence of the biocompounds in *S. birrea* (Sène *et al.*, 2018; Niang *et al.*, 2021). These biocompounds identified in *S. birrea* extracts are reputed to have numerous therapeutic virtues. For instance, the alkaloids, tannins and phenolic compounds are known for their antimicrobial, analgesic and antitumoral properties (Sarode *et al.*, 2023; Shaikh and Patil, 2020; Taylor *et al.*, 2006; Togola *et al.*, 2023).

Total polyphenol and flavonoid contents

The Table 2 shows the results of the determination of polyphenols and flavonoids levels in the *S. birrea* extracts. The results show that the polyphenol (p-value = 0.001E-20 < 0.05) and flavonoid (p-value = 0.002E-8< 0.05) contents varied significantly according to the solvents and the organs. The highest levels of the total polyphenols and flavonoids were obtained with the ethanolic root extracts, at 841.63±7.05 mg GAE/100 g and 105.92±1.36 mg QE/100 g respectively.

Otherwise, the lowest contents were recorded in the aqueous leaf extracts with 66.00±0.88 mg GAE/100 g and 38.33±6.80 mg QE/100 g, respectively for the polyphenols and flavonoids. Similarly, the work of Niang *et al.* (2021) on the same species reported the phenolic compound richness of bark with contents of 37.48±1.33 mg GAE/g DM and 34.95±0.29 mg GAE/g DM for infused and hydroalcoholic extracts respectively.

Furthermore, the involvement of tannins and flavonoids in reducing the permeability of blood capillaries and enhancing their resistance to hemolysis have been highlighted (Sarode *et al.*, 2023). The use of the species in the traditional management of high blood pressure by the Malian local populations (Dembélé *et al.*, 2020; Sanogo *et al.*, 2009) could be linked to these properties. Other works have mentioned the ability of *S. birrea* to regulate the cholesterol level in the human body, as result enhance healthily the heart and blood vessels (Abdelwahab *et al.*, 2024; Ngueguim *et al.*, 2015).

Table 1. Phytochemical screening

Chemical groups	Leaves			Trunk bark			Roots		
	Aqueous	Ethanolic	Acetate	Aqueous	Ethanolic	Acetate	Aqueous	Ethanolic	Acetate
Alkaloids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	-	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	-

Presence: +; Absence: -

Table 2. Total polyphenols and flavonoids amount

Organs	Extracts	Polyphenols (mg GAE/100 g)	Flavonoids (mg QE/100 g)
Leaves	Aqueous	66.00±0.88^{h}	38.33±6.80^{e}
	Ethanolic	355.71±15.66^{d}	87.35±3.12^{bc}
	Ethyl acetate	125.47±7.24^{g}	58.94±4.43^{d}
Trunk bark	Aqueous	422.64±14.09^{c}	58.86±3.39^{d}
	Ethanolic	705.40±23.50^{b}	79.65±3,12^{c}
	Ethyl acetate	348.32± 14.35^{d}	90.33±9.05^{b}
Roots	Aqueous	161.63±11.74^{f}	27.88±2.04^{f}
	Ethanolic	841.63±7.05^{a}	105.92±1.36^{a}
	Ethyl acetate	288.54±6.78^{e}	61.21± 2.96^{d}
p-value		0.001E-20	0.002E-8

The means that do not share the same letters are considered to be significantly different at the 0.05 threshold.

Antioxidant potential in vitro

Two independent methods were used to assess the antiradical activity of the plant extracts: Phosphomolybdate test or Total Antioxidant Capacity (TAC) and the DPPH test. The results obtained are translated into the Fig. 1 and 2.

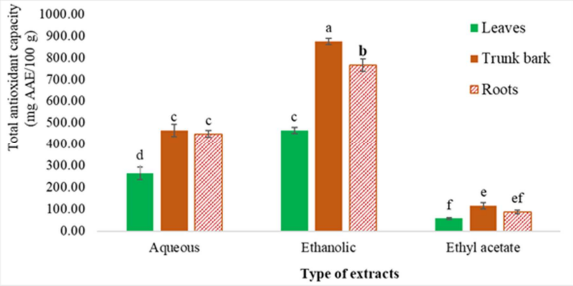

Fig. 1. Total antioxidant capacity of extracts

The means that do not share the same letters are considered to be significantly different at the 0.05 threshold.

The results obtained with both tests show that the anti-free radical power varied from one extract to another (p-value < 0.05). For each extract, the bark showed the greatest anti-free radical potential compared with the leaves and roots. In terms of solvents used, the ethanolic extracts showed the best antioxidant potential, followed by the aqueous and ethyl acetate extracts respectively (Fig. 2). The ethanolic extracts from the bark were the richest in total antioxidants with 874.17±14.43 mg AAE/100 g, while the lowest total antioxidant capacity was recorded in the leaves with ethyl acetate (57.50±50 mg AAE/100 g) (Fig. 1).

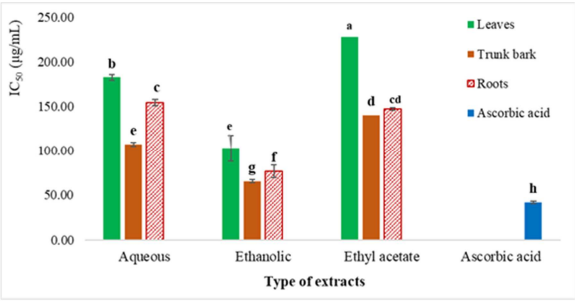

Fig. 2. IC_{50} values for anti-free radical DPPH scavenging of extracts

The means that do not share the same letters are considered to be significantly different at the 0.05 threshold.

As IC_{50} is inversely proportional to free radical scavenging capacity, the highest DPPH radical reducing potential (IC_{50} = 65.57±2.34 µg/mL) was presented by the hydroethanolic bark extracts and the lowest potential (IC_{50} = 227.61±14.65 µg/mL) by ethyl acetate leaf extracts. Our results are in line with those of Niang *et al.* (2021) on the antioxidant activity of hydro-acetone, hydro-methanol and aqueous extracts of *S. birrea* leaves and bark, which showed that for all extracts, the bark exhibited the highest activity. This richness of hydroethanolic extracts of *S. birrea* bark had already been reported by Niang *et al.* (2021) with IC_{50} = 0.156±0.0007 mg/mL. These data corroborate those of numerous studies that have demonstrated a high content of antiradical substances in trunk bark of other medicinal species. For instance, with *Anacardium occidentale* Da Silva *et al.* (2016) and Togola *et al.* (2020) reported IC_{50} values of 1.12 µg/mL and 5.24±0.34 µg/mL respectively; with *Ficus platiphylla* Konaré *et al.* (2020) found IC_{50} =

5.10±0.25 µg/mL. This could explain the preference for the trunk bark and the leaves by the patients with high blood pressure (Sène *et al.*, 2018).

The literature reported that the free radicals contribute to the occurrence of many diseases (cancer, skin cell diseases, diabetes, hypertension and other cardiovascular diseases (Drame *et al.*, 2022; Niang *et al.*, 2021). So, the patients who suffered from these pathologies need antioxidants to better manage the excess of free radicals generated. Thanks to their richness in antioxidant, the plant extracts are helpful to fight against these diseases linked to the free radicals (Niang *et al.*, 2021; Togola *et al.*, 2023). The works carried out by Drame *et al.* (2022) demonstrated a normalization of total oxidant levels in type 2 diabetic patients, from 52% to 87%, six months after the introduction of *Moringa oleifera* leaf powder treatment. This radical-reducing capacity of *S. birrea* extracts would explain its incorporation in the antihypertensive phytomedicine "Nitrokoudang" developed in Mali by the Department of Traditional Medicine (DMT) (Sanogo *et al.*, 2009) and its use against many other pathologies by the African populations (Dembélé *et al.*, 2020; Niang *et al.*, 2021; Sène *et al.*, 2018).

Previously studies have shown that the plant extracts contribute to maintaining the balance of oxidative stress due to their trace element content, which is necessary for the functioning of physiological antioxidant enzymes (Glutathione peroxidase (GPx), Superoxide dismutase (SOD)) (Razis *et al.*, 2014). So, this recorded antioxidant capacity of *S. birrea* extracts would be due to its richness in minerals, polyphenols, vitamin C (Sène *et al.*, 2018). The same authors had demonstrated that *S. birrea* with its low iodine (6.5g/100g) and peroxide (4.35) indices could induce a good oxidative stability. These findings strongly support its traditional use and beneficial contribution in the metabolic diseases such as type 2 diabetes and hypertension (Drame *et al.*, 2022; Razis *et al.*, 2014; Sanogo *et al.*, 2009). These recorded data showed that *S. birrea* extracts could usefully contribute to the betterment traditional management of hypertension and other pathologies linked to the excessive free radical release.

Conclusion

The present work highlighted the richness of *S. birrea* leaf, bark and root extracts in bioactive substances and free radical scavengers. If the roots were found to be richer in polyphenols and flavonoids, the bark showed the best antiradical potential. The ethanol proved to be the best solvent for extracting phenolic compounds and antioxidants agents. This richness of bioactive substances and antioxidants would support the use of *S. birrea* in traditional medicine against hypertension, diabetes and other pathologies induced by the oxidative stress. To enhance the value of this species, this study needs to be extended by evaluating the antihypertensive and anti-inflammatory activities of hydroethanolic extract of *S. birrea*.

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