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Antioxidant and Antidiabetic Potential of Extracts from *Anacardium occidentale* and *Sclerocarya birrea*

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Abstract

Anacardium occidentale and *Sclerocarya birrea* are two medicinal plant species highly coveted for the traditional treatment of diabetes in Mali. This study aimed to investigate the chemical constituents and the anti-radical and antidiabetic potential of extracts from these two species. The phytochemical characterization tests identified several prominent chemical groups in the extracts. The best solvent for extracting polyphenolic and flavonoid compounds was 70% ethanol. The highest levels of polyphenols were presented by the leaves of *A. occidentale* (3.85±0.49 mg GAE/100) and the bark of *S. birrea* (2.58±0.08 mg GAE/100 g). As for flavonoids, the bark presented the best levels. The aqueous bark extracts of *S. birrea* and the hydroethanolic leaf extracts of *A. occidentale* with respective IC_{50}=174.61±2.77 µg/mL and 178.64±3.42 µg/mL showed the best anti-radical activity. Regardless of the species, the hydroethanolic extracts exhibited the highest inhibition potential of α-amylase, i.e., the lowest IC_{50}. These IC_{50} were 316.8±31.4 µg/mL for the bark of *S. birrea* and 4201±254 µg/mL for the leaves of *A. occidentale*. The kinetic parameters (Michaelis-Menten constants) found the inhibition mode non-competitive against α-amylase for each species. The current work reveals the richness of *A. occidentale* and *S. birrea* in bioactive compounds which would offer them antiradical and antidiabetic potential. These species should be more valorized for the management of diabetes in Mali.

Keywords: *Sclerocarya birrea*, *Anacardium occidentale*, Phytochemical composition, Antidiabetic potential, Antioxidant activity

Introduction

Diabetes is a medical condition that has different causes and is characterized by long-term high blood sugar levels. It is associated with a disruption in the way the body processes carbohydrates, lipids, and proteins. This disruption is caused by a defect in the action and/or secretion of insulin (DeFronzo *et al*., 2015; Skalli *et al*., 2019). This chronic hyperglycemia is associated with numerous long-term complications, especially affecting the eyes, kidneys, nerves, heart, and blood vessels (Drame *et al*., 2022; Goïta *et al*., 2023).

Diabetes mellitus is a major public health problem, that has reached a worrisome level. Globally, the number of diabetic patients has been increasing exponentially in recent years. Based on data from the International Diabetes Federation, the occurrence of diabetes in adults aged 20-79 (Diawara *et al*., 2023; Goïta *et al*., 2023) increased by over two-fold between 2000 and 2021, rising from 151 million individuals (equivalent to 4.6% of the global population) to 537 million individuals (10.5% of the global population) in 2021. Without sufficient action, we will expect 643 million people to be affected in 2030 (or 11.3% of the population) (IDF, 2021). At the rate of this trend, the figures will increase to 783 million (or 12.2%) by 2045. At the same time, Africa records 24 million cases, with a forecast of 55 million by 2045 (IDF, 2019; IDF, 2021; Sun *et al*., 2022). Epidemiological studies have shown that this pathology affects all categories of populations indiscriminately. For example, in Mali, 1.2 million young people under the age of 20 were victims compared to 152,500 adults (Martini *et al*., 2022; Diawara *et al*., 2023).

The use of insulin and other medications such as biguanides, α-glycosidase inhibitors, and sulfonylureas is one of the current therapeutic approaches for treating this illness. Therefore, numerous therapeutic measures have emerged to control this pathology. While these medications have visibly helped to provide relief to those affected, numerous adverse effects caused by their regular intake have been documented. Among these side effects are hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea (Le Jeunne & Vital Durand, 2022). Therefore, managing diabetes with very few or no side effects remains a challenge for the scientific and medical community (Saxena & Vikram, 2004; Lee *et al*., 2021). With this in mind, numerous studies have been conducted on the continent to identify the plants used in the traditional treatment of this pathology (Diallo *et al*., 2004; Asir *et al*., 2014; Skalli *et al*., 2019; Lee *et al*., 2021; Kashtoh & Baek, 2023). These studies revealed that in addition to conventional diabetes management, many patients use plants.

Herbal remedies are frequently thought to be less harmful and cause fewer side effects when compared to synthetic medications (Asir *et al*., 2014; Haidara *et al*., 2024). Given that it incorporates knowledge passed down orally and generation to generation to a select group of people known as traditional healers and herbalists (Makhkamov *et al*., 2024), the art of plant healing has been known and practiced for a very long time in Africa (NoÃ *et al*., 2020; Togola *et al*., 2020; Goumou *et al*., 2022). Many traditional

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remedies are based on medicinal plants, and research carried out in Mali has made it possible to confirm its antidiabetic properties and characterize certain active antidiabetic ingredients, such as Diabetisane, which is based on *Sclerocarya birrea* marketed by the Department of Traditional Medicine (DMT) (Keita *et al*., 1998). The organs of *Anacardium occidentale* are also mentioned by many authors in Mali for managing diabetes (Olatunji *et al*., 2005; Togola, 2014; Togola *et al*., 2020). In addition to their antidiabetic properties, the different parts of *S. birrea and A. occidentale* are endowed with analgesic, antiplasmodial, anti-inflammatory, antidiarrheal, antimicrobial, antihypertensive, anticonvulsant, gastroprotective and antioxidant properties (Araújo *et al*., 2015; Séne *et al*., 2018; Togola *et al*., 2020).

Despite these numerous advantages, as far as we are aware, these two kinds of plants (*S. birrea* and *A. occidentale*) belonging to the Anacardiaceae family have not been well investigated for their pharmacognostic properties in Mali. The current study focused on the evaluation of their phytochemical profiles, and antioxidant and antidiabetic potential.

Materials and Methods

Material

The plant material consisted of the bark and leaves of *S. birrea* and *A. occidentale*, collected in the locality of Béléko (Koulikoro region, Mali). After identification and authentification by the Botanists from the Laboratoire de Botanique et d'Ecotoxicologie (Labotec) of the University of Sciences, Techniques and Technologies of Bamako (USTTB) for the identification and authentification of plant species, these samples were dried at room temperature under shade in the Laboratory of Food Biochemistry and Natural Substances (LBASNa).

Extract Preparation

Ten grams (10 g) of powdered coarse from each organ (leaves and bark) was macerated in 200 mL of solvent (water or 70% ethanol) for two hours before vacuum-filtering. The filtrates were combined after this process was carried out three times. The filtrate was kept cold before being used for future qualitative and quantitative investigations.

Determination of Phytochemical Composition

Phytochemical Screening

Phytochemical characterization tests were carried out on the different extracts utilizing qualitative methods following the guidelines provided by Shaikh and Patil (2020) and Konaré *et al*. (2020). The results obtained were evaluated as follows: (+) for a positive test and (-) for a negative test. The phytochemical groups were detected by different tests or reagents. The alkaloids were detected by the Dragendorff and Mayer reaction / Kraut test while the flavonoids were detected by the alkaline reagent test; the tannins by the Braymer test; the coumarins by the NaOH test; the sterols and terpenoids by the Salkowski test; the anthraquinones by Borntrager test; and the saponins by the foam test.

Determination of Total Phenolic and Flavonoid Compounds

With minor variations, the spectrophotometric approach suggested by Konaré *et al*. (2023) was followed in the quantification of polyphenols and flavonoids.

Determination of Total Polyphenols

In summary, 200 µL of Folin-Ciocalteu reagent was combined with 200 µL of extract. After 5 minutes, a volume of 600 microliters of disodium carbonate (Na_2CO_3) solution with a concentration of 7.5% was introduced. The reaction mix was allowed to react for 2 hours at room temperature without exposure to light. Afterward, the absorbance of the mixture was measured at a wavelength of 765 nm using a spectrophotometer (Thermo Scientific, Biomate 3S). Under the same working conditions, a calibration curve was made using different amounts of gallic acid, from 20 to 120 µg/mL. It is measured in milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Calculations

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

$$m = \frac{C \times Vf}{Ti} \times Fd \quad (1)$$

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)

Dosage of Total Flavonoids

800 µL of distilled water and 50 µL of a 5% sodium nitrite ($NaNO_2$) solution were mixed with 200 µL of each extract. It took 5 minutes, and then 50 µL of 10% aluminum chloride ($AlCl_3$) was added. Six (6) minutes later, 400 µL of 1 M sodium hydroxide (NaOH) was added. Finally, 1 mL of distilled water was introduced into the tube containing the mixture. The mixture was well homogenized before reading its absorbance at 510 nm using a spectrophotometer (Thermo Scientific, Biomate 3S). Quercetin concentrations ranging from 20 to 120 µg/mL were used to create a calibration curve under identical operating conditions. The findings were expressed as milligrams of catechol equivalent per gram of extract (mg CE/g).

The same formula described above was used to calculate the total flavonoid content.

Determination of Antioxidant Activity

The antioxidant activity was performed by the DPPH (2.2-diphenyl-1-picryl-hydrazyl) test according to the protocol used by Togola *et al*. (2019).

To begin, add 50 µL of each extract at varying concentrations to 1.95 mL of a methanolic DPPH solution (0.024 g/L). To create a

negative control, 50 µL of methanol was mixed with 1.95 mL of the DPPH solution. After 30 minutes of dark incubation at room temperature, the absorbance was measured at 515 nm against a blank using a spectrophotometer (Thermo Scientific, Biomate 3S). Ascorbic acid, a standard antioxidant, served as the positive control, and its absorbance was measured under the same conditions as the samples.

The antioxidant capacity associated with the ability to capture the DPPH radical was quantified as a percentage of inhibition, which was calculated based on the absorbances obtained using the following formula:

$$\text{Inhibition (\%)} = [1 - \frac{Asborbance\ of\ sample}{Asborbance\ of\ negative\ control}] \times 100 \quad (2)$$

Determination of Antidiabetic Potential

The alpha-amylase activity inhibition test was used to assess the antidiabetic potential according to the protocol described by Konaré *et al*. (2023). The mode of inhibition or action of this enzyme was evaluated according to the method reported by Ali *et al*. (2006).

Potential for Inhibiting α-Amylase Activity

In summary, 100 µL of each extract and 100 µL of a sodium phosphate buffer (pH 6.9) containing 100 µL of α-amylase solution at a concentration of 10 µg/mL were combined in a tube and incubated at a temperature of 25ºC for 10 minutes. A substrate consisting of 100 µL of 1% starch dissolved in 0.02 M sodium phosphate buffer (pH 6.9) was introduced and then incubated at 25ºC for 10 minutes. Afterward, 200 µL of dinitrosalicylic acid reagent (DNS) was added to stop the reaction. Subsequently, the tubes were placed in a water bath that was heated to its boiling point and kept there for 5 minutes. Following the cooling process, the reaction mixture was diluted with 2 mL of distilled water. The absorbances were then measured at a wavelength of 540 nm using a spectrophotometer (Thermo Scientific, Biomate 3S). The negative control was prepared using distilled water instead of the extract, following the same procedure. The activity of the positive control was evaluated by substituting the extract with a solution of acarbose (a reference molecule) at concentrations ranging from 150–1200 µg/mL. The α-amylase inhibitory activity was calculated and was expressed as a percentage inhibition.

$$\%\ \text{Inhibition} = [1 - \frac{\text{Asborbance of sample}}{\text{Asborbance of negative control}}] \times 100 \quad (3)$$

The IC_{50} values, which represent the concentrations of the extracts that result in 50% inhibition of the enzyme, were determined using Microsoft Excel through graphical analysis.

Mode of Inhibition of α-Amylase

The ethanolic leaf extract of *A. occidentale* and the aqueous leaf extract of *S. birrea* were selected to determine the mode of inhibition. These extracts were chosen due to their strong ability to inhibit the amylase activity.

An amount of 250 µL of the extract, which had a concentration of 100 µg/mL, was mixed with 250 µL of α-amylase solution. The mixture was then incubated for 10 minutes at a temperature of 25 ºC in a sealed tube. To initiate the reaction, a starch solution with varying concentrations (ranging from 0 to 2.5 mg/mL) was added to each mixture, with a volume of 250 µL. The mixtures were subjected to incubation at a temperature of 25 degrees Celsius for a duration of 10 minutes. Subsequently, 500 µL of DNS was added to halt the reaction, and the mixtures were then heated in a water bath for 5 minutes. The amount of reducing sugars released was quantified using spectrophotometry at a wavelength of 540 nm.

For the negative control, the extract was replaced with phosphate buffer (pH 6.9).

A Lineweaver-Burk plot was created to analyze the mode of inhibition by plotting the reciprocal of the reaction rate (1/v) against the reciprocal of the substrate concentration (1/[S]). The reaction rate (V) was calculated using the following formula.

$$\text{Reaction rate (V)} = \frac{\text{Absorbance at 540 nm}}{\text{Reaction time (min)}} \quad (4)$$

Data Analysis

The statistical analysis of the data was performed using Minitab 18.1 software.

Results and Discussion

Phytochemical Composition

The phytochemical studies revealed the presence of many major chemical groups in the leaf and bark extracts of *S. birrea* and *A. occidentale*, as depicted in **Table 1**.

All the extracts contained tannins, flavonoids, and terpenoids. However, coumarins were only found in the aqueous leaf extracts of the two species. Anthraquinones were detected only in the bark extracts of *A. occidentale*.

Table 1. Chemical constituents of the samples

	Aqueous extracts				**Hydroalcoholic extracts**			
	S. birrea		*A. occidentale*		*S. birrea*		*A. occidentale*	
Chemical groups	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf
Alkaloids	+	+	+	-	-	+	-	-
Tannins	+	+	+	+	+	+	+	+

Flavonoids	+	+	+	+	+	+	+	+
Coumarins	-	+	-	+	-	+	-	+
Saponines	-	-	-	+	-	-	-	-
Sterols and triterpenes	+	+	+	+	+	-	+	+
Anthraquinones	-	-	+	-	-	-	+	-
Terpenoids	+	+	+	+	+	+	+	+

Note. Presence (+); absence (-)

Table 2 shows the results of the determination of total polyphenols from the gallic acid calibration curve (y = 0.0452x - 0.0115; R^2 = 0.9901) and those of flavonoids from the catechol calibration curve (y = 0,002x - 0,004; R^2 = 0,99). The highest levels of phenolic compounds were obtained from the hydroethanolic extracts for each species and organ ($p<0.05$). The polyphenol levels for *S. birrea* were 2.58±0.08 mg GAE/100 g for the bark and 0.94±0.03 mg GAE/100 g. Regardless of the species, the barks were the richest organ in terms of phenolic compounds, except for *A. occidentale*. With this species, the leaves were richer in polyphenols ranging from 3.85±0.49 to 4.08±0.29 mg GAE/100 g.

Table 2. Comparison of the phenolic compound amounts

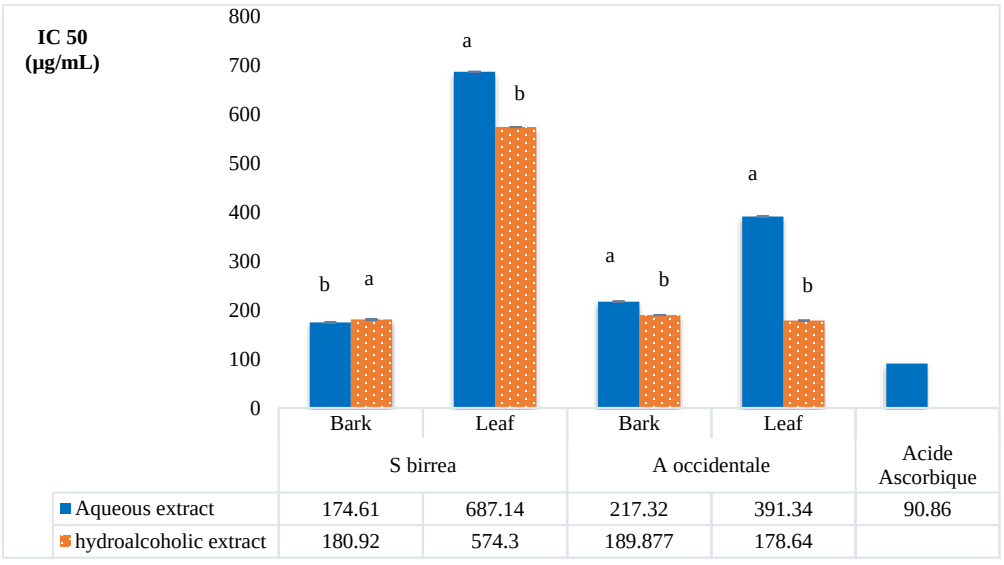
		S. birrea		***A. occidentale***	
Phenolic compounds	**Organs**	**Aqueous extracts**	**Hydroethanolic extracts**	**Aqueous extracts**	**Hydroethanolic extracts**
Polyphenols (mg GAE/100 g)	Bark	0.86 ± 0.16^{aB}	2.58 ± 0.08^{aA}	2.02 ± 0.12^{bA}	2.08 ± 0.03^{bA}
	Leaf	0.12 ± 0.00^{bB}	0.94 ± 0.03^{aA}	3.85 ± 0.49^{aA}	4.08 ± 0.29^{bA}
Flavonoids (mg CE/100 g)	Bark	0.37 ± 0.01^{aB}	0.81 ± 0.02^{aA}	0.35 ± 0.04^{aB}	0.64 ± 0.01^{aA}
	Leaf	0.08 ± 0.01^{bB}	0.56 ± 0.05^{bA}	0.18 ± 0.00^{bB}	0.24 ± 0.00^{bA}

Note. For each phenolic compound and each organ of the same extract and from the same plant, at 0.05, there is a significant difference between the averages that don't contain any lowercase letters. The mean values that do not share any bold, capital, or italic letters are significantly different at 0.05 for every extract from the same organ and plant.

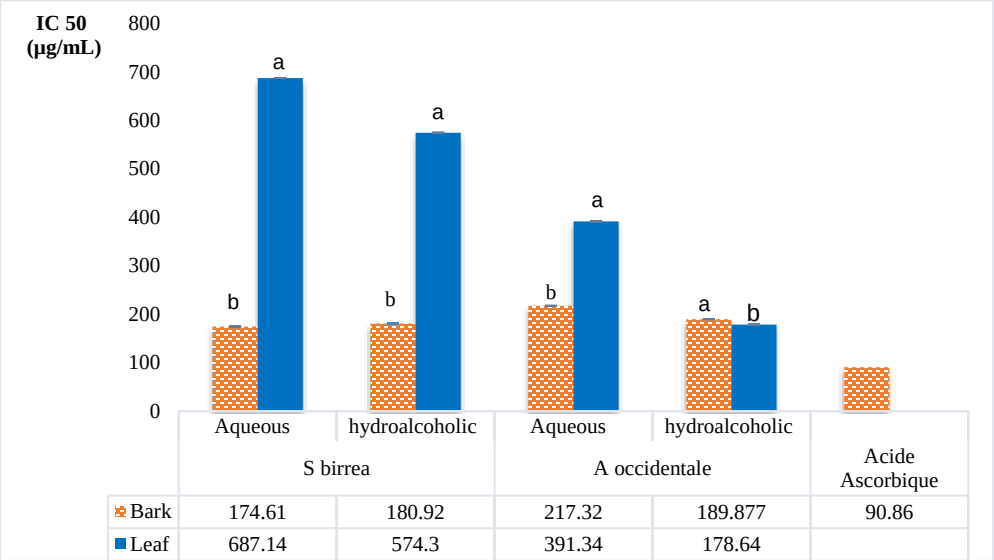
Antioxidant Potential

The antiradical potential of the extracts was evaluated by the DPPH method. The results obtained are expressed as the IC_{50} **(Figure 1)**.

Figure 1a shows the antiradical activity expressed as the IC_{50} (µg/mL) per species and organ. The results showed that the antiradical potential varied from one extract to another ($p<0.05$). **Figure 1b** shows that regardless of the species and extract (aqueous or hydroalcoholic), the barks possessed greater activity than the leaves. The lowest activity was obtained by the aqueous extract of the *S. birrea* leaves (687.14 µg/mL), and the highest activity was obtained by the aqueous bark extract of *S. Birrea* (174.61 µg/mL).



a)



b)

Figure 1. Comparison of inhibitory concentrations reducing 50% of DPPH (IC_{50}): according to the type of extracts (a), and to the plant parts (b)

Note. For each extract from the same plant and the same organ, at 0.05, there is a significant difference between the averages that don't contain any lowercase letters **(Figure 1a)**. For each organ of the same plant and the same extract type, at 0.05, there is a significant difference between the averages that don't contain any lowercase letters **(Figure 1b)**.

Antidiabetic Potential

The results of the inhibition rate and mode α-amylase assays are represented in the following paragraphs.

Potential for İnhibiting α-Amylase

Figure 2 shows the comparisons of the α-amylase inhibition rate depending on the organ and the extract type.

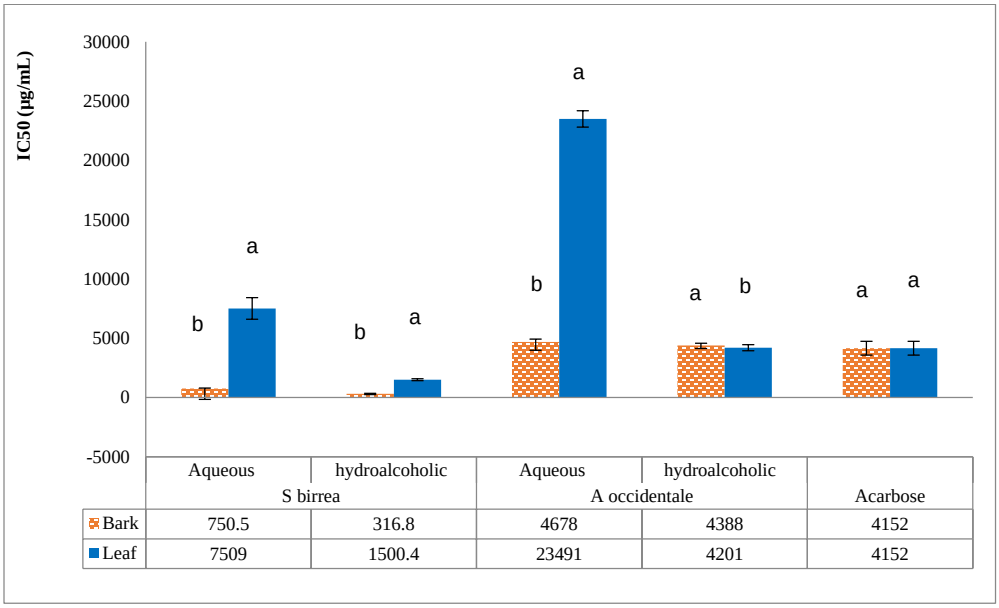


Figure 2. Comparison of the inhibitory concentrations 50 (IC_{50}) of the organs of each extract and each plant on α-amylase

Note. For each organ of the same type of extract and the same plant, at 0.05, there is a significant difference between the averages that don't contain any lowercase letters.

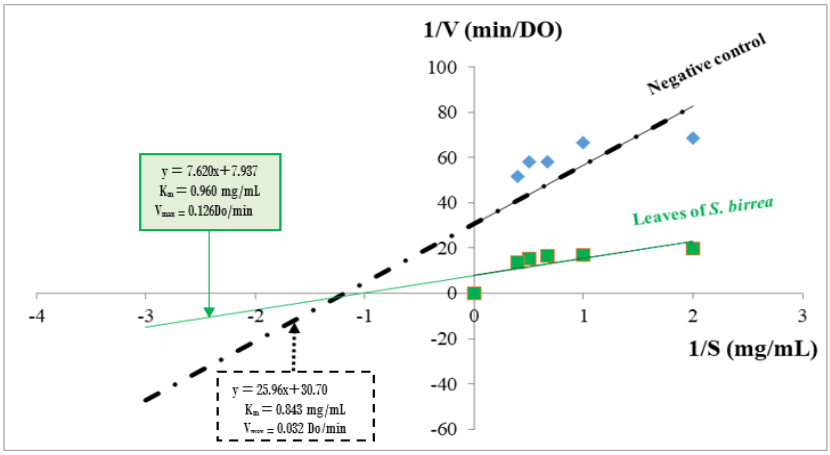
The results show that this potential varies from one solvent to another and from one organ to another (***ρ<0.05***). The highest inhibitory activity was obtained from the hydroalcoholic extracts, and that of the barks of *S. birrea* was the greatest (316.8 µg/mL) **(Figure 2)**. For *A. occidentale*, the greatest inhibitory activity against amylase (4201 µg/mL) was recorded for the hydroethanolic leaf extract **(Figure 2)**.

Mode of α-Amylase İnhibition

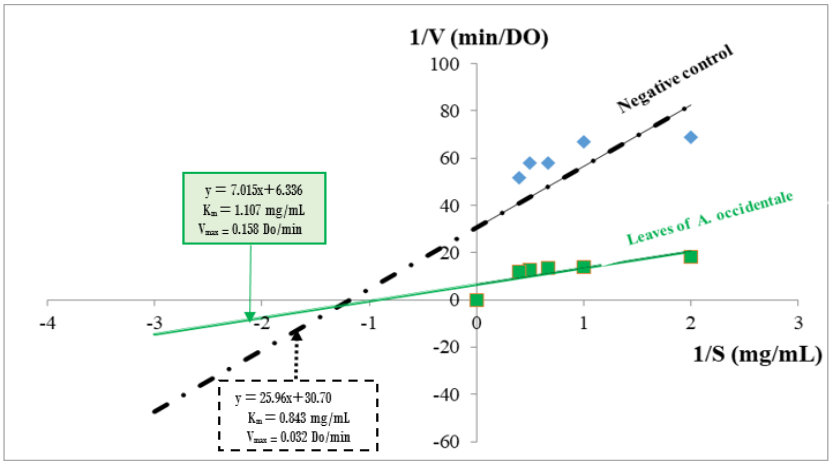
The kinetics of inhibitory effects of the aqueous leaf extracts of *S. birrea* and the hydroethanolic leaf extracts of *A.* occidentale on α-amylase are shown in **Figure 3**.

The Lineweaver-Burk plots obtained from the reciprocal of the reaction rate (1/v) against the reciprocal of the substrate concentration (1/[S]) generated the following regression equations: y = 25.96x+30.70; y = 7.620x+7.937, and y = 7.015x+6.336 for the positive control, the aqueous leaf extract of *S. birrea*, and the leaf hydroethanolic extract of *A. occidentale*, respectively (where x is the inverse of the substrate concentration and y the inverse of the reaction rate). The Michaelis-Menten constants (V_{max} and K_m) deduced from these equations were for the different leaf extracts.

Based on the results represented in **Figures 3**, the x-axis approximately at -1 corresponded to Km = 1 mg/mL, as mentioned in **Table 3**. Thus, the inhibitory mode of the investigated extracts on α-amylase was found to be non-competitive. At different crossing points with the y-axis indicating different maximum speeds, the *A. occidentale* extracts have presented the highest reaction rate (0.158 Do/min) **(Figure 3b)**, followed by those of *S. birrea* (0.126 Do /min) **(Figure 3a)** and the positive control (0.032 Do/min). All of these extracts exhibited the same affinity for the tested enzyme, with Km values ranging from 0.960 to 1.107 mg/mL.



a)



b)

Figure 3. Lineweaver-Burk curve of the aqueous leaf extract of *S. birrea* on α-amylase (a) and the leaf hydroethanolic extract of *A. occidentale* on α-amylase.

Table 3. Values of Km and Vmax obtained from the inhibition curves

	Positive control	***S. birrea***	***A. occidentale***
Regression equation	y = 25,96x+30,7	y = 7,620x+7,937	y = 7,015x+6,336
K_m (mg/mL)	0,843	0,960	1,107
V_{max} (Do/min)	0,032	0,126	0,158

Note. x: the inverse of the substrate concentration and y: the inverse of the reaction rate.

The presence of tannins, flavonoids, and terpenoids was revealed by the phytochemical screening of leaf and bark extracts. Numerous earlier works have also reported the presence of these metabolites (Togola *et al*., 2020; Konaré *et al*., 2024). This study revealed that the organs of *S. birrea* and *A. occidenta*le are very rich in secondary metabolites. The antihyperglycemic power due to the synergic action between these highlighted biocompounds were reported in the literature (Kashtoh & Baek, 2023).

Phenolic compounds were found in the hydroalcoholic extracts at the highest concentrations (2.58 and 0.94 mg GAE/100 g) of *S. birrea*, and regardless of the extract, there was no significant difference for *A. occidentale*. Depending on the organ, the maximum was recorded for the barks (from 0.86 to 2.53 mg GAE/100 g) of *S. birrea*. In contrast, *A. occidentale* where the greatest level was found in the leaves (from 3.85 to 4.07 mg GAE/100 g). For the flavonoids, the greatest levels were recorded for the hydroalcoholic bark extracts of *S. birrea* (0.81 mg CE/100 g) and *A. occidentale* (0.64 mg CE/100 g). Our results are similar to those of numerous other works reporting the best yields of phenolic compounds with hydroalcoholic extracts (Togola *et al*., 2020; Metri *et al*., 2024). Compared to our results, Togola *et al*. (2020) obtained significantly lower results, with 211.2 mg GAE/g for polyphenols and 58.64 mg EQ/g for flavonoids in the methanolic leaf extracts of *A. occidentale*. For *S. birrea*, we recorded a slight difference compared to the results of Konaré *et al*. (2024) in which the ethanolic extracts of the roots had the best yields of polyphenols and flavonoids, with 822.6 mg GAE/100 g and 112.59 mg EQ/100 g, respectively; followed by those of the barks with 761.4 mg GAE/100 g and 85.1 mg EQ/100g. Differences in total phenolic content can be attributed to genetic variation, and different environmental and climatic conditions (Hamata wallet *et al*., 2020; Metri *et al*., 2024).

The results of antioxidant determination expressed as the IC_{50} (µg/mL) revealed a significant reduction in DPPH radical. The antiradical activity varied from one extract to another ($p<0.05$). We note that whatever the extract (aqueous and hydroalcoholic), the bark of *S. birrea* had a stronger anti-radical activity with 174.61 and 180.92 µg/mL in contrast to the foliage. Additionally, a notable variation in anti-radical potential was noted depending on the organ of *A. occidentale*, while the aqueous bark extracts demonstrated the greatest potential ($\rho=0.005$ E-3<0.05), the ethanolic leaf extracts provided a high activity ($\rho=0.005<0.05$). Our results agreed with those of Togola *et al*. (2020) for *A. occidentale*, who observed the best activity with the alcoholic bark extracts. As for, *S. birrea*, we observed a slight difference from the results of Konaré *et al*. (2024), who found that *S. birrea*'s potential to scavenge DPPH radicals was comparable to that of the ethanolic extracts from the bark, with an IC_{50} = 65.57±2.34 µg/mL. A recently study showed, in addition to its antihyperglycemic and antioxidant potential, treatment with *A. occidentale* also offers lipid-lowering properties in diabetic patients (Olatunji *et al*., 2005). Due to this lipid-lowering power, these extracts could be useful to prevent atherosclerotic cardiovascular disease (ASCVD) which is accelerated in people living with diabetes, and the major cause of morbidity and mortality in patients living with diabetes (Zivkovic *et al*., 2023; Chait *et al*., 2024).

Globally, regardless of the species, the best anti-radical potential was found in the bark. Given that flavonoids and total polyphenols are more common in this reaction, their high concentration may contribute to their exceptional ability to lower the DPPH radical (Olatunji *et al*., 2005; Konaré *et al*., 2023).

The results of the enzymatic activity inhibition test revealed that all the extracts of the two plant species can inhibit the activity of α-amylase and therefore have antihyperglycemic power. Overall, the statistical tests revealed that the inhibitory potential of α-amylase activity varied significantly ($\rho<0.05$) according to the extraction solvent and the plant organ (AlShammasi *et al*., 2024; Gehrke *et al*., 2024; Ludovichetti *et al*., 2024; Menhadji *et al*., 2024; Ravoori *et al*., 2024).

The hydroethanolic bark extracts of *S. birrea* were the most active, with the lowest IC_{50} (316.8±31.4 µg/mL) while the aqueous leaf extracts of *A. occidentale* (IC_{50} = 4201 µg/mL) exhibited the strongest anti-amylase activity. Compared with that of standard acarbose, the IC_{50} of *A. occidentale* extracts in inhibiting α-amylase may be greater, indicating moderate inhibition of the enzyme, which is ideal for effective diabetes medication (Bajalan *et al*., 2016). This would avoid excessive inhibition of α-amylase (Mbhele *et al*., 2015). Many secondary metabolites identified in our extracts (particularly phenolic compounds) are known to have hypoglycemic effects (Kazeem & Ashafa, 2017). Recent works have demonstrated the strong involvement (R^2 = 0.94) of polyphenols and flavonoids in the inhibition of amylase activity (Yahia *et al*., 2020; da Veiga Correia *et al*., 2022; Anarado & Iziga, 2023; Konaré *et al*., 2023). Especially, flavonoids are reputed to be the most ubiquitous group of secondary metabolites found in plants (Zhu *et al*., 2020; Kashtoh & Baek, 2023). They are broadly studied due to their large range of bioactivities, which include anti-oxidant (Hoang Anh *et al*., 2020), anti-inflammatory (Maleki *et al*., 2019), anti-microbial (Górniak *et al*., 2019), and anti-diabetic properties (Proença *et al*., 2022; Konaré *et al*., 2023). Various molecules belonging to the groups of flavonoids have the potential to inhibit α-amylase enzymes due to their non-covalent binding ability to the active site residues of the enzyme (da Veiga Correia *et al*., 2022). Otherwise, Konaré *et al*. (2023) have also highlighted a strong correlation (R^2 = 0.91) between the antioxidant potential (DPPH) of the plant extract and the ability of the extract to reduce the activity of amylase on starch.

With this ability to inhibit α-amylase (a starch metabolizing enzyme), these extracts could help slow carbohydrate digestion, which prevents hyperglycemia.

To understand the mode of inhibition of this enzyme, a Lineweaver–Burk plot was generated, which allowed us to obtain curves that intersect on the abscissa axis with those of the control, thus indicating noncompetitive inhibition. The hydroalcoholic leaf extracts of *A. occidentale* exhibited the greatest reaction rate (Vmax = 0.158 Do/min), followed by the aqueous leaf extracts of *S. birrea* (Vmax = 0.126 Do/min). These data showed that the extracts from the two species bind to the enzyme without preventing the binding of the substrate (starch), i.e., they have their binding site on the enzyme different from that of the substrate. Our results support those obtained by Kazeem *et al.* (2017), who also

revealed a non-competitive inhibition with the *Eucomis humilis* species. However, Meng *et al*. (2016) found competitive inhibition with other medicinal species (*Hovenia dulcis*). Based on the affinity parameter of Michaelis-Menten (Km), all the tested extracts presented similar Km values ranging from 0.960 to 1.107 mg/mL, which are close to that of the reference molecule, acarbose (Km = 0.843). Thus, with this inhibitory effect on amylase, these two plant species could be potential sources of bioactive molecules useful for the treatment of diabetes.

Conclusion

The present study investigated the phytochemical richness and the anti-radical and antidiabetic potential of the leaf and bark extracts from *S. birrea* and *A. occidentale*. The barks of *S. birrea* and the leaves of *A. occidentale* presented the greatest levels of phenolic compounds. The highest levels of flavonoids were detected in the bark of the two species. Globally, regardless of the species, hydroethanolic extracts are the most active at reducing the radical DPPH and inhibiting amylase activity on starch; therefore, hydroethanolic extracts have the best antidiabetic potential. Furthermore, whether the hydroalcoholic leaf extracts of *A. occidentale* exhibited the fastest mode of action on α-amylase, followed by the aqueous leaf extracts of *S. birrea*, showed that both extracts had the same affinity. The mode of action on the tested substrate (starch) of these extracts was found to be non-competitive with α-amylase to reduce blood sugar, which would explain their anti-diabetic effect. In conclusion, the two plant species under investigation may be potential sources of bioactive molecules useful in diabetes treatment.

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