

Comparative Study of the Phytochemistry and Antioxidant Activity of *Anacardium occidentale* (L.) Leaf and Stem Bark Extracts

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Abstract: The use of plant roots and stem barks is a common practice in traditional medicine. This practice could lead to the disappearance of some plant species. *Anacardium occidentale* (L.) is a plant whose stem bark is commonly used for the traditional management of diabetes and hypertension in Mali. In the hope of replacing this organ with the leaves, a comparative phytochemical study of the two organs was carried out. The Phytochemical screening was carried out through colouring and precipitation reactions. The Folin-Ciocalteu reagent was used to determine the content of total phenolic compounds whereas the flavonoids were determined using aluminium trichloride. Antioxidant activity was evaluated by the TAC and DPPH methods. The results obtained have shown that both organs were rich in secondary metabolites with a similar phytochemical profile. Also, it was found that regardless of the solvent used, the leaves contained the highest levels of total phenols and flavonoids. Thus, the contents of total phenols in methanolic extracts have been 211.2±21.8 mg GAE/g and 129.72±5.15 mg GAE/g for leaves and stem barks respectively. As for those of flavonoids, they have been 59.02±5.88 mg QE/g for leaves and 31.30±2.74 mg QE/g for stem barks. However, the stem barks showed the higher antioxidant activity than the leaves, which is also appreciable. In sum, other studies such as toxicological one must be conducted before replacing stem barks with leaves in the traditional management of these two pathologies.

Keywords: *Anacardium occidentale*, Phytochemistry, Antioxidant Activity, Leaf and Stem Bark

1. Introduction

African populations are facing the emergence of diabetes and high blood pressure (hypertension), diseases that are said to be new to traditional African medicine [1]. In Sub-Saharan Africa, the prevalence of hypertension is high among adults aged 18 years and older, ranging from 16% to 40%. In some studies, the prevalence exceeds 60% among people aged 65 years and older [2]. In 2013, there were 19.8 million people

with diabetes, and this number is expected to increase to 41.4 million in 2035 [3]. In Mali, these two pathologies are increasingly becoming a real public health problem. Indeed, the prevalence of hypertension varies according to the studies from 20.83% to 39.4% [4]. According to the International Diabetes Federation, in 2019 in the 20-79 age group, there were 2,669 deaths due to the diabetes in the country and the number of people living with diabetes was 157,600 [5]. The treatment and monitoring of these diseases is an additional economic problem. In addition, there is a lack of specialists

in the area, remoteness, the scarcity or absence of health centres in villages, and the unavailability and excessive cost of pharmaceutical products [6]. More than 80% of the population uses medicinal plants for health care [7, 8]. *Anacardium occidentale*, a plant belonging to the Anacardiaceae family, is frequently used by the African population in the management of diabetes and hypertension [6, 9, 10, 1]. In Mali, the stem barks of the plant are the most appreciated organs and this practice is not safe for the plant. Indeed, the debarking of the plant has harmful effects such as blocking the transport of sap, increasing the risk of insect attack and limiting the plant's survival rate [11]. Moreover, the method of harvesting of stem bark and the increased use by the population make the species more threatened with extinction [12]. In the hypothesis that the stem barks of *Anacardium occidentale* could be substituted by its leaves, in the interest of preserving biodiversity, this work was carried out in order to compare the phytochemical compositions, as well as the antioxidant activity of the different extracts of the stem barks and leaves of this plant.

2. Material and Methods

The plant material used was the stem barks and the leaves of *Anacardium occidentale* harvested at Tousséguéla in Mali, coordinates are 11°03'14''North, 6°35'10''West. The samples were identified at the Laboratory of Tropical Ecology, University of Sciences, Techniques and Technologies of Bamako.

2.1. Preparation of Extracts

Twenty-five grams of powder of each organ (stem barks and leaves) were dissolved by magnetic stirring for 48 h at 30°C in 250 ml of different solvents (Methanol, Water and Ethyl Acetate). After filtration of the resulting mixture, the solvent was evaporated under reduced pressure. The dried extracts were recovered and stored in the freezer for analyses.

2.2. Phytochemical Screening

The extracts were analysed to highlight the different phytochemical groups. The protocols described by Harborne and Srivastava *et al.*, [13, 14] were used to carry out this work.

2.3. Dosage of Total Polyphenols

The contents of phenolic compounds in the different extracts of *Anacardium occidentale* were estimated by the Folin-Ciocalteu method described by Singleton *et al.*, [15]. Thus, 500 µL of Folin-Ciocalteu reagent (diluted 10% in distilled water) is added to 100µL of extract and 400 µL of disodium carbonate (Na₂CO₃) at 75mg/mL was added to the reaction mixture. After an incubation of 2 hours at room temperature and protected from light, the absorbance was read at 765 nm. A calibration curve was performed under the same operating conditions using a gallic acid dilution series. The results were expressed in milligram equivalent of gallic acid per gram of extract (mgGAE/g).

2.4. Dosage of Total Flavonoids

The estimation of total flavonoids was carried out according to the method described by Chang *et al.*, [16]. To 1000 µL of each extract to be analysed, 1500 µL of 95% methanol, 100 µL of 10% AlCl₃ (w/v), 100 µL of 1 M sodium acetate and 2.8 mL of distilled water were added. The mixture was stirred and incubated in the dark at room temperature for 30 min. The blank was made by replacing the extract with 95% methanol and the absorbance was measured at 415 nm using a UV spectrophotometer. The results were expressed in mg quercetin equivalent per gram of dry weight.

2.5. Antioxidant Activity

The DPPH radical scavenging test and the phosphomolybdate test were used to evaluate *in vitro* the antioxidant activity of the different extracts.

2.5.1. DPPH Radical Scavenging Activity

The ability to trap the stable free radical 1,1-diphenyl 2-picrylhydrazyl (DPPH) of *Anacardium occidentale* stem barks and leaves extracts was evaluated using the spectrophotometric method described by Brand-Williams *et al.*, [17]. Briefly 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of extract at various concentrations (2-12 µg/mL). At the same time, a mixture of 1 mL methanol and 1mL DPPH solutions was used as a control. The reaction was performed in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes incubation of the samples in the darkness. The positive control was ascorbic acid whose absorbance was measured under the same conditions as the samples. The antioxidant activity related to the scavenging effect of the DPPH radical is expressed as percentage inhibition (PI) calculated from absorbances obtained according to the following formula:

$$[PI] = \frac{A_0 - A_1}{A_0} \times 100$$

A₀=DPPH absorbance; A₁: sample absorbance.

IC₅₀ (concentrations that inhibit 50% of the DPPH radical) were inferred from the linear regression line obtained from the graph representing the percentage inhibition of DPPH.

2.5.2. Phosphomolybdate Test or Total Antioxidant Capacity (TAC)

The total antioxidant capacity was determined using the spectrophotometric method described by Prieto *et al.*, [18]. Thus, to 1ml of extract at concentrations ranging from 10 to 100 µg/ml was added the reagent composed of H₂SO₄ (600 mM), NaH₂PO₄ (28mM) and ammonium molybdate (4mM). The tubes were then incubated in a water bath at 95°C for 90 minutes. After cooling at room temperature, the absorbance was read at 695 nm. Ascorbic acid was used as standard and antioxidant capacity was expressed in mg ascorbic acid equivalent per gram of dry weight (mgAA/g DW).

2.6. Data Analysis

The data obtained were processed using Excel® version

2013 and Minitab 18.1 software. Analyses were run in triplicates and the results were expressed as mean values with standard error mean. The one ANOVA test using the Fisher's test was chosen to compare the levels of polyphenols, flavonoids and antioxidant activity of different types of extracts (ethyl acetate, water and methanol) from the stem barks and the leaves of *Anacardium occidentale*. P-values less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening

The Table 1 presents the results of the phytochemical screening of the different extracts from the stem barks and leaves of *Anacardium occidentale*. The table shows the presence of alkaloids in the stem bark extracts (aqueous and methanol extracts) and in the methanol leaf extract. The absence of tannins was found in the aqueous and methanol leaf extracts. The flavonoids and coumarins were detected in all tested samples. Except the aqueous extracts of the two organs, the terpenoids were observed in the other samples. The saponins were absent in the methanol stem bark extract and the ethyl acetate leaf extract while their presence was observed in the other plant extracts. According to the Table 1 the phytochemical profile of the two organs (stem barks and leaves) of *Anacardium occidentale* are almost similar. Our findings are close to those of some previous work [19-21]. According to Ifesan et al [22], the presence of these bioactive compounds in the organs of *Anacardium occidentale* is a strong indication that this plant has medicinal potency. Indeed, many studies have shown that the secondary metabolites contained in *Anacardium occidentale* extracts have hypoglycemic and anti-hypertensive effects [23-25].

Table 1. Phytochemical screening of *Anacardium occidentale* stem bark and leaf extracts.

Chemical Groups	Stem bark			Leaf		
	EAE	WE	ME	EAE	WE	ME
Alkaloids	-	+	+	-	-	+
Tannins	+	+	+	+	-	-
Flavonoids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Terpenoids	+	-	+	+	-	+
Saponins	+	+	-	-	+	+

Legend: +=positive. -=negative. EAE=Ethyl acetate extract. WE=Water extract. ME=Methanol extract.

3.2. Total Polyphenol and Flavonoid Contents

The levels of total phenolic compounds and flavonoids in the different types of stem bark and leaf extracts are shown in the Tables 2 and 3. Thus, the maximum of total phenolic compounds were obtained with the methanol extract from the leaves (211.2 ± 21.8 mgGAE/g) and the minimum with the aqueous extract from the stem barks (77.70 ± 1.06 mg GAE/g). The results in Table 2 showed that overall there is a significant difference between the samples ($p=2.09E-8$) and regardless of the solvent used, *Anacardium occidentale* leaves are richer in

total polyphenols than stem barks.

Table 2. Total phenol contents of *Anacardium occidentale* stem bark and leaf extracts.

Solvents	Total phenol (mg GAE/g)	
	Stem bark	Leaf
Ethyl Acetate	99.53 ± 0.84^c	138.47 ± 6.27^b
Water	77.70 ± 1.06^d	96.82 ± 7.14^c
Methanol	129.72 ± 5.15^b	211.2 ± 21.8^a

* For each parameter, the averages of each row that do not share any letters are significantly different at the threshold of 0.05.

Table 3. Flavonoids contents of *Anacardium occidentale* stem bark and leaf extracts.

Solvents	Flavonoids (mg QE/g)	
	Stem bark	Leaf
Ethyl Acetate	32.49 ± 0.53^d	44.09 ± 2.46^b
Water	25.02 ± 2.49^e	36.74 ± 1.32^c
Methanol	31.30 ± 2.74^d	58.64 ± 2.62^a

* For each parameter, the averages of each row that do not share any letters are significantly different at the threshold of 0.05.

Table 3 provides information on the levels of flavonoids in the leaves and stem barks of *Anacardium occidentale*. It shows that with the solvents used, the leaves performed the highest flavonoid contents while the stem barks exhibited the lowest values. Overall, there is a significant difference in flavonoid levels ($p=4.7E-9$). This fact has already been shown for total polyphenol contents. In this study, the results obtained with total polyphenols and flavonoids are different from those reported in previous studies [26-28]. This may be related to the genetic variation and geographical origins of the plant species [29].

3.3. In Vitro Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

Figure 1 shows that the different extracts from leaves and stem barks of *Anacardium occidentale* have antioxidant activities. The highest activity of the barks was observed with ethyl acetate extract with an IC_{50} of 5.24 ± 0.34 μ g/ml. For leaves, methanol and ethyl acetate extracts have shown similar antioxidant activity ($p=0.169$).

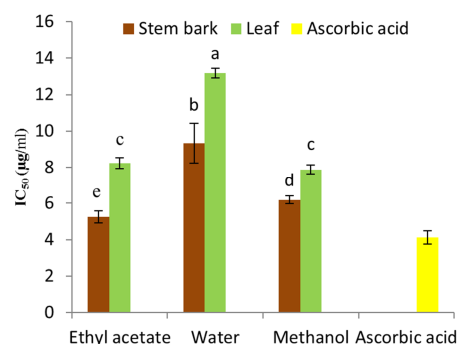


Figure 1. Reduction capacity of the DPPH radical expressed in IC_{50} (μ g/ml) as a function of the organs and the extraction solvent.

*Each value is expressed as a mean \pm standard error. The bar charts for each extract with different letters are significantly different ($p < 0.05$; $n=3$).

In 2016, a study conducted by Da Silva *et al.*, [30] showed that *Anacardium occidentale* stem barks and leaves have the similar antioxidant activity with IC₅₀ of 1.12 µg/mL and 1.47 µg/mL, respectively. However, the findings of Sija *et al.* [31] have shown that the young leaves have a higher antioxidant activity than stem barks.

3.3.2. Total Antioxidant Capacity (TAC)

In addition to the DPPH method, the antioxidant activity was evaluated by the phosphomolybdenum reduction test. Therefore, the total antioxidant capacity of the different leaf and stem bark extracts of *Anacardium occidentale* is summarized in the Table 4.

Table 4. Total antioxidant capacity of *Anacardium occidentale* stem bark and leaf extracts.

Solvents	Total antioxidant capacity (mg AAE/gDW)	
	Stem bark	Leaf
Ethyl Acetate	92.81±1.83 ^a	54.34±2.93 ^c
Water	37.83±1.26 ^e	18.12±1.23 ^f
Methanol	79.36±1.89 ^d	49.85±3.05 ^d

* For each parameter, the averages of each row that do not share any letters are significantly different at the threshold of 0.05

Data analysis showed a significant difference between the samples ($p=2.3E-13$). Ethyl acetate extracts exhibited the best antioxidant activities (92.81±1.83 mg EAA/gDW and 54.34±2.93 mg EAA/gDW for stem barks and leaves respectively). The water extract showed the lowest values with 37.83±1.26 mg EAA/gDW for the stem bark and 18.12±1.23 mg EAA/gDW for the leaves, the aqueous extracts of *Anacardium* organs showed low TAC. The different trends observed with DPPH radical scavenging was confirmed by this second method. The different stem bark extracts showed higher antioxidant activities than leaf extracts ($p<0.05$). Also for each organ considered, ethyl acetate extracts showed the strongest antioxidant activities. According to some authors, the antioxidant power of *Anacardium occidentale* depends on the extraction solvent [32-34]. For others, the antioxidant activity of *Anacardium occidentale* could be attributed to its phenolic constituents [30, 35]. However, this study has shown that the antioxidant activity of *Anacardium occidentale* is not only due to the activity of phenolic compounds. Indeed, the leaves had the highest levels of total phenolic compounds and flavonoids while the best antioxidant activity was observed in the stem barks. That may explain the preference for stem barks by the patients with diabetes and hypertension. However, in Chad and Cameroon the leaves of this plant are also used in the traditional management of these two diseases [6, 9]. It is necessary to note that both organs have proven antioxidant activity according to this study.

4. Conclusion

In this study, a phytochemical comparison of *Anacardium occidentale* leaves and stem barks was carried out. The screening of the extracts showed that the two organs have a

similar phytochemical profile. Leaves have the highest levels of total phenols and flavonoids. The antioxidant activity determined by the TAC and DPPH methods have indicated that the two organs have an appreciable reducing power and that the antioxidant activity of the stem barks is significantly higher. The results from the current work revealed that *Anacardium occidentale* leaves could be a potential source of antioxidant molecules. Our findings have shown that the leaves could validly replace the stem barks. That could be helpful for protecting better this species from an eventual extinction. However, more research is needed before advising the leaves in the management of diabetes and hypertension.

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