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Research Article

Phytochemical Screening and Antioxidant Activity of *Annona Senegalensis* Extracts (Leaves and Stem Bark) Collected from Three Regions of Senegal

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Abstract

The main objective of the present study is to focus on evaluation of the secondary metabolites composition and antioxidant activity of hydro – ethanolic extracts of *Annona senegalensis* leaves and stem bark which used in traditional medicine against several kind of diseases. The yields of hydro-ethanolic extracts of organs (leaves and Stem bark) were determined, followed by phytochemical screening and amount of secondary metabolites, by using conventional analytical methods. Anti-radical activity was carried out by DPPH (2, 2-diphenyl-l-picrylhydrazyl) reduction tests. The best extraction rates, whatever the plant organ or origin, were obtained with leaves (15.5% on average). Phytochemical screening revealed the presence of polyphenols, flavonoids and tannins. The highest levels of total polyphenols were obtained with leaves extracts [83.931 ± 0.018 g EGA/100 g Dry Matter (DM)] compared with stem bark (77.886 ± 1.001 g EGA/100 g DM). The highest tannin and flavonoid contents are 1.093 ± 0.006 g EC/100 g DM and 0.195 ± 0.004 g EC/100 g DM, respectively. Moreover, the extracts have a high antioxidant capacity with high inhibition percentages of 95.06 ± 0.10% for the leaves and 92.87 ± 0.10% for the bark. These results highlight the presence of bioactive compounds and potential antioxidant activity that have been shown to be efficient in the treatment of certain diseases, in accordance with ethno - botanical survey.

Keywords

Annona Senegalensis, Hydro-ethanolic Extracts, Phytochemical Screening, Antioxidant Activity

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1. Introduction

In West Africa, medicinal plants are widely used in the treatment of many pathologies. Their high content of bioactive molecules which are part of secondary metabolites make them very particular molecules [1]. These kinds of molecules contribute broadly to the treatment of serious illnesses such as cancer, diabetes and cardiovascular disease [2]. As a result, over 80% of the world's population use plants as an alternative to conventional medicines to satisfy primary health problems, dietary problems and, above all, the recurrent chemoresistance of pathogens to pharmaceutical drugs [3-6]. The antivenom properties of *Annona senegalensis* bark and roots against snakebites have already been reported in Senegal [7]. Various therapeutic properties of the same plant have also been reported in Nigeria by Alqasim [8]. In Senegal, leaves, stems and roots of *Annona senegalensis* are frequently used such as phytomedicine to relieve disease such as diarrhea, dysentery, stomach and head aches, in addition the plant is very accessible [6]. However, despite the considerable contribution of traditional medicine to phytotherapy of this plant in Senegal, it remains relatively unexplored by scientist researchers. To the best of our knowledge, there are few scientific publications relating the use of seeds and the oil it would contain, even though leaves, stem bark and roots are commonly used in pharmacopoeia [9, 10]. In this context, the objective of this study is to evaluate the chemical composition of secondary metabolites and the antioxidant activity of hydro-ethanolic extracts of *Annona senegalensis* leaves and stem bark, in order to justify their traditional use by senegalese society, particularly for medicinal and dietary purposes.

2. Materials and Methods

2.1. Plant Materials

Annona senegalensis, better known as wild apple or wild soursop, is a plant in the Annonaceae family. The plant is a shrub or sub-shrub measuring 1 - 4 m high and around 30 cm of diameter. The bark of the stem is smooth or coarse and silver-gray or gray-brown. Leaves are alternate, simple, oblong, oval or elliptical, green to bluish-green, almost hairless on the upper surface but often with brownish hairs on the underside.

The leaves and stem bark making up the plant material were collected in the mornings during the summer of 2022 in three regions of Senegal: Kolda (Saré Bounda village), Ziguinchor (Mangaloulack village) and Thiès (village Fandène). The samples were identified and authenticated at the Botanic Laboratory of the Fundamental Institute of Black Africa at the Cheikh Anta Diop University of Dakar.

Figure 1. *Photographs of Annona Senegalensis (a: plant of Annona senegalensis; b: leaves; c: stem husks).*

2.2. Extraction and Calculation of Extraction Rates

After washing with distilled water (to remove dust) and oven-drying at 65 ℃ in the laboratory for 24 hours, the leaves and stem bark were pulverized using an electric grinder (Kenwood, France). The powders were then extracted by decoction at a ratio of 10 g/100 ml solvent. Decoction extraction was carried out with a water/ethanol (80/20; V/V) solvent mixture under stirring, for thirty minutes. After cooling, the mixture is clarified in a Hittich Universal 16A centrifuge at 3,000 rpm for 10 minutes, then vacuum filtered on Wattman No.1 paper. The solvent is evaporated at 78 ℃ using a rotary evaporator, and dried for 24 hours at 40 ℃. Extracts are stored in a hermetically sealed sterile glass vial at 4 ℃ after determining extraction rates (Rdt) using the following formula:

$$Rdt(\%) = 100 * (\frac{P_1 - P_2}{PE}) \quad (1)$$

P1: mass of flask with dry extract; P2: mass of empty flask; PE: mass of dry matter

2.3. Chemical Composition of Metabolites

2.3.1. Phytochemical Screening

The presence of main families of secondary metabolites were analyzed in dry hydroethanol extracts of *Annona senegalensis* leaves and stem bark. Qualitative phytochemical analysis was carried out according to standard methods, using several tests: flavonoids (Shibata test), tannins (Stiasny reaction followed by ferric chloride reaction), alkaloids (Dragendorff and Mayer reagent), sterols (Liebermann-Buchard reaction) and saponosides (foam index) [11, 12]. Once the chemical compounds has been identified, total polyphenols, flavonoids and tannins were quantitatively determined.

2.3.2. Determination of Total Polyphenols

Total polyphenol content was determined using the Folin-Ciocalteu method [11]. Results are expressed in gram equivalent of gallic acid (g EGA) per hundred grams of dry matter (DM), using a calibration curve.

2.3.3. Determination of Total Flavonoids

The flavonoid content of extracts is determined using the colorimetric method described by Kim et al [12]. Results are expressed in grams of catechin equivalent (g CE) per hundred grams of DM, using a calibration curve.

2.3.4. Tannin Determination

Tannins are determined using the colorimetric method of Folin Denis [13, 14]. Results are expressed in gram equivalents of gallic acid (g EGA) per hundred grams of DM, based on a calibration curve.

2.4. Evaluation of Antioxidant Power by the DPPH Method

Antioxidant activity was assessed with DPPH (2, 2-diphenyl-1-picrylhydrazyl, Sigma, chemical company, USA) with a few modifications to the method [15, 16]. The method is based on the ability of an extract to donate a single electron to the dark purple DPPH free radical, stabilizing it as yellow-green DPPH. Thus, 100 µL of extracts are introduced into a test tube containing 1300 µL of DPPH (0.004% prepared in methanol). The negative control is prepared by mixing 100 µL of methanol with 1300 µL of DPPH methanolic solution. Absorbance is read against a blank prepared for each concentration at 517nm after 30 min incubation in the dark at room temperature. Samples were kept in the dark for 30 min at room temperature as well, and absorbance was measured at 517 nm using a UV/visible light spectrophotometer (Spectronic Genesys 8, Rochester, USA). All measurements were performed three times. Anti-free radical activity is expressed as percentage inhibition (PI) using the equation below:

$$PI(\%) = 100 * \left(\frac{A_{Co} - A_{Sa}}{A_{Co}}\right) \quad (2)$$

PI (%): Percentage of DPPH inhibition; A_{Co}: Control absorbance; A_{Sa}: Absorbance of sample.

2.5. Statistical Analysis

Analytical results obtained from three independent trials are subjected to analysis of variance (ANOVA) using STATISTICA 7.1 software. Statistical differences with a probability value of less than 0.05 are considered significant.

3. Results and Discussions

3.1. Rate of Extraction

Extraction rates for *Annona senegalensis* leaves and stem bark harvested in the three regions (Thiès, Kolda and Ziguinchor) are represented in Figure 2 below. The results show that the highest extraction rates (16.5% and 8.95%) were obtained with leaves and stem bark harvested in Kolda and Ziguinchor respectively. Similarly, the extraction rates for *Annona senegalensis* leaves were higher than those for stem bark.

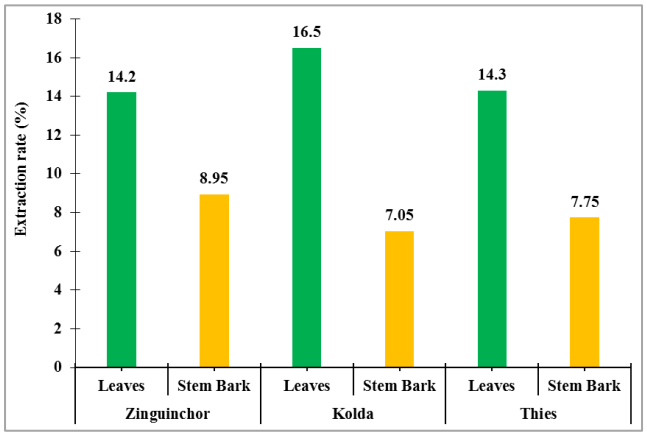

Figure 2. *Hydroethanol extraction rates for Annona senegalensis leaves and stem bark.*

Analysis of the results shows that extraction rates depend on the organ studied. Indeed, the best extraction rates were obtained with leaf extracts. These hydroethanolic extracts of *Annona senegalensis* leaves and stem bark were prepared by decoction of the powder of each organ in ethanol/water (80/20 V/V). The aim of using this solvent was to extract as many polar compounds as possible, as well as medium- and low-polarity compounds.

3.2. Phytochemical Screening

Phytochemical screening of hydroethanolic extracts obtained from leaves and stem bark of *Annona senegalensis*, revealed the presence of polyphenols, tannins, flavonoids and alkaloids. However, sterols and polyterpenes were absent, as were traces of saponins, as summarized in the following Table 1.

Table 1. *Phytochemical screening of Annona senegalensis leaves and stem bark extracts.*

Origin	Extracts	Flavonoids	Tanins	Alcaloids		Saponins	Sterols Triterpenes	Polyphenols
				MR	DR			
Kolda	Le	+	+	+	+	+	±	+
	Sb	+	+	±	+	+	-	+
Ziguin-chor	Le	+	+	+	+	±	±	+
	Sb	+	+	+	+	+	±	+
Thiès	Le	+	+	-	-	-	±	+
	Sb	+	+	±	+	-	-	+

MR: Mayer reagent; DR: Dragendorff reagent; Le: leaves; Sb: stem bark
+: Presence; ±: Trace presence; -: Absence

These results are consistent with other studies relating to *Annona*'s organs [16-22]. These metabolites are also frequently found in plant extracts such as *Sebastiania chamaelea* [23] and *Ziziphus mauritiana* Lam [24]. These various metabolites are known for their remarkable medicinal and physiological activities [25].

3.3. Total Polyphenol, Total Flavonoid and Total Tannins Contents

Table 2 shows that polyphenol, flavonoid and tannin contents vary significantly according to plant organ and origin. Quantitative estimation of certain metabolites present in the hydroethanol extract of *Annona senegalensis* shows that total polyphenol contents are the highest (31, 393 ±0.020 to 83.931 ± 0.018 g EGA /100 g DM), followed by tannins (0.849 ± 0.014 to 1.093 ± 0.006 g EGA /100 g DM) and flavonoids (0.0953 ±0.0001 to 0.1952 ±0.004 g EC/100 g DM). The best total polyphenol contents were obtained with leaves and stem barks extracts from Ziguinchor, with 83.931 ± 0.018 g EGA /100 g DM and 77.886 ± 1.001 EGA /100 g DM respectively. However, the lowest levels were obtained with samples from Thiès, with 39.118 ± 0.020 g EGA /100 g DM (leaves) and 31.393 ± 0.020 g EGA /100 g DM (bark). This difference could be explained by the nature of soils in relation to the plant's geographical location or climatic conditions. In Ziguinchor, for example, the weather is wetter than in the rest of the country, which means that pests are more likely to proliferate rapidly, prompting plants to synthesize secondary metabolites to defend themselves. The high quantities of secondary metabolites found in this study would justify the antibacterial activities demonstrated by Johnson and al. [26] and, Awa and al. [27]. Polyphenols are considered a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups. Low levels of flavonoids were found in *Annona senegalensis* leaves and stem barks extracts (Table 2). In addition, low tannin contents ranging from 0.849 ±0.014 to 1.093 ± 0.006 g EGA/ 100 g DM were noted for all samples. Flavonoids generally serve as flavouring ingredients in plants and are also expressed in plants in response to microbial infection, suggesting their antimicrobial activity [28]. Flavonoids are also antioxidants, such as those found in tea, which are thought to reduce oxidation of low-density lipoproteins and lower blood cholesterol and triglyceride levels [29]. The quantity of tannin is relatively low, and is practically the same in leaves and stem barks for all samples, regardless of origin. We note the same tendence for the quantity of flavonoids.

Table 2. *Amount of hydro-ethanolic extracts of Annona seneganensis.*

Origin	Extracts	Total polyphenol *(g EAG/100 g DM)*	Total flavonoid (g EC/100 g DM)	Total tannins (g EGA / 100 g DM)
Kolda	Le	49,363 ±0,018^{a}	0,095 ±0,001^{a}	0,995 ±0,011^{a}
	Sb	42,018 ±0,013^{b}	0,194 ±0,002^{b}	1,053 ±0,003^{b}
Ziguinchor	Le	83,931 ±0,018^{c}	0,099 ±0,004^{c}	1,093 ±0,006^{c}
	Sb	77,886 ±1,001^{d}	0,195 ±0,004^{d}	1,036 ±0,001^{d}

Origin	Extracts	Total polyphenol *(g EAG/100 g DM)*	Total flavonoid (g EC/100 g DM)	Total tannins (g EGA / 100 g DM)
Thiès	Le	39,118 ±0,020^{e}	0,096 ±0,004^{e}	0,849 ±0,014^{e}
	Sb	31,393 ±0,020^{f}	0,194 ±0,001^{b}	0,858 ±0,003^{f}

Le: leaves; Sb: stem bark; g EGA: gram equivalents of gallic acid; g CE: grams of catechin equivalent; DM: dry matter
In the same column, means bearing the same letter are not significantly different at the 5% threshold.

The assay results show that the total polyphenol content of extracts varies significantly with organs. Total polyphenol content is higher in leaves than in bark. This disparity is a phenomenon reported by several authors [30, 31]. Flavonoid contents are lower than those found in Euphorbia hirta L (4.14 ± 0.50 mg EQ/g) [32] and Ziziphus mauritiana Lam (5.94 ± 0.23 mg EQ / g) [24]. Tannin content is low, particularly in bark extracts.

3.4. Antioxidant Power by DPPH·

Hydro-ethanolic extracts showed significant antioxidant potential, irrespective of the organ, with significant variation (Table 3). In fact, the extracts inhibited the DPPH radical, as in the case of hydro-ethanolic extracts of Piliostigma thonningii Schumach leaves and bark, and hydro-acetone extracts of Sclerocarya birrea. All inhibition percentages obtained were high, ranging from 89.36% ±0.25% to 95.06% ±0.10%. Leaves extracts had significantly higher antioxidant activity than stem barks extracts, whatever the region of origin, which correlates with the variation in polyphenol content. The synergistic action of tannins, total phenols and flavonoids would explain the high antioxidant power values of plant extracts.

Table 3. *Anti-radical power of Annona senegalensis leaf and stem bark hydroethanol extracts.*

Origin	Kolda		Ziguinchor		Thiès	
Extracts	Le	Sb	Le	Sb	Le	Sb
PI (%)	94,41 ±0,11	89,47 ±0,64	94,84 ±0,53	89,36 ±0,25	95,06 ±0,10	92,87 ±0,10

PI: percentage inhibition

DPPH reduction reveals the antioxidant activity of all the extracts tested. Leaves showed a greater inhibitory power than stem barks for all samples, regardless of the harvesting area, so we can conclude that *Annona senegalensis* leaves are more rich in antioxidant substances than stem barks. This greater anti-free radical activity in the leaves could be linked to the polyphenols and flavonoids presence at higher levels in this organ. This difference between the two organs has been observed in anti-inflammatory and analgesic activity [33]. Indeed, several authors have reported the antioxidant properties of flavonoids, as well as tannins [34].

4. Conclusion

In this study, we have investigated the leaves and stem bark of *Annona senegalensis* collected from three regions of Senegal. A phytochemical screening and antioxidant activity of all these samples were carried out. The results highlight the presence of bioactive compounds such as flavonoids, tannins and polyphenols. Specifically, the analyzed samples exhibit high level of polyphenol, particularly in the leaves and in damp areas. In addition, all the extracts exhibit a very high power of inhibition of free radicals, thus highlighting a great antioxidant potential. These findings showcase the use of the organs of *Annona* in the traditional pharmacopoeia. In perspective, antimicrobial tests of these extracts would undoubtedly allow us to better understand their values for the pharmacopoeia.

Abbreviations

ACo	Control Absorbance
ASa	Absorbance of Sample
DM	Dry Matter
DPPH	2, 2-diphenyl-l-picrylhydrazyl
DR	Dragendorff Reagent
g EC	Grams of Catechin Equivalent
g EGA	Gram Equivalents of Gallic Acid
g CE	Grams of Catechin Equivalent
Le	Leaves

MR	Mayer Reagent
P1	Mass of Flask with Dry Extract
P2	Mass of Empty Flask
PE	Mass of Dry Matter
PI	Percentage of DPPH Inhibition
Rdt	Extraction Yield
Sb	Stem Bark

Author Contributions

Diawo Diallo: Data curation, Formal Analysis, Investigation, Resources, Software, Writing – original draft, Writing – review & editing

Abdoulaye Drame: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Lahat Niang: Formal Analysis, Investigation, Methodology, Visualization

Edmond Antoine Badock: Formal Analysis, Investigation, Methodology, Validation, Visualization

Ibrahima Diallo: Methodology, Visualization, Writing – original draft

Salif Sow: Visualization, Writing – original draft, Writing – review & editing

Nicolas Cyrille Ayessou: Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing

Conflicts of Interest

The author declares no conflicts of interest.

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