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Secondary Metabolites and Antioxidant Activity of Different Parts of the Baobab Fruit (*Adansonia digitata* L.)

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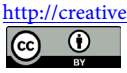

Abstract

The objective of this study was to determine the polyphenol, flavonoid and tannin content and the antioxidant power of methanolic extracts from the different parts of the Senegalese baobab fruit. Phytochemical screening revealed the presence of saponosides, tannins, alkaloids, sterols, flavonoids, coumarins and total sugars in all extracts from the fruit parts. The total polyphenol content was determined by the folin-ciocalteu method. This method is based on the quantification of the total concentration of hydroxyl groups present in the extract. In an alkaline medium, the reagent of folin-ciocalteu, oxidizes the phenols to ion phenolates and partially reduces its hetero-polyacids, hence the formation of a blue complex. The absorbance is read at 765 nm against a control. The Flavonoids vere determined using aluminum trichloride and sodium hydroxide. Aluminum trichloride forms a yellow complex with flavonoids and sodium hydroxyde forms a pink complex absorbing in the visible range at 510 nm. The alternative colorimetric method based on reactions with vanillin in an acidic medium made it possible to determine the tannin content. Absorbances were measured at 500 nm. The results show that the methanolic extracts of the fibers and shell have very high polyphenol, flavonoid and tannin contents. In fact, the polyphenol contents of the fiber (159.00 ± 0.93 µg EAG/mg extract) and shell (155.39 ± 0.89 µg EAG/mg extract) were much higher than those of the pulp (27.21 ± 0.26 µg EAG/mg extract) and seeds (18.36 ± 0.07 µg EAG/mg extract). In addition, the flavonoid contents of the fibers (97.64 ± 0.40 µg EQ/mg) and of the shell (86.18 ± 0.46 µg EQ/mg) were

higher than those of the seeds (12.82 ± 0.04 µg EQ/mg) and pulp (5.66 ± 0.18 µg EQ/mg). The tannin contents of the fibers (256.65 ± 1.45 µg EC/mg) and of the shell (196.05 ± 25 µg EC/mg) are higher than those of the pulp (103.09 ± 0.62 µg EC/mg extract) and seeds (1.09 ± 0.04 µg EC/mg extract). The antioxidant activity of extracts from different parts of the baobab fruit has Also been achieved using two different methods (DPPH and FRAP). The trapping capacity of the DPPH radical is very advantageous for the fibers (IC50 = 2.27 µg/mL) and the shell (IC50 = 1.52 µg/mL). The FRAP test has shown that the extracts from the shell (18.47 µg/mL) and fibers (20.00 µg/mL) have a greater iron reduction capacity than that of the standard ascorbic acid (45.64 µg/mL).

Keywords

Adansonia digitata L., Fruit, Pulp, Seeds, Shell

1. Introduction

Secondary metabolites are compounds that are biosynthesized naturally by plants, but which do not directly participate in plant metabolism. They are used in traditional medicine thanks to their therapeutic properties [1]. These compounds participate in the preservation of food but also protect the human body by opposing oxidation by inhibiting the effect of free radicals [2]. A free radical is a molecule or an atom having one or more unpaired electrons, which makes it extremely reactive [3]. The term reactive oxygen species (ERO) often refers to all free radicals and their precursors. The overproduction of these components during metabolism can cause oxidative stress, contributing to several pathologies such as cardiovascular dysfunction, inflammation, chronic degenerative diseases, and atherosclerosis [4]. Oxidation is a chemical phenomenon generated by unstable free radicals that seek to recover an electron from their environment in order to regain their stability. The target molecular species are fatty substances but also proteins [3] [5]. In a process of promotion and development of forest resources in Senegal, we are interested in the species *Adansonia digitata* L. which belongs to the family of Bombacaceaes and to the order of Malvales better known by the name of African baobab. The baobab (*Adansonia digitata* L.) is a massive tree that is very popular in Senegal and Africa [6]. It plays an important role in social, economic, and environmental terms. It is widely used in food, cosmetics [1], and traditional medicine. The fruit it produces is wrapped in a hard brown shell in which there are black seeds covered with white pulp and reddish fibers.

Several studies have been carried out on baobab but few have focused on secondary metabolites as well as on the antioxidant activity of all parts of the fruit: Anti-inflammatory and antioxidant activities of polysaccharide from (*Adansonia digitata*) an in vitro study [7] Ibrahima *et al.* phenolic content and antioxidant capacities of (*Parinari curatellifolia*), (*strychnos spinosa*) and (Adansonia digita-

ta) [8]; Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi [9]; Antibacterial, antioxidant and phytochemical analysis of edible parts of potent nutraceutical plant (*Adansonia digitata*) [10].

The objective of this study is to quantify the polyphenols, flavonoids and tannins of methanolic extracts from different parts of the baobab fruit and to assess in vitro the antioxidant activity of these compounds.

2. Materials and Methods

2.1. Plant Material

The plant material is made up of the various components of the baobab (*Adansonia digitata* L.) fruit that are the shell, the fibers, the pulp that surrounds the seeds. These fruits were harvested in the Tambacounda region of Senegal.

2.2. Extraction of the Baobab Parts

The different parts of the fruit (Hull, Fibers, Seeds and Pulp) were mechanically separated and then purified before being reduced to powders by a laboratory-type mill (MONBROY, BMS-6). A first extraction for 8 hours by Soxhlet's method with hexane will allow removing the oil. The obtained residue is dried for 24 hours. The second extraction with methanol will allow extracts of the different parts of the baobab fruit to be obtained. These extracts obtained are stored in dark bottles and then stored at 4˚C until the time of analysis.

2.3. Phytochemical Screening

The detection for groups of chemical compounds was carried out on the extract fractions according to the protocols described in the work of N'Guessan *et al.* [11] and de Békro *et al.* [12].

2.4. Determination of Total Polyphenols

The content of total polyphenols in the various parts of the baobab leak was determined according to the method adopted by Javanmardi *et al.* [13] which uses the Folin-ciocalteu reagent and gallic acid as standard. To a test tube containing 0.5 mL of the mother solution (1000 µg/mL), 4 mL of Na_2CO_3 (7.5%) is added. After stirring, 2.5 mL of the Folin-ciocalteu solution was added. The whole is incubated in a water bath at 45˚C for 30 minutes. The absorbance is read at 765 nm against a control without extract. The total polyphenol content of the extracts was determined from the standard gallic acid curve and the results are expressed in micrograms of gallic acid equivalent per milligram of extract (µg EAG/mg extract).

2.5. Determination of Flavonoids

The flavonoid content was determined by the aluminium chloride method using quercetin as standard [14]. 6.4 ml of distilled water and 0.3 ml of 5% sodium ni-

trite ($NaNO_2$) solution are added to a test tube containing 1 ml of the metered extract. Everything is well mixed. After 5 minutes, 0.3 mL of 10% aluminium trichloride solution (m/v) is added to the mixture which is incubated at room temperature for 6 minutes. To this mixture is added 2 mL of sodium carbonate (1 M). The mixture is completely agitated in order to homogenize the content. After 30 minutes of incubation at room temperature, the absorbances are read using a visible UV spectrophotometer (LLG-uniSPEC 2) at 510 nm against a control (without extract). The calibration curve obtained with quercetin as standard made it possible to calculate the concentrations of flavonoids contained in our extracts (seeds, pulp, fiber, and shell of the baobab fruit). The results are expressed in µg quercetin equivalent per milligram of extract (µg EQ/mg of extract).

2.6. Determination of Tannins

The determination of the tannins was carried out by the method described by Rebaya *et al.* [4] using catechin as standard. 0.1 mL of the extract was mixed with 3 mL of 4% methanolic vanillin solution. Then 1.5 mL of hydrochloric acid is added, the mixture is stirred and left to stand for 20 minutes. The absorbances were measured at 500 nm. The results are expressed in µg equivalent of catechin per milligram of extract (µg EC/mg of extract).

2.7. Antioxidant Activity Evaluation

In order to enhance the value of the baobab fruit, we have evaluated the antioxidant activity of the different parts of the fruit with a view to using them as a natural antioxidant. This evaluation of the antioxidant activity was carried out using two complementary methods: the DPPH (2,2-diphenyl-1-pierylhydrazyl) free radical reduction test and that of FRAP (Ferric Reducing Antioxidant Power).

2.7.1. DPPH Radical Scavenging Activity

The evaluation of the anti-free radical activity was carried out by the method of DPPH (2,2-diphenyl-1-pierylhydrazyl), according to the protocol described by Brand-Williams *et al.* [15] with some modifications. The principle of this test is based on the ability of the extract to reduce the dark purple DPPH free radical which turns yellow after reduction. A series of different concentrations (2.5 to 100 µg/mL) of the extracts is prepared in methanol. 0.5 ml of the DPPH methanolic solution (0.2 mM) is added to a tube containing 2.5 ml of the sample.

After incubation in the dark for 30 minutes, the absorbances are measured at 517 nm against a blank. The negative is represented by a standard antioxidant, ascorbic acid. The neutralizing power of DPPH free radicals in percentage (I%) was calculated as follows:

$$I\% = \left(\left(A_0 - A_1 \right) / A_0 \right) 100$$

where A_0 is the absorbance of the Negative Control and A_1 is the absorbance of the sample.

A curve of the concentrations of the extracts as a function of I% was drawn, making it possible to obtain the IC50 index which is defined as being the concentration of the substrate in µg/ml which causes the loss of 50% of the activity of DPPH.

2.7.2. Ferric Reducing Antioxidant Power (FRAP) Iron Reduction Test

The reducing power of the extracts is determined by the method described by M'barek *et al.* [16]. This technique is based on the ability of the extracts to reduce ferric iron (Fe^{3+}) present in the potassium ferricyanide complex K_3Fe (CN) 6 to ferrous iron (Fe^{2+}). To 0.5 ml of each extract are added 1.5 ml of tapon phosphate solution (0.2 M; pH = 6.6), then 1.5 ml of potassium hexacyanoferrate [$KFe(CN)_6$] at 1%. The whole is heated to 50˚C in a water bath for 20 minutes.

A volume of 1.25 mL of 10% trichloroacetic acid is then added and the mixture is centrifuged at 3000 rpm for 10 minutes. Finally, 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% ferric chloride [$FeCl_3$]. All incubated in the dark for 30 minutes. The absorbance of the reaction medium is determined at 700 nm against a blank. The standard curve used ascorbic acid solution (0.5 to 100 µg/mL) and the reducing power was expressed in equivalent concentrations (EC50). The EC50 concentration corresponds to the effective concentration at which the absorbance is equal to 0.5.

2.8. Statistical Analyses

One-factor analysis of variance (ANOVA) and the Fischer LSD test at the 5% significance level were performed. The results obtained represent the average of three analyzes and the Origin 6.0 software was used.

3. Results and Discussion

3.1. Yield of Extraction

The results obtained with methanol extraction are presented in (Table 1). The yield of methanolic extract was higher with the pulp than with the fibers, the shell or the seeds. Indeed, these extraction yields were respectively 57.13%; 17.14%; 8.60% and 3.85% with pulp, fibers, shell and seeds. Therefore, the pulp would contain more polar substances than the other parts of the baobab fruit.

3.2. Phytochemical Screening

The results of the phytochemical screening of methanolic extracts from the different parts of the baobab fruit (*Adansonia digitata* L.) presented in (Table 1).

Table 1. Efficiency of methanolic extraction of the different parts of the baobab fruit.

	Seeds	Pulp	Fibers	Shell
Yield (%)	3.85 ± 0.02	57.13 ± 1.93	17.14 ± 0.07	8.60 ± 0.12

Analysis of the results shows the presence of alkaloids, saponosides, flavonoids and total sugars in all extracts of parts of the fruit. However, the presence of catechic and Gallic tannins was noted respectively in the methanolic extracts of the seeds and the shell.

Coumarins are absent in the fiber extract. Except for the methanolic extract from the shell, sterols and polyterpenes are abundantly present in all other extracts from the fruit. Furthermore, the presence of phenolic compounds (polyphenols, flavonoids and tannins) in these extracts suggests biological activities such as antioxidants [7] [17], anticancer agents [15], antimicrobials [18] [19] and anti-inflammatories [7].

3.3. Total Polyphenols, Flavonoids and Tannins Contents

The polyphenol, flavonoid and tannin contents of the methanolic extracts from the different parts of the baobab fruit (*Adansonia digitata* L.) have been determined (Table 3). The results obtained reveal that the polyphenol contents of the shell (155.39 ± 0.89 µg EAG/mg of extract) and of the fibers (159.00 ± 0.93 µg EAG/mg of extract) are much higher seeds (18.36 ± 0.07 µg EAG/mg extract) and pulp (27.21 ± 0.26 µg EAG/mg extract). Consequently, the methanolic extracts from the fibers and the shell have more antioxidant activities than those from the seeds and pulp.

Indeed, phenolic compounds, secondary metabolites widely present in the plant kingdom, participate in the plant's defence system and in the nutritional value of food [2] [20]. These phenolic compounds can also help the body to

Table 2. Phytochemical screening of extracts from different parts of the baobab fruit.

	Seeds	Pulp	Fibers	Shell
Coumarines	+	+	−	+
Alkaloids	+	+	+	+
Saponosides	+	++	++	+
Sterols and polyterpenes	++	++	++	−
Flavonoids	+	+	++	++
Catechic tannins	+	−	−	−
Gallic tannins	−	−	−	+
Sugars	+	++	++	+

(+) = Presence; (++) = Abundant presence; (−) = Absence.

Table 3. Content of polyphenols, flavonoids and tannins of methanolic extracts from different parts of the baobab fruit (*Adansonia digitata* L.)

	Seeds	Pulp	Fibers	Shell
Total polyphenols (µg EAG/mg of extract)	18.26 ± 0.07	27.21 ± 0.26	159.00 ± 0.93	155.39 ± 0.89
Total Flavonoids (µg EQ/mg of extract)	12.82 ± 0.04	5.66 ± 0.18	97.64 ± 0.40	86.18 ± 0.46
Total tannins (µg EC/mg of extract)	1.09 ± 0.04	103.09 ± 0.62	256.65 ± 1.45	196.05 ± 25

strengthen its defence system against dysfunctions caused by oxidative stress [4] [21]. These polyphenols are abundant in fruits (grapes, apples, and cherries) and their contents can reach 500 mg EAG/100g [19].

Consequently, the results obtained make it possible to classify the baobab fruit among the richest in polyphenols (27.21 µg EAG/mg of extract for the pulp). In addition, the pulp of the Adansonia digitata species from Senegal which we have analyzed contains more total polyphenols than that of Zimbabwe (50 mg/100g), Ivory Coast (1084 mg/100g) and Malawi (1870 mg/100g) [9] [19] [22].

Flavonoids are also a class of secondary metabolites found widely in nature [23]. Indeed, they play an important role in the defence of plant cells against microorganisms, insects and UV irradiation [18]. The results obtained indicate differences, depending on the extract studied.

Indeed, the flavonoid contents of the shell (86.18 ± 0.46 µg EQ/mg) and of the fibers (97.64 ± 0.40 µg EQ/mg) are greater than those of the seeds (12.82 ± 0.04 µg EQ/mg) and pulp (5.66 ± 0.18 µg EQ/mg). The flavonoid content in the shell (86.18 µg EQ/mg) and fiber (97.64 µg EQ/mg) of baobab fruit is three times that found in the leaves of the Japanese blackberry (26.6 mg EQ/g) [24].

Also, the tannin contents of the methanolic extracts of the different parts of the baobab fruit are very varied. In fact, the results reveal that the tannin contents of the shell (196.05 ± 25 µg EC/mg) and of the fibers (256.65 ± 1.45 µg EC/mg) are higher than those of the seeds (1.09 ± 0.04 µg EC/mg of extract) and of the pulp (103.09 ± 0.62 µg EC/mg of extract). However, compared with catechin (1052.16 ± 0.00 µg EC/mg), the levels determined remain very low.

3.4. Antioxidant Activity

The evaluation of the antioxidant activity of the methanolic extracts of the different parts of the baobab fruit was carried out using two different methods (DPPH and FRAP). The results obtained are listed in **Table 4**. The antioxidant properties of the extracts were evaluated according to their concentration IC50 and EC50. Extracts from the shell (1.52 µg/mL) and fibers (2.27 µg/mL) showed much greater inhibition of the DPPH radical than those from the pulp (18.42 µg/mL) and seeds (20.19 µg/mL).

Ascorbic acid, the standard antioxidant, showed very high anti-free radical activity with an IC50 of 1.74 µg/mL. Therefore, these recorded IC50 indicate that the methanolic extracts from the fibers and seeds have anti-free radical activities comparable to that of the reference molecule (ascorbic acid).

On the other hand, the shell extract displayed a higher antioxidant power than the standard. In addition, our extracts from the IC50 results obtained for the pulp and seeds are clearly better than those reported in the work of Gahane and Kogje, [10] (50 µg/mL for the pulp and 94 µg/mL for the baobab seeds). However, the extracts from the different parts of the baobab fruit showed fairly significant antioxidant activity compared to the extracts from the leaves of Moringe oleifera (IC50 of 87.86 µg/mL) and Saba senegalensis (18.4 µg/mL) [25] [26].

Table 4. Antioxidant activity of methanolic extracts from different parts of the baobab fruit (*Adansonia digitata* L.).

	Seeds	Pulp	Fibers	Shell	Standard
DPPH (IC_{50} µg/mL)	$20.19^{a} \pm 0.48$	$18.42^{a} \pm 1.17$	$1.52^{b} \pm 0.004$	$2.27^{b} \pm 0.025$	$1.74^{c} \pm 0.21$
FRAP (EC_{50} µg/mL)	$>100^{a}$	$>100^{a}$	$18.47^{b} \pm 0.38$	$20.00^{c} \pm 0.20$	$45.64^{d} \pm 0.14$

Also, the evaluation by the FRAP test showed that the extracts of the shell (18.47 µg/mL) and of the fibers (20.00 µg/mL) have a greater reducing activity than that of the standard ascorbic acid (45.64 µg/mL). However, the extract from the pulp and seeds has very low activity (> 100 µg/mL) (Table 4).

The results obtained show that whatever the evaluation method used, the shell and fiber extracts have a higher antioxidant activity than that of the seeds and pulp. In addition, we note a correlation between the contents of polyphenols, tannins and flavonoids with the determined antioxidant activities.

These results are in agreement with the data in the literature. Indeed, several authors have reported that there is a close relationship between phenolic compounds and antioxidant activities [12] [27]. The finding of Besco *et al.* [28] confirm the results obtained and indicate that, whatever the preferred evaluation method (DPPH, FRAP, TRAP, ORAC), the fibers had the most antioxidant activity than the other parts of the fruit (pulp, seeds).

Thus, he found that the integral antioxidant capacity (CAI) of the fibers (1617.3 µmol/g) of baobab was 66 times greater than that of orange pulp (24.3 µmol/g). The results show that the shell and fibers of baobab which are almost not valued have a remarkable antioxidant power. They could be used as natural antioxidants.

4. Conclusions

Based on this study, phytochemical screening, extraction yield, total polyphenol contents and antioxidant activity were determined. Phytochemical screening reveals the presence of secondary metabolites in the different parts of the baobab fruit (shell, fibers, seeds, pulp).

Also, the methanolic extracts from these different parts contain high amounts of phenolic compounds (polyphenols, flavonoids and tannins). These results show that the components of the fruit could be applied in the pharmaceutical field. However, the isolation and characterization of the compounds responsible for the antioxidant activity are necessary for better development.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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