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Bioactive Compounds and Scavenging Capacity of *Adansonia digitata* L. (Baobab Fruit) Pulp Extracts against ROS and RNS of Physiological Relevance

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Abstract: Background: Baobab fruit is valued for its nutritional and medicinal benefits. Although it is acknowledged that baobab pulp is beneficial for health, studies that link its nutraceutical properties to the ability to eliminate reactive species (ROS and RNS) are scarce. Methods: The nutritional profile and the antioxidant properties of baobab pulp extracts from Angola were evaluated. Thus, for the first time, the evaluation of in vitro scavenging capacity against the most physiologically relevant reactive oxygen species (ROS) and reactive nitrogen species (RNS) were the focus of investigation. Results: Angolan fruit pulp presented high contents of ash (8.0%) and total dietary fiber (52%). Vitamin E content was reported for the first time in fruit pulp. Green solvents were used to quantify bioactive compounds and antioxidant activity. Hydroalcoholic extracts exhibited the highest contents of phenolics (1573.0 mg/100 g) and flavonoids (768.7 mg/100 g). Thus, hydroalcoholic extracts showed higher antioxidant activity, and higher scavenging capacity for ROS ($O_2^{\bullet -}$, H_2O_2, HOCl, ROO$^\bullet$) and RNS ($^\bullet$NO, $ONOO^-$), being most active for $^\bullet$NO and $ONOO^-$. Conclusion: For the first time, Angolan baobab fruit was described in respect to its nutritional contribution as well as its positive antioxidant effects, both as a functional food and as a nutraceutical ingredient.

Keywords: baobab fruit; nutritional profile; dietary fiber; vitamin E (HPLC-DAD-FLD); phenolics; flavonoids; antioxidant activity; ROS and RNS scavenging assays

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1. Introduction

Baobab (*Adansonia digitata* L.) is native to Sub-Saharan Africa. It is widely recognized for its socioeconomic significance for the local populations due to the plant's many useful organs, which can be used for food, or for traditional medicine [1]. The Adansonia genus, a member of the Malvaceae family, encompasses eight identified species spread across numerous African countries [2]. Besides their nutritional importance, the protection of these wild fruits not only contributes to health but also yields economic advantages, as certain wild edible fruits are actively traded in various markets. Native plants, including fruits, are now used in several food recipes, feeds, medicinal applications, household products, and ritual applications, by the local population [3]. Thus, they play an important role in global food security, particularly in agricultural societies. Focusing on human health applications, several ethnobotanical studies have recorded the worldwide use of *A. digitata* for medicinal purposes [4,5]. Due to the existence of numerous compounds with biological action [6,7], this species, especially the fruit, has been reported to possess both nutritional

and therapeutic value and it is therefore considered a food–medicine. The pulp of baobab fruit is acknowledged to be a valued source of minerals (calcium, potassium, iron, and magnesium), vitamins (ascorbic acid, niacin, and pyridoxine), and phenolic compounds like rutin, quercetin, catechin, gallic acid, and proanthocyanins [8–10]. According to Prior et al. [10], more polyphenols and vitamin C are found in baobab pulp than in similar commonly eaten fruits (kiwis, strawberries, oranges, and apples). However, taking into consideration the biological roles indicated in various research efforts, the fruit pulp lacks categorization regarding its other vitamin contents.

Also, fruit pulp possesses a high content of fibers and is low in proteins and fats [10]. Thus, baobab pulp appears to be ideal for adding fiber to foods, while also improving their nutritional profile [11–13]. Because of its higher content of soluble fiber, as compared to other foods, its nutritional profile indicates that baobab may be able to be categorized as a low-glycemic-index food [4,14]. In 2008, the European Commission added the pulp of baobab fruit to the list of novel food components in the EU, and, 1 year later, the Food and Drug Administration recognized it as a food ingredient [15]. Consequently, the nutritional benefit of baobab fruit has been recognized, and it has been accepted as a food additive in Europe and the United States of America [16]. Since then, an extensive amount of research has been conducted on its biochemical, agronomical, and botanical attributes. A significant variance in nutritional value has been reported, probably due to several factors, such as the origin, quality, production, storage, and so on of the analyzed samples, suggesting a need for more research to promote scientific knowledge on this species.

Baobab edible fruits are high in phytochemical compounds from an assortment of chemical families, including flavonoids, triterpenoids, steroids, and phenolic acids. Because of their antioxidant properties, bioactive compounds change and reduce the risk of chronic pathologies associated with oxidative stress. These compounds are associated with some of the reported biological activities, such as anti-hyperglycemic [15,17,18], analgesic and antipyretic [15,19], antibacterial, antiviral [15,20], antioxidant [18], and anti-inflammatory [4,10] properties. Moreover, polyphenols are well-known for reducing the worsening and/or development of oxidative stress. Reactive pro-oxidant species, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS), are overproduced during oxidative stress, leading to an inequity between pro-oxidants and antioxidants. To mitigate oxidative stress-related diseases, it becomes essential to introduce antioxidant molecules, aiming to restore and maintain optimal levels of antioxidants within the organism [21]. The potential scavenging capacity of methanolic extract of baobab fruit pulp against nitric oxide (NO) has already been reported in the literature [22]. However, despite the phytopharmaceutical interest in the antioxidant effects of baobab fruit pulp, no studies have been reported on ethanolic, aqueous, and hydroalcoholic extracts as to the evaluation of the in vitro scavenging effect against physiologically important ROS and RNS.

In recent years, there has been an increase of interest in baobab fruit, leading researchers to conduct studies on both edible and inedible parts of *Adansonia digitata* L. However, there are differences in the asserted values of baobab parts (pulp, seeds, and leaves), which could be attributed to a variety of factors, including different market samples, plant origin, soil and climacteric properties, and analytical methods, as well as the nature of the solvent extraction and the storage conditions, among other factors.

Therefore, this experimental work meant to analyze and report the proximate composition of baobab fruit pulp, as collected in several municipalities in the province of Luanda, Angola. Much of the recent research on baobab fruit has concentrated on regions in southern and eastern Africa, including Kenya and Sudan [8,19,22–24], South Africa [25], Mali [18], and Nigeria [8,17]. Although the economic interest in baobab fruit continues to increase, there is still a lack of knowledge on Angolan fruits. Vitamin E will be measured in the fruit pulp for the first time, with the goal of improving the antioxidant profile of baobab fruit and expanding its applications. Additionally, this research aims to assess the antioxidant activity in various baobab pulp extracts based on green solvents, and characterized by low toxicity, convenient accessibility, and great efficiency. Although prior

research has been conducted on the antioxidant capacity of this fruit [5,9,15,17,19,22], we believe that this is a pioneering study on the evaluation of the in vitro scavenging effect of baobab extracts against physiologically important ROS and RNS. Furthermore, this study is noteworthy, considering that ROS and RNS are central in the cellular and biochemical process complexity of oxidative stress, which can be physiological (acting pro-hermetic) or pathogenic (producing destructive vicious circles) processes.

2. Materials and Methods

2.1. Raw Material and Reagents

Adansonia digitata L. fruits were collected from traditional local markets in three main municipalities of Luanda, Angola (Quiçama: 9.7984° S, 13.7289° E; Icolo Bengo: 9.1871° S, 13.8216° E; and Belas: 9.0834° S, 13.1279° E). Luanda has a dry tropical climate, and its typical soil consists of luvisols, calcisols, and cambisols (calcareous and calcialitic soils), which give good clayey soils for crops. Before analysis, the fruits were blended to create a representative sample from the Luanda region. A 2 mm sieve was used to separate the fruit pulp from the seeds. Following that, the fruit pulp was kept at room temperature (25–28 °C) in a hermetically sealed vial covered with aluminum foil until analysis.

All chemicals and solvents used were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ferrous sulphate heptahydrate, sodium carbonate decahydrate, sodium nitrite, aluminium chloride, and ferric chloride hexahydrate were all purchased from Aldrich Chemical Co. (Steinheim, Germany). Catechin and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dihydrorhodamine 123 (DHR), sodium hypochlorite solution (NaClO) with 4% available chlorine, 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5), β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulphate (PMS), lucigenin, α,α′-azodiisobutyramidine dihydrochloride (AAPH), fluorescein sodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Tiron, ascorbic acid, and quercetin were all obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). 4,5-Diaminofluorescein (DAF-2), 30% hydrogen peroxide (H_2O_2), Folin–Ciocalteu's reagent, absolute ethanol, sodium acetate, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained using a Milli-Q water purification system (TGI Pure Water Systems, Leominster, MA, USA). The peroxynitrite anion ($ONOO^-$) was obtained by synthesis, as described by Gomes et al. [26]

2.2. Nutritional Profile

The nutritional analyses were performed using AOAC protocols [27]. Moisture content was measured using an infrared balance (Scaltec model SMO01, Scaltec Instruments, Heiligenstadt, Germany). The ashes were measured by direct incineration at 500 °C. Total fat and crude proteins were measured by the Soxhlet extraction and Kjeldahl methods, respectively. Dietary fiber analysis was carried out using enzymatic–gravimetric techniques [27]. The energy evaluation of fruit pulp (kcal/100 g) was calculated using conversion factors based on general Atwater values for food energy content in EU Regulation N. 1169, 2011 [28], being 4.0 kcal/g for protein and carbohydrates, and 9.0 kcal/g for fat.

2.3. Lipidic Fraction Extraction

The lipidic fraction of baobab fruit pulp was extracted according to the procedure validated by Alves et al. [29], with a few modifications. The sample (about 150 mg) was combined with 75 µL of 0.1% BHT (*m*/*v*), 50 µL of tocol (internal standard, 0.1 mg/mL), and 1 mL absolute ethanol. The solution was homogenized for 30 min using an orbital vortex mixer (VV3, VWR International, Darmstadt, Germany). After that, 2 mL of n-hexane (HPLC grade) was added, and the solution was homogenized for 30 min. Next, 1 mL of 1% NaCl (*m*/*v*) was added. The supernatant was collected and preserved following centrifugation (5000 rpm for 5 min) with a Heraeus Labofuge A (Hanau, Germany). The residue was re-extracted with 2 mL of n-hexane for 30 min before centrifugation (5000 rpm

for 5 min). The resultant supernatant was collected and mixed with the previous extract. An appropriate amount of anhydrous sodium sulphate (Na_2SO_4) was added to the solution, which was then centrifuged (5000 rpm for 5 min). After being evaporated to dryness, the supernatant was mixed with 1 mL of solvent and injected into an HPLC-DAD-FLD (high-performance liquid chromatography connected to a diode array detector and fluorescence detector) system for assessment of the vitamin E profile.

Vitamin E Profile by HPLC-DAD-FLD

Vitamin E quantification was carried out through HPLC-DAD-FLD, employing an integrated HPLC system (Jasco, Tokyo, Japan) that included an AS-950 automated injector, a PU-980 pump, and an MD-2015 multiwavelength diode array detector (DAD) coupled to an FP-2020 fluorescence detector (Jasco, Tokyo, Japan). The system was programmed for excitation at 290 nm and emission at 330 nm. Each compound was chromatographically separated on a normal phase SupelcosilTM LC-SI column (75 mm × 3.0 mm, 3.0 µm) (Supelco, Bellefonte, PA, USA), following the method described by Alves et al. [29]. Chromatographic data analysis was conducted using Borwin-PDA Controller Software (Jasco ChromNAV, version 2.02.08, Tokyo, Japan).

Vitamin E vitamers in samples were identified by comparing the retention times with the respective standards (α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols), using UV spectra as a reference. Quantification was made through the analysis of fluorescent signals, and subsequently converted to concentration units using calibration curves obtained from commercial standards for each compound. Tocol was used as the internal standard. The results were expressed as mg/100 g of sample dry weight (dw).

2.4. Total Phenolic and Total Flavonoids Content

2.4.1. Preparation of Baobab Pulp Extracts

Samples (~1 g) were macerated with 20 mL of ethanol, ethanol/water (1:1), or distilled water for 30 min at 40 °C, based on the parameters established out by Costa et al. [30]. The extracts were stored at 4 °C until evaluated. Aqueous filtrates were freeze-dried, and hydroalcoholic and alcoholic filtrates were reduced to residue by a rotary evaporator at 40 °C. The extracts were reconstituted in the extraction solvent to determine the bioactive compounds and antioxidant activity.

2.4.2. Total Phenolic Contents and Total Flavonoid Contents

The total soluble phenolic content (TPC) of the extracts was determined by employing the Folin–Ciocalteu (FC) solution, with some adjustments made according to the approach described by Vinha et al. [31]. Succinctly, 30 µL of each extract was mixed with 150 µL of FC reagent (1:10) and 120 µL of Na_2CO_3 aqueous solution (7.5%, *m*/*v*). The mixture was initially kept at 45 °C for 15 min; this was followed by 30 min of incubation without the presence of light, and at room temperature. A Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to obtain absorbance measurements (765 nm). The amount of TPC was calculated using a gallic acid calibration curve (covering the concentration range between 5 and 300 µg/mL), and results were presented in milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (dw).

A colorimetric test was used to determine the total flavonoid contents (TFC), using the conditions previously published by Costa et al. [32]. To summarize, 1 mL of each sample extract was combined with 4 mL of distilled water and 300 µL of 5% $NaNO_2$. A total of 300 µL of 10% $AlCl_3$ was added after 5 min at room temperature. One minute later, 2 mL of NaOH) (1 M) and 2.4 mL of distilled water were added. Catechin was used to plot a standard curve (2.5–400 mg/L; R^2 = 0.999), and absorbance was measured at 510 nm using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The results were presented in milligrams of catechin equivalent (CAE)/100 g dw.

2.5. Antioxidant Activity

2.5.1. Determination of DPPH Free Radical Scavenging

The DPPH scavenging ability of the different extracts was determined according to the method validated by Costa et al., with modifications [30]. Briefly, 30 µL of Trolox standard (562 µg/L)/blank solution or diluted extract (1:10) were mixed with 270 µL of the DPPH$^{\bullet}$ solution (6.1×10^{-5} M). The kinetic reaction was monitored with a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA), in equal intervals of 10 min, at 525 nm. The reaction endpoint was attained in 20 min. Trolox was used as a standard in the preparation of the calibration curve (2.5–1000 µg/mL, $R^2 > 0.996$) and DPPH$^{\bullet}$ scavenging activity was expressed in mg of Trolox equivalents (TE)/100 g of sample (dw).

2.5.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to Costa et al. [30], with a few adjustments. In short, 35 µL of ferrous sulphate standard (5–600 µmol)/blank/diluted extract (1:10) was combined with 265 µL of the FRAP reagent (containing 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM of $FeCl_3$). The mixture was kept away from light for 30 min at 37 °C, and then the absorbance was measured at 595 nm using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The standard curve was constructed using $FeSO_4$ (150–2000 µmol, $R^2 > 0.996$), and the results were expressed as µmol $FeSO_4$ (FSE) equivalents/100 g (dw).

2.6. ROS and RNS Scavenging Assays

The ROS [($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxyl radical (ROO$^{\bullet}$)] and RNS [nitric oxide ($^{\bullet}$NO), and $ONOO^-$] scavenging assays were performed using a microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT, USA) equipped with a thermostat, for fluorescence, UV/Vis, and chemiluminescence measurements. In these experiments, baobab fruit pulp extracts and the positive controls quercetin, Tiron, Trolox, and ascorbic acid were dissolved in the buffer used for each assay. The results were expressed as IC_{50} values of four independent experiments using five to seven concentrations in duplicate. The IC_{50} values were determined using curves of percentage inhibition versus compound concentration.

2.6.1. $O_2^{\bullet -}$-Scavenging Assay

A non-enzymatic system (NADH/PMS/O2) created $O_2^{\bullet -}$. These radicals form a purple-colored medium via the reduction of the NBT into diformazan [26], enabling the spectroscopic measurement of $O_2^{\bullet -}$ scavenging capacity at 560 nm. Tiron and ascorbic acid served as positive controls. The results were expressed as the inhibition (in IC_{50}, except for ascorbic acid, whose result was presented as a percentage) of the NBT reduction to diformazan.

2.6.2. H_2O_2-Scavenging Assay

The scavenging activity of the H_2O_2 was determined according to a methodology previously described [33]. The effects of the studied extracts were monitored to establish the latter's H_2O_2-scavenging activity (31.25–1000 µg/mL) and the effect of the positive control (ascorbic acid) (31.25–1000 µg/mL) on the H_2O_2-induced oxidation of lucigenin. The results were expressed as the inhibition (in percentage for baobab pulp extracts and in IC_{50} for positive controls) of the H_2O_2-induced oxidation of lucigenin.

2.6.3. HOCl-Scavenging Assay

The HOCl scavenging capacity was measured by a fluorescent methodology described by Gomes et al. [26], one based on monitoring the effects of the extracts (aqueous extract: 31.25–1000 µg/mL; hydroalcoholic extract: 7.81–250 µg/mL; and alcoholic extract: 15.63–500 µg/mL) and the positive controls (ascorbic acid: 0.5–10 µg/mL; and quercetin: 0.08–5 µg/mL) on the HOCl-induced oxidation of DHRc to rhodamine 123. HOCl was

generated by adjusting the pH of 1% NaClO solution (m/v) with H_2SO_4 (10%) to 6.2. The HOCl concentration was obtained in a spectrophotometer at 235 nm. The results were established as the inhibition (in IC_{50}) of HOCl-induced oxidation of DHR.

2.6.4. ROO•-Scavenging Assay

ROO• scavenging capability was assessed by monitoring the effects of the examined extracts (aqueous and hydroalcoholic extracts: 31.25–1000 µg/mL; and alcoholic extract: 15.63–500 µg/mL) and the positive controls (ascorbic acid: 0.05–1.5 µg/mL; and quercetin: 0.006–0.2 µg/mL) on the fluorescence decay resulting from ROO•-induced oxidation of fluorescein [34]. ROO• was produced by the thermal degradation of AAPH at 37 °C. The fluorescence signal was monitored every minute at the emission wavelength of 528 ± 20 nm and excitation was performed at 485 ± 20 nm until the complete discoloration of fluorescence. Trolox was employed as a standard control, and the results were expressed as ROO•-induced oxidation of fluorescein.

2.6.5. •NO-Scavenging Assay

The ability to scavenge the •NO was tested by monitoring the effects of baobab pulp extracts (7.81–500 µg/mL) and the positive control (quercetin: 0.06–2 µg/mL) on •NO-induced oxidation of DAF-2 (non-fluorescent) to triazolofluorescein (DAF-2T) (fluorescent) [35]. The •NO was produced via the degradation of NOC-5. The results are presented as the percentage inhibition of •NO-induced oxidation of DAF-2.

2.6.6. $ONOO^-$-Scavenging Assay

$ONOO^-$-Scavenging capacity was determined by monitoring the effects of the baobab pulp extracts (aqueous extract: 7.81–500 µg/mL; hydroalcoholic extract: 1.95–125 µg/mL; alcoholic extract: 7.81–250 µg/mL) and the positive controls (ascorbic acid: 0.16–2.5 µg/mL; quercetin: 0.31–10 µg/mL) on $ONOO^-$-induced oxidation of DHR (non-fluorescent) to rhodamine 123 (fluorescent) [33]. The experiments were performed in the presence and absence of 25 mM $NaHCO_3$. The results are displayed as the % inhibition of $ONOO^-$-induced oxidation of DHR.

2.7. Statistical Analysis

The collected data were statistically analyzed using IBM SPSS Statistics (version 26 for Windows, IBM Corp., Armonk, 241 NY, USA). One-way ANOVA was used to assess significant differences between samples, followed by Tukey's HSD, to make paired comparisons between means, with a significance level of 5% ($p \leq 0.05$). The correlation values between bioactive substances (TPC and TFC) and antioxidant activity (DPPH and FRAP) were examined using Pearson correlation. Differences of $p \leq 0.05$ were considered significant.

3. Results

3.1. Nutritional Profile

The nutritional composition of baobab fruit pulp was determined, as shown in Table 1.

Table 1. Nutritional composition of baobab fruit pulp. Results are expressed in g/100 g dry weight dw).

Nutritional Composition	Fruit Pulp *
Moisture	12.95 ± 0.04
Ash	8.0 ± 0.1
Crude Protein	2.3 ± 0.1
Total Fat	0.4 ± 0.1
Total Dietary Fiber	52.0 ± 1.0
Remaining Carbohydrates	37.0 ± 1.0
Energy (kcal)	160 ± 0.1

* Values are presented as mean ± standard deviation (n = 3).

The baobab fruit pulp is a rather low-quality source of protein (2.3 ± 0.1%) and lipids, (0.4 ± 0.1%), and its most prevalent macronutrient is total dietary fiber (52.0 ± 1.0%). The ash content is high, indicating a high mineral concentration.

3.2. Vitamin E Profile

Vitamin E is utilized not just as a preservative in animal feed, but also as a medical ingredient, dietary supplement, and antioxidant food stabilizer. Considering the biological properties of vitamin E, as well as its antioxidant activity, this vitamin was quantified, for the first time, in this study in the pulp of the baobab fruit (Table 2, Figure 1).

Table 2. Vitamin E profile of baobab fruit pulp. Results are expressed in mg/100 g dw.

Vitamer	Content *
α-tocopherol	0.54 ± 0.03
β-tocopherol	0.0509 ± 0.0001
δ-tocopherol	nd
γ-tocopherol	nd
α-tocotrienol	nd
β-tocotrienol	nd
γ-tocotrienol	nd
δ-tocotrienol	nd
Total vitamin E	0.59 ± 0.03

* Values are presented as mean ± standard deviation (n = 3); nd—not detected.

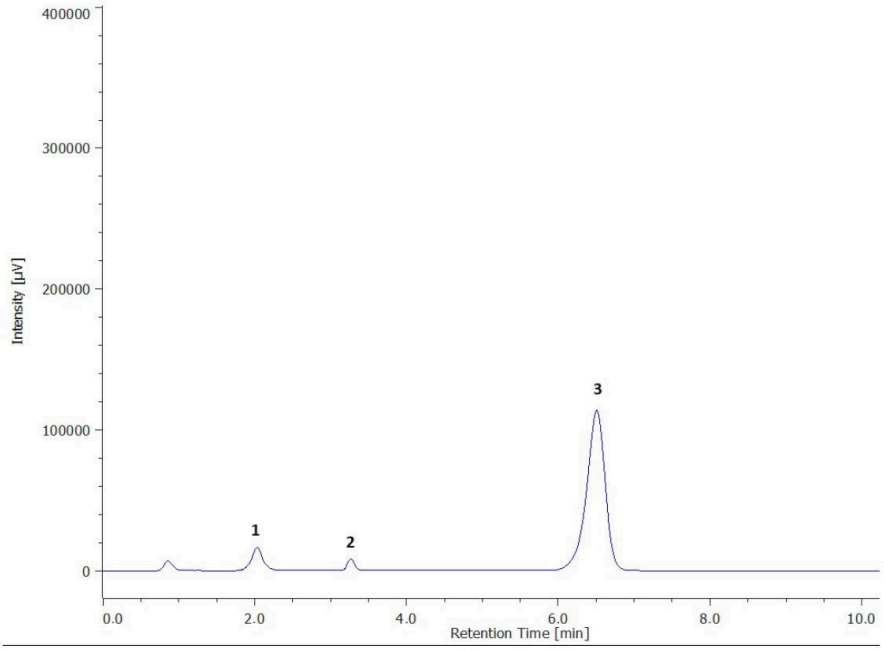

Figure 1. Chromatogram of vitamin E quantification. (1—α-tocopherol; 2—β-tocopherol; 3—tocol-internal standard).

Table 2 shows that baobab fruit pulp presents only two vitamin E isoforms: α-tocopherol (0.54 mg/100 g), and **β**-tocopherol (0.0509 mg/100 g). The chromatographic separation of the α-tocopherol (peak 1), β-tocopherol (peak 2) and tocol-internal standard, 0.1 mg/mL (peak 3) is presented in Figure 1.

3.3. Bioactive Compounds and Antioxidant Activity

The evaluations of total phenolic and flavonoid contents and the antioxidant activity detected in fruit pulp extracts are presented in Figure 2.

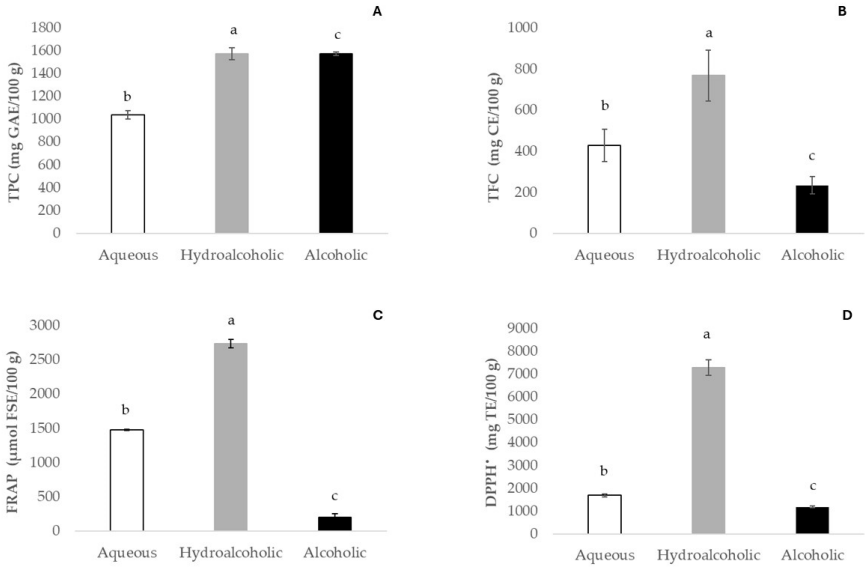

Figure 2. Total phenolic content (TPC, (**A**)), total flavonoid content (TFC, (**B**)), ferric reducing antioxidant power (FRAP, (**C**)), and activity inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH•, (**D**)). Values represent mean ± SD (n = 6). Results are expressed as mg of gallic acid equivalents (GAE), mg of catechin equivalents (CE), µmol of ferrous sulfate equivalents (FSE), and mg of Trolox equivalents (TE) per 100 g of baobab pulp. Different letters denote significant differences ($p \leq 0.05$).

3.4. ROS and RNS Scavenging Assays

Table 3 shows the scavenging activity of baobab pulp extracts against the ROS ($O_2^{\bullet -}$, H_2O_2, HOCl, and ROO•) and RNS (•NO and $ONOO^-$).

Table 3. Superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxyl radical (ROO•), nitric oxide (•NO), and peroxynitrite ($ONOO^-$) scavenging activities of aqueous, hydroalcoholic, and alcoholic extracts from *A. digitata* pulp fruits and positive controls.

	Reactive Oxygen Species (ROS)		
Baobab pulp extracts IC_{50} (µg/mL)	$O_2^{\bullet -}$	H_2O_2	HOCl
Aqueous	235 ± 18	21 ± 2 *	156 ± 16
Hydroalcoholic	114 ± 5	55 ± 1 *	46 ± 4
Alcoholic	338 ± 10	58 ± 0.3 *	85 ± 7
Positive controls			
Quercetin	-	62 ± 4	0.46 ± 0.03
Ascorbic acid	54 ± 3 *	177 ± 9	2.33 ± 0.08
Tiron	83 ± 4	-	-
Baobab pulp extracts	ROO• (Ssample/STrolox)		
Aqueous	0.0030 ± 0.0003		
Hydroalcoholic	0.0026 ± 0.0002		
Alcoholic	0.0067 ± 0.0007		
Positive controls			
Quercetin	4.09 ± 0.52		
Ascorbic acid	0.53 ± 0.06		
	Reactive nitrogen species (RNS)		
Baobab pulp extracts IC_{50} (µg/mL)	•NO	$ONOO^-$ (without $NaHCO_3$)	$ONOO^-$ (with $NaHCO_3$)
Aqueous	54 ± 6	64 ± 1	59 ± 4
Hydroalcoholic	27 ± 5	19 ± 1	17 ± 1
Alcoholic	40 ± 4	46 ± 1	42 ± 3
Positive controls			
Quercetin	0.091 ± 0.009	2.02 ± 0.08	3.67 ± 0.24
Ascorbic acid	-	0.41 ± 0.02	0.78 ± 0.03

IC_{50} = half-maximal inhibitory concentration (mean ± standard error of the mean). * Scavenging effect (mean % ± standard error of the mean) at the highest tested concentration (1000 µg/mL).

4. Discussion

4.1. Nutritional Profile

In this work, the centesimal composition of baobab fruit pulp was analyzed. Despite the high nutritional characterization of this fruit pulp [1,7,14,23,36], it is important to perform this analysis due to the fact that the nutritional content varies, depending on an assortment of factors, including geographic origin, soil and temperature conditions, and fruit maturation index, among other factors [23]. Until now, no research has been conducted on this fruit, as harvested in Luanda (Angola). Our results revealed high contents of total dietary fiber (52%) and total ash (8%) in baobab fruit pulp. Low protein and total fat contents were observed. The high quantity of total ash suggests that the fruit pulp is rich in inorganic matter, which is in accord with its high mineral content. Thus, the European Union has defined a range of 5.5–6.6% ash content for baobab pulp [15]. Our findings are consistent with the levels described in the literature, although slightly higher. According to several studies, baobab pulp contains substantial amounts of iron, zinc [36], magnesium, calcium, and potassium [23]. Considering geographical proximity, Monteiro et al. [14] described lower ash contents (4.71–5.18%) in Angolan baobab fruits collected from the municipalities of Namibe (southern Angola). However, the same authors reported identical contents of dietary fiber, ranging from 51.38% to 56.62%. The inorganic composition variation may be due to the adjustments of plant root architecture and exudation, in response to nutritional needs and soil conditions, with several modifications acting as markers of the plant's and the soil's nutritional status.

According to Muthai et al. [36], the baobab fruit pulp contains higher concentrations of all critical nutrients, compared to regularly consumed fruits including bananas, mangoes, and berries. Our results also showed that baobabs from the Luanda region possess an important economic value and are a good and low-cost source of total dietary fiber (52.0%). Regarding dietary fiber content, Muthai et al. [36] reported fiber levels ranging from 5.4% to 11.15% in several baobab fruits collected from distinct areas (Tanzania, Zambia, Kenya, Malawi, and Mali). Also, lower dietary fiber contents (20.62–24.19%) were described in baobab fruit pulp from two districts of Mozambique [37]. Other studies, however, report values ranging from 45.1% to 52%, values similar to those obtained in this study [9,14,24]. One possible reason justifying this variation may be the different evaluations: crude fiber or cellulose, rather than dietary fibers, which are not the same. Also, several analytical methods are used, like enzymatic gravimetric assay and the AOAC method, which can lead to differing assessments of the results. Despite everything, dietary fiber intake promotes favorable body weight and overall metabolic health. According to Barber et al. [38], increased dietary fiber consumption has also been associated with lower incidence of cardiovascular diseases. Additionally, there is evidence suggesting further health benefits of a diet rich in fiber, such as a lower risk of cancer, and a better gastrointestinal health [36], increasing the interest in baobab fruit. Regarding carbohydrate content, our sample exhibited a value of 37.0%, which is higher than those obtained by Monteiro et al. [14] in Angolan fruits. Higher values were reported in fruit pulp from Mozambique, ranging between 70.74–77.23% [37]. Since carbohydrates are usually determined by difference (indirect method), these variations in the amounts of carbohydrates come from the fact that the fiber content increases the apparent amount of digestible carbohydrates. Despite all of these differences, the baobab fruit pulp provides a balanced nutritional profile, which enhances its incorporation into food supplements, in addition to its other industrial applications. The protein amount of fruit pulp obtained was 2.3%, and therefore inside the range of 2.03–3.24% reported by European Union [38] with reference to a novel food ingredient. Moreover, our results agree with others described in the literature [23]. It should be acknowledged that baobab fruit is recognized for its low protein and fat contents [6]. The total fat content obtained in this work was very low (0.4%), indicating a probable similarity with previous research that did not address the fat content in fruits harvested in Angola and Kenya [14,23].

4.2. Bioactive Compounds and Antioxidant Activity

As a food source, fresh fruits and their derivatives offer vital dietary micronutrients as well as bioactive substances like vitamins and polyphenols. Vitamin C is an essential nutrient and an antioxidant compound described as being found in abundance in baobab fruit powder [23,39,40]. According to research described in the *OJEU* [39], the amount of vitamin C found in baobab fruit pulp is three times greater than that found in oranges. Despite the importance of vitamin C, few or no studies mention vitamin E, an outstanding natural antioxidant. In addition to its antioxidant properties, vitamin E is involved in immunological function, cell signaling, gene expression regulation, and other metabolic processes [41]. Thus, protein kinase C, an enzyme involved in smooth muscle cells, and platelet and monocyte proliferation and differentiation, is inhibited by alpha-tocopherol. Additionally, vitamin E raises the production of two enzymes that limit the metabolism of arachidonic acid, increasing the endothelium's release of prostacyclin, which dilates blood vessels and prevents platelet aggregation [42]. Given its beneficial properties, the content of vitamin E was determined in our study. To our knowledge, no study has reported the vitamin E content in baobab fruit pulp. Only two vitamers were detected, α-tocopherol, and β-tocopherol (0.54 mg/100 g and 0.0509 mg/100 g, respectively). Even though the fruit pulp contained less vitamin E than vitamin C (163.8–288.9 mg/100 g), as described in other baobab fruits from Angola [14], this determination is innovative. Indeed, in addition to the health benefits of vitamin E as to human metabolism, the antioxidant activity of vitamin E may contribute to food preservation, reducing the negative impact of chemical reactions on food systems by improving the safety, nutritional value, and shelf-life of food products. Thus, our results may suggest the use of this fruit pulp as a functional ingredient for food products [43].

Regarding the polyphenolic contents in our samples, substantial variability in the TPC of baobab fruit pulp was observed among the different extracts in our study. The hydroalcoholic extract exhibited the highest concentration (1573.0 mg GAE/100 g), followed by the aqueous one (1038.6 mg GAE/100 g). As for the measurement of bioactive compounds (TPC and TFC), the ethanolic extract was revealed to be the worst extraction solvent. Tembo et al. [44] reported total phenolic contents of Malawi baobab pulp methanolic extracts ranging from 1870 to 4057.5 mg GAE/100 g. Another study on baobab fruit extracts from different locations of Sudan described phenolic contents ranging from 15.50 to 99.66 mg GAE/g [45]. Fruit pulp extracts from Madagascar presented 1090 mg GAE/100 g of total phenolic content [46], and those obtained in Burkina Faso showed a variation in total phenolic content between 3520 and 4060 mg GAE/100 g [15]. However lower total phenolic contents were reported in methanolic baobab fruits collected in Angola, ranging from 460.5 mg GAE/100 g to 972.2 mg GAE/100 g [14]. As previously stated, these differences can be related to both environmental conditions [23] and the nature of the extracting solvent. For instance, Ismail et al. [5] used different solvents on baobab fruit pulp and reported higher TPC values in acetone extract, when compared to methanolic extract. However, for aqueous and ethanolic extracts, the values reported by the same authors were significantly lower than those obtained in this work. According to earlier research, the nature of the solvent applied affects both the effectiveness of the extraction and the amounts of phenolic compounds extracted [15,44]. Additionally, extraction times and temperatures and the maceration or pulverization of the samples possibly influence the TPC results. Currently, there is no suggested solvent for the best recovery of phytochemicals, due to their diverse chemical characteristics and polarities, which may affect their solubility in a particular solvent. Phenolic compounds are usually extracted with organic solvents, referred to as conventional solvents, such as methanol, acetone, benzene, chloroform, petroleum ether, and hexane [47]. However, they are frequently rejected because of their flammability, explosiveness, low biodegradability, and toxicity to the final consumer. Green technologies are being tested, with a focus on efficacy and efficiency, increasing productivity, enhancing process selectivity, and reducing energy use. Alternative solvents, such as ionic liquid

(IL) solvents, deep eutectic solvents (DESs), and natural eutectic solvents (NADESs), are a different option for this problem [47].

Just as with TPC, certain factors are observed with reference to the total flavonoid content (TFC). The profile of TFC extractive capacity recovery followed the same pattern as phenolics, with higher contents in hydroalcoholic extract (768.7 mg CAE/100 g), followed by aqueous (428.4 mg CAE/100 g) and ethanolic (235 mg CAE/100 g) extracts, respectively.

Our study has demonstrated that, on average, the total flavonoid concentration is always lower than the total phenolic concentration. These results contradict those reported by Ismail et al. [5] and Wasihun et al. [48], which reported higher amounts of flavonoids in most of the baobab pulp extracts. However, our results are in accordance with other studies [15,45]. Lamien-Meda et al. [15] reported TFC in baobab pulp of 31.70 ± 3.35 mg QE/100 g and 42.73 ± 0.46 mg QE/100 g, using methanol and acetone solvents, respectively, while total phenolic contents were significantly higher in both solvents (3518.33 and 4057.50 mg GAE/100 g, respectively). Furthermore, fruit ripening is a multifaceted, genetically engineered process that results in notable alterations regarding color, texture, flavor, and chemical composition. Kamatou et al. [49] observed a reduction in phenolic and flavonoid concentrations during the development and maturation of baobab fruit. Despite the above, in general, flavonoid content remains understudied, and the few studies that have described their content levels used different solvents.

The antioxidant activity of baobab fruit pulp extracts was assessed using the free radical scavenging capacity ($DPPH^{\bullet}$) and the ferric reducing antioxidant power (FRAP) assays. The values observed in both antioxidant activity assays were directly related to the bioactive contents obtained in each extract evaluated. Regarding the $DPPH^{\bullet}$ assay, scavenging capacity ranged from 1189.3 to 7288.2 mg Trolox/100 g for alcoholic and hydroalcoholic extracts, respectively. In the FRAP assay, the hydroalcoholic extract exhibited the highest antioxidant activity (2736.7 µmol FSE/100 g), followed by the aqueous and alcoholic extracts (1477.8 and 199.2 µmol FSE/100 g, respectively). Ismail et al. [4] reported lower $DPPH^{\bullet}$ values for baobab pulp extracts from Malawi and South Africa, ranging from 721.78 mg Trolox/100 g (ethanol) to 2089.76 mg Trolox/100 g (hydroalcoholic). Our results showed significant differences ($p \leq 0.05$) in both antioxidant assays in aqueous, hydroalcoholic, and alcoholic extracts. To summarize, the antioxidant activity of baobab fruit extracts is related to total polyphenolic concentrations, and this was additionally found in our investigation. Pearson's correlation was calculated, as polyphenols are predominantly responsible for antioxidant activity. TFC, FRAP, and DPPH all had exceptionally positive correlation coefficients with TPC, of 0.893, 0.991, and 0.836, respectively. Furthermore, the correlations between FRAP and DPPH relative to TFC were 0.980 and 0.941, respectively. Our results suggest that flavonoid contents contribute more to the overall antioxidant activity.

4.3. Reactive Oxygen and Nitrogen Species

One of the novelties of this work reports the evaluation of baobab fruit pulp extracts against reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS play critical roles in the immune system. Moreover, oxidative stress is induced by an imbalance between the systemic synthesis of reactive pro-oxidant species and the biological system's ability to quickly neutralize or repair the ensuing damage. Antioxidant compounds obtained from food or dietary supplements can reduce the generation of reactive species at different stages of oxidative stress and through different pathways [21,50]. Thus, traditional fruits, including baobab, might be acknowledged as significant healthy antioxidant sources [2,9,14].

The extracts' scavenging abilities against several ROS and RNS were assessed. $O_2^{\bullet -}$ is the first reactive pro-oxidant specie produced because of the activity of multiple enzymes in the complex cascade of reactive species production. Once produced, $O_2^{\bullet -}$ can be physiologically dismutated to H_2O_2, either spontaneously under acidic conditions or through the action of the enzyme superoxide dismutase [51]. In turn, H_2O_2 can form hydroxyl radicals ($HO^{\bullet}$) through contact with transition metal ions. $HO^{\bullet}$ is the most reactive and oxidative

ROS, and it can induce lipid peroxidation with polyunsaturated fatty acids, resulting in the production of ROO•. After a reaction with chloride ions and in the presence of myeloperoxidase, H_2O_2 is also used to produce additional microbicide ROS, namely, HOCl [50,51]. A comprehensive review of the results obtained in this investigation revealed that, among the extracts examined, the hydroalcoholic extract has a higher scavenging effect against the assessed ROS and RNS (Table 3). In the $O_2^{\bullet}$—scavenging assay, Tiron was the most effective scavenger, whereas ascorbic acid was the least effective.

Quercetin showed no activity in this assay up to the highest quantity tested (1000 µg/mL). Furthermore, the ROO•—scavenging capacity of baobab pulp fruit extracts was determined using the ORAC test. Nonetheless, none of the extracts demonstrated appreciable scavenging action for this ROS at the highest tested concentration (1 mg/mL). According to several studies, the cellular oxidative stress can be modulated by flavonoids by the scavenging of $O_2^{\bullet-}$, neutralizing the effects of this ROS; through hydrogenation; or by complexing with oxidant species [21,50,52]. In terms of RNS-scavenging capacity, all of the extracts proved to be effective scavengers of both •NO and $ONOO^-$. The hydroalcoholic extract was, once again, the most active of the extracts tested, with IC_{50} values of 27 ± 5, 19 ± 1, and 17 ± 1 µg/mL for •NO and $ONOO^-$ evaluated in the absence and presence of $NaHCO_3$, respectively. The inclusion of $NaHCO_3$ in the assays is crucial because, under physiological conditions, $ONOO^-$ rapidly reacts with CO_2 (in equilibrium with bicarbonate anion), resulting in the formation of the nitrosoperoxycarbonate anion ($ONOOCO_2^-$). The subsequent decomposition of $ONOOCO_2^-$ gives rise to various species, including nitrogen dioxide (NO_2) and carbonate radical ($CO_3^{\bullet-}$). Freitas et al. [53] stated that these substances are the main causes of nitration and/or oxidation processes that occur in vivo. Since ascorbic acid was previously found in significant concentrations in baobab pulp fruit, this isolated compound was also evaluated in similar assays to compare its results to those obtained for the extracts. When compared to the baobab pulp extracts, ascorbic acid demonstrated significantly greater scavenging capacity for almost all of the studied ROS and RNS (excluding $O_2^{\bullet-}$). However, the scavenging capacity of quercetin for all of the assessed ROS and RNS was much greater than the values reported for the extracts. Nonetheless, quercetin was the most effective scavenger of H_2O_2, HOCl, ROO•, and •NO, outperforming ascorbic acid. Some in vitro and in vivo studies have demonstrated the efficacy of quercetin as an antioxidant in decreasing and avoiding damage and oxidative stress caused by free radicals [54]. HOCl, a highly oxidative reactive molecule naturally produced in neutrophils by myeloperoxidase, also yielded intriguing results. Rita et al. [55] reported a high scavenging capacity for $O_2^{\bullet-}$ and •NO radicals (IC_{50}—77.14 ± 3.43 and 39.33 ± 6.89 µg/mL, respectively) in aqueous extracts of baobab fruit pulp, although significantly lower than our results. Ibraheem et al. [22], using methanolic extracts of *A. digitata* pulp, also stated a higher value for •NO inhibitory activity (IC_{50}—36.55 ± 1.79 µg/mL). According to Ntchapda et al. [56], aqueous extract of baobab fruit reduces •NO levels in the aorta, heart, and kidney of hypertensive rats. Thus, according to Ibrahim et al. [57], the polysaccharides of *A. digitata* have stronger $O_2^{\bullet-}$ and •NO scavenging activity, as well as stronger H_2O_2 scavenging activity, than butylated hydroxytoluene (BHT) and ascorbic acid. Therefore, the results support our hypothesis that baobab pulp extracts, mainly aqueous and hydroalcoholic, possess an antioxidant capacity through the inhibition of ROS and RNS, which can lead to oxidative stress prevention. This study provided a novel approach for assessing the Angolan native *Adansonia digitata* L. species by emphasizing its potential as superfruit or alternatively as a functional ingredient.

5. Conclusions

Baobab pulp can be consumed as food or used as a functional ingredient, owing to its high content levels of ashes, dietary fiber, and phenolic and flavonoid compounds. This phytochemical profile is responsible for the high antioxidant activity reported in this work. *Adansonia digitata* L. health benefits encourage large-scale regional and global commercialization of this fruit. However, the quantities of nutrients and non-nutrients can be impacted

by the location of the gathered baobab samples, as well as the extraction solvents and analytical procedures. For the first time in baobab fruit research, results were presented showing that the use of non-toxic green solvents allowed the production of extracts capable of scavenging ROS and RNS. Summarizing, the obtained results demonstrated that *A. digitata* fruit pulp is rich in bioactive compounds that may be related to its biological activity, which is characterized by evident antioxidant properties, corroborating the nutritional value of this delicacy. Further research is suggested, including enhanced sampling of the baobab fruits collected from different geographical areas of Angola. Several factors, like geographical origin, ripening stages, and agrotechnical measures may influence the nutritional and chemical composition of the fruits. Similarly, the identifications of individual phenolic compounds and their individual quantifications are relevant. Also, the in vitro antiproliferative and antitumor activities of fruit pulp extracts should be tested against human cancer cell lines to evaluate the bioactivity of the chemical compounds described in baobab fruit.

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