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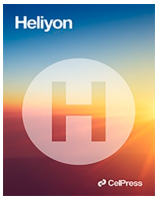

Research article

In-vitro antioxidant, antimicrobial and phytochemical properties of extracts from the pulp and seeds of the African baobab fruit (*Adansonia digitata* L.)

Philip T. Thompson [a], Vivian E. Boamah [b], Mercy Badu [a,*]

[a] *Department of Chemistry, Faculty of Physical and Computational Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[b] *Pharmaceutical Microbiology Section, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

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ABSTRACT

Adansonia digitata, commonly known as the African Baobab plant is used widely in traditional medicine for treating of many diseases. The current study investigates the antioxidant and antimicrobial properties, and nutritional composition of the pulp and seeds from the fruit of African Baobab plant. Matured fruits were harvested and processed by separating the fruit pulp and seeds. Water, 70 % Ethanol/water mixture, and Hexane were used as solvents for extraction. Antioxidant properties of extracts in this study were investigated using the 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxidescavenging assays., Total Flavonoid Content, Total Phenolic Content, Total Tannin Content, and Total Antioxidant Capacity were also investigated. Agar Well Diffusion and Broth Dilution methods were used to estimate the antimicrobial properties of the extracts. The proximate composition of the seeds and fruit pulps was also determined. GC-MS was employed to determine the fatty acid composition. Results obtained showed the presence of Total phenolics (range 4.1–5.5 mg GAE/g), Total flavonoids (range 10.1–16.5 mg QE/g), Total Tannins (range 1.7–15.6 mg CE/g), and Antioxidants (range 2.0–14.5 mg AAE/g). The H_2O_2 and DPPH assays gave IC_{50}s in the ranges of 300–1800 mg/L and 700–1600 mg/L respectively. Extract from the fruit pulp was found to inhibit the growth of a panel of 2 g-positive bacteria, 2 g-negative bacteria, and two fungi microorganisms. Fatty acids such as myristic acid, palmitic acid, and stearic acid were found to be present in oil from the seeds. Proximate components such as crude protein, crude fat, and crude fibre were found to be high. From the results, seeds and the fruit pulp of the African Baobab plant have significant antioxidant properties and can inhibit microbial growth.

1. Introduction

The African Baobab plant also called *Adansonia digitata* L. is ancient andfound in sub-Saharan Africa as well as Western Madagascar areas [1]. The plant is huge and bears many fruits. The leaves, stem bark and roots of the plant are used in the preparation of traditional medicine. Many communities use the fruits as food, and other parts for shelter, clothing, and material for hunting and fishing. Reports in the literature have shown the presence of antioxidant and anti-inflammatory properties found in different parts of the plant [2–10].

* Corresponding author.
E-mail addresses: mbadu0@gmail.com, mbadu.sci@knust.edu.gh (M. Badu).

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Ismail and co-workers have identified some phyto-constituents present in the pulp from the fruit of the Baobab using the LC-MS/QTOF tool. They also investigated how different solvents affected the phenolic content and the antioxidant properties of the pulp [2].

In Ghana, the African Baobab plant grows very well in the savannah regions where, as a result of the harsh climatic conditions, it has been badly affected by climate change [1,11]. A critical effect of climate change seen in the lives of people in these communities is acute hunger, malnutrition, and an increase in disease burden [12].

The African baobab plant's fruit pulp is added to a range of local foodstuffs, including beverages and starchy staples. Their seeds are removed and used to make oils, soups and stews. The fruit pulp is known to possess high protein content and essential amino acids such as tyrosine, glutamine, aspartic acid, glycine, cysteine, and methionine [13]. There are also reports showing the presence of essential mineral elements including sodium, copper, magnesium, potassium, calcium, phosphorus, manganese, zinc, and iron as well as Vitamin C in the fruit pulp [13–15]. With regards to the seeds, oils extracted from them are known to provide a good source of mineral elements and unsaturated fatty acids such as oleic, linoleic, and linolenic acid. Consumption of these fatty acids is beneficial to human health by lowering blood cholesterol, being cytotoxic to human breast cancer cells, immune boosting, and supporting skin and hair health [16–19]. Again, the seed oil has been found to contain vitamins A, D, E, and K. Polyunsaturated fatty acids such as Vitamin A and K are responsible for cell membranes renewal, and vitamin E for oxidative stability and increasing shelf life [20].

Recent research interest is geared towards the full utilization of potential medicinal and nutritious plant-based products which is in line with the Sustainable Development Goal (SDG) number 3 [21]. These plant-based products are expected to be accessible, cheaper, and available to all. The purpose of this investigation is to present data on the nutritional and therapeutic qualities of extracts made from the African baobab plant's seeds and fruit pulp. It is expected that the results obtained will serve as baseline data for the promotion of fruit pulp and seeds as food and nutraceuticals to help reduce malnutrition and diseases in highly affected communities.

2. Materials and methods

2.1. Sample collection and preparation

Fruits of the African baobab plant were obtained from Kpong (near mountain Yuaga) in the Eastern region of Ghana (Latitude: 6.18471° or 6°11′5″ north and Longitude: 0.04465° or 0°2′41″ east) (Fig. 1). Identification and authentication of the plant materials were conducted at the Herbarium of the Department of Herbal Medicine, KNUST Ghana. The dried pulp was manually scraped from the seeds and kept in an air-tight container. The seeds were washed and sun-dried for 10 h and then milled into fine powder.

2.2. Extraction and phyto-constituents determination

2.2.1. Extraction

The bioactive compounds of the fruit pulp were extracted with water to obtain the Baobab Fruit Water Extract (BFWE) using the maceration method of extraction at 27 °C for 72 h. Oil from the seeds were extracted with a non-polar solvent, hexane, in a Soxhlet

Fig. 1. Map of sampling area showing mountain Yuaga in the Eastern region of Ghana – Africa.

extraction at 65 °C for 8 h to get the Baobab Oil (BO). The defatted powdered sample of the seeds was also extracted with 70 % ethanol/water in maceration and labeled Defatted Baobab seed Ethanolic Extract (DBEE).

2.2.2. Glycosides

From the powdered sample, 1 g was suspended in 1 mL glacial acetic acid, filtered, and cooled. To the cooled mixture, three drops of 10 % ferric chloride was added. Two milliliters of concentrated sulfuric acid was gently added to the solution along the walls of the test tube. A reddish-brown ring phased between two layers gave a positive response to the presence of glycosides [22].

2.2.3. Phenols

To 1 g of powdered plant material, 2 mL of distilled water was added after which the mixture was filtered. Ferric chloride (10 %) solution was then added to the filtrate dropwise. A dark green color inferred a positive result for the presence of phenols [16].

2.2.4. Steroids

Five grams of powdered plant material were suspended in 2 mL of chloroform. Concentrated sulfuric acid was added gently in drops along the walls of the test tube. A blowfish green color at the interface indicated a positive result for steroids [23].

2.2.5. Terpenoids

Five grams of the samples was suspended in chloroform (2 mL) after which 3 ml of concentrated sulfuric acid was added to the resulting solution. The formation of a reddish-brown colored interface showed a positive inference of terpenoids [24].

2.2.6. Flavonoids

To 5 g of the sample, 5 ml of methanol was added and filtered. To a portion of the filtrate, 2 mL of sodium hydroxide solution was added. Three drops of diluted hydrochloric acid was added to the resultant mixture. The appearance of a yellow solution when the sodium hydroxide solution was added which became colourless when dilute hydrochloric acid was added, this confirmed that flavonoids are present [22].

2.2.7. Tannins

To 5 g of the sample, 10 ml of distilled water, was added heated, and filtered. One milliliter of $FeCl_3$ (0.1 %) was added to the filtrate. A blueish-green precipitate shows the presence of tannins [23].

2.2.8. Anthraquinones

To 5 g of sample, 10 ml of toluene was added, shaken, and filtered. Five milliliters of ammonia were added to the filtrate. A greenish-yellow coloration showed the presence of anthraquinones [23].

2.2.9. Saponins

Two grams of powdered plant material were suspended in 5 mL distilled water. The mixture was vigorously shaken for 1 min. Persistent copious lather for more than 5 min shows the presence of saponins [18].

2.2.10. Coumarins

Powdered plant material was suspended in 1 N NaOH solution in a test tube. The test tube was then enclosed using filter paper and placed in a bath of boiling water for 5 min. After that, the filter paper was taken off and fluorescence was detected under a UV lamp. The presence of coumarins was established by a yellow fluorescence [24].

2.2.11. Alkaloids

One milliliter of aqueous extract of the sample was suspended in 1 ml of distilled water and drops of diluted hydrochloric acid (2 M). To the resulting mixture 1 mL Dragendorff's reagent was added. An orange-red precipitate shows the presence of alkaloids [25].

2.2.12. Proteins

A gram of the powdered plant material was boiled in 2 ml Ninhydrin solution (0.2 %). The presence of amino acids or proteins was confirmed by the appearance of a violet color [20].

2.2.13. Carbohydrates

A mixture of Fehling A and Fehling B (equal portion) reagents was prepared. Two milliliters of the mixture were gently boiled with a gram of powdered plant material. A brick-red precipitate at the bottom of the test tube gave a positive test for carbohydrates [26].

2.3. Antioxidant properties

2.3.1. Total Phenolic Content

The Folin-Ciocalteu method was used to quantify thephenolic content of the extracts as described by Ganamé et al. with slight adjustment [27]. One milliliter of the test solution, (gallic acid; 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.125 μg/ml) and (extracts; 1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml) were transferred separately into capped test

tubes. To each tube Folin-Ciocalteu reagent (1 ml) was added and allowed to incubate at 27 °C for 6 min. Two milliliters of 7.5 % sodium carbonate (*m/v*) were added to the solutions and further incubated at 50 °C for 10 min. The absorbance of all prepared solutions was taken at 765 nm against a blank by a spectrophotometer (SPECTROstar NANO, BMG LABTECH GmbH Allmendgrun 877799, Ortenberg, Germany). A standard calibration curve of gallic acid was plotted ($y = 0.0159x - 0.0407$; $R^2 = 0.9964$). The total phenolic content was expressed in mg equivalent of gallic acid per 100 g of the raw extract (mg GAE/100 g).

2.3.2. Total flavonoid content

One milliliter of quercetin (ranging 3.125 μg/ml to 100 μg/ml)) and extract (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml) was transferred into a capped test tube. 0.3 ml sodium nitrate 5 % (*m/v*) and 0.5 ml of $AlCl_3$ 10 % (*m/v*) were added. The solution formed was left to incubate for 6 min at 27 °C. The absorbance of standard and sample solutions were taken at 510 nm with a SPECTROstar NANO, spectrophotometer. The flavonoid content of the extracts is expressed as mg equivalent of quercetin per 100 g of crude extract (mg QE/100 g) [28].

2.3.3. Total Tannins content

To 0.5 ml of catechin concentrations (ranging 3.125 μg/ml to 100 μg/ml) or extract (ranging 31.25 μg/ml to 1000 μg/ml) in a capped test tube 3 ml of n-butanol/HCl (95:5 $^{v}/_{v}$) solution was added. Again, 0.1 ml iron solution (2 % ferric ammonium sulfate in 2 N HCl). The mixture was capped and incubated at 95 °C for 1 h. Absorbance of test solutions was taken at 510 nm with a SPECTROstar NANO, spectrophotometer, The total tannin content of the extracts is expressed as mg equivalent of catechin per 100 g of crude extract (mg CE/100 g) [29].

2.3.4. Total Antioxidant Capacity

A phosphomolybdanum reagent (a mixture of equal volumes of 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid) was prepared and kept in the dark overnight. A 0.1 ml of the standard (ascorbic acid conc. Ranging from 20 μg/ml to 100 μg/ml) or extract (BO/DBEE/BFWE conc. Ranging from 200 μg/ml - 1000 μg/ml) was mixed with 1 ml phosphomolybdanum solution in a test tubes tightly covered and after which the content was thoroughly shaken. The tubes were allowed to incubate for 90 min at 95 °C in a water bath. The reaction mixture was allowed to cool to room temperature, and the absorbances of the resulting solutions were taken at 695 nm with a SPECTROstar NANO, spectrophotometer. The total antioxidant capacity of the extracts is expressed as mg equivalent of catechin per 100 g of crude extract (mg CE/100 g) [30].

2.3.5. DPPH free radical scavenging assay

A DPPH solution (0.2 mM) was prepared (0.0078864 g of DPPH in 100 ml methanol). In a microtiter plate, 150 μl of 0.2 mM DPPH was added and followed by a 50 μl of standard (ascorbic acid; , ranging 40 μg/ml to 200 μg/ml) or extract (BO/DBEE/BFWE; ranging 200 μg/ml to 1000 μg/ml). The mixture was left to stand in the dark at 27 °C for 30 min. From the resulting solution absorbances were taken against a blank at 517 nm with a SPECTROstar NANO, spectrophotometer. The scavenging activity of the extracts against DPPH radical was calculated as scavenging activity (%) = $(DPPH_{abs} - Extract_{abs}/DPPH_{abs}) \times 100$ [25].

2.3.6. Hydrogen peroxide scavenging assay

A 1 mM ferrous ammonium sulfate was prepared by dissolving 0.02841g of $Fe(NH_4)_2(SO_4)_2$ in 100 ml distilled water. From the stock solution a 5 mM hydrogen peroxide solution was prepared. A 200 ml of 1 mM of 1,10-phenanthroline was prepared. Three milliliters of the standard (ascorbic acid ranging 20 μg/ml - 100 μg/ml) or extract (BO/DBEE/BFWE rangin 200 μg/ml - 1000 μg/ml) together with 0.5 ml of 1 mM ferrous ammonium sulfate and 0.13 ml H_2O_2 (5 mM) were capped in test tubes and kept in a dark room for 5 min. Three milliliters of 1,10-phenanthroline were added to the resulting mixture and left in the dark for 10 min at room temperature. Absorbances of the resulting solutions were taken against a blank at 510 nm with a SPECTROstar NANO, spectrophotometer The scavenging activity of the extracts against hydrogen peroxide was calculated as scavenging activity (%) = $(Extract_{abs}/Control_{abs}) \times 100$ [31].

2.4. Antimicrobial activity

2.4.1. Microorganism cultures and inoculum preparation

The Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST – Ghana provided all the test organisms required for the experiment which was carried out in their Microbiology Laboratory. In a single-strength nutrient broth the organisms were cultured and incubated at 37 °C for 24 h. On a nutrient agar they were sub-cultured and again incubated for 24 h at 37 °C. The morphology of the organisms was observed and confirmed after the 24-h incubation period. The Petri dishes containing the sub-cultured organisms on nutrient agar were then stored in a freezer at 0 °C until further use. To prepare a 0.5 McFarland standard solution, a 0.05 mL of 1.175 % barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), and 9.95 mL of 1 % sulfuric acid (H_2SO_4) were mixed. A colony suspension was prepared in Using a sterile normal saline solution colony suspension was prepared and adjusted to the prepared McFarland standard by optical observation and again diluted in sterile double-strength nutrient broth to obtain $\sim 2 \times 10^5$ CFU/ml [32,33].

2.4.2. Gram-positive microorganisms

Staphylococcus aureus ATCC 25923.

Streptococcus pyogenes Clinical strain.

2.4.3. Gram-negative microorganisms

Pseudomonas aeruginosa ATCC 27853.
Escherichia coli ATCC 25922.

2.4.4. Fungi

Candida albicans Clinical strain.
Tinea corporis Clinical strain.

2.4.5. Zone of inhibition

A mixture of each colony suspension (0.1 ml) and 25 ml nutrient agar was prepared in a Petri dish. In a solidified agar, five wells (4 mm diameter) were bored using a 4 mm cork borer. Each eells was filled with 100 μL of the extract at concentrations ranging 12.5 mg/ml - 100 mg/ml and a standard drug (Ciprofloxacin/Clotrimazole; 1 mg/ml). The prepared mixture were then incubated at 37 °C for 24 h. Blanks for various vehicles were also prepared alongside the samples. Analysis of the results were captured as diameter of the zone of inhibition. The diameters were taken with the meter rule from one end of the zone to the opposite end where no visible growth (clear zones) was observed after the 24-h incubation period [34].

2.4.6. Minimum Inhibition Concentration (MIC)

A 2-fold serial dilutions of the extracts and standard drugs (100 μl of each concentration) were directly prepared in the microtiter plate at designated wells. The bacterial inoculum (20 μl) was pipetted into their designated wells. To prepare the nutrient broth (double strength (200 μl)), 1.8 g of the broth was dissolved in 70 ml sterile water, andadded to the wells. The plates were sealed with sterile covers and allowed to incubate at 37 °C for 24 h 20 μl of 1.25 mg/ml 3- (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye were added to each well and incubated at 37 °C for 30 min. The presence of purple coloration in a well indicated microbial growth and otherwise. MIC was estimated as the lowest concentration that prevented microbial growth for both the extract and/or standard drug that. The tests were done in triplicate [35,36].

2.4.7. Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the extracts was arrived at using a similar method as used in the MIC assay protocol. Here, when the 24-h incubation period elapsed, 50 μl portions of the suspensions in wells with extract concentrations greater than the MIC were plated on sterile nutrient agar in Petri-dishes. The agar plates were then incubated for 24 h at 37 °C. MBCs were recorded as the lowest concentration of the extract suspension that did not grow the microorganism on the nutrient agar [36].

2.5. Nutritional composition

2.5.1. Fatty acids analysis by Gas Chromatography-mass spectrometry (GC-MS)

TheGC-MS analysis was conducted on the fatty acid methyl esters (FAMEs) derivatives formed. To achieve this, 0.5 g of hexane extract was taken and a 17.64 ml 2 % (m/v) methanolic sodium hydroxide solution added and heated in a water bath for 10 min by refluxing.30 ml of concentrated HCl and the methanolic mixture of ratio 3:2 respectively were added and allowed to boil for another 10 min. Hexane (10 ml) was added, and further boiled for 2 min. The content was then left to cool to 27 °C and then 30 ml of distilled water was added. The hydrocarbon layer formed was collected and analyzed. The methylated fatty acids were analyzed using a PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HTMS (5 % diphenyl/95 % dimethyl poly-siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm DF) [37].

2.5.2. Proximate composition analysis

The nutritional composition such as moisture, ash, protein, fat, fiber, and carbohydrate content of the seeds and fruit pulp were determined using techniques certified by the Association of Official Agricultural Chemists (AOAC) with slight modifications [38]. Each analysis was done in triplicates. A weighed sample (5 g) was placed in a preheated oven at 105 °C and dried to a contant weight to measure the percentage moisture. The micro-Kjeldahl technique was used to determine the crude protein concentration [39]. Percentage fatin the seeds and fruit pulp were determined by extracting from a 5 g sample in petroleum ether through the Soxhlet extraction method [38]. After digesting a measured amount of the fat-free residue produced from the crude fat determination, the crude fiber was evaluated gravimetrically. With 1.25 % sodium hydroxide and 1.25 % sulfuric acid, the digestion was carried out under reflux. The sample was burned for 10 h at 600 °C in a muffle furnace to ascertain its ash content.

Carbohydrate percentages of the various samples were estimated as follows:

Carbohydrate (%) = 100 - % yield [moisture + fat + ash + protein + fiber]

The Total Energy values of the plant materials were obtained by calculations according to the Atwater factor as shown below:

Energy value (kcal) = 4(% crude protein) + 9(% crude fat) + 4(% carbohydrate), [38].

2.6. Statistical analysis

Each experiment in this study was done in triplicates, and each data was reported in mean values and standard deviations (mean ±

SD). GraphPad Prism 8 software was used for the data analysis. And the Pearson Correlation was used to statistically analyze the correlation between the phytoconstituents and the antioxidant activity.

3. Results and discussion

3.1. The plant samples

The African Boabab plant (Fig. 2D) is a versatile tree plant in the Africa region. When in season, it bears thousands of fruits (Fig. 2C) which has a white pulp (Fig. 2B) and seeds (Fig. 2A). This plant has been reported widely to possess high antioxidant and anti-inflammatory properties from different parts.

3.2. Preliminary phytochemical screening and extraction

To determine the various kinds of chemicals present in *A. digitata*'s fruits and seeds, a preliminary phytochemical screening was carried out (Table 1). The results revealed that both the seeds and fruit pulp contain compounds including Tannins, Glycosides, Steroids, Flavonoids, and Saponins. Alkaloids were found only in the seeds and not in the fruit pulp. Chemical compounds obtained from plants are very important due to their potential pharmacological activities in the body [22,24].

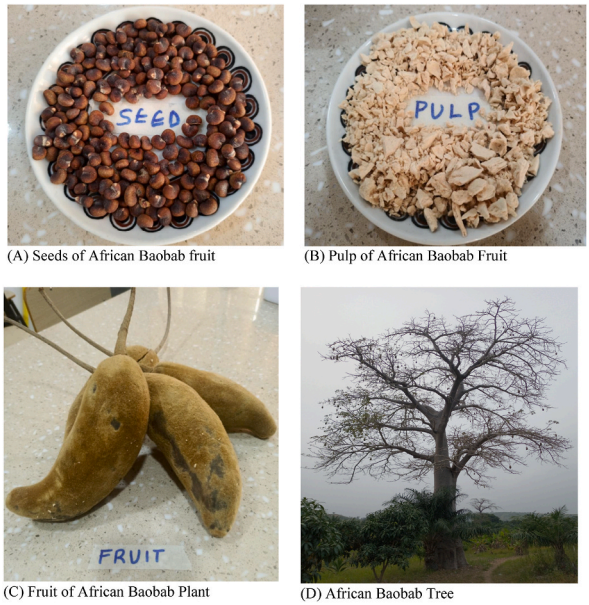
The percentage yield of phyto-compounds which were extracted using the different solvents are shown in Table 2. The results revealed that the seeds contain about 6.20 % non-polar compounds which were extracted into the hexane solvent and 4.88 % polar compounds extracted into the 70 % ethanol solvent. The fruit pulp was found to contain about 27.94 % polar compounds extracted into an aqueous solvent. Since the aim was to mimic the exact conditions of the traditional use, the data from the study shows that the traditional way of preparing the *A. digitata* fruit pulp as a beverage and the alcohol concoction of the seeds will give about 28 % and 5 % respectively extract of polar compounds.

3.3. Antioxidant properties

Secondary metabolites from plants comprise many related compounds that have different physiological properties. The nature of these compounds is dependent on the type of plant as well as the part of the plant from which the compound is obtained [40]. Anti-oxidant properties of the seeds and fruit pulp of the Baobab plant was evaluated by quantifying the presence flavonoids, tannins, and phenols (Table 3).

3.3.1. Total phenolic, flavonoid, and tannin contents

From the results, aqueous extract of the Baobab fruit pulp (BFWE) gave the highest total phenolic content of 5.545 ± 2.79 mg GAE/g, then the defatted Baobab seed ethanolic extract (DBEE) of 5.009 ± 3.26 mg GAE/g, and Baobab seed oil (Hexane extract) (BO) gave 4.102 ± 3.32 mg GAE/g. However, with regards to the flavonoid content, the hexane extract gave the highest yield of 16.512 ± 3.52 mg QE/g followed by the fruit pulp aqueous extract of 14.076 ± 3.77 mg QE/g with the defatted seed ethanolic extract showing the



(A) Seeds of African Baobab fruit
(B) Pulp of African Baobab Fruit
(C) Fruit of African Baobab Plant
(D) African Baobab Tree

Fig. 2. Pictures of sample **(A)** seeds, **(B)** fruit pulp, **(C)** fruits, and **(D)** tree of the African Baobab plant.

Table 1
Phyto-constituents found in baobab seed and fruit pulp.

Phytochemicals Tested	Baobab Seeds	Baobab Fruit Pulp
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	–
Steroids	+	+
Glycosides	+	++
Saponins	+	+
Coumarins	+	+
Phenols	+	++
Terpenoids	+	++
Anthraquinone	+	++
Carbohydrates	+	+
Proteins	–	+

Key: (+) means present (−) means absent (++) means abundantly present.

Table 2
The percentage yield of extracts.

Solvent used	Seeds	Fruit pulp
Hexane	6.20 %	–
70 % Ethanol/Water	4.88 %	–
Distilled Water	–	27.94 %

Table 3
Total Flavonoid, Total Phenolic, Total Tannin, and Total Antioxidant Capacity of various Extracts obtained in the study.

Extract	Total Flavonoid Content (mg QE/g)	Total Phenolic Content (mg GAE/g)	Total Tannin Content (mg CE/g)	Total Antioxidant Capacity (mg AAE/g)
BFWE	14.1 ± 3.8	5.5 ± 2.8	11.2 ± 3.3	9.5 ± 3.9
DBEE	10.1 ± 3.1	5.0 ± 3.3	1.7 ± 0.8	7.4 ± 2.4
BO	16.5 ± 3.5	4.1 ± 3.3	15.6 ± 4.0	2.6 ± 0.6

lowest flavonoid content. For the total tannin content, again, the Baobab seed oil (hexane extract) gave the highest total tannin content of 15.569 ± 4.00 mg GAE/g, followed by the aqueous fruit. pulp extract of 11.174 ± 3.32 mg CE/g and the defatted seed ethanolic extract recorded the lowest total tannin content of 1.748 ± 0.82 mg CE/g.

Mechanistically, antioxidants quench the chain reaction of oxidation by donating hydrogens or accepting free radicals but becoming stabler radicals themselves. Secondary metabolites that possess a phenolic structure like flavonoids, tannins, catechins, β-carotenes, and their derivatives can elicit the mechanism of antioxidation [41]. Phenolic compounds such as tannins, saponins, flavonoids, phenolic acids, anthocyanins, etc. are regarded as the most substantial secondary metabolites biochemically synthesized by plants. These substances are dispersed throughout the plant, and their concentrations are significantly influenced by factors such as the type of organ, variety, climate, and location, among many others. The values obtained for the phenols, flavonoids, and tannins in this study are only marginally different from those compared with in the literature. Alessandra Braca and co-workers estimated the total phenolic content of a variety of Baobab fruit pulp from different origins in Mali. They reported that the total phenolic content in the fruit pulp ranged from 120 to 162 mgGAE/g [42]. Mady Cisse and colleagues reported a range of 0.02–0.05 mgEAG/g of polyphenols in various seed extracts from Baobab fruit [43]. These differences compared to our values in Table 3 could be attributed to extraction process, origin of sample, or the time of maturity [44]. Table 3 summarizes the various flavonoid, phenolic, and tannin contents of the extracts as compared to a known standard.

3.3.2. Total antioxidant capacity (TAC)

The total antioxidant capacity results obtained in the study is shown in Table 3. Overall, the fruit pulp extract (BFWE) yielded the highest antioxidant capacity (9.5 ± 3.9 mgAAE/g) as compared to the other seed extracts. The defatted seed extract (DBEE) recorded a TAC of 7.4 ± 2.4 mgAAE/g and the seed oil extract (BO) gave relatively, the least total antioxidant capacity (2.6 ± 0.6 mgAAE/g). This result is consistent with the free radical scavenging activities (DPPH and H_2O_2) in terms of the increasing order of antioxidant activity. Thus BFWE > DBEE > BO. From the correlation study, it was shown that all the polyphenols quantified had a positive correlation with the antioxidant activities of the extracts and therefore can be said to be responsible for the antioxidant properties observed.

3.3.3. DPPH radical scavenging activity

Quantifying the antioxidant activities using the DPPH scavenging activity was done, the results are shown in (Fig. 3 (A-D)). This indicates that the radical scavenging activity of BFWE with an IC_{50} of 787.5 ± 74 is the highest among the extracts as was expected

with reference to the reported high phenolic content, tannin content, flavonoid content, and antioxidant capacity. DBEE was the second-highest free radical scavenging extract with an IC_{50} of 1402.1 ± 36. BO had a relatively lower radical scavenging activity as compared to the other extracts as it recorded an IC_{50} of 1617.8 ± 8. However, all the extracts showed inhibition level below that of the standard Ascorbic acid yielded (IC_{50} = 70.5 ± 2).

3.3.4. *Hydrogen peroxide radical scavenging activity*

Like the DPPH activity, the Hydrogen Peroxide radical scavenging property of the extracts followed a similar trends. BFWE showed the best scavenging activity relative to the other extracts. Followed by DBEE The IC_{50} values are shown in Fig. 4 (A-D). The scavenging observed to be dose-dependent and correlates positively with the TAC and DPPH assay values.

The antioxidant activity of most plant extracts occurs through the formation of an initiator or terminating radicals during chain propagation. Some radical scavenging entities also work indirectly by increasing enzymatic activities that quench reactive oxygen species (ROS) or induce the expression of like proteins [45]. Many reports in the literature have attributed the most antioxidant properties to of phenolic compounds. In this study we looked at the relationship between the antioxidant activity of the extracts and the phenolic content.

3.4. *Correlation analysis*

The correlation coefficients between the total phenolic content, total flavonoid content, and total tannin content and the antioxidant activities such as total antioxidant capacity, DPPH scavenging, and H_2O_2 scavenging activities were used to investigate the antioxidant potential of the extractsfound in baobab fruit pulp and seeds (Table 4). Many authors have reported the synergistic nature of naturally occurring antioxidative compounds and their free radical quenching potential [46–48]. Data obtained from the study revealed thatthe total phenolic content (R^2 = 0.996, 0.823, and 0.955) antioxidant activity of the extracts showed a more significant correlation as compared to the total flavonoid content (R^2 = 0.321, 0.012, and 0.104) and the tannin content (R^2 = 0.262, 0.002, and 0.068). A poor positive correlation between TFC and TPC, and the antioxidant activity was observed. Even though this anomaly could be attributed to the various assays used, it is notable that flavonoids' radical scavenging activity is strongly linked to the hydroxyl groups found at core position of the flavonoid structure. The bond dissociation energy O – H in the hydroxyl group of the flavonoid core, controls the susceptibility of the hydrogen atom to dissociation. This dissociation is chiefly responsible for the inactivation of free radicals [49]. This indicates that not all kinds of flavonoids have the same radical scavenging potential hence the observed reduced TAC and TPC as compared with the TFC.

TAC: total antioxidant capacity; TPC: total phenolic content; TFC: total flavonoid content; TTC: total tannin content; H_2O_2: hydrogen peroxide scavenging assay; DPPH: 2,2-diphenyl-1-picrylhydrazyl assay.

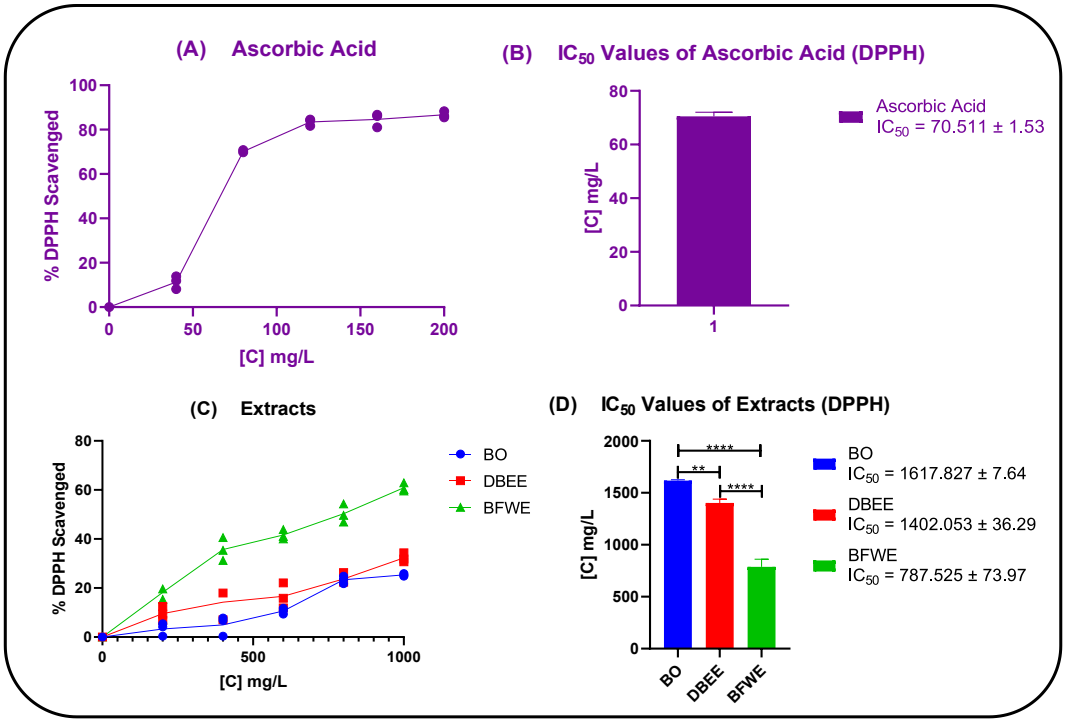


Fig. 3. Graph of % DPPH Scavenged against concentration (mg/L) of **(A)** Ascorbic acid standard and **(C)** Extracts: BO, DBEE, and BFWE. Bar chart representing IC_{50} values of **(B)** Ascorbic acid standard and **(D)** Extracts: BO, DBEE, and BFWE.

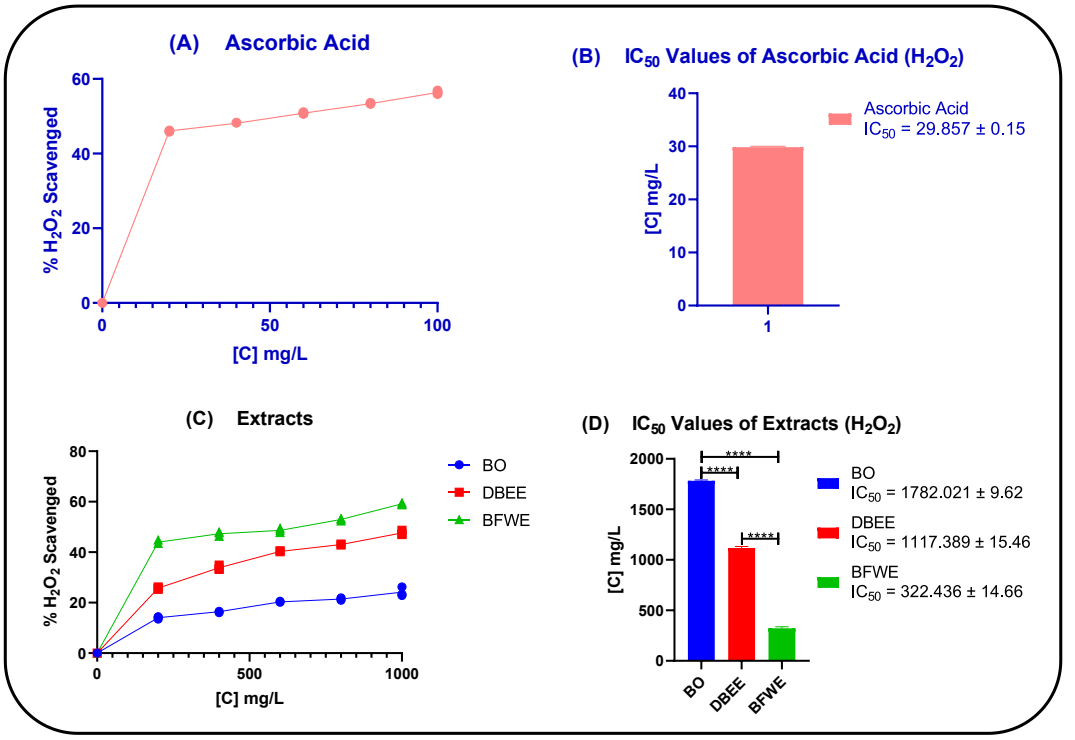


Fig. 4. Graph of % Hydrogen Peroxide Scavenged against concentration (mg/L) of **(A)** Ascorbic acid standard and **(C)** Extracts: BO, DBEE, and BFWE. Bar chart representing IC_{50} values of **(B)** Ascorbic acid standard and **(D)** Extracts: BO, DBEE, and BFWE.

Table 4
Correlations between the phytochemical compounds and Antioxidant properties of extracts of the fruit pulp and seeds of baobab.

Variables	TPC	TFC	TTC
TAC	0.996[a]	0.321	0.262
H_2O_2	0.955[a]	0.104	0.068
DPPH	0.823[a]	0.012	0.002

[a] Correlation is significant at $p < 0.01$ level.

3.5. Antimicrobial bioactivity

3.5.1. Agar Well Diffusion

A pronounced dose-dependent inhibition was seen amongthe fruit pulp extract (BFWE) on *S. aureus, S. pyogenes, P. aeruginosa, E. coli, T. corporis,* and *C. albicans* (Table 6). A zone of inhibitions was not observed at a concentration of 12.5 mg/ml of BFWE on *S. aureus, S. pyogenes, E. coli, T. corporis,* and *C. albicans*. There were no display of anti-microbial effects on the test organisms among the seed extracts (BO and DBEE) (Tables 7 and 8). BO and DBEE recorded utterly no zone of inhibition even at very high concentrations.

Concentrations of the standard drugs (the anti-bacterial Ciprofloxacin and the anti-fungi Clotrimazole) (Table 5) that were utilized for positive controls on the tested organisms were a twelve-fold difference less than the least concentration of the BFWE (Table 6). Even

Table 5
Zone of inhibition (mm) for standard drugs (clotrimazole and ciprofloxacin).

Test Organism	Concentration (1 mg/ml)	
	Ciprofloxacin	Clotrimazole
S. aureus	46.7	
S. pyogenes	42.0	
P. aeruginosa	43.0	
E. coli	47.3	
T. corporis		15.6
C. albicans		14.3

at the twelfth of the concentration of BFWE, the standard drug, Ciprofloxacin, demonstrated a zone of inhibition that was at least thrice as high as that of BFWE for all investigated bacterium species. Clotrimazole, the anti-fungi standard drug, at a twelve-fold lower concentration, also exhibited a higher zone of inhibition for the tested fungi as compared to the BFWE.

3.5.2. Broth dilution

The Fruit pulp extract (BFWE) was the only extractin this study that showed a positive anti-microbial activity from the broth dilution assay. Very small, almost to no changes was observed with the BFWE with regards to inhibition ability on the test organisms. were observed (Table 9). BFWE recorded a Minimum Inhibition Concentration (MIC) of 6250 μg/ml for *S. aureus* and *C. albicans*, it also recorded a MIC of 12500 μg/ml for *S. pyogenes, P. aeruginosa, E. coli,* and *T. corporis* (Table 11).

This study found a correlation between the patterns of the zone of inhibition in the agar diffusion experiment and the qualitative patterns of the MICs obtained using the broth dilution method. For BFWE and the standard drugs, there is good agreedment between results of the broth dilution and agar diffusion assays. The amount of extract needed to suppress microbial growth in the agar diffusion for BFWE was greater (by at least two-fold) than in the broth dilution test, despite the fact that the qualitative pattern of microbial species inhibition in the assay aligns with that of the agar diffusion. The small amount of extract that diffuses from the placement spot through the agar plate to the bacteria hints that greater concentrations are needed for the inhibition in the agar diffusion procedure. The MICs from the broth dilution for the positive control drug used against the bacterial specimenswere higher (by at least 10^3 times) than that of BFWE (values range from 3.906 to 7.813 μg/ml): *S. aureus* (7.813 μg/ml), *S. pyogenes* (3.906 μg/ml), *P. aeruginosa* (7.318 μg/ml), *E. coli* (3.906 μg/ml) (Table 10). Likewise, Clotrimazole displayed MICs against *T. corporis* (15.625 μg/ml) and *C. albicans* (31.250 μg/ml) which were also at least 10^2 times higher than the BFWE (Table 10).

The minimum bactericidal concentration (MBC), the lowest concentration of the extract that can kills 99.9 % of the bacterial inocula after 24-h incubation at 37 °C, was recorded.This helps to appropriately assess the inhibitory effects the BFWE had on the various microorganisms studied. BFWE was found to possess bactericidal effects at two-fold concentrations more than the MICs (Table 12).

The anti-microbial activity of most plant extracts is concentration-dependent. An elevated ratio of the bioactive phytoconstituent to lipid along the microorganism membrane region greatly increases the active compounds' prowess to pierce and damage the integrity of the membrane. Transmembrane pore formation, ion channel formation, and membrane rupture which all help in killing microbes are more common at higher doses of powerful extract.

Although single therapeutic agents may elicit certain effects when used alone, combination therapy is increasingly emerging as the "new school" due to its many benefits. Because combination therapy requires the microbe to become accustomed to two or more medications with completely different modes of action, it can lessen the abrupt upsurge of drug-resistant bacteria. Additionally, the range of pathogens that can be targeted could be expanded based on the individual medications included in that particular combo. Finally, combination therapy may help prevent the toxicity that comes with raising dosage. This is because, under single-drug therapy, lower dosages of the medication would be required to achieve equal levels of efficacy [50]. Supplementation of anti-microbial functional food such as baobab to harness the effects of orthodox medicine to be used for the treatment of infections. For instance, there's a significant chance that COVID-19 will increase the range of treatment alternatives.

3.6. Fatty acids composition of baobab seed hexane extract (BO)

The composition offatty acids found in the hexane extract was identified using a Gas Chromatography (GC) separation method and the various fractions were detected with a Mass Spectrometer (MS). Fig. 5 shows the GC spectrum of BO. The spectrum shows that Myristic acid (0.5 %), Palmitic acid (21.2 %), Alpha-Linoleic acid (62.8 %), Stearic acid (5.1 %), Malvalic acid (2.6 %), *Cis*-10-Nonacednoic acid (0.7 %), and Arachidic acid (1.0 %) as the major fatty acids detected. However, Palmitic acid, Alpha-Linoleic acid, and Stearic acid were the most abundant fatty acids in the oil.

The National Institute of Standard and Technology (NIST) for standard fatty acids data was used to identify the fatty acid composition. To confirm the presence of the identified fatty acids, Msalilwa and co-workers (2020) have reported on identifying twelve different fatty acids from the baobab seed oil using GC-FID and standard fatty acids. The fatty acids identified are myristic, palmitic acid, stearic, arachidic, oleic, linoleic, and linolenic [46]. Additionally, Komanea et al. (2017) reported that the major fatty acids

Table 6
Zone of inhibition (mm) for BFWE.

Test Organism	Concentrations of Extract and Standard Drug (mg/ml)					
	100	50	25	12.5	1	1
	BFWE				Ciprofloxacin	Clotrimazole
S. aureus	16	13	11	–	41	
S. pyogenes	18	14	13	–	40	
P. aeruginosa	17	14	12	11	35	
E. coli	17	13	12	–	50	
T. corporis	16	15	12	–		15
C. albicans	16	13	11	–		13

Note: (−) means no observed zone of inhibition.

Table 7
Zone of inhibition (mm) for DBEE.

Test Organism	Concentrations of Extract and Standard Drug (mg/ml)					
	100	50	25	12.5	1	1
	DBEE				Ciprofloxacin	Clotrimazole
S. aureus	–	–	–	–	45	
S. pyogenes	–	–	–	–	46	
P. aeruginosa	–	–	–	–	47	
E. coli	–	–	–	–	46	
T. corporis	–	–	–	–		16
C. albicans	–	–	–	–		15

Note: (−) means no observed zone of inhibition.

Table 8
Zone of inhibition for BO.

Test Organism	Concentrations of Extract and Standard Drug (mg/ml)					
	100	50	25	12.5	1	1
	BO				Ciprofloxacin	Clotrimazole
S. aureus	–	–	–	–	54	
S. pyogenes	–	–	–	–	40	
P. aeruginosa	–	–	–	–	47	
E. coli	–	–	–	–	46	
T. corporis	–	–	–	–		16
C. albicans	–	–	–	–		15

Note: (−) means no observed zone of inhibition.

Table 9
Broth dilution for BFWE.

Test Organism	Concentration (mg/ml)						
	25	12.5	6.25	3.125	1.5625	0.78125	0.390625
S. aureus	–	–	–	+	+	+	+
S. pyogenes	–	–	+	+	+	+	+
P. aeruginosa	–	–	+	+	+	+	+
E. coli	–	–	+	+	+	+	+
T. corporis	–	–	+	+	+	+	+
C. albicans	–	–	–	+	+	+	+

Note: (−) indicates no observed microbial growth; (+) indicates microbial growth.

Table 10
Broth dilution for standards (ciprofloxacin and clotrimazole).

Standard	Test Organism	Concentration (mg/ml)						
		0.125	0.0625	0.03125	0.015625	0.0078125	0.00390625	0.001953125
Ciprofloxacin	*S. aureus*	–	–	–	–	–	+	+
	S. pyogenes	–	–	–	–	–	–	+
	P. aeruginosa	–	–	–	–	–	+	+
	E. coli	–	–	–	–	–	–	+
		0.5	**0.25**	**0.125**	**0.0625**	**0.03125**	**0.015625**	**0.0078125**
Clotrimazole	*T. corporis*	–	–	–	–	–	–	+
	C. albicans	–	–	–	–	–	+	+

Note: (−) indicates no observed microbial growth; (+) indicates microbial growth.

present in the oil of baobab seeds were myristic acid (0.1 %), palmitic acid (28.5 %), stearic acid (5.9 %), oleic acid (25.7 %), linoleic acid (35.8 %) and arachidic acid (0.7 %) [51]. Triglycerides with a higher proportion of saturated and unsaturated fatty acids are thought to make up fatty acids. These substances are long aliphatic carbon chain-containing esters of higher fatty acids and glycerol. Their fatty acid percentages will determine a range of characteristics that could be advantageous to the skin when used as everyday cosmetics [51]. Because Baobab seed oil contains significant percentages of both polyunsaturated (alpha-linoleic) and unsaturated (palmitic), it may be important as a cosmetic basis to reduce trans epidermal water loss by building a protective barrier on the

Table 11
Minimum inhibition concentration (MIC) values of BFWE and standards.

Test Organism	MIC (mg/ml)		
	BFWE	Ciprofloxacin	Clotrimazole
S. aureus	6.250	7.813×10^{-3}	
S. pyogenes	12.500	3.906×10^{-3}	
P. aeruginosa	12.500	7.813×10^{-3}	
E. coli	12.500	3.906×10^{-3}	
T. corporis	12.500		15.625×10^{-3}
C. albicans	6.250		31.250×10^{-3}

Table 12
Minimum bactericidal concentration (MBC) values of BFWE and standards.

Test Organism	MBC (mg/ml)		
	BFWE	Ciprofloxacin	Clotrimazole
S. aureus	12.500	7.813×10^{-3}	
S. pyogenes	25.000	3.906×10^{-3}	
P. aeruginosa	25.000	7.813×10^{-3}	
E. coli	25.000	3.906×10^{-3}	
T. corporis	25.000		15.625×10^{-3}
C. albicans	12.500		31.250×10^{-3}

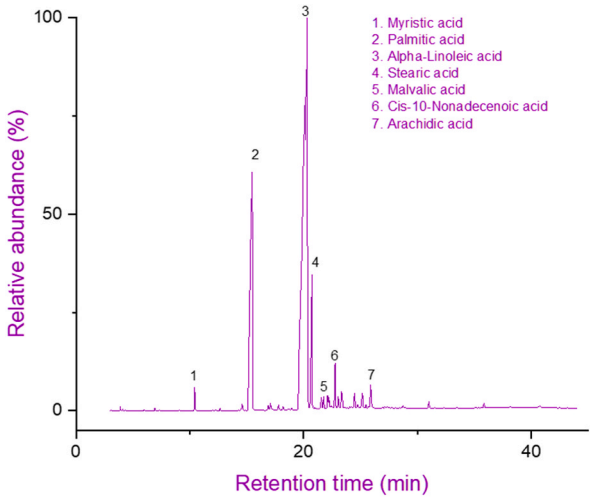


Fig. 5. The Gas Chromatography spectrum of Baobab Seed Oil (BO) is represented as a graph of relative abundance (%) against retention time (min).

epidermis. Results from the study could imply that the oil obtained from the Boabab seed can be used in the cosmetics industry. The presence of high levels of palmitic and alpha-linoleic acids could help prevent trans epidermal lost of water. Alpha-linoleic acid, a polyunsaturated fatty acid that naturally occurs in sebum, also contributes significantly to the improvement of the lipid barrier of the epidermis and the normalization of skin metabolism. Furthermore, there is a clear correlation between the amount of alpha-linoleic acid in the ceramides of the stratum corneum and the barrier of permeability function of the skin [52]. Due to the high concentration of linoleic acid present, Baobab seed oil has been suggested by Kanlayavattanakul and Lourith (2011) as a viable therapeutic topical use for the treatment of acne [53]. The oil sample had a high percentage of omega-6 (alpha-linoleic acid), which has been found to help maintain healthy skin, especially when administered directly to acne instances. Treatment with linoleic acid for oily, troublesome skin could improve sebaceous gland activity to prevent the onset of comedo-acne [52]. The fatty acids; myristic acid, palmitic acid, stearic acid, and arachidic acid play important nutritional and medicinal roles in the human body as those classes of compounds can possess some physiological effects on the human system [54].

3.7. Proximate analysis

From the results, the baobab seeds contain higher crude fat (10.05 ± 0.05 %), crude fiber (11.27 ± 0.33 %), and crude protein

(13.57 ± 0.36 %) content, and hence possess greater energy (341.93 kcal) than the fruit pulp. The fruit pulp, however, contains a higher amount of carbohydrates (70.17 %) than the seed comparably. Similar values were observed for the moisture and ash content of both plant parts. The results are tabulated as seen in Table 13.

The fruit pulp moisture content is significantly higher than what has previously been reported in the literature [14]. The high pulp moisture content recorded may be due to the high altitude, high annual precipitation, and moderate temperature conditions of the sampling area. Ruiz-Rodriguez and co-workers also hint that factors like sunlight and wind exposure account for the dry nature of the pulp [55]. The crude protein content recorded for the pulp was very low (2.19 ± 0.36 %) and it is consistent with reports in the literature [14]. It is however worth knowing that baobab fruit pulp contains relatively, higher protein content than commonly eaten fruits such as oranges, grapes, mangoes, bananas, and pawpaw which have crude protein contents of 0.7 %, 0.5 %, 0.6 %, 1.2 %, and 0.6 % respectively [56]. Ash content is an indicator of inorganic matter in plant material. From the results it can be concluded that fruit pulp from the baobab plant can serve as is a good source of mineral elements which can be essential to the human biological system. The fat content, ash content, fiber content, and protein content recorded for the fruit pulp are consistent with what is stated in the literature [14].

The seed's proximate compositions of this study were found to follow the order: carbohydrate > crude protein > crude fiber = moisture > crude fat > ash. This was consistent with what Parkouda et al. reported in 2012 with minor differences [57]. The fat content, fiber content, and protein content recorded by the seeds were relatively higher than those recorded for the fruit pulp as was suspected and known from the literature [14]. This contributed to the seed (341.93 kcal) expressing a greater energy value than the fruit pulp (295.11 kcal).

4. Conclusion

This study has revealed that the fruit pulp and seeds of *Adansonia digitata* L. possess a range of phenolic compounds with exciting health benefits. The extracts showed high phytochemical diversity with good bioactivities including antioxidant and antimicrobial properties. The study also revealed a significant correlation between phenolic compounds and the associated antioxidant properties of the extracts.

Data availability statement

All data generated in this study has not been deposited into any publicly available repository.
Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this article.

CRediT authorship contribution statement

Philip T. Thompson: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Vivian E. Boamah:** Writing – review & editing, Validation, Resources, Formal analysis. **Mercy Badu:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mercy Badu reports equipment, drugs, or supplies was provided by Organization for Women in Science for the Developing World (OWSD). Mercy Badu reports equipment, drugs, or supplies was provided by Cambridge-Africa ALBORADA Research Fund.

Table 13
Proximate composition of *Adansonia digitata* fruit pulp and seed.

Parameters	Fruit Pulp	Seed
Moisture (%)	16.26 ± 0.11	11.27 ± 0.33
Ash (%)	4.06 ± 0.00	4.54 ± 0.01
Crude Fat (%)	0.63 ± 0.20	10.05 ± 0.05
Crude Fiber (%)	6.69 ± 0.11	11.27 ± 0.33
Crude Protein (%)	2.19 ± 0.36	13.57 ± 0.36
Carbohydrate (%)	70.17	49.30
Energy (kcal)	295.11	341.93

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