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Proximate Composition, In vitro Antioxidant and Anti-inflammatory Properties of *Adansonia digitata and Belanites aegyptiaca* Seed

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Abstract

This study evaluated the nutritional and medicinal properties of seeds from *Adansonia digitata* (BSF) and *Balanite aegyptiaca* (DDSF) plants. Proximate chemical composition, mineral elements composition, flavonoids, phenolics, antioxidant capacity, and anti-inflammatory properties were studied. Results obtained revealed that DDSF had the highest moisture, crude fat, and crude protein content of 7.66 %, 42.80 %, 20.37 %, respectively, whilst BSF gave the highest ash, crude fibre, and carbohydrate content. Elemental analysis revealed BSF had the highest Mg content (313.65 mg/100g) and DDSF gave the highest Ca content (118.62 mg/100g). Additionally, DDSF gave the highest total phenolics (18.89 mg TAE/ 100 g), total flavonoids (8.80 mg QE/ 100 g) as well as the highest total antioxidant capacity of (19.62 mg AAE/ 100 g) dry of extract. Based on results obtained in this study, seeds obtained from the *Adansonia digitata* and *Balanite aegyptiaca* could be a potential source of functional food and antioxidant agents.

Keywords: Underutilized; nutritional content; anti-nutrients; total phenolics; medicinal properties; oil seeds

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1 Introduction

Dietary diversification has been accepted worldwide as an effective and sustainable way of eradicating hunger and malnutrition [1, 2]. African traditional and indigenous plants have been reported to have the potential to help address hunger and its associated health problems, especially in sub-Saharan Africa [3, 4]. Recently, a lot of research has been directed at exploring new and nonconventional edible wild seeds as food or industrial feedstock. Two of such wild seeds are obtained from *Adansonia digitata* and *Balanites aegyptiaca*.

Adansonia digitata, commonly referred to as the Baobab plant, belongs to the family Malvaceae. It is a native of the African continent and grows in the dry and hot savanna regions of Sub-Saharan Africa, and is described as the largest succulent plant in the world [5]. *Adansonia digitata* is a multi-purpose plant that offers protection and provides food, clothing and medicine, and raw material for many useful items. It has hand-sized leaves, divided into 5-7 finger-liked leaflets, and a large egg-shaped capsule fruit, which has been used extensively by many local communities as food [6]; the fruit consist of hard, black, kidney-shaped seeds found in a dry, acidic pulp. The fruit pulp has very high vitamin C, calcium, phosphorus, carbohydrates, fibres, potassium, protein and lipid content, which can be used as a beverage and also in food preparation [7]. The fermented seeds are used as a flavouring for soups, whereas the roasted seeds are pressed for their oil, which is usually used for cooking, pharmaceuticals and cosmetics. The seeds have been reported to contain appreciable quantities of phosphorus, magnesium, zinc, sodium, iron, manganese, whereas they have high levels of lysine, thiamine, calcium and iron [8]. It has been used in the treatment of various conditions such as muscle spasms, varicose veins and wounds by many traditional healers and the indigenous people of communities where the plant is found. However, not much work has been done on the proximate composition and medicinal properties of extracts of the seeds.

Balanites aegyptiaca, commonly known as Desert date, belongs to the family Zygophyllaceae. It is a multi-branched, evergreen shrub native to the Sudano-Sahelian region of Africa, the Middle East, and South Asia. It is a thorny species, spiny shrub or tree with 10 m height [9]. It is reported as one of the most common but neglected wild plant species of the dryland areas of Africa and South Asia. It bears fruits, which contain light brown, fibrous and hard seeds. The leaves, flowers and fruits of *Balanites aegyptiaca* are good sources of proteins, K, Fe, Mn, Zn, and Cu [10]. The fleshy pulp of both ripe and unripe fruit is edible and eaten dried or fresh. The fruit is used as a sweetmeat in Ghana, alcoholic liquor in Nigeria and soup ingredient in Sudan. The seeds have been used as an expectorant, antibacterial and antifungal agents. They have also been used to treat fever [11]. *Balanites aegyptiaca* has a long history of traditional uses for a range of diseases. Studies conducted by Chapagain in 2006 showed that different parts of the plant possess antioxidant, antimicrobial, anticancer, diuretic, wound-healing, antiviral, antidiabetic, hepato-protective, mosquito larvicidal, anti-inflammatory, analgesic, antivenin and cardio-protective properties. The bark, fruits, seeds, the seed oil and leaves have also been widely used in folk medicine [12]. *B. aegyptiaca* has been reported to contain different biologically active compounds, contributing significantly to its role in nutriceutical applications [13,14].

In Ghana, the *Adansonia digitata* and *Balanites aegyptiaca* plants are commonly found in the Northern Savanna regions. There are numerous reported data on the nutritional and medicinal properties of different parts of these two plants [15-17]. They produce large amounts of seeds. However, little is known about their utilization. Therefore, it is essential to research the chemical composition of the seeds to help encourage and promote their utilization, especially in the food and pharmaceutical industry. Once a thorough chemical characterization is done and complete information on the chemical composition of *B. aegyptiaca* and *A. digitata* seeds are available, these seeds will gain economic interest and could be sold on both the local and international markets. This may serve as a source of employment for many of the local communities and will lead to poverty reduction. Additionally, encouraging the use of seeds of *Balanites aegyptiaca* and *Adansonia digitata* will create an avenue for employment for the local inhabitants and hence, poverty reduction. This study was designed to evaluate the proximate composition, mineral content, antioxidant and anti-inflammatory properties of the seeds obtained from *Belanites aegyptiaca* and *Adansonia digitata.*

2 Experimental Section

2.1 Sampling

In this study, mature ripe fruits of *Balanites aegyptiaca* (DDSF) and *Adansonia digitata* (BSF) were collected from farmers in the Upper East

region of Ghana. The fruits were cut open with a sterilized stainless-steel knife, and the seeds removed, washed and air-dried for 72 hours at 35 °C. The seeds were cracked open in a clean sterilized mortar with a laboratory pestle, and the kernels were removed. The kernels were milled into a fine powder, put in clean zip-locked plastic bags and kept in a refrigerator at 0°C until it was ready for analyses.

2.2 Proximate composition of the seeds

The proximate composition of the seeds was analyzed using methods based on the standard procedures recommended for food analysis by Nielsen (2014) with slight modifications [18]. The moisture content was determined using the hot oven method [19]. In brief, a weighed (5 g) sample was put in a pre-heated oven (105 °C) and dried until a constant weight was achieved. The percentage of moisture content was estimated according to the weight loss of the samples. Crude protein content was obtained from the nitrogen content of the samples using the micro-Kjeldahl method [20]. The value obtained for the nitrogen content was then multiplied by a factor of 6.25 to obtain the crude protein content. The crude fat content was determined by solvent (hexane) extraction using a Soxhlet extractor. After the extraction process, the percentage fat content of the seeds was quantified gravimetrically [21]. The crude fibre was also determined gravimetrically after digesting and refluxing weighed amount of fat-free seed flour in 1.25% sulphuric acid and 1.25% sodium hydroxide. The ash content was determined by incinerating the sample in a muffle furnace at 600 °C for 5 hours, according to AOAC (1990) [19]. The carbohydrate content was estimated using the formula below:

% Carbohydrate = [100 – % (crude protein + crude fat + crude fibre + moisture content + ash content)] (AOAC, 1990). (Equation 1)

Total energy value was calculated by the Atwater factors, according to the formula

Energy value = (% crude protein×4)+(% carbohydrate×4)+(% crude fat×9) (Equation 2)

2.3 Mineral composition

Mineral elements were determined using the AOAC method (1990). In this method, approximately 1 g of the ash sample was dissolved in 75 ml of 5 % hydrochloric acid and filtered into a 100 ml volumetric flask. The solution was topped up with distilled water to the mark, covered with a stopper and inverted several times before allowing it to stand. The atomic absorption spectrophotometer (AAS model novAA 400P, Analytik Jena, Germany) was used to analyze the digested sample [19].

2.4 Antioxidant Properties of seeds

The total phenolic content was expressed as tannic acid equivalent. The antioxidant properties of the seeds flour were determined using the defatted seed flour after extracting the crude fat content by soxhlet extraction using hexane as the solvent [21]. From the defatted seed flour, a cold maceration method of 70 % ethanol was used to extract the bioactive compounds. The extracts were concentrated and the crude extract kept in the refrigerator until the various analyses were carried out. The antioxidant properties of the seeds were evaluated by determining the total phenolic content, total flavonoid content, total antioxidant capacity, reducing power assay and the DPPH free radical scavenging activity assay. The total flavonoids content was quantified, and the results expressed as Quercetin equivalent.

The antioxidant assays were conducted using (UVmini 1240, Shimadzu) spectrophotometer.

2.5 Determination of Total Phenols

The total phenolic content was determined according to the Folin-Ciocalteu method [22]. In this method, the reaction mixture was made of 200 µl of extract solution (500 µg/ml) and0.5 ml (10 %) Folin-Ciocalteu phenol reagent and thoroughly mixed together. The mixture was allowed to stand for 3 mins after which 2 ml of 20 % sodium carbonate was also added and allowed to stand for 60 mins in the dark. The

absorbance of the mixture was measured at 650 nm. Varying concentrations of tannic acid standard solutions (0.2 – 1 mg/ml) were prepared. The reaction mixture for the tannic acid standard reagent was prepared as above, and the absorbance measured and the data obtained were used to plot the standard curve. Based on the measured absorbance, the concentration of phenolic compounds present was read (mg/ml) on the calibration curve and, the content of phenols in extracts was expressed as mg tannic acid equivalent per 100 g dry weight of the sample.

2.6 Determination of Total flavonoids

The total flavonoid content was determined by the aluminium chloride colourimetric method [23]. The method is based on the formation of a complex flavonoid–aluminium. In this, 1 ml (0.5 mg/ml) of the extract was mixed with 0.3 ml of 5 % $NaNO_2$, 0.3 ml of 10 % $AlCl_3$ and incubated for 6 mins, after which 2 ml of 1 M NaOH was added. The mixture was allowed to stay for 15 mins before the absorbance was measured at 510 nm. Quercetin and Ascorbic acid standards were used. The standard curve was obtained from 1 mg/ml stock solution and made into concentrations of 0.2 mg/ml – 1 mg/ml. The data obtained from their absorbance measurements were used to plot the standard curve, the concentration of flavonoids was read (mg/ml) on the calibration curve, and the flavonoid content in the extracts was expressed as mg quercetin and ascorbic acid equivalent per 100 g dry weight of the sample.

2.7 Determination of Total Antioxidant Capacity

The total antioxidant capacity was analyzed by the phosphomolybdate method [23]. The phosphomolybdate reagent was prepared by dissolving 28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid. Quercetin and ascorbic acid (0.2 mg/ml – 1 mg/ml) were prepared and used as standards. A concentration of 0.5 mg/ml of the extract was used. The reaction mixture was prepared from 1 ml of the sample solution and 1.5 ml of the phosphomolybdate reagent. The mixture was incubated at 95◦ C for 90 min after which it was cooled to room temperature, and the absorbance was measured at 695 nm. The total antioxidant capacity was quantified and expressed as mg ascorbic acid equivalent per 100 g dry weight of the sample.

2.8 DPPH Free Radical Scavenging Activity Assay

In this method, 0.1 mM solution of DPPH (2, 2′- diphenyl-1-picrylhydrazyl) was prepared in ethanol [24]. To 3 ml of the extract solution at different concentrations (0.1 mg/ml – 0.6 mg/ml), 1 ml of the DPPH solution was added. The mixture was kept in the dark at room temperature for 30 min. The absorbance of each sample was measured at 517 nm. The scavenging effect was expressed as (%) using the expression below.

$$\text{Scavenging effect \%} = [1-(A_{sample}-A_{sample\ blank})/A_{control}]\times 100, \quad \text{(Equation 3)}$$

2.9 Reducing power

The test solution was prepared to a 1 ml volume at different concentrations (0.1 mg/ml – 0.7 mg/ml. The solutions were mixed with 2.5 ml phosphate buffer (0.2 M at pH 7.0) and 2.5 ml potassium ferricyanide (1 %). The solution was mixed thoroughly and incubated at 50 °C for 30 mins. After the incubation period, the mixture was allowed to cool to room temperature, and 2.5 ml of trichloroacetic acid (10 %) was added. The mixture was then centrifuged for 10 mins at 3000 rpm [23].

From the upper layer of the solution, 1.25 ml was taken and mixed with 1.25ml distilled water and 0.25 ml (0.1 %) ferric chloride solution. The absorbance of the solutions was measured at 700 nm.

2.10 Anti – Inflammatory Properties of the defatted seed flour

2.10.1 Inhibition of Albumin (Protein) Denaturation Assay

A stock solution of the ethanolic extract (1 mg/ml) was prepared after which serial dilutions were prepared from the extract solution in different concentrations (0 – 0.5 mg/ml). An aqueous solution (5 mg/ml) of the

bovine serum albumin (BSA) was also prepared. Additionally, stock solution (1 mg/ml) of the standard drug (diclofenac) was also prepared, after which serial dilutions were used to the extract solution in different concentrations (0 – 0.5 mg/ml). The reaction mixture contained 1 ml extract solution and 1 ml of BSA solution. The mixture was allowed to incubate at 57°C for 20 min after which 2.5 ml of 0.5 M phosphate buffer, pH 6.3 was added and the absorbance measured at 660 nm [25].

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Where: Abs- Absorbance

(equation 4)

% Inhibition was plotted against concentration to evaluate the effectiveness of the extract to inhibit protein denaturation.

2.10.2 Membrane stabilization Assay

Fresh whole bovine blood (10 ml) was collected and centrifuged at 3000rpm for 15 min and washed three times with equal volumes of normal saline solution (5 M NaCl solution). The volume of the blood was then measured and reconstituted to 2 % v/v suspension with normal saline. A stock solution of both ethanolic extract and a standard drug (1 mg/ml) were prepared separately after which serial dilutions were used to the extract and standard drug (diclofenac) solution in different concentrations (0 – 0.5 mg/ml). The reaction mixture (about 3 ml) was prepared by mixing 1 ml of 2.5 M hyposaline solution, 0.5 ml of 0.5 M phosphate buffer (pH 6.3), the extract solution (1 ml) in 5 M normal saline solution and 0.5 ml of 2 % RBCs suspension. The mixtures were incubated in a water bath at 56 ^{o}C for 30 min after which the mixture was allowed to cool to room temperature under running water and then centrifuged at 4000 rpm for 15 min. The supernatant was decanted and the absorbance measured at 560 nm [25].

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Abs- Absorbance

(equation 5)

% Inhibition was plotted against concentration to evaluate the effectiveness of the extract to protect the damaging effect of the hypotonic solutions

2.11 Statistical analysis

All measurements were done in 3 replicates, and data expressed as the mean ± standard deviation (SD). Data collected were analyzed using the single factor Analysis of Variance (ANOVA). The mean values were separated using Pearson's correlation and Least Significant Difference. *P* values < 0.05 were regarded significantly different.

3 Results and Discussions

3.1 Proximate composition

The proximate compositions (% dry weight basis) of seeds obtained from *Adansonia digitata* and *Balanites aegyptiaca* are shown in Table 1. The results indicated that *Balanites aegyptiaca* had the highest moisture, crude fat and crude protein content of7.66 %, 42.80 %, 20.37 %, respectively and *Adansonia digitata* seed gave the highest ash, crude fibre and carbohydrate content of 5.73%, 8.80% and 41.71 % respectively. Comparing the proximate compositions of *Adansonia digitata* and *Balanites aegyptiaca* seeds, it is evident that the differences in all parameters are statistically significant ($p < 0.05$). The data obtained are in agreement with the data reported by Osman (2004) and Nkafamiya et al., (2007) in their studies on the characterization of the *Adansonia digitata* seeds [8, 26]. The high oil content of *Balanites aegyptiaca* makes it economically viable and can be used as a potential raw material for many industrial activities such as feedstock for the chemical industry as well as in biodiesel production, cosmetics, soaps and detergents. The crude protein content of both seeds suggests their possible use as a protein supplement for low protein foods and other staples, especially in the production of animal feeds. The crude fibre content of *Adansonia digitata* seed was found to be higher than that of *Balanites aegyptiaca*, and the values obtained are similar to data reported by Muthai et al., 2017 [27]. Research has shown that the fibre in our diets is known to expand the inside walls of

the colon, allowing free bowels and preventing constipation [28-29]. This implies for selection of a seed as edible, a significant amount of fibre in the seed is critical. Again, the percentage ash content of a plant material gives an idea of the levels of mineral elements present in the sample [30]. Therefore, the high amounts of the ash content of the *Adansonia digitata* seed suggest high levels of micro and macro elements in the *Adansonia digitata* seed flour. According to data reported in the literature, the acceptable levels of moisture content in edible oilseeds range between 5-12 % [31]. The lower moisture content found in the *Adansonia digitata* seed promotes good storage advantage and may prevent microbial growth.

Table 1. Percentage composition of proximate parameters of *Adansonia digitata* (BSF) and *Balanites aegyptiaca* (DDSF) seeds flour

Component	BSF	DDSF
Moisture	2.66 ± 0.54^{a}	7.66 ± 0.170^{b}
Ash	5.73 ± 0.18^{b}	3.55 ± 0.22^{a}
Fat	22.82 ± 0.30^{a}	42.80 ± 0.203^{b}
Protein	18.34 ± 0.46^{a}	20.37 ± 0.57^{b}
Fibre	8.80 ± 0.66^{b}	4.31 ± 0.306^{a}
Carbohydrate	41.71 ± 1.34^{b}	21.32 ± 1.05^{a}
Energy Value (kcal)	445.58	551.96

Note: The data are reported as mean ± standard deviation of three replicates. The mean values followed by different superscript in a row are statistically different at (p< 0.05) and same superscript in a row means statistically equal (p> 0.05).

3.2 Mineral composition

Determination of mineral elements in oilseeds is crucial since it gives an idea of the presence of inorganic substances, which are essential constituents of the human diet. Dietary intake of mineral elements by living organisms is needed in many physiological and metabolic processes. They are required in the human body for growth and maintenance of functional activities [32].

In this study, mineral elements such as copper, Magnesium, Zinc, Sodium, Calcium, Manganese, Potassium and Iron were analyzed to estimate their levels in the *Adansonia digitata* and *Balanites aegyptiaca* seed flour and results obtained showed in Table 2. Mg showed the highest concentration (313.65 mg/100g) for BSF, followed by K (135.80 mg/100g). Mn gave the lowest concentration (1.32 mg/100g) in the seed. Strong positive correlations were found between Cu and K (r=0.986) and Fe and Ca (r=0.964). There were also significantly high correlations between Zn and K (r=0.884), Mn and Mg (r=0.819), and Zn and Cu (r=0.796). This gives a clear indication that as Cu concentration increases, K concentration increases by 97.2%, and as Zn concentration increases, K concentration increases by 78.1%; however, Zn concentration increases by 63.4% as Cu increases. K weakly correlated with Na (r=0.496), while Na correlated appreciably with Cu (r=0.632). There was, however, no significant correlation between Na and Zn. This suggests that the Na and K sources for the plant may be different. K may come from the same source as Cu and Zn. Fe strongly correlated negatively with Mg (r=-0.947), indicating that as iron concentration increased, Mg concentration decreased by 89.7% and vice versa.

Table 2. Concentrations of mineral elements present in *Adansonia digitata* (BSF) and *Balanites aegyptiaca* (DDSF)

Mineral (Mean ± SD mg/100 g)	BSF	DDSF
Copper (Cu)	1.55 ± 0.01	0.10 ± 0.01
Magnesium (Mg)	313.65 ± 0.10	285.27 ± 0.05
Zinc (Zn)	5.16 ± 0.04	4.66 ± 0.02
Sodium (Na)	4.11 ± 0.03	17.61 ± 0.09
Calcium (Ca)	4.24 ± 0.01	118.62 ± 0.08
Manganese (Mn)	1.32 ± 0.01	0.97 ± 0.01
Potassium (K)	136.80 ± 0.45	9.95 ± 0.01
Iron (Fe)	26.38 ± 0.13	2.06 ± 0.20

Note: Elemental composition was analyzed in triplicates and reported as the mean ± standard deviation.

According to the results, for the BSF, Mg showed the highest concentration (313.65 mg/100g) followed by K (135.80 mg/100g). Mn gave the lowest concentration (1.32 mg/100g) in the seed. There were strong positive correlations between Cu and K (r=0.986) and Fe and Ca (r=0.964). There were also significantly high correlations between Zn and K (r=0.884), Mn and Mg (r=0.819) and Zn and Cu (r=0.796). This gives a clear indication that as Cu concentration increases, K concentration increases by 97.2% and as Zn concentration increases, K concentration increases by 78.1%; however, Zn concentration increases by 63.4% as Cu increases. K weakly correlated with Na (r=0.496), while Na correlated appreciably with

Cu (r=0.632). There was, however, no significant correlation between Na and Zn. This suggests that the Na and K sources for the plant may be different. The potassium may come from the same source as Cu and Zn. Fe strongly correlated negatively with Mg (r=-0.947), indicating that as iron concentration increased, Mg concentration decreased by 89.7% and vice versa.

Considering *Balanites aegyptiaca* , again, Mg was the highest (285.27 mg/100g) followed by calcium (118.62 mg/100g) with copper as the least mineral element present in the seed. There were strong positive correlations between Ca and Cu (r=0.991), Fe and Zn (r=0.999) and Fe and K (r=0.929). Ca and Mn and Mn and Cu had strong positive correlations of r=0.969 and r=0.926, respectively. These positive correlations could be attributed to the same sources of these elements for the plant. Na and K, Mn and Fe and Mn and Zn had strong negative correlations of r=-0.998, r=-0.975 and r=-1.000, respectively, indicating that the sources of these elements to the plant are completely different. Also, as the concentration of Mn increased, Zn decreased by 100% and vice versa.

Mineral elements in the human body form part of the regulatory compounds, including vitamins, many enzymes, and most hormones, which help in the proper functioning of the human body [33]. Mg, which was found in the seeds to the most abundant mineral element, is very important and required for the activity of over 300 enzymes in the human body. It takes part in many significant physiological activities for good health and glucose homeostasis [34]. According to the report of the joint FAO/WHO expert consultation on human vitamins and mineral requirements, the level found in both seeds is above the recommended daily intake of 100-260 mg/day, according to the report of the joint FAO/WHO expert consultation on human vitamins and mineral requirements [35]. The Mg levels recorded in this study are low compared to the estimated 2-9 mg /day requirement for an adult of average body weight for the proper functioning of the body [36]. Therefore, based on the results obtained from this study, the *Adansonia digitata* seed and *Balanites aegyptiaca* are not the best sources of Mg for the human body. The Cu content found in *Adansonia digitata* seed is within the estimated daily intake whilst that of *Balanites aegyptiaca* fell below the estimated value of Cu for an adult (1-3 mg/day) [36]. According to the Joint FAO/WHO expert consultation on human vitamins and mineral requirements, recommended daily intake of calcium ranges from 300-1300 mg/day for infants and adults, respectively [35]. Considering this range, it is clear the levels of calcium found in both seeds are low. Fe, which is an essential mineral and required for several biological activities, was found to be present in the seeds. In this study, *Adansonia digitata* seed recorded 26 mg/ 100g Fe whilst *Balanites aegyptiaca* recorded 2.06 mg/100g Fe. Based on this result, every 100 g of flour of *Adansonia digitata* seed will meet the recommended daily intake (9-17 mg/day) of Fe for both males and females, while flour from the *Balanites aegyptiaca* seed will not meet the required [37]. Zinc levels recorded in the study for every 100 mg of seed flour were below the recommendation made by the US National Academy of Sciences, which estimates that every adult of average body weight consumes 15-20 mg zinc a day [38]. In order to meet the daily requirement of Zn, large quantities of the seeds should be consumed, or they should be added to other Zn-containing foods. Zinc plays a crucial role in antioxidant defense diseases such as diabetes. Zinc deficiency has been associated with metabolic abnormalities, such as impaired glucose tolerance [39-40].

3.3 Total Antioxidant Capacity

Antioxidants have been widely used as food additives to help manage or reduce the risk of certain types of cancers, coronary heart diseases, and cardiovascular diseases as well as ageing [23]. Epidemiological studies have shown that dietary intake of antioxidant substances reduces death associated with chronic degenerative diseases [41].

In this study, the phenolic content; flavonoid content, total antioxidant capacity, the DPPH free radical scavenging and reducing power activity were used to estimate the antioxidant property of the seeds obtained from the *Balanites aegyptiaca* and the *Adansonia digitata* plants. Results obtained for total phenolic; total flavonoid and total antioxidant capacity are shown in Table 3.

Table 3. Levels of phenolic compounds and total antioxidant capacity of ethanolic extracts of *Adansonia digitata* (BSF) and *Balanites aegyptiaca* (DDSF)

Sample ID	Total phenolics (mg TAE/ 100g extract)	Total flavonoid (mg QE/ 100g extract)	Total antioxidant capacity (mg AAE/ 100 g extract)
BSF	13.56±0.81[a]	7.40±0.16[b]	13.49±2.16[c]
DDSF	18.89±3.89[b]	8.80±0.19[a]	19.62±1.82[d]

Note: The data are reported as mean ± standard deviation of three replicates. The mean values followed by different superscript in a row are statistically different at (p< 0.05) and same superscript in a row means statistically equal (p> 0.05).

From the study, *Balanites aegyptiaca* gave a total phenolic content of 18.89 mg TAE/ 100 g extract and *Adansonia digitata* seed gave a total phenolic content of 13.56 mg TAE/ 100 g extract. The total phenolic content of the two seeds was statistically different with $p < 0.05$. Phenolic compounds are very important compounds due to their ability to scavenge free radicals, superoxide and hydroxyl radicals [41]. Their presence in natural products has been reported to be responsible for the antioxidant activities of natural products. Again, the antioxidant activity of the phenols is due to the redox property, which allows them to act as a reducing agent, hydrogen donors, and singlet oxygen quench [42-44]. They may also have a metal chelating potential which contributes significantly to their antioxidant activity. The total flavonoid content in *Balanites aegyptiaca* was 8.80 mg QE/ 100 g whilst that of *Adansonia digitata* was 7.40 mg QE/ 100 g. Flavonoids are important phenolic compounds which have been reported to exhibit a number of biological activities that contribute significantly to their anti-inflammatory properties [45]. Therefore, the determination of the total phenolic content and the total flavonoid content in this study can be used as a basis to assess the preventive or reductive property of the seeds against many chronic diseases. Comparing the total phenolic content and the total flavonoid content in this study, it was observed that the total phenolic content of the extracts was significantly higher than the total flavonoid content which implies the presence of other phenolic compounds in the seeds other than flavonoids.

Additionally, to estimate the antioxidant properties of the seeds, the free radical scavenging activities, the reducing power assay and total antioxidant capacity were measured. The total antioxidant capacity was quantified and expressed as ascorbic acid equivalent Table 3. From the results, *B. aegyptiaca* gave the highest total antioxidant capacity of 19.62 mg AAE/ 100 g dry extract whilst *A. digitata* gave 13.49 mg AAE/ 100 g dry extract. In comparing the total flavonoid and total phenolic content with the total antioxidant capacity of the seeds, it was observed that *B. aegyptiaca* which had the highest total phenolic and flavonoid content again had the highest antioxidant capacity. This may be true because the estimation of the total antioxidant capacity of extracts from the seeds is a measure of the ability of the extracts to reduce or prevent oxidative stress reaction and also their ability to quench free radical reactions.

From the transformation of ferric to ferrous iron reducing power assay, the electron-donating ability of the extracts of the seeds was estimated and compared with Ascorbic acid and Quercetin standard reagent. It was observed that the reducing power of the seed extracts increased with increasing concentration of the extracts Figure 1. However, the reducing power potential at the different concentrations for the seeds was lower than the Ascorbic acid and Quercetin standard reagents used. Again, DPPH, which is a stable free radical, and has the ability to accept hydrogen radical or an electron to form a stable diamagnetic molecule was also employed to assess the antioxidant activities of the seed extracts. The reducing power of the DPPH radical is characterized by a decrease in its absorbance at 517 nm in the presence of an antioxidant molecule. From the results obtained Figure 2, it is clear that as the concentration of the extract increases, there was a marginal increase in absorbance; hence, an increase in percentage inhibition.

3.4 Inhibition of albumin denaturation

The protein denaturation assay was used to evaluate the anti-inflammatory activity of ethanolic extracts of seeds from the *A. Digitata*and *B. egyptiaca*. The mechanism of the protein denaturation involves the loss of tertiary and secondary structures of proteins by application of external stress. Proteins denaturation has been reported as a major cause of inflammation [21,46]. In this study, the

anti-inflammatory properties of the ethanolic seed extract were studied. Table 4 shows the absorbance measured and their corresponding percentage inhibition of the protein denaturation assay of the ethanolic extract of *A. digitata* (BSF) *and B. egyptiaca* (DDSF) defatted seed flour and diclofenac standard drug at different concentrations. The results obtained showed that *B. egyptiaca* exhibited the highest inhibition effect between the two seeds with a maximum inhibition of 39.15 % at 400 mg/ml concentration whilst, *A. digitata* gave a maximum inhibition of 16.52 % at 500 mg/ml concentration. Diclofenac, which was used as the standard drug, showed a very high percentage inhibition of proteins denaturation as compared to the seeds extract.

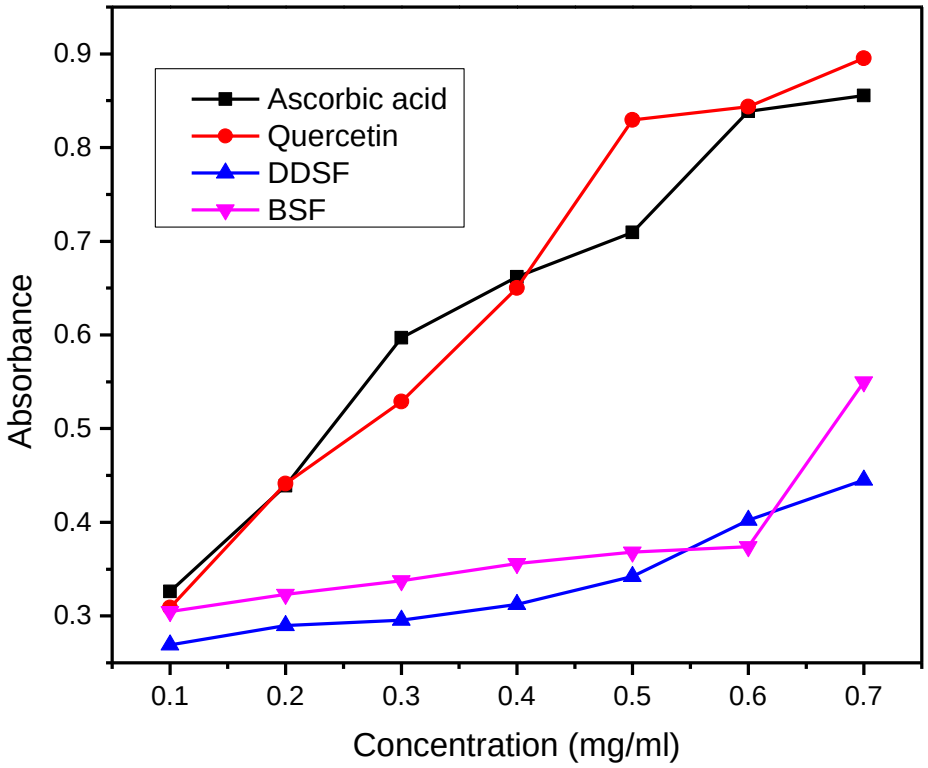

Figure 1. Reducing power assay of ethanolic extract of *Adansonia digitata* (BSF) and *Balanites aegyptiaca* (DDSF) compared with ascorbic acid and quercetin standard reagents at varying concentrations. Data represents the mean value of triplicate analysis.

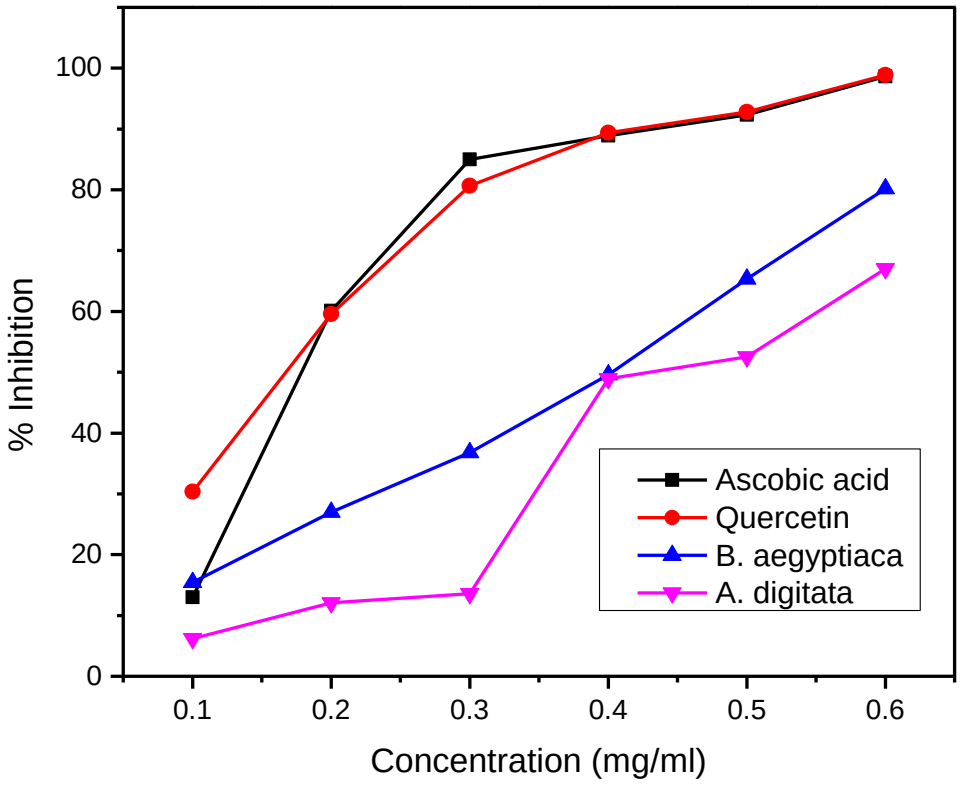

Figure 2. DPPH Scavenging activity of ethanol extract of *Adansonia digitata* (BSF) and *Balanites aegyptiaca* (DDSF) compared with ascorbic acid and quercetin standard reagentsat varying concentrations. Data represent the mean value of triplicate analysis.

Table 4: Absorbance and percentage inhibition of the protein denaturation assay of the ethanolic extracts of *A. digitata* (BSF) *and B. aegyptiaca* (DDSF) defatted seed flour and diclofenac standard drug

	BSF		DDSF		Diclofenac	
Conc. (mg/ml)	Abs. at 660 nm	% Inhibitn.	Abs. at 660 mn	% Inhibition.	Abs. at 660 nm	% Inhibition.
control	0.39±0.00	-	0.31±0.02	-	0.42±0.02	-
0.1	0.035±0.01	9.72	0.23±0.01	28.19	0.35±0.03	17.01
0.2	0.35±0.00	9.21	0.22±0.01	28.40	0.19±0.04	55.04
0.3	0.36±0.01	8.00	0.20±0.04	34.79	0.08±0.07	80.86
0.4	0.34±0.01	13.17	0.19±0.04	39.15	0.04±0.03	90.90
0.5	0.32±0.01	16.52	0.20±0.00	35	0.01±0.01	97.00

Note: The data are reported as mean ± standard deviation of three replicates.

Table 5: Absorbances and their corresponding percentage inhibition of membrane stabilization assay ofextracts of *A. digitata* (BSF) *and B. aegyptiaca* (DDSF) defatted seed flour and diclofenac

	BSF		DDSF		Diclofenac	
Conc. (mg/ml)	Abs. at 560 nm	% stabilisatn.	Abs. at 560 nm	% stabilisatn.	Abs. at 560 nm	% stabilisatn.
Control	0.13±0.03	-	0.15±0.06	-	0.35±0.05	-
0.1	0.13±0.01	3.99	0.10±0.01	29.57	0.17±0.03	51.82
0.2	0.12±0.01	7.23	0.10±0.02	29.12	0.15±0.03	58.21
0.3	0.11±0.01	14.96	0.11±0.00	26.41	0.19±0.01	44.56
0.4	0.09±0.01	29.43	0.12±0.00	16.70	0.21±0.01	38.93
0.5	0.09±0.01	34.66	0.09±0.01	40.41	0.23±0.03	35.31

Note: The data are reported as mean ± standard deviation of three replicates.

3.5 Membrane Stabilisation Assay

The Red Blood Cells (RBCs) membrane stabilization assay is a method used to estimate the in-vitro anti-inflammatory activity of extracts. In this method, the ability of the extracts to stabilize erythrocyte membranes was measured [21]. In the study, combined heat and hypotonic induced haemolysis were employed to evaluate the ability of the extract to stabilize the erythrocytes membrane at different concentrations. Results obtained showed that extracts from both seeds gave an increase in protection of the erythrocytes membrane against the damaging effect of heat and hypotonic solution with increasing concentration of the extract. However, results obtained from the diclofenac standard drug showed a high increase in stabilization with increasing concentration until it reached a threshold of 58.21 % stabilization at 200 ug/ml, after which an increase in concentration reduced the stabilization effect of the standard drug Table 5. Comparing *A. digitata and B. egyptiaca,* it was observed that *B. egyptiaca* gave the highest stabilization potential of 40.41 % at 500 ug/ml while *A. Digitata* gave 34.66 % stabilization at 500 ug/ml concentration.

4 Conclusion

This study sought to estimate the nutritional composition and medicinal properties of the seeds obtained from *Balanites aegyptiaca* and *Adansonia digitata*. Results obtained revealed the presence of high amounts of crude fat and protein for *B. aegyptiaca* whilst *A. Digitata* was found to contain high amounts of crude fibre and carbohydrate. The increased amount of crude fat (over 40 %) obtained from *B. aegyptiaca* seeds gives it the advantage to be exploited as an economically viable industrial crop. From the mineral elements analysis, *B. aegyptiaca* was found to contain high levels of calcium, and *A. digitata* had high levels of zinc. Based on the concentrations of the different mineral elements analyzed, it is clear that the consumption of *B. aegyptiaca* and *A. digitata* seeds may contribute significantly to the daily supply of the mineral elements to the body. The results revealed that ethanolic extract of the seed flour from both plants possess significant antioxidant and anti-inflammatory properties, and this may be due to the presence of high levels of phenolic and flavonoid compounds. Based on the results from the study, it can be concluded that ethanolic extracts from both *B. aegyptiaca* and *A. digitata* can be used as free radical scavengers and inhibitors to heat-induced albumen denaturation. The extracts can also stabilize the Red Blood Cells membrane according to data obtained from the study. However, comparing the two plants, *B. aegyptiaca* was more potent in both antioxidant and anti-inflammatory activities than that of *A. digitata.* Considering the high carbohydrate, Crude protein, and crude fat content, the BSF could serve as a good source of food for humans and feed for different classes of livestock.

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6 Daftar Pustaka

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