Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2022-141

Research Article

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Comparative Evaluation of Amino acid profile and *In vitro* antioxidant Activity of *Balanites aeqyptiaca* (L.) Delile meal, Defatted and Concentrate

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Received: October 11, 2022
Accepted: December 24, 2022
Published: December 26, 2022

Citation: Friday OA, Ojotu EM, Abraham GT, Oneh AJ. 2022. Comparative Evaluation of Amino acid profile and In vitro antioxidant Activity of Balanites aeqyptiaca (L.) Delile meal, Defatted and Concentrate. *J Food Chem Nanotechnol* 8(4): 201-210.

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Abstract

The need to screen potential antioxidant and expose better amino acid functional foods from plant based continue to increase. However, these plant-based food depends on their ability to proffer antioxidant ability. This study, we investigated amino acid profile and the antidiabetic resolved toasted seed from *Balanites aeqyotiaca* (L.) Delile comparatively. In this work we evaluated the total phenolic, flavonoid, amino acid profile and invitro antioxidant properties of *Balanites aeqyptiaca* (L.) Delile seed meal, defatted meal, and concentrates sample. The results revealed high total phenol (2.0mg/100g), hydrophobic amino acid (40.95%), Tryptophane (1.16%), **(1,1**-diphenylpicrylhydrazine radical scavenging activities **(DPPH** ,80%) and the hydroxyl radical scavenging activities (HRSA ,320%) was high in *Balanites aeqyptiaca* (L.) Delile **protein concentrate (**APC) sample. However, flavonoids (7mg/100g) , Superoxide radical scavenging activities (SORCA,35%) and total antioxidant power **(**ABTS,8.5%) of *Balanites aeqyptiaca del* **protein concentrate** (APC) were below the reference standard .On the other hand the *Balanites aeqyptiaca del* **defatted meal** (DAM) sample has high flavonoids (12mg/100g), Aromatic amino acids **(**AAA) (10.42%), total antioxidant power (12%),Ferric reducing activity (0.6%) and metal chelating (65%) while the *Balanites aeqyptiaca* (L.)Delile meal (AM) sample show high flavonoid content (17mg/100g), Essential amino acid (EAA,47.84%), leucine (12.01%) content and lipid peroxidation (0.055%) ability The excellent ability of the *Balanites aeqyptiaca* (L.)Delile protein concentrate and the *Balanites aeqyptiaca* (L.)Delile **defatted meal (** DAM) to inhibit , scavenge free radical and appreciable content of flavonoids could be part of the possible door way to which resolved materials from Aduwa seed could be used as potential bioactive to help in the management of chronic disease often associate with free radical build up in the human system.

Keywords

Phytochemical, *Balanites aeqyptiaca* (L.) Delile meal, Defatted meal, Concentrate, Amino acid profile, Antioxidants

Introduction

Balanites are attributed to the genus of flowering plants. *Balanites aegptiaca* (L.) Delile is a desert plants called Aduwa in Nigeria popularly known as desert date. This multipurpose evergreen bearing seed plant is common in Africa and Arabian countries.The use of *Balanites aeqyptiaca* (L.)Delile seed is popular in traditional medicine for treating ectoparasite, diabetes, sore throat, constipation, and eye irritation [1].It is a historical folkloric medicinal plant due to its curative nature towards diverse fatal diseases and has been associated with the manage-

ment of a myriad of diseases such as jaundice, intestinal worm infection, wound healings, malaria, syphilis, epilepsy, dysentery, stomach aches, constipation, diarrhea, hemorrhoid and asthma [2].The bark, fruit, seed and oil of Balanites seed tree have been used to treat various disorders including cancer [3], tuberculosis, HIV/AIDS [4],sleeping sickness [4], diabetes ,wounds healing [5], colds, syphilis, liver and spleen disorders [6].The infusion of *Balanites aeqyptiaca del* root bark has been used cure diarrhea and hemorrhoid [7]. As a result of the presence of several bioactive ingredients in the various parts of *Balanites aeqyptiaca* (L.) Delile, it could serve as antioxidant, anthelminthic, antimicrobial, antihypertensive therapeutic food. These desert resistance seed bearing plant is widely neglected; however, its fruits are edible, the leaves and seed usually boiled and could be mixed with cereals like sorghum to make family meal especially during famine periods. The nutritional profile, amino acid profiles and phytochemicals of the leaves, flower and seed have been opined by [8-11]. Similarly, the anti-nutritional properties at various processing approaches have been reported and were below lethal dose when the seed are either boiled, fermented, or roasted in the processing [11, 12]. Recent studies on *Balanites aeqyptiaca* (L.) Delile essential and non-essential amino acid profile as well as invitro antioxidant properties of the kernel when boiled and when in raw states respectively have been studied [8, 13, 14]. These authors revealed the high essential amino acid presence and antioxidant potentials of Aduwa seed kernel. Plants, especially nuts are potential source of antioxidant and study on their phenolics bioactivity, acting as antioxidant potential scavenging free radicals, for instance in cashew nuts and peanuts have been reported [15-17]. *Balanites aeqyptiaca* (L.) Delile seed nuts are also rich in minerals, proteins fibre, unsaturated fatty acids and phytochemical together could reduce risk of metabolic diseases such as diabetes and hypertension by acting as antioxidant or anti chronic disease triggers. The advocacy on use of plant nuts to alter the risk of coronary disease and obesity also to promote cell longevity is on the rise [17, 18]. Plant phenolics and flavonoid, a derivative has synergistic function and are dominant in nuts. The hydrophilic configured phenols in nuts have been attributed to boost immune system -hence could help in COVID -19 infection management because phenolics have antioxidant potentials to mitigate lipid oxidation and to scavenge free radicals [19, 20]. Although work on the phytochemical screening, amino acid profile has been carried out on the Aduwa seed but little or no work have been done on the toasted seed and their comparative study. In most evolving cultures Aduwa kernels are being processed before use, hence the motivation to evaluates their comparative properties when the toasted seed are been resolved from whole meal through defatted meal to concentrate.

Source of raw materials

The matured *Balanites aeqyptiaca* (L.) Delile *seeds* were purchased at Gashua modern market The sample was conveyed the biology laboratory of the Federal university Gashua in Yobe state. Where it was identified **as** *Balanites aeqyptiaca* (L.) Delile, belonging to the family Zygophyllaceae (Balanitaceae) called desert dates. The sample was given a LOT No 20GASHUA10. About nine hundredth grams (900) grams mesocarp kernels moisture value was ascertain at 15% by wooden solar cabinet dryer before processing.

Aduwa seed processing

The seed kernels were toasted before milling. The milled meals were pressed using centrifugal screw which is automated for making meals from seed nuts and for making and oil [12]. The cake made were sealed and transported to the Human Ecology College, Cereal laboratories for analysis.

Defatting meal (DAM)

Preparation of Defatted *Aduwa* sample were prepared from seed made into meal sample by toasting. The method by Sathe, as modified by [21] was adopted in defatting *Balanites aeqyptiaca* (L.) Delile. Cold (4 ^{0}C) acetone was used to defeat the sample using 1:5 w/v sample to solvent ration. The mixture was stirred over a magnetic stirred for 4 Hrs. The final slurry was then filtered using Whatman 1 filter paper. Residue was re-extracted twice in a similar fashion. The defatted sample was left in a fume hood at room temperature and finally made into powder using a blender and stored in air-tight container.

Production of meal concentrates (MC)

Aduwa Meal concentrate (MC) was produced by the modified approach of [22]. Briefly, two hundredth grams defatted meal dissolved in 200 ml distilled 200 ml water were made to final 1:10 ration concentrations. The mixture was properly mixed on a magnetic stirrer for 10 min, and the pH of the resultant mix adjusted with 0.1 M HCl to pH 4. The process was allowed to proceed on stirring for 2 Hrs. at constant pH. The mix was centrifuged at 3,500 × g for 30 min and the concentrate washed with distilled water twice and then centrifuged again at 3,500 × g for 10 min. The concentrates were collected and dried in an oven at 45 °C for 8 hr and stored in freezer for analysis.

Materials and Methods

Phytochemical determination

Phenolic content TPC analysis was carried out by folin-ciocalteu's phenol reagent reaction, according to [16]. The sample was prepared to final concentration in water to 1.0 mg/mL and then centrifuged to collect supernatants. A calibration curve of gallic acid solution was prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, and1.0 ml to obtain a final concentration of (1.0 mg/mL garlic acid) in triplicate. About 1 ml of the sample and the standard were pipetted inside clean test-tubes in triplicate, and 1.5 mL folin-ciocalteu reagent was added to each tube. This was incubated at room temperature for 5 min and the addition of 1.5 mL of 10% (w/v) sodium trioxocarbonate solution to give a final volume of 4.0 mL. The mixture was allowed to be incubated for one and half hours (90 min) and the absorbance read at 725 nm against the blank. The standard curve was obtained from absorbance and concentration. The phenolics content in the meal sample was extrapolated from standard Gallic acid equivalent per g of extract (mg GAE/g extract*)*.

The flavonoid content

The total flavonoid content of the meal and concentrate were determined using modified method reported by [16, 17]. Briefly, zero point five (0.5 mL) of diluted sample was mixed with 0.5 mL methanol plus 50 µL of 10% AlC_{13}, and with 50 µL of 1 mol/L potassium acetate and 1.4mL distilled water. The mixture was incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using the JENWAY UV–Visible spectrophotometer. The total flavonoid, quercetin was referenced as standard.

Amino acid profile of whole aduwa meal, defatted meal, and concentrates

The essential and non- essential amino acid profile of the samples were determined as described by [23, 24]. Three same samples were ampulated using HPLC system, after hydrolyzing with 6 M HCl. Performic acid oxidation was used to determine cysteine and methionine and alkaline hydrolysis used to determine tryptophane a [23-25].

In-vitro antioxidants

Radical scavenging activity (DPPH) (2,2 -Diphenyl-1-Picryl Hydrazyl)

The DPPH radical scavenging activity of the meal defatted and concentrated were determined by the modified method of [26] The DPPH (0.02 mg/ml) was mixed with various concentration of sample extracts (0 to 5 mg/mL). The extracts were dissolved in methanol and thereafter the mixture was made to stand for 60 minutes at 25 °C and absorbance read at 517 nm using a spectrophotometer. GSH was a positive control. The percentage inhibition was calculated using the formula:

% Inhibition = (A0– A1)/A0) x 100
where,
A0= Absorbance of control
A1= Absorbance of sample

Supraoxide radical scavenging activity (SORCA)

The superoxide scavenging activity methods described by [27] was adopted. An aliquot (80 µL) of the sample and glutathione as a control both at a concentration of 1 mg/mL were mixed with 80 µL of 50 mM Tris-HCL buffer at (pH 8.3) containing 1 mM EDTA. The mix was piped into a clear bottom 96-well plate in the dark. Pyrogallol (1.5 mM) was dissolved in 10 mM HCl and 40 uL added to each well in the dark. Absorbance was measured at 420 nm for 4 min at room temperature.

$$\text{Superoxide Scavenging Activity (\%)} = \frac{(\Delta \text{ A420 nm/min blank} - \Delta \text{ A420 nm/min sample})}{\Delta \text{ A420 nm/min blank}} \times 100\%$$

The metal ion chelating activity (MCA)

The Fe^{2+} chelating activity of the three samples were determined using modified method by [28]. Freshly prepared 500 mol $L^{-1}1FeSO_4$ (0.15mg) quantity added to a reaction mixture containing 0.168 mg of 0.1 mol L^{-1} Tris–HCl at (pH 7.4) plus 0.218 mg saline and the sample concentration between (0–0.025 mg). The reaction mixture was incubated for 5 min, and 0.013 mg of 0.25% 1,10-phenanthroline (w/v) added. The absorbance was measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was calculated with respect to the control.

$$\text{Percentage } Fe^{2+}\text{chelating ability (\%)} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

where Abs control = absorbance of the control (reacting mixture with-out the test sample)

Abs test sample = absorbance of reacting mixture with the test sample

Hydroxyl (OH) radical scavenging assay (HRSA)

The ability of the aqueous Aduwa samples to prevent Fe^{2+} and hydrogen peroxide (H_2O_2) induced decay of deoxyribose was carried out using the method of [29]. Briefly, freshly prepared aqueous sample concentration (0.0–0.10mg) was added to a reaction mixture containing 20 mM deoxyribose, 04 mg 0.1 M phosphate buffer, 20 mM hydrogen peroxide and 0.040 mg 500 Mm $FeSO_4$, and the volume made to 0.8mgL with distilled water. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was stop by the addition of 0.5 mL of 2.8% TCA (trichloroacetic acid), this was followed by the addition of 0.4 mL of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer.

$$\text{OH, radical scavenging ability (\%)} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

Where Abs control = absorbance of the control (reacting mixture with-out the test sample).

Abs test sample = absorbance of reacting mixture with the test sample.

FRAP -ferric antioxidant power

Reducing property of sample was determined by assessing extract ability to reduce $FeCl_3$ solution described by [30, 31]. Sample volume of 2.5 mL aliquot was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL,1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min, and then 2.5 mL, 10% TCA added. The mix was then centrifuged at 650 rpm for 10 min. 5 mL of the supernatant was taken mixed with an equal volume of distilled water and 1 mL plus 0.1% ferric chloride. Similar treatment was performed to standard ascorbic acid solution and the absorbance taken at 700 nm. The ferric reducing power was calculated and expressed as ascorbic acid equivalent.

ABTS-antioxidant power

Total antioxidant power of sample was assessed using the ABTS method described by [30]. Radicals containing ABTS were generated by reacting 7 mmol/L of ABTS aqueous solution with 2.45 mmol/L of potassium thiosulphate solution in the dark for 16 Hrs. and reading at 734 nm.

Lipid peroxidation

Lipid peroxidation measurement was by modified method of [32]. One hundredth microliter of sample solutions were mixed with reaction mixtures containing 30 microliter of 0.1 M Tris-HCl buffer (pH 7.4), sample concentration of (0.0–100 µL) plus 30 microliters of the 7 µM sodium nitroprusside. The final volume of the mix was made up to 300 microliters by water before incubation at 37 °C for 2 h. Color reaction developed by adding 300 microliters of 8.1% SDS to the reaction mixture which contains sample, followed by the addition of 600 microliter of acetic acid/HCl at (pH 3.4) plus 600 microliters of 0.8% thiobarbituric acid. This mixture was incubated at 100 °C for 1 Hr. The absorbance of thiobarbituric acid reactive radical produced was measured at 532 nm in UV–Visible spectrophotometer. Malondialdehyde (MDA) as percentage control.

Statistical analysis

Three readings were analyzed for mean ± standard deviation from three readings. Data were subjected to analysis of variance (ANOVA) followed by Duncan Multiple Range Test to compare treatment means. Differences were considered significant at $p < 0.05$ using the statistical package for social sciences for window program (SPSS V23) software.

Discussion

Phenolic and flavonoid contents

Phenolic compounds play important function in the stability of lipid layer against peroxidation and inhibits oxidizing enzymes that promote oxidative stress [33, 34]. Phytochemicals could stop autoxidation of fat, and this can stop accumulation of radicalized low-density lipoprotein (LDL) that could induce heart disease [33]. Phenols are a phytochemical with the characteristic as a Polyphenol compound is a subject of interest due to their health benefits [27, 28, 35]. Numerous evidence has attributed the invitro antioxidant activities of plants food to total phenolic compounds [36, 37] and maybe due to certain level of phenol redox activities.

Phenol content of the meal, defatted, and meal concentrate are presented in figure 1. Result revealed total phenol content of the meal ranged between 1.9 mg/100g-2.0mg/100g for protein meal and defatted meal 1.21 mg/100 g to 2.1 mg/100 g. The defatted meal and protein concentrate samples are higher in phenolic activities than the *Balanaites aegypyiaca* del meal (AM). These values are greater than values reported by [19] on peanut phenol when finally processed to concentrate, this significance increase ($p>0.05$) was also observed in defatted meal (DAM .2.0mg/100g) and protein concentrate (AC,2.0mg/100g) when compared to the reference (1.21 mg/100g), these upsurge in total phenol agrees with [37], asserting that heat does increases phenol content in tomatoes. The basis of the increase in phenolic in the meal, defatted meal and concentrated could be due to material annealing during processing and breaking down of the tannins [38]. This could also be reasons behind defatted meal and concentrate behaviors. This work revealed that concentrate has higher total phenol content, followed by, defatted meal and the least was in meal. There is further evidence that there is a correlation between the phenolics contents, and the biological remedies studied [23]. Hence, total phenol in the meal and concentrate may be part of the active compound responsible for the antioxidant activities envisage in Aduwa meals The phenolics in Aduwa seed may have advanced their scientific report of their use in folklore medicine [10, 39, 40]. It is therefore worth noting that there is high phenolic reflection in defatted meal and the proteins concentrate.

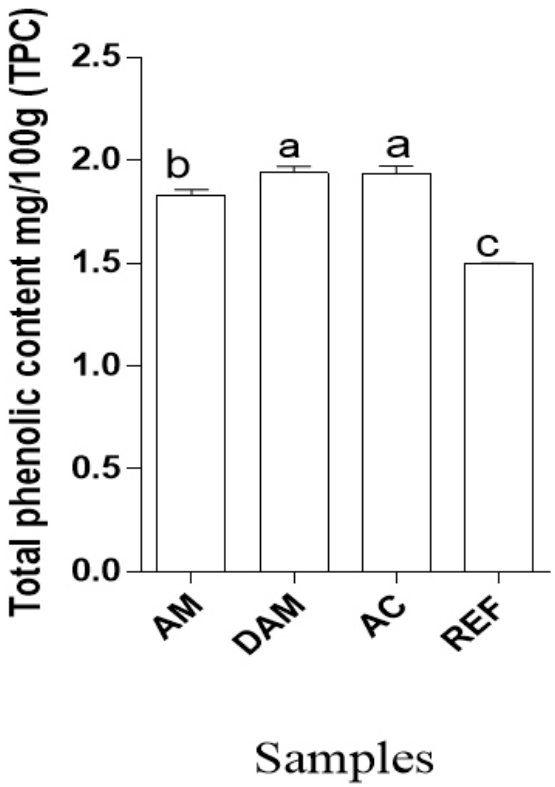

Figure 1: Phenolic Content (PC) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at $p<0.05$ Key:AM= meal, DAM =defatted meal, AC =concentrate.

Flavonoids of *Balanites aegyptiaca* (L.) Delile meal defatted and concentrates

Flavonoid (TF) contents in *Balanites aegyptiaca* (L.) Delile meal, defatted protein meal, protein concentrate is shown figure 2. Flavonoid content in food system has been attributed to antioxidant and microbial inhibitory potential [33]. Flavonoid content in the sample reduced significantly as AM sample was resolved to protein concentrate. High flavonoid content was observed in AM (15.2mg/g) and this was followed by defatted meal (DAM ,11.0mg/g). There exist no significant differences at $p>0.05$ among samples. The value of flavonoids reported by [41] in soaked and boiled *Balanites aegyptiaca* cake samples were (13.40 mg/100 g) and (12.40 mg/100 g) respectively, however differ from the values under study. Because antioxidant capacity is an index of the scavenging compounds like flavonoids, thus, it can be concluded that *Balanites aegyptiaca* (L.) Delile meal and concentrate samples may have the highest total antioxidant competency.

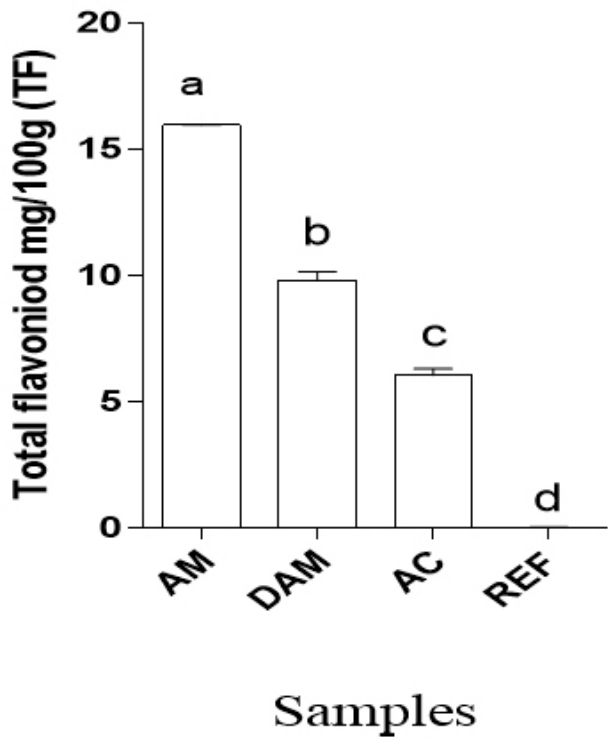

Figure 2: Total Flavonoid (TF) **of meal, defatted and concentrates.** Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key:AM= AM= meal, DAM =defatted meal, AC =concentrate.

Amino acid profile in *Balanites aegyptiaca* (L.) Delile meal, defatted protein meal, and protein concentrates

Amino acid has been defined as the building blocks of proteins and it is an important parameter to determine the quality of protein in food ingredient [42]. The crude protein content in meal, defatted and concentrate were within 25%.34% and 62% range, respectively. Table 1 showed the essential and non-essential amino acid composition of *Aduwa* meal, defat meal, and concentrate. The essential amino acid (EAA) ranged between 37.35 and 47.84 %, AM has the highest value, followed by the defatted meal and the least was obtained in protein concentrate which may be attributed to extraction process effects. This suggests that *Balanites aegyptiaca* (L.) Delile protein meal and the resolved defatted proteins are good quality source of protein. In another manner, the aromatic amino acids, hydrophobic amino acids, positively charged amino acid, of the samples ranged between 5.24 and 10.42 %, 39.96 to 40.95 % and 15.90 to 17.0 % respectively, with AC having the highest amounts. The high concentrations of the hydrophobic amino acids in the concentrate may have implications on the structural behaviors of the proteins [43].

The values obtained for the Sulphur containing amino acid ranged between 3.22 to 3.15%, with the concentrate having the highest value. The trend of the Sulphur amino acid in the samples could dictate the antioxidant trend of the samples. The tryptophan content of the samples ranged between (1.16 - 1.78%) higher than (0.1 -0.12%) reported for okra seeds flours, isolated proteins, and hydrolysate samples [44]. Similarly, the histidine content of the samples ranged between 3.00 to 3.75 %, with AM having the highest value. However, the differences in the amino acid content of meal and defatted meal when compared with those of concentrate sample may be attributed to extra processing techniques and extraction process applied to producing *Balanites aegyptiaca* (L.) Delile concentrate [45].

Antioxidant properties

GSH as a referral in the comparative analysis of Aduwa samples, is chemically reactive and could maintain oxidative hemostatic environment with cells with the help of its thiol group. This ability is attributed to GSH to sequester ion and scavenge abilities on free radicals.

DPPH in *Aduwa* meal, defatted and concentrates

DPPH Radical Scavenging Activity determines antioxidant capacity of biological materials by scavenging the free radicals formed in the oxidant system [46]. Its measures the decrease in absorbance of the radical ion at 517 nm wavelength [25].

DPPH in Aduwa Meal, Defatted and Concentrates are presented in figure 3. DPPH is not an important scavenging assay, but it is widely used to evaluate the antioxidant properties of natural compounds [47]. DPPH indicate the ability of the antioxidant substances to donate electrons or hydrogen atom, converting free radical to a more stable species [48]. Thus, AM and AC showed the strongest activity which was different at (p>0.05) in comparison to sample DAM but significantly (p>0.05) lower than GSH. Moreover, AM and AC has similar DPPH scavenging activity but low than the GSH. These indicated that Aduwa protein meals

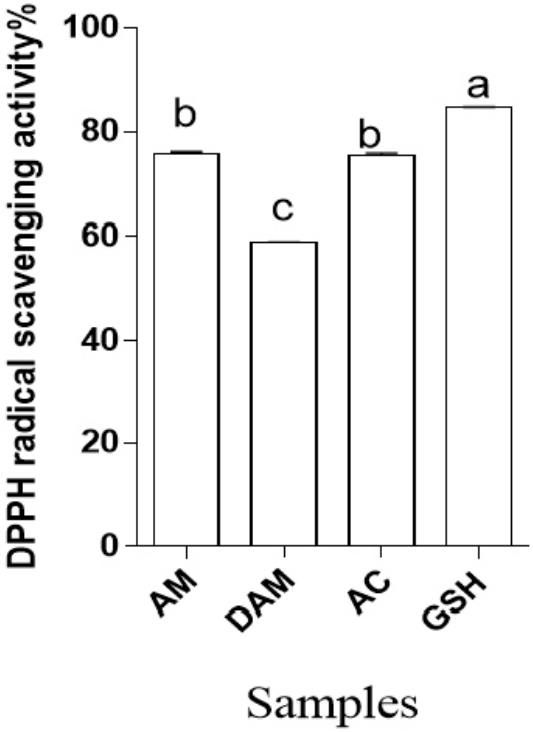

Figure 3: DPPH graph of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM = meal, DAM =defatted meal, Ac = Concentrate.

could contribute to DPPH scavenging activity as observed in AM sample. The ability of AM to scavenge free radicals similar with AC could be due to synergistic protein interaction with possible inherent polyphenol [48, 49] and may also be due to hydrophobic amino acids which may be present in the AC samples [50, Table 1].

Metal ion Chelating Activities (MCA) in *Balanites aegyptiaca* (L.)Delile meal, defatted and concentrates

Metal Chelation Activity.The generation of peroxides to form ROS species is very slow but in presence of metals ion such as cupper and iron catalysis the production of ROS. Therefore, sequestering this metal is also pivotal.

Figure 4 shows the metal ion Chelating activities (MCA) in *Balanites aegyptiaca del* seed Meal, defatted Aduwa meal and Concentrate. Metal ions do participate in Haber Weis reaction producing super oxides radicals thus, resulting in hydroxyl radicals [51]. The antioxidant chelates the Fe 2+ by reducing the ions and reduces the triggers of OH radical by this distorting the propagation of hydroxyl ions into delicate living cells system. Figure 2, GSH (80%) is significant p>0.05 high than M 78%, DAPM 65% APC 22% respectively. There was decrease in Metal Chelation for concentrate as protein materials were resolved through defatting, solvation, and precipitation, respectively. The *Balanites aegyptiaca* meal and defatted meal revealed higher Metal Chelation (MCA) activities compared with concentrate sample. The decrease in Metal Chelation activity in concentrate may be due to presence of histidine in the protein meal samples [48-50].

Table 1: Amino Acid profile of *Balanites aegyptiaca* (L.) Delile defatted and concentrate.

Amino acid	APM	DAPM	APC
Leucine	12.01	8.60	8.47
Lysine	8. 41	5.32	5.35
Isoleucine	5.62	4.50	4.55
Phenylalanine	2.50	5.15	5.06
Tryptophan	1.78	1.18	1.16
Valine	7.49	5.02	5.06
Methionine	2.21	1.58	1.62
Proline	1.59	4.01	4.10
Arginine	3.75	8.07	8.26
Tyrosine	0.96	4.09	4.12
Histidine	3.75	3.00	3.39
Cystine	1.01	1.52	1.53
Alanine	4.80	5.02	5.27
Glutamic acid	8.65	18.22	18.08
Glycine	8.21	4.11	4.26
Threonine	5.86	4.81	3.86
Serine	7.49	4.82	4.93
Aspartic acid	13.93	10.99	10.93
AAA	5.24	10.42	10.34
BCAA	25.12	18.12	18.08
HAA	39.96	40.67	40.95
PCAA	15.90	16.39	17.00
NCAA	35.93	38.84	37.80
SCAA	3.22	3.10	3.15
EAA	47.84	37.98	37.35

Key: *(AAA) = phenylalanine, tryptophan and tyrosine,* ***Branched chain amino acids*** *(BCAA) = leucine, isoleucine, valine,* ***Hydrophobic amino acids*** *(HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine and cysteine,* ***Positively charged amino acids*** *(PCAA) = arginine, histidine, lysine,* ***Negatively charged amino acids*** *(NCAA) = aspartic ,glutamic , threonine, serine, (SCAA) = methionine, cysteine, (EAA) = histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.*

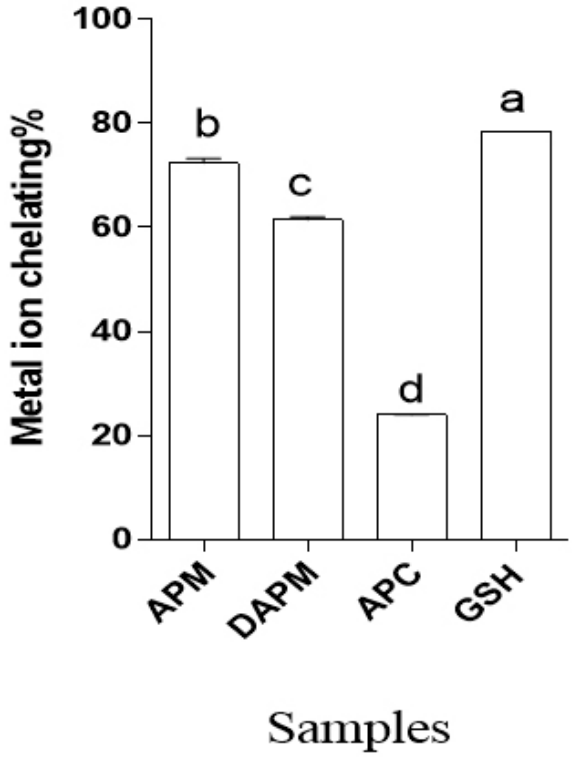

Figure 4: Graph on Metal ion chelating (MCA) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM= Meal, DAM =defatted meal, AC =concentrate.

Hydroxyl Radical Scavenging Activities (HRSA) in *Balanites aegyptiaca* (L.) Delile meal, defatted and concentrates

Figure 5 showed the OH radical scavenging activity. The OH radical scavenging activity of *Balanites aegyptiaca* (L.) Delile meals AM and concentrate AC had high (p>0.05) percentage absorbance of (300%) compared to GSH control and samples in this study. The hydroxyl Radical Scavenging Activities **(**HRSA) of meal, defatted and concentrate were 40 % suggesting their better hydroxyl Radical Scavenging Activities **(**HRSA) and plausible reason for the high hydroxyl Radical Scavenging Activities **(**HRSA) observed in these samples and might be due to concentration of HAA in the samples. [45-48] reported that phenylamine can increase **hydroxyl radical scavenging activities** because phenylaniline can react to form stable substituted derivatives of phenylaniline.

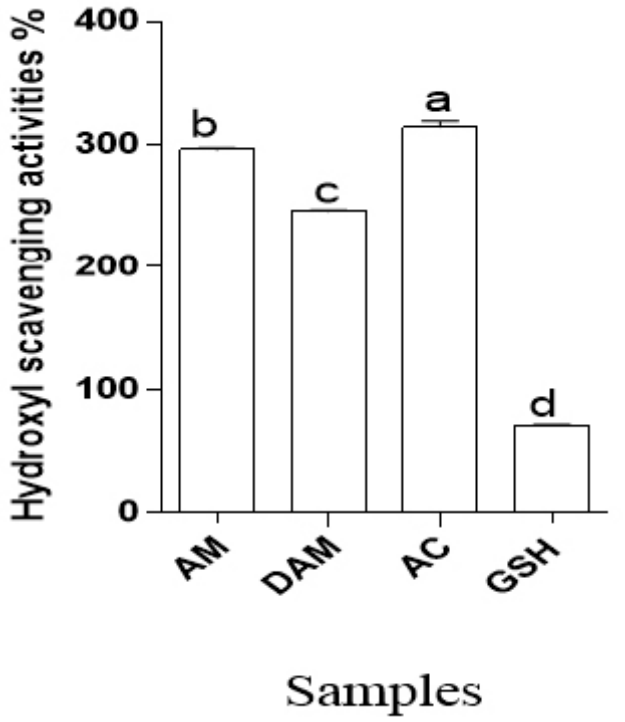

Figure 5: Graph on Hydroxyl radical scavenging activities (HRSA) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM= Meal, DAM = Aduwa meal, AC =concentrate.

Superoxide Radical Scavenging Activities (SORSA) of *Balanites aegyptiaca* (L.) Delile meal, defatted meal, and concentrates

Superoxide Radical Scavenging Activity in vivo are responsible for generating superoxide radicals, which are toxic species that could damage human health when they encounter delicate cells like DNA, live via hydroxyl radical production [52].

Superoxide Radical Scavenging Activities (SORSA) in *Balanites aegyptiaca* (L.) Delile Meal, Defatted and Concentrate are presented in figure 6. Superoxide radicals are a concerned because they are precursors to hydroxyl and hydrogen peroxide radicals. These radicals target DNA, protein, and lipid cells. to protect biological human health's, these radicals must be scavenged. As shown in figure 4, GSH (70%) exhibited (p>0.05) higher SRSA when compared with meal, defatted, and concentrated protein concentrate which ranged from 22-10% when compared to that of GSH. This slight increase in in superoxide radical scavenging activities (SRSA) in meal and defatted meal compared to AC could be duo to lysine and leucine amino acid moieties (Table 1) protein structures present in the samples [45].

Ferric Reducing Antioxidant Power (FRAP) in *Balanites aegyptiaca* (L.) Delile meal, defatted and concentrates

Ferric Reducing Antioxidant Power FRAP is a method based on the ability of an antioxidant compound to reduce ferric ion (Fe^{3+}) into the ferrous (Fe^{2+}) form. Ferric reducing auto oxidation power breaks chain reactions by donating hydrogen atom. The ability to donate hydrogen atom to reduce or form stable product measures ferric reducing auto oxidation

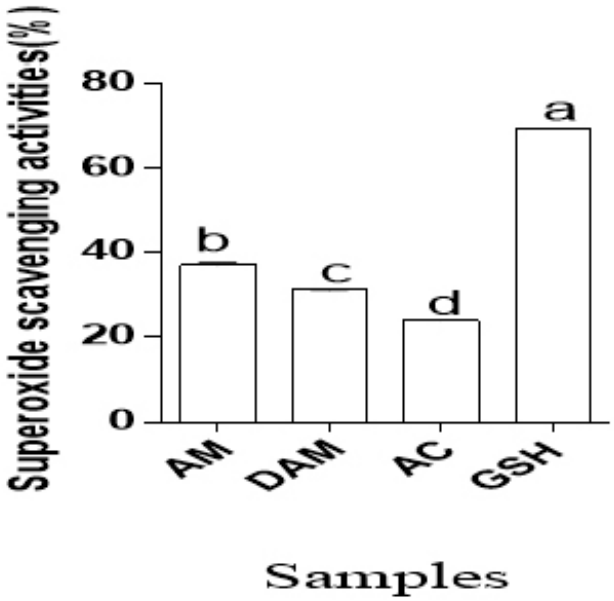

Figure 6: Superoxide radical scavenging activities (HRSA) of meal, defatted and concentrate. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM= Meal, DAM =defatted meal, AC = concentrate.

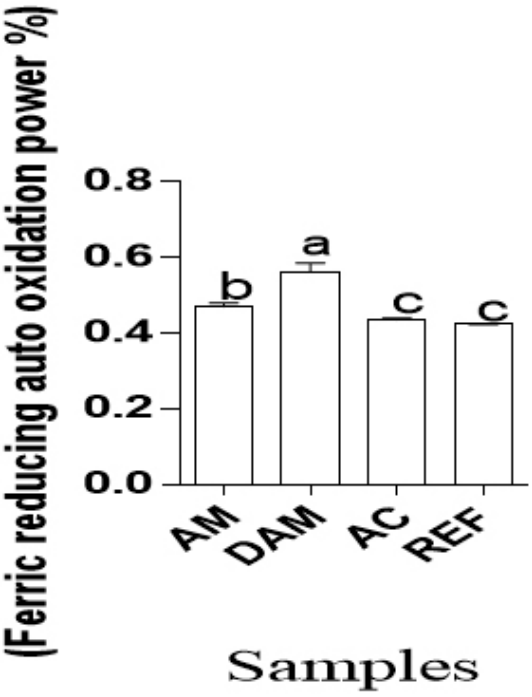

Figure 7: Graph of Ferric reducing antioxidant power (FRAP) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM = Meal, DAM = defatted meal, AC = concentrate.

potency of materials as shown in figure 7. All the sample under this study revealed higher ferric reducing antioxidant power (FRAP ,0.19-0.6%) compare with reference standard (0.44%). ferric reducing antioxidant power from defatted meal (0.6%) was different at (p>0.05) compared to meal and concentrate samples under study. The defatted meal (DAM,0.6%) exhibited greater ferric reducing antioxidant power (FRAP) activity when compare with meal, concentrate and the reference.

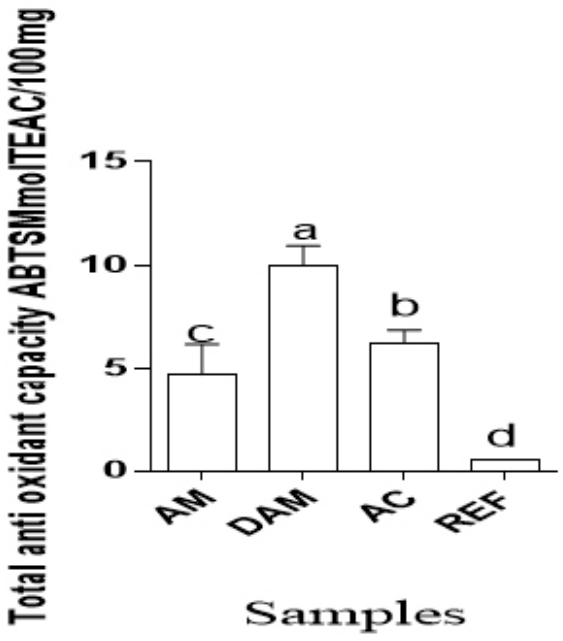

Figure 8: Total antioxidant power (ABTS) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM = Meal, DAM =defatted meal, AC =concentrate.

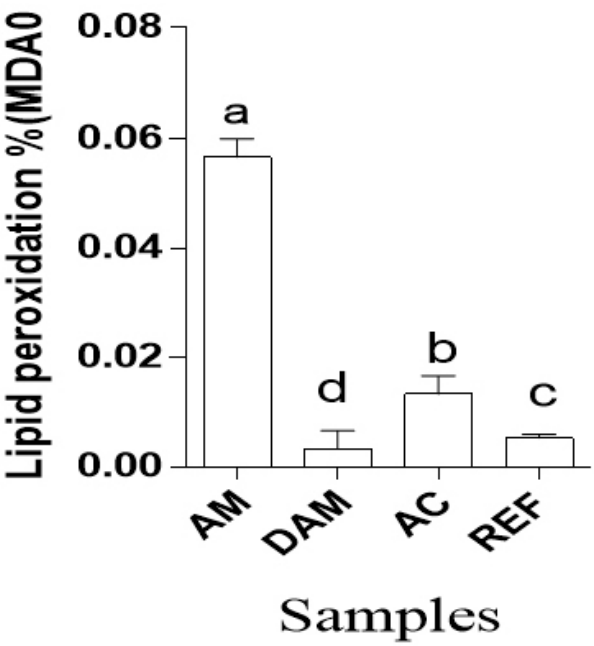

Figure 9: Lipid peroxidation (MDA) of meal, defatted and concentrates. Readings from triplicate determinations; Means followed by same alphabetic on the bar are not significantly different at p<0.05 Key: AM = Meal, DAM = defatted meal, AC =concentrate.

Total antioxidant power (ABTS) of *Balanites aegyptiaca* (L.) Delile meal, defatted meal, and concentrates

The ABTS scavenging ability of the *Balanites aegyptiaca del* meal, defatted meal and concentrate samples presented as TEAC in figure 8, revealed scavenging ABTS activities. However, the defatted meal (DAM ,10.0 mmol TEAC/mg), followed by concentrate (8 mmol TEAC/mg) had (P > 0.05) higher total antioxidant scavenging ability compared the meal (5.0 mmol TEAC/mg) and reference (1.50 mmol TEAC/mg) samples. These values disagreed with total antioxidant scavenging ability value from *Balanites aegyptiaca* (L.) Delile leaves and gall reported by [24] from *Balanites aegyptiaca* (L.) Delile *in-vitro* antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities of gall and leaves during their studies. Total radical scavenging activities in defatted meal was observed to be significantly higher than sample meals and reference used.

Lipid peroxidation or Malondialdehyde (MDA) in *Balanites aegyptiaca* (L.) Delile protein meal, defatted meal, and concentrates

This is one of many reactive electrophile species that cause toxic stress in cells and form advanced glycation end products (AGEs) that triggers diseases such as cancer, diabetes, and kidney dysfunctions. Protein cross links could be introduced by MDA, and this could form adducts with amino acids and DNA thus, inducing alterations in physiological properties. The malondialdehyde (MDA) content of the *Balanites aegyptiaca* (L.) Delile meal, defatted and concentrate samples are presented in figure 9. The result revealed (P<0.05) increase in the MDA content in meal than defatted meal and concentrate. This high increase may have resulted from toasting process of the seed to making the protein meal This could be attributed to the breakdown of peroxides to carbonyl and aldehyde compounds such as malondialdehyde, inducible by heating or high milliard reaction temperature [38, 50, 53]. This might be the reason for high MDA in meal due to meal annealing and low MDA values for defatted meal and concentrate samples. Unsaturated aldehydes may undergo further changes by autoxidation producing other volatile compounds. Thus, meal and concentrate sample may have produced, hydroperoxyl aldehydes which might undergo cleavage to give shorter chain aldehydes, and other chemical groups, among these, malondialdehyde (MDA).

Conclusion

Conversion of meal into defatted and protein concentrate improved their phytochemicals, amino acid and antioxidant properties which is reflected in the phenolics and flavonoids, amino acid profile and antioxidants. The protein concentrates also display strong antioxidant due to histidine than meal and defatted meal which employs the imperativeness concerning processing and extraction. However, these may have contributed to better antioxidant property of defatted meal also because of high content in Negatively charged amino acids (NCAA), total antioxidant power (ABTS) activity which enhances metal chelation and metal scavenging activities. Therefore, protein concentrate could be chosen as potent ingredient to make economic functional food formulars.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgements

The authors are grateful to Tet FUND for the Financial support through the Management Federal university Gashua, the Department of Home science and Management Federal University Gashua, Yobe state. Nigeria for the assistance during the research.

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