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Implications of dehusking and aqueous soaking on anti-nutrients, phytochemical screening and antioxidants properties of jack beans (*Canavalia ensiformis* L. DC)

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Abstract
The world's growing population is pushing humans to look for alternative food sources among underutilised or wild plants. One of these food sources has been identified as *Canavalia ensiformis*, or jack beans. The only issue with using jack beans is that they contain anti-nutrient chemicals, which must be removed or diminished in order for them to be fit for human consumption. The objective of this study is to determine the nutritional and industrial utility of *Canavalia ensiformis* by analysing the anti-nutrient, phytochemical, and antioxidant composition of raw whole seed and soaking dehusked seeds using established procedures. Phytate (23.48±0.24, 15.24±0.41 and 14.83±0.00), oxalate (4.32±0.09, 3.96±0.09 and 2.88±0.09), tannins (22.77±0.73, 18.68±0.03 and 17.50±0.46), and lectins (6.67±0.04, 6.20±0.01 and 6.42±0.07) exhibited the highest anti-nutrient values in raw whole seed and, at the very least, in dehusked, soaked seeds. The samples were subjected to phytochemical screening, which detected the presence of cardiac glycosides as well as anthraquinones, alkaloids, tannins, saponins, steroids, flavonoids, terpenoids, phlobatannins, and flavonoids. Due to the reduction in phytochemical contents quantified as a result of dehusking and soaking, phlobatannins and anthraquinones were not found in the samples. The research findings also demonstrated elevated concentrations of several plausible phytochemical components with potential medical value, with the raw whole seed exhibiting the greatest capacity to scavenge free radicals. Accordingly, the study's findings validate the seed's therapeutic applications and imply that it might be an inexpensive source of antioxidants for humans and animals alike.

Keywords: Dehusking, soaking, anti-nutrients, antioxidants

Introduction
Wild legumes are essential for food security, nutrition, agricultural development, and crop rotation all contribute to the nation's economic growth. Many wild legumes are noted for their low protein, caloric value, essential amino acids, vital fatty acids, fibre, and vitamins. The development of products derived from *Canavalia ensiformis* is projected to substitute, diminish, or even replace soy protein, hence reducing the need for and dependence on soybeans [1]. *Canavalia ensiformis* (Jack bean), also known as feijão-de-porco ("pigbean") in Brazil, is a legume used for animal fodder and human nutrition. The food sector requires a novel way to delivering new materials, technologies, and fresh, healthy, and sustainable food items [2]. Legumes are cheaper than animal products, hence they are used globally as a major source of protein, especially in developing countries, or particularly in poor countries where animal protein consumption may be restricted due to economic, socio-cultural, or religious beliefs [3].

Anti-nutrients or anti-nutritional factors are substances produced in natural feedstuffs by species' normal metabolism and various mechanisms (for example, inactivation of some nutrients, reduction of the digestive process, or metabolic utilisation of feed) that have an effect contrary to optimal nutrition. Anti-nutrients are most concentrated in grains, beans, legumes, and nuts, but they can also be found in the leaves, roots, and fruits of other plant species. Plant-based foods contain a variety of antinutrients, including phytates, tannins, lectins, and oxalates. Anti-nutrients in vegetables, whole grains, legumes, and nuts are of concern when a person's diet consists mainly of unprocessed plant foods.

The ability of lectins to bind to glycoprotein receptors on epithelial cells that line the intestinal mucosa hinders growth by interfering with nutrition absorption. Phytic acid can reduce mineral availability. Some phenolic compounds can additionally influence protein digestibility and mineral bioavailability [4]. For example, oxalate binds to calcium to produce complexes (calcium oxalate crystals) which restrict calcium absorption by the body, resulting in disorders such as rickets osteomalacia [4].

Phytochemicals are bioactive substances found in fruits, vegetables, cereal grains, and organic beverages such as tea and wine. Phytochemical intake is linked to a lower risk of multiple kinds of chronic diseases due to their antioxidant as well as free radical scavenging properties [5].

Plants are a rich source of organic antioxidant compounds, which are frequently utilized in nutritional supplements. Based on their mechanism of behavior, the association between the consumption of foods rich in antioxidants and the alleviation of oxidative damage-related conditions has become a significant area of study in the food and medicine disciplines.

Antioxidants can be classified in several ways. Enzymatic and non-enzymatic antioxidants are distinguished by their activity. Enzymatic antioxidants function by breaking down and eliminating free radicals. The antioxidant enzymes convert harmful oxidative products to hydrogen peroxide (H_2O_2) and then to water in a multi-step process requiring cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants inhibit free radical chain reactions. Vitamin C, vitamin E, carotenoids, and other antioxidants are few examples of non-enzymatic antioxidants [6]. Thus, this study's objective is to assess how dehusking and aqueous soaking impact Jack bean (*Canavalia ensiformis*) seeds.

Materials and Methods

Collecting and preparation of the sample

Canavalia ensiformis, (jack beans) seeds were collected from a local farm in Akure, Ondo State, Nigeria in March 2022. After being removed the pods, the seeds of *Canavalia ensiformis* were air dried for a few days and then separated into three groups: raw whole seeds, raw dehusked seeds, and aqueous soaking dehusked seeds (1: 4 beans, water) at room temperature for approximately two hours in order to facilitate dehusking. At 400 °C, the soaked and dehusked seeds were oven dried for an entire night. Subsequently, the seeds were separately processed into powder: raw whole seeds, raw dehusked seeds, and soaked dehusked seeds. Each pulverised seed weighed 20 grammes. 100 mL of distilled water was added and the mixture was shaken lightly overnight so as to extract it effectively. The resultant mixtures were filtered by means of muslin cloth. The powdered raw whole, raw dehusked, and soaked dehusked samples underwent anti-nutrient treatment, and the filtrates of the powdered seed samples were used for phytochemical and antioxidant analyses.

Anti-nutrient analyses

Measurement of Phytate

Phytate was calculated using Wheeler and Ferrel's technique [7]. After soaking a 4 g sample for 3 hours in 100 ml of 2% HCl, it was filtered through No. 1 Whatman filter paper. A conical flask was filled with 25 ml of the filtrate after 5.0 ml of 0.3% ammonium thiocyanate solution was added as an indicator. Afterwards, 53.5mL of purified water were added to give it the optimum acidity, and this was titrated against a standard iron (III) chloride solution containing 0.00566 g/mL, which had around 0.00195 g of iron per millilitre, until a persistent brownish-yellow hue lasted for five minutes.

Quantification of oxalate

For the purpose of determining the oxalate content, 1g of the sample was soaked in 75 ml of 1.5 N H_2SO_4 for an hour, use No. 1 Whatman filter paper to filter through. 25 ml of the filtrate was removed, and it was placed inside a conical flask and then titrated hot (between 80 and 90 °C) against 0.1mM $KMnO_4$ until a pink color persisted for 15 seconds [8].

Determination of Lectins

Measuring 1.0 g of each seed sample, 9ml of Normal Saline (0.9 g of NaCl in 100 ml of H_2O) was added, and the mixture was left for an hour. 200 mL was pipetted out of each sample, and then 2mL of reagent C (the Lowry method) and 5 mL of the follin reagent were added, respectively, and kept for an hour. At 700 nm, the samples' absorbency was measured.

Qualitative phytochemical analyses

Test for terpenoids

After filtering 0.5 ml of the extract and 20 ml of chloroform, 3ml of concentrated H_2SO_4 was added to the filtrate to create a layer. At the interface, a reddish-brown hue was noticed, indicating the presence of terpenoids.

Test for alkaloids

On a steam water bath, 0.5 ml of the extract and 5ml of 1% aqueous HCl were mixed. 1 ml of the filtrate was treated with a few drops of the Dragendorff reagent. Blue-black turbidity was considered an initial measure an indication of the presence of alkaloids.

Test for saponins.

In order to produce foaming, which indicates the presence of saponins, 5mL of seed extract and 20mL of water were combined and shaken briskly.

Test for tannins

100 mL of distilled water was mixed with 0.5mL of the extract, filtered, and then the filtrate was mixed with ferric chloride reagent. The precipitate's blue-green color was interpreted as tannin present.

Test for phlobatannins

Phlobatannins were determined to be present based on the red precipitate that formed when 0.5 mL of the extract was heated in 1% aqueous HCl.

Test for anthraquinone

The anthraquinone test was performed using Borntrager's method. 10 ml of benzene and 0.5 ml of the extract were agitated, filtered, and 5 ml of a 10% ammonia solution was included into the filtrate. After shaking the mixture, the ammonia layer's violet color was observed, indicating the existence of free anthraquinone.

Test for flavonoids

After 5.0 mL of the extract was mixed with 20 mL of diluted ammonia solution, a yellow coloration was

observed. The addition of 1 mL of strong H_2SO_4 caused the yellow coloration to disappear, indicating the presence of flavonoids.

Test for steroids
After filtering 0.5 ml of the extract with 20 ml of acetic anhydride, 2 ml of concentrated H_2SO_4 was added to the filtrate. Green instead of violet was the color that changed, signifying the presence of steroids.

Test for Cardiac Glycosides: To test for cardiac glycosides, the following procedures were used.

1. **Legal's test:** After dissolving the extract in pyridine, small amount of 20% NaOH was added along with a few drops of 2% sodium nitroprusside. The presence of cardenolides is indicated by a deep red hue that gradually fades to a brownish yellow color.
2. **Lieberman's test:** 0.5 ml of the extract was mixed with 20 ml of acetic anhydride, filtered, and then 2 ml of concentrated H_2SO_4 was added to the filtrate. The existence of steroids nucleolus was indicated by a colour change from violet to blue (i.e. a glycone portion of the cardiac glycosides).
3. **Salkowski's test:** 3 ml of concentrated H_2SO_4 was added to the filtrate to create a layer after 0.5 ml of the extract and 20 ml of chloroform were mixed and filtered. There was an apparent reddish-brown coloration at the interface, indicating the presence of a steroidal ring.
4. **Keller-Kaliani test:** 0.5 ml of 2mL of glacial acetic acid with one drop of ferric chloride solution was used to dissolve the extract. After adding 1mL of concentrated sulfuric acid as an underlay, a brown hue was observed at the interface, signifying the presence of a deoxy-sugar, which is expected of Cardenolides.

Quantitative Assessment of some Phytochemicals
Determination of Cardiac Glycosides
The technique proposed by Sofowora [9] was applied. A 250 ml conical flask was pipetted with 10 ml of the extract. After adding 50 ml of chloroform, the vortex mixer was agitated for an hour. The blend was strainated into a 100 mL conical flask. After adding 10 ml of pyridine and 2 ml of 29% sodium nitroprusside, the mixture was well shaken for 10min. To get a brownish yellow color (Glycosides standard (Digitoxin), 3 mL of 20% NaOH were added. A concentration that which were made from stock solution and had an absorbance range of 0 to 50 mg/ml; the absorbance was measured at 510 nm.

Determination of Terpenoids
The procedure proposed by Sofowora [9] was applied. A 50 ml conical flask containing 0.5 g of finely ground material was filled with 20 ml of chloroform: methanol (2:1), shaken well, and left to stand at room temperature for 15 mins. At 3000 rpm, the suspension was centrifuged. After discarding the supernatant, the precipitate was subsequently washed using 20 mL of a 2:1 chloroform: methanol solution and centrifuged once more. A 10% sodium dodecyl sulphate (SDS) solution in 40 mL was used to dissolve the precipitate. After adding 1ml of 0.01 M Ferric chloride, the mixture was let to stand for a period of 30 mins, and the absorbance at 510 nm was measured. The terpenoid (alpha terpineol) concentration working standard from the standard solution, ranging from 0-5 mg/ml.

Determination of Saponins
Saponin was determined using Brunner *et al*.'s spectrophotometric technique [10]. A 250 ml beaker was filled with 2 g of the finely ground material and 100 ml of isobutyl alcohol, often known as But-2-ol. To ensure uniform mixing, the mixture was shaken for 5hours using a shaker. Using No. 1 Whatman filter paper, the mixture was filtered into a 100 ml beaker containing 20ml of a 40% saturated magnesium carbonate ($MgCO_3$) solution. To get a clear, colorless solution, the combination was filtered through No. 1 Whatman filter paper. Using a pipette, 1ml of the colorless solution was transferred into a 50 ml volumetric flask. After adding 2 ml of 5% iron (III) chloride ($FeCl_3$) solution, distilled water was used for preparing the remaining amount. To allow the colour to develop, it was left to stand for 30 mins. At 380 nm, the absorbance was measured in relation to a blank.

Determination of alkaloids
200ml of 10% acetic acid in ethanol was added to a 250 ml beaker containing 5g of the sample, which was then weighed, allowed to stand for 4mins, and then filtered. On a water bath, the extract was concentrated to a quarter of its initial volume. The extract was gradually mixed with concentrated ammonium hydroxide until the precipitation was fully formed. After leaving the entire mixture to settle, the precipitate was gathered, cleaned with diluted ammonium hydroxide, and filtered. Next, the residue was alkaloid that was weighed after drying. Harbone [11].

Determination of steroids
The number of steroids was measured by weighing 5g of the finely milled sample into a 100 ml conical flask, adding 50ml of pyridine, shaking it for 30mins at room temperature, adding 3 ml of 250 mg / ml metallic copper powder, or copper (1) oxide, and letting it sit in the dark for 1hr. After that, the absorbance was measured at 350nm against the reagent blank [9] Sofowora.

Determination of tannins
A 50ml sample container was filled with 0.2 g of the finely ground sample. A full 10ml of 70% aqueous acetone was added and sealed. The bottle was placed in an ice bath shaker and allowed to shake at 300 ºC for 2 hrs. After centrifuging each solution, the supernatant was frozen in ice.
After pipetting 0.2 ml of each solution into the test tube, 0.8ml of distilled water was added. Standard tannin acid solutions was created by adding distilled water to 0.5mg/ml of the stock to make 1ml of the solution. Both the sample and the standard were mixed with 0.5 ml of Folin-Ciocateau reagent and 2.5 ml of 20% Na_2CO_3. After vortexing the solution, it was left to incubate at room temperature for 40mins. The concentration of the sample solution was determined using a standard tannin acid calibration curve, and the absorbance of the solution was determined at 725nm in comparison to a reagent blank [12].
A few *in vitro* evaluations of the seed samples' antioxidant content.

Determination of DPPH free radical scavenging ability
The capacity of 1, 1-diphenyl-2-picryhydrazyl (DPPH) to

scavenge free radicals. The extract was determined with Gyamfi *et al*.'s modified methodology [13].

1.0 ml of the extracts at three different concentrations (20, 40, and 80 mg/ml) was added to the appropriate test tubes. The samples were mixed with 1.0ml of a 0.4mM methanolic DPPH solution. Before the absorbance at 516nm was measured, these samples were vortexed and allowed to sit at room temperature for 30mins in the dark. A decreased sample absorbance suggests that the DPPH free radical scavenging capacity is present.

Determination of Nitric oxide (NO) radical scavenging ability

Utilising modified techniques from Jagetia and Baliga [14], the Nitric oxide radical was ascertained capacity for scavenging. Sodium Nitroprusside in physiological pH 7.0 aqueous solution produces NO on its own, which combines with oxygen to create nitrite ions, which may be measured using 1.0ml of Greiss reagent (0.33% produced in 20% glacial acetic acid, room temperature for a period of 5mins, and 1ml of 0.1% w/v naphthylethylenediamine dichloride). Because nitric oxide scavengers compete with oxygen for available oxygen, nitric oxide synthesis is decreased. The extract was combined with 5mM sodium nitroprusside in phosphate-saline and incubated for 150mins at 25ºC. The reaction mixture was then added to the Greiss reagent, and the absorbance at 546nm was measured in relation to the absorbance of a reference potassium nitrate solution that had undergone the same treatment with the Greiss reagent [14].

ABTS scavenging ability

2, 2'-azino-bis-3-ethylbenthiazoline-6-sulphonic acid (ABTS) scavenging ability: Re *et al*. [15] method was used to test the extract's ABTS scavenging ability. The ABTS was generated by combining a 7mM ABTS aqueous solution with 2.45 mM/l of $K_2S_2O_8$ (final concentration) and leaving it to react in the dark for 16hours. Then, use ethanol to raise the absorbance at 734 nm to 0.700. After 15 mins, the absorbance at 732 nm was measured after 0.2 ml of the extract was added to 2.0ml of ABTS solution. subsequently, the antioxidant capacity corresponding to TROLOX was estimated.

Peroxidation of lipids

Rats were given a little ether anaesthesia before their heads were chopped off, and the liver tissues were quickly removed, chilled, and weighed. The tissues were homogenised right away in a cold solution of 50mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged at 4000 grams for 10 mins to produce a low-speed supernatant and a pellet that was discarded. A 100 ml supernatant aliquot was incubated at 37 °C for one hour and the total amount of sodium nitroprusside (SNP) was 3mM in the presence of extracts, both with and without pro-oxidants. This method was employed to determine lipid peroxidation. With the exception of the pH 3.4 buffer used for the colour reaction, the production of thiobarbituric acid reactive species (TBARS) was measured as stated by Ohkawa *et al*. [16]. 500 ml of acetic acid/HCl (pH 3.4) and 500 ml of 0.8% thiobarbituric acid (TBA) were added sequentially to the supernatant to generate the colour response after 300 ml of 8.1% Sodium Dodecyl Sulphate (SDS) was added. An hour was spent incubating this combination at 95^0C. In order to compare the absorbance of the TBARS produced with the controls, measurements were taken at 532 nm.

Absorbance Capacity of Hydroxyl (OH) Radicals.

Deoxyribose's breakdown caused by Fe^{2+} and H_2O_2 was prevented by the extract was conducted utilising Halliwell and Gutteridge's approach [17]. A reaction mixture comprising 120 µl, 20 mM deoxyribose, 400 µl, 0.1 M phosphate buffer pH 7.4, 40 µl, 20 mM hydrogen peroxide, and 40 µl, 500 µM $FeSO_4$ was supplemented with freshly produced extract (0-100 µl). The volume was then increased to 800 µl using distilled water. After 30 minutes of incubation at 37 °C, the reaction was halted by adding 0.5 ml of 2.8% trichloroacetic acid (TCA) to the reaction mixture. The addition of 0.4 ml of a 0.6% TBA (thiobarbituric acid) solution came next. After that, the tubes were incubated for 20 minutes in boiling water. In a spectrophotometer, the absorbance was measured at 532 nm.

Ferric Reducing Antioxidant Power (FRAP) Determination

The altered method identified the extract's decreasing property using Pulido *et al*.'s technique [18]. The decrease of (Fe^{3+}) ferricyanide in stoichiometric excess in relation to the antioxidants served as the foundation for this procedure. The sample's methanolic extract and its different fractions (10-50µg/ml) were added in varying amounts to 1.0 ml/0.25 ml of 200 mM sodium phosphate buffer pH 6.6 and 1.0 ml/0.25 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was left to incubate for 20 minutes at 50 °C. After that, 1.0 ml/0.25 ml of freshly made 10% Trichloroacetic Acid (TCA) was added, and the mixture was centrifuged for 10 minutes at 2000 rpm. The supernatant was then combined with 1.0 ml of distilled water and 0.25 ml of 0.1% $FeCl_3$ solution. With the exception of the 0.1% potassium ferricyanide, all other reagents existed in the control solution, which was collapsed using distilled water without the test sample. Absorption of these Spectrophotometer measurements were made of mixtures at 700 nm. Reduced absorbance suggests that the sample has the ability to reduce ferric iron.

Determination of Fe^{2+}Chelation ability of the seed aqueous extract

Puntel *et al*. [20] adopted a modified version of Minotti and Aust [19] to assess the extract's capacity to chelate Fe^{2+}. A reaction mixture comprising 168 μL of 0.1 M Tris-HCl (pH 7.4), 218μL saline, and the various quantities of extracts (0-25 μL) was further enriched with freshly made 500 μM $FeSO_4$ (150 μL). Thirteen microliters of 0.25% (w/v) 1, 10-phenanthroline were added to the reaction mixture after it had been incubated for 5 minutes. After that, the absorbance was measured in a spectrophotometer at 510 nm.

Quantification of the Sample's Total Phenolic and Flavonoid Contents

Calculating the Whole Phenolic Content

The concentration of extractable phenol was calculated on the extracts using the Singleton *et al*. [21] technique. A mixture of 2.0 ml of 7.5% sodium carbonate and 2.5 ml/1.5 ml of 10% Folin-Ciocalteau's reagent was combined with 0.2 ml of the extract. Garlic acid was employed as the standard phenol, and the reaction mixture was then incubated at 45 °C for 40 minutes. The absorbance was then measured at 700 nm in the spectrophotometer.

Determination of Total Flavonoid: Bao's colorimeter

assay was used to calculate the extract's total flavonoid content [22]. At zero-time, 0.2 ml of the extract was mixed with 0.3 ml of 5% $NaNO_3$. 0.6 ml of 10% $AlCl_3$ was added after 5 minutes, and after 6 minutes, 2.0 ml of a 1M NaOH solution and 2.1 ml of distilled water were added to the mixture. The capacity to absorb was compared to the reagent blank at 510 nm, and the flavonoid content was reported in milligrammes of gallic acid equivalent.

Ascorbic acid determination with the 2, 4-dinitrophenylydrazine (DNPH) reagent: The modified spectrophotometric technique outlined by Roe and Keuther [23] was used to analyse ascorbic acid. Sample preparation involved homogenising 10 grammes of powdered material with roughly 50 millilitres of a 10% acetic acid solution. Once a homogenous dispersion was achieved, it was quantitatively placed into a 100 ml volumetric flask and gently shaken. The 10% acetic acid solution was then added to dilute it to the appropriate level. After that, the mixture was filtered, and the clear filtrate was saved so that the amount of vitamin C in that sample could be measured.

Estimation of vitamin C

To the filtered sample solution's 0.5 and 1.0ml a few drops of bromine water were added to separate standard volumetric flasks until the solution took on colour, signifying that the ascorbic acid's oxidation to dehydro-ascorbic acid had been completed. To get rid of the excess bromine, a few drops of 10% thiourea solution was added and the clear solution was the result. After adding and thoroughly mixing 0.5 ml of 2, 4-dinitrophenyl hydrazine (DNPH) reagent (2% in 9N H_2SO_4) with the oxidised ascorbic acid, the solution was topped off with 10% acetic acid solution to reach the 2.0 ml threshold. Volumes of 0.2-1.0 ml standard ascorbic acid (2 mg/ml) solution were handled in the same way as the samples. A spectrophotometer was used to measure the absorbance at 520 nm. The standard was used to calculate the ascorbic acid concentration in the sample calibration curve expressed in mg/g of sample and in linear regression mode.

Results

Table 1: *Canavalia ensiformis* seed composition of several anti-nutrients

Parameters	Raw whole seed	Raw dehusked seed	Raw Seed husk	Soaked dehusked seed	Soaked Seed husk
Lectins mg/g	6.67±0.04	6.42±0.07	2.48±0.02	6.20±0.01	1.82±0.00
Phytate mg/g	23.48±0.24	15.24±0.41	4.12±0.00	14.83±0.00	3.30±0.00
Tannins mg/g	22.77±0.73	18.68±0.03	14.82±0.22	17.50±0.46	14.47±0.08
Oxalate mg/g	4.32±0.09	3.96±0.09	1.26±0.00	2.88±0.09	0.86±0.06

Table 2: Phytochemical screening of the aqueous extracts of *Canavalia ensiformis*

Parameters	Raw whole seed	Raw dehusked seed	Raw Seed Husk	Soaked dehusked seed	Soaked Seed husk
Saponins	++	++	++	++	+
Tannins	+	+	+	+	+
Phlobatannins	ND	ND	+	ND	+
Steroids	++	+++	+	+++	+
Flavonoids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Alkaloids	++	++	+	+	+
Anthraquinones	ND	ND	ND	ND	ND
Cardiac glycosides					
Legal test	+	+	+	+	+
Keller kiliani test	+	+	+	+	+
Salkwoski test	+	+	+	+	+
Lieberman test	+	+	+	+	+

Table 3: Some Phytochemical compositions of seed of *Canavalia ensiformis*

Parameters	Raw whole seed	Raw dehusked seed	Raw Seed Husk	Soaked dehusked seed	Soaked Seed husk
Saponins mg/g	60.27±0.82	57.18±0.64	59.00±0.82	56.65±0.45	49.09±0.18
Terpenoids mg/g	31.95±0.02	26.41±0.04	16.65±0.03	20.29±0.02	14.20±0.03
Alkaloids %	23.25±0.03	18.05±0.05	8.88±0.03	12.30±0.02	6.58±0.03
Glycosides mg/g	33.73±0.03	27.04±0.03	15.24±0.04	19.65±0.03	12.28±0.02
Steroids mg/g	9.65±0.01	6.67±0.01	4.89±0.01	3.70±0.01	2.35±0.01

Table 4: Some antioxidant properties of seed of *Canavalia ensiformis*

Parameters	Raw whole seed	Raw dehusked seed	Raw Seed Husk	Soaked dehusked seed	Soaked Seed husk
Flavonoids mg/g	0.36±0.01	0.21±0.01	0.20±0.00	0.14±0.00	0.12±0.00
Total phenols mg/g	15.20±0.50	13.87±0.08	12.42±0.08	12.12±0.04	6.67±0.27
FRAP mg/g	26.81±0.01	26.23±0.11	19.94±0.14	19.12±0.16	11.20±0.55
Vit. C mg/g	18.18±0.01	14.19±0.91	9.91±0.24	11.68±0.55	7.12±0.48

Table 5: Percentage free radical scavenging ability of seed of *Canavalia ensiformis*

Parameters	Raw whole seed	Raw dehusked seed	Raw Seed Husk	Soaked dehusked seed	Soaked Seed husk
TBARS %	190.78±0.46	149.78±0.49	113.59±0.97	100.82±0.68	91.75±0.49
Fe^{2+}Chelation %	73.42±0.51	73.00±0.61	65.75±0.41	68.17±0.13	50.63±0.29
DPPH %	75.22±0.01	74.40±0.06	48.11±0.02	65.20±0.06	19.36±0.08
NO Radical %	67.48±0.46	54.63±0.38	17.97±0.24	27.07±0.08	6.02±0.60
OH Radical %	64.95±0.34	58.41±0.33	23.26±0.80	56.07±0.67	2.34±0.16
ABTS %	88.99±0.02	88.05±0.01	70.60±0.05	83.36±0.03	76.78±0.07

Discussion

Due to the presence of epicarp in the samples, the quantitative estimation of the anti-nutrients of *Canavalia ensiformis* (Table 1) revealed that the raw whole seed for lectins has the highest value than raw dehusked seed and soaked dehusked seed, which is consistent with other researchers Oseni *et al.* [3]. The raw whole seed had a greater phytate value (23.48±0.24 - 14.83±0.00 mg/g) than the other samples. The value range was lower than the 37.7 mg/g to 29.0 mg/g range value for dry cowpeas (*Vigna unguiculata*), a legume family, published by Schlemmer *et al*., [24]. The value decreased to 15.24±0.41 after being dehusked, and it decreased even more to 14.83±0.00 after being soaked. This suggests that both dehusking and soaking decreased the phytate concentration, which is consistent with the report from Ogbonna *et al*., [25] and Oseni *et al*., [3]. Following dehusking and soaking, the values of lectins and oxalate (6.67± 0.04 mg/g and 4.32± 0.09 mg/g), respectively, decreased as shown in Table 1. Because of the seed coat, the raw whole seed had a high tannin value of 22.77±0.73 mg/g; this value decreased to 18.68±0.03 mg/g in raw dehusked seed and further decreased to 17.50±0.46 mg/g in soaked dehusked seed. One of the main evidences that the *Canavalia ensiformis* seed has potent anti-nutrient properties is the high level of tannin present in the raw whole seed. The finding by Morteza and Jamuna [26] that tannin can be neutralised or abolished by dehusking or soaking is supported by the lowered tannin level. The factor can chelate divalent and trivalent cations including Zn^{2+}, Fe^{2+}, Ca^{2+}, and Mg^{2+} and can bind to proteins to block their absorption [3].

The astringent quality of high tannin diets has been attributed to their therapeutic efficacy. When present in excess and unrestricted, they cause irritation to the mucosa. When used in modest amounts, they precipitate a small quantity of proteins in the mucosa's cells, making them impermeable and preventing further irritants from getting to the deeper layers of the damaged mucosa, which helps the healing process [27]. Because some plant tannins have been shown to have hypoglycaemic effects [27], the fact that *Canavalia ensiformis* seeds (Tables 1 and 2) contain tannins supports the plant's usage as an antidiabetic. Additionally, it has demonstrated possible antibacterial, antiviral, and antiparasitic properties [28].

The values of phytate, oxalate, and tannin found in this research are higher than the results obtained in *Canavalia ensiformis* in another site as reported by Usman *et al*., [29]. Due to water leaching the anti-nutrients, there were also decreases in the values of raw seed husk compared to soaked seed husk. The reduction in anti-nutrient levels in this study after soaking for both raw seed and the husk can be attributed to the water-soluble nature of many anti-nutrients, which facilitates their leaching out from foods [30]. Additionally, it activates an inbuilt enzyme called phytase, which lowers the amount of phytate and other anti-nutrients and makes other process easier such as heating or cooking (shortening the cooking time), improving nutritional value and digestibility [31].

Tannins, flavonoids, alkaloids, saponins, steroids, terpenoids, and cardiac glycosides were detected by phytochemical screening of *Canavalia ensiformis* seeds (Table 2), however the anthraquinones test by Bontrager's was negative. Leaching of water-soluble vitamins and minerals from legumes was the reason for the lower values of phytochemical compositions for saponins, terpenoids, alkaloids, glycosides, and steroids in the soaking dehusked seed compared to the raw whole seed [25, 32]. It has been reported that these chemical compounds exhibit pharmacological effects. As a result of their ability to inactivate some nutrients under specific circumstances, such as large quantities, some of them such as tannins, alkaloids, saponins, lectins, oxalates, etc.; are referred to as anti-nutritional factors [27]. For instance, legumes' lectins can obstruct the absorption of calcium, iron, zinc, and Phosphorus. Increased phenolic compounds would reduce the bioavailability of amino acids, cause weight loss, appetite loss, respiratory issues, and heart complications. Oxalates can bond with calcium and prevent it from being absorbed [24, 33, 34].

The samples' presence of cardiac glycosides (Table 3) showed that the soaked dehusked seed had the lowest amount when compared to the raw dehusked seed and raw whole seed. The same pattern was shown by terpenoids, alkaloids, and steroids in Table 3. A class of triterpenoids is known as cardiac glycosides. Although most have pharmacological activity, they are hazardous or toxic. These are the main ingredients that are active in cardiovascular medication [27]. Terpenoids from plants are often used for their aromatic properties. They play an essential role in traditional herbal remedies.

Alkaloids, the biggest single family of secondary plants, are a heterogeneous group with different chemical structures. The chemicals are frequently harmful to humans and cattle, but many have strong pharmacological activity, resulting in their widespread use in medicine [27]. Some alkaloids from *Trigonella Foenum-graecum* (Ferugreek) seeds have been shown to lower blood sugar, cholesterol, and triacylglycerols [27], all of which are often increased in diabetes mellitus. The presence of alkaloids (Table 3) in *Canavalia ensiformis* may explain its application in diabetic therapy. Some of the toxicological symptoms of potato glycoalkaloids involve gastrointestinal upsets and neurological abnormalities, especially at dosages that exceed 0.2 mg/g sample [3], and the level of alkaloids in *Canavalia ensiformis* for raw whole seed, raw dehusked seed and soaked dehusked seed were significantly greater. Saponins' nutritional relevance stems from their hypocholesterolemic activity, which suggests that they may be useful in the prevention and treatment of cardiovascular disease in humans [27]. *Canavalia ensiformis* seed contains

high levels of saponin (Table 3), which supports its usage as an antidiabetic medication. Nimenibo-Uadia [35] investigated the antihypercholesterol and antihypertriacylglycerol properties of an aqueous extract of *Canavalia ensiformis* on diabetic rats.

Steroids in plants have a chemical composition similar to cholesterol and are hypothesised to reduce cholesterol absorption in the intestine. The decrease in cholesterol absorption improves the liver's uptake of LDL cholesterol, and as a result, it can lower blood LDL cholesterol levels without changing the levels of HDL cholesterol or triglycerides. The presence of steroids in *Canavalia ensiformis* demonstrates their efficacy as antihypercholesterol and antihypertriacylglycerol. Plant sterols are the most effective natural way to manage high cholesterol. HDL is beneficial cholesterol because it helps the body eliminate LDL (Low Density Lipoprotein), often known as bad cholesterol (HDL of LDL cholesterol can double the risk of having a heart attack).

Table 4 shows the effects of dehusking and soaking on the total phenol, flavonoids, vitamin C, and ferric reducing antioxidant power (FRAP) of *Canavalia ensiformis*. The parameters revealed a decrease after dehusking and soaking treatment. For example, the data for total phenol demonstrated a decrease from raw whole seed to raw dehusked seed to soaked dehusked seed, which was the trend for the other parameters as well. On this premise, the decrease in total phenols, flavonoids, FRAP, and vitamin C content of *Canavalia ensiformis* (jack beans) after soaking suggests that the components were lost in the soaked water. The results were consistent with those obtained by Eshraq *et al*. [36], who found a higher drop in the content of total phenols and flavonoids.

The raw whole seed of *Canavalia ensiformis* has the highest DPPH antioxidant activity compared to the dehusked and soaked samples (Table 5). The same trend was observed in TBARS, Fe^{2+} chelation, NO radical, OH radical, and ABTS. Both raw and soaked husk had high antioxidant activity, however raw husk had higher values than soaked husk.

Conclusion

The results of the current investigation revealed that the seed of this underutilised legume (*Canavalia ensiformis*) is a valuable nutritional supplement for human use as well as animal feed composition. The study's phytochemical screening revealed the presence of pharmacologically active chemicals, indicating their potential use as an anti-diabetic agent, cardiovascular disease, various oxidative stress conditions, and other medicinal uses. The study found that the seed of *Canavalia ensiformis* contains an important antioxidant component with excellent scavenging capacities (ABTS, DPPH, Fe^{2+} chelation, Nitric Oxides, Hydroxyl, and TBARS radicals). Though this species of bean contains strong antinutritional components and is difficult to prepare for food. As a result, dehusking and soaking *Canavalia ensiformis* seeds are key processing strategies for lowering anti-nutrients and improving the nutritional properties and chemical makeup of this underutilised seed.

According to the findings of this study, additional research is needed using alternative procedures to reduce anti-nutrients to the lowest or zero level while perfectly improving the nutritional value/status. Individual phenolic compounds in the examined legume must be structurally elucidated, isolated, identified, and functional groups, as well as analysed *in vivo*, to support the mechanism of their antioxidant effect.

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Conflict of Interest

The Author declares no conflict of interest exists.

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