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## Effects of processing techniques on phytochemical content and nutritional composition of *Entada gigas* seeds

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### Abstract

The effects of different processing methods (soaking, cooking and autoclaving) on the nutritional, phytochemical and anti-nutritional content of *Entada gigas* (cacao) seeds were investigated. The nutritional composition of the unprocessed *E. gigas* seeds corresponds with most edible legumes containing: carbohydrate (54.5%), crude protein (21.41%), fibre (5.29%), crude fat (4.96%), moisture (5.85%) and ash (2.74%). Essential minerals including calcium (84.87 mg/L), iron (3.24 mg/L), potassium (793 mg/L), magnesium (112 mg/L), sodium (7.24 mg/L) and zinc (1.49 mg/L) were also detected. Flavonoids, glycosides, steroids, terpenoids, saponins, tannins and phenols were among the phytochemicals present in the seeds. The most effective method of reducing the anti-nutritional constituents, while still preserving the beneficial compounds, was soaking the seeds for 21 days. Due to the high nutritive values, utilization of *E. gigas* is encouraged provided that an appropriate processing method is used to reduce the anti-nutrient content.

**Keywords:** Nutrients, *Entada gigas* seeds, mineral, phytochemical, anti-nutritional factor

### 1. Introduction

Much of early traditional medicine relied on specific plants and herbal treatments and to date, a large number of individuals, particularly in under-developed and developing countries still rely on folklore medicine to satisfy their primary health needs. This is not surprising, given that several active constituents of conventional drugs were first discovered and extracted from plants. These discoveries have led to the production of chemically synthesized drugs that have revolutionized global healthcare systems. Epidemiological evidence has established that there is a strong association between poor nutrition and health problems. However, the consumption of plant based foods (diet rich in vegetables, fruits and grains) has been linked to disease prevention, chiefly because the health benefits gained from food are not solely due to the nutritional content, but may be attributed to the presence of valuable phytochemicals. Phytochemicals, also referred to as secondary metabolites, are defined as biologically active natural and non-nutritive compounds produced by plants that provide protection against pathogenic and environmental stressors [1]. Given that the medicinal value of many plants lies in the chemical substances that they produce, phytochemical screening is considered as a prerequisite in assessing plants for the establishment of new drugs, cosmetics and nutraceuticals, one such group of plants that has been evaluated for their phytochemical properties, is the legumes. These plants are excellent sources of inexpensive and widely available protein, dietary fibre, and a variety of micronutrients and bioactive compounds. In Jamaica, the use of traditional therapies using plant material is deeply entrenched. During the eighteenth century, an indigenous group known as the Maroons, relied heavily on the use of herbal medicines for the treatment of various ailments, as that was the primary resource available to them. Consequently, they became skilled at using plants for medicinal purposes. One of the well-known historical maroon settlements that currently exist is Accompong Town, found in the hills of St. Elizabeth, in western Jamaica. Presently, members of this community still utilize the available local plant resources in order to meet their medicinal and nutritional needs. One commonly used plant is *Entada gigas*. *Entada gigas* (Linn.) is a flowering woody liana that belongs to the Fabaceae family. This vine is widely distributed along rivers and estuaries of tropical and subtropical regions of Central America, the Caribbean, northern South America and Africa. This is due to its sea drifting seeds that possess a thick and durable seed coat. In Nigeria, the seed is used in the preparation of traditional concoctions for curing diseases such as gastrointestinal disorders, especially diarrhoea and ulcer; but, it is believed to

be toxic in nature [2-4]. The leaves are commonly used as a traditional vegetable by members of Kenge City in Bandundu [5]. In Jamaica, the Accompong Maroons use the seeds for their nutritional and medicinal (treatment of urinary infection) value. However, the maroons consider the raw seed flesh of *E. gigas* to be poisonous, but it is rendered edible by prolonged soaking and roasting method, prior to its use as an additive to local dishes such as 'run dung.' Although information exists on the phytochemical content of *E. gigas* leaf extract, limited information could be obtained on the phytochemical content of *E. gigas* seed extract. Given that the seeds are used by the maroons in Jamaica, the purpose of this study was to evaluate the phytochemical and nutritional composition of raw and differentially processed (cooked, soaked and autoclaved) *E. gigas* seeds, with the aim of reducing the anti-nutritional compounds and to assess the effects of the processing techniques on nutritional composition. Knowledge of these plant characteristics will aid in the development of nutritious value-added products and promotion of the consumption of *E. gigas* seeds as an inexpensive food supplement.

## 2. Materials and Methods

### 2.1 Sample collection and verification

Mature *Entada gigas* seeds were obtained from Accompong Maroon village located in the hills of St. Elizabeth, Jamaica and authenticated at the Department of Life Sciences, University of the West Indies, Kingston, Jamaica

### 2.2 Preparation of samples

The seeds collected were separated into five batches (Batch 1: unprocessed; Batch 2: soaked - 24 hrs; Batch 3: soaked - 21 days; batch 4: soaked and cooked; Batch 5: autoclaved) as described below. A mortar and pestle was used to break the hard, dark brown seed coats of the seeds from each batch, to allow for removal of the inner whitish flesh (kernel).

#### 2.2.1 Unprocessed

For batch 1, the dried kernel was ground into flour using a blender (Waring commercial laboratory blender/Model 31BL91), packaged and refrigerated (at 4 °C) prior to analyses.

#### 2.2.2 Soaked

Whole seeds were divided into two different batches for soaking at 24 hrs and 21 days at room temperature, in a seed: water ratio of 1:10 (w/v). After soaking, the water was drained and the seeds were dried at 50-55 °C for 6 h in a drying oven (Fisher Econo Temp Laboratory Oven/Model 55G).

#### 2.2.3 Soaked and cooked

For the fourth batch, whole seeds were soaked in distilled water for 24 h at room temperature: water ratio of 1:10 (w/v) then cooked (on a hot plate) in distilled water (100 °C) in a seed: water ratio of 1:10 (w/v) for 20 min. The cooked seeds were rinsed with distilled water and oven-dried at 50-55 °C for 6 h.

#### 2.2.4 Autoclaved

The fifth batch of seeds was processed in an autoclave (Tuttnauer 3870EA Autoclave) at 15psi (121 °C) in distilled water at the seed: water ratio of 1:10 (w/v) for 30 min. Subsequently, the seeds were rinsed with distilled water and dried at 55 °C for 6 h in a hot air oven.

## 2.3 Qualitative Phytochemical Screening

Qualitative detection of active phytochemical constituents of *E. gigas* seed extracts was carried out using standard procedures [6-9].

## 2.4 Quantitative Phytochemical Screening

### 2.4.1 Tannins

Total tannins in the aqueous *E. gigas* seed extracts were estimated according to the indigo carmine method as outlined by Rajpal [10], with minor modifications. The tannins content of the raw and processed *E. gigas* seed samples was estimated by taking 5 g of air dried seed flour and dissolving in distilled water (100 mL). The samples were shaken on a reciprocating shaker (New Brunswick Scientific, USA) for 24 h at room temperature. After which the contents were filtered by vacuum filtration with the use of a Buchner funnel. The supernatant was collected and used for further analysis. The aqueous *E. gigas* seed extracts (1 mL) were separately added to distilled water (75 mL), followed by the addition of indigosulphonic acid solutions (2.5 mL). This was then titrated with constant stirring against potassium permanganate solution (0.0002 N) to a golden yellow colour. Titration of indigosulphonic acid (2.5 mL) in water (75 mL) facilitated the blank test. The concentration of tannin was estimated using the following relationship: 1mL of N/10 potassium permanganate is equivalent to 0.004157 g of tannin compounds calculated as tannic acid. Total tannins were expressed as tannic acid equivalent (mg/g dry *E. gigas* seed powder). Samples were analysed in triplicates.

### 2.4.2 Total phenols

The total phenol content was determined spectrophotometrically using a modified version of Folin-Ciocalteu's method with gallic acid standard, in accordance with the International Organization of Standardization (ISO) 14502-1 [11]. Aqueous ethanol *E. gigas* seed extracts were prepared using 10 fold dilutions. To each sample, 1 mL of 10% diluted Folin-Ciocalteu's reagent was added and mixed. After 5 mins, 7% (w/v) sodium bicarbonate solution (10 mL) was added and the solutions were diluted with distilled water to a final volume of 25 mL. Triplicates of each sample were measured for optical density at 750 nm after 30 mins. Duplicate solutions of gallic acid (0.2, 0.4, 0.6, 0.8, and 1.0 mL of 0.1 mg/mL) were used to prepare a standard curve. The total phenol content was expressed as gallic acid equivalents (GAE) in mg/g dry *E. gigas* seed sample.

### 2.4.3 Saponin

Saponin content was determined based on standard methods described by Obadoni & Ochuko [12]. *E. gigas* seed powder (20 g) was dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath (about 55 °C) for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. Combined extracts were reduced to 40 ml over a water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel, to which 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. n-butanol extracts (60 mL each) were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath at 90°C to allow for evaporation. After which, samples were oven dried to constant weight. All determinations were performed in triplicates and the saponin content was calculated in

percentage.

#### 2.4.4 Total flavonoids

Total flavonoid content was determined by the aluminium chloride colorimetric assay, adapted from Marinova *et al.*, [11]. Aqueous ethanol *E. gigas* seed extracts (50%) were prepared using dilutions of 1:10. An aliquot of the seed extract (1 mL) was added to a 10 mL volumetric flask containing 4 mL of distilled H<sub>2</sub>O and 0.3 ml 5% sodium nitrite. After 5 minutes, 10% aluminium chloride (0.3 ml) was added. Following 6 min, 1 M sodium hydroxide (2 ml) was added to the mixture, which was then made up to a final volume of 10mL with distilled water. The absorbance was determined at 510 nm against a prepared reagent blank. Total flavonoids were calculated from a catechin hydrate standard curve and reported as mg catechin hydrate equivalents (CAE)/g of dried weight. The linearity range of the calibration curve was 0.2 to 1 mg/ml. All determinations were performed in triplicates.

#### 2.5 Proximate analysis

Moisture, ash, crude fat, crude fibre and protein content of the samples were determined according to standard procedures of the Association of Official Analytical Chemists [13]. Crude protein content was obtained by calculation of the nitrogen

content (Kjedahl method) and multiplication of the result by the conventional factor 6.25. The carbohydrate value was calculated by difference.

#### 2.6 Mineral analysis

The seeds were evaluated for quantities of calcium (Ca), iron (Fe), lead (Pb), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), zinc (Zn) and selenium (Se) [14].

#### 2.7 Statistical analysis

Results were expressed as mean values  $\pm$  standard deviations. Data was analyzed using the Statistical Package Version 20 Software (SPSS Inc, Chicago Illinois, USA). Variation among test groups was evaluated using the one-way analysis of variance (ANOVA). *Post hoc* analysis was carried out using Duncan's multiple range test to assess the significant difference among the means ( $p < 0.05$ ). Descriptive statistics were utilized for the analysis of some data.

### 3. Results

#### 3.1 Qualitative phytochemical screening

Preliminary qualitative phytochemical screening of *E. gigas* seed extracts revealed the presence of glycosides, steroids, terpenoids, saponins, tannins and phenols (Table 1).

**Table 1:** Phytochemical constituents of *Entada gigas* seed extracts

Phytochemical	Type of test	Indications
Alkaloid	Wagners	Absent
Glycosides	Fehling's test	Present
Tannins	Braemer's test	Present
Tannins	Lead Acetate test	Present
Steroids and Terpenoids	Liebermann-Burchard's Test	Present
Steroids and Terpenoids	Salkowski's Test	Present
Phlobatannins	Hydrochloric acid	Absent
Antraquinone glycoside	Borntrager's test	Absent
Cardiac glycoside	Keller-Killani test	Absent
Saponins	Frothing	Present
Saponins	Foam test	Present
Phenol	Ferric chloride test	Present
Flavonoids	Alkaline reagent Test I	Present
Flavonoids	Alkaline reagent test II	Present
Flavonoids	Sulphuric acid	Present
Flavonoids	Zinc Hydrochloride reduction test	Present
Protein and amino acid	Ninhydrin test	Present

#### 3.2 Quantitative phytochemical screening

A range of processing methods such as soaking, cooking and autoclaving were investigated for their effects on the levels of phytochemicals in *Entada gigas* seeds (Table 2). Soaking the seeds for 21 days, (a processing method adopted from the maroons), was found to be the most effective method at significantly ( $p < 0.05$ ) reducing the levels of the various phytochemicals including the anti-nutritional components, tannin and saponin. The percentage reduction of

phytochemicals, compared to unprocessed seeds ranged from 72.9 to 97.96% for: saponin (72.9%), tannins (93.3%), total phenol (97%) and flavonoid (97.96%). The autoclaved and soaked & cooked samples both showed significantly lower phytochemical content except for saponin when compared to the unprocessed seeds. Soaking the seeds for 24 hours had no effect on the content of these phytochemicals compared to the unprocessed seeds.

**Table 2:** Quantitative phytochemical constituents of raw and differentially processed *Entada gigas* seed extracts.

Samples	Tannin (mg tannic acid eq./g)	Total Phenol (mg gallic acid eq./g)	Saponin (%)	Flavonoids (mg catechin eq./g)
Unprocessed	16.7 $\pm$ 0.46 <sup>d</sup>	40.96 $\pm$ 0.67 <sup>d</sup>	20.29 $\pm$ 1.71 <sup>b</sup>	22.56 $\pm$ 0.95 <sup>d</sup>
Soaked (21 days)	1.11 $\pm$ 0.27 <sup>a</sup>	0.84 $\pm$ 1.54 <sup>a</sup>	5.5 $\pm$ 0.45 <sup>a</sup>	0.46 $\pm$ 0.04 <sup>a</sup>
Soaked (24 hrs)	17.1 $\pm$ 0.33 <sup>d</sup>	40.10 $\pm$ 1.28 <sup>d</sup>	20.57 $\pm$ 1.47 <sup>b</sup>	22.17 $\pm$ 0.52 <sup>d</sup>
Soaked & cooked	8.7 $\pm$ 0.11 <sup>b</sup>	28.83 $\pm$ 0.63 <sup>c</sup>	19.00 $\pm$ 1.82 <sup>b</sup>	15.30 $\pm$ 0.23 <sup>c</sup>
Autoclaved	9.89 $\pm$ 0.07 <sup>c</sup>	17.15 $\pm$ 0.16 <sup>b</sup>	18.00 $\pm$ 2.36 <sup>b</sup>	13.22 $\pm$ 0.34 <sup>b</sup>

The data is expressed as mean  $\pm$  SD. Values were compared along each column. Values carrying different superscripts in each column are significantly different ( $p < 0.05$ ).

### 3.3 Proximate analysis

Assessment of the proximate composition of raw and differentially processed *Entada gigas* seed kernel (Table 3), indicated that all processing techniques resulted in reduction

in carbohydrate and ash content compared to the unprocessed seeds. Whereas, crude protein and fat content were higher in the differentially processed material; ranging from 24.3-26% and 15.9-20%, respectively.

**Table 3:** Proximate analysis of raw and differentially processed *Entada gigas* seed extracts.

Proximate Composition (%)	unprocessed seeds	Processed seeds			
		Soaked (21 days)	Soaked (24 hrs)	Soaked & cooked	Autoclaved
Moisture	5.85 ± 0.02 <sup>b</sup>	5.27 ± 0.03 <sup>b</sup>	5.69 ± 0.02 <sup>b</sup>	4.90 ± 0.05 <sup>a</sup>	4.42 ± 0.03 <sup>a</sup>
Ash	2.74 ± 0.06 <sup>e</sup>	0.42 ± 0.42 <sup>a</sup>	2.63 ± 0.03 <sup>d</sup>	1.90 ± 0.01 <sup>b</sup>	2.22 ± 0.02 <sup>c</sup>
Crude protein	21.41 ± 0.19 <sup>a</sup>	24.50 ± 0.27 <sup>b</sup>	26.01 ± 0.1 <sup>c</sup>	24.31 ± 0.25 <sup>b</sup>	25.85 ± 0.07 <sup>c</sup>
Crude lipid	14.96 ± 1.11 <sup>a</sup>	15.85 ± 1.76 <sup>a</sup>	16.26 ± 0.99 <sup>a</sup>	20.03 ± 2.29 <sup>b</sup>	18.09 ± 0.14 <sup>ab</sup>
Carbohydrate	54.54 ± 1.05 <sup>b</sup>	53.09 ± 0.65 <sup>b</sup>	49.36 ± 1.01 <sup>a</sup>	48.99 ± 2.41 <sup>a</sup>	49.41 ± 0.02 <sup>a</sup>
Crude fibre	5.29 ± 0.01	-	-	-	-

The data is expressed as mean ± SD. Values were compared along each row. Values carrying different superscripts in each row are significantly different ( $p < 0.05$ ).

### 3.4 Mineral analysis

Evaluation of the mineral composition of unprocessed *E. gigas* seeds revealed the presence of a range of minerals (Table 4) including calcium (Ca), iron (Fe), lead (Pb), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), zinc (Zn) and selenium (Se).

Of the assessed minerals, K was the most abundant (793 ± 0.01 mg/L) and Mn was the least abundant (~0.94 ± 0.11 mg/L).

**Table 4:** Mineral content of unprocessed *Entada gigas* seed.

Parameter	mg/L
Calcium (Ca)	84.87 ± 6.31
Iron (Fe)	3.24 ± 0.63
Lead (Pb)	<0.2
Potassium (K)	793 ± 1.01
Magnesium (Mg)	112 ± 0.00
Manganese (Mn)	0.94 ± 0.11
Sodium (Na)	7.24 ± 3.46
Zinc (Zn)	1.49 ± 0.16
Selenium (Se)	0.047 ± 0.006

## 4. Discussion

Since ancient times, plants have been important sources of medicine for the management of numerous diseases due to the presence of phytochemicals such as tannins, glycoside, saponins, and alkaloids among others. These medicinally active substances have definite physiological action on the human body and are usually found in high concentrations in plant storage organs [15], including those of leguminous plants like *E. gigas*. The seeds of the *Entada gigas* are consumed by the Accompong maroons and are believed to evoke various therapeutic effects. As such, preliminary phytochemical screening was conducted to determine the bioactive principles present in the seeds. This could subsequently lead to the discovery of potential pharmacologically active chemical compounds and provide justification for the plant's utilization by the maroons. Furthermore, the results could facilitate quantitative estimation of active secondary metabolites. Qualitative phytochemical screening of several extracts of *E. gigas* seed has revealed the presence of glycosides, steroids, terpenoids, saponins, tannins and phenols which may account for its medicinal value. Flavonoids and phenolic compounds are known to possess antioxidant properties, which can improve the body's health by assisting in scavenging free radicals, thus minimizing oxidative stress and decreasing the risk associated with certain diseases such as heart diseases and cancers. Additionally, plant based antioxidants are

believed to be safer than synthetic ones.

Flavonoid is the most common group of polyphenolic compounds in the human diet. The total phenolic content of unprocessed *E. gigas* seed (40.96 ± 0.67 mg GAE equivalent/g sample) was similar to that reported in soya beans (39.50 ± 1.96 mg GAE equivalent/g sample). However, the total flavonoid content was lower (54.35 ± 8.21 mg quercetin equivalent/g sample, compared to 22.56 ± 0.95 mg catechin eq./g respectively, [16]). The auto claved and soaked (24 hrs) & cooked samples both showed significantly lower phenol and flavonoid content when compared to the unprocessed seeds. However, soaking for 21 days resulted in lower levels of phenol and flavonoid levels. Studies by Akindele *et al.*, [17] suggested that the aqueous seed extract of *Entada gigas* possess gastroprotective effects possibly mediated via cytoprotective and anti-secretory activities which may be associated with the presence of flavonoids, proanthocyanidins and phenolic compounds detected in the extract. Anti-nutrients are compounds that interfere with the absorption of nutrients. The quantity of anti-nutritional compounds such as saponin and tannin of raw and processed *E. gigas* seed samples was determined. It was found that unprocessed *E. gigas* seed contained 1.67% tannin which was much higher than the levels (0.26%) previously reported [3]. Tannin are water-soluble phenolic astringents which are present in most plant-derived foods that play key roles in a wide range of applications. Tannins are known to exert physiological benefits on human health such as antihypercholesterolemic, antioxidant, antihypertensive, antidiarrheal, antitumour, anticarcinogenic and antidiabetic effects [18-20]. While tannins are beneficial, they possess anti-nutritional properties depending on the chemical structure and dosage. High levels are said to reduce feed efficiency and are not desirable for human consumption [21]. Tannins have the ability to bind to and react with proteins and other organic compounds to form stable water-insoluble co-polymers, hence its use in the production of leather. It reduces the bioavailability of proteins and amino acids during digestion, thus, consumption of foods containing high levels of tannins should be avoided [22]. Soaking for 21 days, 24 hours & cooking and autoclaving the seed samples significantly reduced the tannin content (0.11%; 0.87%; 0.98%, respectively). Currently, there is no scientifically established toxic level of tannin for human consumption. However, based on the reviewed literature on tannin composition in edible seeds, it is safe to conclude that the reported levels of tannin present in the processed *E. gigas* would have little or

no adverse effect on human health and may be beneficial to consumers. Studies have demonstrated that low doses of tannin (0.15 – 0.2%) enhanced growth performance and well-being of chickens [23]. Additionally, studies have demonstrated that the tannin content of commercially important edible nut seeds ranged between 0.01-0.88% [24]. In this study, the tannin levels of *E. gigas* were well below that reported for edible cooked walnut and faba beans (2.33% and 2.6% respectively) [25, 26].

Based on the results, *E. gigas* seeds possessed elevated saponin levels. Saponins are naturally occurring glycosides which have the ability to form stable, soap-like foams in aqueous solutions and are found in a wide variety of plants. Saponins comprise a large family of structurally diverse compounds. Many are responsible for the bitter and irritating taste of some seeds. While low doses of saponin have therapeutic properties, at high levels the compound can be harmful. Studies have shown that high levels of saponin in feed affect growth rate and feed intake in poultry [21, 27]. It can reduce the bioavailability of protein and cholesterol [28]. Other studies have found that it has haemolytic activity against erythrocytes and interferes with the digestive process [29]. Phytochemical studies on related *Entada* species revealed the presence of several types of saponins in the seeds and other parts of the plants [30, 31]. These studies indicated that the saponin structures possessed anti-inflammatory, anticarcinogenic and antioxidant properties, which could be a potential source of medicine that may result in the production of chemically synthesized drugs. Some protein rich seeds such as quinoa (*Chenopodium quinoa*), soybeans (*Glycine max*) and beans derived from *Phaseolus vulgaris*, contain high saponin levels in some varieties. The saponin content of quinoa varies between 0.1-5% and soybean varies between 0.05% to 2% (commercial varieties) and 4 – 7% (wild varieties) [32-34]. Reports suggest that the saponin content can be significantly reduced by soaking due to the leaching out of saponin into water [35].

In this study, soaking the seed samples for 21 days significantly reduced the saponin content in the *E. gigas* seed to 5.5%. Soaking for short duration (24hrs) did not completely hydrate the seed material and so, the saponin content was comparable to the unprocessed seed. Several studies have reported a reduction in saponin content of autoclaved legumes [36]. However, in this assessment, autoclaving the samples for 30 minutes did not significantly reduce the saponin content.

Nutritional composition of *E. gigas* seeds corresponds with that of most edible legumes such as kidney beans (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) containing: carbohydrate 50-55%, protein 21-26%, fat 15-20%, crude fibre 5.3%, and moisture 4.4 -5.9%. The results are similar to those found by Ogungbenle & Oyadipe indicating that, *E. gigas* could potentially be manipulated in formulations of food products [3]. Comparatively, soybeans contain about 40% protein, 35% carbohydrate and 7% fibre, which are higher than that found in *E. gigas* seeds [37]. The crude fat ( $47.00 \pm 0.03\%$ ) and protein ( $38.6 \pm 0.07\%$ ) content of *Arachis hypogaea* (peanut) are higher than *E. gigas* seed samples while crude carbohydrate ( $1.81 \pm 0.02\%$ ) and fibre ( $3.7 \pm 0.03\%$ ) content is lower [38]. Crude fibre plays the physiological role of regulating peristaltic action of the intestinal tract and plays a key role in the prevention of constipation.

It is important to know the effects of various processing methods on the availability of the nutritional components of

the seeds. Seeds soaked for 21 days had significantly lower ash content (0.42%) compared to raw seed and other processed samples (2-3%) and may be due to the leaching of both micro and macro minerals during this prolonged soaking period. Ash content provides an approximate measure of mineral and inorganic matter. The autoclaved ( $4.42 \pm 0.03\%$ ) and soaked & cooked ( $4.90 \pm 0.05\%$ ) samples had significantly lower moisture content compared to the other seed samples. Moisture provides the environment for bacterial growth which can produce undesirable changes; hence, low moisture content is desirable. Heat, acid and alcohol treatments are principal methods used to concentrate protein [39]. In this study, the processed (soaked or heated) *E. gigas* seeds had significantly higher protein content (24-26%) compared to the unprocessed ( $21.41 \pm 0.19\%$ ) seeds. The soaked (24 hrs;  $26.01 \pm 0.1\%$ ) and autoclaved ( $25.85 \pm 0.07\%$ ) samples showed significantly higher increase in protein content than the 21 days soaked ( $24.50 \pm 0.27\%$ ) and soaked and cooked ( $24.31 \pm 0.25\%$ ) *E. gigas* seed samples. *E. gigas* is also a great source of anti-oxidant micronutrients such as potassium, magnesium, calcium, iron and zinc. Potassium ( $793 \pm 1.01$  mg/L) was the most abundant mineral while Selenium ( $0.047 \pm 0.006$  mg/L) was the least abundant one.

## 5. Conclusion

*Entada gigas* seeds possess nutritive values and can serve as a cheap source of high protein, energy, as well as antioxidants and micronutrients. Among the various processing techniques employed, soaking the seeds for 21 days was the most effective in reducing the anti-nutritional compounds (saponin and tannin) without altering its nutritional profile. Soaking the seeds for a period of 21 days is very time consuming and require preparation in advance. If adequate care is not employed, prolonged soaking without consistent changing of the water can expose the food product to high levels of microbes, which can affect human health. Thus, an alternative, safe and more efficient processing technique could be explored.

Further studies are ongoing to determine a more convenient processing technique that will significantly reduce the high saponin content without affecting the nutritional quality of the seed and to assess the biological value and protein quality.

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