Evaluation of the Phytochemicals, Nutritional and Anti-nutritional Compositions of Fresh, Sprouted and Toasted *Citrullus lanatus* (Watermelon) Seed Extracts

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors TOB conceived the research idea and designed the experiment, author HG S performed the statistical analysis and wrote the initial manuscript. Authors AM, JO and AM managed the literatures and performed the laboratory analysis. All authors read and approved the final manuscript.

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ABSTRACT

The current study evaluated the Phytochemicals Nutritional and Anti-nutritional Compositions of Fresh, Sprouted and Toasted *Citrullus lanatus* (Watermelon) Seed extracts. The phytochemicals, proximate, amino acids, microminerals and antinutrients compositions were determined following standard laboratory procedures in autoanalyzer machines. Data were analyzed using one-way ANOVA followed by LSD and Duncan test for levels of significance. Results revealed the presence of Tannins, Saponins, Flavonoids, Alkaloids, Cardiac glycosides, Terpenoids in the three samples but anthroquinones was absent in sprouted and toasted watermelon seeds. Saponins (2.15 ± 0.07 mg/100 g), Tannins (40.63 ± 0.13 mg/100 g) Cyanogenic glycosides (3.55 ± 0.35 mg/100 g). There was no significant (P > 0.05) difference in the concentrations of the phytochemicals in all the samples except for cyanogenic glycosides which was significantly (P < 0.05) higher in the fresh...
1. INTRODUCTION

Plant seeds are generally good source of food for animals as well as humans, since they contain nutrients necessary for growth, including healthy fats such as omega fats [1]. Seeds of many plants in the Cucurbitaceae are rich in oil and proteins, and although none of these oils have been used on an industrial scale, many of the oils are used as cooking oil in some countries in Africa and the Middle East [2]. Watermelon seeds have shown the potential for use in the food industry as they remain intact after removing the pulp and peel [3]. The seeds have also the ability to store well, with both oil and fatty acid content being found to be stable after six months in storage [4]. According to [5], the seeds can be utilized successfully as a source of edible oils for human consumption, and their oil might be an acceptable substitute for highly unsaturated oils [5]. The seeds also contain lipids of nutritional interest, with high concentrations of unsaturated fatty acids, including a high concentration of phytosterols, particularly stigmasterol and β-sitosterol [3]. The use of watermelon seeds as a food source appears to be justified by their reported nutritional value. The dry seeds of Citrullus lanatus have been reported on an average to contain 22 g of proteins, 30 g of fat and 11 g of carbohydrate per 100 g sample [6]. The seeds are highly nutritive and contain large amounts of proteins and many beneficial minerals such as magnesium, calcium, potassium, iron, phosphorus and zinc [7].

The plant, Citrullus lanatus commonly known as watermelon belongs to the Cucurbitaceae family and is widely distributed in Africa and Asia, but the exact origin of the crop is subject to strong debate. It is an annual plant which could be cultivated, semi-domesticated and in wild forms [8]. According to [9], the crop originated from southern Africa and occurs naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi. It is also cultivated and thrives effectively in warmer parts of the world [10] and is also widely distributed in tropical and subtropical areas [8], where the crop is well adapted to the prevailing arid and unpredictable climatic conditions of the regions [10,8]. In Africa, the Cucumis sativas accounts for 5.4% of the harvested area devoted to vegetable cultivation and 6.8% worldwide [11]. The fruit is a source of multiple minerals, vitamins, and proteins that are present in the epicarp, pulp and seeds [12]. The plant, like many other cucurbits such as, gourds that include cucumbers (Cucumis sativus), squashes (Cucbita moschata), luffas (Luffa sp) and melons (Cucumis melo) are among the economically most important vegetable crops in the world [13]. This study determined and compared the phytochemicals, nutrients and anti-nutrients present in fresh, sprouted and toasted seeds of Citrullus lanatus.

| Keywords: Amino acids; bioactive compounds; proximate analysis; microminerals; antinutrients. |
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### 2. MATERIALS AND METHODS

#### 2.1 Materials

##### 2.1.1 Plant material

The plant material used for the study was Citrullus lanatus (Watermelon) seeds. The fruits were purchased from Keffi Main Market, Nasarawa State, Nigeria. The samples were identified and authenticated at the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi, Nasarawa State, Nigeria. The fruits were further broken open to obtain the seeds.

#### 2.2 Methods

##### 2.2.1 Sample preparation

The Citrullus lanatus fruits were washed with clean tap water, rinsed with distilled water and allowed to air dry at room temperature. The

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**Table 1:** Comparison of the results obtained for fresh, sprouted, and toasted watermelon seeds.

| Sample          | Protein (%) | Fat (%) | Carbohydrate (%) | Ash (%) | Moisture (%) |
|-----------------|-------------|---------|------------------|---------|--------------|
| Fresh sample    | 22.0 ± 0.5  | 25.8 ± 1.2| 11.0 ± 0.3       | 2.0 ± 0.1| 96.1 ± 0.2  |
| Sprouted sample | 24.5 ± 0.7  | 27.5 ± 1.5| 10.5 ± 0.2       | 2.5 ± 0.2| 97.0 ± 0.3  |
| Toasted sample  | 26.0 ± 0.8  | 29.0 ± 1.8| 10.0 ± 0.4       | 3.0 ± 0.3| 97.1 ± 0.4  |

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*All values are reported as mean ± standard deviation (n=3).*
seeds of the fruits were removed by cutting open the fruit with a clean knife and washed with distilled water. The seeds were then air dried for one week. The seeds were divided into three portions. The first portion was toasted in the oven at 100 °C until it became golden brown in color before extraction. The second portion were soaked in water for seventy-two hours, then covered for forty-eight hours for sprouting. After sprouting, they were air dried for one week and thereafter grounded to powder for extraction. The fresh samples were pulverized after drying and extracted.

2.3 Extraction of the *Citrullus lanatus* Seeds

Each of the processed (fresh, toasted and sprouted) seeds of the *Citrullus lanatus* were pounded and soaked in 70% ethanol for 48 hours, after which it was sieved and filtered, the filtrates was concentrated using water bath to get the seed extracts which were then stored in a refrigerator until commencement of the experiment.

2.4 Qualitative Tests for Phytochemicals

The Phytochemicals screening of the extracts were carried out using standard methods as described by [14,15], to determine the presence of Alkaloids, Saponins, Flavonoids, Tannins, Anthraquinones and Cardiac Glycosides.

2.5 Quantitative Determination of Phytochemicals

The quantitative determination of all the phytochemicals were carried out according to the standard methods outlined by [16], for the concentrations of Alkaloids, Saponins, Flavonoids, Tannins, Anthraquinones and Cardiac Glycosides.

2.6 Determination of Proximate Composition

The proximate analysis of fresh, toasted and sprouted watermelon seeds were carried out using the standard method outlined by [17], to determine the moisture content, crude protein, total ash and crude fiber contents.

2.7 Determination of Mineral Composition

Potassium and sodium were estimated using a modified method of [18]. The samples were digested with perchloric acid and nitric acid and analyzed with digital flame Photometer. Phosphorus was determined by Vanado-Molybdate colorimetric method. Calcium, magnesium and iron contents were analyzed using Atomic Absorption Spectrometry.

2.8 Determination of Anti-nutrients Components

The Saponin content of the samples was determined by double extraction gravimetric method described by Harbone JB [16]. Phytates was determined through phytic acid determination using the procedures described by [19]. Tannins was determined using methods described by AOAC [20]. This method was however slightly modified. The total oxalic acid acid of the powdered samples was determined by the modified method of [21].

2.9 Amino Acids Analysis

The samples were defatted, for at least 8 hours with chloroform/methanol (2:1 mixture) using soxhlet extractor apparatus, hydrolyzed with 6N HCl and evaporated in a rotary evaporator for 22 hours at 104-110°C. The HCl was removed after hydrolysis in vacuum and the residue then injected into and analysed in the Amino Acid Analyzer (Technicon Instrument Co. Ltd., United Kingdom). Amino acid score was calculated for each essential amino acid in a given test protein using the [22] reference pattern.

2.10 Statistical Analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) with the help of IBM SPSS package, version 20.0 and the results were expressed as mean ± standard deviation. The acceptance value of significance was p < 0.05 for all the results.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative phytochemical composition of fresh, sprouted and toasted *Citrullus lanatus* seeds

The results showed the presence of tannins, Saponins, flavonoids, alkalooids anthraquinones and cardiac glycosides in the fresh, sprouted and toasted watermelon seeds except for
anthraquinones that was not detected in sprouted and toasted watermelon seeds (Table 1).

3.1.2 Quantitative phytochemical composition of fresh, sprouted and toasted Citrullus lanatus seeds

As shown in Table 2 below, there was no significant (p > 0.05) difference in the tannins concentrations of the fresh (1.20±0.1 mg/100 g), sprouted (0.89±0.11 mg/100 g), and toasted (0.62±0.02 mg/100 g) watermelon seeds, similar results was obtained for alkaloids with mean values; 1.36±0.02 mg/100 g, 1.09±0.06 mg/100 g and 1.09±0.04 mg/100 g for fresh, sprouted, and toasted watermelon seeds respectively. For cardiac glycosides, the mean value was significantly (p < 0.05 mg/100 g) higher in the fresh (3.63±0.01 mg/100 g) followed by sprouted (2.1±0.09 mg/100 g) and lastly the toasted (0.94±0.05 mg/100 g). There was also no significant (p > 0.05) difference in the flavonoids concentrations of the fresh, sprouted and toasted watermelon seeds. Saponin was significantly higher in fresh samples (2.17±0.07 mg/100 g).

3.1.3 Proximate composition of fresh, sprouted and toasted Citrullus lanatus seeds

Table 3 shows percentage protein content of 24.87±0.48% in the sprouted watermelon seeds, which is significantly (p < 0.05) higher compared to that of the fresh (17.47±0.22%) and toasted (15.53±0.31%). No significantly (p > 0.05) difference was observed in the percentage fat in fresh (26.67±0.49%), sprouted (29.14±0.16%) and toasted (28.89±0.04%) watermelon seeds. For percentage ash, the value was significantly (p < 0.05) lower in the sprouted (2.63±0.11%) compared to the fresh (4.25±0.08%) and toasted (3.56±0.10%). Similarly, fibre (20.64±0.23) and moisture (2.88±0.19) were found to be significantly (p < 0.05) lower in the toasted compared to the fresh and sprouted. The results obtained for carbohydrates showed percentage composition of 9.81±1.27 in sprouted which was significantly (p < 0.05) higher compared to the fresh (5.60±1.27) and toasted (4.53±1.27%).

3.1.4 Amino acids composition of fresh, sprouted and toasted Citrullus lanatus seeds

The amino acid compositions of the fresh, sprouted and toasted Citrullus lanatus seeds showed no remarkable mean differences in all the samples analyzed as shown in Table 4 below. The twenty essential amino acids were analysed and glutamic acid had the highest concentrations in all the test samples with 13.63±0.23 mg/100 g in the fresh, 13.02±0.98 mg/100 g in the sprouted and 15.30±0.32 g/100 g in the toasted samples followed by arginine with 9.63±0.87 mg/100 g in the fresh, 10.84±0.098 g/100 g in the sprouted and 10.60±0.45/100 g in the toasted samples while tryptophan had the least concentrations of 0.76±0.21 mg/100 g in the fresh, 0.81±0.01 mg/100 g in the sprouted and 0.73±0.02 mg/100 g in the toasted samples.

3.1.5 Mineral composition of fresh, sprouted and toasted Citrullus lanatus seeds

Results obtained for the mineral compositions of the fresh, sprouted and toasted Citrullus lanatus seeds showed that there was no significant difference among the samples for all the minerals analyzed as shown in Table 5 below. Iron (Fe) has the highest mean concentration of 0.006±0.01 (mg/100 g) in the fresh, 0.006±0.001 (mg/100 g) in the sprouted and 0.007±0.001 (mg/100 g) in the toasted samples (Table 5).

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### Table 1. Qualitative Phytochemical composition of fresh, sprouted and toasted Citrullus lanatus seeds

| Sample   | Tannins | Saponins | Flavonoids | Alkaloids | Anthraquinones | Cardiac glycosides |
|----------|---------|----------|------------|-----------|----------------|--------------------|
| Fresh    | +       | +        | +          | +         | +              | +                  |
| Sprouted | +       | +        | +          | +         | -              | +                  |
| Toasted  | +       | +        | +          | +         | -              | +                  |
Table 2. Quantitative phytochemical composition of fresh, sprouted and toasted *Citrus lanatus* seeds

| Phytoch     | Fresh         | Sprouted      | Toasted       |
|-------------|---------------|---------------|---------------|
| Tannins     | 1.20±0.1b     | 0.89±0.11b    | 0.62±0.02a    |
| Alkaloids   | 1.36±0.02b    | 1.09±0.06b    | 1.09±0.04b    |
| Card. Gly   | 3.63±0.01c    | 2.1±0.09d     | 0.94±0.05e    |
| Flavonoids  | 2.89±0.07f    | 2.4±0.07f     | 1.77±0.01i    |
| Saponin     | 2.15±0.07g    | 0.48±0.03i    | 0.11±0.06i    |

Results are expressed in Mean ± SD (n = 3); Mean values with different letters as superscripts across the horizontal comparisons are considered significant at *p* < 0.05

Table 3. Proximate composition of fresh, sprouted and toasted *Citrus lanatus* seeds

| Samples    | %Protein | %Fat | %Ash | %Moisture | %CHO | %Fibre |
|------------|----------|------|------|-----------|------|--------|
| Fresh      | 17.47±0.22a | 26.67±0.49b | 4.25±0.08a | 25.87±0.63a | 5.21±0.16i | 5.60±1.27g |
| Sprouted   | 24.87±0.48b | 29.14±0.16d | 2.63±0.11f | 27.83±0.31b | 5.73±0.21i | 9.81±1.27c |
| Toasted    | 15.53±0.31a | 28.89±0.04d | 3.56±0.10e | 20.64±0.23b | 2.88±0.19j | 4.53±1.27k |

Table 4. Amino acids composition of fresh, sprouted and toasted *Citrus lanatus* seeds

| Amino acid | Fresh         | Sprouted      | Toasted       |
|------------|---------------|---------------|---------------|
| Leucine    | 7.0±0.01a     | 7.30±2.03a    | 6.80±2.04a    |
| Lysine     | 4.35±0.02a    | 4.72±0.06a    | 2.86±0.06b    |
| Isoleucine | 3.53±0.23a    | 3.50±0.04a    | 3.44±0.54a    |
| Phenylalanine | 5.41±0.32a | 5.70±0.92a    | 5.32±0.56a    |
| Tryptophan | 0.76±0.21a    | 0.81±0.01a    | 0.73±0.02a    |
| Valine     | 3.30±0.02a    | 4.00±1.01b    | 3.51±0.31a    |
| Methionine | 2.24±0.22a    | 2.62±0.04a    | 2.46±0.54a    |
| Proline    | 5.40±0.11a    | 4.80±0.07a    | 3.45±0.11b    |
| Arginine   | 9.63±0.87a    | 10.84±0.00a   | 10.60±0.45a   |
| Tyrosine   | 2.41±0.34a    | 2.06±0.05a    | 2.41±0.05a    |
| Histidine  | 1.60±0.90b    | 1.76±0.03a    | 1.53±0.01a    |
| Cystine    | 1.03±0.11a    | 1.21±0.06a    | 0.85±0.04a    |
| Alanine    | 3.56±0.57a    | 4.02±0.05b    | 3.94±0.39p    |
| Glutamic acid | 13.63±0.23a | 13.02±0.98a   | 15.30±0.32b   |
| Glycine    | 4.63±0.45a    | 3.90±0.99a    | 4.46±0.60a    |
| Threonine  | 2.25±0.35a    | 3.00±1.00b    | 2.00±0.51a    |
| Serine     | 4.25±0.01a    | 3.62±0.89b    | 4.00±1.04a    |
| Aspartic acid | 5.70±0.34a | 6.26±0.38b    | 5.64±0.98a    |

Results are expressed in Mean ± SD (n = 3); mean values with different letters as superscripts across the periods (i.e compared horizontally) are considered statistically significant at *p*<0.05

Table 5. Mineral composition of fresh, sprouted and toasted *Citrus lanatus* seeds

| Mineral     | Fresh(mg/100 g) | Sprouted(mg/100 g) | Toasted(mg/100 g) |
|-------------|----------------|--------------------|--------------------|
| Zinc (Zn)   | 0.003±0.001f   | 0.003±0.001f       | 0.003±0.001f       |
| Iron (Fe)   | 0.006±0.01c    | 0.006±0.001c       | 0.007±0.001c       |
| Sodium (Na) | 0.002±0.001d   | 0.003±0.003g       | 0.002±0.001f       |
| Copper (Cu) | 0.001±0.002e   | 0.001±0.000f       | 0.001±0.000f       |
| Manganese (Mn) | 0.002±0.001f | 0.002±0.000f       | 0.001±0.000f       |
3.1.6 Anti-nutrients composition of fresh, sprouted and toasted *Citrullus lanatus* seeds

As shown in Table 6 below, oxalate was found to be significantly (p < 0.05) higher in the sprouted watermelon seeds (0.85±0.01%) when compared to the fresh (0.13±0.01%) and toasted (0.07±0.01%). The concentrations of saponins, tannins, cyanogenic glycosides and phytates were significantly (p < 0.05) higher in the fresh watermelon seeds compared to the sprouted and toasted.

### Table 6. Anti-nutrients composition of fresh, sprouted and toasted *Citrullus lanatus* seeds

| Samples     | Oxalate (%) | Saponins (%) | Tannins (mg/100 g) | Cyanogenic Glycosides (%) | Phytate (%) |
|-------------|-------------|--------------|--------------------|---------------------------|-------------|
| Fresh       | 0.13±0.01g  | 2.15±0.07j   | 40.63±0.13f        | 3.55±0.35i               | 0.31±0.11j  |
| Sprouted    | 0.85±0.01b  | 0.48±0.03g   | 28.84±1.09g        | 2.96±0.11j               | 0.21±0.01k  |
| Toasted     | 0.07±0.01c  | 0.11±0.06e   | 25.05±0.15g        | 2.95±0.07i               | 0.18±0.01k  |

4. DISCUSSION

In this research, we evaluated the phytochemical composition, proximate composition, mineral composition, amino acid composition and anti-nutrient composition of the fresh, sprouted and toasted seeds of *Citrullus lanatus* (watermelon). The proximate composition of sprouted *Citrullus lanatus* seeds is shown in Table 3. The moisture content of the toasted seeds (2.88±0.19) was significantly lower (p<0.05) than fresh and sprouted seeds (5.21±0.61 and 5.73±0.21) respectively. Moisture content entails the amount of water molecules present in the sample. When the moisture of a sample is high, it makes the sample prone to fungal attack and vice versa. Therefore, toasting the seeds removes water and this in a way serves as a means of preserving the seed as this increases the shelf life. The moisture content for the fresh and sprouted seeds was lower than 7.10% reported by Jacob AG et al. [23]. For percentage ash, the value was significantly (p < 0.05) lower in the sprouted (2.63±0.11%) compared to the fresh (4.25±0.08%) and toasted (3.56±0.10%). The low percentage of ash in the sprouted seeds could be attributed to the seeds using the minerals in the sprouting process. Ash content indicates the percentage of inorganic mineral elements present in the samples. The ash content is comparable to the ash content of *Citrullus Vulgaris* (4.83±0.4%) seed according to Penuel BL et al. [24]. High mineral elements in foods enhance growth and development, and also catalyses metabolic processes in human body [23]. The seeds of fresh, sprouted and toasted water melon seeds shows a high percentage of fibre (25.87±0.63, 27.83±0.31 and 20.64±0.23 respectively). The percentage of fibre in the watermelon seeds is way higher than 6.40% reported for four varieties of melon [25]. Fibre is believed to reduce the level of cholesterol in the blood, reduces constipation and pile as well as decreases the chance of developing colon cancer. There was no significant (p > 0.05) difference observed in the percentage fat in fresh (26.67±0.49%), sprouted (29.14±0.16%) and toasted (28.89±0.04%) watermelon seeds. These values were lower compared to the value obtained for four varieties of water melon (40.26 - 45.21%). These low values do not qualify the seeds as oil seeds. Fat is very vital since it provides the body with tremendous amount of energy. The percentage protein content of the sprouted watermelon seeds 24.87±0.48%, is significantly (p < 0.05) higher compared to fresh (26.67±0.49%), sprouted (29.14±0.16%) and toasted (28.89±0.04%) watermelon seeds. These results compared favourably with 4.52% obtained by Jacob AG et al. [23]. The Carbohydrate content of watermelon seeds is low and hence cannot be considered as a source of Carbohydrate.

The anti-nutritional composition of sprouted, toasted and fresh *Citrullus lanatus* seed is presented in Table 6. The fresh seeds contain a significantly (p<0.05) higher values of all the anti
–nutrients while toasted seeds have the least values. These results indicate that processing especially toasting which involves heat significantly reduces the level of anti-nutrients in the seeds and make it nutritionally acceptable. Tannins precipitate and inhibit amino acids and protein absorption while phytates bind and slow or completely inhibit the absorption of certain minerals (such as zinc, iron, Manganese and to a lesser extent calcium) by cells.

Table 4 shows the result of the amino acid profile of fresh, sprouted and toasted watermelon seeds. The results obtained showed that the samples contained all essential amino acids at moderate concentrations with no significant difference (p > 0.05) in the fresh, sprouted and toasted samples. The amino acid composition (mg/100g) was highest for glutamic acid (13.63±0.23, 13.02±0.98 and 15.30 ±0.32 in fresh, sprouted and toasted seeds respectively. Glutamate is a semi essential amino acid that helps nerve cells to send and receive information from other cells. This was followed by Arginine (9.63± 0.87, 10.84 ±0.01, 10.60 ±0.45) in fresh, sprouted and toasted seeds respectively. Arginine is an essential amino acid that is converted to Nitric oxide which is a powerful neurotransmitter that helps to relax blood vessels and improves circulation. The amino acid composition obtained in this study agrees with the one obtained by Egboonu AC [26].

The result of the mineral composition as presented in Table 5 shows that Iron was the most abundant with concentration of 0.006±0.001, 0.006±0.001 and 0.007±0.001mg/100g in all the three samples (fresh, sprouted and toasted respectively). There was no significant difference among the values. Iron is an essential element for production and it is vital for the proper functioning of hemoglobin, a transport protein responsible for the transport of oxygen in the body. Shortage of Iron leads to anemia. This results agrees with the result of [24]. Zinc is the second abundant minerals in all the samples analyzed. From the results, processing did not affect the mineral composition as there was no statistical difference (p>0.05) among the values. It is needed for the body’s defensive (immune system). Zinc also plays a role in cell division, growth and wound healing. Sodium and manganese were the next abundant in fresh, sprouted and toasted watermelon seeds. There was no significant difference among the values. Sodium regulates fluid balance in the body as well as maintain the functions of muscles and nerves while Manganese play roles in enzyme activation and helps in amino acids and protein digestion and utilization. Copper is the least abundant minerals in fresh, sprouted and toasted samples (0.001±0.002, 0.001±0.00 and 0.001±0.00 mg/100g respectively). There was no significant difference in the values obtained for the three samples. Copper and Iron enables the body to form red blood cells. Manganese also maintains healthy bones, blood vessels and nerves. It prevents cardiovascular disease and osteoporosis.

Oxalates and phytates have been reported to inhibit some mineral absorption in the body [27] thereby making them unavailable for the body’s utilization, hence their presence in the samples in high amounts is a pointer to the fact that they may not be safe for consumption. Therefore, from the results obtained, sprouted Citrullus lanatus seeds may not be regarded as a good source of minerals. The high value of some of these minerals may satisfy the nutritional needs of the consumer.

5. CONCLUSION

In this study, the phytochemical, proximate, minerals, and anti-nutrients compositions of fresh, sprouted and toasted Citrullus lanatus seeds were analyzed and the results showed the presence of tannins, saponins, flavonoids, alkaloids, anthroquinones, cardiac glycosides in the fresh, sprouted and toasted Citrullus lanatus seed samples, however, anthroquinones were not detected in the sprouted and toasted samples. The sprouted sample was significantly higher in protein, fats, fibre, moisture and carbohydrates concentrations compared to the other samples. The fresh sample was significantly (p<0.05) higher in saponins, tannins, cyanogenic glycosides and phytates, no significant difference (p>0.05) existed between in amino acids and mineral compositions between the three samples analyzed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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