Research Paper

Characterization of Seed Oil from *Citrullus lanatus* (Watermelon)


Department of Chemistry, Faculty of Science, University of Abuja, Abuja, Federal Capital Territory, Nigeria.

*Corresponding author E-mail: sadam4rich@gmail.com.

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Large varieties of the pumpkin family such as watermelon seed oil have been previously explored for their medicinal conventions due to the presence of bioactive metabolites such as cucurbitacin, triterpenes, sterols, alkaloids, vitamins and minerals, but none of these products have gained utilization on industrial scale. This research is to verify the ongoing experimental and biological analysis to corroborate its ethno-medicinal utilization. It centers on the investigation of the phytochemical composition and nutritional bioassay of the indigenous watermelon (*Citrullus lanatus*) seed oil sold in Gwagwalada market, Gwagwalada Abuja district Nigeria (Longitude 8°N and 7°E). Results of the investigation revealed that the anti-nutritional components such as saponins, alkaloids, phenols, flavonoids and tannins were detected in the sample using n-hexane and ethanol solvent. There are major elements such as Zn (4.8 mg/100g), Ca (27.0 mg/100g) and Mg (29.8 mg/100g). Furthermore, the proximate analysis shows that there are presence of crude protein (19.43±0.38%), ash (5.03±0.05%) crude fibre (26.10±0.03%), crude lipid (21.54±0.2%), moisture (10.92±0.02%) and carbohydrate (16.98%) respectively. Although there have been significant nutritional value in the seed which is the parts always subverted on the basis of acclaimed toxicity, these in turns can be recommended on daily allowance, maintenance of good nutritional status and good health.

**Keywords:** Characterization, *citrullus lanatus*, seed oil

INTRODUCTION

Botanically, Watermelon is known as *Citrullus lanatus*, it is a tropical fruit which can be taxonomically classified as the gourd family native to Southern Africa (Kalahari Desert). Water melon is a monoecious fruit which is cultivated for its large and delicious juicy edibility, usually ripened in August, they are warm season (frost intolerant) stretching annual fruit with stems as long as 5 meters with tendrils at the node and shallow root system. They have a general broad and lobed Leaves. Flowers are large, yellow which occur at the nodes. Watermelon seeds are the most discarded oil seeds. The seeds have nutritional values that are often compared favourably as well as soybean, sunflower and ground nuts (Van der Vossen *et al.*, 2004; Fursa 1981; Maynard, 2001; Oyolu, 1977). The climatic condition of the tropical environments are characterized majorly with high transpiration rate owing to insufficient rainfall, therefore inhabitants of this regions rapidly rejuvenate their adequate supplies of vitamins, minerals, fibres and water from water melon fruit because larger percentage of the fruit consist of water (over 90%) as shown in (Figure 1) (Gabriel *et al.*, 2017) and are also recommended for the control of weight (de Conto *et al.*, 2011). The absorption of the antioxidant–rich food and energy formulation of Nigerian diets has been fundamentally based on fruits and vegetables but inappropriately, only the fleshy pulp (Figure 1) of these fruit have gained edibility attention leaving the seed (Figure 2) and the rind undesirable. The nutritional contents of watermelon seeds are of immense quality; they are rich sources of proteins, vitamins B,
minerals such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese, copper and lipid, carbohydrates, fibre and the likes. The fibre content of the fruits and vegetables has been investigated to have beneficial effects on blood cholesterol and they prevent large bowel diseases (Godwin et al., 2008). It also contains some phytochemicals including saponins, alkaloids, phenols, flavonoids and tannins (Braide, 2012). These bioactive components are responsible for the antimicrobial, antimalaria, anti-inflammatory, anticancer, anti-infection (Adesanya et al., 2011) and antioxidant activities (Loiy et al., 2011). Although in view of its numerous pharmacological and biological activities, randomized clinical trials are yet to established the fact that these bioactive components including antioxidant(β-carotene) have therapeutic potentials (Gonzalez-Aguilar et al., 2008) but traditionally, it has been reported to be of therapeutic use such as, antihypertensive and anti diarrhoeal despite the toxicity levels yet to be studied in-depthly. The watermelons seeds are known to have economic benefits especially in countries where cultivation is on the increase. Snacks milled into flour and used for sauces have been prepared from the seed. Oil from the seeds is used in cooking and constitutes the production of cosmetics (Jensen et al., 2011). Seed oils have varying important nutritional sources of oils which have the industrial and pharmaceutical ethno-medical use (Nzikou et al., 2010). A large percentage of vegetable oils have been derived from various sources, Which includes seed oil, soybean oil, cottonseed oil, peanuts and sunflower oils, palm oil, castor oil, rapeseed oil, palm kernel oil, coconut oil and others (Nzikou et al., 2010). The utilization of these oils in various applications except edibility is characterized by their yields, compositions, physical and chemical properties (Aluyor and Ori-Jesu, 2008). This characteristic of oil from different sources depends majorly on their composition and no oil from the same source can be appropriate and sufficient for all purposes (Mohammed and Jorf-Thomas, 2003). This paper tends to report the phytochemical composition and nutritional bioassay of the indigenous watermelon (Citrullus lanatus) seed oil.

MATERIALS AND METHODS
Sample collection and preparation

The water melon seed was purchased from Gwagwalada market, Gwagwalada Abuja district Nigeria (Longitude 8°N
and 7°E).

Materials used

Round bottom flask, conical flasks, spatula, pipettes, beakers, capillary tube, funnel, filter paper, measuring cylinder, water bath, sample bottles, test tube, syringe, UV-lamp, and AAS

Chemicals

All reagents used are of analar grade. N-hexane, distilled water, ethanol, methanol, ethyl acetate, hydrochloric acid, sodium hydroxide, sulphuric acid, acetic anhydride, chloroform, ferric chloride solution, ferric III chloride solution, Mayers reagent.

Production of watermelon seeds flour

The seeds were washed and air-dried (at 50°C) for 24 h, it was then divided into two parts (A and B). The whole seeds were milled into flour in an electronic blender, kept in an air-tight plastic container at 4 °C prior to analysis.

Extraction

20 g of sample B was extracted with 200 ml of n-hexane for maceration and then allowed to stand for 48 h, it was then filtered to obtain a white residue. The filtrate has intense orange coloration which fades upon continuous extraction until a light orange coloration was obtained. The residue was returned into the container for further extraction using ethanol; this process was repeated for sample B until a light orange coloration was also obtained for the ethanolic extraction. After the extraction, the filtrates were then concentrated using water bath to obtain the oil crude extract weighing 23.70 g of oil (ethanol solvent) and 16.00 g (n-hexane solvent).

Qualitative phytochemical analysis

The phytochemical assays of both extracts (ethanolic and n-hexane) were carried out using (Trease and Evans, 2002; Tiwari et al., 2011; Boakye et al., 2015) to identify the phyto-constituents present in them.

Total Phenol: method of Bray and Thorpe
Tests for alkaloids: Mayer’s test, Dragendorff’s test, Wagner’s test.
Tests for steroids and sterols: Liebermann Burchard’s and Salkowski test.
Tests for saponins: Foam test.
Test for flavonoids: Shinoda test.
Tests for tannins: Lead acetate test and gelatin test.
Test for triterpenoids: Tin and thionyl chloride test.

Proximate analysis

The proximate compositions of the dried Watermelon seed were determined using standard analytical methods. All measurements were done in duplicates and values presented in percentage.

Moisture content

This is a measure of the percentage moisture lost to drying at oven temperature of about 105°C (AOAC, 1999). 2 g of the sample A was oven-dried in a crucible at 105°C overnight. The dried sample was then cooled in desiccator for 1 h and weighed to a constant weight. The percentage loss in weight was expressed as percentage moisture content.

Ash content

The residue remaining was weighed after the ashing of 2 g dried grounded seed in a crucible. The ashing was done in a muffle furnace of temperature 550°C for 6 h. The ashed sample was cooled in a desiccator and weighed. The percentage residual weighed was expressed as ash content (AOAC, 1999).

Crude Lipid content

Continuous extraction of lipid was done for 5hours with petroleum ether in a soxhlet extractor. 2.00 g of the sample was used for determining crude lipid (Udo and Oguwele, 1986).

Crude protein content

Kjeldahl, (1883) method was used to determined total protein. 1g of the sample was put into a filter paper and put into a Kjedahl flask, 10 cm³ of concentrated H₂SO₄ were added and digested in a fume cupboard until the solution becomes colorless. The distillation was carried out with 15 mL of 50% of NaOH. The tip of the condenser was dipped into a conical flask containing 6cm³ of 4% boric acid in a mixed indicator until a green coloration was observed. Titration was done in the receiver flask with 0.01 M HCl until the solution turned red.

Crude fibre content

Estimation of the crude fibre was done by acid and alkaline digestion methods 2.00 g of each sample were used with 20% H₂SO₄ and NaOH solution.

Carbohydrate content

The carbohydrate content of the test sample was determined by estimation using the arithmetic difference method (de Conto et al., 2011; James, 1995).

%CHO = 100 - (% fat + % ash + % fibre + % protein).

Mineral analysis
**Table 1.** Proximate analysis result of watermelon seed.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>19.43±0.38</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>26.10±0.03</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>21.54±0.02</td>
</tr>
<tr>
<td>Ash</td>
<td>5.03±0.05</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.92±0.02</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>16.98</td>
</tr>
</tbody>
</table>

Key: Data are mean triplicates determinations ± standard deviation.

**Table 2.** Qualitative phytochemical analysis of Ethanolic and N-hexane extract of watermelon seeds.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>N-hexane extract of watermelon seeds</th>
<th>Ethanolic extract of watermelon seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Present, - Absent

**Determination of mineral content**

Mineral analysis was determined using 10 g of the sample A and subjected to dry ashing for 5 h in well-cleaned porcelain crucibles at 550°C. The residue ash was dissolved in 5 ml of HNO₃/HCl (1:2) and heated gently on a hot plate until brown fumes disappeared and white coloration was formed. The solution on each sample was dissolved in 5 ml of HNO₃ and gently on a hot plate until brown fumes disappeared and white coloration was formed. The solution on each sample was subjected to dry ashing for 5 h in well-cleaned porcelain crucibles. The ash was filtered into 100 ml volumetric flask and the solution was made up to 100 ml with deionized water. The zinc element was determined from the solution using flame photometer (Perkin-Elmer model 52A) (Shahid et al., 1999).

**Determination of Ca and Mg content**

This was determined by complexometric titration method. 20 ml of the extract was pipette into a conical flask and 2-3 ml of 0.5 M KOH was added. After shaking the solution, 4-5 drops of solochrome blue T was added and the sample was titrated against 0.05 M EDTA solution in the burette, until a colour change from wine red to blue appeared to indicate the end point (Mendham et al., 2000).

**RESULTS AND DISCUSSION**

From (Table 1), the proximate analysis shows that there are presence of crude protein (19.43±0.38%), crude fibre (26.10±0.03%), crude lipid (21.54±0.2%), moisture (10.92±0.02%), carbohydrate (16.98%) and ash with value of (5.03±0.05%) respectively. The moisture content of Sudanese watermelon seeds obtained from western Sudan is 2.8% as determined by Yousuf, (1992), whereas Mustafa et al. (1972) reported a value of 4.94% for another type obtained from kordufan in the vicinity of Elobied. The moisture content of two Nigerian varieties of watermelon seeds reported by Ogunsua and Badifu, (1984) were found to be 7.9% and 5.6% respectively, therefore the values obtained were found to be of acceptable limits. The differences could be attributed to methodology, soil, storage and variety. Moisture content of the watermelon fruit is affected by storage and preservatives as reported by Gabriel et al., (2017). From (Figure 3), the crude fibre was analyzed to have 26.10%; this value is much higher compared to 6.46% obtained for that of pumpkin seed. Therefore, watermelon seed can be recommended for the lowering of cholesterol levels in the blood and reduce risk of various cancers. The results also indicated that watermelon seeds contained appreciable amount of crude protein content (19.43.9 ± 0.38%) making it a good source of supplementary protein for man and livestock feeds. The proximate composition of the samples shows that the crude lipid extracted had the second higher amount with value of 21.54% showing that watermelon seeds can be utilized for the production of oil to substantiate that of palm oil, coconut oil and the likes. Mabalaha et al. (2007); Madaan Lai, (1984) also reported oil content values of 41.0-56.6% and 24.8-30.0% in melon seeds. These values are within range of little difference which might be attributed to methodology or variety. The crude protein content showed values of 19.43%, this was less when compare to previous works on varieties of C. vulgaris seed which has high protein value that qualifies the seed as a valuable and good source of protein. The carbohydrate content is 10.92% as shown in (Table 1 and Figure 3). This is lower as compare to that obtained from different varieties and was also lower as compared to 26% reported for sunflower. Other reports of carbohydrate content ranged from 7.08-14.15%. The least was in Leganaria siceraria while Chebil et al. (2006) reported the highest value in Cucumeropsis edulis.

The roots, stems, leaves, flowers, fruits or seeds are the major source of phytochemical accumulation (Costa et al., 1999). The quality of foods can be quantified by the presence of primary components of plants(protein), this in turns could increase the bioactive compound that could also be isolated in future (Thomsen et al., 1999). From (Table 2), there are presence of alkaloid, phenol, tannin, saponin, steroid and flavonoids in the ethanol extract while there is absence of terpenoid in both solvent extract. This may be due to the affinity of the solvent for hydrophillicity while the hexane extract shows the presence of alkaloids, phenol, tannin and saponin while there is absence of steroid, flavonoid and terpenoid as shown in (Table 2). Phenolic compounds (flavonoids) and tannins are plant secondary metabolites which possess...
Table 3. Elemental analysis.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Concentration (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>4.8</td>
</tr>
<tr>
<td>Ca</td>
<td>27.0</td>
</tr>
<tr>
<td>Mg</td>
<td>29.8</td>
</tr>
</tbody>
</table>

Figure 3. Graph of Proximate analysis on watermelon seed.

Figure 4. Graph of elemental analysis on watermelon seed.

multiple biological and therapeutic activities such as treatment of neurodegenerative diseases, vasodilatory action, antimicrobial, antibacterial, antiviral and anti-inflammatory (Tsuchiya, 2010), Asian medicine (Japanese and Chinese) utilizes tannin for natural healing especially in treating tumors (Cushnie and Lamb, 2005; Murray, 1998; Funatogawa et al., 2004). Phenolics are also antioxidant which neutralizes diseases related to oxidative stress. A vast majority of researchers have reported alkaloids as an antimicrobial by inhibiting DNA topoisomerase also, pharmacologically against chronic diseases including hypertension, arrhythmic effect and antimalarial (Yao et al., 2004; Akiyama et al., 2001). Due to the higher concentration of calcium and magnesium as well as Zinc minerals in the water melon seed as shown in (Table 3 and Figure 4), it should be recommended as
an element of bone and teeth strengthening. Also as a potential ingredient in cosmetics for stimulation and revitalization.

Conclusion
The phytochemical contents coupled with proximate and elemental analysis of watermelon seed oil constituents studied underline the potency of watermelon seed oil rather than disposing the seed as a waste product after consuming the pulp. The seed could be used in the development of new functional foods to promote good health. Thus the watermelon seed oil extraction is found to possess some excellent source of secondary metabolites that provides them with an ability to be used as an indigenous ethno-medicine by traditional healers. This can further be investigated in a wide scale for the purpose of drug development against various diseases and possibility of incorporating the oil in the manufacturing of soaps.

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