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Research Article
Evaluation of the Medicinal Properties and Possible Nutrient Composition of *Citrullus lanatus* (Watermelon) Seeds

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Abstract

Background and Objective: In countries where cultivation of *Citrullu slanatus* (watermelon) is on the increase, watermelon is known to have invaluable benefits. Watermelon seeds are often discarded while the fleshy fruit is eaten. This study aimed at determining the medicinal and nutritive bioactive components of *Citrullus lanatus* through proximate, mineral, vitamin, amino acid and phytochemical analysis. **Materials and Methods:** In this study, seeds of watermelon were analyzed for proximate, minerals, phytochemicals and vitamin content. The proximate analysis, minerals content, vitamins, amino acids and phytochemicals screening were performed using standard guideline of AOAC, GC-MS, AAS, HPLC and the statistical analysis was carried out using the Microsoft excel software 2010 version. **Results:** The proximate analysis results indicated that the watermelon seeds had moisture 48.7%, ash content 0.96%, fat 22.77%, carbohydrate 13.99% and protein content 8.9%. The amino acid profile showed the seeds were rich in phenylalanine, arginine, valine, glutamate and serine content. Vitamins A and C at 68.13 and 19.45 mg kg^{-1}, respectively were the most abundant vitamins in the seeds. The seeds also contained appreciable mineral elements such as; Fe, Mg, Na and K with K (18.189 ppm) being the highest. The phytochemistry of the samples showed a lot of compounds with known medicinal effects. **Conclusion:** The present findings suggested watermelon seeds as considerable source of nutrients in the diet and may have health and economic benefits due to its vitamins, minerals and phytochemicals with high level of antioxidant activities and consequently a very useful potential nutraceutical.

Key words: Watermelon seed, proximate, vitamins, amino acids, phytochemicals, nutraceutical

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits and vegetables are known to contain a variety of natural bioactive compounds[1]. The seed, peel and rind of some fruits have greater vitamins, fibres, minerals and other essential nutrients than the pulp[2]. One such medicinal plant is *Citrullus lanatus* (watermelon).

Citrullus lanatus (watermelon), a fruit crop, is a herbaceous creeping plant and belongs to the family Cucurbitaceae, also referred to as cucurbits[3]. It is a tropical fruit which can be found in most parts of Africa and requires a lot of sunshine and high temperature of over 25°C for optimum growth especially along river beds in the Northern Savannah areas[4]. Every aspect of the fruit has nutritional value, including the rind, peel and the seeds. The most common way of watermelon is eaten, is the consumption of the pink flesh watermelon lemonade and fresh salad[5]. It is known to be low in calories, highly nutritious and thirst quenching[6]. The pigment extracted from watermelon acts as functional ingredient and can be incorporated into breakfast cereals, frozen dairy desserts, yoghurts, spreads, candy and carbonated beverages. The seeds are used locally, to produce a sweetener which is locally known as "ogiri" or they are boiled with leaves wrap to make another sweetener called "igbãlo"[7]. Also, oil from the seeds are used in cooking and incorporated into the production of cosmetics and pharmaceutical industry[8]. For their high protein and fat content seeds are also used in the improvement of local infant formula. The seed has been shown to possess anti-inflammatory activity[9], anti-ulcer activity[10], anti-microbial and antifungal activity[11,12] anti-oxidant activity potential[13] and hepatoprotective activity[9]. However, the seeds are not routinely eaten with the pulp, they are simply thrown away. The aim of this research work was to determine the proximate components, vitamins phytochemicals, minerals and amino acids present in the seed of *Citrullus lanatus* in-order to create awareness of its potential nutritional values and increase its consumption.

MATERIALS AND METHODS

Sample collection: Matured whole watermelon fruits were purchased from Eke Awka Market, Awka, Anambra state, Nigeria. The seeds were harvested, dried and dehusked. The dehusked seeds were further dried and ground to powder and stored in an air tight container for analysis.

Study area: The study was carried out from June-August, 2019 at the Applied Biochemistry Department, Research Laboratory, Nnamdi Azikiwe University Awka, Springboard Research Laboratory, Awka, in Anambra State, Nigeria and the Department of Chemical Engineering, Ahmadu Bello University, Zaria, Kaduna state, Nigeria.

Proximate analysis of seeds: Moisture content, ash content, crude fibre and crude proteins contents were determined using methods as described by AOAC[14].

Determination of crude fat (Soxhlet fat extraction method): Some 250 mL clean boiling flasks were placed in oven at 110°C for about 30 min and then transferred into a desiccator and allowed to cool. Correspondingly labeled, cooled boiling flasks were weighed and filled with about 300 mL of petroleum ether (boiling point 60°C). The extraction thimble was plugged lightly with cotton wool and the soxhlet apparatus assembled and allowed to reflux for about 6 hours. The thimble was carefully removed and cooled petroleum ether was collected in the top container of the set-up and drained into a container for reuse. When flask was almost free of petroleum ether, it was removed and dried at 105°C for 1 h and then transferred from the oven into a desiccator, allowed to cool and then weighed[14].

Determination of vitamins: Appropriate colorimetric and titration methods were applied for the determination of the vitamin concentrations in watermelon seeds. Vitamins A, C and E were assayed as described by Kirk and Sawyer[15]; vitamins B1, B2, B12 and B9 as described by Rajput *et al.*[16], vitamins B3 and B6 were assayed by the spectrophotometric methods of Khateeb *et al.*[17], while vitamins D and K were analyzed according to the description of Zakaria *et al.*[18].

Heavy metal analysis: Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer (AAS) according to the method of APHA[19] (American Public Health Association).

Amino acid composition: The composition of amino acid in the seed was determined using HPLC as described by Sarwar and Botting[20]. The HPLC equipment consisted of a Spectra Physics (San Jose, CA) HPLC apparatus comprising an 8700 XR ternary pump, a 20 µL Rheodyne (Cotati, CA) injection loop, an SP 8792 column heater, a 8440 XR UV-Vis detector and a 4290 integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250×4.6 mm column packed with 5 µm Spherisorb C_{18} (Sugelabor, Madrid, Spain) was used.

Phytochemical components: The GC-MS analysis of the watermelon seed extract was performed on the GC-MS equipment, the GC-MS analysis of bioactive was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length×250 μm in diameter×0.25 μm in thickness of film). The bioactive compounds extracted from different extracts of *Citrullus lanatus* were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards.

Statistical analysis: Obtained data were analyzed using the Microsoft excel software 2010 version and was presented as Mean±SD.

RESULTS AND DISCUSSION

Watermelons contain significantly large amounts of bioactive components. The result of the proximate composition of watermelon seeds are shown in Table 1. The proximate analysis of the cucumber seeds showed that the seeds have a nutritional index. The seeds have high moisture content (48.68%), appreciable crude fat (22.77%), carbohydrates (13.99%), proteins (8.9%) and fibre (2%) content. These results are in agreement with the study of Rekha and Rose[21] that showed the presence of the proximate components analyzed. Nwachoko and Owhonda[22] also showed that a watermelon seed has fat content, carbohydrate and protein content. Although, Alka *et al.*[7] reported about 92-93% water and about 6% sugar in the whole fruit. The composition of dried watermelon seed without shell reported[7,21] previously, include protein 28.3, fat 47.4, water 5.1 g, energy 2340 kJ (557 kcal), carbohydrate 15.3 g. The results of Tabiri *et al.*[23] indicated that the watermelon seeds had moisture content in the range of 7.40-8.50% fat, 26.50-27.83% protein, 16.33-17.75% fibre, 39.09-43.28% ash, 2.00-3.00% carbohydrate, 9.55-15.32% and energy value of 354.05-369.11 kcal/100 g. Opara *et al.*[24] showed that the mean nutritional content of the *Citrullus lanatus* seeds contain (4.20) moisture, (2.70) ash, (7.20) crude fibre, (32.08) crude protein, (30.30) crude lipid and (23.52) carbohydrates contents. The lipid content found in the seed is an indication that the seed could be source for edible vegetable oil. Oil are known for their ability to provide the body with maximum energy[25]. The seeds are a potential additional dietary fibre source in the diet. The ash content of *C. lanatus* seeds indicates the total inorganic matter from which the mineral content could be obtained. The carbohydrate content of the seed are polar compounds that can be converted into glucose as energy source.

Table 1: Proximate and nutrient analysis of watermelon seeds

Parameters	Amount (%)
Moisture content	48.680±0.55
Ash content	00.960±0.66
Fibre content	02.175±0.46
Fat content	22.770±3.30
Protein content	08.925±0.25
Carbohydrate	13.990±0.31

Table 2: Vitamin composition of the watermelon seeds

Vitamin	Amount (mg kg^{-1})
Vitamin A	69.128±0.57
Vitamin B_1	00.967±0.00
Vitamin B_2	00.796±0.12
Vitamin B_3	00.915±0.05
Vitamin B_6	00.053±0.00
Vitamin B_9	01.843±0.05
Vitamin B_{12}	01.092±0.01
Vitamin C	19.450±0.49
Vitamin D	08.760±0.71
Vitamin E	03.533±0.01
Vitamin K	01.437±0.06

The vitamin composition of the watermelon seeds are shown in Table 2. Vitamins A, C at 69.13 and 19.45 mg kg^{-1}, respectively were significantly higher in concentration in the seeds than the other vitamins. Results of vitamin determination revealed the seeds had an abundance of vitamins of significant amount where vitamins A and C at 68.13 and 19.45 mg kg^{-1} were significantly higher than the values reported by Alka *et al.*[7]. The result for vitamin D (8.76 mg kg^{-1}) content was the highest ever reported by a research on the seeds so far. The seeds also contained other vitamins ($B_{1,2,3,6,9,12}$, E and K) in different quantities showing that the seeds of water melon are very good sources of vitamins. This may account for its antioxidant effects[13]. Rekha and Rose[21] and Olamide *et al.*[26] have reported the presence of thiamin, riboflavin, niacin and folate in watermelon seed.

The mineral composition of the watermelon seeds are shown in Table 3. The highest occurring minerals were potassium, sodium and iron at 18.189, 16.416 and 15.19 ppm, respectively followed by magnesium (10.63 ppm). The remaining minerals were present but in varied smaller quantities except for vanadium, arsenic, calcium, silver and selenium which were absent in the seeds. The mineral composition of the seeds showed that watermelon seeds are a good source of minerals. The most important mineral in the seeds were potassium, sodium, iron and magnesium at 18.189, 16.416, 15.19 and 10.63 ppm. Except for the absence of calcium, the previous work[21] has shown that the seed of watermelon contains calcium 54, Phosphorous 755 and iron 7.3 mg. The finding of this research showed that the results obtained were in agreement with previous work done by

Table 3: Mineral analysis of watermelon seeds

Parameters	Concentrations (ppm)
Manganese	00.301±0.01
Lead	04.559±0.26
Zinc	00.905±0.01
Iron	15.190±0.27
Copper	00.243±0.00
Chromium	00.683±0.00
Magnesium	10.637±0.51
Aluminium	00.015±0.01
Selenium	00.000±0.00
Calcium	00.000±0.00
Potassium	18.189±1.83
Sodium	16.416±0.61
Arsenic	00.000±0.00
Vanadium	00.000±0.00
Silver	00.000±0.00
Molybdenum	00.015±0.01

Table 4: Amino acid profile of watermelon seeds

Amino acid	Amount of protein (g/100 g)
Glycine	3.355
Alanine	3.786
Serine	4.470
Proline	4.142
Valine	4.572
Threonine	4.249
Isoleucine	4.100
Leucine	1.723
Aspartame	1.148
Lysine	0.315
Methionine	1.371
Glutamate	4.462
Phenylalanine	5.158
Histidine	2.887
Arginine	4.977
Tyrosine	3.298
Tryptophan	1.037
Cysteine	1.285

Alka *et al.*[7] and Tabiri *et al.*[23]. Seeds also contained appreciable minerals (Ca, P, Mg, Na, K and Zn) with K (3.40-3.5/mg 100 g) being the highest while Na (0.07-0.08 mg/100 g) was the least. Abu *et al.*[27] showed the presence of sodium (30.60 mg kg^{-1}), calcium (0.97 mg kg^{-1}), zinc (0.25 mg kg^{-1}), magnesium (5.98 mg kg^{-1}) and potassium (20.12 mg kg^{-1}). Potassium and sodium were present in the highest quantity, while zinc occurred in the least amount. Lead and cadmium were not detected[27].

The presence of these mineral elements indicated that the seeds can serve as good source of minerals in feeds formulation. The seeds could also contribute to the iron needs of consumers especially in meeting the recommended dietary allowance for iron in adult and children. The role of macro elements such as zinc, copper and iron in human health have been considered[28] and their use in the preparation of herbal drugs was recommended[29]. Mineral elements speed up metabolic processes improves growth and development.

The results of the amino acid profiling of watermelon seeds in Table 4 showed Phenylalanine (5.158 g/100 g) to be the highest occurring amino acid followed closely by arginine (4.977 g/100 g), valine (4.572 g/100 g), glutamate (4.462 g/100 g) serine (4.47 g/100 g) and threonine (4.25 g/100 g). The least abundant amino acid was lysine (0.315 g/100 g). Amino acid profiling of the seed showed that the protein was rich in phenylalanine, arginine, valine, glutamate and serine which is supported by the work done by Alka *et al.*[7]. Rekha and Rose[21] also reported that the protein was rich in arginine, glutamic acid, aspartic acid and leucine amino acids. According to Mogotlane *et al.*[30], the amounts of amino acids varied significantly and leucine was found to be the most abundant amino acid, threonine was the second most abundant in water melon seed followed by methionine. Usman *et al.*[31] reported the prevalence of glutamic acid and aspartic acid in watermelon seeds. Amino acids, especially the sulfur-containing amino acid play essential roles in the synthesis of proteins and stabilization of interactions within proteins and protein complexes. The presence of this essential amino acid demonstrates the potential role of watermelon seeds in biological activities. These observations indicated that watermelon seeds have a good amino acid profile and greater potential for use as food supplements.

The results of the phytochemical analysis of watermelon seed is shown in Table 5. The results showed that the seeds contain many phytochemicals and their retention time on GC-MS, with 2-Propenenitrile having the least retention time of 5.321 min^{-1} and 11-Dodecenol having the highest retention time of 67.272 min^{-1}. Phytochemical analysis of the seeds showed many compounds, apparently with diverse pharmacological and biological significance. However, thiirane one of the phytochemicals present in the seeds is a known anti-oxidant with anti-bacteria properties. This may also account in part for its use in traditional medicine[13].

The phytochemical profile of the watermelon seed indicated the presence of various bioactive compounds which can be utilised for medicinal purposes. The GC-MS constituents obtained from GC-MS analysis are given in Table 5. The identified compounds have several biological properties. The results from the current study indicate that the phytochemistry of seed of watermelon carried out by GC-MS analysis contained various types of compounds with potential pharmacological activity that play a central role in traditional medicines and also as cosmetics commercial commodities in the markets. Literature indicated that plants are the backbone of traditional medicine and the medicinal activity of plant extract is due to different chemical agents in the extract with potential bioactive compounds[32].

Table 5: Phytochemical composition of water melon seeds

Retention time (min^{-1})	Phytochemicals
05.0321	2-Propenenitrile
55.0293	N-Ethylformamide
55.0719	Isoxazole
55.7972	Pyrrole
56.0611	Methyl guanidine
56.0805	N-ethylformamide
57.0076	Oxazole
57.0231	Propiolamide
57.0688	2-Butamine
57.0813	1-Isocyanopropane
58.0046	3-Fluoro-2-propenenitrile
58.0278	Methanesulfonyl fluoride
58.0898	1-Azabicyclo(3,1,0) hexanepyridine
59.0053	Ethyleniminoacetonitryl
59.0480	Thiirane
59.0829	Fluoramine
60.0100	Silamine
60.0488	Fomepizole
61.0031	3-Hexen-1-ol
61.0302	3-Methyl-1,3-pentadiene
61.0884	Ethyl neopentyl carbonate
62.0659	2-Methyl-2-oxiranyl cyclobutane
63.0008	8-Nonenoic acid
63.0318	1-Cyclohexyl-1-propyne
63.0628	11-Dodecen-1-yl acetate
63.0977	Oxirane
64.0093	4-Chloro-3-cyclohexyl tetrahydropyran
64.0675	6,6-dimethyl chloromethyl-6-chloroundecanoate
65.0024	Dodecenol
65.0644	1,6-dichloropyruvic acid
66.0458	Cyclopropanecarboxylic acid
66.0652	Oxirane carboxaldehyde
66.0962	Trimethylsilyl di(trimethylsiloxy) silane
66.0962	Arachidonic acid
67.0272	11-Dodecenol

The plants contain large amounts of bioactive compound that exert a wide range of biological activities on physiological systems. The activities of watermelon seed constituents such as; alkaloids, 2-Propenenitrile, N-Ethylformamide, Isoxazole, Pyrrole, Methyl Guanidine, N-Ethylformamide, Oxazole, Propiolamide, 2-Butamine, 1-Isocyanopropane, 3-Fluoro-2-propenenitrile, Methanesulfonyl fluoride, 1-Azabicyclo (3,1,0) hexanepyridine, Ethyleniminoacetonitryl, Thiirane, Fluoramine, Silamine, Fomepizole, 3-Hexene-1-ol, 3-Methyl-1,3-pentadiene, Ethylneopentyl carbonate, 2-Methyl-2-oxiranyl cyclobutane, 8-Nonynoic acid, 1-Cyclohexyl-1-propyne, 11-Dodecen-1-yl acetate, Oxirane, 4-Choro-3-cyclohexyl tetrahydropyran, 6,6-dimethyl chloromethyl-6-chloroundecanoate, Dodecenol, 1,6-dichloropyruvic acid, Cyclopropanecarboxylic acid, Oxirane carboxaldehyde, Trimethylsilyl di (trimethylsiloxy) silane, Arachidonic acid, 11-Dodecenol as antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic, immunomodulatory and anticoronary[33].

The results obtained from GC-MS analysis shows that most of the phytochemical groups in the watermelon seed have pharmacological activities. Butamine has been used as a drug for the treatment of cardiogenic shock and severe heart failure. Silamine provides conditioning, lubricity, softening to hair and is used as a hair growth stimulant and potent additive to creams and gels. Oxazole have anti-cancerous, anti-viral, anti-diabetic and antibiotic activity. Isoxazole and its derivatives act as regulators of immune functions. Methyl guanidine exhibits anti-inflammatory effect. Pyrrole is a heterocyclic compound found in the structure of macrocycles such as porphyrins of heme. Fomepizole is used in the treatment of methanol and ethylene glycol poisoning, it acts as inhibitor of alcohol dehydrogenase. Phytol acts as an anti-inflammatory agent[34]. The major components based on their functional groups, such as carboxylic acids (Cyclopropanecarboxylic acid), aldehydes (Oxiranecarboxaldehyde), aromatics (4-Choro-3-cyclohexyltetrahydropyran), alkenes (3-Methyl-1,3-entadiene), tertiary alcohols (3-Hexene-1-ol), alkanes (1-Isocyanopropane) and alkynes (1-Cyclohexyl-1-propyne) might be accountable for the pharmacological properties of watermelon seed. Alkenes are used as anaesthetics. Carboxylic acids help in maintaining the cell membrane and control nutrient use along with metabolism. Phenol is used as an antiseptic and is also used as a preservative in some vaccines. Alkynes have antifungal and antitumor activities. Phenol spray is used medically to resolve sore throat[35]. Thus, the results implied that the watermelon seed have antiseptic, anaesthetic, antimicrobial and antitumor activities.

These phytocompounds are responsible for various pharmacological actions of the seeds of the watermelon. Bioactive compound of the watermelon seed are beneficial in repelling harmful organisms, serve as phytoprotectants and respond to environmental changes[36]. The presence of various bioactive compounds justifies the use of watermelon seed for various ailments by traditional practitioners.

The seeds of *C. lanatus* are a rich source of proximate components, vitamins, amino acid, phytochemicals, macro and micro elements. Differences in the amount of proximate components, vitamins, amino acid, phytochemicals, macro- and micro elements contents in comparison to the previous findings could be attributed to soil type, time of harvest, regional differences, genotype and the geographical and environmental conditions in which watermelons are grown.

CONCLUSION

This study provided evidence that watermelon seeds can serve as a good source of vitamins and minerals in human diet and animal feed. In particular it is a very good source of iron needed for blood haem formation. The presence of antioxidant vitamins and phytochemicals attests to its antioxidant bioactive potency.

SIGNIFICANCE STATEMENT

This study discovered that the edible watermelon seed underutilized has a rich amount of valuable ingredients that are beneficial for health and also has therapeutic and medicinal activity. The isolation and purification of bioactive components from watermelon are readily available natural resource and their utilization as potential natural therapeutic agents could be of high economic value. Also, it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drug. This study will help the researcher to uncover the critical area of *in vitro* and *in vivo* investigations to establish which components of the extract are biologically active against disease causing human pathogens.

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