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Nutriceutical potentials of Nigerian grown *Citrullus lanatus* (Watermelon) seed

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Abstract
Several plants and their parts have been used both as food and as medicines since ancient times, but their safety and efficacy still need to be scientifically investigated. This study evaluated the mineral and the phytochemical composition of watermelon (*Citrullus lanatus*) seed-pulp and shell using standards methods of analysis. The phytochemical screening indicated the presence of alkaloids, cardiac glycoside, flavonoids, steroids, saponins and tannins in the seed-shell while alkaloids, cardiac glycoside, flavonoids, steroids and reducing sugar in the seed-pulp. The mean concentrations of the minerals determined: calcium (Ca), copper (Cu), iron (Fe), potassium (K), manganese (Mn), sodium (Na), lead (Pb) and zinc (Zn) in the seed-pulp were found to be 733.4±5.02, trace, 1.85±0.07, 580.925±273, 798.15±1.84, 107.08±1.77, 5.3±0.64 and 15.625±1.24 µg/g respectively. In the seed-shell, the values were 593.13±2.65, 12.65±0.07, 14.48±2.09, 13302.273±4.56, 403.58±0.81, 174.35±1.12, 10.03±0.67and 50.575±0.60 µg/g respectively. The concentration of lead (Pb) in the seed-shell was slightly above WHO permissible limit (10 µg/g). The study revealed that watermelon seed is a rich source of both beneficial minerals and phytochemicals, which serve nutritional and medicinal purposes. It is therefore, advised that watermelon fruit be consumed along with the seed in order to fully benefit from its nutritional and medicinal values.

Keywords: Watermelon; Phytochemicals; TLC fingerprint; Minerals

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a herbaceous plant species in the family of Cucurbitacea, a vine like flowering plant with its origin from West Africa; it is grown usually for its fleshy fruits. It was cultivated more than 5,000 year ago. It flourishes in dry climates and require only limited rainfall [1]. In Nigeria, it grows better in the northern part of country due to the favorable weather of the region. It is usually propagated by seeds. It requires a drained fertile soil of fairly acidic nature in order to grow well [1]. Watermelon fruits are used in fresh salad, snacks, and prepared as juice drinks. It is rich in antioxidants called carotenoids such as lycopene, phytofluene, phytoene, beta-carotene, lutein, and neurosporene. These antioxidants and fibers, citrulline [2], and amino acids fight free radicals that contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis [3]. The seeds are used to prepare snacks, sauces and the oil is used in cooking

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and cosmetics. It is rich in minerals such as zinc, manganese, phosphorous, potassium and copper) and fat [4]. The seeds are also used by tradition medicine practitioners to treat prostate cancer, urinary tract infections, bed-wetting, dropsy, renal stones, alcohol poisoning, hypertension, diarrhoea and gonorrhea [4].

Watermelon seeds contain phytochemicals, due to the fact that, the phytochemicals accumulate in different parts of plants, such as in the roots, stems, leaves, flowers, fruits or seeds [5]. Levels of phytochemicals vary from plant to plant depending upon the variety, processing and growing conditions [6]. Despite the nutritional and medicinal potentials of watermelon seeds, in Nigeria, the seeds are usually discarded as the fruit is eaten. This is because, its major benefits are yet to be fully understood. In this study, phytochemical, TLC fingerprint and mineral constituents of commonly consumed watermelon seeds were analyzed for their chemical profile.

EXPERIMENTAL

Class A glassware of appropriate sizes was washed with detergents, rinsed with deionized and dried prior to use and reagents of Analar grades were used throughout the study.

Sample preparation. Available varieties of watermelon fruits were purchased from Karmo market, FCT, Abuja, Nigeria. The seeds were removed from the fruits, washed and air-dried. The dried seeds were de-shelled where the seed-pulp and the seed-shell were separated and milled into fine powder using blender and stored in clean capped, air-tight plastic containers for subsequent analysis..

Extraction. of The powdered sample (1500 g) was extracted using hexane, ethyl acetate and methanol after soaking it in each of the solvents and made to stand for 24 hours. The solutions were decanted, filtered and concentrated to dryness using rotary vacuum evaporator and kept for further analysis.

Determination of proximate content. Moisture and ash content of the samples were analyzed according to AOAC methods [7,8].

Phytochemical screening. The methods used by Boakye *et al.* [9] and Betty *et al.* [1] were adopted for phytochemical screening of the samples for alkaloids, flavonoids, tannins, reducing sugar, steroids, saponin, and cardiac glycosides.

TLC fingerprint. TLC fingerprint of the extracts were determined by dissolving each extract in appropriate solvent and applying them to the adsorbent silica layer (activated TLC plate) as spots, using capillary tubes. The TLC plate was air-dried and propped vertically in a Chromatank saturated with appropriate eluting solvent (mobile phase) and allowed to travel up the layer of adsorbent by capillary action where the compounds in the mixture move upwards on the plate at different rates, resulting in separation of the compounds until the eluting solvent reached the marked solvent front. The developed plate was removed, air-dried, visualized under UV-lamp at 256 and 365 nm, sprayed with 10% sulphuric acid and oven-heated at 100ºC until colours developed. The identified spots were marked-out and their R_f calculated using the formula:

$$Rf = \frac{\text{distance travlled by spot}}{\text{distance travelled by solvent}}$$

Determination of minerals. The ash of each sample was digested with a mixture of concentrated nitric acid and hydrochloric acid in the ratio of 1:10 and filtered into 25 cm^3 volumetric flask, made-up to mark with deionized and transferred into caped sample bottle. Blank sample was prepared following the same sample preparation process. This study analyzed watermelon seed-pulp and seed-shell for calcium (Ca), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), potassium (K), sodium (Na) and zinc (Zn) using Atomic Absorption Spectrometer (AAS), GBC Avanta Model version 2.0. The equipment was optimized based on the operating condition in Table 1 and calibrated using prepared reference standard solutions of element of interest followed by blank and samples analysis respectively. The concentrations were determined and calculated using the formula:

$$Metal\ (\mu\frac{g}{g}) = \frac{C \times V \times d.f}{W},$$

where C is the concentration of the sample solution in µg/mL; V is the volume of the prepared sample solutions in mL; W is the weight of the sample in grams and d.f is the dilution factor, if applied [10].

Statistical analysis. Microsoft excel spread sheet was used to analyse the data and the results were presented as mean and standard deviation.

RESULTS AND DISCUSSION

Watermelon crude seed-pulp and seed-shell were analyzed for moisture, ash, mineral content and fractionated extracts of the seed-pulp were screened for phytochemicals and subjected to TLC fingerprint. The results obtained for each of the parameters are presented in Tables 2-5.

Table 1: Operating Conditions for the Instrument

Element	Wavelength (nm)	Detection limit (µg/ml)	Slit width (nm)	Lamp current (mA)	Nebulizer uptake	Gas flow (Air/acetylene)
K	324.7	0.025	0.5	3.0	5ml/min	13.5/2.5
Fe	372.0	0.45	0.2	7.0	5ml/min	13.5/2.5
Pb	217.0	0.06	1.0	5.0	5ml/min	13.5/2.5
Zn	213.9	0.008	0.5	5.0	5ml/min	13.5/2.5
Na	330.2	3.00	0.5	5.0	5ml/min	13.5/2.5
Mn	279.5	0.45	0.2	7.0	5ml/min	13.5/2.5
Cu	324.7	0.02	0.5	3.0	5ml/min	13.5/2.5
Ca	239.9	2.00	0.5	5.0	5ml/min	13.5/2.5

Table 2: Moisture and Ash Content

Parameters	Seed-pulp (% w/w)	Seed-shell (% w/w)
Moisture	9.9± 0.10	9.9±0.11
Total ash	7.92±0.05	0.98±0.02

Table 3: Mineral content

Minerals	Mean Concentration (µg/g)	
	Seed-pulp	Seed-shell
Ca	733.4±5.02	593.13±2.65
Cu	Trace	12.65±0.07
Fe	1.85±0.07	14.48±2.09
K	580.925±273	13302.273±4.56
Mn	798.15±1.84	403.58±0.81
Na	107.08±1.77	174.35±1.12
Pb	5.3±0.64	10.03±0.67
Zn	15.625±1.24	50.575±0.60

Table 4: TLC Fingerprint of the extracts

Fractions	No. of spots	Rf values
Methanol	1	0.36
Ethyl acetate	2	0.63, 0.94
Hexane	1	0.5

Table 5: Phytochemical composition of extracts

Phytochemicals	Ethyl acetate	Hexane
Alkaloids	+	++
Cardiac glycoside	+	+
Flavonoids	++	+
Reducing sugar	+	-
Saponin	-	+
Steroids	+	+
Tannins	-	++

Key: + (present), ++ (highly present), - (Absent)

Ash content. The percentage ash content of a sample could serve as a gauge of inorganic constituents such as silica materials or a reflection of the mineral content of that particular sample. In this study, the percentage ash (Table 2) in the seed-pulp (7.92%) was higher than in the seed-shell (0.98%), this was not relative to the concentration of the mineral content obtained (Table 3).

Mineral content. Minerals are required by both plants and animals, but the requirement varies with type and concentration. This is because some are required in large quantities (e.g. the macro-minerals), some in trace quantities (e.g. the micro-minerals) while others are not required at all, even at trace level (e.g. the toxic ones such as lead). The mineral content of the crude seed pulp and shell of watermelon analyzed are presented in Table 3. The concentrations of the macro (Ca, K and Na), micro (Cu, Fe and Zn) and the toxic (Pb) minerals obtained in both the seed-shell and the pulp indicated high concentrations of the macro-minerals in the seed-shell than the seed-pulp and as compared with the micro-minerals apart from Mn, which was higher than Na. The result of this study indicates that, the watermelon seed analyzed can effectively meet children and adult daily intake needs of Na (1.0 to 1.5 g/day), Ca (700 to 1,300 mg/day), Mn (1.2 to 18 mg/day), Zn (3 to 13 mg/day), Fe (7 to 27 mg/day) but cannot be depended upon as a source of Cu [11]. The concentration of Pb detected in the seed-shell at 10.03 µg/g was just slightly higher than WHO [12] permissible limit of lead in edible foods (10 mg/kg). It has been established that, both macro-minerals and micro-minerals are required by the body to maintained good health status, but lead (Pb) as a toxic mineral is not required at all by either plant or animal due to its effects, which ranges from inhibition of enzymes to the production of marked morphological changes and death [13]. Contamination of the watermelon seed observed could be due to use of agrochemical for weed or pest control [14]. Acar *et al*. [15] in his similar study reported that, variation of mineral content of similar samples depended on whether the seed were de-hulled or not. The result of this study indicated higher concentrations of Ca, Fe, K, Mn and Zn as compared to the results obtained by Betty *et al*. [1] from the seeds of three varieties of watermelon. Braverman and Pfeiffe [16] in a study suggested that zinc and manganese can prevent growth of cancer cells. Presence of iron, zinc and the antioxidants are necessary requirements for optimum functioning of red blood cell and overcoming anemic conditions

[17]. The most important health benefits of Ca are maintaining bone and dental health, prevention of colon cancer and reduction of obesity; it is required from infant stage to the older age by all sex groups. Iron acts as oxygen carrier to different body cells, an important facilitator for regulating body temperature, directly related to brain health and its functions provide the supply of oxygen required for contraction of muscles and formation of hemoglobin. Potassium acts as an electrolyte this mineral is required for keeping the heart, brain, kidney, muscle tissue and other important organ systems of the human body in good condition. Manganese is required for proper functioning of the thyroid gland and sex hormones, regulating blood pressure and insulin release, formation of connective tissues, antioxidant that seeks out the free radicals in the human body and neutralizes these damaging particles, absorption of calcium and metabolism of fats and carbohydrates [18].

TLC fingerprint. Thin-layer chromatography (TLC) is a technique used for separation and identification of compounds in mixtures [19]. In this work, it was used for fingerprint profiling of chemical constituents of extracts. The results of the TLC fingerprint profile of the extracts are presented in Table 4. The R_f values of the spots obtained from methanol, ethyl acetate and hexane are 0.36, 0.63 and 0.94, and 0.5 which indicated that more of less polar compounds were present in the fractions, because the higher the R_f value the less polar the compound and the less the R_f value the more polar the metabolites.

Phytochemical constituents. Phytochemicals are naturally occurring chemical compounds, found in plants, with biological activities and health benefits to both humans and plants [19,20]. In this study the, the phytochemicals obtained in the fractions of the seed extracts (Table 5) indicated the presence of alkaloids, flavonoids, steroids, and cardiac glycosides in both the ethyl acetate and the hexane extracts, while tannins and saponins were detected only in the hexane extract and reducing sugar only in ethyl acetate extract. High concentrations of alkaloids and tannins were found in the hexane extract, while the ethyl acetate extract indicated higher concentration of flavonoids. King and Young, [6] reported that, plant varieties, processing and growing conditions are factors responsible for variation in levels of phytochemical contents. Each of these phytochemicals exhibits specific roles. For example, alkaloids are antibacterial, antiplasmodic and analgesic; tannins are antidiabetic, antiproliferative, antihaemorrhage, antitumor, antimycolic and antimutagenics [1,22,23]; glycosides are antidiarrhoeal [24]; flavonoids were reported to exhibit antimicrobial, anti-inflammatory, cytotoxicity and antitumor activities [20]. Saponins chemically, include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Researchers have reported immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins [25,26].

Conclusion. The study revealed that watermelon seed-pulp and seed-shell, which are usually discarded during consumption of the fruit juice, are rich source of minerals and phytochemicals required for healthy living. It is therefore, advised that both the juice and the seed be consumed together in order to fully benefit from its nutritional and medicinal values.

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