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Analysis of Mineral Elements, Proximate and Nutritive value in *Citrullus vulgaris Schrad*. (Watermelon) seed extracts

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Abstract
Dietary elements, commonly known as dietary minerals are chemical elements required by living organisms other than the elements present in the common organic molecules. Dietary fibre and minerals are important for healthy functioning of the human body. The mineral nutrition is an important aspect and its pivotal role in human life provides healthy growth. The present study was undertaken to analyze the mineral contents and nutritive value of the seeds of *Citrullus vulgaris Schrad*. The dried and powdered seeds of watermelon were subjected to various sample preparation stages for the proximate analysis such as Fat, Moisture, Ash and Fibre and the dietary minerals such as Iron, Phosphorus and Calcium from seed sample and sample ash were estimated using various biochemical and titration methods under standard laboratory protocols. From the study results, the estimated mineral composition and nutritive value in the seeds of watermelon were found in good amounts which can suggest that the established role of essential dietary elements in the physiology of human life and the impact of watermelon seeds on health and hazards.

Keywords: Minerals, *Citrullus vulgaris Schrad*, Nutritive value, Physiology, Proximate analysis.

1. Introduction
Medicinal plants are in the food obtained from the vegetation. It is useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body. Therefore medicinal plants come into preparation of various drugs singly or in combination or even are used as the source of raw material for the other medicines [1].

In most developing countries most of the flora remains virtually unexplored from the point of view of the medicinal plants utilizing through traditional system of medicines that strongly upholds the use of the mineral elements for curing many diseases [2]. In order to face the problem of malnutrition and food scarcity in the society, fruits can be utilized for the good source of nutrients and food supplements. Fruits are commonly well known for the excellent source of nutrients such as minerals [3].

Mineral elements though usually form a small portion of total composition of most plant materials and of total body weight; they are nevertheless of great physiological importance particularly in the body metabolism. Besides several organic compounds, it is now well established that many trace elements play a vital role in general well-being as well as in the cure of diseases [4, 5]. Several studies have reported elemental contents in plant extracts, which are consumed by us either as herbal health drink or medicine [6-8]. These elements are present at varying concentrations in different parts of the plants, especially in roots, seeds and leaves which are used as a dietary item as well as ingredient in the medicinal preparation [9].

Macro and microelements influence biochemical processes in the human organism. Active constituents of medicinal plants i.e. metabolic products of plant cells and a number of mineral elements play an important role in the metabolism [10]. Some mineral elements remain chelated with organic ligands and make them bioavailable to the body system [11].

Determination of mineral elements in plants is very important since the quality of many foods and medicines depends upon the content and type of minerals [12]. In this way, not only must the absolute amounts of minerals be estimated in the edible portions of foods, but these minerals must also be in forms that are bioavailability for organism. In recent years, scientists and nutritionists have started believing in the therapeutic role of metals in human health [13]. Ashes give us an idea of the mineral matter contained in a plant because mineral matter of the reality may be the cause of a pharmacological effect [14].

Minerals are required by living organisms and can help to prevent occurrence of some diseases. Some plants contain significant amount of minerals, the presence and quantity depend on plant family, history and phytochemical properties of the plant [15]. Medicinal value of the plant is due to presence of a variety of phytochemical and elemental composition. Therefore, it is essential to investigate the phytoconstituents, elements and vitamin supplements present in the medicinal plant to assess their medicinal values.

Watermelon (*Citrullus vulgaris Schrad*.) is a warm season crop in the Cucurbit family. There are prospects for use of watermelon seeds in the improvement of infant nutrition in view of their high protein and fat content [16]. The seeds of watermelon fruit are a good source of various bioactive compounds and its importance being much unaware among the society. The present study has been framed to estimate the nutritive value and mineral elements in the seeds of *Citrullus vulgaris Schrad*.

2. Materials and Methods

2.1 Plant Material and Processing of seed samples

Seeds were collected from the watermelon fruits which were purchased from Coimbatore market, Tamilnadu during April to June 2014. The seed samples were washed and shade dried at room temperature. The dried seed samples were powdered using mechanical grinding mortar for effective extraction. The shade dried powdered seed material was subjected to pressurized hot aqueous extraction.

2.2 Pressurized Hot water extraction

It was carried out in pressurized extractor at the ratio of 10g seed powder with 100 ml distilled water. The extracts were then filtered through Whatmann filter paper and concentrated to dryness by evaporation of liquid solvents on petri dishes. The obtained concentrated seed extracts were then used for the mineral elements estimation.

2.3 Proximate analysis of Fat, Moisture, Fibre and Ash

This denotes the evaluation of the nutritional value and organic content of the plant materials. It was achieved by the measure of percentage proximate composition which includes the quantification of the amount of lipid, moisture, fiber and ash by the method of Raghuramula *et al*., [17]. Determinations of all parameters were carried out in triplicates.

2.4 Determination of Moisture Content

For determination of moisture content 10 g powdered seed material is kept in pre-weighed watch glass/moisture box and dried at 100 – 105 °C over night in an oven. The sample with watch glass is cooled at room temperature in a desiccator and final weight is taken after achieving constant weight. The weight loss in sample regarded as moisture content. The moisture content was calculated using the formula as follows

% Moisture = (Total weight – Final weight) / Weight of the sample x 100

2.5 Determination of Crude Fat

Crude fat were determined by extracting 10 g of moisture free seed material with petroleum ether in a soxhlet extractor for 10-16 hours. This petroleum ether extract that contained crude fat, was taken in a pre-weighed beaker (W_1) and petroleum ether was evaporated. The weight of beaker along with the residual extract after evaporation (Crude fat, W_2) was taken and crude fat content of the sample was calculated using the formula [26].

% Crude fat = (W_2 - W_1) / Fresh sample weight x 100

2.6 Determination of Crude Fibre

For determination of Crude fibre, 2 g of moisture and fat free seed material were treated with 200ml of 1.25% H_2SO_4 with 30 min boiling. After filtration and washing, the residue was treated with 1.25% NaOH with 30 min boiling, then filtered, washed with hot distilled water. The residue was dried overnight at 80-100 °C and weighed (W_1). It was then ignited and the ash weighed (W_2). Loss in the weight gives the weight of crude fiber calculated using the formula.

% Crude fibre = [100 – (Moisture + Fat)] x [W_1-W_2] / Weight of moisture and fat free sample

2.7 Determination of Ash Content

For determination of ash content, 5 g of powdered seed material weighed and taken in porcelain crucible and heated first over a low flame till all the material was completely charged, followed by heating in a muffle furnace for about 3-5 hrs at 600 °C. Then the sample was cooled in a desiccator and weighed. It was again heated in muffle furnace for 1 hour, cooled and weighed. This was repeated consequently till the weight of sample became constant (Ash became white or grayish white). The loss in weight of the sample gives the ash content calculated using the formula as follows.

% Ash = Weight of ashed sample / Weight of sample taken x 100

2.8 Determination of Nutritive value

The nutritional value of watermelon seeds was calculated as per the formula used by Nile and Khobragade [18].

Nutritive value = (4 x percentage of protein) + (9 x percentage of fat) + (4 x percentage of carbohydrate)

2.9 Ash solution for mineral estimation

Ash represents the inorganic part of the plant. The composition of ash depends on part of the plant material. The quantity of minerals in ash would be much more accurate than extracted sample as they may contain an array of various other organic compounds.

Moisture obtained ash with 1 ml distilled water. Added 5 ml HCl, evaporated to dryness on boiling water bath and this step is repeated again. Then added 4 ml HCl and few ml distilled water and warm. Filtered into 50 ml volumetric flask cooled and made up the volume. The aliquots were used for the estimation of Iron, Phosphorus and Calcium in ash solution.

2.10 Estimation of Iron

Iron content was estimated by the method of Raghuramula *et al*., [17]. Taken 1.5 ml of extracted seed sample and 1.5 ml of prepared ash solution in test tubes, added 1.0 ml of 30% H_2SO_4 and 1.0 ml of 7% potassium per sulphate solution and 1.5 ml of 40% potassium thiocyanate solution are added. The red color developed was read at 540 nm within 20 minutes. The standard aliquots with concentration corresponding to 10-50 µg were treated similarly. The estimation was done in triplicates and the results were expressed mg/g sample.

2.11 Estimation of Calcium

The estimation of Calcium was done by method of Raghuramula *et al*., [17]. Taken 2 ml of extracted seed sample and 2 ml of prepared ash solution in test tubes, added 2.0 ml of distilled water and 1.0 ml of 4% ammonium oxalate, mixed well and allowed to stand overnight. After calcium precipitation, centrifuged and removed the supernatant fluid without disturbing the precipitate. To this 3.0 ml of 2%

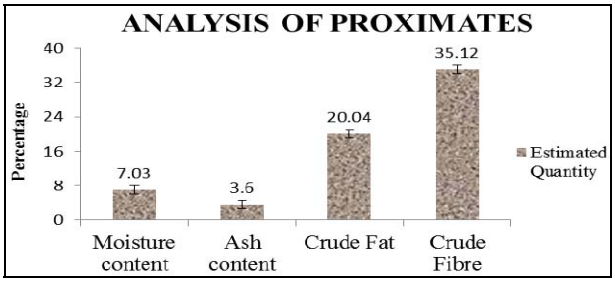
ammonia was added along the sides of the tube, mixed well and centrifuged again. Supernatant fluid was poured off.
This was repeated until the supernatant gave no precipitate with calcium chloride solution. Added 2.0 ml of 1N H_2SO_4 and mixed the precipitate well, placed in boiling water bath for few minutes. Keeping the mixture at 70–75 ^oC, titrated against 0.01 N $KMnO_4$, to a faint pink color, which persisted for about a minute. Titrated 2.0 ml of 1N H_2SO_4 as blank to the same endpoint. The difference between the titration gives the volume of 0.01N $KMnO_4$ required to titrate the calcium oxalate. The estimation was done in triplicates and the results were expressed mg/g sample.

2.12 Estimation of Phosphorus
The estimation of Phosphorus was done by method of Fiske and Subbarow [19]. Taken 1.0 ml of extracted seed sample and 2 ml of prepared ash solution in test tubes, added 5 ml of 10% TCA. Centrifuged and to 3.0 ml of supernatant, added 1 ml of prepared ammonium molybate solution and 0.4 ml of ANSA (Aminonapthol sulphonic acid). The standard aliquots with concentration corresponding to 8-40 µg were treated similarly. Made up the volume in all tubes to 10 ml, mixed well and after 20 min, the color developed was read at 660 nm against reagent blank. The estimation was done in triplicates and the results were expressed mg/g sample.

2.13 Statistical analysis
All the estimations were done in triplicates and the results were analyzed statistically. It was expressed as mean (n=3) ± standard deviation.

3. Results
The results obtained in the present study quantified the nutritive value and mineral elements present in the watermelon seed sample. The results obtained are given in tables and figures under each of its respective topics as follows. All the experiments performed were under standard laboratory conditions with standard protocols.

3.1 Proximate analysis and Nutritive value
Generally, the nutritional quality of food materials may be evaluated by biochemical analysis of the food for proximate composition. The quantification of Moisture, Crude fat, Crude fibre and Ash were done to figure out the nutritional and medicinal importance of the watermelon seed sample. The results were given in the figure and the proximate composition is expressed as a measure of percentage (%) in the sample.
The crude fibre content was found to be higher 38.12% followed by crude fat 26.04%. The moisture content was found to be lower 7.03% followed by ash 1.60%. The nutritive value was calculated to be 465.68 K Cal/ 100 g for the seed sample as given in Table 1.



Values are expressed by mean ± SD of three samples

Fig 1: Analysis of Proximate in hot aqueous seed extracts of *Citrullus vulgaris Schrad.*

Table 1: Nutritive value of *Citrullus vulgaris Schrad.* (Watermelon) seeds

Parameters	Estimated Quantity (K Cal/100g)
Nutritive value	465.68±1.04

3.2 Mineral element estimation
The mineral elements such as Iron, Calcium and Phosphorus in the extracted seed sample and ash solution were estimated and tabulated in Table 2. Mineral ions are of prime importance in determining the nutritional value of foods. The results were expressed mg/g sample and Iron was found to be higher in extracted sample than in ash solution whereas Phosphorus and Calcium were found higher in ash solution than in the extracted seed sample.

Table 2: Estimation of Mineral elements in hot aqueous seed extracts and ash solution of *Citrullus vulgaris Schrad.*

Mineral elements	Estimated Quantity in aqueous extracted sample (mg/g)	Estimated Quantity in ash (mg/g)
Iron	2.29±0.13	2.25±0.12
Phosphorus	0.83±0.19	1.07±0.16
Calcium	29.63±0.38	45.14±0.19

Values are expressed by mean ± SD of three samples

4. Discussion
Plants are the rich source of all the elements essential for human beings. The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history but has increased substantially in the last decades [20, 21].
The current study provides the proximate analysis of Moisture content, Ash content, Crude fibre and Crude fat and estimated amounts of mineral elements such as Iron, Calcium and Phosphorus present in the hot aqueous seed extract and ash solution of watermelon. This may provide knowledge on the biological activities of watermelon seeds. Further the proximate analysis and mineral elements estimation may aid in the detection of the bioactive dietary elements that are responsible for the therapeutic properties of watermelon seeds.
Ash contains inorganic material of the plant because ashing destroys all the organic material present in the sample. Ash is also indicative of high digestibility of the plant [22]. A strong correlation may be suggested between moisture contents and fiber, which could be of interest to human health as the fibrous are easily digested and disintegrated [23]. Fibers in the diet are necessary for digestion and for effective elimination of wastes. It can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer [24].
Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. Mineral elements also are needed in minute quantities for the proper functioning of the human system, health growth and development [25]. The content of mineral elements in plants depends to a high degree on the soils abundance, including the intensity of fertility [26].
Calcium is one of the mineral believed to be an important factor governing fruit storage quality [27]. Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality [28]. Calcium is essential for healthy bones, teeth and blood [29, 30]. The health of the muscles and nerves depends on

calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter.

Iron is the most well known in biological system. It performs a wide range of biological functions. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells [31]. Iron deficiency is the most prevalent nutritional deficiency in humans [32]. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes [33]. Phosphorous maintain blood sugar level, normal heart contraction dependent on phosphorous [34] also important for normal cell growth and repair. It helps in the process of ossification of bones by getting deposited in the form of Calcium Phosphate [35].

5. Conclusion

The knowledge of element concentrations in the plant gives a new insight into their potential use in therapeutics. Proximate analysis and mineral elements estimation of medicinal plants is very important in identifying new sources of nutritionally important compounds.

Mineral elements possess a very important role in human nutrition. Though they are required in minute quantities they are essential for proper functioning of the entire human system. This can further be investigated in a wide scale for the purpose of drug development against various deficiencies. The nutritional value and mineral analysis of watermelon seeds may therefore yield to the conclusion that it may act as a good source of diet to fight against deficiency disorders of Iron, Calcium and Phosphorus.

The watermelon seed extract was quantified for proximate composition analysis of Moisture content, Ash content, crude fibre and crude fat that are found to be essential to maintain good health. The quantitative estimation of the crude fibre may suggest that it can be a diet source which provides good digestion and palatability. Moisture and ash content estimation may give a feedback about plant digestibility. The estimated mineral compounds may act as a base for the future work on their underlying role in deficiency disorders.

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