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Estimation of some phytoconstituents in the aqueous extract of the endocarp, seeds and exocarp of watermelon (*Citrullus lanatus*) fruit

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Abstract
Watermelon (*Citrullus lanatus*) is an indigenous fruit consumed in Nigeria and the world over. This study was aimed at comparatively evaluating phytoconstituents in the endocarp, seeds and exocarp of this fruit. Phytochemical tests revealed that, terpenoids, flavonoids and saponins were significantly (p<0.05) higher in the endocarp, whereas, alkaloids, total phenols, cyanogenic glycosides and anthraquinones were significantly (p<0.05) higher in the exocarp. Proximate analysis revealed that crude lipids and proteins were significantly (p<0.05) higher in the seeds; crude fibre and ash significantly (p<0.05) higher in the exocarp, while the moisture and carbohydrates were significantly (p<0.05) higher in the endocarp. Minerals like phosphorus, magnesium, chloride, iron, calcium and potassium were significantly (p<0.05) higher in the exocarp while sodium and iron were significantly (p<0.05) higher in the endocarp. The entire fruit may be consumed since every part had phytoconstituents of medicinal relevance; instead of the common practice of discarding the exocarp and seeds.

Keywords: *Citrullus lanatus*, phytoconstituents, proximate analysis, minerals/elements, nutraceuticals

Introduction
The Watermelon plant with scientific name *Citrullus lanatus* belongs to the cuccurbitaceae family. It is a large, sprawling annual plant with coarse, hairy, pinnately-lobed (3 to 7 lobes) dark green, long-stalked, oblong-ovate leaves (8 to 20cm) with prominent veins and white to yellow monoecious flowers (about 2 cm in diameter) occurring singly in axils [1, 2]. It is grown for its edible fruit, also known as watermelon, which is a special kind of berry botanically known as pepo. The fruit is very large and ellipsoid to oblong with light or deep green or yellow coloured, smooth thick exterior exocarp with irregular grey or light green vertical stripes, sometimes covered with a white waxy bloom, about 30 centimeters long. The fleshy endocarp is either white, pink, red or even yellow and usually crispy, soft and juicy with many compressed seeds which can be black, brown, white, green, or yellow embedded in the middle third layer; some are however seedless [3, 4, 2]. Generally, watermelon endocarp is the main consumable portion; however, the outer exocarp or rind is also used in some parts of the world [5, 6].
Watermelon is generally used as thirst quencher and in the very dry areas and desert of Africa; it is usually relished by both humans and animals as a source of water. The fruits are generally eaten raw and the seeds are sometimes grounded to form flour. The young leaves are also cooked or added to soup. Watermelon seed oil is edible oil quite similar to sunflower oil [7].
Citrullus lanatus (watermelon) with the red fleshy endocarp is a rich source of phytochemicals especially lycopene, vitamin C as well as the amino-acid citrulline. After the consumption of several kilograms of this fruit, an elevated concentration of citrulline is measured in the blood plasma; which could be mistaken for citrullinaemia or other urea cycle disorders [8, 9]. Preliminary researches in human nutrition indicate that, this fruit has antihypertensive effects and contributes to the integrity of the blood vessels [10].
Phytochemicals also known as secondary metabolites e.g. tannins, phlobatannins, quinones, alkaloids, saponins, terpenoids, saponins, flavonoids and cardiac glycosides are often found in disposable parts of plants, like stem and root barks, leaves, flowers, fruits and seeds [11]. Currently this term is being used only for those plant chemicals that may have health-related effects but are not considered essential nutrients like proteins, carbohydrates, fats, minerals and vitamins [7, 12]. They are thought to protect the cells from damage, stimulate anticancer enzymes and help remove carcinogens from the body. They have been found in large quantities in fruits, vegetables and even whole grains where they contribute to the colour, flavour and fragrance of plants.

Watermelon fruit consumption in various regions of the world is done differently. While some eat only the fleshy endocarp, others consume the entire fruit i.e. the endocarp, seed and exocarp. There are however, various claims by locals in those regions of the therapeutic importance of eating the whole fruit. This study therefore is an attempt to scientifically verify those claims by qualitatively and quantitatively estimating the phytoconstituents in parts of the watermelon fruit (endocarp, exocarp and seeds) to either debunk or support the claims of those that consume the whole fruit while establishing whether all the parts of the watermelon fruit may be eaten for either therapeutic or nutritional purposes.

Materials and Methods

Reagents/chemicals

All reagents and chemicals used were of analytical grade and products of Aldrich Laboratory, Germany), British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company, USA.

Collection and identification of the watermelon fruit

The watermelon whole fruit was purchased from Okada market, Edo state, Nigeria. The ripe one was confirmed by a hollow sound when hit with the palm. Field identification of the sample was carried out by a Botanist Prof. J. E. Ehiagbonare, of the Department Biological Sciences, College of Natural and Applied Sciences, Igbinedion University Okada, Edo State, Nigeria and identified as *Citrullus lanatus* (watermelon).

Preliminary treatment and extraction procedure of the watermelon fruit

The watermelon fruit was washed thoroughly with tap water and cut into pieces to obtain the various samples i.e. endocarp, seeds and exocarp (rind + the white part) for analysis. The seeds were washed again separately to remove all traces of the endocarp. All the fresh samples were separately ground with distilled water into a viscous liquid using an electric blender. Thereafter filtration was carried out using porcelain and eventually clearer filtrates were obtained using Whatman's No.1 filter paper. This served as the aqueous extracts that were evaluated for varied phytoconstituents.

Phytochemical analysis of the endocarp, seeds and exocarp of the watermelon fruit

Qualitative phytochemical tests were carried out on the aqueous extracts of the endocarp, seeds and exocarp using standard conventional procedures to identify the phytochemicals as described by [13-17].

Quantitative phytochemical analyses were carried out as follows:

1. Total phenolics was determined using Folin-Ciocalteau reagent (FCR) with slight modifications [18].
2. Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins [19].
3. Flavonoid content was estimated according to a slightly modified method [20].
4. Estimation of alkaloid and saponins contents [21].
5. Estimation of anthraquinone and cardiac glycoside contents [15].
6. Estimation cyanogenic glycoside content [22].
7. The cyanide content of each sample was determined using the alkaline titration method [23].

Estimation of minerals/elements in the endocarp, seeds and exocarp of the watermelon fruit

The double acid (9 ml of conc. HCL and 3 ml of HNO_3) extraction method was used to estimate the presence of minerals/elements (Phosphorus (P), Sodium (Na), Magnesium (Mg), Chloride (Cl^-), Zinc (Zn), Iron (Fe), Calcium (Ca), Potassium (K), Cadmium (Cd), Lead (Pb) in each of the watermelon filtrate samples using the atomic absorption spectrophotometer (AAS). In this technique the samples were completely digested and fractions of the metals were in the extracted solution [24].

Proximate analysis in the endocarp, seeds and exocarp of the watermelon fruit

This was done following the methods recognized by the Association of Official Analytical Chemists (AOAC) to test for the presence of ash, nitrogen free extract (carbohydrate), fibre, lipids, moisture and protein [23].

Estimation of antioxidant activity in the endocarp, seeds and exocarp of the watermelon fruit

Free radical scavenging activity in the various parts of the fruit aqueous extract was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) referred to as the DPPH radical scavenging method [25].

Statistical Analysis

The data collected from this study were subjected to statistical analysis using SPSS version 22 (IBM, 2014) and the results expressed as mean ± SEM. One way analysis of variance (ANOVA) was also used to compare the means of all the parameters measured and where significant differences were observed at 95% confidence level i.e. $p<0.05$, Duncan's New multiple Range test was used to identify them.

Results

Qualitative phytochemical content in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

The qualitative phytochemical composition of the watermelon fruit determined are summarised in Table 1. It was generally observed that in all parts of the watermelon fruit used (endocarp, seed and exocarp) there were no steroids, tannins and anthocyanins. However in the endocarp of the fruit there were high levels of terpenoids, saponins and carotenoids but alkaloids and cyanogenic glycosides were absent. The exocarp on the other hand indicated relatively high levels of alkaloids and cardiac glycosides. Whereas moderate amounts of terpenoids, alkaloids, cardiac glycosides and cyanogenic glycosides were observed in the seed.

Table 1: Qualitative phytochemical content in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

Parameter	Endocarp	Seed	Exocarp
Steroids	-	-	-
Terpenoids	+++	++	+
Alkaloids	-	++	+++
Saponins	+++	+	++
Tannins	-	-	-
Carotenoids	+++	+	++
Anthraquinones	++	-	+
Phlobatannins	+	-	-
Cardiac glycosides	+	++	+++
Cyanogenic glycosides	-	++	+
Anthocyanins	-	-	-

Key: + Low; ++ Moderate; +++ High; - not detected

Quantitative phytochemical content in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

The quantitative phytochemical composition of the endocarp, seed and exocarp of the watermelon fruit evaluated are summarised in Table 2. It was generally observed that all the phytochemicals estimated were present in varied amounts in all the different parts of the fruit but tannins were visibly absent. Alkaloids, total phenols, cyanogenic glycosides and anthraquinones were significantly ($p<0.05$) higher in the exocarp when compared to the endocarp and seeds. The level of saponins, flavonoids, terpenoids and antioxidants were significantly ($p<0.05$) higher in the endocarp when compared to the seeds and exocarp but alkaloids and cardiac glycosides were absent. However no significant ($p>0.05$) differences were observed in the level of cardiac glycosides and phlobatannins in all parts of the fruit used, but were present in trace amounts.

Table 2: Quantitative phytochemical content in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

Quantitative Determination (mg/100 g)	Endocarp	Seeds	Exocarp
Alkaloids	0.00 ± 0.00^c	19.73 ± 0.23^b	37.42 ± 0.35^a
Total phenols	33.53 ± 1.07^c	42.10 ± 0.50^b	52.02 ± 0.79^a
Saponins	25.55 ± 0.91^a	0.08 ± 0.00^c	17.79 ± 0.65^b
Tannins	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cardiac glycosides	0.00 ± 0.00^a	2.92 ± 2.21^a	1.61 ± 0.14^a
Flavonoids	11.44 ± 0.80^a	3.98 ± 0.05^b	2.32 ± 0.31^b
Terpenoids	7.48 ± 0.76^a	3.78 ± 0.04^b	1.56 ± 0.10^c
Cyanogenic glycoside	3.95 ± 0.17^c	7.63 ± 0.39^b	9.49 ± 0.37^a
Anthraquinones	0.18 ± 0.03^b	0.00 ± 0.00^c	5.61 ± 0.05^a
Phlobatannins	1.38 ± 0.08^a	1.46 ± 1.46^a	0.00 ± 0.00^a
Antioxidants	7.42 ± 0.50^a	4.96 ± 0.29^c	6.43 ± 0.35^b

Values represent mean ± SEM of triplicate determinations. Means of the same row followed by different lettered superscripts differ significantly ($p<0.05$). Means of the same row followed by the same lettered superscripts are similar or differ insignificantly ($p>0.05$).

Mineral/elemental content in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

Elemental composition in three parts of the watermelon fruit: endocarp, seed and exocarp expressed as percentage is shown on Figures 1, 2 and 3. It was observed that the content of phosphorus, magnesium, potassium, iron, calcium and zinc were significantly ($p<0.05$) higher in the exocarp followed by the seeds and least in the endocarp. However the sodium and chloride levels were significantly ($p<0.05$) higher in the endocarp compared to what was obtained in the exocarp and the seed. There were however trace amounts of lead but not significant ($p>0.05$) in the exocarp, whereas it was not detected in the seed and the endocarp. There was however complete absence of cadmium in all parts of the entire fruit.

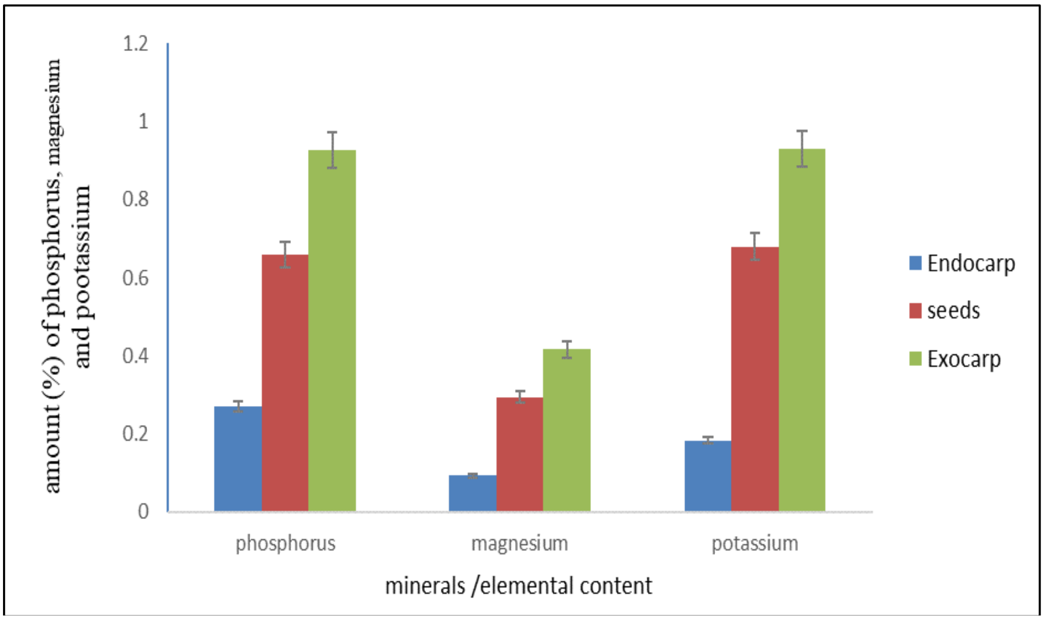

Fig 1: The amount (%) of phosphorus, magnesium and potassium in the aqueous extract of the endocarp, seeds and exocarp of the watermelon fruit. All values represent mean ± standard error of mean (SEM) of triplicate determinations (n = 3) and $p<0.05$ was considered significant.

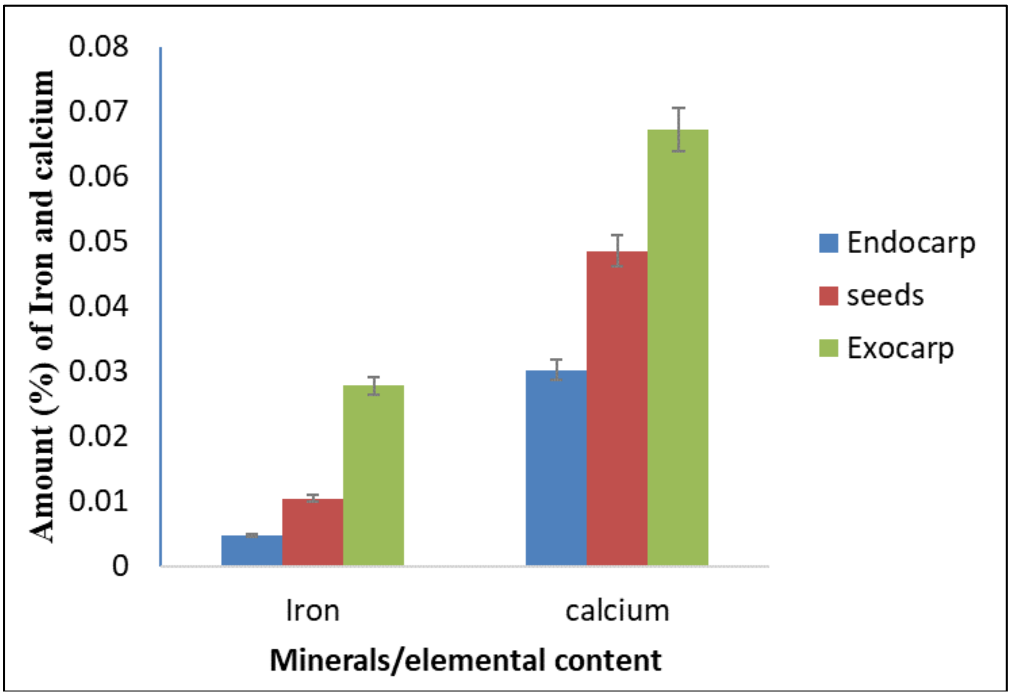

Fig 2: The amount (%) of iron and calcium in the aqueous extract of the endocarp, seeds and exocarp of the watermelon fruit. All values represent mean ± standard error of mean (SEM) of triplicate determinations (n = 3) and $p<0.05$ was considered significant

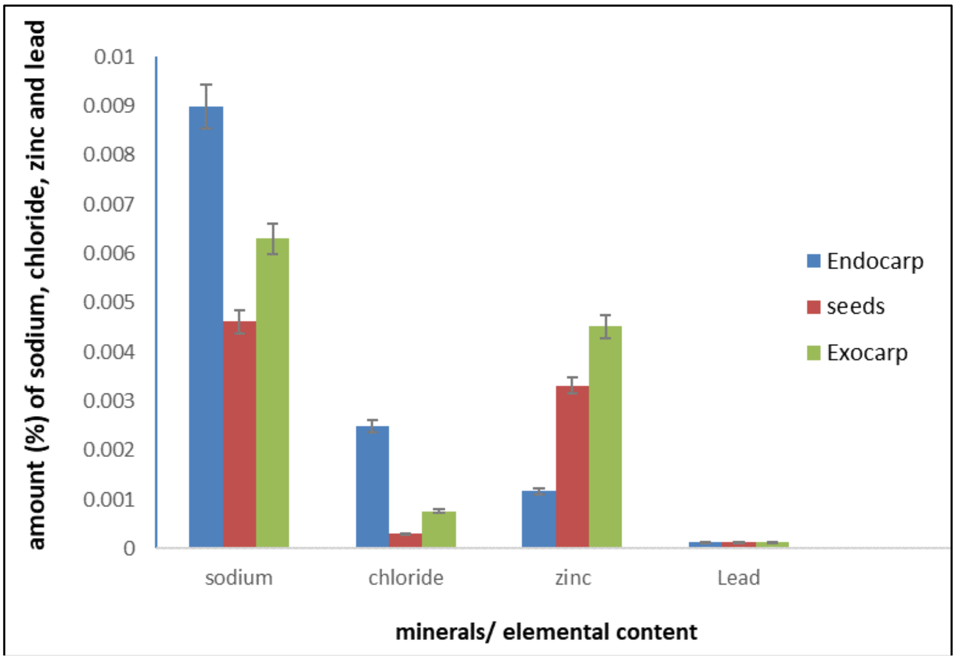

Fig 3: The amount (%) of sodium, chloride, zinc and lead in the endocarp, seeds and exocarp of the watermelon fruit. All values represent mean ± standard error of mean (SEM) of triplicate determinations (n = 3) and $p<0.05$ was considered significant.

Proximate contents in the aqueous extract of the watermelon fruit endocarp, seed and exocarp

The proximate constituents in all parts of the watermelon fruit analysed and expressed in percentage are outlined on Figures 4 and 5. It was observed that all the various constituents analysed were differentially present in all the parts (endocarp, seed and endocarp) of the watermelon fruit. The moisture and carbohydrate contents were seen to be significantly ($p<0.05$) higher in the endocarp when compared to the exocarp and seed. The crude protein and crude lipid contents were significantly ($p<0.05$) higher in the seed compared to that obtained in the exocarp and endocarp. The ash and crude fibre contents were significantly ($p<0.05$) higher in the exocarp when compared to that obtained in the seed and endocarp.

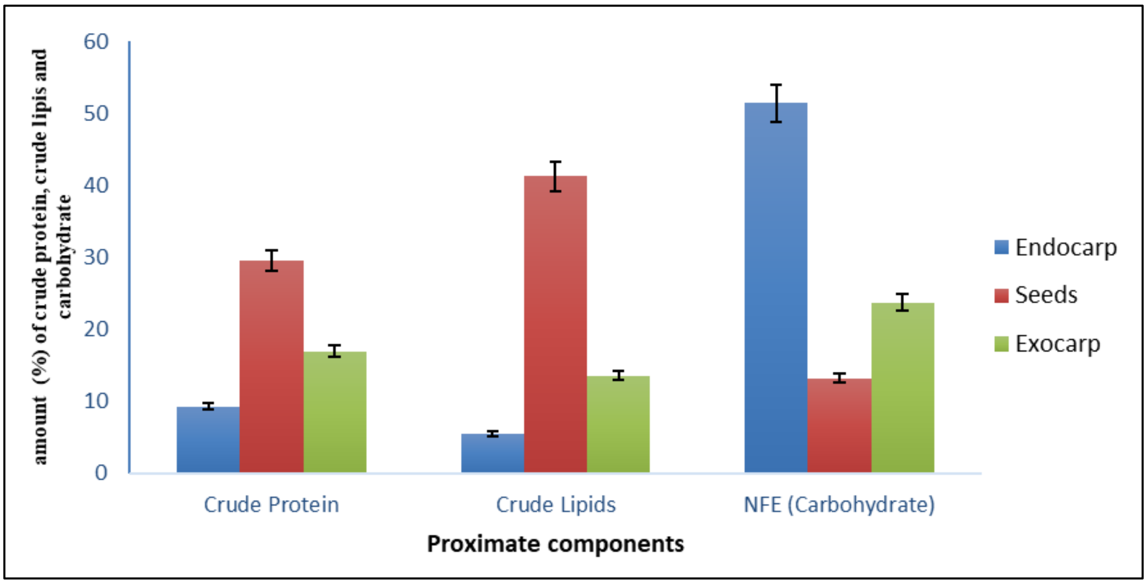

Fig 4: Proximate composition of crude protein, crude lipids and NFE (Carbohydrate) in the endocarp, seeds and exocarp of the watermelon fruit. All values represent mean ± standard error of mean (SEM) of triplicate determinations (n = 3) and $p<0.05$ was considered significant.

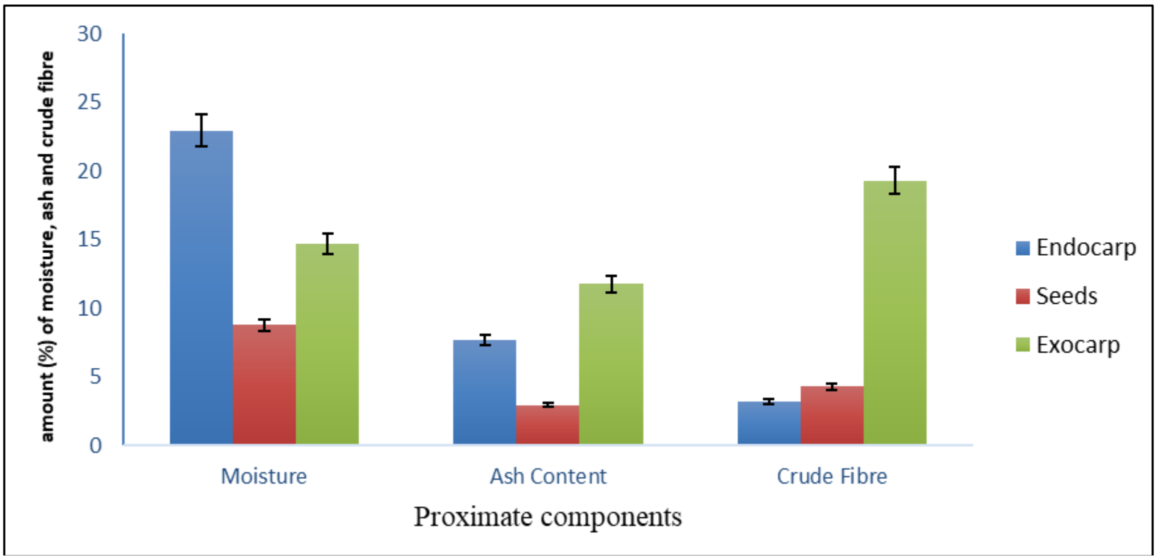

Fig 5: Proximate composition of moisture, ash content and crude fibre in the endocarp, seeds and exocarp of the watermelon fruit. All values represent mean ± standard error of mean (SEM) of triplicate determinations (n = 3) and $p<0.05$ was considered significant.

Discussion

Phytoconstituents in plant parts like leaves, fruits, seeds, flowers, roots, stem, and bark has attracted the attention of plant researchers due to their biological activities as well as efficacies relevant in the development of new and modern drugs for the pharmaceutical industry [26]. Numerous techniques are available to carry out phytochemical studies that play a significant role in the search for additional sources of secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, cyanogenic glycosides, saponins etc., [27, 28]. Many plant secondary metabolites are known to provide protection and treatment roles against various human disorders, including but not limited to microbial infections, cancer, diabetes, hypertension and Alzheimer's disease [27]. There are also phytochemicals with relevant physiological properties which may be elements/minerals rather than complex organic molecules.

The qualitative phytochemical analysis obtained in this study (Table 1) revealed that in all parts of the watermelon fruit used (endocarp, seed and exocarp) there were no steroids, tannins and anthocyanins. However in the endocarp, there were high levels of terpenoids, saponins and carotenoids but alkaloids and cyanogenic glycosides were absent. The exocarp on the other hand indicated relatively high levels of alkaloids and cardiac glycosides. Whereas moderate amounts of terpenoids, alkaloids, cardiac glycosides and cyanogenic glycosides were observed in the seed. These results agree with previous authors [29], who reported that the aqueous extract of the mesocarp, juice and seeds of the watermelon fruit qualitatively contained terpenoids, cardiac glycosides and saponins on the one hand, but on the other hand lacked steroids and tannins.

The quantitative phytochemical screening of the aqueous extracts of the endocarp, seed and exocarp of the watermelon fruit (Table 2) revealed that the concentration of alkaloids, total phenols, cyanogenic glycosides and anthraquinones were significantly higher in the exocarp relative to the fleshy endocarp and the seeds while saponins, flavonoids, antioxidants and terpenoids were significantly higher in the endocarp. These results partially agrees with the report by previous authors [30], who established that the watermelon rind (exocarp) comparatively contained a higher concentration of alkaloids, and phenols compared to the seeds although the values obtained were obviously lower. A previous author [30] also established that tannins were present in both the rind and seed, which was not the case in the present study as well as that reported previous authors [29]. The likely reason for the variations in the results obtained in the different studies might have been due to differences in the methods of analysis of the phytochemicals or the source of the samples used. Alkaloids are organic nitrogenous compounds, said to exhibit remarkable physiological activity in animals [31]. Isolated plant alkaloids and their synthetic derivatives are basic medicinal agents used as central nervous system stimulants, topical analgesics, ophthalmologic, antispasmodic and bactericidal effects [31]. The absence of tannins in this study may suggest that the samples lack astringent and antimicrobial properties [32], but it is worthy of note that other available phytochemicals might have these functions e.g. phenolic compounds function as antimicrobial compounds that can get rid of plant pathogens [31].

In this study, the flavonoid concentration in the endocarp was significantly higher when compared to the seed and exocarp and this agreed favourably with the report by a previous author [29]. However, the flavonoid level in the seed was insignificantly higher compared to the exocarp in this study, but the reverse was the case in the report of a by previous author [30]. Flavonoids are water-soluble plant phenolics that have been shown to have antibacterial, antifungal, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory activity [33]. The potent antioxidant activity of flavonoids and their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals may aid in related disorders [32]. Thus the availability of flavonoids in the samples suggests that they may offer protection against ailments related to free radicals, bacterial and fungal activities [32].

There were no cardiac glycosides in the endocarp while the amount in the exocarp and seed were low and hence in agreement with the report of a previous author [34] who suggested that for *Tapinanthus preussii* leaves it was quite minimal in both the aqueous and ethanolic extracts. They are important in ethno medicine due to their action in managing congestive heart failure as well as cardiac insufficiency [35]. They inhibit the Na^{+}/k^{+} pump resulting in the elevation of sodium ions in the myocytes, leading to a rise in the level of calcium ions [36].

Inorganic mineral elements are considered to be essential in human nutrition and generally, those from plant sources are less-bioavailable than those from animal sources. In this study, potassium, phosphorus, magnesium, zinc and calcium were higher in the exocarp, hence correlating with the ash content of the exocarp (which indicated high mineral content). The results obtained by a previous study that agreed favourably with this study [37]. The minerals present in all the samples are vital for the overall mental and physical wellbeing, and are important constituents of bones, teeth, tissues, muscles, blood and nerve cells [38].

Magnesium is a component of chlorophyll and it is an important content in connection with Ischemic heart disease and calcium metabolism in bones. Zinc is associated with protein metabolism and also involved in the normal functioning of the immune system. Iron is an essential trace element for haemoglobin formation as well as other hemoproteins, normal functioning of central nervous system and in the oxidation of carbohydrate, protein and fats. Deficiencies of these minerals are known to adversely affect the performance and health of humans.

The proximate composition of this study revealed that the crude protein and crude lipid contents in the seeds were significantly higher when compared to the endocarp and exocarp. However, there was a higher carbohydrate and moisture content in the endocarp relative to the other parts. The report by a previous study revealed that the Rosmas watermelon seed variety contained more moisture, crude fibres and crude fats than the Sassako variety [37]. The moisture content of any food substance is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination, hence spoilage. These slightly high moisture contents also mean that dehydration would increase the relative concentration of other food nutrient and therefore improve the shelf-life or preservation of the fruit.

The crude fiber content was very high in the exocarp. Crude fiber is the part of food that is not digested by humans but the normal functioning of the intestinal tract depends upon the presence of adequate fiber. It increases stool bulk and decreases the time that waste materials spend in the gastrointestinal tract. Fiber helps to reduce cholesterol level of the body hence the maintenance of human health [39]. A low fiber diet has been associated with heart disease, cancer of the colon and rectum, varicose veins, phlebitis, obesity, appendicitis, diabetes and even constipation [40, 41]. Hence watermelon exocarp could be recommended as a veritable crude fiber source in the diet as a result of its relatively high fiber content.

The proportion of ash content is a reflection of the mineral contents present in a food material [42]. The results obtained from this study therefore, suggests a high deposit of mineral elements in the watermelon exocarp since the ash content was higher in this part relative to the endocarp and seed. The amount of crude protein was highest in the seed of the present study as was the case in the Sassako variety of the *Citrullus lanatus* seeds [37]. It was previously reported that a diet is nutritionally satisfactory if it contains high calorie value and a sufficient amount of protein [43]. It was stated in a previous in a study that any plant food that provide about 12% of their caloric value from protein are considered good sources of protein [44]. Based on the afore mentioned report, watermelon seeds meet this requirement.

Conclusion

Knowledge of the phytochemical, proximate and mineral composition in a food substance provides an idea of its pharmacologic, dietary and toxic potentials. It was on this premise that the quantitative analysis on parts of the watermelon fruit (endocarp, seed and exocarp) was carried out and it revealed a comparatively, significant concentration of several important and powerful phytoconstituents whose relevance cannot be overemphasized. The exocarp and seed of watermelon (*Citrullus lanatus*) are generally discarded as food wastes in Nigeria and most parts of the world where the fruit is consumed. Therefore, utilizing assumed plant wastes in ethno medicine and advisable healthy diets, could improve healthcare, food supply and the environment. It can therefore be suggested that the whole fruit may be utilized as neutraceuticals, food additives and supplements to prevent or manage some human health disorders through its remarkable antioxidant potentials. Hence, this study concludes that it is beneficial to consume the watermelon fruit whole to derive maximum nutrients and relevant phytoconstituents from it.

Recommendations
The entire watermelon fruit was found to be rich in several relevant phytoconstituents, implying that, the whole fruit may be consumed contrary to the common practice of discarding the exocarp and seeds. Further studies therefore needs to be carried out in order to isolate, identify, characterize, elucidate the mode of action as well as the toxicological effects of the whole wide range of bioactive constituents implicated in ameliorating several disorders. Eventually there will also be a need to standardize, formulate and commercialize these phytomedicines to health care providers.

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