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Evaluation of Bioactive Components, Antioxidant Potentials, and Mineral Composition of Aqueous Extracts of Watermelon Pulp, Seed, & Rind (*Citrallus Lanatus*)

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Abstract - Watermelon is a sweet juicy fruit, containing mainly three parts (pulp, seed and rind), which is consumed due to its thirst-quenching ability and low in calorie nutritional status. However, many consumers only consume the pulp, neglecting other parts (seed and rind), turning them to waste products, and these neglected parts have been reported to possess some nutritional potentials. Hence, this study sought to investigate the nutritional (mineral) compositions, bioactive compounds, and antioxidant abilities of the aqueous extracts of watermelon fruit (pulp, seed and rind). In the mineral composition studies, Ca, Mg, K, Na, Mn, Fe, Cu, P, and Zn were analysed, the studies revealed that potassium (K) was the most abundant mineral in the fruit with watermelon rind having the highest value (32625mg/l), watermelon pulp was higher with the value (13757mg/l) than watermelon seed having the least value (6053mg/l). Copper (Cu) was the least abundant mineral in the fruit, watermelon seed had the highest value (17.1mg/l), watermelon pulp had higher value (6.20mg/l) than watermelon rind with least value of 6.00mg/l. Furthermore, the antioxidant potentials (2,2- azinobis 3-ethylbenzo-thiazoline -6-sulfonate (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Total phenol, and Total flavonoid) of the aqueous extracts of watermelon fruit (pulp, seed and rind) were carried out and the results showed that watermelon seed was the richest in total phenolic content having (4.50 ± 1.17mg GAE/100g), watermelon rind was richer (0.56 ± 0.13mg GAE/100g) than watermelon pulp (0.30 ± 0.07mg GAE/100g). The total flavonoid assay showed the seed extracts with highest value (8.29 ± 2.06mg QE/100g), the pulp extract was higher (5.07 ± 0.71mg QE/100g) than the rind extract (4.71 ± 1.36mg QE/100g). The ABTS assay also revealed that watermelon pulp, seed, and rind had (32.14 ± 2.16mmol TEAC/100g, 11.4 ± 4.63mmol TEAC/100g, and 5. 36 ± 2.12mmol TEAC/100g) respectively. The scavenging abilities of the extracts showed that watermelon rind had highest scavenging ability (49. 77 ± 3.04 mgMAE/ml), while watermelon seed had higher scavenging ability (51.59 ± 7.86mgMAE/ml) than watermelon pulp having (59.37 ± 4.77 mgMAE/ml). Bioactive compounds such as flavonoid, saponin, alkaloids, cardiac glycosides & cardinocides and terpenoids were present in the whole watermelon fruit (pulp, seed and rind) while tannin and chalcone were not present in the whole fruit. The studies revealed that the neglected parts (seed and rind) exhibited significant antioxidant potentials which may inhibit overproduction of oxidants involved in the pathogenesis of many chronic diseases.

Keywords: watermelon pulp, watermelon rind, watermelon seed, antioxidants, phytochemicals

1. Introduction

Watermelon (*Citrallus lanatus*) is an important horticultural crop, mostly known for its sweet juicy fruit (Munisse *et al.*, 2011). It belongs to the genus *Citrullus*, which has four species (*C. lanatus*, *C. ecirrhosus*, *C. colocynthis*, and *C. rehmi*) (Shimotsuma, 1963). The sugar content and sweetness are the critical factors in determining the quality of many watermelon varieties. It is known to be low in calories but highly nutritious and thirst quenching. Watermelon can be used as fresh salad, dessert, snack, and for decorations. Drinks can also be made from the juice. In Namibia, the juice is fermented into a refreshing,

lightly alcoholic drink (Okonmah, *et al*. 2011). In some parts of Africa, the rind is sliced, dried, cooked and eaten. Pickled watermelon rind is widely eaten in some parts of USA. The fruit is known to be a good source of lycopene and carotenoid. It helps quench the free radicals that contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis. It is also high in fibre and citrulline; an amino acid the body uses to make arginine (Oyeleke, *et al*, 2012). Watermelon seeds are known to be highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals (Braide *et al*, 2012).

The knowledge of the nutritive and the anti-nutritive content of various parts (seeds, rind and pulp) of the watermelon fruit will encourage their consumption in diverse ways and re-utilisation of the vast amounts of seeds discarded as waste. The nutrient and anti-nutrient value of many fruits, seeds and their rinds have not received much attention and these are at times discarded, even with their hidden nutrients (Johnson *et al.,* 2012). There is also limited literature on the effect of variety on the nutritional, phytochemical and antioxidant properties of the watermelon seeds, rind and pulp. Hence, this study investigated *in vitro* antioxidant activities in the seeds, rind and pulp of watermelon. The seeds, rind and pulp were also screened for the presence of some phytochemicals (qualitatively & quantitatively) and minerals contents.

2.0 Materials & Method

2.1 Samples collection

3 watermelon fruits were bought at capital market in Osogbo, Osun state, Nigeria. The watermelon fruits were cut into small pieces, the seeds were removed from the pulp and finally, the pulps were carefully removed from the rind. The 3 samples (watermelon pulp, rind and seed) were oven-dried at 50ºc for 48hrs, 24hrs and 4hrs respectively. The watermelon dried samples were pulverised to powdery substances by using electric blender.

2.2 Sample preparation

2.2.1 Aqueous extraction of the samples

5g each of the powdery substance of watermelon samples (pulp, rind, and seed) was weighed on analytical weighing balance and dissolved in 50ml of distilled water. It was poured in a reagent bottle and placed on HY-BII speed governing multi-purpose oscillator/shaker for 24hrs for thorough shaking and mixing. Then, each dissolved sample was filtered, using a whatmann No1 filter paper and funnel. The filtrate was centrifuged in medical low speed centrifuge at the speed of 2500 for 10mins. The supernatant was poured into a clean reagent bottle and kept in the refrigerator for further analysis while the sediments were discarded.

2.3 *in vitro* antioxidant assays

2.3.1 Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions of the sample extracts were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as Gallic acid equivalent.

2.3.2 Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. (2005). Briefly 0.5 mL of appropriately diluted sample extracts was mixed with 0.5 mL methanol, 50 µL 10% $AlCl_3$, 50 µL 1 M Potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). The total flavonoid content was subsequently calculated using quercetin as standard.

2.3.3 ABTS radical scavenging ability

The $ABTS^{\cdot}+$ (2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging ability of the watermelon samples (pulp, rind, and seed) extracts were determined according to the method described by Re et al. (1999). The $ABTS^{\cdot}$ was generated by reacting an (7 mmol/L) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in the dark for 16hrs and adjusting the Absorbance at 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extract was added to 2.0 mL $ABTS^{\cdot}+$ solution and the absorbance were measured at 734 nm after 15min. Trolox was used as standard and trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

2.3.4 Determination of ferric reducing antioxidant property (FRAP)

The reducing property of the aqueous extracts from watermelon samples (pulp, rind, and seed) were determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). 2.5ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50^0 C for 20 min. and then 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

2.3.5 1, 1-diphenyl–2 picrylhydrazyl radical scavenging ability (DPPH)

The free radical scavenging ability of the extracts against 1,1-diphenyl–2 picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi et al. (1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated as percentage of the control.

2.4 Qualitative phytochemical analysis

Qualitative phytochemical analysis of aqueous extracts of watermelon samples (pulp, rind, and seed) were carried out on the extracts using standard procedure to detect the bioactive constituents as described by Sofowora (1993), Trease and Evans (1989a) and Harbone (1973).

2.4.1 Test for Taninns: 1ml of each extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue – black coloration which confirmed the presence of tannin.

2.4.2 Test for Saponin: About 5ml of each extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filterate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirmed a positive presence of Saponin.

2.4.3 Test of Flavonoids: 3ml of 1% Aluminium chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution was added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicated a positive test for flavonoids.

2.4.4 Test for Steroids: 2ml of acetic anhydride was added to 2ml extract of each watermelon samples (pulp, rind, and seed), followed by careful addition of 2ml H_2SO_4. The change in color from violet to blue or green indicated the presence of steroids.

2.4.5 Test for Terpenoids (Salkowski test): 5ml of each the extract was mixed with 2ml of chloroform, and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration on the interface was formed to show positive results for the presence of terpenoids.

2.4.6 Test for Cardiac Glycosides and Cardenolides: 5ml of each watermelon extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristics of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glycoside.

2.4.7 Test for Alkaloids: 1ml of each extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of Wagner's reagent (solution of iodine in Potassium iodide). The formation of a cream colour gave a positive test for alkaloids.

2.4.8 Test for Chalcones: 2ml of ammonia solution was added to 5ml of watermelon extracts. Formation of a reddish color confirmed presence of chalcones.

2.4.9 Test for Phenol: 5ml of each extract was pipetted into a 30ml test tube, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added and left to react for 30min. Development of bluish -green color was taken as a positive presence of phenol.

2.5 Quantitative phytochemical analysis

2.5.1 Determination of total alkaloids: It was carried out according to the method of Harborne, (1973). 5g each of watermelon sample (pulp, rind, and seed) was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

2.5.2 Determination of total saponins: It was carried out according to the method of Obdoni et al; (2001). 20g of each watermelon sample (pulp, rind, and seed) was put into a conical flask and 100cm3 of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated.

2.6 Mineral composition analysis.

Standard preparation: Standard solutions were prepared before metal concentrations were determined and were used to eliminate sample standard matrix indifferences. They were also used to calibrate the AAS instrument for all the analytes and also to prepare their calibration curves one after the other using $C_1V_1=C_2V_2$ from the stock solution.

Digestion: The digestion process was carried out by using the method of Sharma et al; (2009). 1.0g of each of the watermelon fruit sample was weighed and transferred into a separate 50ml conical flask. 10.0ml of Concentrated $HOCl_3$ and HNO_3 (1: 3) was added to it and heated to about 115^oc for about 90 minutes until it gave a clear solution. The solution was allowed to cool and filtered before it was made up to the mark of 25.0ml of volumetric flask. Blank was also prepared in the same way but the sample was

replaced with distilled water. Lanthanum was added to these solutions to prevent potential anionic inference. The absorbance was read at 690nm using Atomic Absorption Spectrophotometer (AAS).

3.0 Results

Table 1. Total phenol, total flavonoid, ABTS, and FRAP of aqueous extracts of watermelon fruit (pulp, seed & rind).

Sample	Total Phenol (mgGAE/100g)	Total Flavonoid (mgQE/100g)	ABTS (mmol/TEAC/100g)	FRAP (mmolAAE/g)
Watermelon Pulp	0.30 ± 0.07	5.07 ± 0.71	32.14 ± 2.16	0.81 ± 0.06
Watermelon Seed	4.50 ± 1.17	8.29 ± 2.06	11.4 ± 4.63	1.04 ± 0.01
Watermelon Rind	0.56 ± 0.13	4.71 ± 1.36	5. 36 ± 2.12	0.97 ± 0.09

Values represent means ± standard deviation of triplicate readings.
GAE = Gallic Acid Equivalent
QE = Quercetin Equivalent
AAE = Ascorbic Acid Equivalent
TEAC = Trolox Equivalent Antioxidant Capacity

Table 2. 1,1-diphenyl-picrylhydrazyl (DPPH) radicals scavenging abilities of the aqueous extracts of watermelon fruit (pulp, seed & rind).

Assay	Watermelon Pulp	Watermelon Seed	Watermelon Rind
DPPH (mgMAE/ml)	59.37 ± 4.77	51.59 ± 7.86	49. 77 ± 3.04

Values represent means ± standard deviation of triplicate readings.

Table 3. Mineral composition results.

Sample	Ca (mg/l)	Mg (mg/l)	K (mg/l)	Na (mg/l)	Mn (mg/l)	Fe (mg/l)	Cu (mg/l)	P (mg/l)	Zn (mg/l)
W. Pulp	1110.75	1520.15	13757	1205.5	5.65	81.75	6.20	150.50	22.15
W. Seed	190.15	1900.55	6053	902.00	19.55	60.05	17.1	234.75	36.4
W. Rind	2157.9	2757.50	32625	2119	22.45	104.25	6.00	136.31	32.9

Values represent means ± standard deviation of triplicate readings.
W= Watermelon

Table 4. Qualitative phytochemical screening of the aqueous extracts of watermelon fruit (pulp, seed & rind).

Phytochemical Assay	Watermelon Pulp	Watermelon Seed	Watermelon Rind
Saponin	+ve	+ve	+ve
Tannin	-ve	-ve	-ve

Alkaloid	+ve	+ve	+ve
Cardiac glycoside & Cardinocides	+ve	+ve	+ve
Steroids	+ve	+ve	-ve
Terpenoids	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve
Phenol	+ve	-ve	+ve
Chalcone	-ve	-ve	-ve

+ve = Present
-ve = Not present

Table 5. Quantitative phytochemical screening of the aqueous extracts of watermelon fruit (pulp, seed & rind).

Phytochemical Assay	**Watermelon Pulp**	**Watermelon Seed**	**Watermelon Rind**
Total saponin (%)	1.39	0.90	1.80
Total Alkaloid (%)	0.80	0.81	0.22

4.0 Discussion

Overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body is responsible for the pathogenesis of some diseases. The scavenging effects of these oxidants are thought to be an effective measure to depress the level of oxidative stress of organisms (Wang *et al*., 2013). The neglected parts (seed and rind) of the watermelon fruit exhibited significant higher antioxidant abilities than the usual consumed part (pulp) and it has been reported by Deng et al; (2012) that fruits and vegetables that are rich in antioxidants have health benefits due to important role they play in the prevention and management of chronic diseases caused by oxidative stress. They are also the basis of other bioactivities and health benefits, such as anti-inflammatory action, anti-cancer, anti-aging, and protective action for cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative diseases. Oxidative stress has been implicated as one of the culprits in the pathogenesis of neurodegenerative diseases ensuing atrophy of neurons and degeneration of the brain tissue (Oboh *et al*., 2012). Neuronal cells and sensitive organs in the body are to be protected against exacerbating effects of free radicals and reactive oxygen species because once the cells and organs are damaged, they can hardly be regenerated. Therefore, augmenting the body's antioxidant status through dietary means could be a practical and economical means of attenuating oxidative stress (Deng *et al*., 2012). This present study revealed that watermelon seed and watermelon rind extracts had higher scavenging abilities than watermelon pulp extracts, which could mop up free radicals that may initiate processes leading to several chronic and degenerative diseases. The study also correlated with the submission reported by Mohd Khairul Amran Mohammad et al; (2014), that watermelon juice possessed nutritional and medicinal benefits in modulating the oxidative damage induced by low dose X-ray exposure in terms of suppressing the level of malondialdehyde (MDA) while enhancing the levels of superoxide dismutase (SOD) and glutathione (GSH) activities in mice.

The neglected parts (seed and rind) may have protective roles against chronic diseases due to the presence of some bioactive compounds such as saponin, alkaloids, flavonoids, cardiac glycoside & cardinocides, terpenoids, and phenols. In medicine alkaloid-containing plants, e.g quinines are used as antimalarial drugs, reserpines are used as antihypertensive drugs, morphines are used as analgesic drugs, etc. (Manfred, 2002). This present study is in line with the previous study reported by Gill et al; (2011), he documented the presence of alkaloids, steroids, terpenoids, and flavonoids in the methanolic and hydromethanolic extracts of watermelon seed extracts. Clinical studies had investigated the relationship between flavonoid consumption and cancer prevention, the studies revealed that dietary flavonoid intake can be associated with reduced gastric carcinoma risk in women and reduced aero-digestive tract cancer risk in smokers. (Gonzalez *et al*., 2013 and Woo *et al*., 2013). Bhattacharya et al; (2010) reported that fruits or plants that contain phenol can act as protective agents and inhibitors against toxins that could cause cell damage in the body. Active compound like saponin and alkaloids have been discovered to be

very potent against clinical pathogens such as *Escherichia coli*, *Salmonella typhi* and *Staph aureus* (Ajibade and Falegan, 2007).

Micronutrients and macronutrients are essential nutrients required by the body in small and large quantities respectively throughout life to orchestrate a range of physiological function to maintain good health. (Gernad *et al*., 2016 and Tucker, 2016). The watermelon seed and rind exhibited much mineral compositions which make them essential as part of watermelon fruit. In this study, potassium was the most abundant mineral in the watermelon fruit with watermelon rind having the highest potassium content (32625mg/l) and watermelon pulp having higher potassium content (32625mg/l) than watermelon seed (6053mg/l). The neglected parts of the watermelon fruit (seed and rind) exhibited significant mineral compositions which make them essential dietary mineral sources. Aburto et al; (2013) and D'Elia et al; (2011) reported that diets low in potassium increase risk of hypertension, stroke and cardiovascular disease. Nabila et al; (2013) also reported that a severe shortage of potassium in the body fluids may cause a potentially fatal condition known as hypokalemia, which results from loss of potassium through diarrhea, dieresis and vomiting, this may lead to muscle weakness and cramps, respiratory paralysis, and paralytic ileus.

5.0 Conclusion

The study has been able to reveal the bioactive compounds that are present in the watermelon whole fruit (pulp, seed and rind) which may be responsible for their potencies in prevention and management of some chronic and degenerative diseases caused by oxidative stress. The neglected parts (seed and rind) exhibited significant antioxidant potentials which may inhibit overproduction of oxidants involved in the pathogenesis of many chronic diseases.

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