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Research article

Studies of Phytochemical and Antioxidant properties of the Fruit of Watermelon (*Citrullus lanatus*). (Thunb.)

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Abstract:

The present study was undertaken to investigate the medicinal effect of the fruit of watermelon (*Citrullus lanatus*, family *Cucurbitaceae*). The fruit was obtained from Owo market in Ondo State and analyzed qualitatively and quantitatively for some basic phytochemicals of antioxidant importance in the various parts of the fruit using standard methods. The results of the antioxidant properties showed that the highest dose of 200 μ L possessed higher concentrations of flavonoids and total phenol in all extracts of the various parts investigated while lower dose of 50 μ L produced the highest percentage of iron chelation ability in all the parts investigated. Similarly, in this study it was also confirmed that the fruit possess the ability to scavenge free radicals and reactive oxygen species with the seed possessing the highest ability against DPPH at the least concentration of 150 μ L from the range of (150 – 500) μ L investigated. The results obtained from this study however indicate the possibility of utilizing the fruit as nutraceuticals or functional food to prevent or manage some critical diseases of man through scavenging or inhibition of some destructive species or radicals.

Key words: Diseases, antioxidant, concentration, consequence, medicine.

Introduction:

Watermelon (*Citrullus lanatus*, family *Cucurbitaceae*) is a vine-like (scrambler and trailer) flowering plant originally from southern Africa. Its fruit, which is known as a watermelon is a special kind of fruit known by botanists as a pepo, a berry which has a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Watermelon is one of the major under-utilized fruits grown in the warmer part of the world. The juice or pulp from watermelon is used for human consumption, while rind and seeds are major solid wastes^[4,21,1]. The rind is utilized for products such as pickles and preserves, as well as for extraction of pectin^[20,10]. The therapeutic effect of watermelon has been reported and has been ascribed to antioxidant compounds^[22,23]. Among them, citrullin and lycopene have been demonstrated to play a prominent role in the treatment and management of ailments such as cancer and cardiovascular diseases^[37]. The rind is usually discarded, applied to feeds or fertilizer. But they are also edible, and sometimes used as a vegetable. In China, they are stir-fried, stewed, or more often pickled^[3]. When stir-fried, the de-skinned and de-fruited rind is cooked with olive oil, garlic, chili peppers, scallions, sugar and rum. Pickled watermelon rind is also commonly consumed in the Southern US, Russia, Ukraine, Romania and Bulgaria^[35]. In traditional Chinese medicine, watermelon rind is extensively applied to clear away heat to eliminate toxic substances and its extract is available in powdered forms^[38]. In Nigeria, watermelon rinds are fermented, blended and consumed

as juice. High antioxidant activities have been reported on food products via microbial fermentation^[29,39]. Antioxidant protects against many ailments caused by reactive oxygen species (ROS)^[39]. Watermelon is a very rich source of vitamins, can be served for breakfast, as an appetizer or snack, depending on how it is prepared^[14]. It also serves as a good source of phytochemical and lycopene, a red carotenoid pigment which acts as antioxidant during normal metabolism and protects against cancer^[27]. One generous slice of watermelon (about 1/16th of a melon) contains large amounts of vitamin C and beta-carotene which may help protect against various forms of cancer due to their antioxidant properties. Watermelon is also high in potassium which helps regulate

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heart functions and normalize blood pressure. It is a good source of fiber which helps maintain bowel regularity and works to prevent colon and rectal cancer. Watermelon seeds contain cucurbitocitrin to aid in lowering blood pressure and improve kidney function. The sweet watermelon surprisingly has only half the sugar content (5 percent) of an equivalent-sized apple. It tastes sweeter because the sugar is its main taste-producing agent. Two cups of watermelon has only 80 calories, no fat, and no cholesterol^[9]. This study aims at investigating the medicinal consequence of the various parts of the watermelon fruit by qualitatively and quantitatively estimates some antioxidant parameters and scavenging ability of the fruit against free radicals.

Material and methods

Materials

Citrulus lanatus was collected from Owo market, Ondo State. They were stored in a conducive atmosphere prior to analysis.

Sample extracts of watermelon fruit for analysis

The water melon fruits were divided into different parts: the seeds, mesocarp, juice and a mixture of the whole fruit. The seeds were grinded fresh and extracted with distilled water; aqueous extract was obtained by filtration. The juices were also filtered using filter paper to remove traces of the mesocarp. The mesocarp was grinded into viscous liquid and finally, the whole fruit was grinded into a viscous liquid. On extraction, clear filtrates were obtained by filtering through Whitman's No1 filter paper.

The phytochemical screening of the various parts of the watermelon fruit were carried out to ascertain the qualitative composition.

Quantitative phytochemical estimation

Alkaloids determination

0.2ml dilution of each extract was measured into a 250ml beaker and 50ml of 10% acetic acid in ethanol was added and allowed to stand for some minutes. This was filtered and the extract was concentrated on a water bath for 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 is the alkaloid which was dried and weighed and calculated method^[13].

Saponin determination

0.2ml dilution of each extracts was placed into a test tube with propan-2-ol for a day in the ratio 1:10 for each sample, 2ml of 40% solution of magnesium sulphate ($MgSO_4$) solution was added. The mixture obtained was filtered through No 1 Whatman filter paper obtaining a clear colorless solution. 5% $FeCl_3$ solution was added and allowed to stand for 30 minutes, a red color was observed and the absorbance was read at 380nm and saponin content was calculated accordingly.

Determination of antioxidant properties

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by [15]. Each extracts was measured into three test tubes in the range of 50, 100, and 200 μ L and each was mixed with 500 μ L of methanol. Water was added to mark up to 200 μ ml. 50 μ ml 10% $AlCl_3$ followed by 50 μ L of 1M potassium acetate and 1400 μ L water was added and allowed to incubate at room temperature for 30mins. The absorbance of the reaction mixture was subsequently measured at 415nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

Iron ion (Fe^{2+}) chelation determination

The Fe^{2+} chelating ability of the extracts were determined using a modified method of^[17] with a slight modification by^[28]. 25, 50, 100 μ L of each extract was measured into three test tubes. 168 μ L of 0.1M Tris HCl buffer pH 7.4 ($C_6H_{11}NO_3$) was added and made up with water in the range of 657, 632, 552, 432 μ ml. 150 μ L of freshly prepared 500 μ mol L^{-1} $FeSO_4$ was added to all, and incubated for 5mins at room temperature. 13 μ L of 0.25%1,10 ortho-phenanthrolin ($C_{12}H_8N_2$) was added and traces of pink color was observed which indicate Fe^{2+} chelation and these was read at 510nm using spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated with respect to the reference (which

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contains all the reagents without the test sample).

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.*,^[32]. Briefly, appropriate dilution of the extracts were oxidized with 2.5ml of 10% Foli-Ciocalteau's reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate to pH 7.4. The reaction mixture was incubated for 40mins at 45°C and the absorbance was read at 765nm in the spectrophotometer. The total phenol content was subsequently calculated as Gallic acid equivalent.

for 30mins and the absorbance was taken at 516nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Statistical analysis:
Statistical significance was

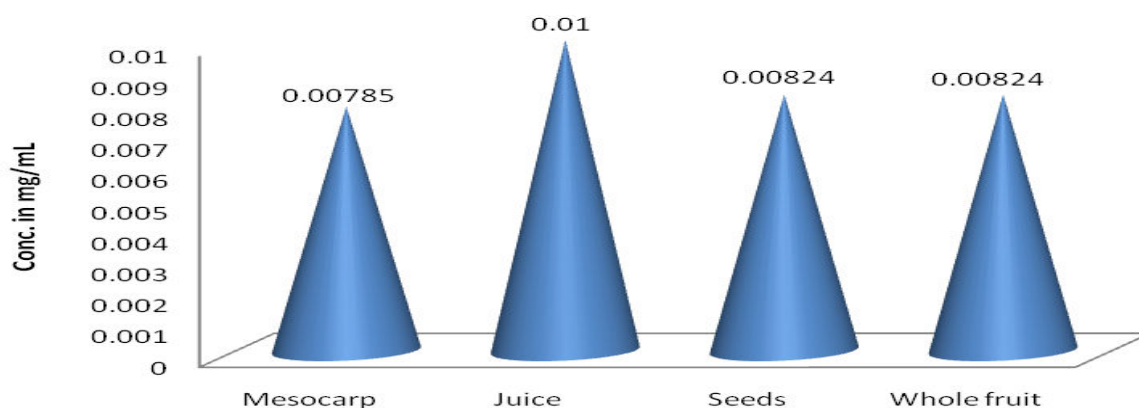


Figure1. Total flavonoid content of the various parts of watermelon

1, 1-Diphenyl-2 picrylhydrazyl free radical scavenging ability (DPPH ASSAY)

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by^[12]. Appropriate dilution of each of the extracts was measured in the range 150µL, 300µL, 400µL, 500µL into four different test tubes and water was added to make up the mark to 500µL. these was mixed with 1ml of 0.4mM methanolic solution containing DPPH radicals, the mixture was incubated in the dark

established using One-Way Analysis of Variance (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 17.0 (SPSS Inc. Chicago, IL,USA).

Results:

Table 1. Qualitative phytochemical composition of various parts of watermelon

PHYTOCHEMICALS	SAMPLES			
	Mesocarp	Juice	Seeds	Whole fruit
Saponnin	+	+	+	+
Tannin	-	-	-	-
Anthraquinone	+	+	-	+
Flavonoids	+	+	+	+
Steroids	-	-	-	-
Terpenoids	+	+	+	+
Alkaloids	+	-	+	+
Phlobatannin	-	+	-	+
Cardiac glycosides				
Keller killiani	+	+	+	+
Legal test	+	+	+	+
Steroid	-	-	-	-
Flavonoid	+	+	+	+

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Table 2. Some quantitative phytochemical contents of the various parts of watermelon

Parts of the fruit	Phytochemical Concentrations	
	Saponin (mg/mL)	Alkaloids (%)
Mesocarp	1.56±0.04	71.2±0.4
Juice	2.08±0.03	ND
Seeds	1.55±0.05	33.77±0.4
Whole fruit	1.22±0.09	50.8±0.35

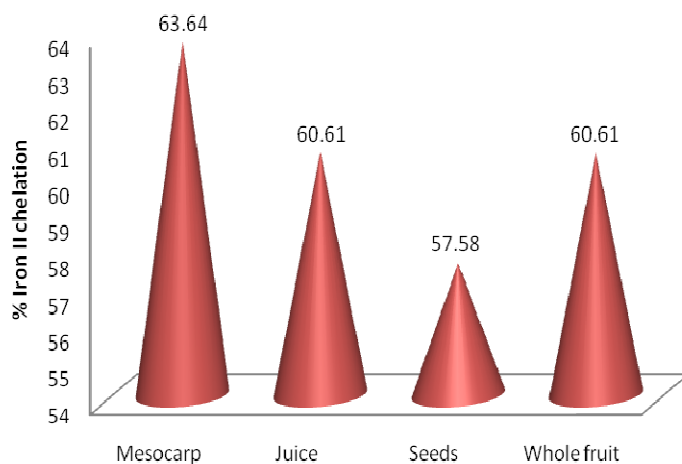


Figure 2. Fe²⁺ chelating ability of the various parts of watermelon

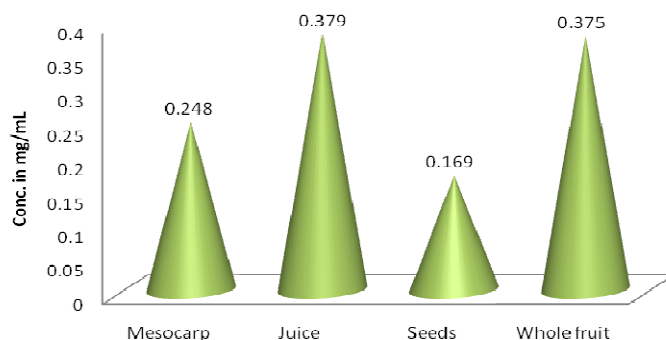


Figure 3. Total Phenol content of the various parts of watermelon

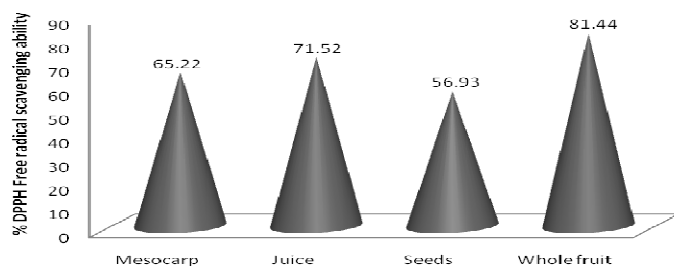


Figure 4. DPPH free radical scavenging ability of the various parts in 150µL of the extract

Discussion:

The phytochemical screening of the various parts of the watermelon showed that saponin, flavonoids, terpenoids and some basic cardiac glycosides were present as tannin and steroids were not detected in all the parts investigated while alkaloid, phlobatanin and anthraquinone were not detected in the juice, mesocarp and seed respectively in Table 1. The presence of cardiac glycosides which several mechanisms have been proposed with the most widely accepted involves the ability of cardiac glycosides to inhibit the membrane bound Na⁺-K⁺-ATPase pump responsible for Na⁺-K⁺ exchange, and are used as heart drugs^[6]. The quantitative estimation in percentage and mg/mL of some of these crude chemical constituents of the watermelon. Table 2 showed that it is rich in alkaloids (33.70–71.20%) with the highest concentration in mesocarp followed by whole fruit and the seed, though alkaloids was not detected in the juice of the fruit, it has been speculated widely that alkaloids ranked the most efficient therapeutically significant plant secondary metabolite and are widely used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects^[36,34]. While the estimation of saponins in this work revealed that the juice contained the highest content with 2.08±0.03mg/mL followed by the mesocarp, seed and the whole seed with (1.56±0.04; 1.55±0.05 and 1.22±0.09)mg/mL respectively. Saponin has been reported to show medicinal as well as exhibiting physiological activities. A high saponin content of 11.48± 0.10 and 1.24±0.30mg/100g had earlier

been reported for *G. kola* and *A. melegueta* respectively which were observed to possess inhibiting roles on microorganisms that explained their utilization in traditional medicine as a cough suppressant, an anti-tumor agent and an aphrodisiac^[19]. The saponin reported in this work is however higher than the previously reported which implied that the fruit of watermelon have high quantity of saponin content with general characteristics that include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness^[18,33] and hence its usefulness and importance in preventing some diseases in man. The quantitative estimation of flavonoids as antioxidant in this work ranged from (0.00785- 0.01mg/mL) which showed that the juice of the fruit contained the highest concentration 0.01mg/mL compared with least concentration of 0.00785mg/mL in the juice as observed in Figure 1. The biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors^[19]. Flavonoids represent the most common and widely distributed groups of plant phenolics. Flavonoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis^[31,19]. It has been reported that flavonoids in intestinal tract lower the risk of heart disease and as antioxidants flavonoids from *G. kola* and *A. melegueta* seeds provide anti-inflammatory action^[19]. However, the flavonoid content of this fruit of watermelon quit outweighs that reported for *G. kola* and *A. melegueta* consequently, there is possibility of utilizing the fruit of watermelon as nutraceuticals or functional food to prevent or manage some critical diseases of man through scavenging or inhibition of some destructive species or radicals.

The reducing powers of various parts of the watermelon were assessed based on their ability to reduce Fe (III) to Fe (II) and the results are presented in Figure 2. The reducing power which is a novel antioxidation defense mechanism was determined by measuring the percentage iron chelation of the various parts of the watermelon and as was observed in this investigation it revealed the range of (57.58-63.64%) with highest concentration in mesocarp (63.64%) and least in the seed (57.58%) as showed in Figure 2. The high content of reducing power observed in this work however, explains the medicinal importance and usefulness of watermelon. Figure 3 showed the amount of total phenol obtained in this investigation which ranged from (0.169-0.379mg/mL) with the juice having the highest amount of 0.379mg/mL than all the other parts while the seed recorded the least concentration of 0.169mg/mL, Phenolic compounds have been reported to protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of total phenols and flavonoids is principally based on the structural relationship between different parts of their chemical structure^[30]. Natural polyphenols are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α -tocopherol radicals, and

inhibiting oxidases^[2]. However, the results obtained in this investigation are higher than that reported earlier for some Nigerian medicinal plants ranging from (0.06-0.81%)^[7].

This allusion however suggests the medicinal corollary of the fruit of watermelon. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging ability of extracts from the various parts of the fruit of watermelon as shown in Figure 4 ranged from (56.93-81.44%) which revealed the highest ability for the whole fruit (81.44%) and seed with the least (56.93%). The antioxidant activities of plant phytochemicals occur by preventing the production of free radicals or by neutralizing or scavenging free radicals produced in the body or reducing and chelating the transition metal composition of foods^[24,26]. The prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action^[5].

This high DPPH radical scavenging ability of this fruit extracts could be attributed to the high total phenol and flavonoid content of the fruit. The total antioxidant capacity is a combination of different antioxidant mechanisms, including free radical scavenging ability, reducing power and Fe (II) chelating ability.

Conclusion:

The qualitative screening of the various parts of the fruits revealed some powerful and important phytochemicals of pharmaceuticals and medicinal importance. Since the various antioxidant parameters monitored in the fruit of the watermelon were observed

higher in comparison to some other medicinal plants, it could be inferred therefore that the fruit will be of medicinal importance which suggests the possibility of utilizing the fruit as nutraceuticals or functional food to prevent or manage some critical diseases of man through scavenging or inhibition of some destructive species or radicals.

Recommendation

Based on the findings from this study that showed the whole fruit of water melon including the rind and the seeds to be rich in some of the important phytochemicals, it is recommended that the whole fruit of watermelon (the outer skin, mesocarp, endocarp and the seeds) should be consumed after through washing which is contrary to the practice in the consumption of watermelon especially in Nigeria where only the center (mesocarp and endocarp) are consumed without the outer skin and seed of the fruit. However, further works are to be carried out on this fruit of watermelon to exploit other aspects of benefits to human race.

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