

Quantitative Phytochemical Evaluation and Phenolic Contents Of Extracts Of *Citrullus Lanatus* seed

Research Article

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Abstract

Background: *Citrullus lanatus* (watermelon), a member of the Cucurbitaceae family, contains antioxidant molecules. It is a good source of carotenoids, vitamins, and minerals.

Aim: The present study investigated the phenolic contents of aqueous and methanol extracts of *C. lanatus* seed.

Methods: Aqueous and methanol extracts of *C. lanatus* seed were prepared using standard method. The phenolic contents of the plant extracts were determined using standard procedures.

Results: Of the four phytochemicals quantified alkaloids were present in the highest amount, while phytate was completely absent. The methanol extract had significantly higher total phenol, tannins, flavonoids, and proanthocyanidin contents, relative to the aqueous extract ($p < 0.05$).

Conclusion: These results indicate that *C. lanatus* seed is a good source of phenolic compounds and could be used as a natural constituent of food and medicines.

Keywords: Citrullus Lanatus; Flavonoids; Phenols; Proanthocyanidin; Tannins.

Introduction

There has been an intense search for novel compounds with potent biological effects over the last few decades. Plant-derived drugs have the added advantage of being readily available, effective, and offering a broad spectrum of biological effects. Medicinal plants exert their pharmacological effects via the numerous phytochemicals they contain [1-3].

Citrullus lanatus (watermelon), a member of the Cucurbitaceae family, has a deep green- or yellow-colored smooth thick exterior rind, with gray or light green vertical stripes. The fruit is pink, red or yellow inside with small black seeds embedded in the middle third of the flesh. Generally, *Citrullus lanatus* flesh (juice or pulp) is the main consumable portion; however, the outer rind is also consumed in some parts of the world [4]. *Citrullus lanatus* flesh contains antioxidant molecules such as carotenoids (lycopene and β -carotene), citrulline, minerals like potassium, and superoxide dismutase [5-7]. The rind contains alkaloids, saponins, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber and

carbohydrates [8]. Lycopene from this medicinal plant has been shown to protect against a growing list of cancers [9]. *Citrullus lanatus* seeds are rich sources of protein, B-group of vitamins, minerals (such as magnesium, potassium, phosphorus, sodium, iron, zinc, manganese and copper), as well as fat [10]. The seeds are used to prepare snacks, milled into flour and used for sauces. Oil from the seeds are used for cooking and production of cosmetics [11].

Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. When cells are exposed to oxidative stress a defense system ensures the expression and regulation of antioxidant enzymes as a defense mechanism to protect them from the damaging effect of free radicals. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components [12]. They act by reducing the energy of the free radical or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain re-

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action to minimize the damage caused by free radicals. It has been reported that a substantial link exist between free radicals and more than sixty different health conditions, including aging, cancer, diabetes mellitus, Alzheimer disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, the body's potential to reducing the risk of free radical-related health problems is made more palpable [13]. The aim of this study was to investigate the phenolic contents of aqueous and methanol extracts of *C. lanatus* seed.

Materials and Methods

Plant Sample Collection and Preparation

The plant seeds were obtained from New Benin Market, and identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Preparation and extraction was carried out using standard method [14]. The aqueous and methanol extracts were concentrated using rotary evaporator and made into powder via lyophilisation.

Quantitative Phytochemical Screening

A portion of the pulverized seeds (2g) was defatted with 100 mL of diethyl ether for 2h using a soxhlet apparatus.

Determination of Alkaloids Content

Portion of the defatted sample (5g) was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered, and allowed to stand for 4 h. It was then filtered, and the filtrate was concentrated on a water bath to one-quarter of the original volume. Concentrated NH_4OH was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH , and then filtered. The residue is the alkaloids, which was dried and weighed [15]. The alkaloid content was calculated as shown in Equation 1:

$$\% \text{ Alkaloids} = (W_2 - W_1)/W \times 100 \text{ ----- (1)}$$

Where W = weight of sample; W_1 = Weight of empty filter paper; W_2 = Weight of paper + precipitate.

Determination of Saponins Content

Portion of the defatted sample (20 g) was ground and put into a conical flask, and mixed with 100 mL of 20 % ethanol. The mixture was heated over a hot water bath at about 55°C for 4 h with continuous stirring. The mixture was then filtered and the residue re-extracted with another 200 mL of 20 % ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred to a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded. The process was repeated and 60 mL n-butanol was then added. The combined n-butanol extracts were washed twice with 10 mL of 5 % aqueous NaCl. The remaining solution was concentrated in a water bath, and the resultant concentrate was dried in the oven to constant weight [16]. The saponins content

was calculated as shown in Equation 2:

$$\% \text{ Saponins} = (W_2 - W_1)/W \times 100 \text{ ----- (2)}$$

Where W = weight of sample; W_1 = Weight of empty filter paper; W_2 = Weight of paper + dried saponins.

Determination of Oxalate Content

Exactly 75 mL of H_2SO_4 was added to 1 g of pulverized sample and the mixture was carefully stirred intermittently with a magnetic stirrer for 30 min, and then filtered using Whatman No. 1 filter paper. Aliquot of the filtrate (25 mL) was titrated against 0.05 M KMnO_4 until the appearance of a faint pink color which persisted for 30 sec [17].

$$\text{Oxalate content (mg)} = \text{titre} \times 2.2 \text{ ----- (3)}$$

Determination of Phytate Content

Exactly 100 mL of 2% HCl was added to 0.2 g of pulverized plant sample in 250 mL conical flask for 3h, and then filtered. Aliquot of the filtrate (50 mL) was diluted with 10 mL of distilled water and titrated against standard iron (III) chloride solution which consisted of 0.00195 g FeCl_3 per 1 mL. Appearance of yellow color which persisted for 5 min was taking as the endpoint. Ten (10) mL of 0.3 % ammonium thiocyanate solution was used as indicator [18].

$$\% \text{ Phytic acid} = (\text{Titre value} \times 0.00195 \times 1.19 \times 100)/2 \text{ ---- (4)}$$

Estimation of Total Phenolic Content (TPC)

Total phenolic content was determined according to the Folin and Ciocalteu's method as described by Cicco et al., [19]. Varied concentrations of gallic acid (0.2 - 1 mg/mL) were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin- Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate. The mixture was allowed to stand for 30 min at room temperature, then absorbance was read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the control.

Estimation of Total Tannins Content

Total tannins content was determined using Folin-Denis method with slight modification [20]. Exactly 0.5 mL of 1 mg/mL extract was added to a solution of 0.5 mL Folin-Denis reagent and 1 mL of 7.5 % Na_2CO_3 and mixed thoroughly. Absorbance of the resultant solution was read at 700 nm after dilution with 3.4 mL of distilled water. The total tannins content was expressed as mg tannic acid equivalent (TAE)/g of extract.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the method described by Ayoola et al., [21]. Briefly, 2 mL of 2 % AlCl_3 in ethanol was added to 2 mL of extracts. A concentration of 1 mg/mL of the extract prepared in methanol was used. Similar concentrations of quercetin, the standard control were used. The absorbance was measured at 420 nm after 1 h of incubation at room temperature.

Determination of Proanthocyanidin Content

The determination of proanthocyanidin was carried out according to the method of Sun et al., [22]. To 0.5 mL of 1.0 mg/mL of each extract was added 1 mL of 4 % methanol solution and 0.75 mL of concentrated hydrochloric acid. The mixture was left undisturbed for 15 min and the absorbance was read at 500 nm. Ascorbic acid was used as standard.

Statistical Analysis

Data are expressed as mean \pm SEM. Statistical analysis was performed using SPSS (21.0). Groups were compared using Student's t-test. Statistical significance was assumed at $p < 0.05$.

Results

Results of Quantitative Phytochemical Screening

As shown in table 1, of the four phytochemicals quantified alkaloids were present in the highest amount, while phytate was completely absent.

Phenolic Contents of Extracts of *C. lanatus* seed

The methanol extract had significantly higher total phenol, tannins, flavonoids, and proanthocyanidin contents, relative to the aqueous extract ($p < 0.05$). These results are shown in Figures 1 – 4.

Discussion

Fruits, vegetables and seeds are the main sources of antioxidants in the diet. Oxidative stress results in the damage of biopolymers such as nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates [23]. Bioactive metabolites in plants contribute to their medicinal effects [24]. Antioxidants of nutritional origin play key roles in complementing in vivo antioxidant enzymes and Molecules in the fight against free radicals. Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to antioxidant effect [21, 25]. Phenolics possess diverse biological activities, such as antiulcer, anti-inflammatory, antioxidant, antitumor and antidepressant properties [26]. Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This

Table 1. Results of Quantitative Phytochemical Screening.

Phytochemical	Composition (%)
Alkaloids	24.50 \pm 3.50
Saponins	13.31 \pm 1.48
Oxalate	0.66 \pm 0.13
Phytate	0.00 \pm 0.00

Data are phytochemical composition of *C. lanatus* seed, and are expressed as mean \pm SEM.

Figure 1. Total Phenol Content of Extracts of *C. lanatus* Seed.

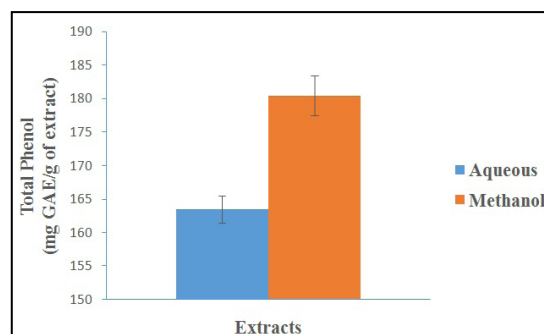


Figure 2. Total Tannins Content of Extracts of *C. lanatus* Seed.

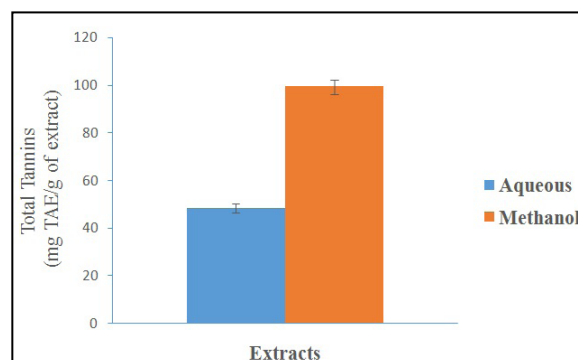
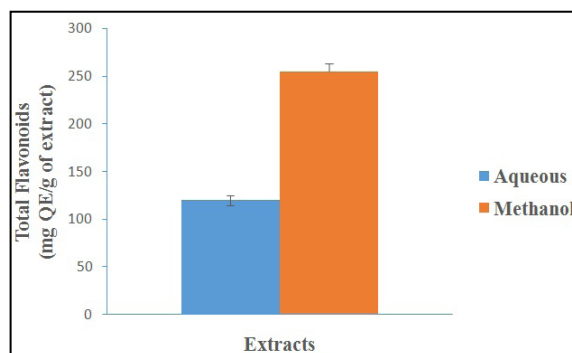
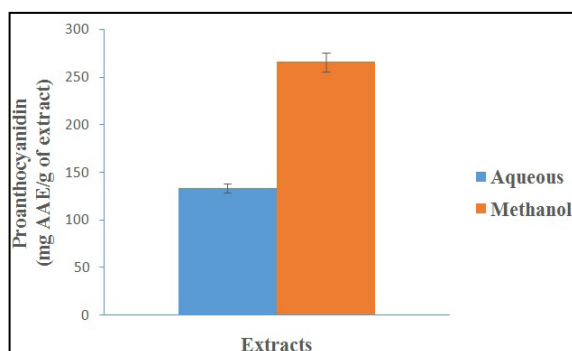


Figure 3. Total Flavonoids Content of Extracts of *C. lanatus* Seed.Figure 4. Proanthocyanidin Content of Extracts of *C. lanatus* Seed.

makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells [27]. Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants. High phenolic content-containing plant materials have high radical scavenging capacities [21, 28, 29].

Flavonoids possess potent and appreciable antioxidant, anti-inflammatory and anticancer effects [30, 31]. In this study, the total flavonoid content of methanol extract of *C. lanatus* seed was significantly higher than that of the aqueous extract.

Proanthocyanidins are a class of polyphenols found in a variety of plants. Chemically, they are oligomeric flavonoids. Many are oligomers of catechin and epicatechin and their gallic acid esters. More complex polyphenols, having the same polymeric building block, form the group of tannins. Plant proanthocyanidins are involved in induced defense mechanisms against plant pathogens and predators. They possess vasodilatory, anti-carcinogenic, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immunostimulating, antiviral and estrogenic effects [32]. The results of this study showed that the proanthocyanidin content of the methanol extract was significantly higher than that of the aqueous extract, an indication that the methanol extract may be a better antioxidant.

Conclusion

The results obtained in this study indicate that *C. lanatus* seed is a good source of phenolic compounds and could be used as a natural constituent of food and medicines.

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