A comparative analysis of the antioxidant potential of watermelon (*citrullus lanatus* (thunb.)) mesocarp extract fractions evaluated by the DPPH method

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Abstract. Watermelon mesocarp contains bioactive compounds with antioxidant properties that hold significant scientific and industrial interest. Antioxidants from natural sources are increasingly explored for applications in food preservation, cosmetics, and pharmaceuticals due to their efficacy and safety. This study aimed to identify fractions with strong antioxidant potential and explore their industrial applications. The crude extract was fractionated into water, ethyl acetate, and n-hexane fractions, and their antioxidant activity was evaluated using the DPPH method. The ethyl acetate fraction exhibited the strongest antioxidant activity, with an IC50 value of 7.68 ppm, which, while slightly less potent than ascorbic acid (IC50 = 4.09 ppm), demonstrated substantial free radical scavenging ability. This was followed by the water fraction (IC50 = 34.9 ppm), with the n-hexane fraction showing moderate activity (IC50 = 52.07 ppm). The ethyl acetate fraction of watermelon mesocarp extract demonstrated the strongest antioxidant activity, highlighting its potential for applications in food preservation and developing cosmetic formulations as a natural antioxidant source.

1 Introduction

Watermelon (*Citrullus lanatus* (Thunb.)), originating from Africa [1], is among the most cultivated horticultural crops in the world. Watermelon farming takes up roughly 7% of the global area utilized for vegetable production, with China as the main producer (67%) [2]. The fruit can range in shape from spherical to oval, and its exocarp is usually green with darker mottling or striping. The pulp (including the endocarp) of the fruit is watery, has a

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mild taste and pink to red color. The mesocarp, the thick inner layer of the rind, is moist, white, and tough [1] .

Watermelon contains a variety of bioactive chemicals that possess antioxidant activity. The color of the pulp is primarily due to carotenoids. Carotenoids, such as lycopene and beta-carotene, are the primary antioxidants in red-fleshed watermelons [3,4], with lesser levels of phytofluene, phytoene, γ-carotene, ζ-carotene and α-carotene. The primary pigments in orange-fleshed watermelons were beta-carotene, prolicopene, phytoene, and ζ-carotene, with lycopene in trace levels [5]. The primary hydrophilic chemicals contributing to the antioxidant activity in may be the phenolic compounds, which are distributed differently in the flesh, rind (mesocarp and exocarp), seeds, and leaves. In an experiment, the total phenolic content was higher in the rind than in the flesh (458 vs 389 mg chlorogenic acid equivalent kg^{-1} fresh weight) [6–8]. Flavonoids (quercetin and myricetin) caffeic [9], *p*-coumaric, sinapic, cinnamic and hydroxycinnamic acids were reported from watermelon rind [2]. While these methods provide a general quantification of phenolic compounds, our approach focuses specifically on fractionation and antioxidant activity, measured through the DPPH method, offering a complementary perspective to the existing data. Ascorbic acid has also been reported from different parts of the fruit, including the rind [10–12].

Agricultural waste from the fresh-cut food industry, including watermelon rind and seeds, constitutes a significant environmental challenge. Approximately 53% of fresh-cut watermelon is inedible waste, with 44% being rind and 3% seeds [13]. The global antioxidant market was valued at USD 3.5 billion in 2020, driven by rising demand for natural food additives and cosmetic ingredients. However, the use of these underutilized agricultural wastes as a source for food additives might be a solution to the environmental issue. Fruit waste and by products rich in antioxidant polyphenols may be recovered for use in culinary or cosmetic products [6,14].

Previous studies have reported the antioxidant activity of watermelon mesocarp extract, with an IC50 value of 31.42 ppm using the ABTS method [15]. Despite previous studies identifying phenolic compounds and carotenoids in watermelon rind, limited research has focused on the systematic fractionation of mesocarp extracts to evaluate their antioxidant potential. This study addresses this gap by employing a comparative analysis of solvent fractions (water, ethyl acetate, and n-hexane) to identify the most potent antioxidant fraction. This analysis not only highlights the potential of watermelon mesocarp as a sustainable source of antioxidants but also provides insights into its applications in food preservation, cosmetics, and pharmaceuticals.

The objective of this study is to evaluate the antioxidant potential of watermelon mesocarp extract fractions using the DPPH method, identify the most potent fraction, and explore its potential industrial applications.

2 Materials and Methods

2.1 Chemicals and Reagents

This experimental research utilized high-purity reagents, including ethanol 96% (Merck, Germany, ≥99.8%), ethyl acetate (Merck, Germany, ≥99.5%), n-hexane (Merck, Germany, ≥99%), ascorbic acid (Merck, Germany, analytical grade), and DPPH reagent (Sigma-Aldrich, ≥97%).

2.2 Extraction

The watermelon mesocarp was cleansed of dirt and reduce in size before undergoing drying. The resulting dried plant material was powdered and subjected to extraction with 1 liter of ethanol 70% by remaceration for 3 x 24 hours. At the end of extraction, the obtained filtrates were pooled and evaporated (0.5 g). The mesocarp extract was sequentially partitioned with n-hexane, ethyl acetate, and water [16,17]. Solvent selection during the extraction process followed the order of increasing polarity, starting from n-hexane for non-polar compounds, followed by ethyl acetate for semi-polar compounds, and concluding with water, the most polar solvent[18–24]. The rationale for solvent selection was based on their polarity gradient: n-hexane (non-polar) for extracting lipophilic compounds, ethyl acetate (semi-polar) for flavonoids and phenolics, and water (polar) for hydrophilic antioxidants. This approach maximizes the efficiency of compound separation. The use of 70% ethanol in the initial extraction ensures the capture of a broad spectrum of bioactive compounds, balancing the solubility of both lipophilic and hydrophilic antioxidant [18, 25].

Furthermore, the mesocarp extract was fractionated using a liquid-liquid extraction method. Initially, the concentrated extract was dissolved in distilled water, followed by liquid-liquid extraction using n-hexane, resulting in the isolation of the n-hexane fraction. Subsequently, the aqueous fraction underwent further liquid-liquid extraction, employing ethyl acetate. Ultimately, this process yielded three distinct fractions: n-hexane, ethyl acetate, and water fractions [16].

2.3 Preparation of a 50 ppm DPPH solution

Absolute ethanol was used to completely fill a 25 mL volumetric flask after dissolving a total of 1.25 mg DPPH in it. The maximal DPPH wavelength was then determined to be between 450 and 550 nm [26].

2.4 Preparation of fraction series concentrations

Each fraction was weighed up to 2.5 mg and placed in a 25 mL measuring flask, then dissolved in ethanol solvent and the volume was adjusted to 25 mL. Following that, 0.1 mL of each extract solution was obtained; 0.2 mL; 0.3 mL; 0.4 mL and 0.5 mL were then diluted again in a 10 mL volumetric flask to create extract solutions with concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm [26].

2.5 Preparation a series of ascorbic acid concentrations as a positive control

In a 25 mL volumetric flask, 2.5 milligrams of ascorbic acid (0.01%) were mixed with ethanol. Then, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL of the comparison ascorbic acid solution are placed in a 10 mL measuring flask, and a solution with concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm is created [26].

2.6 Determining operating time

For each fraction, the lowest concentration, 1 ppm, is used, and the operating time is completed. The operational time of the DPPH assay was determined to establish the optimal incubation period for accurate antioxidant activity measurement. A 1 ppm solution of each fraction was mixed with 50 ppm DPPH, and absorbance was monitored every 5 minutes for 90 minutes. The time at which the reaction reached equilibrium, determined to be 47 minutes, was selected as the operational time [26].

2.7 Determination of antioxidant activity by DPPH Assay

A total 2 mL of the DPPH 50 ppm solution was added to 2 mL of mesocarp fractions with various concentrations (1 ppm – 5 ppm). The resulting solution's absorbance was meticulously measured at 518 nm using a UV-Vis spectrophotometer. The absorbance was measured at 518 nm using a UV-Vis spectrophotometer, as this wavelength corresponds to the maximum absorption peak of the DPPH radical, ensuring accurate and consistent measurements [26]. Subsequently, an incubation period of 47 minutes was observed, following a prior investigation into the optimal operational time. This protocol was meticulously replicated across varying concentrations, specifically ranging from 2 µg/mL to 5 µg/mL, encompassing different fractions such as the ethyl acetate and n-hexane fractions, in addition to the positive control samples [26].

2.8 Data analysis

The results of the sample absorbance are used in calculating the percentage of free radical inhibition of DPPH with the following formula:

$$\%\ Inhibisi\ = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance}\ x\ 100\%$$

The percent inhibition value was used for linear regression analysis. Linear regression analysis was carried out by connecting the sample concentration value (x) with the sample inhibition value (y), so the intercept value (a) and slope value (b) were obtained, then a linear regression equation, namely y = bx + a. The antioxidant activity was then seen from the IC_{50} value obtained by entering 50% into the linear regression equation which has been obtained as the y value.

3 Result and Discussion

Based on the extraction process, 25 grams of the viscous extract was obtained from 86 grams of dried plant raw material. The principle of the DPPH method is the reduction of radical compounds into non-radical compounds by antioxidants by donating H atoms which were marked by a color change from purple to yellow (a typical color of the picryl group) [27]. The decline intensity of the color is directly proportional to the amount of DPPH free radical compounds, which can be dampened by antioxidant compounds so that the absorbance becomes lower [28].

The antioxidant activity of a compound as shown in the **Table 1.** can be expressed in the Inhibitory Concentration (IC_{50}) value. The IC50 value represents the concentration at which a sample reduces 50% of DPPH activity. Based on classification, very strong antioxidants exhibit an IC50 value of less than 50 parts per million (ppm), strong antioxidants have IC50 values between 50–100 ppm, moderate antioxidants have IC50 values between 101–150 ppm, weak antioxidants have IC50 values between 150–200 ppm, and antioxidants with values greater than 200 ppm are considered very weak [29].

In this study, ascorbic acid was used as a positive control. Ascorbic acid is a natural antioxidant that has a good ability to ward off free radicals [30,31].

The ethyl acetate fraction exhibited the lowest IC50 value, with antioxidant activity comparable to that of the positive control, ascorbic acid. The water and n-hexane fractions demonstrated less remarkable activities. This can be explained by the solubility of flavonoids, which are more soluble in ethyl acetate than in water or n-hexane. Flavonoids, such as quercetin and myricetin, are more soluble in ethyl acetate compared to water and n-

hexane[32,33]. hese findings corroborate our results and support the role of ethyl acetate in extracting bioactive flavonoids that contribute to the observed antioxidant activity.

Table 1. Antioxidant activity strength

Sample	IC_{50}(mg/mL)	Antioxidant activity strength
Ascorbic acid	4.09	Very strong
Water fraction	34.9	Very strong
Ethyl acetate fraction	7.68	Very strong
n-Hexane fraction	52.07	Strong

The IC50 values for the watermelon mesocarp fractions were reported as 7.68 ppm (95% CI: 7.60–7.76 ppm) for the ethyl acetate fraction, 34.9 ppm (95% CI: 34.5–35.3 ppm) for the water fraction, and 52.07 ppm (95% CI: 51.8–52.3 ppm) for the n-hexane fraction. All experiments were performed in triplicate to ensure precision and repeatability. To enhance the robustness of our findings, we calculated the 95% confidence intervals for these IC50 values. The IC50 values for each fraction were determined in triplicate, and the confidence intervals were statistically analyzed to provide more precise interpretations of the data. These additional statistical details ensure more reliable data representation.

The ethyl acetate fraction demonstrated strong antioxidant activity, comparable to ascorbic acid (IC50: 4.09 ppm), indicating that the phytochemicals in the ethyl acetate fraction may offer significant biological benefits. These include mitigating oxidative stress, which plays a role in aging and various diseases such as cancer and cardiovascular diseases [34]. The high antioxidant activity of the ethyl acetate fraction suggests its potential for use in protecting cells from oxidative damage, with applications in both food preservation and health maintenance. The observed differences in antioxidant activity between the watermelon mesocarp fractions and previous studies could be attributed to several factors, including variations in watermelon cultivars, geographical origins, and extraction methodologies. Different extraction techniques, such as solvent polarity or extraction time, as well as watermelon varieties, may yield different profiles of bioactive compounds, influencing antioxidant activity. Additionally, environmental factors such as climate and soil conditions may affect the phytochemical composition of watermelon [35]. These factors warrant further investigation to better understand the variability in antioxidant potential across different watermelon samples. The antioxidant potential of watermelon mesocarp fractions, particularly the ethyl acetate fraction, presents significant industrial applications. These fractions could serve as natural preservatives in food products, helping to extend shelf life by preventing oxidative spoilage. Furthermore, the strong antioxidant capacity of these fractions makes them promising candidates for incorporation into cosmetic products, where they can protect the skin from oxidative stress, a major contributor to aging and skin damage.

While this study focused on antioxidant activity, future research could explore additional bioactive properties of the watermelon mesocarp fractions, such as antimicrobial, anti-inflammatory, and anticancer activities. Investigating the phytochemical profiles of the fractions through advanced techniques like mass spectrometry could help identify specific compounds responsible for these bioactivities. Moreover, testing the stability and bioavailability of the bioactive compounds in different formulations could provide valuable insights for industrial applications. Utilizing watermelon mesocarp, a byproduct of the food industry, as a source of bioactive compounds offers significant environmental benefits. This approach not only helps reduce agricultural waste but also promotes the sustainable use of natural resources. By repurposing waste materials for value-added applications, such as

natural antioxidants in food and cosmetics, we can contribute to waste minimization and support sustainable industrial practices

4 Conclusion

The ethyl acetate fraction of watermelon mesocarp exhibited robust antioxidant activity, comparable to ascorbic acid, highlighting its potential as a valuable source of natural antioxidants for industrial applications. These findings underscore its utility in developing antioxidant-based formulations for food preservation, cosmetics, and health supplements. Future research should focus on isolating and identifying individual bioactive compounds within the fraction and evaluating their specific applications in pharmaceutical and nutraceutical industries.

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