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Determination of Total Phenols, Total Flavonoids and Antioxidant Activity of Watermelon Peel and Rind from Several Cultivation Areas in Indonesia

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ABSTRACT

Background: Watermelon (*Citrullus lanatus*) consists of peel and rind (30-40% of total fruit weight), commonly discarded as waste. Nonetheless, several studies have shown the potential benefit of these watermelon parts. This study aimed to determine the correlation between the watermelon cultivation areas and their chemical composition, especially phenols and flavonoids. Watermelon peel and rind were obtained from Jember (WJ), Sragen (WS), Langkat (WL), Hulu Sungai Tengah (WHST) and Lombok Tengah (WLT).

Methods: The total phenols and flavonoids were determined by using the colorimetric method. Furthermore, the antioxidant activity was determined by using DPPH and ABTS methods.

Result: The highest total flavonoids, *i.e.* 41.86±1,65 mgQE/100 g watermelon, was observed in the WHST rind. While WL peel showed the highest total phenols, *i.e.* 3.97±0.02 mgGAE/g. The correlation analysis for all samples showed no significant relationship between the antioxidant activity and total flavonoids. However, a significant relationship was found between the DPPH-IC50 and total phenols with a correlation value of 0.47 ($p<0.01$) for both watermelon parts peel and rind. In addition, watermelon peel contains higher total flavonoids compared to rind. But, there was no significant difference in the total phenols between the watermelon peel and rind. To conclude, both watermelon peel and rind obtained from five cultivation areas showed antioxidant activities contributed by the phenolic compounds.

Key words: Antioxidant, Flavonoid, Peel, Phenolic, Rind, Watermelon.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a popular fruit valued for its refreshing taste and high water content. While the juicy flesh is commonly eaten, the skin is often discarded (around 30-40%). This fruit is grown extensively in various regions worldwide and it has been observed that the location of cultivation affects its chemical composition. The chemical compounds found in watermelon play a role in its nutritional qualities, taste and potential health benefits (Manivannan *et al*., 2020; Ashoka *et al*., 2022).

The environment where watermelon is cultivated, including factors like climate, soil conditions and farming practices, can impact the presence of different chemical compounds in the fruit, such as phenolic compounds and flavonoids (Martínez *et al*., 2022; Singh *et al*., 2022). These bioactive compounds possess antioxidant properties and potential health advantages, such as lowering the risk of diabetes and heart disease, reducing inflammation and preventing cancer (Tungmunnithum *et al*., 2018). Variations in these compounds can result in differences in the flavor, scent and nutritional value of watermelons from various regions (D'Eusanio *et al*., 2023).

Several studies have investigated the correlation between where watermelon grows and its chemical composition (Bazié *et al*., 2022). These studies have explored the influence of environmental factors, such as temperature, sunlight, rainfall and soil characteristics, on the synthesis and accumulation of certain chemical compounds in watermelons (Kyriacou *et al*., 2018). Other findings have shown that different growing conditions can result in variations in the concentration and profile of phenolic and flavonoid compounds in watermelon. In other case, the different geographical location give the significant result of phenolic and flavonoid content (Lahrizi *et al*., 2023). However, such research has not been done on watermelons that are grown in Indonesia.

Globally, watermelon production will increase to reach 100 million tons ini 2022. This regardless of market demand for watermelon. Indonesia is one of the largest watermelon producing countries in the Southeast Asia region, with total watermelon production reaching 560

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thousand tons in 2022 (BPS, 2022). There are 5 main watermelon producing areas in Indonesia spread over several islands, specifically in Jember, Sragen, Langkat, Hulu Sungai Tengah and Lombok Tengah.

Understanding the correlation between where watermelon grows and its chemical composition is important for various stakeholders, including farmers and consumers. Farmers can optimize cultivation practices and select suitable varieties based on the desired chemical composition of watermelons and consumers can make informed choices based on their preferences and the potential health benefits associated with certain chemical compounds in watermelon.

This study aims to find out more about the relationship between the place where watermelon grows and its chemical composition, especially phenolic compounds and flavonoids. By analyzing watermelon samples from different regions and carrying out chemical analyses, valuable insights can be gained regarding the site-growth impact on the composition of these bioactive compounds.

MATERIALS AND METHODS

Plant material

Watermelons (*Citrullus lanatus*) were obtained directly from farmers in several regions in Indonesia. The fruits were obtained from March to June 2023 (Fig 1). Watermelon fruit is harvested at 60-70 days of planting age. The choice of watermelon used is a watermelon with red colour and has seeds. The watermelon comes from Langkat, (3°14′00″-4°13′00″ North Latitude, 97°52′00″-98°45′00″ East Longitude and 4-105 m above sea level), Lombok Tengah, (82°7′-8°30′ South Latitude and 116°10′-116°30′ East Longitude and 107 m above sea level), Hulu Sungai Selatan, (2°29′ 59″-2°56′10″S and 114°51′19″-115°36′19″E and), Jember, (7059′6″ to 8033′56″ South latitude and 113016′28″ to 114003′42″ East Longitude) and Sragen, (110 45″ and 111 10″ E and 7 15″ and 7 30″S), the fruits were then identified at the School of Life Sciences and Technology, Bandung Institute of Technology with letter number 1086/IT1.C11.2/TA.00/2023.

Each fruit was weighed and peeled into several parts. The peel was obtained by peeling off the outer green skin using a sharp knife at a thickness of 1-2 mm. The rind part was obtained by separating the peel and the flesh so there were no red and green parts left. The rind part was then cut thinly using a meat slicer speed up the drying process. The peels were dried using an oven with a temperature of 50°C for 3 × 24 hours. All dry samples were then weighed and ground using a coffee grinder.

Sample extraction

Dried peels and rinds were soaked in demineralized water for a day using the maceration method in separate glass containers. Fifty grams of sample were soaked in 150 mL of demineralized water and stirred until homogenized. The

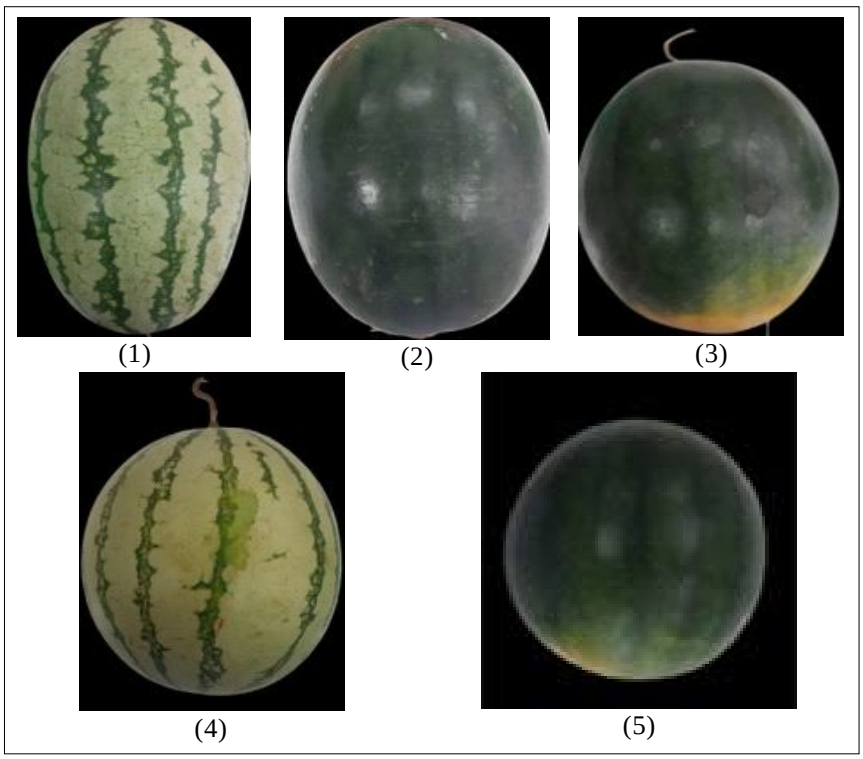

Fig 1: Watermelon originating from 5 regions in Indonesia (1) Langkat Watermelon (WL) (2) Central Lombok Watermelon (WLT) (3) Hulu Sungai Tengah Watermelon (WHST) (4) Jember Watermelon (WJ) (5) Sragen Watermelon (WS).

resulting solution was filtered using Whatman® filter paper to separate the liquid from the residue. The water extract obtained was then analyzed for total phenol, total flavonoid and antioxidants using the DPPH and ABTS methods.

Total phenol and flavonoid contents

The total phenolic content (TPC) of plant extracts was determined using the Folin-Ciocalteau reagent spectrophotometrically, following the method described by (Kähkönen *et al*., 1999) with slight modifications. Samples of three repetitions of 300 microliters each were placed in a test tube. Then, 1.5 ml of Folin-Cioçalteau reagent (diluted 10 times) and 1.2 ml of sodium carbonate (7.5 g/100 ml) were added. The contents of the tube are mixed and kept in the dark for 30 minutes. Then the absorbance was measured at a wavelength of 765 nm using a Hitachi U-1900 uv vis Spectrophotometer. TPC is calculated as milligrams of gallic acid equivalent (GAE) per 100 grams of dry matter.

The determination of total flavonoid content (TFC) was conducted following the methodology outlined by (Ordonez *et al*., 2006), employing aluminum chloride. Each extract (250 µL) was combined with AlCl3 (10%) (150 µL), sodium carbonate (Na2CO3, 1M) (75 µL) and sodium hydroxide (500 µL), with the total volume adjusted to 2.5 mL using distilled water. The mixture was then incubated for one hour in darkness. Optical density was measured at 510 nm using a UV spectrophotometer. TFC levels were quantified as milligrams of quercetin equivalent per gram of dry weight (mg QE/g dw).

Antioxidant analysis

The method described by (Stratil *et al*., 2006) was used to assess the antioxidant activity of the investigated plant extracts against ABTS (2,2′-azino-bis-(3 ethylbenzothiazoline)-6-sulfonic Acid). By oxidizing ABTS with potassium persulfate, ABTS radicals were created. Potassium persulfate (4.95 mM) and ABTS (7 mM) were combined in a 1:1; v/v ratio and left at room temperature for 16 hours. After that, methanol was added to the mixture to dilute it until it had an absorbance value of 1.1-1.5 at a wavelength of 734 nm. Each sample's methanol extract was added to 3.9 mL of ABTS dilution for a total of 0.1 mL. The UV-30 spectrophotometer (Hitachi U-1900 uv vis spectrophotometer, Japan) was used to measure the reduction in absorbance at 734 nm. Using ABTS, a blank solution was created. The principle of the ABTS method is to look at the ability of antioxidants to stabilize free radicals which is characterized by color fading. The greenish blue color of the $ABTS^{+}$ cation radical will be reduced by antioxidants to a colorless non-radical form.

The antioxidant activity of plant extracts against DPPH (2,2-diphenyl-1-picrylhydrazy) was determined using the method proposed by (Katalinic *et al*., 2006). Dilution of methanol DPPH 1 × 10-4 M. A total of 1 mL of each sample was collected and 2 mL of methanol DPPH dilution was added. The mixture was kept in the dark at room temperature for 16 minutes and the absorbance was measured at 517 nm on a UV-30 spectrophotometer (Hitachi U-1900 uv vis spectrophotometer, Japan). A blank solution was prepared by diluting DPPH methanol. The results were expressed in milligrams of quercetin equivalent per milligram dry weight. The calibration line was established using quercetin concentration. The DPPH method works based on an oxidation-reduction reaction, where DPPH is a synthetic free radical that can dissolve in polar compounds such as ethanol and methanol. Antioxidant compounds will react with DPPH by donating hydrogen atoms to obtain electron pairs. The antioxidant activity was determined using the spectrophotometric method, reported as vitamin C standard by reference to the standard curve (y = 13,228× + 4,1657 and R^2 = 0.9974)

Statistical analysis

All data were expressed as mean ± standard of eror mean (SEM). Statistical tests were carried out to see differences in TFC and TPC compounds in watermelon parts and their sources using two-way ANOVA with a confidence level (<0.01). Furthermore, to see the relationship of compounds to antioxidant activity, Pearson Correlation testing was carried out with a significance value of >0.01. Statistical analysis was done using IBM SPPS software version 25.

RESULTS AND DISCUSSION

Total flavonoid

The total TFC was determined using the aluminum chloride spectrophotometric method, reported as quercetin equivalent standard (QE) by reference to the standard curve (y = 0.007x + 0,0214 and R^2 = 0.9922). The results of the analysis showed that the majority of flavonoid compounds were found in the peel section, namely 73.79±1,35 (WLT); 65.74±1,62 (WJ); 57.07±2,04 (WL); and 49.42±1,9 (WS) mg QE/100g respectively, only in WHST which showed the highest flavonoid compounds in the rind section (41.86± 1,65 mg QE/100 g).

Statistical analysis showed that regional factors had a sig value of (0.000) <0.01. It was said that different regions had an effect on the value of flavonoid compounds at the 99% confidence level. The same thing also applies to the fruit portion factor with a sig value of (0.000) <0.01. The peel and rind parts affected the value of flavonoid compounds at the 99% confidence level. Both of these factors have a sig value of (0.000) <0.01, which indicates that there is an interaction between regional factors and sub-factors on the value of flavonoid compounds at the 99% confidence level.

The TFC in the WLT peels was higher than other areas, while the highest TFC in the WHST rinds came. In all samples, it illustrated that the content of flavonoids is found more in the peel part, but this did not apply to WHST. This difference indicated the effect of growing area and environment on flavonoid compounds.

Flavonoids are secondary metabolites with antioxidant activity whose potency depends on the number and position of free OH groups (Panche *et al*., 2016). (Augustia *et al*.,

2020) reported the TFC value obtained in the rind section was 0.71-1.63 mg/L. This value is equivalent to the report by (El-Behairy *et al*., 2022), which was 0.732 mg/L. Meanwhile, Dieng *et al* reported that the TFC in the dry peel section was 1.10±0.14 mgRE/g. Another report on dry peel ethanol extract showed a TFC value of 1.12 mg CE/g.

Extraction results of flavonoid compounds vary based on different sources and solvents (Èukanović *et al*., 2020). Using water as a solvent to extract flavonoid compounds from rind samples is the best choice (Ho *et al*., 2018). According to the literature, genetic diversity and variations in biological, environmental, seasonal and annual factors significantly affect the TFC of vegetables. Davies and Hobson found that tomatoes grown in open fields with more sunlight and UV radiation contained more flavonoids than those grown in greenhouses with artificial lighting. This explains why more flavonoids accumulate in the watermelon peel. Watermelons from the Hulu Sungai Tengah region showed unique results not seen before. Further observation may be needed to determine if other factors contribute to the increase in total flavonoids in the rind.

Total phenol

Phenolic compounds are important plant constituents with redox properties that are responsible for antioxidant activity (Soobrattee *et al*., 2005). The hydroxyl group in plant extracts is responsible for facilitating free radical scavenging. As a basis, the TPC was measured using the Folin-Ciocalteu reagent in each extract. The results were obtained from the calibration curve of gallic acid (20-100 mg/L) and expressed in gallic acid equivalent (GAE) per gram of dry extract weight by reference to the standard curve ($y = 0.0114\times$ + 0,0904 and $R^2 = 0.9978$) (Table 1). The content of phenolic compounds was found more in the rind part than in the peel. This is not the same as TFC, which showed higher levels in the peel.

As is the case with the statistical analysis of flavonoids, statistical analysis shows that there is a significant value for the regional factor and the watermelon section on the phenol sig (0.000) <0.01 at a 99% confidence level. The highest TPC value was found in the peel, as much as 3.97±0.02 mgGAE/g, originating from Langkat, while in the rind section the highest TPC value was 3.57±0.12 mgGAE/g originating from WHST. This finding differs from the reports of Al-Nablsi *et al*., 2022) and (Neglo *et al*., 2021) whereas when compared between peel and rind, the TPC level should be higher in the peel section. However, the TPC levels for both peel and rind reported by Neglo *et al.* (2021) (0.087±0.002 mgGAE/g) were much lower than in the samples. Values close to the findings have been reported by (Naguib *et al*., 2019) (120.83±0.038 µg/g) and (Ho *et al*., 2018) (218.39±0.34).

Phenolics in both fruit peel and rind play a crucial role in growth, development and protection processes (Šamec *et al*., 2021). Phenolic compounds are sensitive and prone to degradation under various environmental conditions such as light, pH, oxygen, temperature and ions due to their unstable nature (Ali *et al*., 2018). The TPC and antioxidant compounds depend on environmental factors (Mahajan *et al*., 2020). The average overall TPC of winter fruit was significantly greater than that of summer fruit, whereas the reverse was observed for overall antioxidants; they were higher in summer than in winter.

Another study reported that plants grown at different times of the year may have significant differences in their chemical content (Lemos *et al*., 2017). For example, the main tea flavanol and polyphenol content was significantly higher in the warm summer months than in the colder months. This is due to higher temperatures, higher light intensity and longer day length during summer (Yao *et al*., 2005).

In the process of phenolic analysis on the sample, drying at 50°C on the rind samples did not damage the flavonoids and phenolic compounds. This is in line with Ho's research which compared 3 processes of drying rind samples at 40°C; 60°C; and freeze drying. Furthermore, the drying process using an oven with a temperature of 40°C resulted in higher content than other drying. In the measurement results, the phenolic values of the 4 rind and peel samples showed higher results than (Ho *et al*., 2018).

Antioxidant activity

The antioxidant potential of watermelon's rind and peel extracts was assessed using the ABTS and DPPH methods. The ABTS test was chosen due to its higher sensitivity compared to DPPH for analyzing antioxidants in food. These methods differ in their reaction mechanisms; DPPH assesses antioxidant ability based on hydrogen donation, while ABTS evaluates the ability to stabilize free radicals by donating proton radicals. Together, these methods complement each other in assessing antioxidant potential (Pokorná *et al*., 2015).

The highest DPPH activity of the aqueous extract on the peel section was recorded at 3.44 mg/mL DW on WL. While the lowest ability came from WJ of 21.63 mg/mL DW. The results for the DPPH radical scavenging capacity of rind sections varied significantly between the studied accessions, from 7.24 mg/mL dw for WJ to 22.79 dw for WL (Fig 2). Among the five sources of watermelon, three areas show better antioxidant potential in the peel part than the rind.

The results showed that the peel has better antioxidant power than the rind, which is in line with reports from (Neglo *et al*., 2021). The inhibitory properties of the watermelon peel were 55.75±2.44%. As for the antioxidant potential shown in the rind of WHST and WJ, it was possible because the TPC levels in the two samples were higher in the rind than in the peel.

Similar results were shown in the results of the ABTS test that high antioxidant potential is found in the peel part.

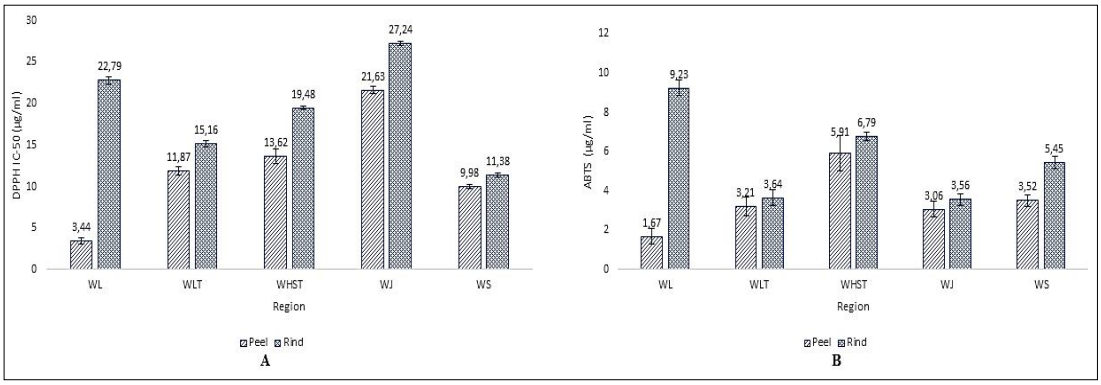

Fig 2: Results of antioxidant testing for peel and rinse samples using the DPPH and ABTS methods.

Table 1: Total flavonoid and total phenolic values on peel and rind watermelon from five regions.

Region	Flavonoid (mg QE/100 g)		Phenolic (mg GAE/g)	
	Peel	Rind	Peel	Rind
WL	57.07±2.04	25.45±1.59	3.97±0.02	3.17±0.04
WLT	73.79±1.35	16.82±1.1	2.38±0.017	1.63±0.02
WHST	18.21±0.52	41.86±1.65	1.76±0.012	3.57±0.12
WJ	65.74±1.62	29.26±0.16	2.32±0.006	3.29±0.03
WS	49.42±1.9	36.15±0.82	2.48±0.003	3.01±0.05

This can be seen in Table 1. Three regions that have good antioxidant activity in the peel (WL; WLT; WS). While the rest seemed better on the rind (WHST; WJ). If seen from the classification of the IC-50 values in the sample, the peels from the Langkat and Sragen areas are quite promising as they have strong antioxidant abilities.

With these results, the peel and rind samples which were considered as waste showed promising sources of antioxidants that can be used as raw materials for food processing such as flour, developing nutraceuticals and developing anti-aging cosmetic product. The peel and rind parts of the watermelon can be consumed after going through processing, such as by drying the fruit and then grinding it into powder. The heating process at moderate temperatures does not damage the important components of compounds that act as antioxidants. The peel and rind parts of the watermelon are also safe for consumption.

Relationship of antioxidant activity to total TFC

To identify possible flavonoid compounds that contribute to the antioxidant activity of watermelon peel and rind extracts, Pearson's simple linear correlation coefficient between total TPC and its antioxidant activity.

The sig value (0.469) >0.01 indicates that there is no correlation between the DPPH-IC50 test and flavonoid compounds. While the sig value (0.009) <0.01 indicates that there is a correlation between the DPPH-IC50 test and phenolic compounds. The resulting correlation was 46.7% with an inverse or negative relationship where the higher the DPPH test value, the smaller the phenolic compound. A value of 46.7% means that the level of relationship between the DPPH test and the Phenolic compound is quite strong.

It is not easy to obtain information about the relationship between TFC and TPC in antioxidant activity in aqueous extracts of peel and rind watermelon from previous studies. However, the findings indicate that the main antioxidant responsible is phenolic in both parts of the watermelon.

CONCLUSION

For the first time, this study reported the TPC and TFC content of the peel and rind parts of watermelon obtained from various regions in Indonesia, as well as their antioxidant abilities. The peel and rind parts of watermelon are known to contain phenolic and flavonoid compounds and have antioxidant activity. The TPC and DPPH value showed a correlation in both parts of the watermelon. Therefore, there is no significant difference in TPC in the two parts of the watermelon. While significant differences were seen in the peel and rind sections of the flavonoid compounds. Therefore, it is necessary to further examination if the two are combined.

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Conflict of interest

All the authors have no conflict of interest to declare.

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