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ANTIOXIDANT ACTIVITY TEST OF EXTRACT AND FRACTIONS OF RED GUAVA (*Psidium guajava* L.) PEEL AND FRUIT

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ABSTRACT

In the modern era, alongside advancements in technology and science, lifestyle changes have negatively impacted public health. These changes include unbalanced dietary habits, insufficient rest and physical activity, as well as smoking and alcohol consumption. Furthermore, environmental pollution has exacerbated health deterioration by reducing the production of essential biochemical compounds that help maintain bodily functions. Natural antioxidants play a crucial role in counteracting free radicals originating from sources such as radiation, pollution, and harmful chemicals (Arnanda & Nuwarda, 2019). Cellular damage caused by free radicals can be mitigated through the intake of antioxidant compounds found in fruits (Verdiana et al., 2018). Red guava (*Psidium guajava* L.) contains natural antioxidants that help neutralize free radicals originating from radiation, pollution, and harmful chemicals. This study aimed to identify secondary metabolites, determine the IC_{50} values of ethanol extract and various fractions of red guava peel and fruit. Four solvents were used ethanol extract, ethyl acetate fraction, n-hexane fraction, and water fraction. Each sample was tested at concentrations of 20, 40, 60, 80, and 100 ppm. Vitamin C, at concentrations of 2, 4, 6, 8 and 10 ppm, served as a positive control. Antioxidant activity was evaluated using the DPPH method with a UV-Vis spectrophotometer at a wavelength of 516 nm and an incubation time of 30 minutes. Phytochemical screening showed that all extracts contained alkaloids, flavonoids, and tannins. The IC_{50} values obtained were: ethanol extract peel 62,4719 ppm, ethanol extract fruit 44,0035 ppm, ethyl acetate fraction peel 56,7955 ppm, ethyl acetate fraction fruit 46,4394 ppm, n-hexane fraction peel 113,9311 ppm, n-hexane fraction fruit 114,4767 ppm, water fraction peel 104,0284 ppm, and water fraction fruit 76,5801 ppm.

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Introduction

In the modern era, alongside advancements in technology and science, lifestyle changes have had a negative impact on public health. These changes include the habit of consuming nutritionally unbalanced foods, lack of rest and physical activity, and the prevalence of smoking and alcohol consumption. Furthermore, environmental pollution, such as high levels of air contamination, has also contributed to the decline in public health by reducing the body's ability to produce beneficial compounds essential for maintaining health. Natural antioxidants play an important role in combating free radicals originating from various sources, including radiation, pollution, and other harmful chemicals (Arnanda & Nuwarda, 2019). Cellular damage caused by free radicals can be mitigated through the consumption of antioxidant-rich compounds found in fruits (Verdiana et al., 2018).

Free radicals are reactive compounds or molecules with unpaired electrons that readily attack and bind to electrons in surrounding molecules. While ionic interactions with free radicals may have limited harmful effects, covalent bonding with free radicals can cause significant damage, including disruption of cellular and tissue function. This damage is associated with the development of chronic diseases such as cardiovascular disorders, cancer, chronic respiratory diseases, diabetes, and kidney disease (Arifin et al., 2019).

One fruit known for its high antioxidant content is red guava (Psidium guajava L.), a tropical fruit widely cultivated and consumed in Indonesia. Red guava has a sweet to slightly acidic flavor and offers multiple health benefits, including alleviating digestive issues, improving respiratory function, and promoting skin health. Additionally, it has been reported to possess antioxidant and anticancer properties, lower cholesterol levels, prevent heart disease, and be safe for individuals with diabetes mellitus (Evelyn, 2022).

The selection of solvents in the extraction process of red guava peel and pulp is crucial to obtaining optimal yields of active compounds. An ideal solvent should efficiently dissolve target metabolites, separate them from other components, and yield extracts rich in the desired bioactive compounds. In total extraction, the solvent must be capable of dissolving almost all secondary metabolites present. Factors influencing solvent choice include selectivity, ease of use, cost-effectiveness, environmental safety, and non-toxicity (Ikhlas, 2013).

Fractionation of ethanol extracts from red guava peel and pulp can be performed using solvents of varying polarity—such as n-hexane, ethyl acetate, and water—to obtain simpler component fractions. Phytochemical screening of the ethanol extract and its n-hexane, ethyl acetate, and aqueous fractions can reveal the types of secondary metabolites present. Typically, n-hexane is used to isolate non-polar compounds, ethyl acetate for semi-polar compounds, and water for polar compounds (Febrianti, 2021).

Antioxidant activity can be measured using various methods, such as Oxygen Radical Absorbance Capacity (ORAC), Total Radical-Trapping Antioxidant Parameter (TRAP), Trolox Equivalent Antioxidant Capacity (TEAC), Peroxyl Radical Scavenging Capacity (PSC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Total Oxyradical Scavenging Capacity (TOSC), and Ferric Reducing Antioxidant Power (FRAP). In this study, the antioxidant activity of ethanol extract and its ethyl acetate, n-hexane, and aqueous fractions from red guava peel and pulp was evaluated using the DPPH method. This method was selected because it is rapid, simple, sensitive, and requires only small

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sample quantities, while the radical source is readily available without the need for substrate preparation (Rahman, 2023).

To date, no studies in Indonesia have reported on the antioxidant activity of ethanol extract and its n-hexane, ethyl acetate, and aqueous fractions from red guava peel and pulp. Therefore, this study aimed to evaluate the antioxidant activity of ethanol extract and its fractions using the DPPH assay.

Materials and Methods

Location and Time of Research

This research was conducted in Duta Bangsa University of Surakarta. This research was conducted over a period of two months starting from April 2025 to June 2025.

Materials and Methods

The tools used in this study were a UV-Visible spectrophotometer (Thermo Scientific Genesys 10S UV-Vis), rotary evaporator (RE 100-Pro), drying oven (Binder), desiccator, moisture analyzer, analytical balance (Fujitsu), blender (Miyako), maceration jars, mesh no. 40 sieve, water bath, Buchner funnel, Erlenmeyer flasks (Pyrex), beakers (Pyrex), test tube rack, test tubes (Pyrex), measuring cylinders (Pyrex), volumetric flasks (Pyrex), amber glass vials, porcelain crucibles, cuvettes, weighing bottles, porcelain dishes, glass stirring rods, dropper pipettes, micropipettes, and aluminum foil. The materials used in this study were the peel and fruit of red guava (*Psidium guajava* L.), 96% ethanol (pro analysis), n-hexane (pro analysis), ethyl acetate (pro analysis), methanol (pro analysis), distilled water, vitamin C (Merck), DPPH (Sigma-Aldrich), sulfuric acid (H_2SO_4, Merck), acetic acid (CH_3COOH, Merck), potassium chromate (K_2CrO_4, Merck), sodium hydroxide (NaOH, Merck), hydrochloric acid (HCl, Merck), ferric chloride ($FeCl_3$, Merck), Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, and Liebermann–Burchard reagent.

Data Collection Techniques

Sample Preparation

A total of 15 kg of red guava fruits were washed thoroughly. The peel and pulp were separated and sliced to a thickness of 2–3 mm. The slices were oven-dried at 60°C until completely dry and brittle. The dried samples were ground into powder using a blender and passed through a 40-mesh sieve to obtain a fine and uniform powder. The powdered samples (simplicia) were stored in airtight containers with silica gel to protect against moisture, sunlight, and insects (Rahmawati *et al*., 2023).

Simplicia Standardization

a. Drying Loss

1 gram of red guava peel and fruit powder was weighed in a pre-dried and pre-weighed stoppered weighing bottle. The powder was spread into a 5–10 mm layer and dried in an oven at 105°C until a constant weight was achieved. After cooling in a desiccator, the bottle was reweighed (Charolina, 2020). The acceptable moisture content was ≤10% (Depkes RI, 2008)

b. Water Content

Water content was determined using a moisture balance. 2 grams of the powdered sample were placed on the sample pan, and the temperature was set to 105°C for 5 minutes. The result was recorded directly as a percentage (Larasati, 2023). The acceptable moisture content was ≤10% (Depkes RI, 2020).

c. Total Ash Content

2 grams of sample were placed in a porcelain crucible previously ignited and weighed. The sample was charred and incinerated gradually at 500–600°C until a constant weight was obtained. The total ash content was calculated as

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a percentage of the dried sample (Depkes RI, 2017).

Extraction

Extraction of the red guava peel and pulp was performed by maceration using 96% ethanol. Two hundred grams of each powdered sample were soaked in 2000 mL ethanol for 3 × 24 hours with regular stirring. Re-maceration was conducted for 1 × 24 hours for the fruit and 2 × 24 hours for the peel. The resulting filtrates were concentrated using a rotary evaporator at 70°C to obtain viscous extracts (Rusmiyati, 2023; Salsabila *et al*., 2024).

Extract Standardization

a. Ethanol Residue Test

2 milliliters of the viscous extract were placed in a test tube, followed by the addition of 2 drops of H_2SO_4 and 2 drops of acetic acid. The mixture was heated, and the absence of a fruity ester odor indicated that the extract was free from ethanol (Ballo *et al*., 2021).

b. Water Content

2 grams of each extract were measured and tested using a moisture balance at 105°C until a stable result was displayed. The water content was recorded in percentage (Oktaviani, 2023). The acceptable limit was ≤10% (Depkes RI, 2008).

c. Heavy Metal Test

1) Lead (Pb) : 1 mL of the sample solution was added with 0.5 mL of K_2CrO_4. A yellow precipitate indicated the presence of Pb (Fatmawati *et al*., 2021).
2) Cadmium (Cd) : 1 mL of the sample was mixed with 5 drops of NaOH reagent. A pink color indicated the presence of Cd (Arifiyana & Fernanda, 2018).

Fractionation

10 grams of thick extract were dissolved in 100 mL of distilled water. The solution was partitioned with *n*-hexane using a separatory funnel. The resulting upper and lower phases were collected, and the aqueous phase was further extracted with ethyl acetate. The ethyl acetate and aqueous layers were collected separately. The process was repeated several times. Each collected fraction was concentrated using a rotary evaporator at 40°C for 10 minutes (Lengkoan *et al*., 2017).

Phytochemical Screening

a. Alkaloid Test

5 mL of ethanol extract and fractions (ethyl acetate, *n*-hexane, water) were dissolved in 5 mL of 2N HCl and heated. The mixture was divided into three test tubes for the following tests:

1) Mayer's reagent: White or yellow precipitate indicates alkaloid presence.
2) Bouchardat's reagent: Brown to black precipitate.
3) Dragendorff's reagent: Brown or orange-brown precipitate.

The presence of alkaloids was confirmed if at least two tests showed precipitate formation (Laia, 2019).

b. Flavonoid Test

2 mL of each sample were dissolved in ethanol, followed by the addition of magnesium powder and 3–5 drops of HCl. The formation of an orange or yellow color indicated the presence of flavonoids (Siregar, 2023).

c. Tannin Test

2 grams of each sample were dissolved in 2 mL of 96% ethanol and mixed. Then, 3 drops of $FeCl_3$ were added. A blue, blue-black, green, or green-black color with precipitate indicated tannin presence (Maulida, 2020).

d. Triterpenoid Test

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2 mL of each sample were mixed with 1 mL of Liebermann–Burchard reagent. A greenish-black or dark green color indicated the presence of triterpenoids (Siregar, 2023).

Antoxidant Activity Assay Using DPPH

a. Preparation of 50 ppm DPPH Solution

5 mg of DPPH was dissolved in 30 mL methanol and transferred to a 100 mL volumetric flask. Methanol was added to volume, and the solution was mixed until homogeneous (Tristantini *et al*., 2016).

b. Blank Solution Preparation

1 mL of the DPPH solution was mixed with 4 mL of methanol to serve as a blank (Tristantini *et al*., 2016).

c. Determination of Maximum Absorption Wavelength

1 mL of DPPH 50 ppm solution was mixed with 4 mL methanol in a brown vial, covered with aluminum foil, and incubated. The absorbance was measured between 500–600 nm using a UV-Vis spectrophotometer to determine the maximum wavelength (Putri *et al*., 2019).

d. Operating Time Determination

4 mL of DPPH 50 ppm solution were measured at the determined wavelength every minute for 60 minutes. The time at which the absorbance stabilized was considered the optimal incubation time (Pujiastuti & Zeba, 2021).

e. Preparation of Vitamin C Standard Solution

A vitamin C stock solution (1000 ppm) was prepared by dissolving 50 mg of vitamin C in 50 mL of analytical grade methanol, ensuring complete dissolution. From this stock, aliquots of 100, 200, 300, 400, and 500 µL were pipetted into separate volumetric flasks and diluted with distilled water to a final volume of 5 mL. These dilutions yielded standard solutions with concentrations of 2, 4, 6, 8, and 10 ppm, respectively (Kiromah *et al*., 2021).

f. Antioxidant Assay of Vitamin C

A volume of 4 mL from each vitamin C solution (2, 4, 6, 8, and 10 ppm) was mixed with 1 mL of DPPH solution. The mixtures were homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance of each mixture was measured using a UV-Vis spectrophotometer at the previously determined maximum wavelength (Kiromah *et al*., 2021).

g. Preparation of Extract and Fraction Test Solutions

The extract and fractions of red guava peel and fruit were dissolved in methanol to prepare stock solutions with a concentration of 1000 ppm by weighing 10 mg of each sample and dissolving it in 10 mL of methanol. Serial dilutions were then carried out by pipetting 0.1, 0.2, 0.3, 0.4, and 0.5 mL of the stock solution and diluting each with 96% ethanol to a final volume of 5 mL. This resulted in test solutions with concentrations of 20, 40, 60, 80, and 100 ppm (Andriani & Murtisiwi, 2020).

h. Antoxidant Assay of Extract and Fractions

The antioxidant activity was evaluated by adding 4 mL of each extract or fraction solution (20, 40, 60, 80, and 100 ppm) into test tubes, followed by the addition of 1 mL of DPPH solution (50 ppm). The mixture was vortexed until homogeneous and then incubated

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at room temperature for the previously determined optimal time. After incubation, the absorbance of each solution was measured at the maximum wavelength obtained (Mahmudah, 2021).

Data Analysis

1. **Extract Yield Calculation**
   The percentage yield of the extract was calculated using the following formula:
   $$\% \text{ Yield} = \frac{Weight\ of\ extract\ dish - Weight\ of\ empty\ dish}{Weight\ of\ simplicia} x\ 100\ \%$$
   (Huljanah, 2023)
2. **Moisture Loss Calculation**
   Moisture loss was calculated using the following equation:
   $$\% \text{ Moisture Loss} = \frac{Initial\ weight\ of\ simplicia - weight\ after\ drying}{Weight\ of\ simplicia} x\ 100\ \%$$
   (Rusmiyati, 2023)
3. **Water Content Calculation**
   The water content was determined using the following formula:
   $$\% \text{ Water Content} = \frac{b-(c-a)}{b} x\ 100\ \%$$
   Where :
   a = weight of dry crucible (constant)
   b = initial weight of sample
   c = weight of crucible and dried sample (constant) (Huljanah, 2023)
4. **Total Ash Content Calculation**
   Total ash content was calculated using the equation below:
   $$\% \text{ Total Ash} = \frac{W2-W0}{W1-W0} x\ 100\ \%$$
   W0 = weight of empty crucible
   W1 = weight of crucible + sample
   W2 = weight of crucible + ash (Widhi, 2022)
5. **Determination of Antioxidant Activity**
   The antioxidant activity was expressed as the percentage of inhibition using the following equation:
   $$\% \text{ Inhibition} = \frac{A\ blank - A\ sample}{A\ blank} x\ 100\ \%$$
   Where:
   A_{blank} = absorbance of DPPH at the maximum wavelength
   A_{sample} = absorbance of the sample with DPPH at the same wavelength
   The % inhibition values were then plotted against sample concentrations (ppm), with concentration as the X-axis and % inhibition as the Y-axis. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was calculated from the linear regression equation:
   $y = bx + a$
   Where:
   y = % inhibisi
   x = concentration (ppm) (Lubis *et al*., 2022)
6. **IC_{50} Determination**
   The IC_{50} value, representing the concentration required to inhibit 50% of free radicals, was calculated using the following formula:
   $$IC_{50} = \frac{50-a}{b} x\ 100\ \%$$
   Where:
   a = intercept of the linear curve
   b= slope of the linear curve (Nathania *et al*., 2020)

Results and Discussion

Plant Determination

The plant used in this study was identified at the UPF Yankestrad Laboratory in Tawangmangu, which specializes in medicinal plant identification. Macroscopic and morphological characteristics of the peel and fruit samples—suspected to be red guava—were compared to standard botanical references. The results confirmed that the specimen was *Psidium guajava L.* (red guava), validating its use for subsequent experimental stages.

Standardization of Simplicia and Extracts

Drying Loss

Drying loss is a critical non-specific parameter in the standardization of simplicia and natural extracts, representing the amount of water and volatile substances

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lost during drying (Utami *et al.,* 2020). The results of the drying loss test for the simplicia of red guava peel and fruit are presented in Table 1.

Table 1. Drying Loss of Red Guava Peel and Fruit Simplicia

Replication	Results (%)	
	Peel	Fruit
1	9,471	9,95
2	9,576	9,6
3	9,123	9,123
Average	9,39 ± 0,2371	9,5577 ± 0,4151
Good Standard	< 10%	

Determination of drying loss is an essential step in the quality evaluation of simplicia, aimed at identifying the percentage of compounds that may evaporate or be lost during the heating process, including water and other volatile components. This test provides insight into the stability of the simplicia during storage and its susceptibility to microbiological contamination. In addition to water, other volatile substances such as essential oils, alcohol, and residual organic solvents may also be lost during the drying process, and all of these are reflected in the obtained drying loss values. The results indicate that both the peel and fruit simplicia of red guava fall within the permissible maximum moisture content limit, which is less than 10%, as specified in the simplicia quality standards. These findings suggest that the drying process was optimal and effectively reduced the moisture content and other volatile compounds to a safe level, thereby ensuring good stability of the simplicia for storage and suitability for use in further product formulation.

Water Content

Water content refers to the amount of water contained in a material. It can be determined by measuring the difference in the weight of the material before and after heating at a specific temperature during the testing process (Winarno, 2002). The results of the water content test for the simplicia of red guava peel and fruit are presented in Table 2.

Table 2. Water Content of Red Guava Peel and Fruit Simplicia and Extracts

Replication	Results (%)			
	Peel Simplicia	Fruit Simplicia	Peel Extracts	Fruit Extracts
1	7,25	6,64	3,94	2,30
2	7,75	6,75	3,84	2,80
3	7,70	6,35	4,29	2,00
Average	7,5667 ± 0,2754	6,58 ± 0,2066	4,0233 ± 0,2363	2,3667 ± 0,4041
Good Standard	< 10%			

The determination of moisture content in the standardization process aims to assess the amount of water remaining in the extract after the concentration process following maceration. High moisture content can promote the growth of microorganisms such as fungi, bacteria, or molds, which may pose health risks. The principle of

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moisture content determination involves comparing the initial mass of the sample with its mass after the water has been evaporated through heating at a temperature above the boiling point of water, typically 105°C, until a constant weight is achieved. This determination is crucial for maintaining the quality of the material during storage. To reduce moisture content, a more optimal drying process with a longer duration is required (Rosyidah, 2016). Based on the data in Table 8, the results of the moisture content determination of both simplicia and extracts from the peel and fruit of red guava (*Psidium guajava* L.) indicate that all samples meet the maximum allowable moisture content requirement, which is less than 10%. These values demonstrate that both the simplicia and extracts of red guava peel and fruit comply with the established moisture content standard of a maximum of 10%. Therefore, it can be concluded that the drying and concentration processes applied in this study were effective in reducing the moisture content to a safe level for storage and further utilization.

Total Ash Content

Ash refers to the residual inorganic substances remaining after the combustion of plant materials. Ash content is used as one of the methods to identify specific medicinal plant species, as each plant type has a characteristic ash composition (Persagi, 2009). The results of total ash content test for the red guava peel and fruit simplicia are presented in Table 3.

Table 3. Total Ash of Red Guava Peel and Fruit Simplicia

Replication	**Results (%)**	
	Peel	**Fruit**
1	8,6414	9,4311
2	8,8235	9,2861
3	8,7606	9,3267
Average	8,7418 ± 0,0925	9,348 ± 0,0748
Good Standard	< 10,6%	

The basic principle of this method involves heating the sample at a high temperature, approximately 600°C, using a furnace or muffle. This heating process continues until all organic components in the material are decomposed and evaporated, leaving behind ash as the residual inorganic components, such as minerals. The process is carried out until the ash reaches a constant weight, indicating completion. The result is expressed as a percentage of ash relative to the initial weight of the sample. Determination of ash content is crucial to ensure the quality of the material, as excessively high ash levels may indicate contamination or unhygienic handling during processing (Depkes RI, 2000). Based on the data in Table 3, the results of total ash content determination in the peel and fruit simplicia of red guava (*Psidium guajava* L.) show that all samples meet the maximum permitted ash content requirement, which is less than 10.6%. This indicates that the red guava peel and fruit simplicia have good purity levels and were not subjected to excessive mineral contamination during material preparation. Therefore, it can be concluded that the washing, sorting, and drying stages were carried out optimally.

Ethanol Residue Test

The ethanol residue test on the thick extract was carried out by adding acetic acid and sulfuric acid, followed by heating. The extract is considered free of ethanol if no

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distinctive ester aroma is detected, which would otherwise indicate the presence of ethanol (Larasati, 2023). The results of the ethanol residue test for the peel and fruit extracts of red guava (*Psidium guajava* L.) are presented in Table 4.

Table 4. Ethanol Residue Test of Red Guava Peel and Fruit Extract

Extract	Results	Interpretation
Peel	No ester odor detected	Negative (-)
Fruit	No ester odor detected	Negative (-)

Based on the data presented in Table 4, the extracts of red guava peel and fruit did not exhibit any detectable ester or alcohol odor. This indicates that no residual ethanol was detected in either extract, suggesting that the evaporation or concentration process was carried out optimally and successfully removed the solvent completely. These results also support the effectiveness of using a low-temperature yet prolonged concentration method, such as a rotary evaporator at 40°C, in eliminating solvents without degrading the active compounds within the extract. Therefore, the absence of residual ethanol in the red guava peel and fruit extracts demonstrates that the extraction and purification processes were conducted properly, and the resulting extracts meet the quality standards required for further research or development of natural product-based formulations.

Heavy Metal Free Test

Standard testing of extracts for heavy metal content is conducted to ensure that the resulting extracts are not contaminated with heavy metals that could pose health risks (Depkes RI, 2000). The results of the heavy metal free test for red guava peel and fruit extracts are presented in Table 5.

Table 5. Heavy Metal Free Test of Red Guava Peel and Fruit Extract

Extract	Type	Results	Interpretation
Peel	Pb	Brown solution with no precipitate observed	Negative (-)
	Cd	Dark brown solution	Negative (-)
Fruit	Pb	Brown solution with no precipitate observed	Negative (-)
	Cd	Brown solution	Negative (-)

The standardization process of natural materials and medicinal plant extracts is a crucial step in ensuring the quality, safety, and consistency of the final product. One parameter that must not be overlooked is the testing for heavy metal contamination, as the presence of such metals in extracts can pose serious health risks if consumed continuously in amounts exceeding the permissible limits (Rahmiani, 2019). Based on observations presented in Table 5, it was found that both the peel and fruit extracts of red guava produced a brown-colored solution without any precipitate after the addition of reagents for Pb testing. This color indicates no formation of a precipitate, which means that no lead (Pb) ions were detected in the extract. Therefore, both types of extracts are considered negative for Pb contamination,

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suggesting the products are safe from this heavy metal risk. Meanwhile, in the test for cadmium (Cd), the peel extract of red guava resulted in a dark brown solution, while the fruit extract produced a brown solution. The absence of a pink coloration or any precipitate, which would indicate the presence of Cd, confirms that the extracts are also negative for cadmium. This suggests that throughout the cultivation, harvesting, and processing stages, no heavy metal contamination occurred, whether from environmental sources or equipment.

Phytochemical Screening

Phytochemical screening is a method used to detect the presence of bioactive compounds that are not directly observable, through rapid testing or examination that can distinguish natural materials containing specific phytochemical compounds from those that do not. This stage serves as an initial step in phytochemical research, aiming to provide preliminary information about the types of compounds present in the plant being studied. The screening process is carried out by observing color changes that occur as a result of reactions with specific color reagents (Sahara, 2019). The results of the phytochemical screening of the extracts and fractions of the peel and fruit of red guava are presented in Table 6.

Table 6. Phytochemical Screening of Red Guava Peel and Fruit Extract and Fractions

Type	Test	Reagent	Results	Good Standard	Interpretation
Peel Extract	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Black precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Orange solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
Fruit Extract	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Brown precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)

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	Flavonoid	Ethanol + HCl + Magnesium powder	Brownish orange solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
N-Hexane Fraction of Peel	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Brown precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Yellow solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Blackish brown precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
N-Hexane Fraction of Fruit	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Brown precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Yellow solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)

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Ethyl Acetate Fraction of Peel	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Black precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brownish orange precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Yellow solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
Ethyl Acetate Fraction of Fruit	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Black precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Yellow solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
Water Fraction of Peel	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Brown precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Orange solution	Orange or yellow solution	Positive (+)

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	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)
Water Fraction of Peel	Alkaloid	Mayer	Yellow precipitate	White or yellow precipitate	Positive (+)
		Bouchardat	Brown precipitate	Brown to black precipitate	Positive (+)
		Dragendorff	Brown precipitate	Brown or orange-brown precipitate	Positive (+)
	Flavonoid	Ethanol + HCl + Magnesium powder	Orange solution	Orange or yellow solution	Positive (+)
	Tanin	Ethanol + $FeCl_3$	Black precipitate	A blue, blue-black, green, or green-black color with precipitate	Positive (+)
	Triterpenoid	Lieberman-Burchard	Brown precipitate	A greenish-black or dark green solution	Negative (-)

Alkaloids are a group of compounds commonly found in nearly all types of plants. These compounds typically contain at least one nitrogen atom, are generally basic in nature, and form heterocyclic ring structures (Sahara, 2019). Flavonoids belong to the largest group of natural phenolic compounds and are characterized by a basic structure consisting of 15 carbon atoms. This structure follows a C6-C3-C6 pattern, comprising two aromatic rings connected by a three-carbon chain that may form a third ring. Additionally, flavonoids are polar due to the presence of hydroxyl groups or sugar components within their structures. Tannins are a class of compounds containing phenolic components with specific chemical and physical. characteristics. They typically possess aromatic rings with one or more hydroxyl groups as substituents Triterpenoids are chemical constituents found in plants and are known for their distinctive aroma. They generally have a cyclic structure and may exist as alcohols, carboxylic acids, or aldehydes. These compounds are often present in seed-bearing plants in glycoside form. The presence of triterpenoids can be identified by the formation of a brown-colored ring when treated with concentrated sulfuric acid (Sholikhah, 2016). Based on the data presented in Table 6, the extracts and fractions of red guava (*Psidium guajava* L.) peel and fruit tested positive for alkaloids, flavonoids, and tannins, while triterpenoids were not detected.

Antioxidan Activity Assay

The antioxidant activity assay began with the preparation of a 50 ppm DPPH solution as a stable free radical reagent. The

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solution was then diluted with methanol, and its absorbance was measured in the wavelength range of 500–600 nm using a UV-Vis spectrophotometer, with methanol serving as the blank. The result showed that the maximum absorbance wavelength (λmax) of DPPH was observed at 516 nm with an absorbance value of 0.825. Operating time determination was carried out to identify the optimal reaction time at which the absorbance becomes stable, ensuring that the interaction between DPPH and antioxidant compounds had reached completion before measurement. Absorbance was recorded at 1 minute intervals for 60 minutes. The results indicated that absorbance stabilized between the 17th and 19th minute, with a value of 0.860. This time interval was therefore selected as the optimal measurement time. The antioxidant activity of the samples was evaluated using the IC_{50} value (Inhibitory Concentration 50), which represents the concentration of a sample required to reduce 50% of the initial DPPH radical concentration. A lower IC_{50} value indicates a stronger antioxidant capacity. The IC_{50} was calculated based on the percentage of inhibition at various sample concentrations, followed by linear regression analysis (Pusmarani *et al*., 2022). The results of the antioxidant activity test for vitamin C (as a reference standard), ethanolic extracts, and various solvent fractions of red guava peel and fruit are presented in Table 7.

Table 7. Antioxidant Activity of Vitamin C, Ethanolic Extract, and Solvent Fractions of Red Guava Peel and Fruit

No	Sample	IC_{50}	Interpretation
1.	Vitamin C	1,1321	Very strong
2.	Peel Extract	62,4719	Strong
3.	Fruit Extract	44,0035	Very strong
4.	Ethyl Acetate Fraction of Peel	56,7955	Strong
5.	Ethyl Acetate Fraction of Fruit	46,4394	Very strong
6.	N-Hexane Fraction of Peel	113,9311	Medium
7.	N-Hexane Fraction of Fruit	114,4767	Medium
8.	Water Fraction of Peel	104,0284	Medium
9.	Water Fraction of Fruit	76,5801	Strong

The antioxidant activity assay on extracts and fractions of red guava peel and fruit showed a color change in the DPPH solution from purple to light purple or yellow after reacting with the sample. This change occurs due to the donation of hydrogen atoms from the sample, which react with DPPH radicals to form a stable compound, 1,1-diphenyl-2-picrylhydrazine (DPPH-H) (Hasan *et al*., 2022). An incubation period of 17 minutes was applied to ensure sufficient time for the reaction between DPPH and the sample to proceed optimally.

In this study, vitamin C was used as the standard antioxidant solution. Vitamin C is commonly used as a reference in antioxidant assays because it functions as a secondary antioxidant capable of neutralizing free radicals, preventing chain reactions, and exhibiting very strong antioxidant activity. Additionally, vitamin C is readily available and possesses more polar characteristics compared to other vitamins. The presence of free hydroxyl groups in vitamin C plays a crucial role in neutralizing free radicals (Damanis *et al*., 2020). Vitamin C demonstrated a very strong antioxidant activity with an IC_{50} value of 1.1321 ppm, as it falls within the $IC_{50} < 50$ ppm category (Syaiffudin, 2015).

The antioxidant activity test results of extracts and solvent fractions from both peel and fruit parts revealed significant

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variations in antioxidant potency depending on the type of sample and solvent used. Antioxidant activity was measured in terms of IC_{50} (ppm), which indicates the concentration required to reduce 50% of DPPH free radicals. The lower the IC_{50} value, the higher the antioxidant potency of the sample.

The ethanolic extract of the peel showed antioxidant activity with an IC_{50} value of 62.4719 ppm, which falls under the strong category. In contrast, the ethanolic extract of the fruit exhibited an IC_{50} value of 44.0035 ppm, classified as very strong. These results indicate that antioxidant compounds soluble in ethanol are more abundant in the fruit than in the peel. Ethanol is capable of dissolving both polar and semi-polar compounds, such as phenolics and flavonoids, which are commonly found in fruit tissues (Hakim & Saputri, 2020).

In the ethyl acetate fractions, which are semi-polar, the peel showed an IC_{50} value of 56.7955 ppm (strong), whereas the fruit showed 46.4394 ppm (very strong). The n-hexane fractions, being non-polar, demonstrated the weakest antioxidant activity among all fractions. The peel yielded an IC_{50} value of 113.9311 ppm, and the fruit 114.4767 ppm both categorized as moderate. For the aqueous fractions, which are highly polar, the peel showed an IC_{50} of 104.0284 ppm (moderate), while the fruit had 76.5801 ppm (strong).

Overall, the data suggest that the fruit part tends to possess stronger antioxidant activity than the peel, particularly in ethanolic and ethyl acetate solvents. This may be attributed to the higher content of bioactive compounds such as flavonoids, tannins, and other phenolics in the fruit. Furthermore, the type of solvent greatly influences the effectiveness of antioxidant compound extraction, with polar and semi-polar solvents like ethanol and ethyl acetate yielding higher antioxidant activity than non-polar solvents like n-hexane.

Ethyl acetate fractions exhibited better antioxidant activity compared to water and n-hexane fractions. This may be due to the presence of bioactive compounds such as flavonoids and tannins, both of which play significant roles in antioxidant mechanisms. Flavonoids can scavenge and neutralize free radicals through hydrogen atom donation, while tannins act as metal ion chelators and inhibitors of radical formation (Mahmudah, 2022). Tannins are natural antioxidant compounds capable of neutralizing free radicals, binding metal ions, and inhibiting oxidative enzymes. Due to these properties, tannins help protect body cells from oxidative stress-related damage (Fajriati, 2006). The combination of these compounds in the ethyl acetate fraction is presumed to contribute synergistically, enhancing its ability to inhibit free radical activity and strengthening its overall antioxidant potential (Mahmudah, 2022).

Conclusion

Based on the results of the study, the following conclusions can be drawn:

1. The secondary metabolites identified in the ethanolic extract and the ethyl acetate, n-hexane, and water fractions of red guava (*Psidium guajava* L.) peel and fruit include alkaloids, flavonoids, and tannins.
2. The ethanolic extract and the ethyl acetate, n-hexane, and aqueous fractions of the peel and fruit exhibited antioxidant activity, as indicated by the fading of the DPPH solution color after reacting with the samples within the designated time.
3. The IC_{50} values obtained were as follows: ethanolic extract of peel: 62.4719 ppm, ethanolic extract of fruit: 44.0035 ppm, ethyl acetate fraction of peel: 56.7955 ppm, ethyl acetate fraction of fruit: 46.4394 ppm, n-hexane fraction of peel: 113.9311 ppm, n-hexane fraction of fruit: 114.4767 ppm, aqueous fraction of peel:

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104.0284 ppm, and aqueous fraction of fruit: 76.5801 ppm.

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