

Antioxidant Activity Testing of Extract Kweni Peel (*Mangifera odorata* Griff)

Yolanda Dwi Lestari*, Suci Permatasari* & Ade Oktasari*

* Chemistry Study Program, UIN Raden Fatah Palembang

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*Corresponding Author:

Yolanda Dwi Lestari,
Chemistry Study Program
UIN Raden Fatah Palembang
Email: yolandadwil12@gmail.com

ABSTRACT

This research aims to test the antioxidant activity of extract kweni peel (*Mangifera odorata* Griff). The antioxidant testing was performed by maceration using the solvents of n-hexane and ethanol 96% after that it was fractioned using ethyl acetate solvent. The phytochemical tests yield positive results from ethyl acetate extract were flavonoids and tannins. The antioxidant testing was carried out using the DPPH method (2,2, diphenyl-1-picrylhydrazyl) based on the inhibition concentration test parameters (IC₅₀). The result showed that the antioxidant activity (IC₅₀) found to be 169.43 µg/mL which was classified as moderate antioxidant activity intensity. Therefore, it can be concluded that the extract of kweni peel (*Mangifera odorata* Griff) has potential antioxidant activity that could be used as an inhibitor of free radicals.

Keyword: antioxidant, kweni peel, phytochemicals

1. INTRODUCTION

Life in society is never separated from free radical compounds, such as solar radiation, cigarette smoke and environmental pollution such as vehicle fumes (Wahdaningsih, 2011). One of the effects of the free radicals is premature skin aging. Premature skin aging is the process of accelerating cell degeneration which is characterized by wrinkled, dull, and dry skin. If the premature skin aging occurred in a long period of time, it could cause the skin cancer (Dewiastuti, 2016).

One of the efforts to prevent the free radicals is by using an antioxidant. Antioxidants are compounds that are able to prevent and inhibit free radical reactions by donating one electron to free radical compounds (Murwanto, 2012). The benefit of antioxidants is that they are able to reduce the poor effects of free radicals by binding them and turning them into compounds that do not have a poor impact on the body (Wulandari, 2013).

People usually consume drugs or supplements that contain antioxidants to meet their body's needs. However, the use of these synthetic antioxidants has side effects on body health. The synthetic antioxidants are carcinogenic to the body (Jami'ah, 2018). Carcinogenic compounds can trigger cancer, so it is necessary to find ways to reduce the long-term effects of synthetic antioxidants by utilizing natural antioxidants derived from natural ingredients such as plants. Plants contain phenolic or polyphenolic compounds which can be in the form of flavonoids, cinnamic acid derivatives, coumarins, tocopherols which can be used as natural antioxidants (Nurhasnawati, 2017). One of the plants that can be used as natural antioxidants is the mango species of kweni (*Mangifera odorata* Griff). According to Prihatiningtyas (2018), the kweni plant belongs to the Anacardiaceae family group, which is known to have phenolic compounds that have potential as medicinal compounds and natural antioxidants. Therefore, this study aimed to determine the antioxidant

activity of kweni fruit peel extract (*Mangifera odorata* Griff) using ethyl acetate as a solvent through the DPPH (2,2, diphenyl-1-picrylhydrazyl) method.

2. RESEARCH METHOD

a. Time and Place

This research was carried out at the Chemistry Laboratory of the Faculty of Science and Technology of Uin Raden Fatah in February 2020 - December 2020.

b. Tools and Materials

The tools that used were a set of maceration tools, glassware (Pyrex and Iwaki), stirring rods, analytical balance (OHAUS), blender (Maspion MT-1207), glass bottles, separating funnel (Pyrex), filter paper, rotary evaporator (Yamato RE301C-W), UV-Vis spectrophotometric instrument (biochrom Libra S12) and FTIR instrument (Bruker Alpha 2). While, the materials that used were kweni fruit peel (*Mangifera odorata* Griff), 96% ethanol (C₂H₅OH) (Merck), n-hexane (Merck), ethyl acetate (C₂H₅CH₃COOH) (Merck), aquades (Merck), DPPH powder (2,2 diphenyl-1 -picrylhidrzyl) (merck), aluminum foil, 1% HCl (merck), concentrated HCl (merck), mayer's reagent (merck), Wagner's reagent (merck), FeCl₃ (merck), H₂SO₄ (merck), Mg powder (merck).

c. Research Procedures

1) Sample Preparation

The peel of the kweni fruit (*Mangifera odorata* Griff), were washed to remove the dirt, drained, and then cut into smaller pieces. The small pieces of the kweni peel were dried in the sun until completely dry. After that, it mashed until became a fine powder.

2) Extraction of Kweni Peel (*Mangifera odorata* Griff)

200 grams of kweni fruit peel powder was macerated and put into a closed glass bottle, added with n-hexane solvent for 3x24 hours, the resulting residue was dried and extracted with 96% ethanol solvent for 3x24 hours, filtered to produce a filtrate. After that, the filtrate was concentrated with a rotary evaporator to obtain a thick extract of ethanol which was then carried out for phytochemical screening and characterized by FTIR. The ethanol extract was isolated using ethyl acetate solvent. It put into a separating funnel and shaken until homogeneous. The extract was let in a moment until the ethanol phase and ethyl acetate phase were formed. The ethyl acetate phase was evaporated using a rotary evaporator. Furthermore, phytochemical screening and characterization were carried out with the FTIR instrument (Nugraha, 2017).

3) Phytochemical Screening Test

The aim of testing the content of secondary metabolites in chemical compounds was to determine the secondary metabolites contained in the thick ethanolic extract of kweni fruit peel and ethyl acetate extract.

a) Flavonoid Test

The extract sample was mixed with Mg powder and a few drops of concentrated HCl. If the mixture was yielding a pink, magenta, and orange colot, it indicates flavonoid compounds.

b) Alkaloid Test

The extract sample was added with a small amount of 1% HCl, then 1mL of Mayer reagent was added. If there is a white precipitate, it indicates a positive alkaloid compound. Then with the same treatment, Wagner reagent was added, if there was a brown precipitate, it was positive for alkaloid compounds.

c) Saponin Test

The extract sample was added with 10 mL of distilled water and then shaken vigorously for 30 seconds. If it yields a stable foam, then it indicates a saponin compound.

d) Steroid Test

The extract sample was added with a little of acetic anhydrous and 1 drop of H₂SO₄ (Lieberman Buchard reagent). If it produces a blue-green color, it indicates a positive steroid compound

e) Terpenoid Test

The extract sample was added with a little acetic anhydrous and 1 drop of H₂SO₄ (Lieberman Buchard reagent). If there is a brownish red color or a brownish pink ring, it indicates a positive terpenoid compound.

f) Tanin Test

The extract sample was added with 10 mL of distilled water and then boiled. Add a few drops of FeCl₃. If the presence of a brownish green or bluish black color, it indicates a positive tannin compound.

4) Antioxidant Activity Testing with DPPH Method

a) DPPH Solution Preparation

A total of 1.98 mg of DPPH powder was dissolved in 96% ethanol and poured into a 50 mL volumetric flask. After that, it filled with 96% ethanol until the mark, then put into a dark color of bottle.

b) Determination of the Maximum Absorption Wavelength of DPPH

A total of 2 mL of 0.1 mM DPPH solution was taken into a test tube and added with 2 mL of 96% ethanol. After that, it homogenized with a vortex, then the maximum absorption was measured using UV-Vis spectrophotometry at a wavelength range of 400-800 nm.

a) Preparation of Blank Solution

A total of 2 mL of 0.1 Mm DPPH solution was taken and put into a test tube and added with 2 mL of 96% ethanol, covered with aluminum foil. After that, it homogenized with a vortex and incubated in the dark environment for 30 minutes. Next, the absorption was measured by UV-Vis spectrophotometry at the maximum wavelength that was obtained.

b) Preparation of Test Solution

Preparation of 1000 ppm mother solution of ethyl acetate extract of kweni peel taken as much as 50 mg dissolved with 96% ethanol. It was poured into a 50 mL volumetric flask, then filled with 96% ethanol to the limit mark. Furthermore, the mother solution was diluted to make variations in the concentration of the test solution, namely 10 ppm, 20 ppm, 30 ppm and 40 ppm.

c) Antioxidant Activity Testing

The absorbance of each concentration of the test solution was measured by UV-Vis spectrophotometry by taking 2 mL of the test solution into a test tube that had been covered with aluminum foil and added with 2 mL of 0.1 mM DPPH solution. The solution was homogenized with a vortex and incubated in the dark environment for 30 minutes. After obtaining the absorbance value of the sample, the % value of inhibition was calculated (Akmal, 2014).

d. Data Analysis

Antioxidant activity is expressed in terms of the percent of DPPH radical that calculated using the following formula:

$$\% \text{ DPPH inhibition} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\%$$

Description:

Blank absorbance: DPPH absorbance value

Sample absorbance: absorbance of ethyl acetate extract sample in DPPH at maximum wavelength (Najihudin, 2017).

In addition, the calculation of the IC₅₀ value of the sample indicated the concentration of sample extract that required to capture the DPPH radicals is 50%. It obtained from the linear regression equation between the percent radical reduction and various test concentrations. The smaller the IC₅₀ value, the higher the antioxidant activity. The IC₅₀ value can be determined by the following formula:

$$Y = ax + b$$

Description:

a= concentration constant

b= constant

x= concentration

Y=% attenuation

3. RESULTS AND DISCUSSION

3.1 Preparation and Maceration

The preparation process began by cleaning as much as 872 grams of wet kweni fruit peel samples (*Mangifera odorata* Griff) using water. After that, the samples were dried under the sun for 8 days. The drying process was carried out to reduce the moisture content in the kweni fruit peel samples. Therefore, the fungi and bacteria were not easy to grow. A total of 416 grams of dried kweni fruit peel were produced.

Samples that have been dried were mashed using a blender. The mashing process aims to expand the surface so that the maceration process would runs optimally. The sample was sieved to obtain a fine powder of 295 grams of kweni peel. The following steps is the extraction process of the fine powder of the kweni peel which serves to extract the chemical compounds contained in the kweni fruit peel sample.

The results of extract kweni peel were macerated by immersion using n-hexane solvent for 3 days and followed by immersion with 96% ethanol solvent for 3 days. The evaporation was carried out to obtain saturated extract. The use of n-hexane as a solvent was to attract non-polar compounds, while the use of ethanol as a solvent because was to attract polar compounds. The results of maceration of kweni fruit peel (*Mangifera odorata* Griff) shown in Table 1.

Table 1. The results of maceration of kweni fruit peel

No	Maceration Step	Results
1	Wet weight	872 grams
2	Dried weight	416 grams
3	Powder weight	295 grams
4	Ethanol saturated extract	26.26 grams
5	% yield	13.13 %

3.2 Liquid-Liquid Extraction

The next test carried out was liquid-liquid extraction using a separating funnel. This process aims to obtain the desired secondary metabolite compounds. The sample of the saturated extract of the kweni fruit peel was dissolved with distilled water and extracted with ethyl acetate as a solvent to attract the compounds with lower polarity. Therefore, it was expected to attract the desired compound. This process used a separating funnel which was carried out by shaking and disposing the gas contained in the separating funnel while it was being reacted. It was allowed to stand until two layers were formed. The upper part was the clear green ethyl acetate phase, and

the lower part was the brown aquadest phase. The process of shaking and disposing gas aims to reduce the air pressure contained in the funnel in the testing process. Then evaporation process take place at room temperature of the extraction with ethyl acetate solvent to obtain a saturated extract. The results that were obtained can be seen in the Table 2.

Table 2. The result of ethyl acetate solvent extraction of kweni fruit peel

No	Extraction Step	Results
1	Ethanol saturated extract	8.50 grams
2	Extraction with ethyl acetate	170 mL
3	Dry filtrate results	1.13 grams
4	% yield	13.29 %

3.3 Phytochemical Test

The results of phytochemical tests on kweni fruit peel can be seen in the Table 3.

Table 3. Phytochemical content of kweni fruit peel

Compound Group	Changes	Ethanol	Ethyl Acetate
		Extract Test Results	Extract Test Results
Flavonoids	Orange color	+	+
Saponins	Have a stable foam	+	-
Alkaloids	White precipitated (Mayer's reagent)	-	-
	Chocolate precipitated (Wagner's reagent)	-	-
Terpenoids	Red color and produce ring	-	-
Steroids	Turquoise color	-	-
Tannins	Deep black color	+	+

Notes: (+) contain compound; (-) not contain compound

Based on the data from the phytochemical test results on Table 3.3, the ethyl acetate extract of the kweni fruit peel was positive for flavonoid compounds which were indicated by the formation of an orange color. It was produced when Mg and HCl were added in order to reduce and get colored complex compounds. The resulting color indicates the presence of flavonoids as a result of reduction by HCl and Mg (Ergina, 2014). The reaction between flavonoids with Mg and HCl can be seen in Figure 1.

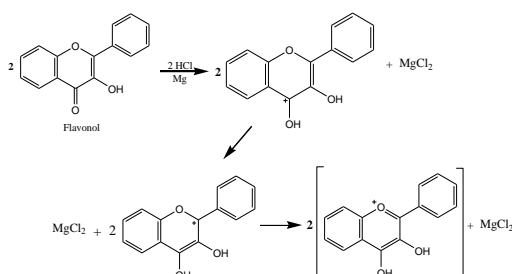


Figure 1. Reaction of Flavonoids with Metals Mg and HCl (Ergina, 2014)

The ethyl acetate extract contains tannin compounds which are characterized by the formation of a dark black color in the sample test. The formation of the black color after being added to FeCl₃

solution caused by the tannins will form a complex compound with Fe^{3+} ions. This shows that the ethyl acetate extract sample of kweni fruit peel contains polyphenol compounds which are thought to be tannin compounds.

The formation of complex compounds in the reaction of tannins and FeCl_3 caused by the presence of Fe^{3+} ions as the central atom while tannins have an O atom which has a lone pair of electrons that can coordinate to the central atom as a ligand (Ergina, 2014). The reaction can be seen in Figure 2.

Based on the results of phytochemical tests that have been carried out, it shows that the ethyl acetate extract of kweni fruit peel contains flavonoid compounds and tannins. This means that the ethyl acetate extract of kweni fruit peel has the potential to have antioxidant activity.

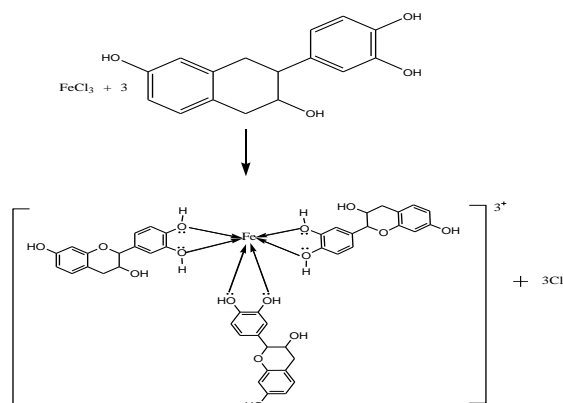


Figure 2. The reaction between tannins and FeCl_3 (Ergina, 2014).

3.4 FTIR Analysis of Kweni Peel Extract

The results of FTIR analysis of kweni fruit peel extract showed that it contained flavonoid phenolic compounds which were shown to have O-H, aliphatic C-H, C=O carbonyl, C=C aromatics, C-O alcohol group absorption. It also contained tannin compounds which were characterized by functional groups O-H, C-H, C=C aromatic, C-O alcohol and C-O-C ether. In the range of wave number of 3266 cm^{-1} was the absorption of the O-H group. At the wave number of 1701 cm^{-1} was the C=O carbonyl. While, in the wave number of 2992 cm^{-1} is the aliphatic C-H functional group. At wave number of 1602 cm^{-1} was an aromatic C=C group. The absorption of the C-O-C ether group at a wave number of 1245 cm^{-1} . Still, at the wave number of 1066 cm^{-1} was the absorption of the functional group C-O alcohol. The results of testing the kweni fruit peel extract using FTIR spectrophotometry can be seen in Figure 3.

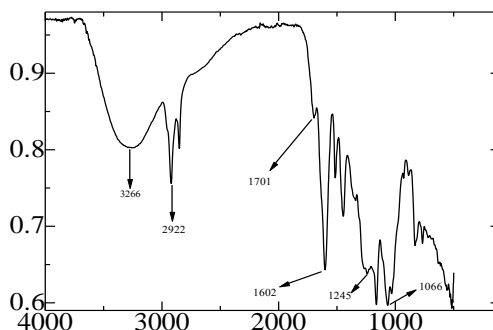


Figure 3. Infrared spectrum of ethyl acetate extract of kweni fruit peel

3.5 Antioxidant Activity Testing

Testing of antioxidant activity was carried out by reacting samples of kweni fruit peel extract with free radicals in the form of DPPH solution. The sample will experience DPPH color fading. The test was carried out in 4 variations of concentration, namely 10 ppm, 20 ppm, 30 ppm and 40 ppm.

The color change that occurs in the DPPH radical solution is due to the addition of compounds that act as antioxidants. The interaction between antioxidants and DPPH can occur by electron transfer or hydrogen radicals to DPPH which can neutralize the free radical character. If all the electrons in the DPPH free radical are paired, then the color of the solution will change from a dark purple to a yellow color (Murwanto, 2012).

Testing the antioxidant activity of kweni peel extract with the DPPH method needs to be carried out in several stages such as (a) determining the maximum wavelength and (b) measuring the antioxidant potential of the kweni peel extract sample.

a. Determination of Maximum Wavelength DPPH

The determination of the maximum wavelength was done by measuring the absorbance of DPPH (2,2-diphenyl-1-picrylhydrazyl) at a wavelength range of 400-800 nm. The maximum wavelength was determined by the largest absorption results of DPPH. The maximum wavelength that signified in this step was 516 nm. This maximum wavelength will provide optimal absorption from the test solution, so that the optimal absorbance value can be obtained in the sample.

b. Antioxidant Activity Testing

Testing the antioxidant activity of ethyl acetate extract of kweni fruit peel done by making stock solution of DPPH. It began by weighing a total of 19.8 mg of DPPH powder and dissolved in ethanol 96% in a 50 mL volumetric flask, so that 0.1 mM DPPH solution was obtained. Furthermore, stock solutions were made from the sample results with various concentrations of 10 ppm, 20 ppm, 30 ppm and 40 ppm. Then 2 mL of DPPH was added and the volume was made up with 96% ethanol in a 10 mL volumetric flask, then homogenized with a vortex and incubated in a dark room for 30 minutes so that the sample can react with DPPH solution.

The test was carried out in a room protected from direct sunlight so that the sample and DPPH remained stable. Sunlight can decompose the solution and can affect the results. Absorbance measurements were carried out using UV-Vis spectrophotometry at a wavelength of 516 nm, then the test was repeated three times in order to obtain more accurate results. The results of testing the relationship between absorbance and concentration in the ethyl acetate extract of kweni fruit peel can be seen in the Figure 4.

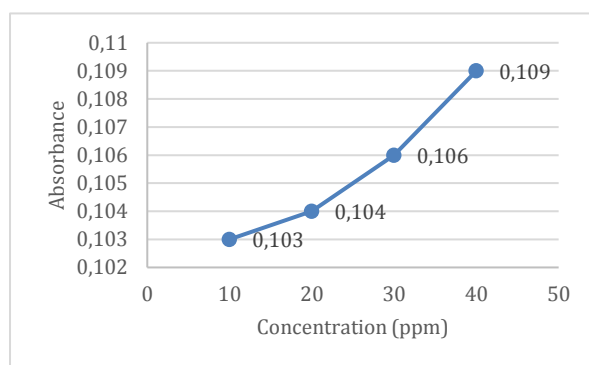


Figure 4. Absorbance of kweni fruit peel extract

Based on Figure 4, it can be seen that the higher the concentration, the higher the absorbance value. This is possible because the concentration range chosen in the study is not significant so that the absorption values obtained are almost visible at the same level. In testing the antioxidant activity, the sample reacted with the absorbance free radical compound obtained must experience a decrease trend in the absorption concentration. Therefore, it can be said that the increasing concentration of the sample which is reacted with free radical compounds is expected to yield in a decreasing

absorption value. Where the decrease in the absorption value indicates that the sample which is thought to contain antioxidant compounds is able to oxidize these free radical compounds. It can be seen that the absorbance results between concentrations have values that are not too far apart, this is presumably because the range of variations in the concentration chosen is not so significant.

Furthermore, after obtaining the absorbance value of the sample, it can be obtained the percent value of free radical inhibition (% inhibition). The percentage value of free radical inhibition of kweni fruit peel extract can be seen in the Table 4.

Tabel 4. The results of antioxidant activity test of kweni fruit peel extract

No	Concentration	% Inhibition	Blank	IC ₅₀ (µg/mL)
1	10 ppm	61.71		
2	20 ppm	61.33		169.43
3	30 ppm	60.59	0.269	(Moderate)
4	40 ppm	59.47		

Based on Table 4, the higher the concentration, the lower the % inhibition value produced. The decrease in the value of % inhibition was thought to be because antioxidant compounds were not optimal in inhibiting and stabilizing free radicals. It is suspected that the possibility that can occur is that the compound is pro-oxidant. This is in accordance with Gordon's research, which states that the greater the concentration of added antioxidants can affect the rate of oxidation. At high concentrations, the antioxidant activity of the phenolic group often disappears and can even turn into pro-oxidants (Rohmawati, 2019). In samples of kweni fruit peel extract produced the highest % inhibition at a concentration of 10 ppm with a value of 61.71%. This indicates that at a concentration of 10 ppm, the % inhibition value has reached the optimum level.

The antioxidant test results obtained the value of % inhibition as the one of the test parameters to show the ability of an antioxidant to inhibit a free radical. Where the higher the concentration containing more antioxidant active compounds, the greater the value of % inhibition or the increased ability to inhibit free radicals. However, this is thought to be inconsistent with the results obtained in this study.

Then to get the IC₅₀ value, the linear equation obtained by using the Microsoft Excel signified the equation of $y = -0.0746x + 62.64$. The y -coefficient has a value of 50 which is the IC₅₀ coefficient, the x -coefficient is the concentration of the extract to be looked for, the x -value obtained is the amount of concentration needed to soak 50% of the DPPH radical activity. By having a correlation value (R^2) of 0.953, the value of R^2 is the linearity of concentration to % inhibition. If the R value is 1 or close to 1, then the research data obtained are very good (Masrifah, 2017).

Based on the results of this research, the antioxidant activity test of kweni fruit peel extract has the potential as an antioxidant with an IC₅₀ value of 169.43 g/mL which indicates a moderate intensity category, because it is in the IC₅₀ range of 101-250. The smaller the IC₅₀ value, the higher the antioxidant value. A compound can be said to have antioxidant activity if it has antioxidant intensity. If IC₅₀ value < 50 ppm indicates very strong antioxidant, while if the IC₅₀ value 50-100 ppm indicates strong antioxidant, still if the IC₅₀ value 101-250 ppm indicates moderate antioxidant, the IC₅₀ value 250-500 ppm shows weak antioxidants, and if the IC₅₀ values >500 ppm indicates inactive antioxidants (Akmal, 2014). Based on the results of this research, the antioxidant activity test of the kweni fruit peel extract (*Mangifera odorata* Griff) has the potential as an antioxidant with an IC₅₀ value of moderate intensity. The reaction of reducing free radicals by antioxidant compounds can be seen in the Figure 5.

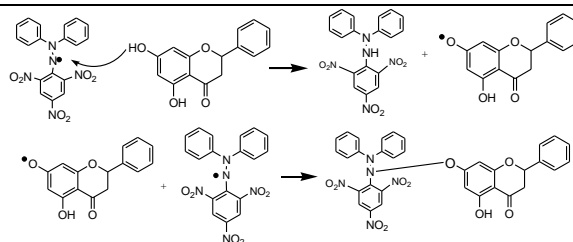


Figure 5. DPPH reduction reaction of free radical scavenging compounds (antioxidants)

In this study, the compounds that act as antioxidants are flavonoids. Flavonoid compounds belong to derivatives of phenolic compounds that have many hydroxyl groups (OH), where phenolic compounds are able to donate hydrogen atoms so that the free radical DPPH can be reduced to a more stable form. Flavonoid compounds are a group of secondary antioxidants with a mechanism of action, namely cutting the chain oxidation reactions of free radicals or capturing free radicals so that free radicals cannot react with other components. The mechanism of action of flavonoids as antioxidants is by donating hydrogen ions hence, they can stabilize the reactive free radicals (Rohmawati, 2019).

4. CONCLUSION

Based on the findings on this research, the peel of kweni fruit (*Mangifera odorata* Griff) contains secondary metabolites that can act as antioxidants. The secondary metabolites contained in the kweni peel are flavonoids and tannins. Based on the test parameters, the IC_{50} value of kweni fruit peel extract has a moderate antioxidant intensity of 169.43 g/mL. This shows that the kweni fruit peel extract has antioxidant activity that can inhibit free radicals.

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