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# Uncovering the Antioxidant Power: Investigating the Skin and Flesh of Crystal Guava with Chloroform and Methanol Extractions and DPPH Assay

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**Abstract**

Crystal guava (*Psidium guajava* var. crystal) is one type of guava plant that has high economic value due to its thick flesh and few seeds. The crystal guava plant is believed to have antioxidant activity, which is a substance that can prevent the formation of free radicals in the body. This research aims to determine the level of antioxidants in chloroform and methanol extracts of the skin and flesh of crystal guava fruit using the DPPH method. Samples were taken through a stepwise maceration process and solvents of chloroform and methanol, then analyzed using probit analysis and SPSS 25 software. The results of the study showed that both chloroform and methanol extracts of the skin and flesh of crystal guava fruit have antioxidant activity. The results showed that the $IC_{50}$ value of the chloroform extract of crystal guava fruit skin is 218.88 ppm and is classified as moderate, the methanol extract of crystal guava fruit skin is 89.78 ppm and is classified as strong, the chloroform extract of crystal guava fruit flesh is 270.56 ppm and is classified as weak, and the methanol extract of crystal guava fruit flesh is 185.72 ppm and is classified as moderate.

**Keywords:** Antioxidant; DPPH; Chloroform; Crystal guava; Fruit flesh; Methanol; Peel.

## INTRODUCTION

One horticultural product that is widely found and used in tropical countries is the crystal guava. This guava plant is widely cultivated by farmers and bears fruit throughout the year. In addition to its fruit, almost every part of the guava plant can be utilized. Guava fruit is commonly consumed to help improve digestion and lower blood sugar levels. Guava leaves can even be used as medicine to treat diarrhea, vomiting, and sore throat. Guava is also easily found in the market at affordable prices (Rustani and Susanto, 2019). Crystal guava, which is rich in vitamin C and has a high economic value, is very suitable for agro-tourism development programs (Haidawati et al., 2015). Vitamin C is one of the natural antioxidants found in fruit, in addition to vitamin E, polyphenols, carotenoids, and flavonoids (Febrianti et al., 2016). It can not only be consumed directly but crystal guava can also be used for other purposes and has its own advantages compared to other types of guava.

Crystal guava is believed to have a lot of phytochemicals such as flavonoids, polyphenols, isoflavonoids, and tannins in its leaves and fruit, providing natural antioxidants. Flavonoids are the main bioactive compounds found in guava leaves, while the majority of vitamin C content is found in the fruit skin (Jamieson et al., 2021). Guava leaves also contain quercetin, which has antioxidant properties and is considered the most active and strong antioxidant in guava seed leaves (Sharma and Borah, 2021). The presence of natural antioxidants plays an important role in managing various types of degenerative diseases caused by free radicals.

Free radicals are small molecules with unpaired electrons, making them highly reactive (Hartati et al., 2020). Their reactive nature causes free radicals to seek out electrons in other compounds, forming new chains of radicals and damaging the structure of certain compounds (Setiabudi et.al., 2020). Free radicals can also continuously form in the body, so antioxidants are needed to balance them. Antioxidants can reduce the negative impact of many free radicals. Free radicals can be neutralized by antioxidants through electron donors, making free radicals more stable and less reactive. The presence of antioxidant compounds prevents free radicals from bonding with electrons in other compounds, as their electrons are already bonded with electrons from the antioxidants (Handayani et al., 2020). Therefore, antioxidant compounds play an important role in the human body.

The testing of antioxidant levels in crystal guava is widely conducted. Typically, the antioxidant levels test uses ethanol as the solvent in extraction and the maceration method. In this research, extraction was conducted using chloroform and methanol solvents. The use of chloroform and methanol as solvents is based on their different polarities. Chloroform is non-polar, while methanol is polar. The principle of solubility says that polar solvents will dissolve polar compounds, and non-polar solvents will dissolve non-polar compounds. With the use of different solvents, it is hoped that the observed components will be separated according to their level of polarity (Taroreh et al., 2015).

The compounds that have the potential as antioxidants are flavonoids and phenols, which are included in the polar fraction (Yusriyani and Syarifuddin, 2021). Plants also contain non-polar compounds such as waxes, lipids, and proteins (Dewitasari, 2020). Non-polar solvents such as chloroform are needed to cleanse polar antioxidant compounds to be more optimally absorbed by polar solvents such as methanol. If non-polar compounds such as proteins and lipids are not cleaned by non-polar solvents, it will interfere with the process of capturing free radicals by flavonoid compounds (Rahmadani and Nasution, 2021). The maceration method used is a stepwise maceration, so this is one of the innovations in this research. This study aims to determine the antioxidant level in chloroform and methanol extracts of crystal guava skin and flesh using the DPPH method.

## MATERIALS AND METHODS

### Materials

The equipment used in this research includes an oven, electric balance, filter paper, hot plate, blender, reaction tube, dropper, maceration bottle, knife, distillator, rotary evaporator, UV-Vis spectrophotometer (Shimadzu), vortex, reaction tube rack, and plastic bags. The materials used in this research include the skin and flesh of crystal guava, methanol, p.a ether, water, ethyl acetate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, and chloroform**.** The guava fruit used in this study was those with a green-yellow and green maturity level. This is because the fruit with green-yellow maturity has the highest vitamin C content compared to fruits that are green and bright green. (Dewi et al., 2017)

**Figure 1**. Crystal guava (*Psidium guajava* var. crystal) (*Source: Personal documentation*).

### Procedures

#### *Preparation of Simplicia and Extraction*

20 kg of crystal guava fruits is peeled and separated between the skin and flesh. The skin and flesh are washed and drained. Then, they are cut and dried in an oven at 450°C until dry. In the next step, the dried samples are blended into a fine powder and sieved using a 25-mesh sieve. The prepared crystal guava skin and flesh simplicial will be extracted using a sequential maceration method. The solvents used for sequential maceration are chloroform and methanol. Both solvents are used with a ratio of each solvent to a simplicial of 1:2. Extraction is performed by weighing 125 grams of guava skin and flesh simplicial and placing them into a container. Then, it is poured with 250 ml of chloroform solvent, covered and allowed to stand for 24 hours in a light-protected condition, and stirred every 8 hours. Afterward, the macerate is collected and concentrated using a rotary evaporator to form a concentrated extract. To make a methanol extract, the dried chloroform extract residue is processed in the same way as the chloroform extraction, but using methanol solvent. The extract is then concentrated again using a rotary evaporator to make a concentrated methanol extract. The yield of the extract was calculated using the formula (Edison et al. 2020):

$$\%\ \text{Yield} = \frac{\text{weight of extract } (g)}{\text{weight of sample } (g)} \text{x } 100\%$$

#### *Antioxidant Testing*

25 mg of each dried sample was weighed and dissolved in p.a methanol to make the volume 50 mL (500 ppm). Then, a series of concentrations of the three samples

were created, namely 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm. The testing process was carried out by adding 0.5 mL of the solution from the two sample extracts with various concentrations. Then, each was added with 3.5 mL of 50 ppm DPPH. The mixture was then vortexed and incubated for 30 minutes in a dark environment and at 37°C. Its absorbance was then measured at a wavelength (λ) of 517 nm. An anti-oxidant control test was used using ascorbic acid at 10 mg weighed and dissolved in p.a methanol to have a concentration of 100 ppm. Then, a series of ascorbic acid concentrations were made with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. The test was then carried out by pipetting 0.5 mL of the sample solution from different concentration series. Each solution was then added with 3.5 mL of 50 ppm DPPH. The mixture was then vortexed and incubated for 30 minutes at 37°C and in a dark room. After that, its absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The anti-oxidant activity was calculated using the following formula (Febrianti and Ariani, 2020):

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of blank} - \text{absorbance of sample}}{\text{Absorbance of blank}} X\ 100\ \%$$

The $IC_{50}$ value was obtained from linear regression analysis by substituting the value of y as 50 from the equation y = a + bx. The smaller the $IC_{50}$ value, the greater the antioxidant activity of a substance (Martiningsih et al., 2016).

**Data analysis**

The UV-Vis spectrophotometry measurement data ~~was~~ were analyzed using the linear regression analysis method (ANOVA) with the help of SPSS 25 software at a 5% level to decide on the hypothesis proposed. A graph was made using the probit analysis between the log concentration of the sample as the x-axis and the percentage of antioxidant activity as the y-axis, to find the linear regression equation. Thus, the $IC_{50}$ can be found in the methanol and chloroform extracts.

## RESULTS AND DISCUSSION

**Extraction Results**

The extraction conducted in the research produced four types of concentrated extracts, namely chloroform extract of the fruit skin, chloroform extract of the fruit flesh, methanol extract of the fruit skin, and methanol extract of the fruit flesh. The obtained concentrated extracts were then calculated for yield values as shown in Table 1. The highest yield value was in the sample of chloroform extract of the fruit skin (5.92%), followed by chloroform extract of the fruit flesh (5.8%), methanol extract of the fruit skin (2.08%), and methanol extract of the crystal fruit flesh (1.68%). This is not directly proportional to the weight of the obtained concentrated extract. The result of the extraction from each 125 gram of the plant material showed that the highest extract was obtained from the methanol extract of the fruit skin (7.4 g), followed by the methanol extract of the fruit flesh (5.8 g), chloroform extract of the fruit skin (2.6 g), and chloroform extract of the fruit flesh (2.1 g). This result indicates that the extract produced from chloroform solvent resulted in a higher yield value compared to the yield value obtained from the extraction using methanol solvent. Chloroform is nonpolar, while methanol is polar and a compound is separated based on its polarity (Taroreh et al., 2015). Therefore, it is possible that the group of compounds in both samples that were well absorbed in chloroform solvent were nonpolar compounds.

**Table 1.** Extraction results using chloroform and methanol on skin and flesh of crystal guava fruit.

| No | Sample Name | Weight of | | | Chloroform Extract Yield (%) | Methanol Extract Yield (%) |
|---|---|---|---|---|---|---|
| | | Simplicia (grams) | Chloroform Extract (grams) | Methanol Extract (grams) | | |
| 1 | Skin fruit | 125 | 2,6 | 7,4 | 5,92 | 2,08 |
| 2 | Flesh fruit | 125 | 2,1 | 5,8 | 4,64 | 1,68 |

**Antioxidant Test Results**

The IC50 value was obtained by first conducting a probit analysis. The results obtained from the r value show that the probit data from the chloroform and methanol extracts from the skin and flesh of crystal guava are very good, as they approach the value of 1 (Fadiyah et al., 2019). A positive value of +1 indicates that the higher the extract concentration, the higher the antioxidant activity. Antioxidant activity is usually expressed in percentage of DPPH reduction or with the $IC_{50}$ value. The line equation obtained was used to calculate the $IC_{50}$ value. After the calculation, the $IC_{50}$ value for the skin of the crystal guava fruit is 218.88 ppm for the chloroform extract and 89.78 ppm for the methanol extract.

Meanwhile, for the flesh of the crystal guava fruit, the $IC_{50}$ value is 270.56 ppm for the chloroform extract and 185.72 ppm for the methanol extract, and 18.09 ppm for ascorbic acid. The results of the antioxidant test can be seen in Table 2.

**Table 2.** $IC_{50}$ values of antioxidants in chloroform and methanol extracts from skin and flesh of crystal guava fruit.

| Sample | Concentration | % Inhibition | Log Concentration | Probit | $IC_{50}$ Value (ppm) |
|---|---|---|---|---|---|
| Skin fruit extract (C) | 50 | 26,82 | 1,6990 | 4,36 | 218,88 |
| | 100 | 27,78 | 2,0000 | 4,39 | |
| | 150 | 29,94 | 2,1761 | 4,45 | |
| | 200 | 30,23 | 2,3010 | 4,48 | |
| | 250 | 31,62 | 2,3979 | 4,50 | |
| Skin fruit extract (M) | 50 | 30,91 | 1,6990 | 4,48 | 89,78 |
| | 100 | 33,95 | 2,0000 | 4,56 | |
| | 150 | 37,90 | 2,1761 | 4,67 | |
| | 200 | 39,46 | 2,3010 | 4,72 | |
| | 250 | 45,57 | 2,3979 | 4,87 | |
| Flesh fruit extract (C) | 50 | 31,20 | 1,6990 | 4,50 | 270,56 |
| | 100 | 31,79 | 2,0000 | 4,50 | |
| | 150 | 33,75 | 2,1761 | 4,56 | |
| | 200 | 34,23 | 2,3010 | 4,59 | |
| | 250 | 35,18 | 2,3979 | 4,61 | |
| Flesh fruit extract (M) | 50 | 32,96 | 1,6990 | 4,53 | 185,72 |
| | 100 | 33,83 | 2,0000 | 4,56 | |
| | 150 | 34,50 | 2,1761 | 4,59 | |
| | 200 | 38,08 | 2,3010 | 4,69 | |
| | 250 | 38,99 | 2,3979 | 4,69 | |
| Ascorbic acid | 5 | 44,19 | 0,6990 | 4,85 | 18,09 |
| | 10 | 60,78 | 1,0000 | 5,25 | |
| | 15 | 77,28 | 1,1761 | 5,74 | |
| | 20 | 88,98 | 1,3010 | 6,18 | |
| | 25 | 96,39 | 1,3979 | 6,75 | |

*(C) = Chloroform, (M) = methanol

The $IC_{50}$ value shown in Table 2 indicates that ascorbic acid as a comparison has the strongest antioxidant activity, this is due to ascorbic acid being a pure antioxidant compound. Methanol extract from the skin and flesh of crystal guava fruit has a higher $IC_{50}$ value compared to the chloroform extract from the skin and flesh of crystal guava fruit, as shown in Figure 2.

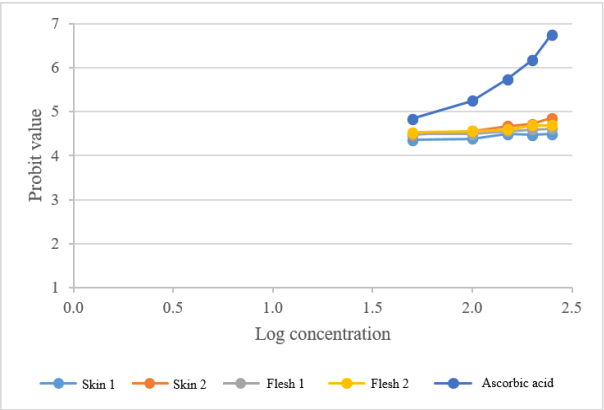


**Figure 2.** The relationship between the concentration of chloroform and methanol extracts from the skin and flesh of the crystal guava fruit (*Psidium guajava* var. crystal) and antioxidant activity. **Note** Skin 1= Chloroform extract from the skin of the fruit; Skin 2= Methanol extract from the skin of the fruit; Flesh 1= Chloroform extract from the flesh of the fruit; Flesh 2: Methanol extract from the flesh of the fruit.

## Discussion

In the drying process, both samples were dried using an oven at a temperature of 45 degrees Celsius for 150 hours until the dried simplicia, seen from the brownish color and easy to break. Then, these dried samples were blended and filtered with the aim of breaking down the cells and expanding the sample surface so that it is easier to extract. The size of the fine powder produced makes it easier for the solvent to extract the contents directly because the larger the surface area and the more effective the interaction with the solvent (Pertiwi et al., 2022).

In this study, extraction was carried out using a multistep maceration method. Maceration means soaking and is a method for isolating active substances using a solvent and stirring at room temperature several times over a certain period. Maceration is a cold method that can protect secondary metabolites such as flavonoids from the effects of heating. The solvents used in the extraction are 75% chloroform and 75% methanol. The

extraction was carried out through a multistep maceration method, which is slightly different from the ordinary maceration method. In this multi-step method, solvents with different polarity levels are used. The first extraction was carried out using a non-polar solvent, chloroform, and then the residue from the chloroform maceration was processed again using methanol as the solvent.

The use of the multistep maceration method aims to extract active substances (antioxidants) more optimally. In addition, the use of solvents with different polarity levels also aims to extract active substances with different polarities, so that the extraction results can be better (Kuspradini et al., 2016). Antioxidants have polar properties, so the use of non-polar chloroform solvent in the initial extraction is intended to pull non-polar substances from the sample, so the sample will be free of non-polar substances. The second extraction using methanol will optimally pull polar substances, so when the DPPH test is performed, non-polar substances will not interfere and affect the results that are not maximal. The extraction yields a concentrated extract which is then calculated for its yield value.

The yield in this study was measured by comparing the dry extract mass (grams) with the initial mass of the material before the extraction process (grams). The dry extract was obtained after the sample was dried in an oven until it had a constant weight. This calculation was performed to determine the percentage of material that remained after the extraction process and to determine the level of effectiveness of the process produced (Senduk et al., 2022). The yield value can also indicate the number of secondary metabolite compounds present in the sample. Therefore, this calculation can serve as a reference in antioxidant testing or as a comparison of antioxidant testing results.

The second antioxidant test of the two sample types used chloroform and methanol solvents and produced positive results. This is indicated by the color change produced during testing with DPPH. In the antioxidant activity study, the addition of DPPH solution to the sample was marked by a change in color from purple to yellow. This indicates the occurrence of free radical capture. The change in intensity of the purple color is due to the free radical scavenging produced by the reaction of the DPPH molecule with hydrogen atoms released by the sample molecule. This causes the fading of the DPPH color from purple to yellow. DPPH, which has unpaired electrons, will give a purple color, and the color will change to yellow when the electrons have been paired (Konda et al., 2020). The test was performed using a UV-Vis spectrophotometer with a wavelength of 517 nm, which is the maximum wavelength of DPPH. This wavelength will provide the best absorption results from the test solution and provide high sensitivity, thus it is expected to obtain optimal absorbance values for the sample (Konda et al., 2020)

The samples tested for antioxidant activity include chloroform and methanol extracts of the skin and flesh of crystal guava fruit and ascorbic acid (vitamin C) as a reference. Vitamin C was used because it has an antioxidant function, capturing free radicals and preventing chain reactions. Vitamin C has free hydroxyl groups that function as free radical captures, and if it has polyhydroxy groups it will increase antioxidant activity. The $IC_{50}$ values for the skin and flesh of the crystal guava fruit show that the antioxidant activity in the skin of the crystal guava fruit is stronger than in the flesh of the crystal guava fruit. This is due to the higher vitamin C content in the skin of the fruit (Jamieson et al., 2021). A smaller $IC_{50}$ value indicates stronger antioxidant activity, while a larger $IC_{50}$ value indicates weaker antioxidant activity (Andriani and Murtisiwi, 2020). Research has been conducted on the health benefits of guava leaves, which are attributed to the various phytochemicals they contain such as quercetin avicularia, apigenin, guaijaverin, kaempferol, hyper in, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid. The extracts from guava leaves (GLs) have been investigated for their biological properties such as anticancer, antidiabetic, antioxidant, antidiarrheal, antimicrobial, lipid-lowering, and liver protection activities (Kumar et al., 2021).

Various factors can affect the antioxidant content in fruits, such as variety, species, cultivar, harvesting conditions, ambient temperature, photosynthesis process, relative humidity, oxidative stress, and exposure to sunlight. Pollution also plays a role in antioxidant content variation. The ripeness of the fruit also affects the Vitamin C content. The riper the fruit, the higher the ascorbic acid content, but overheating during the washing and cooking process can cause a loss of antioxidants due to their easily soluble and easily damaged nature with heating (Febrianti et al., 2016).

## CONCLUSIONS

The results of the study showed that both chloroform and methanol extracts of the skin and flesh of crystal guava fruit have antioxidant activity. The results showed that the $IC_{50}$ value of the chloroform extract of crystal guava fruit skin is 218.88 ppm and is classified as moderate, the methanol extract of crystal guava fruit skin is 89.78 ppm and is classified as strong, the chloroform extract of crystal guava fruit flesh is 270.56 ppm and is classified as weak, and the methanol extract of crystal guava fruit flesh is 185.72 ppm and is classified as moderate.

***Authors' Contributions:*** All authors are major contributors to this research. AUM, AA, and NW collaborated to design the research and write the initial research draft. AA: executed the experiment, AUM:

wrote the article draft and revised the draft, NW: contributed suggestions for article improvement

***Competing Interests:*** The authors declare that there is no potential for conflicting interests in this research study.

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