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Antioxidant Activity Test of N-Hexane, Ethyl Acetate, and Aqueous Fractions of Santang Mandarin Orange Peel (Citrus reticulata) Using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Method

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A R T I C L E I N F O

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A B S T R A C T

This study aims to determine the antioxidant activity of the n-hexane fraction, ethyl acetate fraction, and water fraction from santang orange peel using the DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) method and to determine the IC50 (Inhibitory Concentration 50) value. The Santang orange peel was extracted using the maceration method with ethanol as the solvent, followed by a multi-step fractionation process using solvents with different levels of polarity, namely the n-hexane fraction, the ethyl acetate fraction, and the water fraction. Antioxidant activity assays against DPPH radicals were conducted on the n-hexane fraction, ethyl acetate fraction, and water fraction to determine the comparative inhibitory effects of the antioxidants. The results of the study showed that the ethyl acetate fraction and the water fraction generally had higher antioxidant activity compared to the n-hexane fraction due to the presence of more polar phenolic and flavonoid compounds. The IC50 values obtained represent the concentration required to scavenge 50% of DPPH free radicals; the lower the IC50 value, the higher the antioxidant activity.

INTRODUCTION

Santang oranges (Citrus reticulata var. nobilis) is one of the local citrus varieties that are popular with the Indonesian people because of their fresh sweetness, distinctive aroma, and high health benefits. This fruit is a seasonal plant that is widely consumed, especially its pulp for juice, snacks, or culinary preparations. According to Saati (2009) in Wahyuni (2010), around 40-50% of the weight of Santang oranges consists of the peel which is often disposed of as waste by most people, even though Santang orange peel contains high-value bioactive compounds such as flavonoids and antioxidants (Goodman, 2007).

Santang Orange Peel (Citrus reticulata) contains natural dyes that are quite high in carotenoids and flavonoids. Carotenoids are dyes that play a role in providing orange-yellow color with the potential to be natural dyes for food and can be used as an alternative to synthetic dyes that are safer for health (Citramukti, 2008). Santang Orange peel has a higher potential as an antioxidant than the meat. As explained from the results of the study, the antioxidant activity between Santang orange flesh and Santang orange peel showed higher results in Santang orange peel (Pujiharjo, 2010).

Antioxidants play an active role in overcoming excess free radicals which generally work as free radical catchers and prevent chain reactions. Antioxidants are present in the enzymes superoxide dismutase (SOD), Glutathione and catalase. Antioxidants that come from outside the body such as vitamin C, vitamin E, β-carotene, xanthophils and flavonoids (Nugraheni, 2007). Santang Orange Peel contains key antioxidant compounds such as hesperidin, naringin, and polyphenols that contribute to higher free radical anti-inflammatory activity than the pulp. This antioxidant plays a role in protecting body cells from oxidative damage, preventing premature aging, and reducing the risk of chronic diseases such as cancer and heart disease (Pujiharjo, 2010). Research shows that Santang Orange peel extract has a lower IC50 (50% inhibition concentration) on the DPPH test than the meat, indicating superior antioxidant potential (Citramukti, 2008).

Free radicals can cause damage to the brain due to harmful chemicals that are easily absorbed by fat, whereas most brain structures are fat. Free radicals that can harm health can be paralyzed by antioxidants, so antioxidants are needed by the body. Free radicals are atoms or molecules that are unstable and highly reactive because they contain one or more unpaired electrons in their outer orbitals. To achieve atomic or molecular stability, free radicals will react with surrounding molecules to obtain electron pairs. This reaction will take place continuously in the body and if not stopped will cause various diseases such as cancer, heart, cataracts, premature aging, and other degenerative diseases. Therefore, the body needs an important substance, namely antioxidants that are able to capture these free radicals so that they cannot induce a disease (Kikuzaki, 2002).

Antioxidants are compounds that can prevent and slow down damage caused by free radicals through inhibition of oxidative mechanisms (Widjaja, 2012). Antioxidants can help shield the human body against damage caused by reactive oxygen compounds ROS (Reactive Oxygen Species) and other free

radicals. Due to high reactivity, free radicals can damage the sharing of macromolecular cells. Free radicals are able to damage molecules and are the cause of various degenerative diseases and chronic diseases (Yuhernita, 2011).

According to research by Pujiharjo (2010) explained that the skin of the santang orange has very strong antioxidant activity, the antioxidants contained in the Santang orange peel (Citrus reticulata var. nobilis) are hesperidin, naringin, narirutin, and polyphenols (main flavonoids) with a total phenolic content of 150-250 mg GAE/100 g dry weight, as well as DPPH activity of up to 85-92% at optimal concentrations (Citramukti, 2008).

According to Sudewo (2005), compounds that have the potential to be antioxidants are generally flavonoids, phenolic, and alkaloid compounds. Flavonoid compounds and polyphenols are antioxidant, antidiabetic, anticancer, antiseptic, and anti-inflammatory, while alkaloid compounds inhibit the growth of cancer cells (Dalilmartha, 2007).

According to Pujiharjo (2010), the skin of the santang orange can be used to treat digestive disorders (such as diarrhea and constipation), oral inflammation (stomatitis), minor wounds, and help lower cholesterol and high blood pressure thanks to the content of hesperidin, flavonoids, and essential oils that are anti-inflammatory, antimicrobial, and hypolipidemic. From some of the facts and scientific sources mentioned in this section, the researcher tried to draw the idea of researching the relationship of free radicals with the activity contained in the peel extract of the santang orange fruit through the DPPH (1,1-Diphenyl-2-Picryl Hydrazil) method. The DPPH method is a method that can measure the effectiveness of antioxidants quickly, simply, and without cost. DPPH has been widely used to measure a compound's ability to inhibit free radicals or as a hydrogen donor, and also to evaluate antioxidant activity in food (Attamimi, 2008).

Orange peel can be useful in food production as well as in industry such as natural dyes in food and beverages. In the field of pharmacology, dragon fruit peel can also be used as an antioxidant or antidote to free radicals (Wahyuni, 2020).

According to research by Ghasemzadeh et al. (2022), The advantages of the skin of the santang orange fruit as an antioxidant are due to the fact that the santang orange fruit is rich in polyphenol compounds. In addition, the skin of the santang orange also contains vitamin C, vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamamin, phenolics, carotene, and phytoalbumin which are also suspected to have benefits as antioxidants (Nurmalasari, 2023). The phytochemical content in the skin of the orangutan fruit also depends on the variety of the orangutan fruit.

THEORETICAL REVIEW

Free Radical and Antioxidant Theory

Free radicals are highly reactive molecules or molecular species that contain one or more unpaired electrons. Their high reactivity enables them to react with biological molecules and may contribute to oxidative damage when their production exceeds the body's antioxidant defense capacity. Antioxidants are compounds capable of inhibiting or reducing oxidation by donating hydrogen atoms or electrons to free radicals, thereby stabilizing the radicals and preventing further oxidative reactions. Phenolic compounds and flavonoids found in plant materials are among the important groups of natural compounds associated with antioxidant activity.

Citrus fruits, particularly their peels, are considered potential sources of natural antioxidants because they contain various phenolic and flavonoid compounds. A study of 19 Citrus reticulata genotypes found that the peel generally had the highest average total phenolic and flavonoid contents among the fruit tissues examined. The peel also showed the highest average antioxidant capacity based on DPPH and ABTS assays (Zhang *et al*., 2018).

A recent review of Citrus reticulata also reported that phenolic compounds are important contributors to the antioxidant capacity of citrus fruits. Several flavonoids, including hesperidin, naringin, neohesperidin, didymin, and poncirin, have been identified in C. reticulata. These compounds may contribute to antioxidant activity through their ability to donate electrons or hydrogen atoms and neutralize free radicals.

Therefore, the presence of antioxidant-related compounds in Citrus reticulata peel provides a theoretical basis for investigating the antioxidant activity of Santang Mandarin Orange peel and its different solvent fractions.

Santang Mandarin Orange Peel (Citrus reticulata)

Citrus reticulata is a citrus species whose fruit peel contains various secondary metabolites, including phenolic compounds, flavonoids, polymethoxyflavones, and other bioactive constituents. These compounds have attracted attention because of their potential biological activities, including antioxidant activity.

The peel is particularly relevant for antioxidant research because studies have shown that citrus peel can contain greater amounts of phenolic and flavonoid compounds than other fruit tissues. Zhang et al. (2018) reported that the peel of C. reticulata had the highest average total phenolic and flavonoid contents among the fruit tissues studied and also demonstrated the highest average DPPH antioxidant capacity.

Previous research specifically examining C. reticulata peel has also demonstrated antioxidant potential. Fadhlillah et al. (2024) investigated ethanol, ethyl acetate, and n-hexane extracts of C. reticulata peel using DPPH and ABTS methods. The study detected phenolic and flavonoid compounds in the peel extracts and demonstrated antioxidant activity, with ethanol extract showing the strongest activity among the extracts examined.

These findings support the assumption that C. reticulata peel contains compounds capable of acting as free-radical scavengers. However, differences in

extraction solvent may result in differences in the type and amount of compounds extracted, which can subsequently affect antioxidant activity.

Extraction, Fractionation, and Solvent Polarity

Extraction and fractionation are important processes in obtaining bioactive compounds from plant materials. Different compounds have different chemical characteristics and solubilities. Consequently, the polarity of the solvent can influence the compounds that are extracted or distributed into a particular fraction. n-Hexane is a relatively non-polar solvent, ethyl acetate is commonly considered a semi-polar solvent, and water is a polar solvent. Therefore, fractionation using solvents with different polarities can separate compounds according to their relative affinity toward the solvents.

This principle provides a theoretical basis for comparing the n-hexane, ethyl acetate, and aqueous fractions of Santang Mandarin Orange peel. Differences in the chemical composition of each fraction may result in differences in their ability to neutralize DPPH radicals.

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Previous research supports the importance of solvent selection in determining antioxidant activity. Fadhlillah et al. (2024) found differences in total phenolic and flavonoid contents and antioxidant activity among ethanol, ethyl acetate, and n-hexane extracts of C. reticulata peel. Ethanol extract showed higher phenolic and flavonoid contents and stronger antioxidant activity than the ethyl acetate and n-hexane extracts in that study

A related study by Fadhlillah et al. (2019) investigated ethanol extract and n-hexane, ethyl acetate, and water fractions of Citrus reticulata leaves using the DPPH method. The study demonstrated antioxidant activity in several of the fractions, supporting the possibility that fractionation based on solvent polarity can produce fractions with antioxidant activity.

However, the antioxidant activity does not necessarily follow one universal order according to solvent polarity. For example, a study of flavonoid-rich fractions from citrus fruits reported that the ethyl acetate fraction contained the highest total phenolic and flavonoid contents and exhibited the highest antioxidant activities among the fractions investigated. This indicates that the antioxidant activity of a fraction depends not only on solvent polarity but also on the specific chemical composition of the plant material and the compounds present in each fraction.

Thus, differences in antioxidant activity among n-hexane, ethyl acetate, and aqueous fractions cannot be assumed in advance and need to be experimentally determined.

DPPH Method for Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is one of the commonly used methods for evaluating the free-radical-scavenging activity of plant extracts and fractions. DPPH is a stable free radical with a characteristic purple color.

When DPPH reacts with an antioxidant capable of donating a hydrogen atom or electron, the radical is reduced and the intensity of the purple color decreases. This change can be measured spectrophotometrically, commonly at approximately 517 nm.

The decrease in absorbance indicates the ability of the tested sample to scavenge DPPH radicals. The percentage of inhibition can then be calculated based on the reduction in absorbance compared with the control. Therefore, the DPPH method can be used to evaluate and compare the antioxidant activity of the n-hexane, ethyl acetate, and aqueous fractions of Santang Mandarin Orange peel.

Mishra et al. (2012) described DPPH as a widely used assay for evaluating antiradical properties and emphasized that the assay is based on the reduction of DPPH by antioxidant compounds. Similarly, Pyrzynska and Pękal (2013) reported that DPPH is widely applied in plant biochemistry to estimate the ability of plant constituents to scavenge free radicals.

Previous research on C. reticulata peel has also demonstrated the applicability of DPPH for evaluating antioxidant activity. Fadhlillah et al. (2024) used DPPH and ABTS assays to evaluate the antioxidant activity of C. reticulata peel extracts and reported measurable antioxidant activity

Nevertheless, not every C. reticulata compound or fraction necessarily exhibits strong DPPH-scavenging activity. Research summarized in a recent review reported that individual compounds from C. reticulata could show relatively weak DPPH-scavenging percentages, indicating that antioxidant activity may vary substantially according to the compounds and sample tested. Therefore, the antioxidant activity of the three fractions in the present study must be determined experimentally rather than assumed solely from the presence of antioxidant compounds.

IC50 of Antioxidant Activity

The IC50 value represents the concentration of a sample required to inhibit or scavenge approximately 50% of the target radical under the specified experimental conditions. In the DPPH assay, IC50 is generally determined from the relationship between sample concentration and percentage of DPPH radical inhibition. A lower IC50 value generally indicates a stronger radical-scavenging activity because a lower concentration of the sample is required to achieve 50% inhibition.

The determination of IC50 is therefore important for quantitatively describing the antioxidant activity of the n-hexane, ethyl acetate, and aqueous fractions of Santang Mandarin Orange peel. The IC50 values obtained from each fraction can be used to characterize and compare their antioxidant activity under the same experimental conditions.

Previous studies have demonstrated that IC50 can be obtained from DPPH antioxidant assays of Citrus materials. For example, Fadhlillah et al. (2024) reported an IC50 value from the DPPH assay for C. reticulata peel extract, demonstrating the applicability of IC50 as a quantitative parameter for antioxidant activity

However, IC50 values from different studies should be compared carefully because DPPH assay conditions can substantially affect the results. Mishra et al. (2012) noted that differences in DPPH concentration, sample concentration, reaction time, solvent, and other experimental conditions can lead to variation in reported antioxidant parameters. Menezes et al. (2021) similarly emphasized that inconsistencies in DPPH methodology and IC50 calculation can make comparisons between studies problematic. Therefore, in the present study, the IC50 values should be determined using consistent experimental conditions for all three fractions.

Development of Hypotheses

Antioxidant Activity of the Fractions

The theoretical basis indicates that C. reticulata peel contains phenolic, flavonoid, and other bioactive compounds that may contribute to free-radical-scavenging activity. Previous studies have demonstrated antioxidant activity of C. reticulata peel and related citrus fractions using the DPPH method. Zhang et al. (2018) reported that C. reticulata peel showed high antioxidant capacity, while Fadhlillah et al. (2024) demonstrated antioxidant activity in ethanol, ethyl acetate, and n-hexane extracts of C. reticulata peel.

Research on C. reticulata leaves using ethanol extract and n-hexane, ethyl acetate, and water fractions also demonstrated antioxidant activity, providing additional evidence that different fractions of Citrus reticulata may possess radical-scavenging properties.

Although some studies have reported differences in antioxidant strength among solvents and some individual C. reticulata compounds have shown relatively weak DPPH-scavenging activity, these findings do not negate the possibility of antioxidant activity in the peel fractions. Instead, they indicate that the activity depends on the chemical composition of each fraction.

IC50 of the Fractions

The DPPH assay allows antioxidant activity to be expressed quantitatively through the IC50 value. Previous research on C. reticulata peel has demonstrated the determination of IC50 using the DPPH assay, while methodological studies have established IC50 as an important parameter for describing radical-scavenging activity.

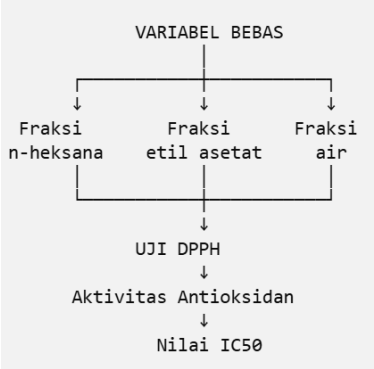

Figure. 1 IC50 of the Fractions

METHODOLOGY

The subject of this study is Santang Orange Fruit with sampling in Sleman, Kulon Progo, Yogyakarta. This research method is descriptive of experiments. The free variables in this study are various solvent variations, namely the N-hexane fraction, the ethyl acetate fraction and the water fraction from the skin of the lemon fruit. The bound variables in this study were the antioxidant power of the skin of Santang Orange Fruit and the IC value$_{of\ 50}$.

Tools and materials

1. Tools

The tools used in this study include maceration vessels, measuring cups, analytical scales, split funnels, sieve number 60, ovens, stirring rods, a set of rotary evaporator tools, Erlenmeyer flasks, flannel cloth, measuring flasks, drip pipettes, volume pipettes, beaker glass, test tubes, filter paper, UV-Vis spectrophotometry.

2. Ingredients

The materials used in this study included samples of Santang Orange Fruit, 96% ethanol, N-hexane, ethyl acetate, aqua distillate, ethanol p.a, DPPH, hot water, magnesium, HCl, acetic acid anhydride, concentrated sulfuric acid, $FeCl_3$, acetic acid.

Working procedure

1. Material Collection

Sample of 20 kg of coconut orange peel from Sleman, Kulon Progo, Yogyakarta.

2. Determination

The determination of the simplicia of the santang citrus fruit plant was carried out by a determination test at the pharmaceutical laboratory, Ahmad Dahlan University.

Sample Collection and Processing

Citrus fruit sampling is carried out in the morning (09:00-11:00), by picking one by one manually using hands. The citrus fruit sample is cleaned from the dirt attached to the fruit, the flesh is separated from the skin, using running water and then poured 1 cm and dried in the sun (if the weather is not possible/not hot, use a drying cupboard). Simplicia that has been dried and mashed using a blender and then sifted is stored in a dry place and tightly closed.

Extraction

This method is extracted by the maceration method, because it is done by soaking simplicia powder in a filter liquid using a 96% ethanol solvent. A sample of 470 grams of the skin of the santang orange fruit was put into a maceration

container. Add 96% ethanol in a ratio of 1:4 (b/v) to the jar filled with dried orangutan fruit peels until all parts are completely submerged. In maceration for 3x24 hours, then filtered with filtrate using whattman filter paper no. 42. The maceration process is carried out repeatedly up to three times or until the filtrate is clear. The maceration results are evaporated using a *rotary evaporator* until all the ethanol evaporates and then dried again with a waterbat and a thick extract of orange peel is obtained. Phytochemical analysis and antioxidant tests were carried out on the condensed extracts obtained.

Fractionation

Fractionation of santang orange peel fruit extract using a mixture of two solvents that have different polarities in partition. Ethanol extract of the banyan orange peel weighing 1.9 grams was dissolved with 50 mL of warm water, and 50 mL of ethanol was added, 96% ethanol As much as 100 mL of N-hexane was added into a separate funnel to dissolve the dissolved extract, whisked vigorously until a layer of N-hexane and a layer of water were obtained, then the two layers were separated. In the water layer, ethyl acetate is added, shaken vigorously until a layer of ethyl acetate and a layer of water are obtained, then the two layers are separated. The process is repeated.

Extract Characteristics Examination

Macroscopic Testing

Organoleptic testing was carried out which included the properties of substances in general, especially shape, color, size, and smell.

Drying Shrinkage

Weighing 2 grams of santang orange peel powder is put into a moisture analyzer. The moisture content requirement should not be more than 10%. Dry shrinkage measurement in this study was carried out using a moisture analyzer and the percentage of moisture content of santang orange peel powder was obtained at 8.9%.

Moisture Rate

Moisture content was determined with a *moisture analyzer*, weighing 2 grams of santang orange peel powder. The moisture content requirement should not be more than 10%. The measurement of the moisture content in this study was carried out using *a moisture analyzer* and the percentage of moisture content from the coconut peel powder was obtained at 8.94%.

Total Ash Content

The determination of the total ash content is carried out with the aim of providing an overview of the mineral content contained in simplicia. Total ash content is related to minerals of inorganic compounds. In the total ash content

test, the total ash content was obtained as much as 10.742 ± 0.108. The amount of ash content is likely due to the large number of minerals and metal compounds contained in simplicia.

Extract Filtration

Alkaloids

Weigh 2 grams of orange peel extract powder, then grind with 5 mL of ammonia. Then 20 mL of N-hexane is added and filtered. The filtrate plus HCl is then taken from the top layer and put into 2 test tubes. It is dripped with Bouchardat LP in test tube 1 and will form a brown deposit if it contains alkaloids. Then in tube 2 it is dripped with Mayer LP and will form a white deposit if it contains alkaloids.

Flavonoids

Weigh 2 grams of coconut orange peel extract powder plus 100 mL of hot water and boil for 15 minutes then filter. A total of 5 mL of filtrate was added with Mg powder and 2 mL of alcohol-HCl solution (1:1). Then add amyl alcohol and then shake it vigorously and let it separate. The formation of an orange or yellow color on the amyl alcohol layer shows positive results.

Saponins

Weigh 2 grams of orange peel extract then add 100 mL of hot water and cool. Put 20 mL of filtrate into the test tube and shake vigorously for 10 minutes, then let it sit and add HCl 2 N. If foam is formed and the foam does not disappear after the addition of HCl, then the sample contains saponins

Tannins

Weigh 2 grams of orange peel extract then add 100 mL of hot water and simmer for 15 minutes then filter. Prepared 2 test tubes containing 5 mL of phytrates each, then 1 tube plus $FeCl_3$ 1% (positively indicated by a blackish-violet green color). Tube 2 plus gelatin (positive indicates the presence of white deposits). Tube 3 is used to distinguish the tannins of galates and catechols by adding filtrate with 3% formaldehyde: HCl (2:1), then heated. Then Stease added that pink deposits will be formed indicating catechol tannin compounds. Then the filtrate is separated by filtering and saturated with Na-acetate and adding $FeCl_3$ 1%. The formation of inky blue or black color indicates the presence of an error tannin compound.

Steroids/ Triterpenoids

Weighing 2 grams of orange peel extract is then macerated using 20 mL of ether for 2 hours, then filtered. Then the filtrate is evaporated until a residue is obtained, which is then dripped with 2 drops of anhydrous acetic acid and 1 drop of concentrated H_{2SO4}. The formation of green or red color indicates the presence of steroid or triterpenoid compounds.

Antioxidant Activity Testing with the DPPH Method

Manufacturing of 50 ppm DPPH Stock Solution

Weighing as much as 5 mg of DPPH was then dissolved with 100 mL of ethanol p.a in a measuring flask.

Manufacture of Vitamin C Stock Solution 1000 ppm

Weighted 50 mg of vitamin C then dissolved with 50 mL of ethanol p.a in a measuring flask

Manufacturing of Orange Fruit Stock Solution 1000 ppm

Weighing 50 mg of ethanol extract of santang citrus fruit was then dissolved with 50 mL of ethanol p.a in a measuring pumpkin.

Blank Absorption Measurement

As much as 3 mL of DPPH stock solution was taken, then wavelength scanning was carried out with a UV-VIS spectrophotometer at a wavelength of 400-800 nm, then absorption was measured using a UV-VIS spectrophotometer at the maximum wavelength obtained.

Measurement of Binding Activity of DPPH with Vitamin C

Measurements were carried out by being pipetted with 20 µL, 30 µL, 40 µL, 50 µL, and 60 µL respectively from the vitamin C stock solution and then suffused up to a volume of 10 mL of measuring gourd with ethanol p.a each so that a solution with concentrations of 2 ppm, 3 ppm, 4 ppm, 5 ppm, and 6 ppm was obtained. From the solution, 1 mL of solution was taken and 2 mL of DPPH stock solution was added. The solution was incubated for 30 minutes and then the sample absorption was measured using a UV-VIS spectrophotometer at a maximum wavelength of 516 nm.

Measurement of DPPH Binding Activity with Santang Orange Peel Extract

Measurements were carried out by being pipetted by 20 µL, 40 µL, 60 µL, 80 µL, 100 µL, and 120 µL respectively from the extract stock solution and sufficient up to a volume of 10 mL of measuring gourd using ethanol p.a so that a solution with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 6 ppm was obtained. From the solution, 1 mL of solution was taken and 2 mL of DPPH stock solution was added. The solution was incubated for 30 minutes and the sample absorption was measured using a UV-VIS spectrophotometer at a maximum wavelength of 516 nm.

Data Analysis

The data of the comparison results of the antioxidant test of Santang Orange Fruit Peels was made with N-hexane fraction, ethyl acetate fraction and water fraction made with concentration through characteristic tests including microscopic testing, drying shrinkage, ash content.

RESULTS AND DISCUSSION

Plant Determination

The test sample of the santang oranges used first has been determined at the Biology Laboratory, Ahmad Dahlan University. The results of the determination that have been made are obtained from the results that the plant used in this study is indeed Citrus Fruit.

Preparation of Santang Orange Fruit Skin Sample

During the drying process there is a change in color, texture and weight. The skin of fresh citrus fruits is orange yellow on the top and orange in the middle. After the drying process, there is a change in color to brownish-yellow, as well as a change in texture to hard and stiff so that it can be broken by hand. The color change is caused by photooxidation of the orange peel, while the change in texture and weight is caused by the loss of several percent of the water content contained in the orange peel. This drying also aims to prevent the sample from being easily damaged by the growth of fungi so that it can be stored for a longer time. The peel of the orange fruit is dried in the sun in the sun.

Table 1. Dry Weight Against Wet Weight Orange Fruit Peels

Wet Weight (kg)	Dry weight (kg)	Yields (%) b/w
20	8	45

Based on table 1. It can be explained from the extraction of the skin of the santang orange fruit at a wet weight of 20 kg after going through the peeling stage of taking the skin from the orange fruit, the dry weight of the orange fruit was obtained which was 8 kg. The yield obtained is 45%.

Simplicia Characteristic Data

Macroscopic Testing

Against simplicia powder of santang orange peels was tested macroscopically. A picture of the results of the macroscopic test of simplicia powder can be seen in the appendix. Macroscopic testing of simplicia powder includes size, shape, color as well as size. The results of the macroscopic testing of simplicia powder can be seen in table 2.

Table 2. Results of Macroscopic Testing of Orange Fruit Peels

Parameters	Simplification
Shape	Powder
Color	Reddish Brown
Size	Small particles
Smell	Sting

Furthermore, simplisia was carried out a test of the characteristics of simplicia powder according to the Indonesian Herbal Pharmacopoeia including the determination of drying shrinkage, determination of moisture content and determination of ash content.

Drying Shrinkage

Dry shrinkage measurements in this study were carried out using *a moisture analyzer* and the percentage of moisture content from orange peel powder was obtained of 8.9%.

Moisture Rate

The moisture content measurement in this study was carried out using *a moisture analyzer* and the percentage of moisture content from orange peel powder was obtained at 8.94%.

Total Ash Content

The total ash content in citrus peel simplicia was 14.12% and in extracts was 9.06%, acid insoluble ash content in simplicia was 7.44% and in extracts was 1%. In the total ash content test, a total ash content of 10.742 ± 0.108% was obtained. The magnitude of the ash content was likely due to the large number of minerals and metal compounds contained in simplicia.

Table 3. Simplicia Characteristic Data

Simplicia Characteristic Test	Results
Drying Shrinkage	8,9 %
Moisture Rate	8,94 %
Total Ash Content	10.742 ± 0.108 %

In the drying shrinkage test, the result was 8.9 %. Dry shrinkage measurements in this study were carried out using *a moisture analyzer.* In the results of the moisture content test, there were results of 8.94% of the total ash content test, there were results of 10.742 ± 0.108%.

1. Extraction

The extraction process in this study was carried out using the maceration method. The maceration results are filtered to separate the filtrate and its residue. The obtained filtrate is collected and filtered. Then the filtrate is concentrated using a *rotary evaporator vacuum* and a water bath at a temperature of 45°C until the solvent evaporates and becomes a viscous extract. The condensed ethanolic extract of red dragon fruit peel (KBNM-Ethanol) obtained was 19,273 grams.

Table 4. Yield of the weight of the extract against the weight of the dragon fruit peel powder

Powder weight(gr)	Extract weight (gr)	Yields (%) (b/w)

470	19,273	4,100

Based on table 4, it can be explained that the weight of orange peel powder is 470 grams. After that, the powder was made from the extract, the weight of the extract was obtained at 19,273 grams. So that the total yield is 4,100% b/b.

2. Fractionation

The ethanol orange peel extract used for liquid-liquid fractionation is only 1.9 grams. During the fractionation process, the mixed fraction of$_{H2O\text{-}ethanol}$ of the ethanolic extract of the orange peel and ethanol is in the upper layer, and the N-hexane fraction of the orange peel is in the lower layer. Because N-hexane solvents have a greater density (1.49 g/cm3) than ethanol (0.7915 g/cm3). The results of the chloroform fraction of orange peel were then concentrated using a *rotary evaporator* at 500°C followed by using a waterbath at 50°C-700°C.

Table 5. Total Result of Red Dragon Fruit Peel Extract Fraction

Extract Weight(gr)	Faction	Fractional weight (gr)	Yields (%) (b/w)
1,9	Chloroform	1,5	78,947
1,9	Ethyl Acetate	1,6	84,210
1,9	Water	1,59	83,684

The N-hexane fraction of orange peel obtained was 1.5 grams and the yield value of the thick fraction of N-hexane of orange peel on dried orange peel was 78.947%. The ethyl acetate fraction of orange peel obtained was 1.6 grams and the yield value of the ethyl acetate fraction of orange peel on dried orange peel was 84.210%, while the water fraction in orange peel extract was 1.59 grams and a yield of 83.684%.

Chemical Compound Content Identification

Alkaloid Test

The results of the research that have been carried out in tube 1 produce an orange-yellow color and no deposits while in tube 2 produce orange so that it can be concluded positively and the orange peel contains antioxidants.

Flavonoid Test

The formation of an orange-yellow color on the amyl alcohol layer shows positive results.

Saponin Test

The results of the research on dragon fruit peel contain saponins so that positive results are obtained, because foam is formed and foam does not disappear after the addition of HCl.

Tannin Test

The results of the study showed that tube 1 was brownish-orange while tube 2 showed a brownish-orange color so that the orange peel extract contained tannins.

Steroids/ Triterpenoid Tests

The results of the study were that tube 1 was yellow while tube 2 was brown deposit, so the results showed negative on the extract or powder of orange fruit peel

Table 8. Results of Identification of Chemical Compound Content of Orange Fruit Powder and Extract

Yes	**Chemical Content**	**Simplification**	**Extract**	**Results**	**Remarks**
1	Alkaloids	+	+	- Yellow-orange no deposits → Dragendrof - Yellow → Mayer	- Yellow-orange no deposits → Dragendrof - Yellow → Mayer
2	Flavonoids	+	+	Yellow Orange	Yellow Orange
3	Tannins	+	+	- orange→ FeCl3 - orange→ Gelatin	- orange→ FeCl3 - orange→ Gelatin
4	Saponins	+	+	Foam formation	Foam formation
5	Steroids/ Triterpenoids	_	_	- Yellow color→ anhydrous acetic acid - Brown-yellow deposits → concentrated acid are formed	- It forms red and then becomes blue and green → anhydrous acetic acid - formed green/red color→ concentrated acid

Antioxidant Activity Test Results
Maximum Wavelength Measurement Results

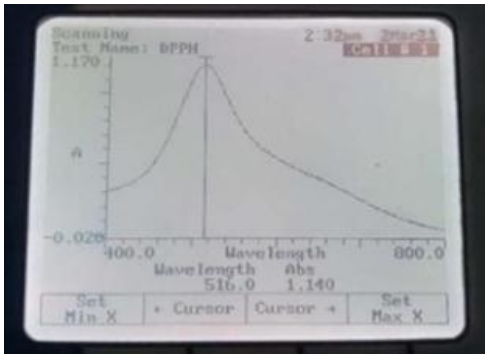

Figure 1. Maximum Wavelength Absorbance

From the results of the maximum wave measurement test, the absorbance was 1.140 at a wavelength of 516 nm. Absorbance measurements in this study were carried out at a wavelength of 516 nm of DPPH solution with an absorbance of 1.140, both wavelengths in accordance with the maximum wavelength range that can be used to measure with the DPPH method.

Results of Determination of Vitamin C Test Levels

The wavelength results are then used to measure the sample absorbent as well as vitamin C to determine the $IC_{value\ of\ 50}$. The IC50 value is a value intended to indicate antiradical activity which is the value of antioxidant concentrations to reduce 50% of free radical activity. The results of the antioxidant activity test on Vitamin C as a comparison and ethanol extract of orange peel can be seen in table 9.

Table 9. Vitamin C Levels Antioxidant Test Results

Test solution	Concentration (ppm)	Average Absorbance	% Inhibition
Vitamin C	20	0,655	42,514
	40	0,578	49,239
	60	0,508	55,380
	80	0,324	71,520
	100	0,268	76,462

To find out the antioxidant activity, it can be seen from the standard IC50 value of vitamin C as a comparison has an IC50 of 3.9986 ppm. Calculations of antioxidant test levels of vitamin C can be seen in the appendix. The smaller the IC50 value means the stronger its antioxidant power.

Antioxidant Activity Test Results

The N-hexane fraction, the ethyl acetate fraction, and the orange peel water fraction with various concentrations are called stock solutions. This test solution is made into 5 concentration series. The concentration is directly proportional to the percentage of damping, the higher the concentration of the test solution, the higher the percentage of damping. The mechanism of radical capture of DPPH from antioxidant compounds that can cause the damping of

radicals that are purple to yellow non-radicals. The process of color change that occurs due to the reduction of conjugated double bonds in DPPH. This occurs when there is an arrest of one electron by an antioxidant substance. The DPPH radical will be stable when reacting with the test agent in the form of an antioxidant compound that donates a hydrogen atom so that it becomes *diphenylpicrylhydrazine*. The change in color from purple to yellow is what can be measured spectrophotometrically. Radical absorption absorption testing is carried out by making a concentration series first on extracts and fractions, then adding DPPH to each concentration series and reading the absorbance at the maximum wavelength that has been determined. The absorbance reading in this study was carried out at a predetermined operating time, namely the 30th minute, and then the percentage of damping was calculated.

Table 10. Average Value of Test Solution Absorbance

Test solution	Concentration (ppm)	Average absorbance	% Inhibition
Chloroform fraction	20	1,438	21,616
	40	1,281	30,154
	60	1,187	35,313
	80	0,860	53,133
	100	0,770	58,001
Faction Ethyl acetate	20	1,346	26,630
	40	1,212	33,932
	60	0,988	46,139
	80	0,857	53,260
	100	0,722	60,635
Faction Water	20	1,425	22,325
	40	1,208	34,132
	60	0,994	45,831
	80	0,909	50,426
	100	0,720	60,726

The results of absorbance measurements in table 10 show that the greater the concentration of the test solution, the less absorbance value will be. In the N-hexane fraction the concentration of 20 ppm shows an absorbance value of 1.438 then at a concentration of 40 ppm there is a decrease in the absorbance value of 1.281. The ethyl acetate fraction with a concentration of 20 ppm has an absorbance value of 1.346, then with an increase in concentration to 40 ppm, there is a decrease in the absorbance value to 1.212. The water fraction with a concentration of 60 ppm has an absorbance value of 0.994 then with an increase in concentration to 80 ppm shows a decrease in the absorbance value to 0.909.

The results of the absorbance measurement are used to obtain the %inhibition value. IC_{50} is the sample concentration needed to capture 50% of DPPH free radicals during operating time. Data from the N-hexane fraction, ethyl acetate fraction and water fraction were then calculated at an $IC_{\text{value of 50}}$ using a linear regression equation based on the formula Y = a + bx

Table 11. IC50 Value

Yes	Test solution	IC50 value (ppm)
1	N-hexane fraction	81,639
2	Ethyl acetate fraction	75,705
3	Water fraction	73,464

The N-hexane fraction has an $IC_{value\ of\ 50}$ of 81.639 ppm, meaning that the N-hexane fraction has the weakest antioxidant activity when compared to the antioxidant activity of the ethyl acetate fraction of 75.705 ppm and the water fraction of 73.464 and in the vitamin C comparison of 3.9986 ppm. However, if you look at the IC $value_{of\ 50}$, the chloroform fraction sample has strong antioxidant activity ($IC_{value\ of\ 50}$ is between 50-100 ppm). The ethyl acetate fraction has the greatest antioxidant activity when compared to the antioxidant activity of the N-hexane fraction with an $IC_{value\ of\ 50}$ of 75.705 ppm, meaning that the ethyl acetate fraction has strong antioxidant activity, this is because in the ethyl acetate fraction there are flavonoid compounds that are able to inhibit oxidation reactions through radical capture mechanisms. The water fraction has an $IC_{value\ of\ 50}$ of 73.464 ppm, which means that the antioxidant activity of the water fraction is greater than the antioxidant activity of the N-hexane fraction. This is because in the water fraction there are triterpenoid compounds, tannins, saponins, and flavonoids that are able to capture radicals.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the research, the antioxidant samples of citrus fruit peels can be concluded as follows:

The antioxidant activity of the N-hexane fraction was 81.639 ppm, the ethyl acetate fraction was 75.705 ppm and the water fraction was 73.464 ppm from the peel of the Orange Fruit using the DPPH method. The water fraction of orange peel extract has the highest antioxidant activity with an ICvalue of 50 which is 73.464 ppm.

FURTHER STUDY

Future research is recommended to identify and characterize the specific bioactive compounds responsible for the antioxidant activity of the N-hexane, ethyl acetate, and aqueous fractions of Santang mandarin orange peel using advanced analytical techniques such as LC-MS or GC-MS. In addition, evaluating antioxidant activity through multiple in vitro and in vivo methods would provide a more comprehensive understanding of the peel's potential as a natural

antioxidant source for pharmaceutical, nutraceutical, and functional food applications.

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