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Characteristics and Antioxidant Activity of Limau Kuit Peel (*Citrus hystrix*) Extract with Variation of Extraction Solvent

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Abstract: Limau kuit (*Citrus hystrix*) has been traditionally utilized for its therapeutic properties, particularly the antioxidant potential of its peel. Extraction is preferred for medicinal use, with solvent selection playing a critical role in determining the yield and concentration of bioactive compounds. This study aimed to assess the characteristics and antioxidant activity (IC_{50} value) of Limau Kuit peel extract extracted with three different solvents. The research methods, starting with sample preparation, were carried out by drying Limau Kuit peel, then extraction using three different solvents: distilled water, ethanol 70%, and ethanol 96%. Extraction with distilled water solvent used the infusion method, while the ethanol solvent used the maceration method. The third extract was then characterized based on the parameters of percent yield, organoleptic testing, phytochemical screening, chromatography profile, and determination of total flavonoid levels. Antioxidant activity testing on the third extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The study results showed that the third extract had the same shape, color, aroma, taste, and compound content: flavonoids, phenolics, alkaloids, and terpenoids. The results of thin-layer chromatography also showed no difference in the third extract. The characteristic differences were found in the parameters of percent yield and total flavonoid content. The highest total flavonoid content of Limau Kuit (*Citrus hystrix*) peel extract was obtained using 70% ethanol (11.47% w/w), followed by 96% ethanol (6.39% w/w), and distilled water (2.82% w/w). Antioxidant activity, evaluated using the IC_{50} value as the parameter, showed that the 70% ethanol extract exhibited the strongest activity (21.26 ppm, very strong), followed by the 96% ethanol extract (31.87 ppm, very strong), and distilled water extract (70.91 ppm, strong). This study concludes that 70% ethanol is the most effective solvent for extracting antioxidant-rich compounds from Limau Kuit peel, supporting its potential use in herbal formulations.
Keywords: Antioxidant; *Citrus hystrix*; limau kuit, solvent.

INTRODUCTION

Limau Kuit (*Citrus hystrix*) is a perennial plant native to Southeast Asia that belongs to the genus Citrus and the family Rutaceae (Halim et al., 2021). Limau Kuit, by the people of South Kalimantan, is generally used as a cooking spice and traditional medicine (Rosida et al., 2023). Although it has the same Latin name as the Jeruk Purut, Limau Kuit has different fruit characteristics. This difference is due to plant adaptation to different environments (Buih & Susandarini, 2023). Limau Kuit plants in traditional medicine can be used for various digestive disorders, nasal congestion, colds, and coughs. The development of research technology reveals that Limau Kuit

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has several significant bioactivities, namely antioxidant, antibacterial, anticancer, and antiviral properties (Halim et al., 2021).

The results of phytochemical tests of Limau Kuit peel extract contain alkaloids, saponins, steroids, triterpenoids, phenolics, and flavonoids (Irwan et al., 2017; Rosida et al., 2023). Flavonoids and phenolics can be body protectors from free radicals and reduce the risk of cancer and inflammation (Fauziah & Mulyani, 2022). Free radicals are a form of reactive oxygen compounds that cause cell damage and various diseases such as cancer, heart disease, cataracts, premature aging, and degenerative diseases (Jubaidah et al., 2024). Antioxidants are substances that can contribute to or prevent chain reactions from free radicals so that they can avoid oxidative damage to target molecules such as proteins, lipids, and DNA (Suryanita et al., 2019). Plants that contain flavonoid compounds, such as Limau Kuit, can function as antioxidants (Jubaidah et al., 2024). Extracts are the primary raw materials for natural medicinal products. Using appropriate solvents to separate compounds from plant materials requires an extraction or withdrawal process of compounds. The extraction separation method uses the principle of like dissolves like, where a solvent will dissolve a compound based on its polarity compatibility with the solvent (Syamsul et al., 2020). Solvents are important in determining an extract sample's chemical compound content (qualitative) and levels (quantitative). Solvents can determine the characteristics of the extract and the pharmacological activity of natural medicinal raw materials (Ariyani et al., 2018).

Limau Kuit peel extract is known to have the ability to inhibit the growth of Escherichia coli and Staphylococcus aureus bacteria (Ariyani et al., 2018), including inhibition of Propinicaterium acne bacteria that cause acne (Nurbidayah et al., 2024). Porphyromonas gingivalis bacteria, which are commonly found in periodontitis, can also be inhibited by Limau Kuit peel extract (Erinda et al., 2022). Limau Kuit peel extract can act as an antifungal, especially Candida albicans (Nurwafa et al., 2023). The Limau Kuit peel extracted with distilled water is known to have antioxidant activity through a free radical neutralization mechanism (Nashucha et al., 2019).

The novelty of this study is that the results will provide an overview of the characteristics of the Limau Kuit fruit peel extract extracted with several different solvents. The extract from the optimum solvent will guarantee natural product ingredients' quality, efficacy, and safety. This will be the basis for the production process on a larger scale. The Limau Kuit also comes from South Kalimantan through extraction using the maceration method, in contrast to many other studies that utilize essential oils in the skin extracted using the distillation method. This study will determine the antioxidant activity of the Limau Kuit fruit peel extract extracted with several different solvents. Other studies generally only examine the antioxidant activity of one solvent (Ariyani et al., 2018; Chairina, 2022), so they do not provide a comprehensive overview of pharmacological activity in different solvents.

MATERIALS AND METHODS

Equipment

The tools used are an infusion tool (Zebra), a UV lamp (Local), a fume hood (Labolytic), a refrigerator (Samsung), a drying cabinet (Local), a micropipette (Dragon Lab), an analytical balance (Ohaus), a UV-Vis spectrophotometer (PerkinElmer), a thermometer (Joil), a vial, a vortex mixer (Lab Companion Jiotech), and a rotary evaporator (IKA® RV 10).

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Materials

The materials used in this study include Limau Kuit (*Citrus hystrix*), aluminum (III) chloride (Merck), aluminum foil, distilled water (Technical), glacial acetic acid (Merck), ethyl acetate (Technical), ethanol (Merck), HCl (Merck), quercetin (Sigma Aldrich), Dragendorff reagent, LB reagent (Liebermann Burchard), GF254 plate (Qingdao Hengze), n-hexane (Technical), DPPH powder (Himedia), and magnesium powder (Merck).

Plant determination

The basic Faculty of Mathematics and Natural Sciences Laboratory, Lambung Mangkurat University, Banjarbaru, South Kalimantan Province, determined Limau Kuit plants.

Preparation of Extract

Limau Kuit fruit samples were taken in Padang Anyar Village, Banjar Regency, South Kalimantan. The Limau Kuit peel is sorted wet, then weighed, followed by the washing process. The Limau Kuit peel is chopped and then dried using a drying cabinet at 50°C for 68 hours. The dry sample was blended into a fine powder and sieved through a mesh 14 sieve before weighing. The dry powder of Limau Kuit peel was extracted with distilled water, ethanol 70%, and ethanol 96% to identify the most effective solvent based on polarity for extracting flavonoids and evaluating antioxidant activity(Rizki et al., 2024). Extraction using ethanol is carried out using the maceration method, while extraction with distilled water solvent uses the infusion method. The maceration process is done by soaking the sample in a solvent with a ratio of 1:20 for 3 x 24 hours, with the solvent replaced every 24 hours. The infusion process is by heating the sample with the solvent in a water bath for 15 minutes, starting when the temperature reaches 90°C. Each filtered liquid extract was thickened using a rotary evaporator at 50°C until a thick extract was obtained, then oven-dried at 50°C until a constant weight was obtained (Rahmi et al., 2021).

Percentage Yield

The percentage yield is calculated based on the percentage of dry extract weight compared to the sample weight before extraction. The percentage yield will indicate the effectiveness of the extraction process (A. K. Sari et al., 2022).

Organoleptic Testing

The organoleptic test of the extract was carried out using the five senses on the three extracts. The organoleptic evaluation was carried out using the five senses: color, odor, taste, and consistency of the extracted extract. Testing was done thrice by replicating each extract (Izma et al., 2023).

Phytochemical Screening

The test solution was made by dissolving the three extracts, each as much as 500 mg, with 50 mL of ethanol solvent p.a, so there were 3 test solutions. Testing was carried out using specific reagents for the flavonoid, alkaloid, phenolic, saponin, steroid, and terpenoid compound groups (Rizki et al., 2021).

Thin Layer Chromatography (TLC) Profile

Samples of viscous extracts of distilled water, 70% ethanol, and 96% ethanol of Limau Kuit peel and quercetin standards were each dissolved with ethanol in a vial. A 10 cm × 10 cm silica gel GF¬254 TLC plate was activated using an oven at a temperature of 110°C for 30 minutes. Samples of extracts and quercetin standards were spotted on the lower limit of the plate and then eluted in a saturated chamber using a mobile phase in the form of a mixture of n-hexane; ethyl acetate with a ratio of 1:1. The plate that had been eluted to the upper limit was then lifted and dried and then the spots were observed under a UV lamp with a wavelength of 254 nm and 366

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nm. Quantitative results can be obtained from the Rf (Retention factor) value (Pratiwi et al., 2023).

Determination of Total Flavonoid Levels

The extracts of distilled water, ethanol 70%, and ethanol 96% of Limau Kuit peel were weighed as much as 10 mg, dissolved with ethanol up to 10 mL, and added with ethanol p.a to the limit mark. The solution of each extract was taken as much as 0.5 mL, 1 mL of AlCl3 10% and 8 mL of acetic acid 5%, then left for the optimum time (operating time). Absorbance will be measured at the maximum wavelength (λmax) identified by scanning the sample using a UV-Vis spectrophotometer from 350 to 450 nm. Quercetin was used to compare, which had been made in concentrations of 20, 40, 60, 80, and 100 ppm. The solution was treated the same as the sample until the comparative absorbance was obtained, and then the standard curve equation was made (Rizki et al., 2023).

Antioxidant Activity Testing

One milliliter of 0.4 mM DPPH solution was mixed with 4 mL of ethanol, homogenized, and left to stand for 30 minutes. The maximum absorbance wavelength (λmax) of the DPPH solution was determined using UV–Vis spectrophotometry in the range of 450–600 nm. All subsequent absorbance measurements were conducted at this wavelength. Quercetin at a concentration of 10 ppm (4 mL) was then mixed with 1 mL of 0.4 mM DPPH solution, and the mixture was homogenized using a vortex mixer. The absorbance of the reaction mixture was measured at the determined λmax every 2 minutes over 60 minutes, representing the operating time established in this study.

Comparative Antioxidant Activity Testing

Quercetin was made into a series of concentrations of 1, 1.5, 2, 2.5, and 3 ppm using ethanol. 1 mL of 0.4 mM DPPH solution was added to each 4 mL quercetin concentration series. The solution mixture was left for the operating time, and then the absorbance was read at the maximum wavelength that had been obtained.

Antioxidant Activity Testing of Extracts

Extracts of distilled water, 70% ethanol, and ethanol 96% of Limau Kuit peel were made in concentrations of 20, 40, 60, 80, and 100 ppm using ethanol. Each solution was taken as much as 4 mL, added with 1 mL of 0.4 mM DPPH, left for operating time, and the absorbance was read at the maximum wavelength. The sentence above explains the explanation (Rizki et al., 2022).

Data Analysis

Flavonoid concentration was determined by a linear regression equation with the formula y = bx + a. The absorbance data from the measurement results were then entered into the linear regression equation, and then the concentration calculation was carried out where y is the sample absorbance. The percentage inhibition value expresses the determination of antioxidant activity, the result of the percentage difference between DPPH absorbance and sample absorbance compared to DPPH absorbance. The percentage inhibition results are used to find the IC_{50} value (Rizki et al., 2022). The category of antioxidant activity can be seen in Table 1.

Table 1. Antioxidant Ability Category (Adawiyah & Rizki, 2018)

No	Category	Concentration (ppm)
1	Very Strong	< 50
2	Strong	50 – 100
3	Moderate	101 – 150
4	Weeak	> 150

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RESULTS AND DISCUSSION

Determination of Limau Kuit

The Limau Kuit plant was taken from Padang Anyar Village, Banjar Regency, South Kalimantan—the Limau Kuit plant was determined with certificate number 006d/LB.LABDASAR/I/2024 states that the Limau Kuit species used as a sample has the Latin name *Citrus hystrix*. Limau Kuit fruit is dark green when young and slightly yellowish when old. The shape of the Limau Kuit fruit is oval with a diameter of 4-5 cm. The peel of the Limau Kuit fruit can be said to be quite thick when it is cut. The peel of the Limau Kuit fruit is classified as rough skin because there are many protrusions on its surface.

Organoleptic and Rendemen of Limau Kuit Peel Extract

Limau Kuit peels simplicia powder from a fresh sample of 3000 grams obtained a powder weight of 961.22 grams with a drying loss of 67.96%. The Limau Kuit peel simplicia powder obtained was a fine yellow powder with a distinctive citrus aroma and a bitter taste. Research conducted by Chairina (2022) showed that the drying shrinkage of the Limau Kuit skin simplicia was obtained at 75.368% from a fresh sample of 4.75 kg with the obtained simplicia powder as much as 1.17 kg, which was yellowish green in color and had a distinctive orange aroma (Chairina, 2022). Table 2 shows the organoleptic test results for the distilled water, ethanol 70%, and ethanol 96% extract of the Limau Kuit peel.

Table 2. Results of the Organoleptic Examination of the Limau Kuit Peel Extract

Sample	Parameter			
	Shape	Color	Aroma	Taste
Distilled watert Extract	Thick	Brown	Typical Orange	Bitter
Ethanol 70% Extract	Thick	Brown	Typical Orange	Bitter
Ethanol 96% Extract	Thick	Brown	Typical Orange	Bitter

The extract produced from the three solvents is a thick extract with a brown color that has a distinctive orange aroma and a bitter taste; this result is in line with Putra's research (2018), which states that *Citrus hystrix* extract is blackish brown with a caramel aroma with a distinctive orange and bitter taste (Putra et al., 2018). Data on the yield of distilled water extracts, ethanol 70%, and ethanol 96% of C. hystrix fruit skin are presented in Table 3.

Table 3. Results of the Yield of Limau Kuit Peel Extract

Sample	Replication	Powder Weight (g)	Extract Weight (g)	Yield (%)	Average (g)
Distilled watert Extract	1	25	7,27	29,09	29,24 ± 0,44
	2	25	7,46	29,83	
	3	25	7,20	28,79	
Ethanol 70% Extract	1	25	7,25	28,98	29,25 ± 0,35
	2	25	7,44	29,75	
	3	25	7,26	29,03	
Ethanol 96% Extract	1	25	6,82	27,26	27,80 ± 0,39
	2	25	6,99	27,94	
	3	25	7,05	28,19	

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The yield results show that solvent types with different polarities can affect the yield (Verdiana et al., 2018). Other studies show that the yield of 96% of the ethanol extract of Limau Kuit is 17.75% (Chairina, 2022) and obtained at 17.59% (Darsono et al., 2022). Research by Putra et al. (2018) with the same sample obtained a yield of 21.403% using ethanol 70% solvent (Putra et al., 2018). The yield of Limau Kuit samples in another study with ethanol 70% and ethanol 96% solvents showed that ethanol 70% extract yield was higher, namely 14.425%, compared to ethanol 96% extract, namely 6.645%. The difference in the yield of the three solvents used also affects the number of compounds extracted in the extract due to the polarity of each solvent (Riwanti et al., 2018). Ethanol is a universal solvent that can attract most polar and non-polar compounds (Verdiana et al., 2018).

Phytochemical Screening of Limau Kuit Peel Extract

The results of the phytochemical screening of distilled water, ethanol 70%, and ethanol 96% extract of Limau Kuit peel can be seen in Table 4.

Table 4. Phytochemical Screening Results of Limau Kuit Peel Extract

No	Test	Distilled watert Extract	Ethanol 70% Extract	Ethanol 96% Extract
1	Flavonoids	Positive	Positive	Positive
2	Alkaloids	Positive	Positive	Positive
3	Phenolic	Positive	Positive	Positive
4	Saponins	Negative	Negative	Negative
5	Steriods	Negative	Negative	Negative
6	Terpenoids	Positive	Positive	Positive

Based on these data, the distilled water, ethanol 70%, and ethanol 96% extract of Limau Kuit peel positively contained flavonoids, alkaloids, phenolics, and terpenoids. These results align with the research of Hesturini et al. (2023), which stated that the ethanol 96% extract of Limau Kuit peel positively contained tannins, flavonoids, alkaloids, terpenoids, and saponins (Hesturini et al., 2023). Research by Aprilyanie et al. (2023) stated that the ethanol 70% extract positively contained flavonoids, alkaloids, and saponins, and the distilled water extract contained alkaloids and saponins. The test results showed that the three extracts did not contain saponins, possibly due to differences in the samples used and the sample extraction process (Pratiwi et al., 2023).

Thin Layer Chromatography Profile

Thin-layer chromatography (TLC) is a method that aims to identify and separate a compound by separating chemical components based on the principles of absorbance and partition (Mustaqimah, 2023). Table 5 shows the TLC profile identification and Rf value calculation results.

The Rf values of distilled water, ethanol 70%, and ethanol 96% extract of Limau Kuit peel have similarities at UV 254 nm of 0.317, 0.55, and 0.75, and at a wavelength of 366 nm, there is fluorescence with Rf values of 0.317; 0.433; 0.55; and 0.867. The Rf values obtained have met the good Rf value range, namely in the range of 0.2-0.8 (Mustaqimah, 2023). Another study by Rahayu et al. (2015), which identified flavonoids in fraction samples using TLC and then identified with a UV-Vis spectrophotometer, stated that spots with Rf values in the range of 0.2-0.75 positively indicate spots containing flavonoids of the flavonol group (Qiu et al., 2020).

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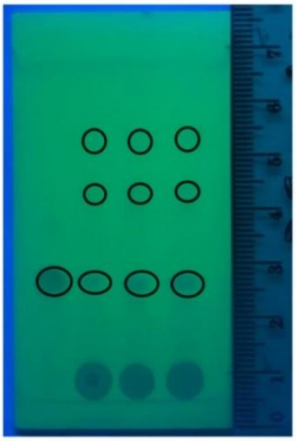
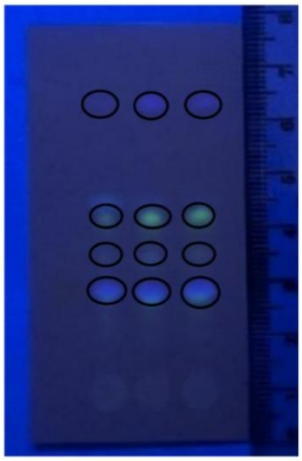
Table 5. Thin Layer Chromatography Results of Limau Kuit Peel Extract

Chromatogram	Nilai Rf	Information
 (a) (b) (c) (d)	UV Lamda 254 nm a. 0,317 b. 0,317; 0,550; 0,750 c. 0,317; 0,550; 0,750 d. 0,317; 0,550; 0,750	Plate size: 4 x 7.5 cm Upper limit: 0.5 cm Lower limit: 1 cm Eluent distance: 6 cm Mobile phase: n-hexane: ethyl acetate (5:5 v/v) a) Quercetin b) Distilled watert Extract c) Ethanol 70% Extract d) Ethanol 96% Extract
 (a) (b) (c) (d)	UV Lamda 366 nm a. – b. 0,317; 0,433; 0,550; 0,867 c. 0,317; 0,433; 0,550; 0,867 d. 0,317; 0,433; 0,550; 0,867	

Total Flavonoid Content of Limau Kuit Peel Extract

The standard compound used is quercetin, which can form a color complex with AlCl3 and has strong antioxidant activity (Mahmudah et al., 2024). The concentration solutions were incubated for 46 minutes according to the operating time and read with a UV-Vis spectrophotometer at a maximum absorption wavelength of 417.4 nm. The results obtained from the creation of the quercetin standard curve are a simple linear regression equation y = bx + a, namely y = 0.0044x + 0.2729 where 0.0044 is the regression coefficient (slope), and 0.2729 is the constant (intercept) with a correlation coefficient value (r) of 0.96519 which means that the effect of concentration on absorbance has a level of accuracy of 96.519%. These results indicate that concentration is directly proportional to the absorbance value, which is in line with the research of Mahmudah et al. (2024), which states that the higher the concentration, the higher the absorbance value obtained (Mahmudah et al., 2024). The correlation coefficient value approaching 1 indicates that the regression equation is linear, so it can be said that absorbance and concentration have a very strong or good correlation(D. Y. Sari & Widyasari, 2022). Determination of total flavonoid levels of C. hystrix fruit peel extract using a concentration of 1000 ppm. The results of determining the total flavonoid content of Limau Kuit peel extract are in Table 6.

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Table 6. The Results of the Total Flavonoid Content of Limau Kuit Peel Extract

Sample	Rep.	Absorbance	Total Flavonoid Content (%w/w)	Average Total Flavonoid Content (%w/w) ± SD
Distilled watert Extract	1	0,3970	2,82	2,82 ± 0,025
	2	0,3972	2,83	
	3	0,3952	2,78	
Ethanol 70% Extract	1	0,7775	11,47	11,47 ± 0,001
	2	0,7776	11,47	
	3	0,7776	11,47	
Ethanol 96% Extract	1	0,5538	6,38	6,39 ± 0,001
	2	0,5539	6,39	
	3	0,5539	6,39	

Based on these results, there are differences in total flavonoid levels between extracts with different solvents. The Limau Kuit peel extract has the highest total flavonoid levels, from the largest to the smallest, and it is 70% ethanol, 96% ethanol, and distilled water extract. Another study that examined Limau Kuit peel extract with ethanol 96% solvent stated that the total flavonoid levels obtained were 2.253 ± 0.006% w/w (Chairina, 2022). This is in line with the research of Riwanti et al. (2020), which examined the total flavonoid levels of *Sargassum polycystum* samples with various ethanol concentrations, stating that the highest flavonoid levels were found in the extract with ethanol 70% solvent (Riwanti et al., 2018). Another study by Verdiana et al. (2018), which examined lemon peel extract, stated that the highest flavonoid levels were found in ethanol 70% extract and the lowest in distilled water extract (Verdiana et al., 2018). Flavonoid levels of the three extracts showed different results, where ethanol 70% extract had higher flavonoid levels than ethanol 96% extract and distilled water extract. Ethanol 70% is a more polar solvent than ethanol 96% and more non-polar than distilled water, so flavonoid compounds tend to dissolve more in ethanol 70%.

Antioxidant Activity of Limau Kuit Peel Extract

Antioxidant activity testing begins with determining the maximum DPPH wavelength. The results of determining the maximum DPPH wavelength were obtained at 516.55 nm with an absorbance of 0.8185. According to Muliani et al. (2024), the maximum DPPH wavelength using UV-Vis Spectrophotometry was carried out in the 450-600 nm wavelength range. The results obtained are consistent with the research of Rizki et al. (2022), which also recorded the maximum DPPH wavelength of 516.5 nm (Rizki et al., 2022). The results of determining the DPPH operating time at minutes 46 to 50 showed a stable absorbance of 0.067. These results are in line with the research of Hasanah et al. (2023), which stated that the stable working time or operating time of the DPPH solution was obtained for 43-46 minutes (Hasanah et al., 2023). The results of stable working or unstable operating time at one number can be caused by compound purity and very high instrument sensitivity (Rahmi et al., 2021).

The regression equation obtained from the relationship between the concentration of the reference solution and the percentage of inhibition is y = 24.35x - 1.965, where 24.35 is the regression coefficient (slope), and -1.9658 is the constant (intercept) with a correlation coefficient value (r) of 0.989. The results of the antioxidant activity of the quercetin are presented in Table 7.

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Table 7. Results of the Antioxidant Activity of the Quercetin

Concentration (ppm)	Percent Inhibition			Average Inhibition	IC_{50} (Category)
	Rep 1	Rep 2	Rep 3		
1	19,64	19,80	19,98	19,81	2,134 ppm
1,5	31,14	30,97	31,14	31,08	(Very Strong)
2	46,54	46,67	46,73	46,65	
2,5	55,56	55,47	55,66	55,57	
3	73,22	73,24	73,26	73,24	

Based on these results, the IC_{50} value of the quercetin comparator was obtained at 2.134 ppm. The lower IC_{50} value observed for quercetin than the extract indicates its superior antioxidant activity. This is primarily attributed to quercetin's purity and well-defined molecular structure, which confers potent free radical scavenging properties. In contrast, crude extracts comprise complex mixtures of bioactive and non-bioactive constituents, potentially leading to dilution of antioxidant efficacy. This shows that at a concentration of 2.134 ppm, the quercetin comparator can inhibit free radicals by 50%. This result is included in the strong antioxidant activity category because it has an IC_{50} value of <50 ppm (Pambudi et al., 2020). This is in line with the research of Adawiyah & Rizki (2018), which obtained the IC_{50} value of quercetin of 2.6 ppm (Adawiyah & Rizki, 2018). The results of the antioxidant activity test of the Limau Kuit peel extract can be seen in Table 8.

Table 8. Antioxidant Activity Test of the Limau Kuit Peel Extract

Sample	Concentration (ppm)	Percent Inhibition			Average Inhibition	IC_{50} (Category)
		Rep 1	Rep 2	Rep 3		
Distilled watert Extract	20	45,02	45,09	45,09	45,07	70,91 ppm (Strong)
	40	47,09	47,14	47,24	47,16	
	60	48,34	48,44	48,45	48,41	
	80	51,43	51,46	51,54	51,48	
	100	52,52	52,59	52,65	52,59	
Ethanol 70% Extract	20	48,18	48,12	48,16	48,15	21,26 ppm (Very Strong)
	40	56,53	56,46	56,50	56,50	
	60	58,94	58,96	58,99	58,96	
	80	63,15	63,29	63,21	63,22	
	100	67,88	67,92	67,88	67,90	
Ethanol 96% Extract	20	47,04	47,10	47,07	47,07	31,878 ppm (Very Strong)
	40	51,49	51,51	51,52	51,51	
	60	56,61	56,63	56,54	56,59	
	80	62,02	62,20	62,17	62,13	
	100	64,10	64,12	64,12	64,11	

The lower IC_{50} value observed for quercetin than the extract indicates its superior antioxidant activity. This is primarily attributed to quercetin's purity and well-defined molecular structure, which confers potent free radical scavenging properties. In contrast, crude extracts comprise complex mixtures of bioactive and non-bioactive constituents, potentially leading to dilution of antioxidant efficacy (Adawiyah & Rizki, 2018).

The IC_{50} value of the distilled water extract of the Limau Kuit peel is 70.91 ppm. The results obtained are included in the category of strong antioxidant activity

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because they have an IC_{50} value of 50-100 ppm (Pambudi et al., 2020). The antioxidant activity of the distilled water extract of Limau Kuit peel is stronger compared to previous research by Moolsup et al. (2023), who studied the distilled water extract of Limau Kuit peel using the DPPH method and stated that the IC_{50} obtained was 117.54 ppm with a moderate antioxidant category (Moolsup et al., 2023). The IC_{50} value of ethanol 70% extract of Limau Kuit peel was 21.26 ppm. The results obtained are included in the very strong antioxidant activity category because they have an IC_{50} value of < 50 ppm (Pambudi et al., 2020). The antioxidant activity of ethanol 70% extract of Limau Kuit peel is very strong compared to previous research by Moolsup et al. (2023), who studied ethanol 70% extract of Limau Kuit peel using the DPPH method, stated that the IC_{50} obtained was 47.95 ppm (Moolsup et al., 2023).

The IC_{50} value of the ethanol 96% extract of Limau Kuit peel was 31.87 ppm. The results obtained are included in the very strong antioxidant activity category because they have an IC_{50} value of < 50 ppm (Pambudi et al., 2020). The antioxidant activity of the ethanol 96% extract of Limau Kuit peel is slightly weaker compared to the study by Chairina (2022), which examined the antioxidant activity of the ethanol 96% extract of Limau Kuit peel, stating that the IC_{50} value obtained was 29.76 ppm (Chairina, 2022). Based on these results, the three antioxidant activities of the extracts have different IC_{50} values depending on the type of solvent used. The largest to smallest IC_{50} values are distilled water extract, ethanol 96%, and ethanol 70%, which show the strongest antioxidant activity in the ethanol 70% extract of Limau Kuit peel. Another study examining lemon peels stated that the highest flavonoid content and strong antioxidant activity were found in extracts with 70% ethanol solvent. The difference in antioxidant activity between extracts is due to differences in the content of active compounds that dissolve in several solvents. Several factors affecting stability in antioxidant tests include temperature, light, pH, oxygen, and metal ions. In addition, a comparison between several similar studies showed a fairly diverse relationship between total flavonoid levels and antioxidant activity, allegedly due to differences in the solvents used, the extraction process carried out, differences in plant growth places and plant species used, and treatments during the extraction process and testing (Suryanita et al., 2019).

The limitation of this study is that the materials used come from the Regency located in South Kalimantan in the wetland area, so differences in growing places can affect the characteristics of active ingredients in plants. Testing of extract characteristics is only limited to five main parameters. Testing of antioxidant activity is limited to in vitro testing using the DPPH method. Despite these limitations, the findings provide a valuable preliminary insight into the antioxidant potential of local plant sources, supporting their future application in developing natural antioxidant-based products.

CONCLUSION

The three extracts have the same shape, color, aroma, taste, compound content, and chromatography profile. The characteristic differences are found in the parameters of percent yield and total flavonoid content. The highest extract yield and flavonoid content are in the ethanol 70% extract of Limau Kuit peel. The highest antioxidant activities are in the ethanol 70%, ethanol 96%, and distilled water extract from Limau Kuit peel at 21.26 ppm (very strong), 31.87 ppm (very strong), and 70.91 ppm (strong). The 70% ethanol solvent has the most optimum extracting ability in Limau Kuit peel. Further investigations are warranted to evaluate the extract's characteristics using non-specific parameters, including drying shrinkage, moisture

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content, ash values (total and acid-insoluble), and heavy metal contamination. Additionally, antioxidant activity should be assessed through in vivo studies in animal models to obtain more comprehensive insights into its biological efficacy. Comparative analysis of Limau kuit sourced from different geographical regions is also essential to elucidate the impact of environmental and agronomic factors on phytochemical composition and pharmacological properties.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to the publication of this paper.

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