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JURNAL TEKNOSAINS

Effect of maceration and mae (microwave assisted extraction) on antioxidant activity of methanol extract of simpur air leaves (*dillenia suffruticosa* (griff.) Martelli)

Indonesian title: Pengaruh metode ekstraksi maserasi dan mae (microwave assisted extraction) terhadap aktivitas antioksidan ekstrak metanol daun simpur air (dillenia suffruticosa (griff.) martelli)

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ABSTRAK

Tanaman simpur air (Dillenia suffruticosa) merupakan salah satu tanaman yang dimanfaatkan dalam pengobatan tradisional untuk merawat berbagai penyakit dan memiliki potensi sebagai sumber antioksidan. Antioksidan adalah senyawa yang menghambat, mencegah dan mengurangi kerusakan oksidatif pada molekul target. Efektivitas ekstraksi senyawa aktif dipengaruhi oleh metode ekstraksi yang digunakan. Maserasi memerlukan waktu yang relatif lama, sehingga dibutuhkan alternatif yang lebih cepat dan efisien seperti MAE. Namun senyawa yang sensitif terhadap panas dapat terdegradasi jika daya yang digunakan terlalu tinggi atau durasi ekstraksi terlalu lama. Oleh karena itu perlu dilakukan perbandingan antara metode maserasi dan MAE. Penelitian ini bertujuan untuk melihat pengaruh metode ekstraksi maserasi dengan MAE terhadap aktivitas antioksidan ekstrak metanol daun simpur air. Rancangan penelitian ini dimulai dengan determinasi tanaman, pembuatan ekstrak metanol dengan metode maserasi dan MAE, kemudian sampel diidentifikasi menggunakan KLT dengan fase gerak toluene : etil asetat : asam format (3:5:1 v/v/v), uji aktivitas antioksidannya dengan metode DPPH (2,2 difenil-1 pikrilhidrazil) dan analisis data menggunakan SPSS. Hasil KLT menunjukkan bahwa ekstrak daun simpur air mengandung flavonoid, yang ditandai dengan warna kuning setelah disemprot dengan $AlCl_3$. Nilai Rf untuk sampel maserasi dan MAE sejajar dengan standar rutin (0,22) dan kuersetin (0,8). Metode ekstraksi MAE menghasilkan aktivitas antioksidan yang lebih baik dibandingkan dengan metode maserasi. Nilai aktivitas antioksidan ekstrak daun simpur air metode MAE adalah 5,942 ± 0,345 µg/ml, sedangkan metode maserasi adalah 10,498 ± 0,213 µg/ml, yang termasuk kategori antioksidan sangat kuat (IC_{50} < 50). Analisis data statistik dengan independent sample t-test menunjukkan perbedaan signifikan antara kedua metode tersebut dengan nilai (p < 0,05).

Keywords: *Dillenia suffruticosa; IC_{50}; Maserasi; MAE.*

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ABSTRACT

The simpur air plant (Dillenia suffruticosa) is one of the plants used in traditional medicine to treat various diseases and has potential as an antioxidant. Antioxidants are compounds that inhibit, prevent, and reduce oxidative damage to target molecules. The effectiveness of extracting active compounds depends on the extraction method used. Maceration requires a relatively long duration, so faster and more efficient alternatives, such as MAE, are needed. However, heat-sensitive compounds may degrade if the power is too high or the extraction time is prolonged. Therefore, a comparison between maceration and MAE methods is necessary. This study aims to determine the effect of maceration and MAE on the antioxidant activity of the methanol extract from the simpur air leaves. Samples were then analyzed using TLC with a mobile phase of toluene, ethyl acetate, and formic acid (3:5:1 v/v/v). Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, and data were analyzed with SPSS. TLC results confirmed the presence of flavonoids indicated by a yellow color after spraying with AlCl3, with IC_{50} values supporting their antioxidant potential. The Rf values of extracts obtained by maceration and MAE with the rutin standard (0.22) and quercetin standard (0.8). The MAE method produced higher antioxidant activity than maceration, with IC_{50} values of 5.942 ± 0.345 µg/mL and 10.498 ± 0.213 µg/mL, respectively, both classified as strong antioxidants (IC_{50} < 50). Independent sample t-test analysis yielded a p-value of less than 0.05, indicating a significant difference between the two methods. The methanol extract of simpur air leaves extracted by the MAE method produces better antioxidant activity than the maceration method.

Keywords: *Dillenia suffruticosa; IC_{50}; Maceration; MAE.*

INTRODUCTION

Antioxidants are stable molecules that neutralize free radicals, reducing their harmful effects (Prasetyaningsih et al., 2022). Although the human body possesses endogenous antioxidant enzymes, their levels are often insufficient to counteract excessive free radicals. Therefore, exogenous antioxidants are required when free radical levels are elevated. Antioxidants are generally categorized as natural or synthetic. Natural antioxidants have low toxicity, while synthetic antioxidants may cause side effects. As a result, natural antioxidants are increasingly preferred due to their safety (M. Kumar et al., 2021; Kurniawati & Sutoyo, 2021).

One promising source of natural antioxidants is simpur Air (*Dillenia suffruticosa*), which is widely found in West Kalimantan. The leaves are the most commonly utilized part, traditionally used to treat stomach pain, wounds, rheumatism, and coughs, while the shoots are consumed as fresh vegetables. Simpur Air leaves also contain secondary metabolites such as flavonoids, phenolics, triterpenoids, steroids, and saponins, contributing to their antioxidant activity (Nurdiani et al., 2023; Utami et al., 2021).

Previous studies have shown that simpur leaves contain flavonoids with antioxidant activity, recording an IC_{50} value of 33.418 µg/ml. The methanol extract of the roots exhibited an IC_{50} of 3.852 µg/ml, and the flower extract showed an IC_{50} of 44.510 µg/mL, confirming the plant's strong antioxidant potential. Antioxidant activity (IC_{50} value) can be influenced by various factors, particularly the extraction method, which affects yield and antioxidant effectiveness. (Nurhasnawati et al., 2017; Utami et al., 2021).

Extraction techniques have advanced from conventional to modern methods to achieve optimal results. Maceration, a widely used conventional method, is simple and inexpensive but time-consuming (Handoyo, 2020). Modern methods such as MAE employ microwave radiation to accelerate selective extraction through rapid Solvent heating, requiring less solvent and offering higher efficiency (Sari et al., 2020).

The selection of variations in extraction methods was conducted because previous research had never compared maceration and MAE extraction methods on simple air leaves. One of the widely used methods for assessing antioxidant activity is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is widely applied due to its simplicity, speed, ease of execution, and sensitivity to low-concentration samples (Rosana et al., 2021).

This study evaluated the impact of maceration and MAE extraction methods on the

antioxidant activity of the methanol extract from simpur Air leaves (*Dillenia suffruticosa*).

Method

The tools used in this study are a UV-Vis spectrophotometer instrument (Shimadzu®), Microwave-Assisted Extraction (MAE) (Electrolux®), food dehydrator (Kris®), UV lamp, sonicator, analytical balance, glassware, micropipette, blender, and Buchner device.

The materials used were simpur air (*Dillenia suffruticosa*) leaves, thin layer chromatography plate (60 F254), technical methanol, methanol p.a, DPPH powder, AlCl3, toluene, ethyl acetate, and formic acid.

Plant Determination

The Simpur Air leaves used in the study were identified at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) at Tanjungpura University.

Preparation of Plant Material (Simplisia)

The samples used in this study were simpur air (*Dillenia suffruticosa*) leaves collected in Sungai Raya District, Kubu Raya Regency, West Kalimantan Province. The leaves selected were fresh and green. 1 kg of leaves was collected and cleaned with clean running water to remove attached impurities. The leaves were then chopped and dried using a food dehydrator at 50 °C, pulverized with a blender, and stored in a tightly closed container.

Extraction Method

The extraction methods used were maceration and MAE. The extraction process of simpur Air leaves followed a ratio (1:10 b/v). The maceration method used 50 grams of simpur air leaf simplisia and 500 ml of methanol solvent, extracted for 3 x 24 hours with a 1 x 24-hour solvent change and occasional stirring. This process was carried out until the solvent was almost clear. Meanwhile, the MAE method uses 30 grams of simpur air leaf simplisia, which was put into a round-bottom flask, and 300 mL of methanol solvent. The extraction was carried out using a microwave oven with a power of 100 watts for 10 minutes. After that, the macerate was taken. The macerate was then collected, filtered using a vacuum buchner, and concentrated with a food dehydrator at 50°C until a thick extract was obtained.

Determination of Water Content

The water content of the sample air is determined by weighing 1 gram of the leaf extract, placing it on a metal plate, and flattening it. The Moisture content was measured using a Moisture Balance, repeated three times. The temperature was set at 105 ° C and the instrument was turned on until a signal indicated the analysis had been completed (Aini et al., 2023).

TLC Identification

The TLC plate was prepared by cutting silica gel 60 F254 into a size of 4 x 5 cm. The plate was then activated at 110°C in an oven for 30 minutes. The quercetin standard, rutin, and methanol extract of simpur air leaves were spotted onto the TLC plate and eluted with toluene: ethyl acetate: formic acid (3:5:1 v/v/v) until the mobile phase reached the upper limit, after which the plate was dried. The elution results were sprayed with AlCl3 reagent and 40 ppm DPPH solution, then observed under UV light at wavelengths of 254 nm and 366 nm (Sayakti & Hidaytullah, 2023; Tuldjanah et al., 2022).

Antioxidant Activity Test with UV-Vis spectrophotometry
Preparation of DPPH Solution

A DPPH stock solution was prepared by dissolving 10 mg of DPPH powder in methanol p.a in a 50 mL volumetric flask, followed by sonication for 5 minutes to ensure complete dissolution. This solution was then diluted to a 40-ppm working solution by taking 10 mL of a 200 ppm DPPH solution and diluting it with methanol p.a. to a final volume of 50 mL in a volumetric flask (Adrianta, 2020).

Determination of Operating Time

A 20-ppm methanol extract solution of simpur air leaves was pipetted and added to a 40 ppm DPPH solution in a ratio of 2:3 into a 5 ml volumetric flask wrapped with aluminum foil. The absorbance was recorded at a wavelength of 517 nm, with measurements taken every 5 minutes over 40 minutes until a stable absorbance value was reached (Arnanda & Nuwarda, 2019).

Determination of Maximum Wavelength (λmax)

The maximum wavelength was determined using a 40 ppm DPPH solution (Isnindar, Sri Luliana, 2025). The maximum wavelength scanning process was carried out in the 400-800 nm (Adrianta, 2020).

Free Radical Reduction Measurement

A 1000 ppm stock solution of methanol extract of simpur air leaves was prepared by dissolving 10 mg of extract in a volumetric flask and adding methanol to the mark. This stock solution was then used to prepare a 5, 10, 15, 20, and 25 ppm concentration series. For analysis, 2 mL of each concentration was reacted with 3 mL of 40 ppm DPPH and incubated. The absorbance of the resulting mixture was then measured at the maximum wavelength using a UV-Vis spectrophotometer. (Arnanda & Nuwarda, 2019).

Analisis Data

The results of antioxidant activity tests were analyzed using the SPSS version 26 program. This analysis determined whether there was a significant difference between maceration and MAE methods on the antioxidant activity of methanol extracts of simpur air leaves.

RESULTS AND DISCUSSION

Plant Determination

Plant determination aims to verify the authenticity of the plants used in the study by matching the morphological characteristics of the simpur air plant (Wang et al., 2018). The results confirmed the plants as *Dillenia suffruticosa* (Griff.) Martelli), a true sympatric species.

Preparation of Plant Material (Simplisia)

The simple air leaves were fresh, green, and not physically damaged. This ensured that the samples obtained had good quality, so the content of secondary metabolites could be maintained. The simple air leaves were then subjected to wet sorting to remove dirt or foreign objects such as pebbles or damaged parts of the leaves. After washing, the leaves were cut to reduce size and facilitate drying. The simpur air leaves were dried using a food dehydrator at 50 ° C until a dry simplisia was obtained. The dried leaves were then ground into powder form using a blender to facilitate the extraction process (Wang et al., 2018).

Preparation of Maceration and MAE Extracts

Simpur air leaves were extracted using methanol solvent through maceration and MAE methods. Methanol was chosen because it is a universal solvent capable of extracting polar and nonpolar compounds. Methanol also has a higher dielectric constant than ethanol solvents; the higher the dielectric constant, the more substances or flavonoids can be extracted. In addition, methanol evaporates quickly; therefore, it was selected for this study (Putri et al., 2023). In the maceration method, the simpur Air leaves were soaked for 3 x 24 hours to extract or isolate active compounds such as flavonoids (Handoyo, 2020).

The MAE method is selected at 100 watts for 10 minutes. These results are from research conducted by Shombing et al. (2022), which compared variations in extraction time of 5, 10, 15, and 20 minutes at 150 watts of power. The results showed that the highest yield was obtained at an extraction time of 10 minutes (Sihombing et al., 2022).

Testing Extract Yield

Yield refers to the proportion of the dry weight of the produced extract to the weight of the raw material used. Table 1 presents the yield test results for Simpur air leaf extract.

Table 1.
Yield Results of Simpur Air Leaf Extract

Extraction Method	Dry Powder	Extract Weight (g)	% Rendemen
Maceration	50	7,9	20
MAE	30	3,4	11,33

Source: Author's Analysis, 2025

Based on Table 1. The yield results of simpur air leaf extract obtained through maceration and MAE methods meet the requirements of good yield, which is not less than 10% (Indonesia, 2022). The amount of solvent used also affects the yield. Since maceration was carried out for 3x24 hours, more solvent was used than in MAE, which lasted only 10 minutes. The results showed that maceration produced higher yields than the MAE method. This indicates that more active components were extracted, which may also be due to the higher maceration water content than MAE, resulting in greater yields. Previous studies have stated that yield is highly dependent on water content. Loss of water molecules in dried material causes a decrease in yield (Wiratara & Ifadah, 2022).

The duration of extraction impacts the outcome, with extended extraction times leading to higher yields and enhancing the solvent's capacity to penetrate the raw material. (Fauziyah et al., 2020). MAE, a modern extraction method, uses microwave energy to quickly generate heat and high pressure on cell walls, causing them to break down quickly. However, some compounds may not completely dissolve in the solvent if the process is too rapid. In contrast, maceration released the compound gradually, providing sufficient time to achieve maximum solubility (Şeyma et al., 2024).

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Water Content Test

The water content of simpur air leaf extract obtained from maceration and MAE methods was 7.45 ± 0.284 and 5.23 ± 0.929, respectively, which met the good water content of <10. Water content parameters are classified into three categories: liquid extracts with water content usually greater than 30%, thick extracts with water content between 5–30%, and dry extracts with less than 5%. Therefore, maceration and MAE extracts are classified as thick (Liwar et al., 2022). The results of water content can be seen in Table 2.

Table 2.
Results of Determination of Water Content of Simpur Air Leaf Extract

Replication	Water Content	
	Maceration	MAE
1	7,76	4,16
2	7,39	5,84
3	7,2	5,69
Mean ± SD	7,45 ± 0,284	5,23± 0,929

Source: Author's Analysis, 2025

TLC Identification

Qualitative TLC tests were carried out to observe the profile of flavonoid compounds and antioxidants. This TLC analysis was a preliminary test using 10% AlCl3 and 200 ppm DPPH. The mobile phase was toluene: ethyl acetate: formic acid (3:5:1 v/v/v). The TLC profile of the simpur air leaf extract can be seen in Figure 1.

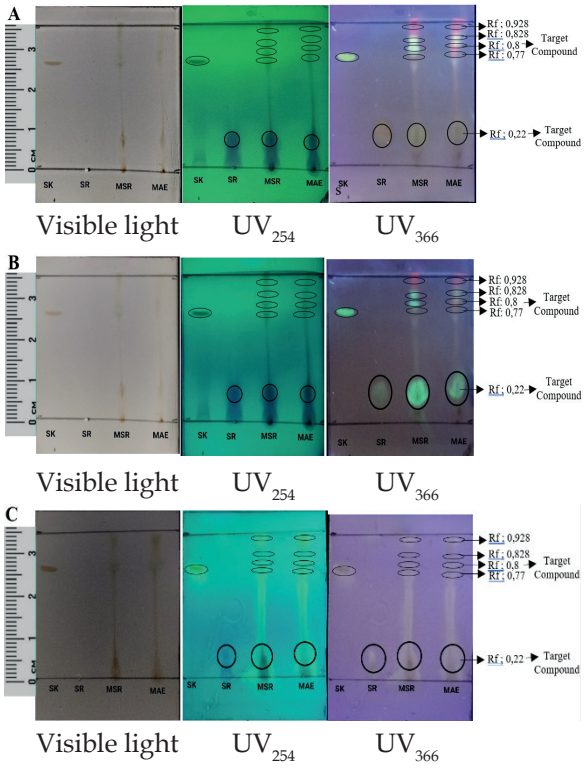

Figure 1.
Flavonoid test profile of simpur air leaf extract with silica gel 60 F_{254} as the stationary phase and toluene: ethyl acetate: formic acid (3:5:1 v/v/v) with $alcl_3$ spotting agent
Source: Author's Analysis, 2025

Where :
SK : Quercetin standard
SR : Rutin standard
MSR : Maceration
A : After elution
B : After adding $AlCl_3$
C : After adding DPPH

Previous studies reported that flavonoid compounds give a yellow color as a positive result (Hasanah et al., 2024). Both maceration and MAE samples showed five spots with Rf values of 0.22, 0.77, 0.8, 0.828, and 0.928, respectively. Spots with lower Rf values are more polar than those with higher values since the plate is polar, causing some compounds to move lowly while others remain.

The first observation was the routine standard. Extracts from maceration and MAE showed the same Rf value of 0.22 with a similar shape and pale-yellow color. These findings are consistent with Hadi & Subekti (2023), who reported rutin in kinca fern tuber extract with an Rf value of 0.24. Singh et al. (2016) also reported that *Dillenia indica* tested positive for rutin. Similar results were also obtained by the research of Kumar et al. (2020), which isolated the plant species *Dillenia pentagyna* and found that the flavonoids contained in the plant were rutin.

The second comparison involved quercetin. The spot from both maceration and MAE samples showed an Rf value of 0.80 with a bright yellow color, closely resembling the quercetin standard at Rf 0.77. When the difference in Rf value between the sample and the standard is less than 0.05, the sample is positive for the compound, the sample can be considered positive for the compound; if the difference is greater than 0.05, the sample is considered negative (Arifah et al., 2023).

Therefore, the simple air leaves from maceration and MAE extraction showed a similar shape and color, with Rf value with an Rf value difference of 0.03 compared to quercetin, which is <0.05, indicating that the samples positively contain the compound. Supported by previous research, which reported that several flavonoid derivatives, including flavonols such as quercetin, have

been identified in *Dillenia suffruticosa* (Sabandar et al., 2016). Based on the preliminary antioxidant test using a DPPH concentration of 200 ppm, it can be concluded that simpur air leaves from maceration and MAE are positive for antioxidants, as indicated by a purple TLC background and pale-yellow spots in Figure 1.

Antioxidant Activity Test

Determination of operating time is necessary to minimize measurement errors. Based on the data in Table 4, stable absorbance values were reached at 25 and 30 minutes. The researcher selected 30 minutes as the optimal time for the reaction between the sample and DPPH. It was also consistent with previous studies that reported 30 minutes as the standard operating time for determining antioxidant activity.

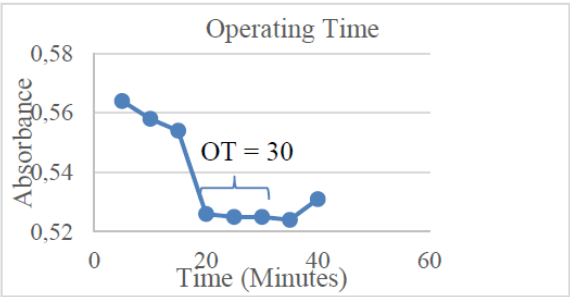

Figure 2.
Graph of Operating Time Determination
Source: Author's Analysis (2025)

The DPPH wavelength was determined to identify the optimal point for accurate and sensitive absorbance measurements. This was carried out using a UV-Vis spectrophotometer to scan a 40 ppm DPPH solution across the 400-800 nm range, and the maximum absorbance (1.034) was observed at 515 nm. This result is consistent with the literature, such as (Tri Saptari et al., 2019), which reports a maximum wavelength range of 515-520 nm. The results can be seen in Figure 3.

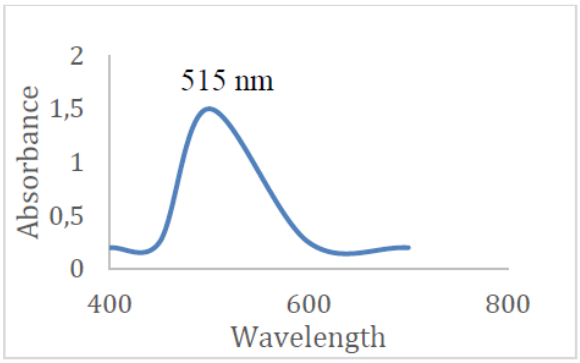

Figure 3.
Maximum wavelength
Source: Author's Analysis, 2025

The Determination of the IC_{50} value for the antioxidant activity test of simpur air leaf extract was carried out using concentrations of 5, 10, 15, 20, and 25 ppm. The results showed that lower absorbance values indicated stronger radical scavenging activity. This is because, at higher concentrations, the ability of antioxidants to capture free radicals increases, resulting in a lower concentration of DPPH and thus decreased absorbance values (Ika Fatmawati, 2023). The antioxidant activity of the methanol extract of simpur air leaves can be seen in Tables 3 and 4.

Table 3.
Results of IC50 Value of Maceration Method

Replication	Maceration	MAE	Category
1	10,579	5,552	Very strong
2	10,66	6,066	Very strong
3	10,297	6,209	Very strong

Source: Author's Analysis, 2025

The methanol extract of simpur air leaves demonstrated very strong antioxidant activity (<50), with IC_{50} values of 10.498 ± 0.213 µg/mL for maceration and 5.942 ± 0.345 µg/mL for the MAE method. These results indicate that the extract effectively inhibits free radicals, even at low concentrations. The IC_{50} values obtained for simpur air leaves fall into the very strong category supported by Utami et al. (2021).

Their study on the antioxidant activity of these leaves resulted in an IC_{50} value of 33.418

µg/ml, while the methanol extract of the roots showed an IC_{50} of 3.852, also classified as very strong. Based on TLC identification, it is suspected that the compounds responsible for the antioxidant activity in simpur air leaf extract are quercetin and rutin, which belong to the flavonoid group. The presence of OH groups in the structure of flavonoid compounds allows hydrogen donors to radical compounds. Flavonoids allow hydrogen donors to occur in free radical compounds. This event causes the radical compound to become more stable. Hence, the presence of OH groups in antioxidant compounds like flavonoids influences their antioxidant activity (Asih et al., 2022).

Method

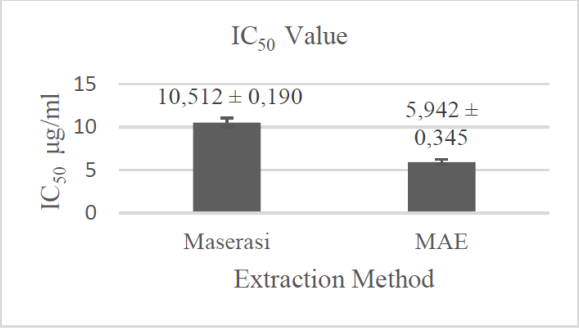

Figure 4.
IC_{50} Values of Maceration and MAE Methods
Source: Author's Analysis, 2025

Based on Figure 4 shows that the IC_{50} value of simpur air leaf extract using maceration and MAE extraction methods showed different antioxidant activity values. The IC_{50} result of maceration is 10.498 ± 0.213 µg/ml, while the MAE method is 5.942 ± 0.345 µg/ml, where the maceration method produces a higher IC_{50} value compared to the MAE method. This factor can be attributed to the advantages of the MAE method in terms of time efficiency during the extraction process, allowing the sample to be extracted more quickly without experiencing oxidation, which can affect antioxidant activity. The maceration method requires a longer extraction time, so the sample is at risk of oxidation, which can impact antioxidant activity (Zhang et al., 2018). These findings align with those of Wahyuni (2021), who reported that the MAE extraction method produces higher antioxidant activity than the maceration method.

The MAE extraction process utilizes heat from microwave radiation. The higher the intensity of electromagnetic waves in MAE, the hotter the resulting temperature. This causes an increase in pressure on the cell wall, which swells, allowing more active compounds to escape from the cell (Utami et al., 2020). This method requires more energy than maceration, resulting in higher antioxidant activity despite the lower yield compared to maceration.

The maceration extraction method yields a higher extract quantity; however, its antioxidant activity is lower than the MAE method. This occurs because the maceration method also has a higher water content than MAE, causing higher yields, more dilute extracts, and less effective in capturing free radicals. MAE produces lower yields but with smaller water content, so the extract is more concentrated and richer in active compounds. The amount of solvent used in maceration is greater, so the drying process of the extract takes longer than the MAE method. Antioxidant activity results are also affected by drying.

The IC_{50} value obtained is then converted into the AAI value, which determines the antioxidant activity index by comparing the DPPH concentration used in the test (ppm) with the IC_{50} value obtained (ppm) from each extract. The AAI value is used to classify antioxidant properties. (Sawiji & La, 2021) The results of the antioxidant activity test of simpur air leaves obtained from maceration and MAE results can suppress free radicals at a very strong level (> 2), specifically (AAI = 3.810) for the maceration method and (AAI = 6.731) for the MAE method. The AAI value of the simpur air leaf extract can be seen in Table 5.

After the IC_{50} value was determined for each extraction method, the data obtained

were analyzed using an independent samples t-test with the help of SPSS. The analysis results showed a significant difference between maceration and MAE methods, with a Sig (2-tailed) value of 0.000 indicating significance ($p < 0.05$). The significant difference in antioxidant activity between the two extraction methods is due to differences in the extraction process, especially in terms of time and temperature, which affect the compounds produced. With the high antioxidant activity in the methanol extract of Simpur Air leaves, this extract has the potential to be developed as an alternative natural antioxidant ingredient.

Table 5.
AAI values of (*Dillenia suffruticosa*) Leaf Extracts

Method	IC_{50}	AAI	Antioxidant Strength
Maceration	10,498	3,810	Very Strong
MAE	5,942	6,731	Very Strong

Source: Author's Analysis, 2025

A Pearson correlation test was conducted using SPSS software to determine the relationship between water content and IC_{50} value. The results showed that the Pearson correlation coefficient was 0.928 with a significance value (2-tailed) of 0.008, smaller than 0.05. This indicates a strong positive correlation between water content and antioxidant activity. In other words, as water content increases, the IC_{50} value also tends to increase, and vice versa. This is due to the results obtained, where the water content in MAE is lower than maceration, so the IC_{50} value in MAE is also lower.

CONCLUSION

Methanol extract of simpur leaves with the MAE method produces better antioxidant activity. This is evidenced by the IC_{50} value of 5.942 ± 0.345 µg/ml. Using the water simpur leaf extraction method with the maceration method produced an IC_{50} of 10.498 ± 0.213 µg/ml. The analysis with an Independent Sample T-Test indicated significant differences between the two extraction methods, with a p-value below 0.05. Based on the findings of this study, it is recommended to analyze the total flavonoid and phenol content and isolate and identify active compounds in simpur air leaf extract using KLT-Densitometry, FT-IR, and LC-MS methods.

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