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Effect of Solvent Polarity on Antioxidant and Antidiabetic Activity of Kesambi Leaf Extract (*Schleichera oleosa* L.)

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Abstract: Kesambi (*Schleichera Oleosa* (L.) is one of the beneficial plants in Indonesia which contains fenolic and flavonoids that exhibit antioxidant properties. The presence of antioxidants in plants could be associated with antidiabetic activity of these plants. This research was conducted to determine the antioxidant and antidiabetic activities of n-hexane, ethyl acetate, acetone and methanol extracts from Kesambi leaves. Extraction was carried out by the *ultrasonic assisted extraction* (UAE) method. The antioxidant activity was determined quantitatively by DPPH and ABTS method, while the antidiabetic activity was evaluated by using α-glukosidase inhibition method. The IC_{50} values from the determination of antioxidant and antidiabetic activities were obtained by using Microplate reader at the maximum wavelength. Results of the antioxidant determination DPPH method showed that IC_{50} values of n-hexane, ethyl acetate, acetone and methanol extracts were 414.1993; 57.7862; 6.33 dan 13.4558 µg/mL, respectively. And for ABTS method showed 162.679; 22.6684; 10.7975; dan 146.869 µg/mL. This indicates the acetone and methanol extracts give the best results in antioxidant activity. Meanwhile, IC_{50} values of in vitro antidiabetic activity of n-hexane, ethyl acetate, acetone and methanol extracts were 99.3235; 272.8723; 1.6799; dan 24.4653 µg/mL, respectively. Acetone and methanol extracts of kesambi leaf give the best results in antidiabetic activity.

Keywords: Antioxidant; Antidiabetic; Kesambi, α-glukosidase.

Introduction

Kesambi (*Schleichera Oleosa* (L.) is a tropical forest tree plant from the Sapindaceae family that is spread across South and Southeast Asia (Cambodia, India, Indonesia, Myanmar, Sri Lanka, Thailand, and Vietnam). The kesambi plant is also known by the regional names: kesambi (Sunda); kesambi, kusambi, sambi (Java and Bali); kesambhi (Madura); kusambi, usapi (East Timor); kahembi (Sumba); kehabe (Sawu); kabahi (Solor); kalabai (Alor); kule, ule (Rote); bado (Makassar); ading (Bugis) (Holil and Griana, 2020). Kesambi leaves contain secondary metabolites in alkaloids, phenolics, tannins, and flavonoids. Phenolics and flavonoids are the most abundant components in kesambi leaves (Holil and Griana, 2020). Kesambi leaves (Schleicheraoleosa) are known to contain flavonoid compounds and Phenolic. The highest phenolic content was obtained in acetone extract, and the highest flavonoid content in ethyl acetate extract (Nursamsiar et al., 2023). The high phenolic and flavonoid content in plants plays a role in providing antioxidant activity.

Antioxidant activity in plants can be associated with the plant's antidiabetic activity. Antioxidants can reduce oxidative stress and prevent diabetes mellitus and complications in diabetics (Wulandari, Nugraha, & Azhari, 2020). Plants that contain phenol as an antioxidant can protect pancreatic beta cells from the

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toxic effects of free radicals produced during chronic hyperglycemia so that insulin levels can be maintained and blood glucose levels remain normal (Dwi Wisudanti, 2016).

Several studies on antioxidants from kesambi plants have been reported. Research on antioxidants of various parts of the kesambi plant showed that ethyl acetate extract of kesambi leaves had better antioxidant activity (IC_{50} 5.4952 µg/mL) compared to bark (IC_{50} 14.3621 µg/mL), roots (43.4159 µg/mL), fruit skin (IC_{50} 159.8782 µg/mL) and fruit flesh (IC_{50} 167.2912 µg/mL).This study was conducted to determine the antioxidant and antidiabetic activities of kesambi leaf extract. The DPPH and ABTS methods were used to decide antioxidant activity. In contrast, the antidiabetic activity test was carried out in vitro using the α-glucosidase enzyme inhibition method with acarbose as a comparator.

Method

Materials

The plant materials used in this study were kesambi leaves (*Schleichera oleosa* L.) taken from Gowa district, South Sulawesi. The chemicals used included methanol, ethyl acetate, acetone, n-hexane, Ethanol p.a (Merck, Germany), distilled water (waterone), Na_2CO_3, (Merck, Germany), Phosphate Buffer (Merck, Germany), PNPG Subtract (Merck, Germany), α-glucosidase enzyme (Merck, Germany), DPPH (1,1-Diphenyl-2-Picryl hydrazil) (Merck, Germany), ABTS (2,2'-azino-bis-[3-ethylbenzothiazoline sulfonate]) (Merck, Germany), Potassium Persulfate ($K_2S_2O_8$) (Merck, Germany).

Sample preparation

Plant samples were obtained from Sicini Village, Siriya Hamlet, Parigi District, Gowa Regency, South Sulawesi Province. Species identification of Kesambi plants was carried out at the Biology Laboratory of Makassar State University with specimen number 051/SKAP/2018. The samples were cleaned by washing them with running water so that dirt attached to them could be removed, and then wet sorting was carried out. The samples were then chopped to reduce the size and dried in a simplicia oven at a temperature of 40 ^oC for 2 x 24 hours (Nursamsiar et al., 2023).

Extraction

Dried kesambi leaf (375 kg) was extracted using various solvents (n-hexane, ethyl acetate, acetone, and methanol) by ultrasonic-assisted extraction (UAE) method. Simplex was put into the extractor container, and then n-hexane solvent was added until all parts of the simplex were submerged. Extraction was repeated until a clear filtrate was obtained. The residue was then extracted further using ethyl acetate, acetone, and methanol solvents using the same method as n-hexane. Each filtrate obtained was concentrated using a rotary evaporator vacuum so that hexane, ethyl acetate, acetone, and methanol extracts would be obtained. The yield obtained was calculated using the following formula (Nursamsiar et al., 2023):

yield = extract weight/dried kesambi leaf x 100%

Antioxidant activity assay by DPPH scavenging

The stock solution of each Kesambi leaf extract (EH = n-hexane extract, EE - Ethyl Acetate Extract, EA = Acetone Extract, and EM = Methanol Extract) with a concentration of 1000 µg/mL was made into a series of concentrations of 1 -1000 µg/mL by inserting a certain volume into a 5 mL flask, then adding 1 mL of DPPH reagent solution (0.4 mM) and making the volume sufficient with ethanol p.a. to 5 ml. Absorbance was measured at the maximum wavelength after being stored at room temperature for 30 minutes. The inhibitory effect was determined based on the percentage of the color change of the DPPH solution fading because the sample was compared to the blank solution (control). The IC_{50} value describes the concentration of the sample solution required to reduce DPPH by 50%, which was obtained from the results of the curve regression between the concentration of the sample solution and the percentage of inhibition. The formula obtained the level of inhibition [$(A_{blank} - A_{sample})/A_{blank} \times 100\%$] (S. Nur et al., 2023).

Antioxidant activity assay by ABTS scavenging

ABTS radicals were prepared by reacting ABTS solution (28 mg ABTS in 10 mL water) with potassium persulfate (15 mg in 10 mL distilled water) in a dark room for 12 hours. The ABTS radical solution was made up to 50 mL with pro-analysis ethanol. Kesambi leaf extract stock solution samples were prepared at various concentrations (1-1000 µg/mL) by taking a certain sample volume and adding one mL of ABTS radical solution. The mixture was made up to 5 mL with pro-analysis ethanol. Then, the mixture was homogenized and incubated in a dark room for 30 minutes, and its absorbance was measured at 752nm. ABTS solution (1 mL ABTS solution into 5 mL distilled water) was used as a blank, and quercetin was used as a positive control. The formula calculated the inhibitory effect [$(A_{blank} - A_{sample})/A_{blank}$ X 100%] (S. Nur et al., 2023).

Optimization of enzyme concentration

30 µL of α-glucosidase enzyme with a concentration of 0.1, 0.075; 0.05 U/mL was inserted into a 96 well plate, then 100 µL was added for fat pH 6.8. The mixture was

incubated for 10 minutes at 37 °C. The mixture was then added with 30 µL of PNPG substrate (5 µM) and then incubated for 10 minutes at 37 °C. After the incubation process was complete, 90 µL of 0.2 M Na_2CO_3 solution was added, and the absorbance was measured using a microplate reader at a wavelength of 405 nm (Syamsu Nur et al., 2021).

Testing of a-glucosidase enzyme inhibitory activity

Into the well plate 96, 130 µL of phosphate buffer solution pH 6.8 (Blank Control) was added, 100 µL of phosphate buffer solution pH 6.8 was added 30 µL of α-glucosidase enzyme 0.075 U/mL (Control), 100 µL of sample and 30 µL of phosphate buffer 6.8 (Blank Sample), 100 µL of sample and 30 µL of α-glucosidase enzyme 0.075 U/mL (Sample). Each mixture was then incubated at 37 °C for 10 minutes. Each mixture was added 30 µL of PNPG substrate (5 µM), then incubated again for 10 minutes at 37 °C. After the incubation process, 90 µL of 0.2 M Na_2CO_3 solution is added, and the absorbance is measured using the microplate reader at a wavelength of 405 nm. (Nursamsiar et al., 2022).

Results and Discussion

Kesambi (*Schleichera oleosa* (Lour) has been reported to have several biological effects such as antiulcer, anticancer, and antimicrobial, and is traditionally used as an antidiabetic (Goswami & Singh, 2019). Research conducted (Nursamsiar et al, 2024) reported that Kesambi leaves have better antioxidant activity than other parts of the Kesambi plant, such as bark, fruit skin, roots, and fruit flesh. The antioxidant activity of kesambi leaves is relatively high, with an IC_{50} of 16.1 ppm. The high antioxidant content in kesambi leaves has led to the development of kesambi leaf extract into a standardized herbal with potential antidiabetic effects (Hartono, Abdul Karim, Nur, Zahra, & Amir, 2021). Kesambi leaves contain secondary metabolites in phenolics, tannins, alkaloids, and flavonoids. The most significant components in kesambi leaves are flavonoids and phenolics. As evidenced by the analysis results using the thin layer chromatography (TLC) method, quercetin was obtained as one of the flavonoid components in kesambi leaves (Holil & Griana, 2020).

In the study, sample processing was carried out through four stages: wet sorting, washing, chopping, and drying. Wet sorting is the stage at which samples are separated from dirt (Kemenetrian Kesehatan Republik Indonesia, 2017). The washing stage uses running water to clean dirt that sticks to the sample. Meanwhile, the chopping stage is carried out to reduce the size of the sample so that the drying process is faster and makes it easier for the compound content in the sample to dissolve into the solvent liquid (Nursamsiar, Khairuddin, Marwati, & Jumarni, 2021). The next stage is drying, which is carried out using a simplicia oven at 40 ° C for 2 x 24 hours. This drying process is carried out to reduce the sample's water content so that damage and quality degradation of the sample can be eliminated even if stored for a long time (Fahmi, Herdiana, & Rubiyanti, 2020).

The extraction process was carried out using the ultrasonic-assisted extraction (UAE) method using solvents with different levels of polarity starting from nonpolar to polar solvents, namely n-hexane, ethyl acetate, acetone, and methanol. Ultrasonic vibrations can produce cavity bubbles that break plant cells so that solvents can enter the cells and dissolve cell components (Şahin & Şamli, 2013). Ultrasonic-assisted extraction is also environmentally friendly, consumes less energy and time, and produces higher phenolic compound yields than conventional methods such as maceration, reflux, and soxhlet (Oroian, Ursachi, & Dranca, 2020). This method can speed up the extraction process, is easy, safe, and efficient (Bimakr et al., 2016).

Based on research conducted by Nursamsiar et al. (2023), it was reported that the yield obtained from the Kesambi leaf extraction process was <10%, with the most significant yield obtained in acetone solvent of 2.52% (table 1).

Tabel 1. Percentage of extraction yield (Nursamsiar et al., 2023)

Solvent	Weight (g)		% Yield
	sample	Extract	
n-hexane	375	4.308	1.15
Ethyl Acetate	375	3.588	0.96
Acetone	375	9.468	2.52
Methanol	375	0.651	0.17

Antioxidant activity

DPPH is a free radical containing unstable nitrogen ions whose absorbance can be measured at a wavelength of 515-520 nm. DPPH radicals that react with antioxidant compounds from the sample will experience a reduction, indicated by a color change from purple to yellow (Molyneux, 2004). Based on the results of the tests carried out, it was obtained that the kesambi leaf extract could reduce DPPH with IC_{50} values (µg/mL) respectively for EH, EE, EA, and EM of 414.1993; 57.7862; 6.33; 13.4558. These results show that the EA of Kesambi leaves has better inhibitory activity against DPPH compared to EM, EE, and EH. The phenolic content found in plants can donate protons to free radicals. Based on research (Nursamsiar et al., 2023), it was reported that the acetone extract of Kesambi leaves has a higher phenolic content compared to n-hexane, ethyl acetate, and methanol extracts. This shows that their

phenolic content influences the antioxidant power of Kesambi leaf samples.

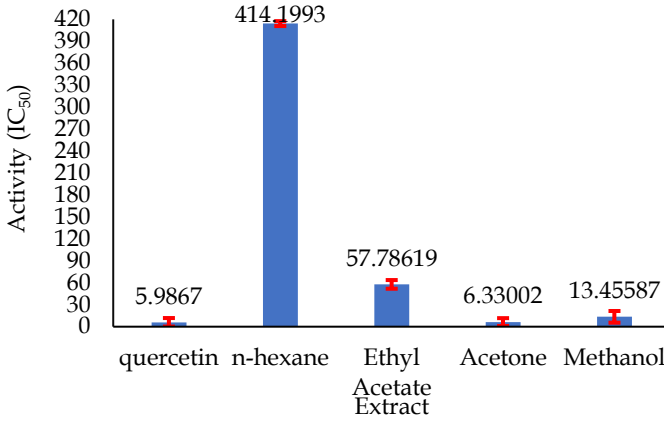

Figure 1. Graph of antioxidant activity of kesambi leaf extract using the DPPH method

The principle of antioxidant testing with ABTS radicals is based on the removal of ABTS cation color after reacting with antioxidant compounds from the sample and compared with ABTS cation radicals that do not respond to antioxidant compounds (Mistriyani et al., 2018 & (Ilyasov et al., 2004). ABTS is oxidized to ABTS cation radicals, and oxidants form ABTS cation radicals. In this study, ABTS cation radicals were obtained from the oxidation of ABTS by $K_2S_2O_8$, then incubated for 12 hours in the dark (Re et al., 1999 & Mistriyani et al., 2018)

Based on the test results, it was obtained that the kesambi leaf extract also had the most potent inhibition of ABTS oxidants. Each sample's antioxidant activity (IC_{50} (µg/mL)), respectively, for EH, EE, EA, and EM, was 162.679, 22.6684, 10.7975, and 146.869. EA kesambi leaves had better antioxidant activity. EA and EE had extreme antioxidant activity. In quantitative testing, a compound is said to have extreme antioxidant power if the IC_{50} value is less than 50 ppm, strong (50-100 ppm), moderate (100-150 ppm), and weak (150-200 ppm) (Molyneux, 2004).

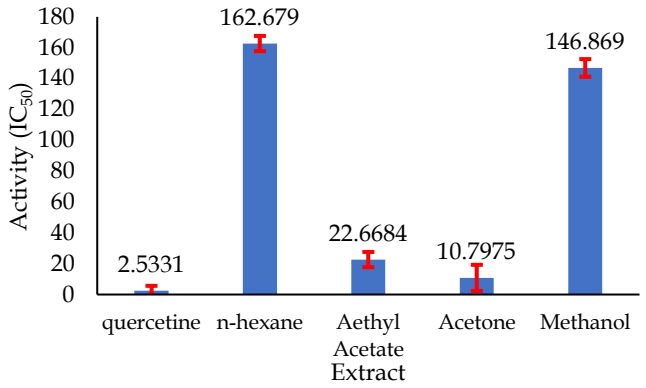

Figure 2. Graph of antioxidant activity of Kesambi leaf extract using the ABTS method.

Antidiabetic Activity

Table 4. Optimization of α-glukosidase enzyme concentration

Concentration of enzyme	Absorbance of Enzyme (K)	Blank Absorbance (BK)	K-BK
0.1 U/mL	1.2370	0.0998	1.1372
0.075 U/mL	0.9178	0.0998	0.8179
0.05 U/mL	0.5654	0.0998	0.4656

Optimization of enzyme concentration was carried out before the α-glucosidase enzyme inhibition test to determine the maximum enzyme concentration that can be used to obtain maximum results. In the optimization of enzyme concentration, it was found that the best concentration of α-glucosidase enzyme that can be used in the test is 0.075 U/mL, which at that concentration gave the best absorption value of 0.8179. The results of enzyme concentration optimization are relevant to the research of (Nur et al., 2021).

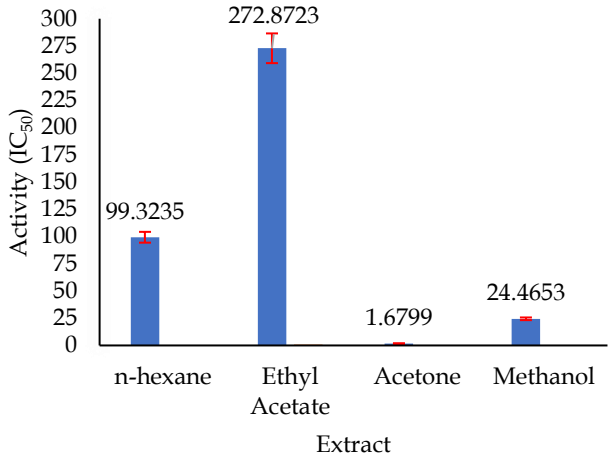

Figure 3. Graph of the inhibitory activity of Kesambi leaf extract against the α-glucosidase enzyme

In the α-glucosidase enzyme inhibition test, the results showed that EA had the best activity compared to EM, EH, and EE with IC_{50} values (µg/mL) of 1.6799, 24.4653, 99.3235, and 272.8723 respectively. Based on these results, it can be seen that EA and EM have better inhibitory power compared to EE and EH. An inhibitor's smaller IC_{50} value indicates the more remarkable ability to inhibit the α-glucosidase enzyme. The difference between the IC_{50} values of acetone and methanol extracts and the IC_{50} values of ethyl acetate and n-hexane extracts is because the compound that acts as an antidiabetic is more easily dissolved in polar solvents such as acetone and methanol.

Based on the results of phytochemical screening, the compounds found in the acetone and methanol extracts are phenolics, flavonoids, and tannins. This

group of compounds is thought to play a role in providing antidiabetic activity. The extract can inhibit the α-glucosidase enzyme due to the presence of flavonoids and phenolic compounds (Moein et al., 2017). Research conducted (Nursamsiar et al., 2023) shows that the acetone extract of kesambi leaves has the highest phenolic content compared to other extracts, so it is predicted that the antidiabetic activity of kesambi leaves is influenced by its phenolic content.

Conclusion

A The antioxidant activity of Kesambil leaf extract using the DPPH and ABTS methods showed that the acetone extract had the best activity with an IC_{50} value of 6.33 µg/mL and 10.7975 µg/mL. Meanwhile, the antidiabetic activity based on the inhibitory power against the α-glucosidase enzyme also showed that the acetone extract had better activity with an IC_{50} value of 1.6799 µg/mL. This shows that there is a correlation between the antioxidant and antidiabetic effects of Kesambi leaf samples.

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Author Contributions
All authors contributed to writing this article.

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Conflicts of Interest
The authors declare no conflicts of interest.

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