Effect of solvent polarity on secondary metabolite content and α-glucosidase enzyme IC\textsubscript{50} of \textit{Dendrophthoe pentandra} (L). Miq leaves extract

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ABSTRACT: Cherry mistletoe (\textit{Dendrophthoe pentandra} (L.) Miq) have an inhibitory effect on the α-glucosidase enzyme, but research related to solvent optimization to find active compound is unclear. Secondary metabolites that can be identified depend on the level of solvent polarity. This study aims to determine the effect of solvent polarity on the secondary metabolite content and the α-glucosidase enzyme by using the polar and nonpolar fractions of the ethyl acetate extract of cherry mistletoe leaves. Cherry mistletoe leaves were extracted using ethyl acetate and were followed by a liquid-liquid fractionation. The polar fraction used ethanol and ethyl acetate as solvents, whereas the nonpolar fraction used n-hexane and ethyl acetate as solvents. Secondary metabolites present in polar fractions were alkaloids, flavonoids, steroids, tannins, and terpenoids, whereas those present in nonpolar fractions were flavonoids and steroids. The IC\textsubscript{50} value of the polar fraction (54.8 ppm) was lower than that of the nonpolar fraction (192.0 ppm). The polar fraction of the ethyl acetate extract of cherry mistletoe leaves could inhibit the α-glucosidase enzyme and, therefore, is classified as active. On the other hand, the nonpolar fraction of the ethyl acetate extract of cherry mistletoe leaves could not inhibit the α-glucosidase enzyme and, therefore, is classified as inactive in general.

KEYWORDS: α-glucosidase; \textit{Dendrophthoe pentandra} (L.) Miq; IC\textsubscript{50}; polarity; secondary metabolites.

INTRODUCTION

The anti-diabetic potential of herbal plants is currently being studied more and more, considering the emergence of the issue of going back to nature. The leaves of cherry mistletoe (\textit{Dendrophthoe pentandra} (L.) Miq) have been shown to have benefits as an antidiabetic, with one of the mechanisms being able to inhibit the α-glucosidase enzyme. The α-glucosidase enzyme works by catalyzing the hydrolysis reaction of disaccharides into monosaccharides. Cherry mistletoe leaves contain flavonoids, alkaloids, saponins, terpenoids, tannins, and steroids, which have the ability to inhibit the α-glucosidase enzyme. These compounds have different polarity levels, so the polarity level of the solvent used in the fractionation process affects the content of secondary metabolites that can be extracted [1],[2].

The ethyl acetate solvent was chosen because it is a semipolar solvent, so it is expected to be able to extract polar and nonpolar compounds. Based on their polarity, flavonoids have hydroxyl or sugar groups, so they tend to dissolve in polar solvents. The active compounds of alkaloids, tannins, and saponins are polar, while steroid and terpenoid compounds tend to dissolve in nonpolar solvents [3].

However, in several previous studies, alkaloids, saponins, and tannins were found in ethyl acetate extract. This is because there is a resonance in the benzene ring, which reduces the polarity of the compound so that it can be extracted by semipolar solvents. Terpenoids and steroids are compounds that tend to be nonpolar. Several types of terpenoid and steroid compounds have hydroxyl groups so that they can form hydrogen bonds with ethyl acetate. The compound can be extracted by ethyl acetate [4].

The ethyl acetate fraction of the ethanol extract was able to inhibit the α-glucosidase enzyme [5]. Another study using the n-hexane fraction of the ethanol extract conducted by Sinulingga, et al. showed that the n-
hexane fraction of the ethanol extract was not active in inhibiting the α-glucosidase enzyme [6]. This study was conducted to determine the effect of solvent polarity on secondary metabolite content and the inhibitory effect of α-glucosidase enzymes on polar and nonpolar fractions of ethyl acetate extract of cherry mistletoe leaves.

**MATERIALS AND METHODS**

**Materials**

The materials needed in this study included cherry mistletoe leaves, ethyl acetate solvent, ethanol solvent, and n-hexane solvent. The materials for the identification of secondary metabolites were HCl solution (Mercks, German), Mayer's reagent (Mercks, German), HgCl$_2$ (Mercks, German), KI (Mercks, German), Dragendorff's reagent, Wagner's reagent, bismuth (III) nitrate (Mercks, German), nitric acid (Mercks, German), FeCl$_3$ (Mercks, German), distilled water (OneMed, Indonesia), magnesium powder (Mercks, German), and concentrated sulfuric acid (Mercks, German). Sodium hydroxide (Mercks, German), potassium dihydrogen phosphate (Sigma, Singapore), bovine serum albumin (Sigma, Singapore), α-glucosidase enzymes (Sigma, Singapore), p-nitrophenyl-α-D-glucopyranose (pNPG) substrate (Sigma, Singapore), acarbose (Kimia Farma, Indonesia), dimethyl sulfoxide solution (DMSO) (Mercks, German), and sodium carbonate (Mercks, German) were used in the α-glucosidase inhibition test.

The equipment used for this study included a blender (Philips, Indonesia), simplicia container, probe, maceration container, macerator, stir bar, micropipette (BioRad, USA), oven (Memmert, German), measuring flask, measuring cup, spoon, separating funnel, cuvette (Shimadzu, Japan), filter paper, mask, rubber gloves, cameras (Samsung, Indonesia), stationery, and UV-Vis spectrophotometers (Shimadzu, Japan).

**Extraction and fractionation**

The simplicia of cherry mistletoe leaves was weighed 3 kg (dry powder) and then macerated using ethyl acetate solvent for 2x24 hours. Solvent replacement was carried out three times every 48 hours. The ethyl acetate viscous extract obtained will be divided into 10 different tubes, and then the liquid-liquid fractionation process will be carried out. For the polar fractions (F1-F5), the liquid-liquid fractionation used a mixture of ethyl acetate and ethanol solvents with a ratio of 9:1 (F1), 7:3 (F2), 5:5 (F3), 3:7 (F4), and 1:9 (F5). For nonpolar fractions (F6-F10), liquid-liquid fractionation uses a mixture of ethyl acetate and n-hexane with the respective ratios of 1:9 (F6), 3:7 (F7), 5:5 (F8), 7:3 (F9), and 9:1 (F10) [3], [4]. Fractionation uses a ratio of extract: solvent mixture of 1:2. The weight of the extract is 10 mg, and the solvent mixture is 20 ml. The range of solvent polarities used ranges from polar (ethanol), semipolar (ethyl acetate) to nonpolar (n-hexane). The dielectric constant of ethanol is 30, ethyl acetate is 6, and n-hexane is 1.89. The higher the dielectric constant value, the more polar the solvent. In this study, the dielectric constant range was 2.3 (F10) - 27.6 (F1) [3],[4].

**Secondary metabolites identification**

Identification of flavonoids was carried out by dissolving 2 ml of the fraction in methanol. A total of 0.5 mg of magnesium powder and 5 drops of concentrated HCl were added and then heated. The presence of flavonoids is indicated by the formation of red-orange to purple-red color [7]. Identification of alkaloids was carried out by dissolving 3 ml of the fraction in 2% HCl in 3 test tubes. In the first tube, 3 drops of Mayer's solution were added. A positive test result is indicated by the presence of a white or yellow precipitate. In the second tube, 2-3 drops of Dragendorff reagent are added. A positive test result is indicated by the presence of a brick red, red or orange precipitate. In the third tube, 3 drops of Wagner's reagent were added. A positive result if there is a brown precipitate [8],[9].

Identification of tannins was carried out by reacting 1 ml of the fraction with 2 ml of 1% FeCl$_3$. The blue-black solution indicates the fraction contains tannins[8]. Saponins were identified by dissolving 10 ml of hot water into 3 ml of the fraction. The solution is cooled for a few minutes at room temperature, and then shaken for 10 seconds. When froth appears, measurements are taken. The fraction contains saponins if the froth size is 1-10 cm[10]. Identification of terpenoids and steroids was carried out by dissolving 1 ml of the fraction in 0.5 ml of chloroform and then adding 0.5 ml of anhydrous acetic acid. Furthermore, the result of this solution is added with 1-2 ml of concentrated H$_2$SO$_4$ through the tube wall. The fraction contains terpenoids if there is a brownish or violet ring at the boundary of the two solvents. The fraction contains steroids if there is a bluish-green color at the border of the two solvents [11].
α-Glucosidase enzyme inhibition test

The polar and nonpolar fractions of the ethyl acetate extract of cherry mistletoe leaves were diluted into four serial concentrations, namely 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm. Acarbose, as a positive control, was also diluted into four serial concentrations. The α-glucosidase enzyme inhibition test procedure was carried out on blanks and samples, as shown in Table 1 [12].

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B₀</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>10</td>
</tr>
<tr>
<td>Phosphate buffer (pH 6.8)</td>
<td>490</td>
</tr>
<tr>
<td>p-NPG 10 mM</td>
<td>250</td>
</tr>
</tbody>
</table>

Incubate at 37 °C for 5 minutes

| Enzyme 0.15 U/ML             |  - | 250|  -  | 250 |
| Na₂CO₃                       | 1000| - | 1000| -   |

Incubate at 37 °C for 15 minutes

| Enzyme 0.15 U/ML             | 250| -  | 250 | -   |
| Na₂CO₃                       |  - | 1000| -   | 1000|

Absorbance measurement at λ 400 nm

The absorbance (Abs) value obtained is used to calculate the percentage of inhibition of the α-glucosidase enzyme with the following formula:

\[
\text{Inhibition} = \left( \frac{\text{Blank Abs} - \text{Sample Abs}}{\text{Blank Abs}} \right) \times 100\%
\]

The results of the inhibition percentage are used to measure the IC₅₀ value. The IC₅₀ value was obtained through a linear regression test with the equation \( y = a + bx \). The letter \( y \) indicates the absorbance, the letter \( x \) indicates the concentration, the letter \( a \) indicates the constant, and the letter \( b \) indicates the slope. The IC₅₀ value can be calculated using the following formula[12]:

\[
\text{IC}_{50} = \frac{50-a}{b}
\]

**RESULTS**

The weight of the ethyl acetate extract obtained was 127 grams, with an extract yield value of 3.2%. The yield values of the polar fraction of the ethyl acetate extract of cherry mistletoe leaves were F1 45% (4.5 g), F2 57% (5.7 g), F3 64% (6.4 g), F4 65% (6.5 g), and F5 64% (6.4 g) respectively. The highest yield value was obtained in F4, namely 65%, while the lowest yield value was obtained in F1, namely 45%. The yield values of the nonpolar fraction of the ethyl acetate extract of cherry mistletoe leaves were F6 60% (6 g), F7 51% (5.1 g), F8 50.2% (5.0 g), F9 46% (4.6 g), and F10 40% (4 g), respectively. The highest yield value was found in F6, namely 60%, while the lowest yield value was found in F10, namely 40%. The percentage yield value of the extract is obtained by dividing the weight of the extract by the initial weight of the simplicia, while the percentage yield value of the fraction is obtained by dividing the weight of the fraction by the initial weight of the extract [4], [5].

In fractions F1-F5, all the same secondary metabolites were identified, namely alkaloids, flavonoids, steroids, saponins and tannins. In the F6-F10 fraction, the same secondary metabolites, namely flavonoids and steroids, were also identified (Table 2). The secondary metabolites identified in the polar fraction of the ethyl acetate extract of cherry mistletoe leaves were alkaloids, flavonoids, steroids, saponins, and tannins, while in the nonpolar fraction of the ethyl acetate extract of cherry mistletoe leaves were flavonoids and steroid compounds. In this study, no further tests were carried out to identify the type of each secondary metabolite. Thus, the suspicion that secondary metabolites have the ability to inhibit the α-glucosidase enzyme is based
on previous studies that secondary metabolites such as flavonoids, alkaloids, saponins, tannins and steroids in several plants can inhibit the α-glucosidase enzyme [13].

Table 2. Secondary metabolite identification results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Terpenoid</th>
<th>Steroid</th>
<th>Saponin</th>
<th>Tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 Fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F2 Fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F3 Fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F4 Fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F5 Fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F6 Fraction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F7 Fraction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F8 Fraction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F9 Fraction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F10 Fraction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>

The IC\textsubscript{50} values for each fraction F1-F10 were, respectively, 61.1 ppm, 56.3 ppm, 45.2 ppm, 58.4 ppm, 53.2 ppm, 49.2 ppm, 104.2 ppm, 194.1 ppm, 206.6 ppm and 406.0 ppm. A comparison of the IC\textsubscript{50} values of the polar fraction, nonpolar fraction, and acarbose is shown in Figure 1.

Figure 1. IC\textsubscript{50} values of polar and nonpolar fractions of ethyl acetate extract of cherry mistletoe leaves.

**DISCUSSION**

The results showed a higher yield value when using a higher ratio of ethyl acetate solvent. The dielectric constants of the solvent mixture are F1 (27.6), F2 (22.8), F3 (18), F4 (10.4), F5 (8.4), F6 (5.6), F7 (4.8), F8 (4.0), F9 (3.1), and F10 (2.3). The higher the value of the dielectric constant, the more polar the solvent. The polarity of the solvent will affect the secondary metabolites, thereby affecting the ability to inhibit the α-glucosidase enzyme. It is suspected that the secondary metabolites that can be withdrawn are at the level of semipolar polarity [3],[4].

The content of active compounds plays a very important role in the mechanism of inhibition of the α-glucosidase enzyme[13]. Identification of positive alkaloids in the polar fraction because alkaloids are polar compounds, so they can be dissolved by a mixture of ethanol and ethyl acetate solvents [13]. Flavonoids are a type of natural compound found in various types of food, such as fruits, vegetables, tea and wine which have many benefits for various nutraceutical, pharmaceutical, medicinal and cosmetic applications [13]. Flavonoids are compounds with sugar groups so they are at the level of polar polarity and can be dissolved by ethyl acetate and ethanol. However, there are several types of flavonoids that tend to be nonpolar, such as flavonones, iso flavonones, alcohols, and flavonols. It is suspected that one of these compounds is contained in the nonpolar fraction of the ethyl acetate extract of cherry mistletoe leaves [13]. Tannins are a group of phenols that are polar, so they can be dissolved in ethanol and ethyl acetate. The isoprene structure in steroids makes it tend...
to be nonpolar, so it can be extracted by ethyl acetate and n-hexane [3],[4]. However, some steroid compounds have hydroxyl groups, which are generally called sterols. The hydroxyl group can form hydrogen bonds, so it tends to be more polar. These compounds can be extracted by ethyl acetate solvent [14]. Saponins consist of sugar and aglycone groups, so they can be dissolved by ethanol and ethyl acetate. The froth formed has different heights, indicating the estimated amount of saponins in each fraction semi-qualitatively [15].

The α-glucosidase enzyme is found in the small intestine, which has a mechanism of action catalyzing the hydrolysis of starch into glucose molecules. Inhibition of this enzyme can reduce postprandial blood sugar levels. Acarbose is one of the diabetes drugs that has been standardized with the mechanism of action of α-glucosidase enzyme inhibition. The positive control of acarbose has an IC50 value of 36.4 ppm and is classified as active, indicating that the method chosen is appropriate [3],[12].

All polar fractions are capable of inhibiting the α-glucosidase enzyme and are classified as active (50-100 ppm) [12],[13]. The highest inhibitory effect was found in F3 with a solvent ratio of ethanol: ethyl acetate of 5:5, while the lowest inhibitory effect was found in F1 with a solvent ratio of ethanol: ethyl acetate of 9:1. In the nonpolar fraction, the inhibitory effect, which was classified as active, was only found in F6 with a solvent ratio of n-hexane: ethyl acetate of 1:9. Inhibition effect of nonpolar fraction of ethyl acetate extract of cherry mistletoe leaves which were inactive (>100 ppm), namely F7 (7:3), F8 (5:5), F9 (3:7) and F10 (1:9) [12],[13]. The IC50 value of the polar fraction (54.8 ppm) is lower than that of the nonpolar fraction (192.0 ppm). In the polar fraction, the inhibitory effect of the α-glucosidase enzyme tends to be greater in samples with the same solvent ratio of ethanol and ethyl acetate. Meanwhile, in the nonpolar fraction, the inhibitory effect of the α-glucosidase enzyme tends to be greater in samples with a higher solvent ratio of ethyl acetate compared to n-hexane. Based on the value of the dielectric constant of the solvent, it is suspected that the position of the secondary metabolites that can inhibit the α-glucosidase enzyme is at the semipolar polarity level. The amount and type of secondary metabolite content extracted by the polar fraction is thought to cause the ability to inhibit the α-glucosidase enzyme of the polar fraction to be better than the nonpolar fraction [3],[4].

Flavonoid compounds basically tend to be polar because they have –OH groups that form hydrogen bonds. However, several types of free flavonoids, such as chalcone, isoflavones, aurons, flavones, methoxylated flavonols, and anthocyanins, are less polar, compounds, so they can be extracted by ethyl acetate solvent [13]. In Saiman's research, a chalcone type of flavonoid compound was identified that was isolated from the ethyl acetate extract of Moringa leaves. Alkaloids tend to be polar, but several types of alkaloids can form a resonance in the benzene ring, which causes a reduced level of polarity of the compound so that it can be extracted by semipolar solvents [16]. Alkaloid compounds can be identified in the ethyl acetate fraction of the earrings plant with, suspected types of alkaloid compounds including sanguinarin, berberine, bazinaprin, caffeine, hydrastin, palmantin, pentypridin compounds, and evosantin [16],[17]. Saponins consist of glycone components, or sugar groups, and aglycones. These components can bind to the active site of the enzyme through hydrogen bonds. The polarity of the sapogenin aglycone compounds varies from the polarity range of semipolar to very nonpolar [17].

Tannins consist of a component called tannic acid. These components can bind to enzymes through hydrophobic and electrostatic interactions. Research by Kusumo, et al. isolated tannins from the methanol, 96% ethanol, ethyl acetate, and n-hexane fractions with the best eluent on n-hexane: ethyl acetate (6:4) [18]. The steroid in this study is thought to be a type of steroid that has a hydroxyl group. The hydroxyl group components can bind to the active site of the enzyme through hydrogen bonds. In Mardaneni's study, it was possible to identify steroid compounds in the ethyl acetate fraction of the methanol extract of red algae (E. cottonii) with suspected steroid compounds of the types β-sitosterol, campesterol, stigmasterol, desmosterol, and fucosterol [19].

**CONCLUSION**

Solvent polarity influences the secondary metabolite content. The solvent used in the polar fraction can attract flavonoids, alkaloids, steroids, saponins and tannins from cherry mistletoe leaves, while the solvent used in the nonpolar fraction can only attract flavonoid and steroid compounds from cherry mistletoe leaves. The polar fraction of the ethyl acetate extract of cherry mistletoe leaves has the ability to inhibit the α-glucosidase enzyme (IC50) better than the nonpolar fraction of the ethyl acetate extract of cherry mistletoe leaves. The use of polar solvents is recommended for the α-glucosidase enzyme inhibition test by extracts or ethyl acetate fractions of cherry mistletoe leaves.
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REFERENCES


