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RESEARCH ARTICLE

Sambiloto (*Andrographis paniculata* Nees.) leaf extract activity as an α -Amylase enzyme inhibitor

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Abstract

Introduction: Sambiloto (*Andrographis paniculata*) is an antidiabetic medicinal plant that acts by inhibiting the α -amylase enzyme. Andrographolide, the active compound of sambiloto leaf, is insoluble in water but dissolves in ethanol. **Aim:** This study compared the *in vitro* activity of aqueous extract and ethanol extract of sambiloto leaf with the α -amylase enzyme. **Methods:** The inhibitory activity test of the α -amylase enzyme was carried out using the ultraviolet-visible spectrophotometric method by measuring the absorbance of remaining starch, which forms a blue complex with iodine-iodide. **Results:** The inhibitory activity of the α -amylase enzyme of the aqueous extract of sambiloto leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL). The results of the statistical tests showed significant differences ($p < 0.05$) between the inhibitory activity of the α -amylase enzyme acarbose and the activity of both extracts.

Introduction

The prevalence of diabetes in Indonesia is relatively high and needs comprehensive prevention efforts. Using medicinal plants that have traditionally been employed by people in Indonesia is a means to overcome diabetes. Antidiabetic medicinal plants help maintain normal blood sugar levels. Inhibition of carbohydrate digestion and absorption is one of the strategies for managing blood sugar levels. The α -amylase enzyme plays a role in converting carbohydrates into sugar; thus, inhibiting its activity can suppress the formation of blood sugar (Saad *et al.*, 2017). Sambiloto (*A. paniculata*) has a high level of bitterness; its main constituents include diterpenoids, flavonoids, and polyphenols. Among the single compounds extracted from *A. paniculata*, andrographolide is the major one in terms of bioactive properties and abundance. It is slightly soluble in ethanol and almost insoluble in water. Andrographolide in leaves, stems, or whole plants can be extracted with ethanol (Chao & Lin, 2010). Andrographolide and ethanol extracts of *A. paniculata* leaf showed antidiabetic, hypoglycemic,

and antioxidant activity in mice with type 2 diabetes mellitus (DM) (Subramanian *et al.*, 2008). Generally, people use the *A. paniculata* as a medicinal plant by boiling it in water or preparing it according to the Indonesian Traditional Medicinal Herb Formulary (Formularium Ramuan Obat Tradisional Indonesia) (Departemen Kesehatan Republik Indonesia, 2017).

Aqueous extract from some plants showed alpha-amylase inhibiting activity (Bhutkar and Bhat, 2012). This study compared the *in vitro* activity of aqueous extract and ethanol extract of *A. paniculata* leaf with the α -amylase enzyme. Various *in vivo* and *in vitro* methods can be used to examine new antidiabetic drugs. *In vitro* test methods were carried out by testing the inhibitory activity of α -amylase and alpha-glucosidase enzymes (Patil *et al.*, 2012).

Methods

The materials used are sambiloto (*A. paniculata*) leaf

obtained from PT HRL Internasional, East Java in the form of powder, α -amylase enzyme (SIGMA), amylum, andrographolide (SIGMA), 70% technical ethanol, ethanol analysis (E. Merck), toluene pro analysis (E. Merck), chloroform pro analysis (E. Merck), methanol pro analysis (E. Merck), double-distilled water, dimethylsulfoxide pro analysis (E. Merck), acarbose tablet, amylum, sodium phosphate, and sodium chloride. The α -amylase enzyme inhibitory activity test was carried out according to Bhutkar and the authors (2018) with few modifications. The potato starch (1% w/v), 1 ml of drug solution (extract, acarbose), 1 ml of α -amylase enzyme (1% w/v) and 2 ml of acetate buffer (0,1M, 7,2 pH) were mixed. The mixture was incubated for one hour, then a 0.1 ml iodine-iodide indicator was added to the mixture. The absorbance measurement used a Shimadzu UV-Vis spectrophotometer using a wavelength of 568.5 nm. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (As - Ac / As) \times 100$$

Ac is the absorbance of the control; As is the absorbance of the sample.

The IC₅₀ calculation was obtained from the linear regression equation after calculating the percentage of inhibition of α -amylase enzyme activity of the test material with a concentration range of 2,5 mg/ml, 5 mg/ml, 7,5 mg/ml, and 10 mg/mL. To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ was considered as statistically significant, alongside the Tukey Post-Hoc Test significance and 95% confidence interval. Linear regression measured the median inhibitory concentration (IC₅₀) to determine the inhibitory activities of α -amylase. IBM SPSS statistics version 22 was used for statistical analysis.

Results

At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α -amylase enzyme activity than the aqueous extract. The additional concentration of the test materials increased the percentage of inhibition of the α -amylase enzyme activity (Figure 1). Acarbose tablet showed a higher percentage of inhibition than both extracts. The aqueous extract and the ethanolic extract of *A. paniculata* leaf showed an *in vitro* inhibitory activity of the α -amylase enzyme with IC₅₀ values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. The thin layer chromatogram of the *A. paniculata* extract showed a spot that is similar to the andrographolide spot (Figure 2).

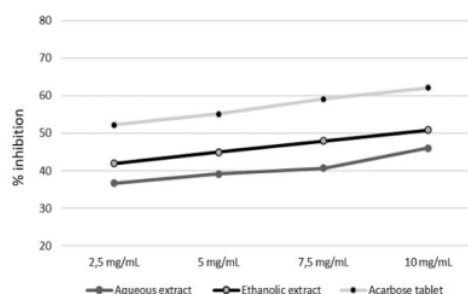
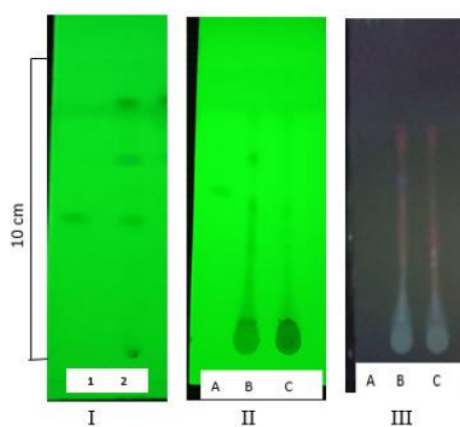


Figure 1: Per cent α -amylase inhibition activity of the aqueous extract, ethanolic extract, and acarbose tablet



(1) Andrographolide; (2) *A. paniculata* leaf powder; (A) Andrographolide; (B) The ethanolic extract of *A. paniculata*; (C) The aqueous extract of *A. paniculata*; (I) and (II) UV₂₅₄ nm detection; (III) UV₃₆₅ nm detection.

Figure 2: Thin layer chromatogram

Discussion

The inhibition of aqueous extract and ethanolic extract of *A. paniculata* leaf against α -amylase enzyme activity was tested *in vitro*, with acarbose being used as a positive control. Acarbose was chosen because of its inhibitory mechanism of action of the carbohydrate hydrolyzing enzyme. The chemical structure of acarbose is similar to that of amylum, which acts as a substrate, where both compounds have a benzene ring and a hydroxyl group that plays a role in binding to the active site of the enzyme; thus, a competitive inhibition mechanism of the enzyme activity can occur (Wright, 2003; Takahama and Hirota, 2017). The decreasing intensity of blue colour in the iodine-amylum complex

is due to the reduced amyllum substrate hydrolyzed by the α -amylase enzyme. The additional concentration of the material tests increased the percentage inhibition of α -amylase enzyme activity (Figure 1). At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α -amylase enzyme activity than the aqueous extract. The ANOVA followed by the Tukey Post-Hoc test ($p < 0.05$) showed a significant difference in the percentages of inhibition of the ethanolic extract of *A. paniculata* leaf, the aqueous extract of *A. paniculata* leaf, and acarbose tablets, on the activity of the α -amylase enzyme.

The level of inhibitory activity against the α -amylase enzyme is expressed as 50% inhibition concentration (IC_{50}), which is the concentration of the test material that can inhibit the enzyme activity by 50% (Subramanian et al., 2008). The value of the percentage inhibition of the test materials is used to calculate IC_{50} by using the linear regression equation formula to determine the equation $y = bx + a$. The IC_{50} value obtained for the aqueous extract of *A. paniculata* leaf was 14.203 ± 0.112 mg/mL, the IC_{50} value for the ethanolic extract of the *A. paniculata* leaf was 9.253 ± 0.116 mg/mL, while the IC_{50} value of the acarbose tablet was 0.983 ± 0.036 mg/mL. The inhibitory activity of the α -amylase enzyme from the ethanolic extract was greater than that of the aqueous extract. It seems that the active compound that functions as an α -amylase enzyme inhibitor in the leaf of *A. paniculata* is in both extracts, but the amount of the compound in the aqueous extract is lower than that in the ethanolic extract. Qualitative testing used the Thin Layer Chromatography method using Chloroform: methanol (9:1) as mobile phase and silica gel GF 254 nm as stationary phase, and it detected the presence of andrographolide (Figure 2).

The *in vivo* antidiabetic activity of *A. paniculata* aqueous extract had also been reported; a significant reduction in blood glucose level was shown when hyperglycemic rats were treated with 50 mg/kg body weight aqueous extract of *A. paniculata* (Husein et al., 2004). Considering that both extracts showed inhibitory activity of the α -amylase enzyme, andrographolide can be in both ethanolic and aqueous extract, thus suggesting that andrographolide is responsible for the inhibitory activity of the α -amylase enzyme. The overall activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity. It seems that they are more effective than purified compounds due to beneficial "synergistic" interactions (Caesar and Cech, 2019). The inhibitory activity of the α -amylase enzyme from the aqueous extract of *A. paniculata* leaf can occur due to the presence of andrographolide compounds itself, or it can be due to compounds other than

andrographolide, present in the extract, that have positive interactions. Further studies are necessary to examine the effect of the combination of compounds in aqueous extract and ethanolic extract of *A. paniculata* leaf as an effort to optimize the safety and benefits of using *A. paniculata* leaf extract. The ethanolic extract of *A. paniculata* leaf had higher α -amylase inhibitory activity than the aqueous extract but lower than acarbose tablets. The aqueous extract and the ethanolic extract of the *A. paniculata* leaf showed *in vitro* inhibitory activity of the α -amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. Although having less activity than ethanolic extract, the aqueous extract showed an *in vitro* inhibitory activity of the α -amylase enzyme; it is thus recommended to prepare *A. paniculata* using water.

Conclusion

Both aqueous extract and ethanolic extract of Sambiloto (*Andrographis paniculata* Nees.) showed inhibitory activity of the α -amylase enzyme. The inhibitory activity of the α -amylase enzyme of the aqueous extract of *A. paniculata* leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL).

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