

***In Vitro* Inhibition of α -Amylase and α -Glucosidase by Different Parts of Amla Plant (*Phyllanthus emblica* L)**

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Abstract

Amla plant (*Phyllanthus emblica* L) has been empirically used to treat diabetes. The aim of this study was to determine the most potential part of amla plant that can be used as α -glucosidase and α -amylase inhibitors. The fruit was subjected to cold extraction method, while the stem underwent soxhlet extraction process. Ethanol was used as solvent for both extracts. In-vitro inhibition against α -glucosidase and α -amylase enzyme was measured by calculating IC_{50} of water fractions of stem and fruits. Fruit fraction exhibited the highest percentage of inhibitory activity on α -amylase (IC_{50} =5.68% w/v), while the strongest inhibition against α -glucosidase was shown by leaves fraction (IC_{50} =0.87% w/v). In conclusion, the stem of *P. emblica* L was potential inhibitor of α -amylase, while the leaves was potential α -glucosidase inhibitor.

Keywords: *Phyllanthus emblica*, α -glucosidase inhibitor, α -amylase inhibitor, fruit, leaves

Introduction

Hyperglycemia is a symptom of diabetes mellitus, characterized by rapid increase of blood glucose levels due to rapid hydrolysis of a complex polysaccharide by the α -amylase enzyme secreted by the pancreas and absorption of glucose in the digestive tract by α -glucosidase enzyme into blood circulation.¹ Glucose absorption is influenced by α -glucosidase and α -amylase enzyme. One of the mechanisms of antidiabetic drug is inhibition of hydrolysis activity by α -glucosidase and α -amylase enzyme in the digestive tract, which can result in the suppression of post-prandial hyperglycemia.^{2,3}

Currently, acarbose has been widely used as α -glucosidase and α -amylase inhibitors. However, the drug was reported to cause various side effects.⁴ Therefore, many efforts were conducted to look for potential candidates of α -glucosidase and α -amylase inhibitors, particularly from natural sources.

One potential plant that can be used as α -glucosidase and α -amylase inhibitor is amla plant (*Phyllanthus emblica* L.). Its activity has been studied in a research performed by Sultana *et al*, showing that amla fruit exhibited hypoglycemic activity

in male rats through inhibition of glucose absorption.⁵ Qureshi *et al* showed that amla fruit water extract (200 mg/kg body weight) had hypoglycemic effect on diabetic-induced rats.⁶ Research from Fauzi *et al* reported that the amla leaves water fraction showed inhibitory activity against α -glucosidase and α -amylase enzymes, with IC₅₀ values of 0.87% and 8.64% w/v, respectively.⁷ The main parts of amla plant include roots, stems, leaves, flowers and fruit. Different parts of the plant often show similar pharmacological activity due to its secondary metabolites. It is important to examine the most potential part of the plant for certain pharmacological activities to select appropriate part than can be used as main source of medicinal product. This study was aimed to determine the most potential part of amla plant that can be used as α -glucosidase and α -amylase inhibitors.

Methods

Extraction and fractionation

Stem and fruit of amla plant were obtained from one of the plantations in West Java, Indonesia. The plant was determined at Jatinangor Herbarium, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Stem and fruit were cleaned, chopped and dried under a lamp with a temperature below 40 °C. It was then crushed using a grinder. Stem was extracted by the soxhlet device using 96% ethanol solvent, while the fruit was extracted using maceration method with the same solvent.

Each liquid extract was then concentrated with evaporator at a temperature of 40-45°C until all ethanol solvents evaporated. Viscous extracts were fractionated using a liquid-liquid extraction method. Each extract was then diluted with 100 ml of warm water (40-70 °C) before being filtered. 100 ml of n-hexane were added and mixed for \pm 1 hour and let it separated into 2 layers. The n-hexane fraction was discarded. The procedure was repeated several times. Fractionation was continued using ethyl acetate solvent with the same process as n-hexane filtration. Water fraction obtained from both parts of the amla plant were then concentrated using an evaporator.

In-vitro assay

The materials used included α -glucosidase and α -amylase enzymes (Sigma®), p-nitrophenyl- α -D-glucopyranoside (p-NPG) (Sigma®), 3,5-dinitrosalicylic acid (DNS) (Sigma®), starch 1%, and acarbose (Glucobay®). The assessment was performed by calculating IC₅₀ against α -glucosidase and α -amylase enzymes with the method based on Sugiwati *et al*.⁸ In the inhibition test, five concentrations of stem (0.6%; 0.8%; 1.0%; 1.2%, 1.4%) and fruit (0, 4%, 0.6%, 0.8%, 1%, 1.2%) water fractions were used. A total of 24 μ l of α -glucosidase enzyme was added to a flask containing 200 μ l of phosphate buffer (pH 6.8) and 40 μ l of p-NPG. Each water fraction was then added and incubated at 37 °C for 20 minutes. The reaction was stopped by adding 200 ml of sodium carbonate 200 mM. p-nitrophenol as the reaction product was measured at λ 400 nm. Similar with

Table 1. Characterization of Amla Extract

Characteristics	Stem	Fruit
Water (%)	5	7.5
Ethanol soluble compound (%)	2.4	31
Water soluble compound (%)	4.4	38
Total ashes(%)	4.75	2.95

Table 2. Phytochemical Screening of Amla Extract

Secondary Metabolites	Stem			Fruit		
	Dried Samples	Extract	Water Fraction	Dried Samples	Extract	Water Fraction
Alckloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Saponins	+	+	+	-	-	-
Quinon	+	+	+	+	+	+
Mono/Sesquiterpenes	-	-	-	-	-	-
Steroid/Triterpenes	-	-	-	-	-	-

the previous method, the inhibition test of α -amylase enzyme activity also used five concentrations of stem (3.1%; 3.8%; 4.5%; 5.2%; 5.9%) and fruit (4.7%, 5.0%, 5.3%, 5.6%, 5.9%) water fractions. A total of 80 μ l of the α -amylase enzyme was added to a flask containing 160 μ l phosphate buffer (pH 6.8) and 80 μ l 1% starch. Subsequently, each water fraction was added and incubated at 37 °C for 15 minutes. The reaction was stopped by heating process (85°C) and addition of DNS. Maltose as reaction product was measured at λ 500 nm. The inhibition percentage of

α -glucosidase and α -amylase enzyme activity was determined using the following equation:

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{Sample Absorbance}}{\text{Control Absorbance}} \right] \times 100\%$$

IC₅₀ values were determined by the linear regression equation.

Results and Discussion

The results of plant determination showed that the stem and fruit used in this study were part of the amla plant (*P. emblica* L.). Characterization test was conducted to assess quality of the extract. The results

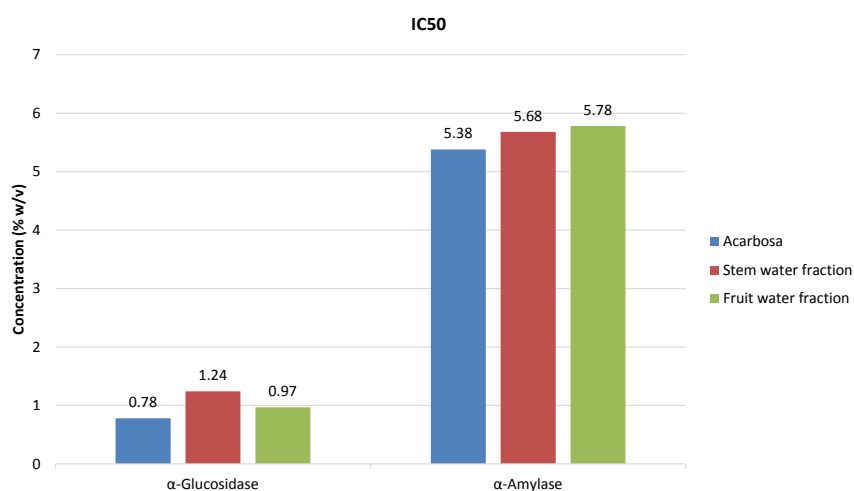


Figure 1. IC₅₀ values of Acarbose, Stem and Fruit Water Fraction of Amla Plant against α -Glucosidase and α -Amylase Enzymes

are presented in Table 1. Characteristic of the extract fulfilled the requirement from *Materia Medika Indonesia*, indicating that water content of the extract should be <10%. Phytochemical screening result is shown in Table 2. The group of secondary metabolites which were found in the water fraction of the stem and fruit were polar compounds. The composition of secondary metabolites among the two fractions were not significantly different. Saponin was not observed in the fruit water fraction.

Assessment of the α -glucosidase and α -amylase inhibition activity were performed by comparing the IC_{50} values of both fractions. Previous study showed that the amla leaves water fraction showed inhibitory activity against both of these enzymes with IC_{50} of 0.87% w/v against α -glucosidase enzymes and 8.64% w/v against α -amylase enzyme activity. The IC_{50} value obtained from the test results is shown in Figure 1. Two fractions showed greater inhibition against α -glucosidase enzyme compared to α -amylase. Nevertheless, its potential was still lower than the acarbose, comparator drug.

The water fraction of the leaves had the highest potential to be used as an α -glucosidase inhibitor, (IC_{50} 0.87% w/v), compared to the fruit (0.97% w/v) and stem (1.24% w/v). The lowest IC_{50} value against α -amylase enzyme was shown by the stem (5.68% w/v) and fruit (5.78% w/v), compared to the leaves (8.64% w/v). The inhibitory activity of these two enzymes is considered necessary in development of anti-diabetic drugs because these enzymes plays vital roles in glucose absorption.⁸⁻¹¹

Conclusions

The leaves of *P. emblica* L. had the most potential activity as an α -glucosidase inhibitor,

while the stem and fruit were potential as α -amylase inhibitor.

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Conflict of interest

None declared

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