Title : Sambiloto (Andrographis paniculata Nees.) leaf extract activity as an alpha-Amylase enzyme inhibitor

Journal Name : Pharmacy Education

[PIT virtual IAI 2020] Decision on Manuscript Sambiloto (Andrographis paniculata Nees.) Leaf Extract Activity as an Alpha-amylase Enzyme Inhibitor

Rudi Hendra <rhendra@iai.id>

Thu 2/18/2021 8:26 AM

To: Yustina Sri Hartini <yustinahartini@usd.ac.id>

Dear Yustina Sri Hartini

Your manuscript entitled "Sambiloto (Andrographis paniculata Nees.) Leaf Extract Activity as an Alpha-amylase Enzyme Inhibitor" which you submitted to the Pharmacy Education Journal in collaboration with The Indonesian Pharmacists association (IAI), has been reviewed and the reviewer comments are attached.

The reviews are in general favourable and suggest that, subject to **major correction**, your paper could be suitable for publication. Please consider these suggestions, and We look forward to receiving your revision.

When you revise your manuscript please highlight the changes you make in the manuscript by using the track changes mode in MS Word or by using bold or coloured text. To submit your revision, please click on the link below:

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Due date: **March 4th 2021**

Thank you

Sincerely

Scientific Committee
PIT Virtual IAI 2020

[PE] New notification from Pharmacy Education

Sherly Meilianti <sherly@fip.org>

Sun 7/25/2021 12:54 PM

To: Yustina Sri Hartini <yustinahartini@usd.ac.id>

You have a new notification from Pharmacy Education:

There is new activity in the discussion titled "[PE] Approval for publication" regarding the submission "IAI CONFERENCE: Sambiloto (Andrographis paniculata Nees.) leaf extract activity as an α-Amylase enzyme inhibitor ".

Link: https://pharmacyeducation.fip.org/pharmacyeducation/authorDashboard/submission/1451

Kind regards

Marwan El Akel (Managing Editor)

Pharmacy Education

Review Form Response Full Article PIT Virtual IAI 2020
Pharmacy Education Journal in collaboration with
The Indonesian Pharmacists Association (IAI)

Manuscript title	:	Sambiloto (Andrographis paniculata Nees.) Leaf Extract Activity as an Alpha-amylase Enzyme Inhibitor
Originality of the work and Scientific merit	:	Good
Title describes the content of paper properly and clearly	:	Good
Appropriateness of abstract	:	Good
Description of the problem and solution offered	:	Good
Description of experimental design	:	Poor
Presentation of experiment results (clear and systematic)	:	Fair
Discussion and interpretation of results	:	Fair
Statistical treatment of data (if necessary)	:	Poor
Relevance of data and conclusion	:	Good
Appropriateness and relevance of citation and references	:	Fair
Quality of figures and tables	:	Fair

Overall quality of the paper	:	Good
RECOMMENDATION	:	Accepted with major revision

Additional Comment:
1. Please use 12 font size.
2. used symbol for alpha.
3. The reference should use APA style.
4. Please explain all the methods in detail.
5. Please explain the statistical analysis used.
6. Please add conclusion section.

Sambiloto (*Andrographis paniculata* Nees.) Leaf Extract Activity as an α-Amylase Enzyme Inhibitor

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Abstract

Sambiloto (*Andrographis paniculata*) is an antidiabetic medicinal plant with the action mechanism of inhibiting the alpha-amylase enzyme. Andrographolide, the active compound of sambiloto leaf, is insoluble in water, but is dissolved in ethanol. This study compared the *in vitro* activity of aqueous extract and ethanolic extract of sambiloto leaf with the alpha-amylase enzyme. The inhibitory activity test of alpha-amylase enzyme was carried out using the ultraviolet-visible spectrophotometric method by measuring the absorbance of the remaining starch which forms a blue complex with iodine-iodide. The inhibitory activity of the alpha-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-value <0.05) between the inhibitory activity of the alpha-amylase enzyme acarbose and the activity of both extracts.

Keywords: alpha-amylase, aqueous extract, ethanolic extract, sambiloto leaf

Commented [AA1]: Used symbol

Introduction

The prevalence of diabetes in Indonesia is relatively high, so that it needs comprehensive prevention efforts. One of the efforts to overcome diabetes is by using medicinal plants that have traditionally been used by people in Indonesia. Antidiabetic medicinal plants are used to help maintain normal blood sugar levels. Inhibition of carbohydrate digestion and absorption is one of the strategies for managing blood sugar levels. The alpha-amylase enzyme plays a role in converting carbohydrates into sugar, inhibiting the activity of the alpha-amylase enzyme can suppress the formation of blood sugar [1]. Sambiloto (*Andrographis paniculata*)/AP has a high level of bitterness, with the main constituents include diterpenoids, flavonoids, and polyphenols. Among the single compound extracted from AP, andrographolide is the major one in terms of its bioactive properties and abundance. Andrographolide is slightly soluble in ethanol, and almost insoluble in water. Andrographolide in leaves, stems, or whole plants can be extracted with ethanol [2]. Andrographolide and ethanol extracts of AP leaf showed antidiabetic, hypoglycemic, and antioxidant activity in type 2 DM mice [3]. In general, people use the AP as a medicinal plant by boiling it in water, or by doing the AP preparation method as stated in the Indonesian Traditional Medicinal Herb Formulary (Formularium Ramuan Obat Tradisional Indonesia) [4]. Aqueous extract from some plants showed alpha-amylase inhibitor activity [5]. This study compared the *in vitro* activity of aqueous extract and ethanolic extract of AP leaf with the alpha-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 alpha-amylase and alpha-glucosidase enzymes [6].

Commented [AA2]: Used *A. paniculata* through to the text

Methods

The materials used are sambiloto (*Andrographis paniculata*) leaf obtained from PT HRL Internasional, East Java in the form of powder, alpha-amylase enzyme (SIGMA), amylum, andrographolide (SIGMA), 70% technical ethanol, ethanol pro analysis (E. Merck), toluene pro analysis. (E. Merck), chloroform pro analysis (E. Merck), methanol pro analysis (E. Merck), aqua bidestilata, dimethylsulfoxide pro analysis (E. Merck), acarbose tablets, amylum, sodium phosphate, and sodium chloride. The alpha-amylase enzyme inhibitory activity test was carried out according to Bhutkar et al. [7]. with few modifications. The absorbance measurement used a Shimadzu UV-Vis spectrophotometer using a wavelength of 568.5 nm. The IC_{50} calculation was obtained from the linear regression equation after calculating the percentage inhibition of alpha-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.

Commented [AA3]: Please explain the method in detail.

Results

At the same concentration, the ethanolic extract of *Andrographis paniculata*/AP leaf showed a greater percentage inhibition of alpha-amylase enzyme activity than the aqueous extract. The additional concentration of the test materials increased the percentage inhibition of alpha-amylase enzyme activity (Fig. 1). Acarbose tablets showed greater percentage inhibition than both extracts. The aqueous extract and the ethanolic extract of AP leaf showed *in vitro* inhibitory activity of the alpha-amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. The thin layer chromatogram of the AP extract showed a spot that is similar with andrographolide spot (Fig. 2)

Discussion

The inhibition of aqueous extract and ethanolic extract of AP leaf against alpha-amylase enzyme activity was tested *in vitro* with acarbose being used as a positive control. Acarbose was chosen because this antidiabetic drug has an inhibitory mechanism of action of the carbohydrate hydrolyzing enzyme. The chemical structure of acarbose is similar to the structure of amylum which acts as a substrate, in which both compounds have a benzene ring and a hydroxyl group that play a role in binding to the active site of the enzyme, so that a competitive inhibition mechanism of enzyme activity can occur [8-9]. The decreasing intensity of blue color in iodine-amylum complex is due to the reduced amylum substrate hydrolyzed by an alpha-amylase enzyme. The additional concentration of the material tests increased the percentage inhibition of alpha-amylase enzyme activity (Fig. 1). At the same concentration, the ethanolic extract of AP leaf showed a greater percentage inhibition of alpha-amylase enzyme activity than the aqueous extract. The statistical test ($P < 0.05$) showed a significant difference among the percentage inhibition of the ethanolic extract of AP leaf, the aqueous extract of AP leaf, and acarbose tablets on the activity of the alpha-amylase enzyme. The level of inhibitory activity against the alpha-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% [3]. 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 $y = bx + a$. It was obtained that the IC_{50} value for the aqueous extract of AP leaf was 14.203 ± 0.112 mg/mL, the IC_{50} value for the ethanolic extract of the AP 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 alpha-amylase enzyme from the ethanolic extract was stronger than that of aqueous extract. It seems that the active compound that functions as an alpha-amylase enzyme inhibitor in the leaf of AP is in both extracts, but the amount of the compound in the aqueous extract is less than 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 (Fig. 2). The *in vivo* antidiabetic activity of AP 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 AP [10]. Considering that both extracts showed the inhibitory activity of the alpha-amylase enzyme, andrographolide can be in both ethanolic and aqueous extract. Therefore, it

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supports the opinion that andrographolide is responsible for the inhibitory activity of the alpha-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.[11] The inhibitory activity of the alpha-amylase enzyme from the aqueous extract of AP leaf can occur due to the presence of andrographolide compounds itself, or it can be due to the presence of compounds other than andrographolide in the extract that have positive interactions. Further study is necessary on the effect of the combination of compounds in aqueous extract and ethanolic extract of AP leaf as an effort to optimize the safety and benefits of using AP leaf extract. The ethanolic extract of sambiloto (*Andrographis paniculata*) leaf showed to have higher alpha-amylase enzyme inhibitory activity than the aqueous extract, but it is lower compared to acarbose tablets. The aqueous extract and the ethanolic extract of the sambiloto leaf showed *in vitro* inhibitory activity of the alpha-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, aqueous extract showed an *in vitro* inhibitory activity of alpha-amilase enzyme, therefore it is recommended for *Andrographis paniculata* preparation using water.

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Figures:

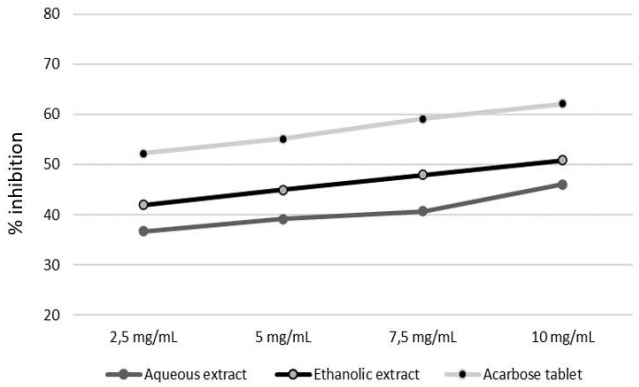

Figure 1. Percent alpha-amylase inhibition activity of the aqueous extract, ethanolic extract, and acarbose tablet

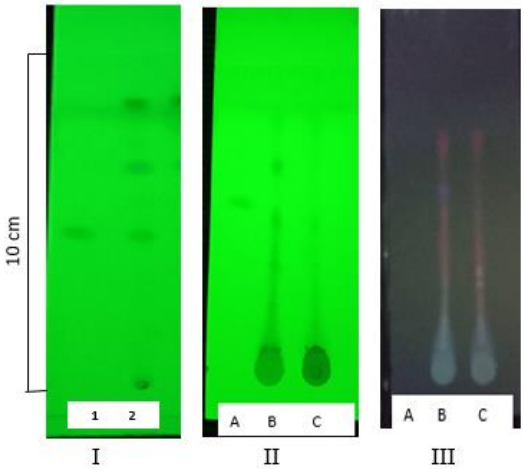

Figure 2. Thin Layer Chromatogram: Andrographolide (1), AP leaf powder (2), Andrographolide (A), the ethanolic extract of AP (B), the aqueous extract of AP (C); UV_{254} nm detection (I), and UV_{365} nm detection (III).