Title : Brotowali (Tinospora crispa L.) steam extract activity as an alpha-Amylase enzyme inhibitor

Journal Name: Pharmacy Education

1/18/23, 10:48 AM

Mail - Yustina Sri Hartini - Outlook

[PE] [PEJ SPECIAL ISSUE] Action to authors - Approval for publication

Emmanuella Nzeribe <nzeribeella@gmail.com> Wed 3/16/2022 10:50 PM

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

Dear Yustina Sri Hartini,

We would like to invite you to do a final review of your manuscript: "IAI SPECIAL EDITION: Brotowali (Tinospora crispa L.) Stem Extract Activity as an α -Amylase Enzyme Inhibitor," before we publish it.

Submission URL: https://pharmacyeducation.fip.org/pharmacyeducation/authorDashboard/submission/1635

May you have a final look and let us know if there are any changes needed by next week?

Please note that if we do not receive any feedback by that time, we will consider this as approval for the publication of your article.

The final galley can be seen in the "copy-edited" section.

Thank you and we look forward to hearing from you. Best,

Pharmacy Education

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1/17/23, 2:14 PM

Mail - Yustina Sri Hartini - Outlook

[PIT Virtual IAI 2021] Revision submission confirmation

rhendra@iai.id <rhendra@iai.id> Wed 10/27/2021 4:42 AM To: Yustina Sri Hartini <yustinahartini@usd.ac.id> Dear Yustina Sri Hartini

We appreciate your submission of your manuscript titled "Brotowali (Tinospora crispa L.) Stem Extract Activity as an α -Amylase Enzyme Inhibitor " We will revise your manuscript in light of the reviewers' comments and will contact you if additional information is required.

Sincerely Scientific Committee PIT Virtual IAI 2021

1/17/23, 2:08 PM

Mail - Yustina Sri Hartini - Outlook

[PIT Virtual IAI 2021] Decision on Manuscript "Brotowali Stem Extract Activity as an α -Amylase Enzyme Inhibitor"

Rudi Hendra <rhendra@iai.id>

Sat 10/23/2021 3:07 AM

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

2 attachments (485 KB)

Brotowali Stem Extract Activity as an α-Amylase Enzyme Inhibitor .pdf; OH11 rev.doc;

Dear Yustina Sri Hartini

Your manuscript, "Brotowali Stem Extract Activity as an α -Amylase Enzyme Inhibitor" which you submitted to the Pharmacy Education Journal in collaboration with The Indonesian Pharmacists Association (IAI), has been peer reviewed, and the reviewer comments are attached.

The reviews are generally favourable, implying that your paper may be suitable for publication with **minor correction**. Kindly consider these suggestions, and we eagerly await your revision. We will revise your revision in light of peer review comments, and once the manuscript satisfies the requirement, it will be submitted to the editor of Pharmacy Education Journal. We will notify you if any changes or additional information become available.

When revising your manuscript, please highlight the changes you make using MS Word's track changes mode or by using bold or coloured text to expedite the processing. Please click on the link below to submit your revision:

https://forms.gle/TbBy9vp1moYUBWvx8

Due date: November 12th 2021

Thank you

Sincerely

Scientific Committee PIT Virtual IAI 2021



Review Form Response Full Article PIT Virtual IAI 2021 Pharmacy Education Journal in collaboration with The Indonesian Pharmacists Association (IAI)

Manuscript title	:	Brotowali Stem Extract Activity as an α-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	:	Good
Presentation of experiment results (clear and systematic)	:	Fair
Discussion and interpretation of results	:	Fair
Statistical treatment of data (if necessary)	:	Good
Relevance of data and conclusion	:	Good
Appropriateness and relevance of citation and references	:	Good
Quality of figures and tables	:	Good
Overall quality of the paper	:	Good
RECOMMENDATION	:	Accepted with minor revision
Additional Comment: Please check my commen in manuscript Please check author guideline Please use reference aplication (mendeley, or another) The manuscript contains: title, abstract, introduction/background, methods, results, discussion, and conclusion. The discussion section should not only tell the results obtained. Manuscripts are double-spaced, with a margin of at least 2.5 cm, and all pages must be numbered. The maximum number of figures and tables is 4, the writing of the table must be in the form of Tables I, II, III, etc. and the images in the form of Figures 1,2,3 and so on. The mumber of manuscripts is 5-6 pages excluding references, figures, and tables. The bibliography must use the APA style		

Pengurus Pusat Ikatan Apoteker Indonesia JI. Wijayakusuma no.17, Tomang - jakarta Barati 14410; +62 21 55962581; +62 21 5671800 Paniteia PIT LAI 2020; fip-pit2020@iai.id

Brotowali Stem Extract Activity as an α-Amylase Enzyme Inhibitor

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ABSTRACT

Introduction: Reducing glucose absorption in the gastrointestinal tract is one of the strategies for treating diabetes mellitus. The condition of treating diabetes mellitus can achieve by inhibiting the activity of the α -amylase enzyme. Brotowali (*Tinospora crispa* L.)/**Tc** has antihyperglycemic activity; compounds contained in the **Tc** stem can inhibit the activity of the α -amylase enzyme. People use **Tc** stem extract in medicine. The extraction of the **Tc** stem used for treatment was done with water and/or ethanol. **Aim:** This study aimed to measure the inhibitory activity of the α -amylase enzyme in both aqueous and ethanolic extract **Tc** stem. **Methods**: The inhibitory activity test of the α -amylase enzyme was carried out using the UV-visible spectrophotometric method. **Results:** The aqueous extract and ethanolic extract of **Tc** stem had α -amylase enzyme inhibitory activity with IC₅₀ values of 11.660 ± 0.310 mg/mL and 10.348 ± 0.313 mg/mL, respectively. The use of **Tc** stem as anti-diabetic drugs can be prepared with water or ethanol.

Keywords: *Tinospora crispa* L. stem, aqueous extract, ethanolic extract, α -amylase enzyme

Introduction

Since ancient times, people have used plants as medicinal ingredients for the treatment of various diseases. Traditionally, diabetes mellitus was among several diseases that can be treated with the stems of brotowali (*Tinospora crispa* L.)/**Tc**. Managing blood sugar levels is one-way prevention of diabetes mellitus. The α -amylase enzyme plays a role in converting carbohydrates into sugar; inhibition of α -amylase enzyme activity can suppress the formation of blood sugar [1]. **Tc** stem is famous as a medicinal ingredient with a very bitter taste characteristic. **Tc** contains more than 65 compounds that have been isolated from various groups of compounds such as furanofditerpenes, lactones, steroids, flavonoids, lignans, and alkaloids [2]. People use medicinal plants by boiling them in water; this statement goes along with the making or the using methods **Tc** stems as stated in the Formulary of Indonesian Traditional Medicines [3]. Aqueous extracts from several plants exhibited α -amylase enzyme inhibitor activity [4]. The test of antidiabetic drugs was done using an *in vitro* test method in the form of an α -amylase enzyme inhibition activity test [5]. This study aims to compare the activity of aqueous extracts and ethanolic extracts of **Tc** stems against α -amylase enzymes *in vitro*.

Material and Method

The materials used are brotowali stem (*Tinospora crispa* L.). The study received the stem from PT HRL Internasional, East Java; α -amylase enzyme (SIGMA Aldrich), Quercetine (E. Merck), ethanol pro analysis (E. Merck), double-distilled water, dimethyl sulfoxide pro analysis (E. Merck), iodine iodide reagent, potato starch, 1N HCL, acarbose tablets . The α -amylase enzyme inhibitory activity test was carried out according to Ononamadu *et al.* [6] with few modifications. The potato starch (1% w/v), 1ml of test material (**Tc** 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 measurement of the inhibitory effect of the sample blank solution was carried out by taking 1 ml of 0.5% potato starch solution into a test tube. The mixture was incubated for one hour, then a 0.1 ml iodine-iodide indicator was added to the mixture. The absorbance measurement used UV-Vis spectrophotometer using a wavelength of 536 nm. The percentage inhibition calculations did as follows:

% 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 4 mg/ml, 8 mg/ml, 15 mg/ml, and 20 mg/mL. To compare the treatment, this research used the analysis of variance (ANOVA) and *p* < 0.05 received the result of 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. This research used IBM SPSS statistics version 22 for statistical analysis.

Results

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The addition of the concentrations of the three test materials (aqueous extract of **Tc** stem, ethanolic extract of **Tc** stem, acarbose tablet) increased the percent inhibition of α -amylase enzyme activity (Fig. 1). The inhibitory activity of the α -amylase enzyme from acarbose was higher than that of the aqueous extract and the ethanol extract of the **Tc** stem. At concentrations of 4 mg/mL and 8 mg/mL **Tc** stem aqueous extract showed a higher percent inhibition of α amylase enzyme activity than ethanolic extract, but at a concentration of 20 mg/mL, it occurred otherwise. At a concentration of 15 mg/mL, aqueous extract and ethanolic extract of **Tc** stem showed the same percent inhibition of α -amylase enzyme activity. Statistical test (P<0.05) showed a significant difference between the percent inhibition of aqueous extract **Tc** stem, ethanolic extract **Tc** stem, and acarbose tablets, on α -amylase enzyme activity. The significant difference existed except between aqueous extract and ethanolic extract at a concentration of 15 mg/mL. The Thin Layer Chromatogram/TLC of the **Tc** did not show a spot that is similar to the quercetine spot (Fig. 2).



Figure 1. Percent inhibition of aqueous extract, ethanolic extract, and acarbose tablet

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Figure 2. Thin Layer Chromatogram: (1) Quercetine, (2) Tc stem powder, (a) Quercetine, (b) Aqueous

extract of Tc stem, (c) Ethanolic extract of Tc stem, (I-II) UV₂₅₄ nm detection, (III) UV₃₆₅ nm detection.

Commented [B37]: author displays the TLC of the sample versus quercetin. but the TLC did not detect the presence of quercetin at the same RF value. So that the TLC analysis is in vain, please the author to reconsider the discussion of the TLC. TLC can be discussed in the article but the author has not discussed it in the discussion section of the article

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Discussion

The inhibitory activity of aqueous extract and ethanol extract of bitter leaf on α -amylase enzyme activity was tested in vitro. As shown in Fig.1 the additional concentration of the material tests increased the percentage inhibition of α -amylase enzyme activity. The level of inhibitory activity against the α -amylase enzyme is expressed as 50% inhibition concentration (IC₅₀). The IC₅₀ value for the aqueous extract of Tc stem was 11.660 ± 0.310 mg/mL, the IC₅₀ value for the ethanolic extract of Tc stem was 10.348 \pm 0.313 mg/mL, while the IC₅₀ value of the acarbose tablet was 5.554 ± 0.380 mg/mL. Acarbose was chosen as a positive control because the chemical structure of this antidiabetic drug is similar to the structure of starch which acts as a substrate. Both compounds have a benzene ring and a hydroxyl group that plays a role in binding the active site of the enzyme. This activity occurred so that a competitive inhibition mechanism of enzyme activity can occur [7]. In vivo antidiabetic activity of Tc stem has been reported. Tinospora crispa L. stems contain alkaloids, flavonoids, glycosides, and terpenoids [8]. In this study, a reference standard compound used flavonoid quercetine. The presence of quercetine in aqueous extract and ethanol extract of Tc stems could not show with the TLC (Figure 2). Several studies reported the presence of quercetine in Tc stem, methods other than TLC are recommended to detect the presence of quercetine in aqueous extracts and ethanolic extracts of Tc stems. The compound borapetoside C is the compound most commonly found in Tc plants and can inhibit the α -amylase enzyme [9]. Compounds in the aqueous extract and ethanol extract of **Tc** stems showed α -amylase enzyme inhibitory activity, this activity could be due to borapetoside C or several compounds, either singly or in a combination of the compounds in the extract. Several studies have shown that the overall activity of botanical extracts can result from mixtures of Commented [B38]: consider to discuss the TLC result

compounds with synergistic, additive, or antagonistic activity. Proponents of the medicinal use of natural product mixtures often claim that they are more effective than purified compounds due to beneficial "synergistic" interactions [10]. The active compound that functions as an inhibitor of the α -amylase enzyme in **Tc** stems can be in the aqueous extract or the ethanolic extract of **Tc** stem so that the use of **Tc** stem as anti-diabetic drugs can be prepared with water or ethanol. Further studies need to focus on the compounds or combinations of compounds in aqueous extracts and ethanolic extracts of **Tc** stems that are responsible for antidiabetic activity through inhibition of the α -amylase enzyme.

Conclusion

As a conclusion, *in vitro* aqueous extract and ethanolic extract of brotowali (*Tinospora crispa* L.) stem showed α -amylase inhibitory activity with IC₅₀ values of 11.660 ± 0.310 mg/mL and 10.348 ± 0.313 mg/mL, respectively.

Acknowledgments

The researcher would like to thank PT HRL Internasional and LPPM Sanata Dharma University for the materials and funds support for this research.

References

1. Saad, B., Zaid, H., Shanak, S., and Kadan, S. (2017). *Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals*. Springer International Publishing. Switzerland.

- Ahmad, W. Jantan, I., and Bukhari, S.N.A. (2016). *Tinospora crispa* (L.) Hook. F. & Thomson: A Review of Its Ethnobotanical, Phytochemical, and Pharmacological Aspects. *Frontier in Pharmacology*, 7 (59), 1-19.
- Ministry of Health Republic of Indonesia, (2017). Formulary of Indonesian Traditional Medicines. Ministry of Health Republic of Indonesia, Jakarta.
- Bhutkar, M.A. and Bhise, S.B. (2012). In Vitro Assay of Alpha-Amylase Inhibitory Activity of Some Indigenous Plants. *Int. J. Chem. Sci*, 12;10(1):457-462.
- Patil, A., Nirmal, S., Pattan, S., Tambe, V., and Tare, M. (2012). Antidiabetic Effect of Polyherbal Combination in STZ Induced Diabetes Involve Inhibition of Amylase and Glocosidase with Amelioration of Lipid Profile. *Phytopharmacology*, 2(1):46-57.
- Ononamadu, C.j., Ezeigwe, O.C., Owolarafe, T.A., Ihegboro, G.O., Lawal, T.A., Salawu, K., Umeoguaju, U.F., and Aminu, I. (2020). Starch-iodine assay method underestimates -amylase inhibitory potential of antioxidative compound and extracts. *BioTechnologia*, 101 (1): 45-54.
- 7. Takahama, U. and Hirota, S. (2017). Interactions of Flavonoid with α -amylase and Strach Slowing Down its Digestion. Food and Function, 1 (3):3-4.
- Elya, B., Handayani, R., Sauriasari, R., Hasyyati, US., Permana, I.T., and Permatasari, Y.I., (2015). Antidiabetic Activity and Phytochemical Screening of Extracts from Indonesian Plants by Inhibition of Alpha Amylase, Alpha Glukcosidase and Dipertidyl Peptidase IV. *Pakistan Journal* of Biological Sciences, 18(6), 279-284.
- 9. Hamid, H.A., Yusoff, M.M., Liu, M., and Karim, M.R., (2015). α-Glucosidase and α-amylase inhibitory constituents of *Tinospora crispa*: Isolation and chemical profile confirmation by

ultra-high performance liquid chromatography-quadrapole time-of-flight/mass spectrometry.

Journal of Functional Foods, 16,74-80.

 Caesar, L.K., and Cech, N.B. (2019). Synergi and Antagonism in Natural Product Extracts: When 1+1 dosen not equal 2. *Natural Product Reports*, 36(6):845-936.

1/17/23, 2:16 PM

Mail - Yustina Sri Hartini - Outlook

[PIT Virtual IAI 2021] Update Pharmacy Education Journal Process

Rudi Hendra <rhendra@iai.id>

Fri 12/17/2021 8:38 AM

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We'd like to inform you that an acceptance letter from the Pharmacy Education Journal (PEJ) has been sent to your email address.

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Regards,

Scientific committee PIT Virtual IAI 2021

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