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RESEARCH ARTICLE

# Brotowali (*Tinospora crispa L.*) 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 be achieved 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. 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 $IC_{50}$ values of 11.660 ± 0.310 mg/mL and 10.348 ± 0.313 mg/mL, respectively. The Tc stem extracted with water or ethanol can be used as an antidiabetic drug.

## Introduction

Since ancient times, people have used plants as medicinal ingredients for the treatment of various conditions. Traditionally, diabetes mellitus was among the diseases that can be treated with the stems of brotowali (*Tinospora crispa L.)*/**Tc**. Managing blood sugar levels is a way to prevent diabetes mellitus. The α-amylase enzyme plays a role in converting carbohydrates into sugar; the inhibition of α-amylase enzyme activity can suppress the formation of blood sugar (Hilallzaid & Slemannkadan, n.d.). **Tc** stem is famous as a medicinal ingredient characterised by a very bitter taste. **Tc** contains more than 65 compounds isolated from various groups of compounds, such as furano-diterpenes, lactones, steroids, flavonoids, lignans, and alkaloids (Ahmad *et al.,* 2016). People use medicinal plants by boiling them in water. This statement goes along with the making or the use methods of **Tc** stems, as stated in the Formulary of Indonesian Traditional Medicines (Keputusan Menteri kesehatan Republik Indonesia, n.d.). Aqueous extracts from several plants exhibited the α-amylase enzyme inhibiting activity (Bhutkar & Bhise, 2012). The antidiabetic activity was tested using an *in vitro* method in the form of an α-amylase enzyme inhibition activity test (Patil *et al.* 2012, Antidiabetic, n.d.). This study aimed to compare the activity of aqueous and ethanolic extracts of **Tc** stems against α-amylase enzymes *in vitro*.

## Material and method

Brotowali stem (*Tinospora crispa* L.) was received from PT HRL Internasional, East Java. The maceration method was used in the compound extraction of **Tc** leaf. The identification of **Tc** leaf methanolic extract compounds was carried out using Thin Layer Chromatography/TLC. The materials used were α-amylase enzyme (SIGMA Aldrich), Quercetin (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 (PT Dexa Medica). The α-amylase enzyme (from porcine pancreas-type VI-B, CAS A3176, SIGMA Aldrich)

inhibitory activity test was carried out according to Ononamadu and colleagues (Ononamadu *et al.,* 2020) with few modifications. The following ingredients were mixed: potato starch (1% w/v), 1ml of test material (**Tc** extract, acarbose), 1 ml of the α-amylase enzyme (1% w/v), and 2 ml of acetate buffer (0,1M, 7,2 pH). 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 a UV-Vis spectrophotometer using a wavelength of 536 nm. The percentage of inhibition was calculated as follows:

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

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

The inhibitory concentration ($IC_{50}$) 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. This research used the analysis of variance (ANOVA) to compare the treatment. A value of $p < 0.05$ was considered statistically significant, alongside the Tukey Post-Hoc Test significance and a 95% confidence interval. Linear regression measured the median $IC_{50}$ to determine the inhibitory activities of α-amylase. This research used IBM SPSS statistics version 22 for statistical analysis.

## Results

The addition of the concentrations of the three test materials (aqueous extract of **Tc** stem, ethanolic extract of **Tc** stem, acarbose tablet) increased the percentage of inhibition of α-amylase enzyme activity (Figure 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 concentrAntituberculosisAntituberculosisations of 4 mg/mL and 8 mg/mL, **Tc** stem aqueous extract showed higher inhibition of α-amylase enzyme activity than ethanolic extract, but at a concentration of 20 mg/mL, it occurred otherwise. At the same concentration of 15 mg/mL, **Tc** stem aqueous extract and **Tc** stem ethanolic extract showed the same percentage of inhibition of α-amylase enzyme activity. Statistical tests ($P<0.05$) showed a significant difference between the percentage of inhibition of α-amylase enzyme activity of aqueous extract **Tc** stem, ethanolic extract **Tc** stem, and acarbose tablets. The TLC of the **Tc** did not show a spot similar to the quercetin spot (Figure 2).

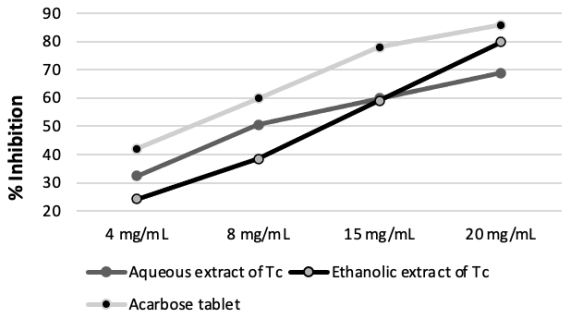
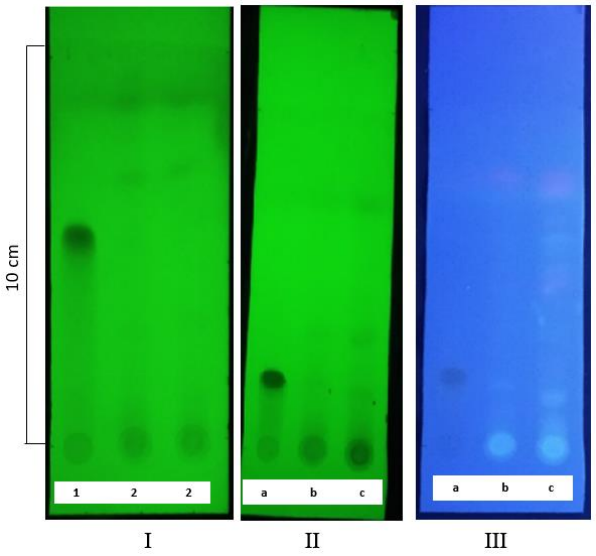



**Figure 1: Percent inhibition of aqueous extract, ethanolic extract, and acarbose tablet**




**Note:** (1) Quercetin, (2) Tc stem powder, (a) Quercetin, (b) Aqueous extract of Tc stem, (c) Ethanolic extract of Tc stem, (I-II) $UV_{254}$ nm detection, (III) $UV_{365}$ nm detection.

**Figure 2: Thin Layer Chromatogram**

## Discussion

The inhibitory activity of aqueous and ethanol extracts of bitter leaf on α-amylase enzyme activity was tested *in vitro*. As shown in Figure 1, the higher 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 ($IC_{50}$). The $IC_{50}$ value were 11.660 ± 0.310 mg/ml, 0.348 ± 0.313 mg/mL, and 5.554 ± 0.380 mg/mL for **Tc** stem aqueous extract, **Tc** stem ethanolic extract, and acarbose tablets, respectively. The antidiabetic drug acarbose was chosen as a positive control because of its chemical structure, similar to that of starch that acts as a substrate. Both compounds have a benzene ring

and a hydroxyl group that play a role in binding the enzyme's active site. This activity occurred so that a competitive inhibition mechanism of enzyme activity could happen (Takahama & Hirota, 2018). *In vivo* antidiabetic activity of **Tc** stem has been reported. *Tinospora crispa L.* stems contain alkaloids, flavonoids, glycosides, and terpenoids (Elya *et al*., 2015). In this study, a reference standard compound used flavonoid quercetin. The presence of quercetin in aqueous extract and ethanol extract of **Tc** stems could not show with the TLC. Even though there are faint spots in the same Rf region, the presence of the same compound with quercetin cannot be asserted. TLC did not detect the presence of quercetin at the same RF value (Figure 2).

Several studies reported the presence of quercetin in **Tc** stems. Methods other than TLC are recommended to detect the presence of quercetin in aqueous extracts and ethanolic extracts of **Tc** stems. Borapetoside C is the compound most commonly found in **Tc** plants and can inhibit the α-amylase enzyme (Hamid et al., 2015). Compounds in the aqueous extract and ethanol extract of **Tc** stems showed α-amylase enzyme inhibitory activity, which 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 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 (Caesar & Cech, 2019). The active compound that functions as an inhibitor of the α-amylase enzyme can be in **Tc** stems aqueous or the ethanolic extracts, so both can be used as antidiabetic drugs. Further studies need to focus on the compounds or combinations of compounds in both aqueous extracts and ethanolic extracts of **Tc** stems responsible for the antidiabetic activity through the inhibition of the α-amylase enzyme.

## Conclusion

In conclusion, *in vitro,* aqueous extract and ethanolic extract of brotowali (*Tinospora crispa* L.) stem showed α-amylase inhibitory activity with $IC_{50}$ values of 11.660 ± 0.310 mg/mL and 10.348 ± 0.313 mg/mL, respectively.

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