α-Glucosidase Inhibitory Activity of Ethanolic Extract of Brotowali Stem (Tinospora crispa Miers.) In Vitro

RISMA MARISI TAMBUNAN¹, KARTININGSIH¹, YESI DESMIATY¹, LOLA DYAH PITHALOKA¹
Fakultas Farmasi Universitas Pancasila
Srengseng sawah, Jagakarsa, Jakarta Selatan 12640
Email: rmu_tambunan@yahoo.com

ABSTRACT

Brotowali (Tinospora crispa Miers.) is a climbing shrub, which empirically has been used to treat various skin problems caused by bacteria, scratchy parasites, burns, stomach disorders, diarrhea, fever, jaundice, and diabetes. Brotowali stem contains the compound of alkaloids, flavonoids (apigenin O - glycosides, palmatin, pikroretosida) and terpenoids, which has potential as an antidiabetic. α-glucosidase Inhibitory activity were conducted to ethanolic extract of brotowali stem with p-nitrophenyl α-D- glucopyranoside as substrate by using Absorbance Reader Microplate ELx800 at λ 405 nm. The result of this study showed that percent inhibition of ethanolic extract of brotowali stem at 450 ppm is 78.34% equivalent to acarbose 81.01%. The IC 50 of ethanolic extract of brotowali stem is 237 ppm and acarbose is 116 ppm. In conclusion, ethanolic extract of brotowali stem has activity as α-glucosidase inhibitor.

Keywords: ethanolic extract, “brotowali” stem, Tinospora crispa Miers., α-glucosidase

INTRODUCTION

Brotowali or Tinospora crispa Miers. (Menispermaceae) is a wooden climbing shrub that thrives in coastal to highlands at tropical areas especially Asia. Brotowali stem are used empirically to overcome various problems of the skin caused by bacteria, scratchy parasites, burns, stomach disorders, diarrhea, fever, jaundice, and diabetes. Some publications claim that ethanolic extract of brotowali stem was given orally to rats showed LD 50 at 40 g/kg and the oral administration of n-hexane, ethyl acetate, and methanol extracts of brotowali stem at 250 mg/kg reduced blood sugar levels in male rats induced by streptozotocin (1,2).

Diabetes mellitus is a disease characterized by hyperglycemic absolute deficiency of insulin or decreased cells sensitivity to insulin. WHO differentiates into type I diabetes mellitus for the absolute lack of insulin, type II diabetes mellitus is independent of insulin in the body, patients can still produce insulin in sufficient amounts but the body loses its sensitivity to insulin, and gestational diabetes mellitus, diabetic state during pregnancy and temporarily (2). This study aimed to test the α-glucosidase inhibitory activity of ethanolic extract of brotowali stem using p-nitrophenyl α-D-glukopiranosida as substrate and acarbose as standard.
MATERIALS AND METHODS

Materials
Powdered crude drug of *Tinospora crispa* Miers. stem (BALITRO), Apigenin Crystalline TLC SA A3145, α-glucosidase (G5003-100UN, Sigma), p-nitrophenyl-α-D-glucopyranoside (N1377-1G, Sigma), bovine serum albumin (Sigma), acarbose (Bayer), ethanol 96%, phosphate buffer pH 7.0, dimethyl sulfoxide, sodium carbonate, sodium hydroxide.

Methods
200 grams of powdered crude drug was macerated in 96% aqueous ethanol to completely extracted. Resemserated for thirteen times by using 5,625 ml ethanol. The extract was collected and evaporated under reduced pressure at < 40°C to dryness and 24,28 of residue was obtained (yield= 12,1%). Phytochemical screening were conducted of the extract include examination of alkaloids, flavonoids, tannins, saponins, steroids / triterpenoids, coumarin and volatile oil, then α-glucosidase inhibitory activity in vitro.

α-Glucosidase Inhibitory Activity of Brotowali’s Extract

The same concentration of ethanolic extract of brotowali stem and acarbose as standard were conducted to α-glucosidase inhibitory activity using p-nitrophenyl-α-D-glucopyranoside as substrate. α-glucodase activity was determined by kawanishi method at 405 nm wave length. 200 mg bovine serum albumin and 1.0 mg α-glucosidase were diluted in buffer phosphat pH (7.0). The mixing solution contains 250 µl solution of 2 mM p-nitrophenyl α-D-glucopyranoside, 400 µl buffer phosphat (pH 7.0), and series of 10 µl, 30 µl, 50 µl, 70 µl and 90 µl sample. Then, it was pre-incubated at 37°C for 5 minutes. Reaction was started by adding 250 µl α-glucosidase, then incubated it at 37°C for 15 minutes. The reaction was stopped by adding 1000 µl Na₂CO₃ solution. The amount of p-nitrofenol obtained was measured at 405 nm wave length. The percentage of the inhibitory activity was counted by using this formula:

$$\text{Inhibitory activity (\%) = \left( \frac{C-S}{C} \right) \times 100}$$

Where C = absorbance of enzyme activity without inhibitor (absorbance of DMSO), and S = absorbance of enzyme activity with sample examined.

RESULT AND DISCUSSION

α-glucosidase would hydrolyze p-nitrophenyl-α-D-glucopyranoside into glucose and p-nitrophenol which color is yellow. α-glucosidase inhibitory activity of ethanolic extract of brotowali stem was determined by the amount of p-nitrophenol obtained, that was measured at 405 nm wave length by using *Absorbance Microplate Reader* Elx800. The percentage of α-glucosidase inhibitory activity of the extract obtained from each concentration is 50 ppm; 27,54%, 150 ppm; 40,91%, 250 ppm; 49,19%, 350 ppm; 61,76%, 450 ppm; 78,34% while acarbose is 50 ppm; 47,05%, 150 ppm; 51,06%, 250 ppm; 58,82%, 350 ppm; 70,05%, 450 ppm; 81,01%. The IC₅₀ of ethanolic extract of brotowali stem is 237 ppm and acarbose is 116 ppm. The graphs are shown in Figure 1 until 4.

Table 1. IC₅₀ ethanolic extract of brotowali stem

<table>
<thead>
<tr>
<th>No.</th>
<th>Inhibitor</th>
<th>IC₅₀ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanolic extract of brotowali stem</td>
<td>237.26</td>
</tr>
<tr>
<td>2.</td>
<td>Acarbose</td>
<td>116.46</td>
</tr>
</tbody>
</table>

Figure 1. The percentage of α-glucosidase inhibitory activity of ethanolic extract of brotowali stem and acarbose as standard
CONCLUSION

The result of this study showed that percent inhibition of ethanolic extract of brotowali stem at 450 ppm is 78.34% equivalent to acarbose 81.01%. The IC₅₀ of ethanolic extract of brotowali stem is 237 ppm and acarbose is 116 ppm. In conclusion, ethanolic extract of brotowali stem has activity as α-glucosidase inhibitor.

REFERENCES