Int. J. Med. Arom. Plants, ISSN 2249 – 4340
Vol. 3, No. 2, pp. 144-150, June 2013

RESEARCH ARTICLE

# Screening of α-Glucosidase inhibitory activity of some Indonesian medicinal plants

**Abdul MUN'IM[1]*, KATRIN[1], AZIZAHWATI[2], Ari ANDRIANI[1], Kun Fitriana MAHMUDAH[1], Maya MASHITA[1]**

[1]*Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, University of Indonesia, Kampus UI Depok 16424 West Java, Indonesia.*

[2]*Department of Biochemistry, Faculty of Pharmacy, University of Indonesia, Kampus UI Depok 16424 West Java, Indonesia*

**Corresponding Author:** Tel.: +62-21-78849002, Fax: +62-21-7863433

**Article History**: Received 28th May 2013, Revised 13th June 2013, Accepted 14th June 2013.

**Abstract**: The aim of this research was to evaluate the inhibitory activity of α-glucosidase in 55 medicinal plants used as antidiabetic agent in Indonesia. Plants materials were extracted using ethanol, and then concentrated under reduce pressure. Inhibitory activity of α-glucosidase was evaluated by measuring the absorbance with spectrophotometry. Acarbose used as a positive control. The higgest α-glucosidase inhibitory activity demonstrated by *Terminallia catappa* L. fruit extract, followed by *Phaseolus vulgaris* L. seed extract, *Ceiba petandra* L. bark extract and *Swietenia mahagoni* (L.) Jacq seed extract. The result of phytochemical screening showed that the extracts with strong α--glucosidae inhibitory activity contain glycosides, flavonoids, terpenoids, and tannins. The higgest α-glucosidase inhibitory activity demonstrated by fruit of *T. catappa* with $IC_{50}$ of 3.02 α-g/ml.

**Keywords**: α-Glucosidase; inhibitory activity; diabetes mellitus; *Terminalia catappa; Phaseolus vulgaris; Ceiba petandra;* glycoside; saponin.

## Introduction

Diabetes is a growing health concern worldwide and now emerging as an epidemic world over. Approximately 171 million people in the world have diabetes in 2000 and these numbers estimated will reach 366 million people by 2030 (WHO 2004). According World Health Organization, Indonesia is fourth largest number people with DM. The prevalence of diabetes mellitus in Indonesia will increase from 8.4 million in 2000 into 21.3 million in 2030 (Wild et al. 2004).

Postprandial hyperglycemic plays important role in development of type II diabetes mellitus and chronic complications associated with the disease, such as micro- and macro vascular disorder or neuropathy (Well et al. 2009). Oral anti diabetic agents are first choice of treatment for controlled diabetes mellitus because they are more convenient for patients. One of the safest oral anti diabetic agent is α-glucosidase inhibitor which controls postprandial blood glucose level by inhibiting α-glucosidase so that reduces glucose intake because of inhibition of carbohydrate breakdown in intestine (Wells et al. 2009).

Screening of α-glucosidase inhibitor from plants and synthetic sources are increasing (Gholamhoseinan et al. 2008). Some researchers focused on unexplorated or ethnomedicinal plants (Elya et al. 2011; Ali et al. 2006). In Indonesia many plants traditionally have been used for treatment of diabetes mellitus (Anonymous 1986; Anonymous 2011). These plants can serve as source for searching of anti diabetic agent from natural products. In this study we try to examine α-glucosidase inhibitory activity from selected medicinal plants in Indonesia. We also identified the phytoconstituent of the plants with the best inhibition against α-glucosidase.

## Materials and methods

### *Plant materials*

*Corresponding author: (E-mail) abdul.munim61 <@> ui.ac.id

http://www.openaccessscience.com
ijmap@openaccessscience.com

Plant materials were obtained from Bogor Botanical Garden and Research Institute for Spice and Medicinal Crops Bogor, Indonesia. The samples were identified by Research Centre for Biology, Indonesian Institute of Science. The sample was rinsed with clean water, and then was sliced into small pieces and stored in drying chamber. Dried sample was grinded. Sample (20 gram) was macerated with 150 ml 80% ethanol for 16 hour and the procedure was repeated two times. The supernatant was concentrated under pressure on rotary vacuum evaporator.

*Phytochemical identification*

Qualitative identification of phytochemical constituent such as alkaloid, flavonoid, terpene, saponin, glycoside and anthraquinone in the extracts was performed by using standard analytical procedures with slight modification (Anonymous 1993; Harborne 1998). The analysis also was performed with TLC using specific spray reagents. Spray reagents used were Dragendorff for alkaloid; $AlCl_3$ for Flavonoid; and $FeCl_3$ for tannin (Waksmundzka-Hajnos et al. 2008).

*α-Glucosidase inhibititory activity assay*

The assay is modification procedure of Dewi et al. (2007). Enzyme solution (0,05 U/mL) was prepared by dissolving 4.0 mg α-glucosidase (*Saccharomyces cerevesiae*, Sigma-Aldrich, USA) into 100 mL phosphate buffer (50 mM, pH 6.8) contained 200 mg bovine serum albumin (Merck, Germany). Sample solution was prepared by dissolving 100 mg extract in DMSO (Dimethyl sulfoxide) and diluted with phosphate buffer (pH 6.8) until get concentration 0.5%, 0.25% and 0.125%.

The assay mixture consisted of 980 µL phosphate buffer (pH 6.8), 500 µL 10 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG, Sigma-Aldrich, Switzerland) and 20 µL extract solution. The assay mixture was incubated for 5 minutes in 37ºC. Then, 500 mL enzyme solution was added and the mixture was incubated for 15 minutes in 37ºC. Enzymatic reaction was stopped by adding 2 mL 0.2 M $Na_2CO_3$ solution. Absorbance value was measured by spectrophotometer UV-Vis on 400 nm.

α-Glucosidase inhibitory activity assay was performed *triplo* (three times) for each extract. Inhibition percentage were calculated by equation,

$$\%inhibition = \frac{B-S}{B} \times 100\% \quad \text{.....(1)}$$

with B is the difference between blank mixture absorbance ($B_1 - B_0$) and S is the difference between assay mixture absorbance ($S_1 - S_0$). $IC_{50}$ value can be calculated by using linear regression where sample concentration is X axis and inhibition percentage is Y axis. From y = a+bx equation, $IC_{50}$ value can be calculated by,

$$IC_{50} = \frac{50-a}{b} \quad \text{.....(2)}$$

*Kinetics of enzyme inhibition*

Michaelis-Menten kinetics was performed for three extracts which have highest inhibitory activity. The assay was performed according the above reaction conditions with inhibitor of various concentration. Michaelis-Menten constant was calculated using Lineweaver-Burk method, in which used linear regression equation, y = a+bx, where x is 1/(extract concentration) and y is 1/absorbance value. Inhibition type can be determined from Lineweaver-Burk plot (Murray et al. 2003).

## Results and discussion

*Alpha-glucosidase inhibitory activity assay*

The results of α-glucosidase inhibitory activity assay can be seen in Table 1. Twenty eight of fifty five extracts demonstrated stronger activity than acarbose, the commercial α-glucosidase inhibitor. Fuits of *Terminallia catappa* L., *Ceiba pentandra* L. barks and *Swietenia mahagoni* seeds, *Arachis hypogea* seeds, *Saccharum officinarum* roots, *Phaseolus vulgaris* seed, and *Anacardium occidentale* leaves extracts demonstrated the best α-glucosidase inhibitory activity. In this study, *Glycine max* seeds and *Momordica charantia* fruits extracts showed no inhibitory activity against alpha-glucosidase.

**Table 1:** Inhibitory activity of the extracts against α-glucosidase.

| S.No. | Extract | Family | $IC_{50}$ (ppm) |
|---|---|---|---|
| 1 | *Barleria prionitis* L. (leaves) | Acanthaceae | 501.37 |
| 2 | *Barleria prionitis* L. (roots) | Acanthaceae | 319.75 |
| 3 | *Barleria prionitis* L. (stems) | Acanthaceae | 837.80 |
| 4 | *Ruellia tuberosa* (leaves) | Acanthaceae | 98.5 |
| 5 | *Sericocalyx crispus* L. (leaves) | Acanthaceae | 275.63 |
| 6 | *Aerva sanguinolenta* Blume. (aerial parts) | Amaranthaceae | 516.68 |
| 7 | *Anacardium occidentale* L (leaves) | Anacardiaceae | 9.11 |
| 8 | *Annona squamosa* L. (leaves) | Annonaceae | 90.47 |
| 9 | *Anethum graveolens* L.(fruits) | Apiaceae | 433.31 |
| 10 | *Coriandrumsativum* L.(seeds) | Apiaceae | 227.38 |
| 11 | *Alstonia scholaris* (L.) R.Br. (barks) | Apocynaceae | 319.08 |
| 12 | *Catharanthus roseus* L. G. Don (leaves) | Apocynaceae | 36.08 |
| 13 | *Ervatamia divaricata* (L.) Burkill (leaves) | Apocynaceae | 137.29 |
| 14 | *Blumea balsamifera* (L.) DC (leaves) | Asteraceae | 28.01 |
| 15 | *Cosmos caudatus* Kunth (aerial parts) | Asteraceae | 58.4 |
| 16 | *Basella alba* L.(leaves) | Basellaceae | 493.47 |
| 17 | *Bixa orellana* L.(seeds) | Bixaceae | 28.61 |
| 18 | *Ceiba pentandra* L. (barks) | Bombaceae | 5.16 |
| 19 | *Brassica oleracea* L. ( leaves) | Brassicaceae | 439.38 |
| 20 | *Brassica juncea* (leaves) | Brassicaceae | 541.71 |
| 21 | *Raphanus sativus*L. (aerial parts) | Brassicaceae | 729.12 |
| 22 | *Beta vulgaris* L.(leaves) | Chenopodiaceae | 339.17 |
| 23 | *Terminalia catappa* L. (fruits) | Combretaceae | 3.02 |
| 24 | *Luffa cylindrical* (L.) M.J. Roemer (seeds) | Cucurbitaceae | 17.46 |
| 25 | *Momordica charantia* L. (fruits) | Cucurbitaceae | 1861.99 |
| 26 | *Sechium edule* (Jacq.) Sw (fruits) | Cucurbitaceae | 484.05 |
| 28 | *Dioscorea hispida* Dennst. (tubers) | Dioscoriaceae | 26.05 |
| 29 | *Jatropha curcas* L. (leaves) | Euphorbiaceae | 29.67 |
| 30 | *Cassia alata* L. (leaves) | Fabaceae | 50.54 |
| 31 | *Glycine max* Merr (seeds) | Fabaceae | 6,645.97 |
| 32 | *Phaseolus vulgaris* L. (seeds) | Fabaceae | 4.83 |
| 33 | *Ocinum americanum* L. (Aerial parts) | Lamiaceae | 80.78 |
| 34 | *Orthosiphon aristatus* Miq. (leaves) | Lamiaceae | 373.91 |
| 35 | *Persea americana* Mill (barks) | Lauraceae | 10.83 |
| 36 | *Allium cepa* L. (tubers) | Liliaceae | 50.58 |
| 37 | *Allium sativum* L. (tubers) | Liliaceae | 193.00 |
| 38 | *Aloe barbadensis* Mill (leaves) | Liliaceae | 98.56 |
| 39 | *Azadirachta indica* A. Juss (leaves) | Melliaceae | 21.94 |
| 40 | *Swietenia mahagoni* (L.) Jacq. (seeds) | Meliaceae | 7.03 |
| 41 | *Tinospora crispa* Miers.(leaves) | Menispermaceae | 68.06 |
| 42 | *Tinospora crispa* Miers. (stems) | Menispermaceae | 22.99 |
| 43 | *Ficus carica* L. (leaves) | Moraceae | 177.92 |
| 44 | *Ficus carica* L.(barks) | Moraceae | 112.84 |
| 45 | *Ficus glomerata* Roxb. (fruits) | Moraceae | 40.62 |
| 46 | *Ficus glomerata* Roxb. (barks) | Moraceae | 183.30 |
| 47 | *Plantago major* L (leaves) | Plantaginaceae | 1,173.17 |
| 48 | *Coix lachryma-jobi* L. (leaves) | Poaceae | 203.00 |
| 49 | *Coix lachryma-jobi* L. (seeds) | Poaceae | 371.08 |
| 50 | *Saccharum officinarum* (roots) | Poaceae | 10.35 |
| 51 | *Punica granatum* L. (pulp-seeds) | Punicaceae | 209.81 |
| 52 | *Nigella sativa* L. (seeds) | Ranunculaceae | 144.13 |
| 53 | *Morinda citrifolia* L. (leaves) | Rubiaceae | 187.19 |
| 54 | *Physalis angulata* L. (leaves) | Solanaceae | 55.89 |
| 55 | *Tectona grandis* L.(leaves) | Verbenaceae | 87.38 |
| 56 | Acarbose | | 117.06 |

*Pytoconstituent assay*

Ethanolic extract of samples which showed the best α-glucosidase inhibitory activity was identified their phytoconstituent. Identification of chemical group was performed by using Bouchardat, Mayer, Dragendorff reagent for

alkaloid; Shinoda and Wilson Toubock reaction for flavonoid; gelatin, gelatin-salt test and ferrous (III) chloride test for tannin; Mollisch test for glycoside; honeycomb froth test for saponin; Brontrager reaction for anthraquinone; and Liebermann-Buchard reagent for terpenoid. and Molisch reagent for glycoside. The results of chemical identification can be seen in Table 2. In this preliminary identification the extracts were detected contained alkaloid, flavonoid, glycoside, tannin, saponin, and terpen. The existence of anthraquinone was not detected in all extracts.

**Table 2:** Phytochemical constituent present in the extract with potent α-glucosidase inhibitory activity.

| No | Extract | Composition | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Alkaloid** | **Flavonoid** | **Terpen** | **Tannin** | **Saponin** | **Glycoside** | **Anthraquinone** |
| 1 | *Anacardium occidentale* L. (leaf) | - | + | + | + | + | + | - |
| 2 | *Arachis hypogaea* L. (seed) | - | + | - | + | - | - | - |
| 3 | *Ceiba pentandra* L. (bark) | + | + | - | + | + | + | - |
| 4 | *Luffa cylindrical* (L.) M.J. Roemer (seed) | + | - | - | - | + | + | - |
| 5 | *Persea americana* Mill (bark) | + | - | - | + | + | + | - |
| 6 | *Phaseolus vulgaris* L. (seed) | - | + | + | + | - | + | - |
| 8 | *Saccharum officinarum* (root) | - | - | + | + | + | + | - |
| 9 | *Swietenia mahagoni* (L.) Jacq. (seed) | - | + | + | - | + | + | - |
| 10 | *Terminalia catappa* L. (fruit) | + | - | + | + | + | + | - |

*Kinetic inhibition mode*

Evaluation of enzyme kinetics was investigated on three extracts which had highest inhibitory activity. The inhibition kinetics of *T. catappa* L. fruit, and *P. vulgaris* L. seed extracts were analyzed by Lineweaver-Burk plots as shown as Figure 1. Extracts derived from *T. catappa* and *P. vulgaris* showed competitive inhibitory mode.

Many Indonesian medicinal plants have been used traditionally to treat diabetes mellitus and some of them had been studied for their activities in controlling blood glucose level in animal model (Anonymous 1986; Anonymous 2011), eventhough mechanism of action from those activities has not yet been studied. One of the mechanisms of action in controlling blood glucose level, especially for postprandial blood glucose level, is inhibition of α-glucosidase in intestine. Concerning this, our research studied the inhibitory activity of α-glucosidase from selected Indonesian medicinal plants.

Sixty four ethanolic extracts prepared from fifty eight plants were tested in α-glucosidase inhibition assay. α-Glucosidase inhibitory activity of the extracts were determined using p-nitrophenyl-α-D-glucopyranoside (p-NPG) as substrate and α-glucosidase from *S. cerevisiae*. More than half on the plants extracts showed stronger inhibition when compared to acarbose as control. Acarbose exhibited only low inhibition activity against *S. cerevisiae* α-glucosidase. This is consistent result as previous studies (Kim et al. 2008; Shinde et al. 2008).

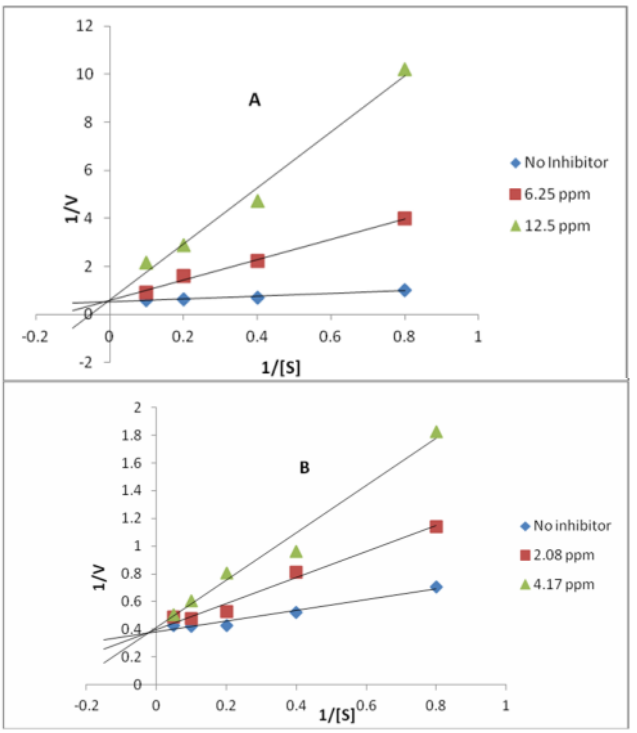


**Figure 1:** Lineweaver-Burk Plot of α-glucosidase inhibition of *T. catappa* (A) and *P. vulgaris* extracts (B) at varied concentrations of PNPG and extracts.

*Ceiba petandra* is tropical tree; the bark empirically was used for treatment diabetes. The antidiabetic activity has been investigated on streptozotocin-induced non-insulin-dependent diabeteic rats (Ladeji et al. 2003). In this study, the extract demonstrated strong α-glucosidase inhibitory activity. Isoflavones, the phytoconstituent of the bark of this plant have been isolated, but those compounds showed no activities against α-glucosidase (Ueda et al. 2002). It is therefore clear that the effective α-glucosidase inhibitory component in this plant is worthy of further studies.

In this study *Swietenia mahogany* seeds extract showed strong α-glucosidase inhibitory activity. The seeds are well known in Indonesia for treatment of diabetes mellitus. The hypoglycemic effect has been scientifically evaluated in streptozotosin and nicotinamid induced type II diabetic rats (Maiti et al. 2008). Based on the significant increase of liver glycogen levels in the treated diabetic animals, the hypoglycemic effect of mahogany seeds extract was suggested due to the reactivation of the glycogen synthase system (Maiti et al. 2008). The results of our study gave addition information about another mechanism of action of mahogany seeds on the reduction of glucose blood level.

Alpha glucosidase inhibitory activity of extract of *T. catappa* was investigated. The extract showed strong activity against α-glucosidase. The fruits of this plant have been reported to produce significant antidiabetic activity on alloxan-induced rats (Naggappa et al. 2003). Their mechanism on lowering blood glucose has not been reported, yet. Until now, there is no study about chemical constituents of this fruits. The isolation and elucidation of potential α--glucosidase inhibitor is promising for further study due to the lack information about the biological activity and chemical content of these fruits.

The fruits of *Momordica charantia* are used as vegetable in many countries. The fruits traditionally have also been used for treatment diabetes mellitus. Their antidiabetic activities were confirmed by *in vivo* and clinical studies (Cathurvedi 2012). However, in this study *M. charantia* extract showed no inhibitory activity against alpha glucosidase. So this plant might have other mechanism for reduction of blood glucose, such as insulin mimetic property (Patal et al. 2012).

On the phytochemical analysis, extracts with the best strong α-glucosidase inhibitory activity were generally identified contain of alkaloid, flavonoid, glycoside, tannin, terpenoid, saponin and glycoside. *T. catappa* and *A. ocidentale* have been known as tannin-rich plants. The plants are rich in tannin contents may have an effect on the enzyme activity. Further study is needed to clarify the effect of tannin on α-glucosidase activity.

Inhibition modes of the extracts which have potential α-glucosidase inhibition were investigated. All the extracts showed competitive inhibition mode. These results indicated that the extracts affected the affinity of the enzyme for the substrate, *p*-NGP, via binding at the active site (Murray et al. 2003).

Inhibition of α-glucosidase enzyme was one of diabetes mellitus treatment approaches by controlling postprandial blood glucose which glucose intake to blood circulation is inhibited. Searching of new α-glucosidase inhibitor or other anti diabetic agents is important to conduct to improve diabetes mellitus treatment. In this study, some extracts showed to be effective inhibitor against α-glucosidase. Those plants are prospective for development new antidiabetic agent via inhibition of α-glucosidase. The isolation and the structural elucidation of active constituents of the extracts will provide useful leads in the development of antidiabetic agents with mechanism α-glucosidase inhibition. Therefore, purification of the effective α-glucosidase inhibitory compound in *T. catappa, S. mahogany* and *P. vulgaris* await further study.

## Conclusion

Some medicinal plants showed potent α-glucosidase inhibitory activity. The results confirmed their usage in traditional medicine for management of diabetes. The highest α-glucosidase inhibitory activity demonstrated by fruits of *T. catappa* with $IC_{50}$ of 3.02 □g/ml. The extract contained alkaloid, flavonoid,

terpene, saponin, tannin and glycoside. Meanwhile, type of enzyme inhibition mechanism from the extract was competitive inhibitor.

**Acknowledgement:** This study was financially supported by Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia through RM UI 2012.

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