International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (4): 277-280, 2009
ISSN 1811-7775

α-Glucosidase Inhibitor Activity of *Terminalia* Species

[1]K. Anam, [2]R.M. Widharna and [1]D. Kusrini
[1]Department of Chemistry, Diponegoro University, Semarang, Indonesia
[2]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

Abstract: *Terminalia* arjuna, *Terminalia ballerica*, *Terminalia chebula*, *Terminalia catappa*, *Terminalia kaerbachii* and *Terminalia microcarpa* leaves were tested for their α-glucosidase inhibitory activity *in vitro*. The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm. *Terminalia kaerbachii* has the highest α-glucosidase inhibitor activity with IC_{50} value of 0.27±0.17 μg mL^{-1} and is a promising antidiabetic herbal medicine candidate. However, most of the *Terminalia* species are also potential as antidiabetic medicine candidates as the IC_{50} values are approximately 5 μg mL^{-1}, which is near the IC_{50} value of 1-deoxynojirimycin, the reference compound, except for *Terminalia microcarpa*, which has IC_{50} value of 25.15±0.04 μg mL^{-1} (above 21 μg mL^{-1}). From the phytochemical screening, *Terminalia kaerbacchi* contains alkaloids, flavonoids and cathechic tannins, but does not contain saponin, quinon, steroid/terpenoids and gallic tannins. It is estimated that there is a correlation between α-glucosidase inhibitory activity and its phytochemical content.

Key words: *Terminalia*, α-glucosidase inhibitor activity, phytochemistry screening

INTRODUCTION

Diabetes is a common metabolic disease characterized by abnormally high plasma glucose levels, leading to major complications, such as diabetic neuropathy, retinopathy and cardiovascular diseases (Gao *et al*., 2008). More than 171 million people worldwide are currently believed to be afflicted with type 2 diabetes and it is estimated that the number will rise to 366 million by 2030 (Shinde *et al*., 2008). The key enzyme which catalyses the final step in the digestive process of carbohydrates in mammalian is α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20), which is located in the brush-border surface membrane of intestinal cells. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppressed postprandial hyperglycaemia (Gao *et al*., 2008). Consequently, α-glucosidase inhibitors, such as acarbose (Balfour and McTavish, 1993) and miglitol (Pogano *et al*., 1995) have been approved for clinical use in the management of type 2 diabetes, as well as the treatment of obesity.

Therefore, the search for effective and safe α-glucosidase inhibitors from natural materials, in order to develop a physiological functional food or lead compounds for antidiabetic agents is necessary.

Terminalia is a flowering plant in family of Combretaceae. Example of traditional use of *Terminalia* species is mentioned as follows, *Terminalia chebula* is used in treatment of fevers, cough, asthma, urinary diseases, piles and worms and is also useful in treating chronic diarrhoea and dysentery, flatulence, vomiting, colic and enlarged spleen and liver (Rao and Nammi, 2006). *Terminalia chebula* extracts had different levels of antioxidant activity for anti-LPO, anti-superoxide radical formation and free radical scavenging activities (Rao and Nammi, 2006). *Terminalia arjuna* is a large tree distributed throughout India and its bark is used as a cardioprotective agent in hypertension and ischaemic heart diseases. The bark powder is reported to exert hypocholesterolaemic and antioxidant effect in humans (Masoko and Eloff, 2007).

Therefore, in order to search a new plant-based medicine to inhibit α-glucosidase in diabetes type II and to find the correlation between the phytochemical compound and its pharmacological activity, this experiment was conducted.

MATERIALS AND METHODS

Materials: *Terminalia arjuna*, *Terminalia ballerica*, *Terminalia chebula*, *Terminalia catappa*, *Terminalia kaerbachii* and *Terminalia microcarpa* leaves were collected from Bogor Botanical Garden Indonesia, in May 2008; ethanol, 1-deoxynojirimycin (Sigma, D9305),

Corresponding Author: Khairul Anam, Department of Chemistry, Faculty of Mathematics and Sciences, Diponegoro University, Jl. Prof. Soedarto SH, Tembalang, Semarang 50275, Indonesia

α-glucosidase (Sigma, G0660-750UN), p-nitrophenyl glucopyranoside [pNPG] (Sigma, N1377) Na_2CO_3 (Sigma, S7795), phosphate buffer (Sigma, P5244).

Equipment: Plant grinding machine, digital scale (Mettler, Toledo AG204), maceration equipments, electric stove, volume pippettes, measuring pippettes, rotavapor (Buchi Rotavapor R-124 Buchi Waterbath B-480), water pump, distillation equipments, glasswares, spektrophotometer UV (Hewlett Packard 8452A).

Extraction methods: One hundred grams of the dried leaves were macerated using 3×500 mL ethanol 96% for 3×24 h. The filtrate obtained was concentrated under vacuum on a rotary evaporator at 40°C and then stored at 4°C for further use.

Inhibition assay for α-glucosidase activity: The inhibition assay for α-glucosidase activity was conducted in June 2008 at Research Center for Chemistry, Indonesian Institute of Sciences, Serpong. α-glucosidase (0.075 unit) was premixed with the extracts at various concentrations (0.01 -200 μg mL^{-1}). About 3 mM p-nitrophenyl glucopyranoside (pNPG) as a substrate in phosphate buffer was added to the mixture to start the reaction. The reaction was incubated at 37°C for 30 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm. The IC_{50} value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions (Kim *et al*., 2004).

Phytochemical screening: The phytochemical screening of the *Terminalia* species was conducted according to Materia Medika Indonesia procedures (Ditjen and Depkes, 1989).

RESULTS AND DISCUSSION

Terminalia species was considered as potential α-glucosidase inhibitor as the IC_{50} values are below or approximately 5 μg mL^{-1} which was comparable to 1-deoxynojirimycin as the reference compound. The result of the α-glucosidase inhibitory activity of the *Terminalia* species is as seen in Table 1.

In order to prove the estimation that there is a correlation between phytochemical content of the *Terminalia* species and its antidiabetic activity, the phytochemical screening of *Terminalia* was conducted. All of the *Terminalia* species tested contains flavonoids and mostly contains steroids/terpenoids except *Terminalia kaerbachii*. The result of the phytochemical screening of *Terminalia* species is are shown in Table 2.

Based on the experiment result, *Terminalia kaerbachii* is the most active α-glucosidase inhibitor, followed by *Terminalia catappa*, *Terminalia arjuna*, *Terminalia chebula* and *Terminalia ballerica*. On the other hand, *Terminalia microcarpa* does not have any α-glucosidase inhibitory effect as the IC_{50} value is above 21 μg mL^{-1}.

Miglitol is one of α-glucosidase inhibitors used to treat diabetes type 2. The chemical compound of miglitol is 1-deoxynojirimycin. In this *in vitro* experiment, 1-deoxynojirimycin is used as a reference compound as it is the most suitable reference compound. Shinde (2008) demonstrated that in *in vitro* α-glucosidase inhibitor activity assay using Baker's yeast and *Bacillus stearothermophilus*, the reference compound 1-deoxynojirimycin had the IC_{50} value of 83.4±2.1 and 0.175±0.003 μg mL^{-1}, respectively. While acarbose showed no inhibition of α-glucosidase in Baker's yeast and *B. stearothermophilus*, but it has the IC_{50} of 210±1.2 and 233±4.0 μg mL^{-1}, respectively for sucrose and maltase in rat intestinal enzymes. It is concluded that acarbose is

Table 1: Inhibitory concentration of *Terminalia* species and 1-deoxynojirimycin as reference compound

Extracts	Average of IC_{50}±SD (μg mL^{-1})
Terminalia arjuna	5.41±0.08
Terminalia bellerica	7.02±0.56
Terminalia chebula	6.32±0.40
Terminalia catappa	3.43±0.98
Terminalia kaerbachii	0.27±0.17
Terminalia microcarpa	25.15±0.04
1-Deoxynojirimycin	5.00±0.00

Table 2: Phytochemical screening of *Terminalia* leaves

Phytochemical content	*T. kaerbachii*	*T. cattapa*	*T. arjuna*	*T. chebula*	*T. bellerica*	*T. microcarpa*
Alkaloids	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	-	+	+	-	-	-
Quinone	-	+	+	+	-	-
Steroids/terpenoids	-	+	+	+	+	+
Gallic taunins	-	-	-	+	+	+
Cathecic tannins	+	+	+	+	+	+

+: Present, -: Absent

appropriate as the reference compound for *in vivo* experiment and 1-deoxynojirimycin for *in vitro* experiment, due to the difference of the enzymes involved and the solvability of acarbose and 1-deoxynojirimycin in the solvent.

Based on the result tabulated in Table 1, *Terminalia kaerbacchii* is the most active α-glucosidase inhibitor and it is estimated due to its chemical contents is different from other *Terminalia* species. This is an important information as *Terminalia kaerbacchii* has not been evaluated for its α-glucosidase inhibitory activity previously.

In order to prove the estimation that there is a correlation between phytochemical content of the *Terminalia* species and its antidiabetic activity, the phytochemical screening of *Terminalia* was conducted.

Gao *et al*. (2008) reported the isolation of maltase inhibitory principles, chebulanin, chebulagic acid and chebulinic acid from the fruits of *Terminalia chebula*. As seen in Table 1, *Terminalia chebula* contains tannins, hence, the finding supports Gao's idea that the antidiabetic activity of *Terminalia chebula* is due to the maltase inhibitory principles.

Bajpai *et al*. (2005) stated that the leaves, bark and fruits of *Terminalia arjuna*, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia muelleri* had high total phenolic contents (72.0-167.2 mg g^{-1}) and high antioxidant activity (69.6-90.6%). Fruits of *T. bellerica* and *T. chebula* were a rich source of gallic acid, meanwhile bark of *T. arjuna*, leaves and fruits of *T. bellerica* and bark, leaves and fruits of *T. muelleri* were a rich source of ellagic acid.

Terminalia bellerica contains triterpenoids and glucosides. It has been used for the treatment of various ailments (Chadha, 1976) and has been reported to contain β-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, mannitol, glucose, galactose, fructose and rhamnose (Nandy *et al*., 1989). It also contains triterpene, belleric acid and its glucoside, bellericoside and arjungenin and its glucoside (Nandy *et al*., 1989). The extracts of *Terminalia bellerica* Roxb fruits contains phenols, flavonoids, glycosides, saponin, tannin. While, the fruits of *Terminalia chebula* Retz. which is also with common name of Harir, contains phenols, glycosides and saponin (Farrukh *et al*., 2006).

Sabu and Kuttan (2002) evaluated the combination of *Terminalia chebula*, *Terminalia belerica*, *Emblica officinalis*, known as Triphala for their antidiabetic activity and their relation with their antioxidant activity. *T. belerica*, was found to be most active plant to reduce serum glucose level followed by *E. officinalis* and *T. chebula*. Triphala which is a combination of all the three produced a significant action in reducing the alloxan induced diabetic. The result is slightly different with the result in our experiment, where in our experiment, *T. chebula* has a higher α-glucosidase inhibitory activity compared to *T. belerica*. This might be due to the location where the plants grew up and the experiment by Sabu and Kuttan (2002) was conducted *in vivo*, while in present experiment *in vitro*.

In another experiment by Rao and Nammi (2006), the chloroform extract of the seeds of *T. chebula* were evaluated for their antidiabetic properties. They are basing their experiment on the facts that *Terminalia chebula* fruits and whole powder of dried ripe fruits are known for their antidiabetic properties. The evaluation data also confirmed the traditional indications. The seed extract of *T. chebula* indicated a potent action in short term study and a prolonged duration of antidiabetic action in long term study and this could be due to multiple sites of action possessed by the active principles of *T. chebula*. The study also revealed that *T. chebula* is more effectively inhibited the incidence of diabetic nephropathy (Rao and Nammi, 2006).

The study by Ahmed *et al*. (2005), indicates that *Terminalia catappa* leaves extracts have antidiabetic activity. Aqueous and cold extracts of *Terminalia catappa* exhibited significant anti hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight. The number of functionally intact β-cells in the islet organ is of decisive importance the development course and outcome of diabetes mellitus. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug and *Vinca rosea* extract have also shown to act by β-cell re-generation (Ahmed *et al*., 2005).

The damage to pancreas in alloxan-treated diabetic control and regeneration of β-cells by glibenclamide was observed. A comparable regeneration was also shown by aqueous and cold extracts of *Terminalia catappa*. This effect may be due to β-carotene, which was reported to be constituents of *Terminalia catappa* (Duke, 1992). Photomicrographical data in the studies confirms healing of pancreas by *Terminalia catappa* leaves extracts, as a plausible mechanism of their anti diabetic activity (Ahmed *et al*., 2005).

Aqueous and cold extract of *Terminalia catappa* leaves exhibited significant anti hyperglycemic activities in alloxan-induced diabetic rats. These extracts showed improvement in parameters like body weight and

lipid profile as well as regeneration of β-cells of pancreas and so might be of value in diabetes treatment (Ahmed *et al*., 2005).

As mentioned earlier, there is a correlation between phytochemical content and antidiabetic properties. In order to have thorough information of the compound responsible for the antidiabetic properties, isolation of the active phytochemical compound of the *Terminalia kaerbachii* is being conducted, which will be followed by the antidiabetic *in vivo* study.

CONCLUSION

Terminalia kaerbacchii is the most active α-glucosidase inhibitor compared to other *Terminalia* species, which are also known to have antidiabetic properties.

REFERENCES

Ahmed, S.M., S.B.M. Vrushabendra, P. Gopkumar, R. Dhanapal and V.M. Chandrashekara, 2005. Anti-Diabetic activity of *Terminalia catappa* Linn. Leaf extracts in alloxan-induced diabetic rats. Iran. J. Pharm. Therapeutics, 4: 36-39.

Bajpai, M., A. Pande, S.K. Tewari and D. Prakash, 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. Int. J. Food Sci. Nutr., 56: 287-291.

Balfour, J.A. and D. McTavish, 1993. Acarbose-an update of its 6. pharmacology and therapeutic use in diabetes-militus. Drugs, 46: 1025-1054.

Chadha, Y.R., 1976. The Wealth of India. Council of Scientific and Industrial Research, New Delhi, pp: 164.

Ditjen, P.O.M. and R.I. Depkes, 1989. Materia Medika Indonesia. Depkes Publishers, Jakarta, pp: 15-19.

Duke, J.A., 1992. Handbook of Phytochemical Constituents of GRAS Herb and other Economic Plants. CRC press, Boca Raton, FL.

Farrukh, A., I. Ahmad and Z. Mehmood, 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol., 30: 177-183.

Gao, H., Y.N. Huang, B. Gao, P. Li, C. Inagaki and J. Kawabata, 2008. Inhibitory effect on α-glucosidase by *Adhatoda vasica* Nees. Food Chem., 108: 965-972.

Kim, Y.M., M.H. Wang and H.I. Rhee, 2004. A novel α-glucosidase inhibitor from pine bark. Car Res., 339: 715-717.

Masoko, P. and J.N. Eloff, 2007. Screening of twenty-four South African combretum and six terminalia species (Combretaceae) for antioxidant activities. Afr. J. Trad. CAM, 4: 231-239.

Nandy, A.K., G. Podder, N.P. Sahu and S.B. Mahato, 1989. Triterpenoids and their glucosides from *Terminalia Bellerica*. Phytochemistry, 28: 2769-2112.

Pogano, G., S. Marena, L. Corgiat-Mansin, F. Cravero, C. Giorda and M. Bozza, 1995. Comparison of miglitol and glibenclamide in diet-treated type 2 diabetic patients. Diabetes Metab., 21: 162-167.

Rao, N.K. and S. Nammi, 2006. Antidiabetic and renoportective effects of the chloroform extract of *Terminalia chebula* retz. Seeds in streptozotocin-induced diabetic rats. BMC CAM, 6: 17-17.

Sabu, M.C. and R. Kuttan, 2002. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethanopharmacol., 81: 155-160.

Shinde, J., T. Taldone, M. Barletta, N. Kunaparaju and B. Hu *et al*., 2008. α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.). Skeels seed kernel *in vitro* and in Goto-Kakizaki (GK) rats. Car Res., 343: 1278-1281.