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Research Article

DIPEPTIDYL PEPTIDASE-IV INHIBITORY ACTIVITY OF SOME INDONESIAN MEDICINAL PLANTS

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ABSTRACT

Objective: The focus of this research is to determine the inhibitory activity of ethanol extracts from 20 plants that have been used traditionally in Indonesia for the treatment of diabetes for dipeptidyl peptidase-IV (DPP-IV) inhibitory activity.

Methods: A crude drug was extracted by reflux methods using 96% ethanol for 1 hr. The assay was performed with modification methods of Al Masri *et al*. using 96 micro well plates, and the absorbance was measured at 380 nm using the microplate reader. Sitagliptin was used as a standard inhibitor DPP-IV.

Results: About 5 of 20 ethanol extracts showed the ability to inhibit DPP-IV activity *in vitro* at a concentration of 2.5 ug/ml. The active extract was obtained from pomegranates rind, bungur leaves, brotowali stem, bodhi leaves, and fenugreek seeds. The inhibitory percentage of the fifth extract was 58.79±2.23, 60.22±2.01, 65.86±0.02, 68.98±1.95, and 71.29±0.33. The IC50 value of sitagliptin was 1.104 ug/ml.

Conclusion: The results of *in vitro* assay showed that some extract inhibited DPP-IV. The fenugreek seed showed the highest inhibition to DPP-IV.

Keywords: Dipeptidyl peptidase-IV, Dipeptidyl peptidase-IV inhibitory, Fenugreek seed, Sitagliptin.

INTRODUCTION

Diabetes mellitus is a global health problem that requires serious treatment. Based on the predictions of the International Diabetes Federation (IDF), patients with diabetes around the world will increase from 366 million, in 2011, to 552 million, in 2030. In Southeast Asia, it was predicted to increase from 71.4 million diabetics, in 2011, to 120. 9 million, in 2030. Diabetes conditions that are not handled properly quite serious complications such as cardiovascular disease, blindness, and kidney damage; therefore, it requires a fairly serious treatment [1].

Dipeptidyl peptidase-IV (DPP-IV, EC. 3.4.14.5) was found, in 1966, under the name glycylprolyl ß-naphthylamidase, which releases glycylprolin that produces ß-naphthylamine which can be detected by a colorimetric method. DPP-IV is a group of serine proteases and present in high concentrations in the luminal membrane of intestinal epithelial cells. DPP-IV work very rapidly degrades the incretin hormones which play a role in the regulation of glucose homeostasis. It is a peptide incretin hormone secreted by the intestinal epithelium and respond to oral glucose. Incretin will increase insulin secretion by activation of a specific receptor on pancreatic beta cells. Glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are metabolized into an inactive form rapidly by the enzyme DPP-IV (about 1-2 minutes), and only about 10-15% of circulating actively stimulate the pancreas. Activation of the GLP-1 receptor will cause a reduction in glucagon secretion, inhibiting gastric emptying and induces a satiety [2,3].

The development of antidiabetic that works by activating the GLP-1 was having problems, because GLP-1 is very rapidly degraded and deactivated by the enzyme DPP-IV [4-6].

Inhibitor DPP-IV group is now widely used for treating type 2 diabetes mellitus. The FDA has approved sitagliptin as an important new therapy for the treatment of type 2 diabetes mellitus, in 2006. DPP-IV inhibitors act by stimulating insulin secretion indirectly by increasing incretin hormones, GLP-1, and GIP. GLP-1 stimulates insulin biosynthesis, inhibits the formation of glucagon and can slow gastric emptying. GLP-1 to stimulate the regeneration and differentiation of pancreatic beta cells [1,7,8]. The treatment of type 2 diabetes mellitus based on incretin hormone is growing, especially sitagliptin. Sitagliptin has some advantages compare to other diabetes therapies such as lowering the risk of hypoglycemia, weight loss and the potential for regeneration and differentiation of pancreatic beta cells [7-9].

Plants as traditional medicine have been used in Indonesia for generations. Some herbs were known to be used in treating diabetes, and some researches were carried out *Coctus speciosus* Smith, *Arthocarpus heterophyllus* Lamk, *Momordica charantia* L., *Ficus religiosa* BL., *Annona squamosa* L., *Pterocarpus indica* Willd, *Allium sativum* L., and *Trigonella foenum-graecum* L. [9]. Some herbs that have been studied as an inhibitor of the enzyme DPP-IV *in vitro* include the leaves of mango (*Mangifera indica* L.) and the bark of *Berberis aristata* [10,11]. However, a few experimental study report on the DPP-IV activities from Indonesian medicinal plants that had been used to lower blood glucose level traditionally. In this research, screening inhibitory activities of DPP-IV from 20 ethanol plant extract that has traditionally been used to lower blood glucose levels. The assay was performed as Al Masri *et al*. with slight modifications, using 96 micro well plates and sitagliptin was used as a standard inhibitor.

METHODS

A total of 20 plants of Indonesian traditional medicines were collected from several place in West Java, Indonesia. *Euphorbia hirta* L. from Cianjur, *Punica granatum, F. religiosa* L. from Dago, Bandung. *Swietenia mahagoni* King, *Andrographis paniculata* (Burm.f.) Wall, *Tithonia diversifolia* (Hemsley) A. Gray, *M. charantia* Descourt, *Azadirachta indica* A. Juss, *Pterocarpus indicus* Willd, *Persea americana* Mill, *A. heterophyllus* Lamk, *Ruellia tuberosa* L, *Euphorbia cotinifolia, Lagerstroemia loudonii* Teijsm & Bin, *Tinospora crispa* (L.) Miers, *Smallanthus sonchifolius*, *C. speciosus, Merremia mammosa* Hall.f., Physalis angulata L., from Manoko Garden, Lembang, and *T. foenum-graecum* L. A part of plant

collecting based on empirical usages. The plant materials were washed, dried, and grounded to small pieces.

Chemical material

DPP4 from human, Gly-Pro-p-Nitroanilide (GPPN), Tris-HCl Buffer, all chemicals purchased from Sigma. Sitagliptin standard (PT. Kimia Farma Tbk, Indonesia).

Preparation of extracts

Each crude drug was extracted by reflux methods using 96% ethanol for 1 hr. Reflux process was repeated up to 3 times; the extract was evaporated to obtain a thick extract.

DPP-IV assay (*in vitro*)

The assay was performed based on Al Masri *et al*. [12] with slight modifications. The assay was performed in micro well plates with 96 holes and read using microplate reader. Sitagliptin as a standard of inhibitor DPP-IV was made in various concentrations (0.2; 0.4; 0.8; 1.6; and 3.2 ug/ml) in a 50 mM Tris HCl buffer (pH 7.5) the volume used was 35 µl, The substrate was chromogenic GPPN (0.2 mM in Tris HCl buffer pH 7.5), cleaved by DPP-IV a serine protease that releases paranitroanilide (pNA) a yellow colored product which was measured at 380 nm. The enzyme solution of DPP-IV (0.05 units/ml) 15 µl was added in micro well plates, and 35 µl of sample extracts with varying concentrations were then incubated for 15 minutes at 37°C. Substrate GPPN 50 µl (0.5 mM) was added to the mixture solution and then incubated for 30 minutes at 37°C. Glacial acetic acid 25% (25 µl) was added to the mixture to stop the enzymatic reaction; the absorbance was measured at 380 nm using the microplate reader. The total volume of the solution in the micro-well plate is 125 mL. The results were then compared with a negative control (without inhibitor). Tests were performed for 3 times. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 µ mol of pNA from the substrate/min in the experimental conditions.

The percentage of inhibition was calculated using the following formula:

$$\%\text{inhibition} = \frac{[\text{absorbance of control} - \text{absorbance of inhibitor}]}{\text{absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

DPP-IV inhibitor

The 20 plant extracts were determined for DPP-IV inhibitory activity. Some of the extracts gave good activities to inhibiting DPP-IV. Some parts of the plant used in this study were the leaves, fruits, seeds, stems, and rind. The plants are selected based on empirical use in the community in dealing with diabetes. The result of DPP-IV inhibition activity of 20 plant extracts was summarized in Table 1. The highest inhibitory percentage was given by the ethanol extract of fenugreek seeds of *T. foenum-graecum* L., followed successively by bodhi leaf *F. religiosa* L., brotowali *T. crispa* Miers ex Hoff.f, bungur leaves of *L. loudonii* Teijsm. & Binn, and pomegranate rind *P. granatum* L. The results explained inhibitory activities on DPP- IV and may have therapeutic potential on type 2 diabetes. Other plant extracts exhibited a very weak inhibitory activities. Inhibitory percentage of sitagliptin as a standard inhibitor was 74.77±0.3 with IC50 1.104 µg/ml trigonelline is the major component of alkaloid fenugreek which has been used in the treatment of diabetes traditionally. The phytochemical constituent present in *Ficus* can impart a significant antidiabetic effect, including phytosterols, flavonoids, tannins and furanocoumarin derivatives (bergapten and bergaptol) [13,14]. Pomegranate is commercially cultivated for its edible fruit. The some part of the pomegranate plant has been investigated as antidiabetic effect. Components in pomegranate (punicalagin and ellagic, gallic, oleanic, ursolic and uallic acids) were found to have antidiabetic effect [15,16]. Bungur (*L. loudonii* Teism. & Binn) belongs to the genus *Lagerstroemia* and family Lythraceae. Other species from the same genus is *L. speciosa* L. was investigated as antidiabetic and capable of inhibiting the activity of the enzyme alpha-glucosidase and alpha-amylase. Compounds responsible for these activities were pentacyclic triterpenoids (oleonic acid, arjunolic acid, asiatic acid, maslinic acid, corosolic acid, and 2,3-hydroxyurosolic acid). Traditionally, genus *Lagerstroemia* has been used to treat several diseases such as diabetes, inflammation and hypertension [17,18]. Phytochemical screening of *L. loudonii* Teijsm. & Binn leaves contain alkaloids, flavonoids, tannins, quinones, monoterpenoid and sesquiterpenoids, steroids and triterpenoids, saponins and polyphenols. Oxidative stress is one of the major etiologies in the pathogenesis and complications of type 2 diabetes. Antioxidant activities of the bungur leaves using 2,2-diphenyl-1-picrylhydrazyl showed excellent result with EC50 14.63 µg/ml and quercetin as a standard antioxidant have EC50 3.64 µg/ml. Based on the biologycal activity and traditionally used in treating diabetes of the genus *Lagerstroemia* and chemotaxonomic approach, bungur (*L. loudonii* Teijsm & Binn) will continue to further research. *Lagerstroemia*, especially extract of *L. speciosa* L. showed in the *in vivo* study are quite good as antidiabetic and can help you lose weight. Tannin molecules contained in *L. speciosa* extract, known as Banaba extract an estimated charge of its activity as a stimulator Insulin-like Glucose Transport [19-21]. Sitagliptin as a standard DPP-IV inhibitor that is used as a therapeutic option in the treatment of type 2 diabetes mellitus has advantages to other diabetes therapies, which lowers the

Table 1: List of plants and percentage of inhibitory activity of dipeptidyl peptidase-IV

Name of plants	Part of plant	% Inhibition
Cecendet (*Physalis angulata* L.)	Leaves	13.94±4.08
Pacing (*Costus speciosus* [J.Koning] sm)	Leaves	25.92±21.60
Sambiloto (*Andrographis paniculata* [Burm.f.] Wall. Ex Nees)	Herb	37.03±0.65
Mahoni (*Swietenia mahagoni* King)	Semen	38.88±22.25
Bidara Upas (*Merremia mammosa* Hall.f.)	Rhizome	17.12±1.95
Paria (*Momordica charantia* Descourt)	Semen	41.66±1.63
Mimba (*Azadirachta indica* A. Juss)	Leaves	17.78±1.02
Angsana (*Pterocarpus indicus* Willd)	Laves	25.0±27.16
Alpukat (*Persea americana* Mill)	Semen	6.48±0.32
Nangka (*Artocaarpus heterophyllus* Lamk)	Laves	30.55±4.90
Kencana wungu (*Ruellia tuberosa* L.)	Leaves	30.09±1.30
Mala (*Euphorbia cotinifolia*)	Leaves	23.61±3.27
Brotowali (*Tinospora crispa* Miers ex Hoff.f)	Stem	65.86±1.02
Bungur (*Lagerstroemia loudonii* Teijsm. & Bin)	Laves	60.22±2.01
Yakon (*Smallanthus sonchifolius*)	Laves	52.84±2.01
Klabet (*Trigonella foenum-graecum* L.)	Semen	71.29±0.33
Bodhi (*Ficus religiosa* L.)	Leaves	68.98±1.95
Paitan (*Tithonia diersifolia* (Hemsley) A. Gray)	Leaves	16.8±1.34
Delima (*Punica granatum*)	Rind	58.79±2.23
Patikan Kebo (*Euphorbia hirta* L.)	Herb	33.52±0
Sitagliptin		74.77±0.3

Value are given as mean±SD. SD: Standard deviation

Table 2: Standardization of crude drugs and extracts of *Lagerstroemia loudonii* Teijsm. & Binn leaves

Determination	Result	
	Crude drugs (%w/w)	Extract (%w/w)
Content of total ash	9.51±0.54	2.31±0.43
Water-soluble ash	2.58±0.19	2.24±0.28
Acid-insoluble ash	3.39±0.16	0.82±0.68
Cotent of water	6.13±0.80(v/w)	14.27±0.84 (v/w)
Water-soluble extractable	17.48±0.53	-
Ethanol-soluble extractable	14.32±0.83	-

Value are given as mean±SD. SD: Standard deviation

risk of hypoglycemia, weight loss and the potential for regeneration and differentiation of pancreatic beta cells [8] (Table 2).

CONCLUSION

The results of *in vitro* test of the activities of some extracts in inhibiting DPP-IV showed that five of the 20 plant extracts had a good activity as DPP-IV inhibitor. Including fenugreek seeds (*T. foenum-graecum* L.); bodhi leaves (*F. religiosa* L.); brotowali (*T. crispa* Miers ex Hoff.f); bungur leaves (*L. loudonii* Teijsm. & Binn) and pomegranate rind (*P. granatum* L.) provided the highest inhibitory activity against DPP-IV enzyme that plays a role in improving diabetes.

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