ABSTRACT

Background: Some edible plants are promising to control blood sugar level. These plants contained phenolic substances that suggested to be able to inhibit dipeptidyl peptidase 4 (DPP IV). Objective: The objective of this study was to investigate the inhibitory effect of several selected Indonesia plants on inhibiting of DPP IV activity and to determine the total phenolic content of the most active extract. Methods: Twelve Indonesia edible plants were macerated using 80% ethanol at room temperature. DPP IV activity was evaluated by using glycyrl-proyl-7-amino-4-methyl coumarin (Gly-Pro-AMC) substrate and the inhibitory effect of extracts were determined based on the level of free AMC group by measuring its fluorescence on excitation wavelength 350-360 nm and an emission wavelength 450-465 nm using a microplate reader. Total phenolic contents of the active extracts were determined with Folin-Ciocalteu 1:4 on 785 nm using microplate reader. Total anthocyanins from extract were determined using the pH differential method. Results: Among the tested samples, the extract of Ipomoea batatas roots at a concentration of 10 µg/mL showed the highest inhibition, followed by Cajanus cajan leaves and Gnetum gnemon rind, with percentage inhibition of 28.8, 24.9 and 24.1, respectively. I. batatas extract have an IC_{50} value of 65.53 µg/mL, while the IC_{50} value of the positive control Sitagliptin 9.37 µg/mL. Total phenolic content from the extracts of I. batatas, C. cajan and G. gnemon rind extract were 279.3; 152.8; and 141.3 mg GAE/gram, respectively. Total anthocyanin from I. batatas extract was 462.14 mg cyanidin-3-glucoside/L. Conclusion: The extract of I. batatas showed the highest inhibition on DPP IV among other plants investigated and showed high content of phenolic compound and anthocyanin that correlated with activity as inhibitor DPP IV.

Key words: Ipomoea batatas, Indonesian edible plant, Dipeptidyl Peptidase 4.

INTRODUCTION

Diabetes mellitus is a common endocrine disease and still a global health problem that requires serious treatment. The International Diabetes Federation (IDF) predicted patients with diabetes around the world will increase from 415 million, in 2015, to 642 million, in 2040.1 Many medical therapeutic strategies are used for effective lowering glucose level in these patients such as (insulin sensitizer like glitazones and biguanides, α-glucosidase inhibitors, secretagogues as sulfonylurea and glinides, inhibitor enzyme dipeptidyl peptidase – IV and incretin mimetics).2 Incretins are hormones that are released from the gut into the bloodstream in response to ingestion of food. Gastric inhibitory polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1) are the two primary incretin hormones secreted from the intestine to stimulate insulin secretion from pancreatic β cells.3 Incretin effect accounts for at least 50% of the total insulin secreted after ingestion. In addition to its insulinotropic effects, it decreases food intake, inhibits glucagon secretion, inhibits gastric emptying and slows the rate of endogenous glucose production.4 It has also been shown to stimulate β-cell proliferation by up-regulation of the β-cell transcription factor pancreatic duodenal homeobox-1 protein (PDX-1) and to protect β cells from apoptosis.5-6

The main biological action of incretin (GIP and GLP-1) depends on their two N-terminal amino acid which is primarily removed by an enzyme dipeptidyl peptidase-4. Incretin (GIP and GLP-1) are metabolized into an inactive form rapidly by the enzyme DPP IV, about 1-2 min and only about 10-15% of circulating actively stimulate the pancreas. DPP IV is a member of the prolyl oligopeptidase family of serine proteases. DPP IV is an ectopeptidase implicated in the degradation of various peptides and hormones including glucagon family peptides, neuropeptides and chemokines.7 Inhibitor DPP IV group is now widely used for treating type 2 diabetes mellitus. These inhibitors promote glucose homeostasis by inhibiting DPP IV, the enzyme responsible for degrading two key glucoregulatory hormones (GIP and GLP-1). Clinical studies have evaluated the potential for DPP IV inhibition to reduce glucagon levels, delay gastric emptying
and stimulate insulin release. DPP IV inhibitors appear to have excellent therapeutic potential in the management of type 2 diabetes. Some plants are known to contain phytochemicals that can help to treat various diseases such as diabetes. These plants that have been studied as inhibitors of the enzyme DPP IV in vitro include seeds of Trigonella foenum-graecum and leaves of Ficus religiosa. Phenolic compound such as flavonoids and stilbenoid, have been suggested as important compounds for diabetes reduction. Plants were selected based on empirical use as antidiabetic and their phytochemical content like stilbenoid, luteolin, apigenin and anthocyanin that reported have related to the inhibitory activity of enzyme DPP IV. The present study aims to screen the potential plants with inhibitory properties on DPP IV activity.

**MATERIAL AND METHODS**

**Chemicals**

DPP IV screening kit and sitagliptin were obtained from (Cayman, US), Folin–Ciocalteu and Gallic acid were purchased from (Sigma Aldrich), Sodium carbonate, potassium chloride and sodium acetate were purchased from (Merck).

**Plant materials**

A total of 12 Indonesian edible plants were collected from several places in Java, Indonesia. Allium sativum and Allium cepa tubers, aerial part of Apium graveolens and Petroselinum crispum, Cinnamomum zaenlicum stem bark, Ipomoea batatas and Rheum officinale rhizoma, Gnetum gnemon rind and seed, Foeniculum vulgare seed, Cajanus cajan leaves and Brassica oleracea flower. All plants were identified by Center for Plant Conservation-Bogor Botanical Garden, Indonesia.

**Preparation of samples**

The plant’s materials were collected, cleaned dried, grounded to small pieces and stored in an airtight glass container. Each sample (200 g) was extracted with 2000 mL of 80% ethanol as the solvent for 2 × 24 hrs for each plant in room temperature. The ethanolic extracts then evaporated using a rotatory vacuum evaporator (Buchi, Switzerland).

**Inhibition of DPP IV assay**

The inhibition of DPP IV activity was performed using the enzyme protocol (Cayman DPP IV screening kit) with slight modification. Sitagliptin was used as the standard inhibitor. Briefly, 30 µL of the buffer solution, 10 µL of enzyme DPP IV, 10 µL of sample solution (100 ppm) and 30 µL Gly-Pro-AMC as the substrate, was added into the well. The mixture was shaken and incubated for 30 min at 37°C to have the complete reaction. In control well, the inhibitor was replaced by aqua bidest. The fluorescence of free AMC group was measured on excitation wavelength 350-360 nm and an emission wavelength 450-465 nm by using a microplate reader (GloMax® Discover System).

The IC<sub>50</sub> value represents the concentration of inhibitor required to achieve 50% enzyme inhibition.

**Phytochemical screening**

Qualitative identification of phytochemical constituent in the extracts was performed by using standard analytical procedures with slight modification. Alkaloid test with Bouchardat, Dragendorff and Mayer reagents; Tannin test with ferrous (III) chloride, gelatin test and gelatin-salt test; Flavonoid test with Wilson-Taoubock and Shinoda reaction; Glycoside test with Molisch reaction; Saponin test with foaming test; Tannin test with ferrous (III) chloride, gelatin test and gelatin-modification. The inhibition of DPP IV activity was performed using the enzyme protocol (Cayman DPP IV screening kit) with slight modification. Sitagliptin was used as the standard inhibitor. Briefly, 30 µL of the buffer solution, 10 µL of enzyme DPP IV, 10 µL of sample solution (100 µL), 1:4 diluted Folin–Ciocalteu reagent and homogenized for 1 min in a flat-bottom 96-well microplate. The mixture was left for 4 min and then 75 µL of sodium carbonate solution (100 g/L) were added and homogenized for 1 min. After 2 hrs at room temperature in the dark, the absorbance was measured at 765 nm using the microplate reader 96 well (VersaMax™ ELISA Microplate Reader, USA).

The yield of GAE in extracts was determined by comparison of absorbance with standards. The calibration curve of standards (gallic acid) was measured by the absorbance from the microplate reader instrument and was calculated using SoftMax 6.5.1 software. The equation formula was:

\[ Y = \frac{6.6409x + 121.82}{R^2 = 0.9947}, \text{where } Y \text{ is the yield of GAE (total polyphenolic content) and } X \text{ is the absorbance of gallic acid or samples.}

**Determination of total anthocyanin from I. batatas root extracts**

The total anthocyanin from extracts were determined using the pH differential method (Lee et al. 2005). Prepared two solutions of extract, the first solution for pH 1 and the second solution for pH 4.5. Take each 1 ml of extract solution and diluted using ph solution into 5 ml. After 20 min at room temperature, the absorbance from each dilution are measured using Spectrophotometer UV-Vis at 520 and 700 nm. To determine its absorbance value by using the following formula:

\[ A = (\frac{\lambda_{520nm} - \lambda_{700nm}}{\lambda_{520nm} - \lambda_{700nm}}) \text{pH 1,0} - (\frac{\lambda_{520nm} - \lambda_{700nm}}{\lambda_{520nm} - \lambda_{700nm}}) \text{pH 4,5} \]

Calculate anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

\[ \text{Total anthocyanin pigment (mg/g)} = \frac{A \times MW \times DF \times 10^4}{\varepsilon \times l \times V \times M_i} \]

where A = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; l = pathlength in cm; V = volume of extract (ml); \( \varepsilon = 26.900 \text{ molar extinction coefficient} \); and 10<sup>4</sup> = factor for conversion from g to mg; M<sub>i</sub> = sample weight (g).

**Chromatogram analysis from PSP extract**

An isocratic Reversed-phase high-performance liquid chromatography (HPLC) using Agilent 1100 (Palo Alto, USA) with UV-Vis diode array detection was used to determined chromatographic anthocyanins from PSP extract. PSP extract was injected into the HPLC with the following operational conditions: mobile phase was 10% glacial acetic acid in distilled water : acetonitrile (85: 15 v/v), Prodigy C18 reversed-phase column (5 mm), 4.6 × 150 mm i.d. (Agilent, USA), volume injection was 20 µL, flow rate was 1 mL/min, detector UV-Vis at 530 nm and column temperature was 30 °C. Separation of anthocyanin was carried out for 20 min.
RESULTS
Inhibition of DPP IV by some Indonesia edible plant

The inhibitory effect on DPP IV activity was performed by in vitro method using Gly-Pro-AMC as a substrate. DPP IV converts Gly-Pro-AMC into Gly-Pro and AMC. The activity on DPP IV inhibition was evaluated based on the level of AMC by measuring its fluorescence using microplate reader (GloMax® Discover System). The activity was measured quantitatively in the presence or absence of the extract. Sitagliptin was used as the positive control that showed percentage inhibition with the value of 90.09 % at 100 μM. The Inhibition of DPP IV by sitagliptin at various concentration is shown in Figure 1.

Only ten from twelve plants showed inhibition activity of DPP IV at 10 μg/mL final concentration. Ethanolic extract of I. batatas, C. cajan and G. gnemon gave the highest activity with percentage inhibition of 24.1 to 28.80 at 10 μg/mL final concentration. I. batatas extract showed the most potent activity with percentage inhibition of 28.80. The extract was further investigated for effect at the various concentration to obtain the IC_{50} value. The percentage inhibition of the extracts and IC_{50} value of the extract is shown in Table 1 and Figure 2.

Phytochemical screening
I. batatas, C. cajan and G. gnemon extracts were further investigated to determine the phytochemical content that responsible for their activity. The common phytochemicals content from the plant such as flavonoid, alkaloid, terpenoid, steroid, tannin, glycoside and saponin have been identified (Table 2).

Total phenolic content
The total phenolic contents from the extracts of I. batatas, C. cajan and G. gnemon were determined with Folin-Ciocalteu method with equivalent to gallic acid. Orders of the total phenolic content from highest lowest were I. batatas > C. cajan > G. gnemon extracts (Table 3).

Total anthocyanin content from I. batatas roots extract
The content of anthocyanins from I. batatas extract was determined using pH differential method to be 462.14 ± 14.27 mg/L (expressed as cyanidin-3-glucoside).

HPLC analysis of purple sweet potato anthocyanins
The chromatogram from the ethanolic extract of I. Batatas is shown in Figure 3.

DISCUSSION
Inhibition of DPP IV by some Indonesia edible plant

In the present study, the effect of extracts on enzyme DPP IV activity is shown in dose-dependent manner. Sitagliptin is the potent DPP IV inhibitor for management T2DM. DPP IV is associated with degradation of (GIP and GLP-1) that contributes to increased insulin secretion, inhibit glucagon secretion, stimulate β-cell proliferation and protect β-cell from apoptosis.

The results demonstrated that 80% ethanol extract of I. batatas, C. cajan leaves and G. gnemon rind gave the highest activity with percentage inhibition of 24.1 to 28.80 at 10 μg/mL final concentration. I. batatas extract showed the most potent activity with percentage inhibition of 28.80. Most of the recent studies revealed that the inhibitory activity of DPP IV is due to the phenolic content in plant. Alkaloid, flavonoid and stilbenoid have been reported to have activity as a DPP IV inhibitor.

The result demonstrated that extract I. batatas roots was the strongest inhibitor of DPP IV activity in vitro, followed by C. cajan leaves and G. gnemon rind. Some extracts showed inhibition activity which indicates that the extracts contained potential compound as DPP IV inhibitor. I. batatas roots was the strongest inhibitor of DPP IV activity in vitro with IC_{50} value 65.53 μg/mL. I. batatas roots have been used as foodstuffs in daily life in Indonesia. This plant also was used as traditional medicine as a substitute source of carbohydrates for diabetes management. But, the scientific data is limited. From this study, we can conclude that I. batatas roots showed a mechanism action as DPP IV inhibitor.

Phytochemical screening
I. batatas, C. cajan and G. gnemon extract were further investigated to determine the source of activity. The common phytochemistry content from plants such as flavonoid, alkaloid, terpenoid, steroid, tannin, glycoside and saponin have identified (Table 2). Most of the recent studies revealed that the inhibitory activity of DPP IV is due to the phenolic content in the plant. Alkaloid, flavonoid and stilbenoid have been reported to have activity as a DPP IV inhibitor.

DPP IV has three active sites (S1, S2, and S3). The specificity S1 is composed of the side chains of catalytic triad (Ser630, Asn710, and His740), which are involved in strong hydrophobic interactions. The active site S2 of DPP IV is cavity near Glu205, Glu206, and Tyr662 residues. And the active site S3 consists of Ser209, Arg358 and Phe357. The inside position of the active site S3 in DPP-IV allows smaller groups access to the site; on the other hand, the outside position of the S3 favors larger groups.19-22 DPP-4 inhibitors interact with the active site S2 at Glu205 and Glu206 by forming salt bridges. This interaction plays an important role in inhibiting the enzyme.19

Based on previous research, these three plants have been reported to have activity as antidiabetes. Administration of G. gnemon extract, Anthocyanin from I. batatas and ethanolic extract C. cajan leaves can decrease blood glucose level in animal test.20-22 Although the antidiabetic activity of G. gnemon, I. batatas and C. cajan has been reported, its ability to inhibit DPP IV activity had not been studied so far. This is the first report indicated that ethanolic extracts of G. Gnemon rind, I. Batatas roots and C. cajan leaves showed significant DPP IV inhibitory activity. The results of this study may explain the mechanism of the extracts in lowering blood sugar levels.

In this study, I. batatas extract showed the highest DPP IV inhibitor activity in vitro with an IC_{50} value of 65.53 μg/mL. Although with IC_{50} value 9.37 μg/mL. Ethanolic extract I. batatas extract contains high amount of total anthocyanins.21 SwissDock computational docking study demonstrated that anthocyanins were able to interact with DPP IV through ligand interaction and therefore potentially inactivate the activity of the enzyme.22 We suggested the anthocyanins contained in I. batatas extract is most responsible for its activity as a DPP 4 inhibitor.

Total phenolic content
Total phenolic content from 80% ethanolic extracts of Ipomoea batatas roots, Cajanus cajan leaves and Gnetum gnemon rind are shown in Table 3. The relationship between DPP IV inhibitory activity of I. batatas, C. cajan and G. gnemon extracts with their phenolic content was established using analysis bivariate pearson comparison. The result significantly indicated a high relationship between total phenolic content with DPP IV inhibitory activity (r = 0.05) with correlation value was 0.916. A number of natural products have previously been found to have DPP-IV inhibitory activities and most of them contain polyphenols, flavonoids and alkaloids as the active components.25

Total anthocyanin content from I. batatas roots extract
The content of anthocyanins from I. batatas extracts were determined using pH differential method to be 462.14 ± 14.27 mg/L. Previous research reported that the highest anthocyanin content from I. batatas was 687.58 mg/L, which was reached at the best condition extraction.26

Table 1. Inhibition (%) of the ethanolic extracts of some Indonesian edible plants on DPP IV Activity

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant Species</th>
<th>Family</th>
<th>Part of The Plant</th>
<th>Inhibition of DPP IV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Allium cepa</em></td>
<td>Liliaceae</td>
<td>Tubers</td>
<td>nil</td>
</tr>
<tr>
<td>2</td>
<td><em>Allium sativum</em></td>
<td>Liliaceae</td>
<td>Tubers</td>
<td>13 ± 4.87</td>
</tr>
<tr>
<td>3</td>
<td><em>Aptium graveolens</em></td>
<td>Apiaceae</td>
<td>Aerial part</td>
<td>6.3 ± 1.98</td>
</tr>
<tr>
<td>4</td>
<td><em>Petroselinum crispum</em></td>
<td>Apiaceae</td>
<td>Aerial part</td>
<td>18 ± 5.93</td>
</tr>
<tr>
<td>5</td>
<td><em>Cinnamomum zeylanicum</em></td>
<td>Lauraceae</td>
<td>Stem barks</td>
<td>8.5 ± 4.24</td>
</tr>
<tr>
<td>6</td>
<td><em>Ipomoea batatas</em></td>
<td>Convolvulaceae</td>
<td>Roots</td>
<td>28.8 ± 7.7</td>
</tr>
<tr>
<td>7</td>
<td><em>Gnetum gnemon</em></td>
<td>Gnetaceae</td>
<td>Seeds</td>
<td>16.0 ± 2.76</td>
</tr>
<tr>
<td>8</td>
<td><em>Gnetum gnemon</em></td>
<td>Gnetaceae</td>
<td>Rinds</td>
<td>24.1 ± 7.7</td>
</tr>
<tr>
<td>9</td>
<td><em>Foeniculum vulgare</em></td>
<td>Apiaceae</td>
<td>Seeds</td>
<td>nil</td>
</tr>
<tr>
<td>10</td>
<td><em>Cajanus cajan</em></td>
<td>Fabaceae</td>
<td>Leaves</td>
<td>24.9 ± 3.2</td>
</tr>
<tr>
<td>11</td>
<td><em>Rheum officinale</em></td>
<td>Polygonaceae</td>
<td>Roots</td>
<td>10.4 ± 4.45</td>
</tr>
<tr>
<td>12</td>
<td><em>Brassica oleracea</em></td>
<td>Brassicaceae</td>
<td>Flower</td>
<td>9.32 ± 2.88</td>
</tr>
</tbody>
</table>

Final concentration of the extract solution in the DPP IV inhibition assay was 10 µg/ml.

Data are mean ± SEM for triplicate measurements.
Nil = no inhibition detected at the assayed concentration

Table 2: Phytochemical screening of the most active extracts

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Ipomoea batatas roots</th>
<th>Cajanus cajan leaves</th>
<th>Gnetum gnemon rind</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponin</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Antraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: - = absent, + = present in small amount, ++ = present in moderate quantity

Table 3: Total phenolic content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic content (mg GAE/gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. batatas</em> extract</td>
<td>279.3 ± 40.3</td>
</tr>
<tr>
<td><em>C. cajan</em> extract</td>
<td>152.8 ± 15.1</td>
</tr>
<tr>
<td><em>G. gnemon</em> extract</td>
<td>141.3 ± 37.02</td>
</tr>
</tbody>
</table>

Data are mean ± SEM for triplicate measurements.
group of phenolic compounds, hundreds of anthocyanins have been isolated from nature. The most common anthocyanins found in fruits and vegetables are cyanidin, malvidin peonidin, and delphinidin. The main anthocyanins in PSP are cyanidin and peonidin which are glycosylated with sugar.29 Tian et al, (2005) showed that glycosylated anthocyanins are generally mono or acylated with hydroxybenzoic, ferulic and caffeic acids.29 This structural complication leads to a major challenge for anthocyanin identification from PSP.

CONCLUSION

The results of in vitro test indicate that out of the twelve plant extracts, ethanolic extracts of Ipomoea batatas extract showed the highest DPP IV inhibitory activity and showed high content of phenolic compound and anthocyanin that correlated with activity as inhibitor DPP IV. The plant may essentially contain phytochemical compounds that can inhibit enzyme activity and further characterization methodologies and structural elucidation have to be carried out in order to identify the bioactive constituents. The expected bioactive components could be anthocyanins or other polyphenols because the literature shows the relationship between polyphenols and inhibition of DPP IV. In conclusion, more research is required for developing a potential DPP IV inhibitor of plant origin.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPP IV: dipeptidyl peptidase iv; Gly-Pro-AMC: Glycy-prolyl-7-Amino-4-Methyl Coumarin; GIP: Gastric inhibitory polypeptide; GLP-1: Glucagonlike Peptide-1; PDX-1: Pancreatic Duodenal Homeobox-1 protein; GAEC: Galic Acid Equivalent; PSP: Purple Sweet Potatoes.

REFERENCES

Amin, et al.: DPP IV Inhibition by Indonesia Edible Plants

**Summary**

- Ten from twelve Indonesian edible plants showed inhibition activity of DPP IV.
- Ethanolic extract of *I. batatas*, *C. cajan* and *G. gnemon* gave the highest activity with percentage inhibition of 24.1 to 28.80 at 10 μg/mL.
- *I. batatas* extract showed the highest DPP IV inhibitor activity in vitro with an IC$_{50}$ value of 65.53 μg/mL.
- The content of anthocyanins from *I. batatas* extracts was 462.14 ± 14.27 mg/L.

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