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Judul Artikel : **Anti-diabetic potential of *Urena lobata* leaf extract through inhibition of dipeptidyl peptidase IV activity**

Tanggal	Activity	Reviewer Comments
21-04-2015	Submission of article	
29-04-2015	Editor responses	Article has been received by editor
06-05-2015	Editor responses	Article is accepted with revision
11-05-2015	Resubmit the revised article	
12-05-2015	Editor responses	Manuscript is accepted with minor modification
29-05-2015	Editor responses	Galley proof sending
30-05-2015	Resubmit the revised of galley proof	
01-06-2015	Editor responses	Revised galley proof is accepted
02-06-2015	Editor responses	Confirmation of the final revision from author
02-06-2015	Resubmit revised article	
03-06-2015	Editor responses	The final correction already made
14-06-2015	Editor responses	Published on line article

Manuscript submission :

Dipeptidyl Peptidase IV (DPP-IV) inhibitory activity of *Urena lobata* leaf extract (Study: in-vitro and in-silico)

Yudi Purnomo^{1,2}, Djoko Wahono³, Sutiman B Sumitro⁴, M. Aris Widodo⁵

¹Doctoral student School of Medicine Brawijaya University
²Pharmacology Department School of Medicine Malang Islamic University
³Internal medicine Department School of Medicine Brawijaya University
⁴Biology Department Science Faculty of Brawijaya University
⁵Pharmacology Department School of Medicine Brawijaya University

Abstract

Objective : Glucagon Like Peptide-1 (GLP-1) is one of incretin hormone which has been proposed as a new treatment for Type 2 Diabetes Mellitus (T2DM). However, this hormone is metabolized excessively by Dipeptidyl Peptidase IV (DPP-IV) into inactive form. The inhibition of DPP-IV can enhance GLP-1 bioavailability and regulate blood glucose level therefore it would be beneficial in the treatment of T2DM. *Urena lobata* is plants growing in Indonesia and has been used to cure many diseases. The objective of this study was to evaluate anti diabetic potency of *U. lobata* leaf extract on DPP-IV inhibitory activity.

Method: *U. lobata* leaf was extracted in hot water and ethanol solvent. The activity of DPP-IV inhibitor was tested by in-vitro study use *Gly-pro-p-nitroanilide* (GPPN) as substrat of DPP-IV and Vildagliptin as reference standard. p-nitroanilide as product reaction between GPPN and DPP-IV was observed with microplate reader λ=405 nm. All data are expressed as the mean ± SD and the IC-50 value was determined by non linear regression curve fit. Active substances in *U. lobata* leaf extract was analyzed by Liquid Chromatography –Mass Spectra (LC-MS). DPP-IV inhibitory activity of active compounds was evaluated by in-silico study used dockingserver software.

Result: The ethanolic extract of *U. lobata* showed DPP-IV inhibitor activity stronger than water extract with an Inhibitory Concentration-50 (IC-50) value of 1654.64 and 6489.88 µg/ml respectively. Vildagliptin used as reference standart of DPP-IV inhibitor activity have IC-50 value 57.44 µg/ml. Mangiferin, Stigmasterol and β-sitosterol in *U. lobata* extract have a strong inhibitory activity on DPP-IV based on in silico test.

Conclusion: *U. lobata* showed DPP-IV inhibitory activity related to it's active compounds Mangiferin, Stigmasterol and β-sitosterol.

Page 1 of 11 3004 Words English (US)

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Anti-diabetic potency of *Urena lobata* leaf extract on the inhibition of Dipeptidyl Peptidase IV (DPP-IV) activity

Yudi Purnomo^{1,2}, Djoko Wahono S³, Sutiman B Sumitro⁴, M. Aris Widodo⁵
¹Doctoral student School of Medicine Brawijaya University
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ABSTRACT

Objective: To evaluate the anti-diabetic potential of leaf extract from *Urena lobata* (U. lobata) through dipeptidyl peptidase IV (DPP-IV) inhibitory activity.

Methods: U. lobata leaf was extracted in hot water and ethanol. The activity of DPP-IV inhibitor was tested by *in vitro* study using gly-pyr-p-nitroanilide as substrate of DPP-IV and vildagliptin, as standard reference. A product of the reactions between gly-pyr-p-nitroanilide and DPP-IV, was observed by microplate readers with $\lambda = 405$ nm. All data were expressed as mean \pm SD and the IC_{50} value was determined by non-linear regression curve fit. Active substances in leaf extract of U. lobata was analyzed by liquid chromatography mass spectrometry. DPP-IV inhibitory activity of active compounds was evaluated *in silico* using docking server.

Results: The ethanolic extract of U. lobata showed stronger DPP-IV inhibitor activity than water extract with the IC_{50} values of 1854.64 and 6489.88 μ g/ml, respectively. Vildagliptin, based on standard reference for DPP-IV inhibitor activity, has IC_{50} value of 57.44 μ g/ml. Based on *in silico* analysis, mangiferin, stigmasterol and β -sitosterol in U. lobata extract have a strong inhibitory activity on DPP-IV.

Conclusion: The results showed that DPP-IV inhibitory activity of U. lobata is related to its active compounds such as mangiferin, stigmasterol and β -sitosterol.

1. Introduction

Recently, the treatment of type 2 diabetes mellitus is focused on incretin hormones. Glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are the major incretin

metabolized by the enzyme called dipeptidyl peptidase IV (DPP-IV) into inactive forms. Therefore, the GLP-1 has a short half life, approximately for 1-2 min. Inhibition of DPP-IV maintains the level of endogenous active (GLP-1) and prolongs its half life. DPP-IV inhibitor has the potential to be a novel, efficient and

experiences, Nigerian people used U. lobata to treat diabetes mellitus because of their biology activities⁶. The study showed that administration of U. lobata roots extract had anti-hyperglycemic effect on rat induced by streptozotocin before⁷. It related to active substances in U. lobata such as steroid groups, alkaloid and flavonoid^{8,9}. Anti-diabetic potential of U. lobata has not been evaluated yet, especially the inhibition of DPP-IV activity. Therefore it is an opportunity to expand herbs that can become candidate of phytopharmaka. The aim of this study was to know the anti-diabetic potential of U. lobata leaf extract through inhibition of DPP-IV activity.

2. Materials and methods

2.1. Chemicals used

DPP-IV was obtained from porcine kidney. Gly-pyr-p-nitroanilide and Tris-HCl buffer were used. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

U. lobata leaf powder was obtained from Materis Medika Beta Makang with certificate number 074027301.8/2015. Then, 50 g of the powdered plant materials were extracted in 250 ml hot water at 90 °C for 30 min. Similarly, 50 g U. lobata powder was extracted in 250 ml ethanol for 4 h by waterbath shaker and repeated 2 times with fresh ethanol. Both of the extracts were then evaporated.

2.3. Identification of active compounds

Both water and ethanol extract were analyzed on a semi qualitative scale by liquid chromatography mass spectrometry (LC-MS) Accela 1250 pump for identification of active compounds. Liquid phase contained 0.1% formic acid in methanol and water.

2.4. DPP-IV assays

The assay was performed in 96 micro well plates. A pre-incubation solution (50 μ l) contained 35 μ l Tris-HCl buffer, 15 μ l DPP-IV enzyme and various concentration (62.5, 1250, 2500, 5000 and 10000 μ g/ml) of the extracts or standard (6.25, 12.5, 25, 50

molecular docking. Free energy binding, inhibition constant and surface interactions were analyzed by this method to measure the DPP-IV inhibitory activity of active compounds.

2.6. Statistical analysis

All data are expressed as mean \pm SD. The IC_{50} was determined by non-linear regression curve fit. The statistical data were analyzed by SPSS (one-way ANOVA test followed by least significant difference test with significant value at $P < 0.05$).

3. Results

3.1. DPP-IV inhibitory activity of U. lobata

Both water and ethanol U. lobata leaf extracts were tested on DPP-IV inhibitory assay by *in vitro* method. The DPP-IV inhibitory activity is shown in Table 1.

Table 1
DPP-IV inhibitory activity of U. lobata leaf extracts and vildagliptin.

Sample (n = 3)	Concentration (μ g/ml)	% Inhibition	IC_{50} (μ g/ml)
Water extract of U. lobata	62.5	00.00 \pm 0.00	6489.88 ^{***}
	1250.00	13.93 \pm 0.00	
	2500.00	25.87 \pm 0.00	
	5000.00	42.22 \pm 3.83	
	10000.00	62.22 \pm 3.83	
Ethanolic extract of U. lobata	62.5	00.00 \pm 0.00	1854.64 ^{***}
	1250.00	48.94 \pm 0.00	
	2500.00	53.32 \pm 0.00	
	5000.00	61.70 \pm 0.00	
	10000.00	74.47 \pm 0.00	
Vildagliptin	6.25	6.99 \pm 0.00	57.44 ^{***}
	12.50	16.97 \pm 4.12	
	25.00	37.20 \pm 0.00	
	50.00	46.81 \pm 3.83	
	100.00	69.71 \pm 0.00	

^{***} Different between showed the differences of the potency ($P < 0.05$, Least Significant Difference test).

The results obtained in the DPP-IV inhibitory assay showed that ethanolic extract of U. lobata showed stronger activity in DPP-IV inhibition, about 4 times folds, compared to water extract ($P < 0.05$). However, the DPP-IV inhibitory activity of both water and ethanolic U. lobata extracts are still lower, approximately 30-100 times folds, compared to vildagliptin as reference drugs of DPP-IV inhibitor ($P <$

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Anti-diabetic potential of *Urena lobata* leaf extract through inhibition of dipeptidyl peptidase IV activity

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