Pharmacogn J. 2018; 10(1):190-193
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Dipeptidyl peptidase IV Inhibitory Activity of Fraction from White Tea Ethanolic Extract (*Camellia sinensis* (L.) Kuntze) *ex vivo*

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ABSTRACT

Background: Treatment for type-2 diabetes mellitus focuses on the incretin hormone, Glucagon-Like Peptide-1 (GLP-1). However, it has a short half-life. Inhibition of the enzyme Dipeptidyl peptidase IV (DPP IV) required maintaining the active form of GLP-1. Based on the previous studies on the highest activity of DPP IV enzyme inhibition of white tea extract, this study conducted on the fraction of white tea extract using rat blood serum (*ex vivo*). **Objectives:** This study aims to evaluate the inhibitory activity of fraction from white tea extract. **Methods:** White tea leaves extracted with ethanol. The inhibitory activity determined by using rat blood serum as DPP IV enzyme source (*ex vivo*), AMC (7-amino 4-methyl coumarin) as fluorescence substrate of DPP IV and sitagliptin as the standard reference. The the cleavage of fluorescence reaction product observed by a microplate reader with λ_{ex} = 360 nm and λ_{em}=460 nm at 37°C. Data expressed as mean ± SD and the IC_{50} value determined by nonlinear regression curve and fit using Prism Graph 7. **Result:** methanol fraction (250 µg/mL) has the greater inhibition percentage (50.487%), and the fraction of n-hexane and ethyl acetate are 32.417% and 36.541%. The methanol fraction IC_{50} value is 227 µg /mL. **Conclusion:** The methanol fraction is the most active to inhibit DPP IV enzyme.

Key words: Antidiabetic, *Camellia sinensis*, Dipeptidyl peptidase IV, DPP IV, Fraction, White tea.

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History

- Submission Date: 18-10-2017;
- Review completed: 08-11-2017;
- Accepted Date: 20-11-2017

DOI : 10.5530/pj.2018.1.32

Article Available online
http://www.phcogj.com/v10/i1

INTRODUCTION

Treatment for type-2 diabetes mellitus depends on the incretin hormone. Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulinotropic Polypeptide (GIP) are the main incretin hormones secreted in the intestine. GLP-1 plays a role in the body's metabolism, such as insulin secretion, increases the mass of β-pancreatic cells, glucagon secretion, reduces gastric emptying and satiety.[1] However, GLP-1 tends with a half-life about 1-2 min due to degradation by the Dipeptidyl peptidase IV (DPP IV).[2] The inhibition of DPP IV required to maintain endogenous GLP-1 inactive form and longer half-life.[3] DPP IV inhibition may also reduce the side effects of hypoglycemia, weight gain, and gastrointestinal disorders.[4,5] Sitagliptin has reported as a potential inhibitor of DPP IV, but this treatment has side-effects on the upper respiratory tract, and the price is relatively high and difficult to obtain.[6]

DPP IV inhibitor from plants expected to reduce side effects, cheaper, and easier to produce. The active compounds in plants have mechanisms in various forms, pancreatic β-cell functions, and glucose absorption on the incretin pathway. Some plants reported as anti-hyperglycemia.[7,8] Studies on different types of tea as anti-diabetic that has done on green tea, black tea and oolong tea, but white tea has not known well. White tea (*Camellia sinensis* (L.) Kuntze) is a tea bud that still rolls processed without fermentation.[9,10] White tea has the highest polyphenol content and highest of catechin derivatives compared with other types of tea.[11,12,13] The differences of bioactive components of each plant provides different antidiabetic activity mechanisms. The content of flavonoids (quercetin and catechin), tannins and polyphenols, have a role in the mechanism of decreasing blood glucose levels against animal testing through inhibition of antibacterial processes.[14]

The study conducted by Elya reported that white tea extract had inhibition of DPP IV enzyme with the highest percentage of inhibition (30.57%) compared to the other plant extracts.[15] Based on the high content of polyphenols and their inhibitory activity against DPP IV enzyme as proposed by Elya, it is necessary to conduct further research on the effect of ethanol extract of *Camellia Sinensis* (L.) Kuntze. Blood serum used as a source of

Cite this article: Ekayanti M, Sauriasari R, Elya B. Dipeptidyl peptidase IV Inhibitory Activity of Fraction from White Tea Ethanolic Extract (*Camellia sinensis* (L.) Kuntze) *ex vivo*. Pharmacog J. 2018;10(1):190-3.

Table 1: Phytochemical screening of fraction.

No	Group of Compounds	Reactor	N-Hexane		Ethyl Acetate		Methanol	
			E	R	E	R	E	R
1.	Flavonoids	Mg + HCl (p)	Colorless	-	Colorless	-	Yellow - Orange	+
2.	Alkaloids	Mayer	White	-	White	-	White	-
		Wagner	Yellow	-	Orange	+	Orange	+
		Dragendorff	no precipitate	-	Brown (p)	+	Brown (p)	+
3.	Tannins	$FeCl_3$ 1%	Yellow	-	Black - blue	+	Black - blue	+
		Gelatin	no precipitate	-	White (p)	+	White (p)	+
4.	Saponins	Heat	Colorless and non-foaming	-	Colorless and non-foaming	-	foaming	+
5.	Steroids / Triterpenoids	Lieberman-burchard	Green	+	Yellow	-	Yellow	-

E: evaluation; R: results; and p: precipitate

Table 2: Inhibition activity and IC50 value of fraction.

Sample	Concentration (µg / mL)	Inhibition Percentage (%)	IC50 (µg / mL)
Sitagliptin	100 mM	93.494 ± 0.503	
n-Hexane	5	7.589 ± 1.687	-
	50	7.79 ± 1.887	
	100	8.111 ± 7.107	
	250	32.417 ± 0.044	
	500	36.557 ± 2.859	
Ethyl Acetate	5	15.54 ± 0.883	-
	50	18,018 ± 7,457	
	100	25338 ± 1.823	
	250	36.541 ± 1.992	
	500	46.463 ± 1.45	
Methanol	5	20198 ± 0.161	227
	50	24,173 ± 4,939	
	100	28.55 + 2,136	
	250	50,487 ± 1,923	
	500	56.948 ± 2.626	

DPP IV enzyme and it has been reported has the identical enzyme specificity and no differences in hydrolysis compared to the kidneys.[16]

MATERIALS AND METHODS

Plant material

Buds of *Camellia sinensis* (Theaceae) were collected from the Research Center for Tea and Quinine, Gamboeng, Indonesia. Plant was authenticated in the Indonesian Institute of Sciences, Bogor, Indonesia.

Chemical used

All chemicals and Dipeptidyl peptidase IV assay kit purchased from Sigma Aldrich (St. Louis, MO, USA).

Plant extraction and fractionation

The white tea leaves powder was weighed 750 g and extracted by reflux method with 70% ethanol (1:10), the temperature maintained 60°C about three h. The filtrate of the first reflux filtered and repeated two times. The filtrate from the reflux and the repetition evaporated at 50°C (MOH, 2000). The ethanol extract fractionated by liquid-liquid partition method by increased polarity of the solvent, i.e., n-hexane, ethyl acetate, and methanol. One kilogram of viscous extract weighed and fractionated by separation funnel, by means of extract dissolved with distilled water until completely dissolved. The extract solution added with n-hexane (1:1), gently shake and released the gas and then kept until it completely separated. The n-hexane and water fractions are separated and collected and then repeated. The non-mixed fraction in the n-hexane solvent was added with ethyl acetate and then carried out the procedure as in n-hexane. Furthermore, the non-mixed fraction in the ethyl acetate solution added to methanol, shaken and then accommodated. Each fraction obtained was then concentrated by a rotatory evaporator until a thickened fraction was obtained.

Phytochemical screening

Parameters examined on phytochemical identification were Flavonoids, alkaloids, tannins, saponins, steroids and triterpenoids by using the specific reagent.

Blood Serum preparation

Blood serum prepared from Sprague-Dawley (SD) rats and obtained from the tail. Previously, tail disinfected with 96% alcohol. The blood sample stored in eppendorf for one h then centrifuged by using Scan Speed, 4000 rpm for ten min. The supernatant (blood serum) pipetted and inserted into a new eppendorf, labeled and stored at -20°C.

Determination of inhibition percentage

The test performed by using DPP IV *Spectrofluorometry Activity Assay Kit* from Sigma Aldrich (St. Louis, MO, USA) with modified.[17] The DPP IV enzyme from the blood serum hydrolyzes the fluorogenic substrate bonds (H-Gly-Pro) conjugated to the AMC group (H-Gly-Pro-AMC) to release the fluorescence product, i.e., the 7-Amino-4-Methyl Coumarin group (AMC) on λ_{ex} = 360 nm and λ_{em} = 460 nm, at 37°C. The inhibitory activity of DPP IV enzyme analyzed by calculating the percent inhibition of each fraction in various concentrations. Tests carried out using microwell plate 96 specifically for fluorescence. The parameters observed in this trial is the amount of fluorescence product released on each sample test then calculated the percentage of inhibition. Data expressed as mean ± SD and the IC_{50} value determined by nonlinear regression curve and fit using Prism Graph 7.

RESULT

Phytochemical screening

The results of the classification of the compounds presented in Table 1. The identification of class compounds indicated by the color changes generated by the addition of specific reagents.

Inhibitory activity

The concentration of sitagliptin in the *well* is 100 mM and generating 93.494% of inhibition. The methanol fraction is more active on inhibiting the DPP IV enzyme with 50.487% of inhibition at 250 µg/mL greater than the n-hexane and ethyl acetate (32.417% and 36.541%). The IC_{50} value of methanol fraction is 227 µg /mL and it shown on Table 2. Based on the other studies, the IC_{50} value of DPP IV enzyme from green tea and black tea were 5.3 mg/mL and 6.4 mg/mL.[18]

DISCUSSION

Phenolic components generally can bind to protein from the enzyme and form enzyme-inhibitor bonds. This condition leads to decrease the activity of the enzyme because of the inability bind its substrate and the enzyme active site has occupied. Based on study, the active compound which plays a role in providing inhibition is catechin (hydrolyzed tannin class). EGCG (*Epigallocatechin gallate*), which derived from catechin also reported having a mechanism as DPP IV inhibitors.[7] However, high concentrations of tannins may provide the experiment bias because of their specific properties that can precipitate proteins. The enzyme is a class of protein that can react with tannins to bind strongly and form a precipitate, so that enzyme activity decreases.

DPP IV catalytic structure can be inhibited strongly by the enzyme serine inhibitor with a covalent bond to modify the active site of the enzyme. Substrate specificity of DPP IV in a mammal relatively limited at P_1 position (proline or alanine). However, studies analogous Growth Releasing Hormone (GRH) indicates that the bond residues P_1 were also hydrolyzed such as serine, valine, glycine, leucine although with the low level.[3]

CONCLUSION

The methanol fraction of the white tea ethanol extracts is more active to inhibit DPP IV enzyme from blood serum.

CONFLICT OF INTEREST

The authors are declaring that have no conflict of interest.

ACKNOWLEDGMENT

The authors are grateful to PITTA Grant, University of Indonesia.

ABREVIATIONS

GLP-1: Glucagon-Like Peptide-1; **DPP IV:** Dipeptidyl peptidase IV; **AMC:** 7-amino 4-methylcoumarin; **GIP:** Glucose-Dependent Insulintropic Polypeptide; **IC_{50}** Inhibition of Concentration at 50%; **EGCG:** *Epigallocatechin gallate*; **GRH:** Growth Releasing Hormone.

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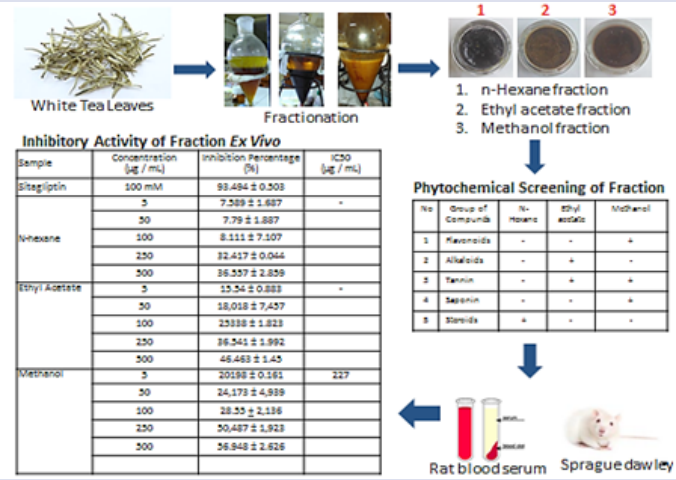
SUMMARY

- White tea leaves extracted with ethanol and fractionated with n-hexane, ethyl acetate and methanol.
- Group compounds examined on phytochemical screening were flavonoid, alkaloid, tannin, saponin, and terpenoid by using the specific reagent.
- The inhibitory activity determined by using rat blood serum as DPP IV enzyme source (*ex vivo*), AMC (7-Amino 4-methyl coumarin) as fluorescence substrate and sitagliptin as the standard reference.
- The methanol fraction is the most active to inhibit DPP IV enzyme from rat blood serum.

GRAPHICAL ABSTRACT



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Cite this article: Ekayanti M, Sauriasari R, Elya B. Dipeptidyl peptidase IV Inhibitory Activity of Fraction from White Tea Ethanolic Extract (*Camellia sinensis* (L.) Kuntze) *ex vivo*. Pharmacog J. 2018;10(1):190-3