Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Antioxidant activity and α-glucosidase inhibition effect of water extract of *Saurauia vulcani* Korth leaves

Tri Nova Lovena[1*], Urip Harahap[1], Panal Sitorus[2] and Denny Satria[2]

[1]*Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.*
[2]*Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.*

Key words: Antioxidant, *Saurauia vulcani* korth, enzymeα-glucosidase, total phenol, total flavonoid.

***Corresponding Author: Tri Nova Lovena,** Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.

Abstract

Objective: *Saurauia vulcani*, Korth leaves contain several compounds such as polyphenols which have activity as the antioxidant. This plant is used traditionally in the treatment of diabetes mellitus and wound healing. **Methods:** *Saurauia vulcani*, Korth leaves powder was extracted by infundation method with water as solvent.This aim of this study was to determined the total content of phenol and flavonoid, antioxidant activity in DPPH method and activity of the α-glucosidase enzyme inhibition of water extract of *Saurauia vulcani* Korth leaf. **Result:** The results were showed that water extract has a total phenol content of 96.75± 0.02 mg/g GAE, a total flavonoid content of 39.50 ± 0.02 mg/g QE, antioxidant activity with DPPH method has an IC_{50} value 22.918 ± 1.32 µg/mL and the highest activity inhibition with IC_{50} value 75.56 ± 1.07µg/mL. **Conclusion:** Based on the results obtained that the water extract leaves of *Saurauia vulcani*has a total phenol content, a total flavonoid content, antioxidant activity with DPPH method and inhibition of enzyme activity α-glucosidase giving synergistic effect.

Introduction

One alternative treatment of diabetes is using a variety of herbs especially containing polyphenols compounds, including flavonoids. These compounds are antioxidants and able to protect pancreatic β cells from the concentration of chain reaction caused by the ROS [1]. In addition to the antioxidant polyphenol compounds are also could bind the protein so that it can inhibit the enzyme α-like carbs parser glucosidase that contributes to *postprandial* hyperglycemia. Polyphenols was contained in various herbs such as green tea, berries, and vines are known to inhibit the enzyme cassava parser carbohydrates such assucrose, α-amylase and α-glucosidase [2]. It also results in many studies to find α-glucosidase inhibitors derived from plants, to develop physiological functions of food for treating diabetes mellitus [3]. *Saurauia* plant is one of natural materials as an alternative medicine that has been widely used to treat a variety of diseases.
Based on empirical data stew leaves *Saurauia* which people believed to have efficacy to heal wounds in a way blackmailed and also diabetes mellitus and how to boil the leaves of *Saurauia vulcani*, Korth. Flavonoid compounds contained in *Saurauia leaf* isolated by extraction and separation method usingthe chromatrography thin layer with n-butanol as developer. TLC produces patches of stain isolated flavonoids on Rf number 0.94 and shows the flavonoids examined are the isoflavones genistein [4]. Genistein can decrease glucose, HbA1c in diabetic rats induced by STZ, reduced glucose tolerance and increased insulin levels [5]. Previoues study showed the biological activity of *Saurauia vulcani* Korth as antihiperlipidemia and hyperglycemic [6]. The description has inspired us to do this research**,** which has been designed to determine the total phenol content, total flavonoids, antioxidant activity in DPPH method and activity of the α-glucosidase enzym of water extract of *Saurauia vulcani*, Korth leaf. This study perform only water extraction which refers to empirical use in community

Materials and methods

Plants material

Leaves of *Saurauia vulcani* Korth were colleted from Simpangan Bolon Village, Girsang Simpangan Bolon Subdistrict, Tapanuli Utara district, North Sumatera Province. It was identified at the laboratory of Herbarium Bogoriense, Botany Research Center for Biology-LIPI, Bogor. Chemical used were DMSO (Merck), enzyma α-glukosidase (Sigma), p-Nitrophenyl-α-D-glucopyranoside (p-NPG) (Sigma), akarbose (Bayer).

Preparation of *Saurauia vulcani,* Korth extract

Saurauia vulcani Korth leaves were dried in oven at 40°C and milled into powder. Then weighed as dry weight.

Dried Simplicia blended into a powder and then stored in a tightly closed container at room temperature to prevent the influence of humid and other doping. Water extract is produced by using the infundance method,50 grams of dried powder leaves inserted into a beaker glass 1000 mland added100 ml water added (1:10), then heated in waterbath at 90°C for 15 minutes (counted since temperatures reach 90ºC) while stirring with a magnetic stirer. The extract was filtered with cotton and concentrated at 55 ºC using a rotary evaporator.

Determination of phytochemical constituents

Phytochemical screening carried out on water extract of *Saurauia vulcani* Korth leaves includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins.

Determination of antioxidant activity with method of DPPH

A total of 25 ml of water extract from each concentration 25, 50, 100, 200 ppm was added 1 mL of solution 1,1-diphenyl-2-picrylhydrazyl (DPPH) 90 µM in methanol and shuffled with vortex for 2 min. Changed the color of technology from purple to yellow shows an antidote efficiency free radicals. Then in 5 minutes last before 60 minutes incubation, absorbance compared toλ 516 nm with using a UV-VIS spectrophotometer. The free radical antidote activity is calculated as a percentage of reduced color DPPH by using the formula :

$$\text{Antioxidant activity } \% = \frac{absorbance \quad sample}{absorbance \quad control} \times 100\%$$

Determination of IC_{50} value

The calculation result of antioxidant sactivityare incorporated into the equation line y = ax +b with concentration (mg/L) as abscissa (x axis) and percentaged value antioxidant sactivity of as ordinate (y axis). IC_{50} value of calculation at percentaged antioxidant activity by 50% will be obtained from the equation of the line [7].

Total Phenol content

The total phenolcontent was tested Folin Ciocalteu method with a slight modification Wolfe et al [8]. The extract of the test as 0.5 mL (1:10 g/L) was mixed with 5 mL, 4 mL of sodium carbonate (75 g/L water) and (1:10 v/v water) of Folin-Ciocalteu reagent. the mixture was shaken for 15 seconds and standed in waterbath for 90 min until the colour was changed. The content of total phenol compounds was expressed as mg of gallic acid equivalents per gram dry matter (GAE). The absorbance was measured at775 nm using a UV-Vis spectrophotometer [9].

Total flavonoid content

The flavonoids content was tested using Ordonez method et al [10]. The water extract as 0.5 mL of (1:10 g/L) was mixed with 0.5 mL of reagent $AlCl_3$ (10% v/v ethanol). The mixture was allowed to stand for 40 min. The color was mixed becomes yellow indicates the presence of flavonoids. The absorbance mix at spectrophotometer at 430 nm. Total flavonoids content was expressed in mg/g have corresponding quercetin.

Inhibitory activity of the enzyme α-Glucosidase assay

The enzyme inhibition activity α-glucosidase was conductedusing Salehi method et al [11]. This study used 20 µL of α-glucosidase (0.5 units/mL) and 120 µL 0.1 M, pH 6.8 phosphate encompasses. were used as a substrate of p-nitrophenyl α-D-glukopiranosida 5 mM in the same encompasses, 10 µL test extracts in different concentrations was dissolved in DMSO solution, was mixed with enzymatic cells were seeded 96-well microtiter plates. The plates was incubated at 37°C for 15 minutes. Then 20 µL substrate solution wasadded and was incubated again at 37°C for 15 minutes. Enzymatic reaction were stopped by the addition of 80 µl 0.2 M sodium carbonate solution. System reaction without sample was used as controls and α-glucosidase without system used as blank. The test wasperformed three times. Absorbance test solution in plates was read at 405 nm in a *microplate reader*, the magnitude of the obstacles ofthe enzyme by a sample test wasexpressed with the following formula:

$$\text{Inhibition } \% = \frac{(\text{A}-\text{B})}{\text{A}} \times 100$$

A: Blank absorbance (DMSO)
B: Sample absorbance (extract/comparison)

Calculated value of IC_{50} sample, the concentration of sample that inhibits 50% enzyme, regression equations through the liner, y = a+bx where x-axis is the concentration of sample and y is the% inhibition.

Inhibitory activity of enzyme kinetics α-glucosidase assay

The inhibition of extract enzyme inhibitory activity that has the highest. The measurement were by increasing the concentration of a given substrate (p-nitrofenil-α-D-glucopyranoside) with there nor without extracts or fractions with different substrate concentration Elya [12]. The determination of the type of inhibition is done with data analysis by the Lineweaver-Burk plot to obtain a Michaelis-Menten equation calculated Elya regression of y = a + *b*x 1/[S] as the x-axis and 1/V as the axis y Murray [13].

Statistics

Analysis of all results was performed using SPSS ANOVA with Tukey's Multiple Comparison Test. *P* values for significance were set at 0.05.

Results and discussion

Phytochemical screening result of water extract of *Saurauia vulcani* Korth leaves

Phytochemical screening result showed that water extract of *Saurauia vulcani* Korth leaves positively contains of Flavonoids, Saponins, Tanins, Glycosides and Steroids/triterpenoid.

Determination of antioxidant activity with the DPPH method

Analysis of DPPH using quercetin comparator. The concentration of extract DPPH free radical impacted immersion, the Concentration of extract higher % free radical DPPH immersion produced. Antioxidant extract was expressed in percentage of DPPH. This number means that the concentration of the extract may lead to more antioxidant activity.

Determination of IC_{50} value

The result of the equation linear regression and the analysis of results obtained from water extracts of IC_{50} leaves *Saurauia vulcani*and quersetin. based on the above results, the value of the antioxidant activity of the water extract of*Saurauia vulcani* IC_{50} values is 22,9182±1.32µg/mL where as IC_{50} produced quercetin value of 4.96±0.02 µg/mL. These results show that the ability to capture free radicals of the water extract of leaves of the *Saurauia vulcani* including a strong antioxidant, due to the strong categories include value is the concentration of 50-100

Total flavonoid content

The determination of total flavonoids content of water extracts using Ordonez method. quercetin with standard raw Curve reagents $AlCl_3$ with regression equation Y = 0, 0188X + 0,0256. This study shows that pirdot leaf extract has the highest number of total flavonoids, namely 39.50± 0.02 mg QE/g extracts. Based on a statistical analysis of total flavonoids content of note that of the whole extract tested.

Total phenol content

The determination of total phenols contentof water extracts using the Folin-Ciocalteu method with a slight modification Wolfe et al. the Method based on the strength of its phenolic hydroxy functional groups in performing the reduction. Standard error acid raw curve with Folin-Ciocalteu reactant with the regression equation Y = 0.0008X + 0.0175. Leave extract contained the highest number of total phenol, i.e. 96.75 ± 0.02 mg GAE/g extract, It is known that statistical analysis of the content of total phenols from the extract that tested.

The results of the activity of the enzyme α-glucosidase inhibition of extracts

Inhibition of this enzyme activity assay performed on water extractof *Saurauia vulcani*. Leaf.Extract weighed of ± 5 mg of DMSO to taste and added in mikrotube. DMSO is useful to help extract the solubility. Next add pH 6.8 phosphate encompasses up to gained the concentration of 1000 µg/mL, with dilution of phosphate pH 6.8 encompasses up to gained 200 µg/mL concentration; 100 µg/mL; 50 µg/mL; 25 µg/mL; 12.5 µg/mL. α-glucosidase enzymes used as blank. Absorbance test of solution in plate read at 405 nm in a microplate reader. The standard acarbose testing done first, It aims to be able to compare between the IC_{50} value of acarbose with the extract. Acarbose selected as a comparison because acarbose is the antidiabetic drugs which work in inhibiting α-glucosidase in circulation in Indonesia, as well as acarbose has already become an internationally recognised benchmark. In addition, in terms of structure, the acarbose has a structure similar to the given substrate of p-nitrofenil-α-D-glucopyranose. Observation on inhibitory activity of these enzymes was performed on all of the extract with concentration 200 µg/mL; 100 µg/mL; 50 µg/mL; 25 µg/mL; 12.5 µg/mL. All activity inhibition of α-glucosidase from the extract compared with the standard (acarbose). The lower the IC_{50} value then the higher α-glucosidase inhibitory activity. The results showed that the extract had a lower IC_{50} value compared with the standard (Figure 1).

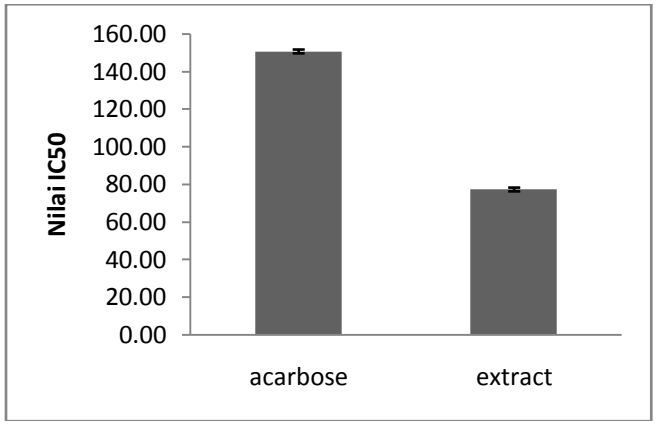

Figure 1. IC50 Value comparison chart from acarbose with extract.

The results showed that the acarbose has the ability inhibits the activity of α-glucosidase with IC_{50} values of 150.63 ± 0.25 µg/mL. While the extract had the ability inhibits the activity of α-glucosidase with lower IC_{50} value i.e. 75.56 ± 1.07 µg/mL. So it can be inferred that the extract has more significant capabilities in inhibits the

activity of α-glucosidase compared to acarbose. Based on the results of Phytochemical screening extract contains flavonoids, tannins, saponins, and glycosides. Flavonoid compounds phenolic lots owned by the plant and serve as an inhibitor of the enzyme α-glucosidase Tadera, *et al.* [14].

Inhibitory Kinetics of enzyme Activity results of α-Glucosidase of extract

The concentration of water extracts used in this test was 100 µg/mL with a wide range of substrate concentration increased from 1; 1.25; 2.5; 5 and 10 mM. Testing without inhibitors also performed with variations of the same concentration. Furthermore, the data acquired is calculated based on the regression equation is y = a + bx, 1/[S] as the x axis and 1/V as the y axis to obtain constants Michealis-Menten kinetics. Based on the plot of Linerweaver-Burk, there is the intersection of two equations of lines on the y axis (Figure 2) and the value of the Vmax is obtained from the equation of a line the third mutually approaching as well as increasing the value of Km (Table 1).

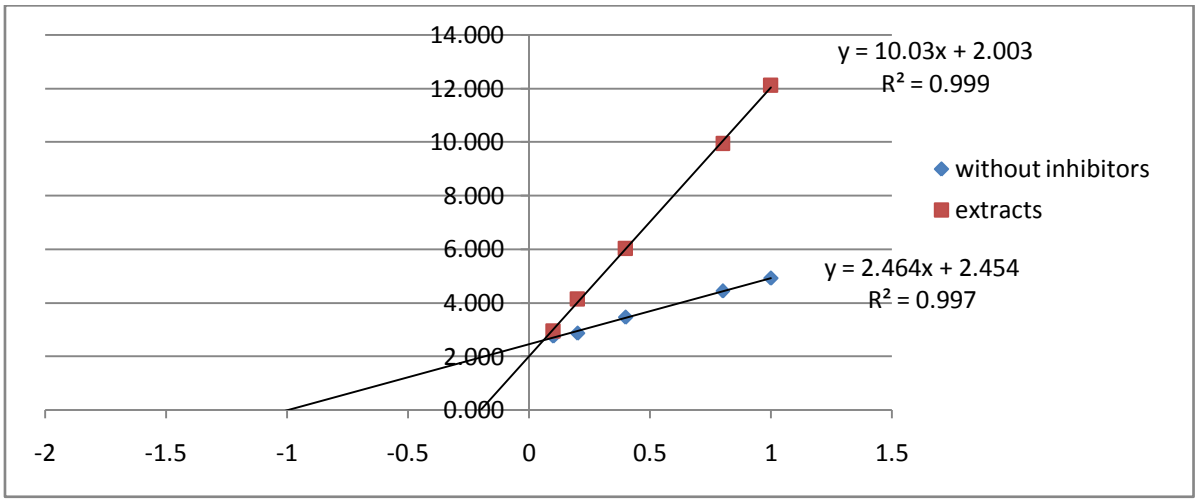

Figure 2. Linerwear-Burk plot Results α Activity Inhibition Kinetics Test-Glucosides of extracts.

Table 1. The results of the calculation of the Michaelis-Menten Constants of extracts.

	a	b	R	V_{max}	K_m
Without inhibitors	2.4643	2.4546	0.9974	0.44	1.17
Extract	10.031	2.0037	0.9995	0.49	4.91

Based on the results of the plot Linerwear-Burk, while 1/[S] close to 0, the maximum speed of reaction (Vmax) is not affected by the presence of inhibitors. Then at the time of the substrate concentration is high, the Vmax at the system with the same inhibitor with or close to Vmax with a system without inhibitor. Inhibitor which works competitively do not affect Vmax values, but increase the value of Km.

Conclusion

Basen on the result, it concluded that water extract of *Saurauia vulcani* korth showed antioxidant activity and effect of α-glukosidase inhibition

References

1. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pacific Journal of Tropical Biomedicine 2012; 2(5):411-420.
2. Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. Journal of Agricultural and Food Chemistry 2001; 49(4):1948-51.
3. Gao H, Huang YN, Gao B, Xu PY, Inagaki C, Kawabata J. α-Glucosidase inhibitory effect by the flower buds of Tussilago farfara L. Food chemistry 2008; 106(3):1195-201.
4. Gupta SK, Dongare S, Mathur R, Mohanty IR, Srivastava S, Mathur S, Nag TC. Genistein ameliorates cardiac inflammation and oxidative stress in streptozotocin-induced diabetic cardiomyopathy in rats. Molecular and cellular biochemistry 2015; 408(1-2):63-72.
5. Fu Z, Zhang W, Zhen W, Lum H, Nadler J, Bassaganya-Riera J, Jia Z, Wang Y, Misra H, Liu D. Genistein induces pancreatic β-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. Endocrinology 2010; 151(7):3026-37.
6. Hutahaean S, Tanjung M, Sari DP, Ningsih VE. Antihyperglycemic and antihyperlipidemic effects of pirdot (saurauia vulcani korth.) leaves extract in mice. In IOP Conference Series: Earth and Environmental Science 2018; 130(1): 012042.
7. Huliselan, Y.M., Runtuwene, M.R.J., Wewengkang. Aktivitas Antioksidan Ekstrak Etanol, Etil Asetat, dan n-Heksan dari Daun Sesewanua (Clerodendron squamatum Vahl.). Pharmacon Jurnal Ilmiah Farmasi 2015; 156:159-162.
8. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. Journal of agricultural and food chemistry 2003; 51(3):609-14.
9. Rebaya A, Belghith SI, Baghdikian B, Leddet VM, Mabrouki F, Olivier E, Cherif JK, Ayadi MT. Total phenolic, total flavonoid, tannin content, and antioxidant capacity of Halimium halimifolium (Cistaceae). Journal of Applied Pharmaceutical Science 2014; 5(1):52-7.
10. Ordonez AA, Gomez JD, Vattuone MA. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food chemistry 2006; 97(3):452-8.
11. Salehi P, Asghari B, Esmaeili MA, Dehghan H, Ghazi I. -Glucosidase and-amylase inhibitory effect and antioxidant activity of ten plant extracts

traditionally used in Iran for diabetes. Journal of Medicinal Plants Research 2013; 7(6):257-66.

12. Elya B, Basah K, Mun'im A, Yuliastuti W, Bangun A, Septiana EK. Screening of *α*-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. BioMed Research International. 2011; 2012.
13. Murray RK, Granner DK, Mayes PA, Rodwell VW. Biokimia Hapler edisi 27 Terjemahan dari Harper's Biochemistry 27 th oleh Brahmu. Jakarta: Penerbit Buku Kedokteran EGC: Halaman 2009; 68.
14. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. Journal of nutritional science and vitaminology 2006; 52(2):149-53.