Advances in Health Sciences Research, volume 40
2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021)

Total Phenolic, Total Flavonoid Content, and α-Glucosidase Inhibitory Activity of *Centella asiatica* (L.) Urb.

Minarti*, Megawati, Sofa Fajriah

Research Center for Chemistry, National Research and Innovation Agency Republic of Indonesia
Kawasan PUSPIPTEK, Serpong, Tangerang Selatan, Indonesia 15314
**Corresponding author: minarti_kuntum@yahoo.com*

ABSTRACT

Public awareness about the side effects of chemical drugs has increased in recent years. This has resulted in the rapid use of natural ingredients as medicine. Diabetes mellitus is one of the leading causes of death in the world. A large number of people with diabetes and the high level of need for drugs encourage efforts to find new sources of drugs. *Centella asiatica* (L.) Urb. has been widely used in folk medicine to treat several ailments, including antimicrobial, antioxidant, and anti-inflammation. However, the potential anti-diabetic activity of *C. asiatica* still needs to be explored. This present study aims to investigate the anti-diabetic potential of the aerial parts of *C. asiatica* (L.) Urb. by determining total phenolic and flavonoid content, also α-glucosidase inhibitory activity from ethanol extract, *n*-hexane, ethyl acetate, and water fractions. Bioassay-guided fractionation has been carried out to identify the bioactive crude fractions responsible for α-glucosidase inhibitory activity using *in vitro* study. The results showed that ethyl acetate fraction of *C. asiatica* afforded the highest total phenolic and flavonoid content by 14.41 ± 0.020 mg GAE/g extract and 48.43 ± 0.052 mg QE/g extract, respectively. In addition, this fraction also demonstrated the highest α-glucosidase inhibitory activity with IC_{50} 47.52 ± 0.030 µg/mL. The results indicated that *C. asiatica* has a potential source as a natural anti-diabetic agent.

***Keywords**: Diabetes mellitus, Centella asiatica (L.) Urb., total phenolic, flavonoid content, α-glucosidase inhibitory activity*

1. INTRODUCTION

Diabetes Mellitus (DM) is a disease or chronic metabolic disorder in which the pancreas does not produce enough insulin or when the body is ineffective in using it [1]. DM is characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both [2]. DM patients will experience hyperglycemia which causes a response to inflammatory compounds through cytokine mediation [2]. Cytokines will impair insulin sensitivity and glucose balance [2]. The increase in DM prevalence is estimated higher in developing countries than in developed countries [2].

Inhibition of the α-glucosidase enzyme is one strategy to control postprandial hyperglycemia glucose content by delaying the absorption of glucose in the intestine [3]. Inhibition of α-glucosidase enzymes can limit blood glucose content by slowing or delaying the process of hydrolysis and carbohydrate absorption [3]. Inhibition of this enzyme is very useful for the therapeutic management of type 2 DM [3].

The existence of these unwanted side effects underlies the emergence of various studies to find alternative therapies for type 2 diabetes, especially through the mechanism of α-glucosidase enzyme inhibition [3]. Therefore, new therapeutic agents are needed through the use of herbal preparations that are effective, relatively inexpensive, and low in side effects and toxicity [2].

Public knowledge in the use of medicinal plants is very diverse, both from the way of processing, how to use it, the parts used, and the efficacy of each type of plant in curing disease [4]. Gotu kola (*Centella asiatica* (L.) Urb.) is a plant that contains antioxidants with the main components being pentacyclic triterpenes (asiatic acid, madecassic acid, asiaticoside, and madecassoside) [2].

People in the Madura area use soaking water from all parts of the plant as a remedy for hemorrhoids and dry coughs, especially for children, it is used for nosebleeds, as well as an appetite enhancer [5]. In the Malay area, Gotu kola is well-known as a medicine for dry coughs and liver diseases [5]. A decoction of the leaves used for sore throat, asthma, colitis and as a mouthwash for canker sores [5]. Gotu kola leaves squeezed on the skin can heal skin inflammation and bruises [5]. In addition, Gotu kola leaves can also be used as a medicine for diabetes [6].

C. asiatica has been shown to have antioxidant, anti-inflammatory, and antimicrobial activities [2]. This plant contains several secondary metabolites, including triterpenoids, saponins, flavonoids, tannins, and alkaloids [4]. These secondary metabolites can treat DM disease [7]. The Gotu kola plant contains antioxidants in all parts of the plant from leaves to roots [8]. The triterpene aglycone compound in Gotu kola is non-polar which when it binds to three sugar molecules it still has little solubility in water and is more soluble in 96% ethanol [1]. The extraction process using ethanol is proven to be better than methanol and water [1]. Ethanol can dissolve triterpene aglycone compounds well, especially phenolic content, asiaticoside, and madecassoside [9].

Thus, this study aimed to evaluate the total phenolic and flavonoid content, also to tested antidiabetic bioactivity *in vitro* against the α-glucosidase enzyme from the ethanol extract of *Centella asiatica* (L.) Urb.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

The aerial parts of the Gotu kola (*Centella asiatica* (L.) Urb. were obtained from the Tawangmangu area and identified by the Research Center for Biology - Indonesian Institute of Sciences. Other materials used include ethanol solvents, *n*-hexana, ethyl acetate, methanol, aquades, dimethylsulfoxide (DMSO), 100 mM pH7 phospat buffer, p-nitorpehenyl α-Dglucopyranoside (PNPG), α-glucosidase enzyme, Na_2CO_3 200 mM. The equipment used in this study were macerator, erlenmeyer, beaker glass, measuring glass, separatory funnel, test tube, micro pipette (Eppendorf, Socorex), analytical scales (Mettler Toledo), rotary evaporator (Buchi), and UV/Vis spectrophotometer (Hitachi U-2000).

2.2. Extraction

The aerial parts of *C. asiatica* (L.) Urb. were washed and dried. Approximately 2700 grams of dried aerial parts of *C. asiatica* were macerated with ethanol, and then the filtrate was evaporated using a rotary evaporator to obtain an extract of 253.24 grams of ethanol extract.

2.3. Fractionation

Approximately 250 grams of ethanol extract was separated by liquid-liquid solvent with ratio a 1:1 with a separating funnel, using *n*-hexane, ethyl acetate, and water as solvents. Each filtrate was evaporated using a rotary evaporator to obtain n-hexane, ethyl acetate, and water fractions.

2.4. Measurement of Total Flavonoid Content

The total flavonoid content in the ethanolic extract and fractions of *C. asiatica* were determined spectrophotometrically with slight modifications in the study of Attanasova et al. [10], [11]. The extract and quercetin as positive control were weighed as much as 4 mg dissolved in 4 ml methanol (main solution 1000 µg/mL). Then standard solutions of 5, 10, 20, 30, and 40 µg/mL were made by pipetting standard solutions of quercetin (25, 50, 100, 150, and 200 µL) into a test tube containing 2 mL of distilled water. Add 150 µL of 5% $NaNO_2$ and shake until homogeneous. After 5 minutes add 10% $AlCl_3$, six minutes later add 2 mL of 1 M NaOH and aquadest until the volume becomes 5 mL. The solution was homogenized and allowed to stand for 5 minutes. The absorbance value of the sample was measured by a spectrophotometer at a wavelength of 510 nm. Calculation of total flavonoid content was based on the regression equation from the quercetin standard calibration curve. Results were expressed as mg equivalent of quercetin per gram dry weight of extract (mg/g extract).

2.5. Measurement of Total Phenol Content

The total phenol content in the ethanolic extract and fractions of *C. asiatica* were determined spectrophotometrically with Folin Ciocalteau reagent using gallic acid as a standard based on the method of De Aguiar et al. [12], [13] with slight modifications. The

extract and gallic acid were weighed as much as 4 mg dissolved in 4 mL of methanol (main solution 1000 µg/mL). Then standard solutions of 5, 10, 20, 30, and 40 µg/ml were made by pipetting the sample solution (0.5 mL) or standard solution of gallic acid (25.50, 100, 150, and 200 µL) into a test tube containing contains 7.5 mL of distilled water. Then 0.5 mL of Folin Ciocalteau was added and shaken until homogeneous. After 8 minutes, the solution was added with 1.5 mL of 20% Na_2CO_3, then incubated for 2 hours in a dark room at room temperature. The absorbance of the sample was measured at a wavelength of 765 nm. Calculation of total phenol content was based on the regression equation of the standard gallic acid calibration curve. Results were expressed as mg gallic acid equivalent per gram dry weight of extract (mg/g extract).

2.6. Antidiabetic Test

In vitro antidiabetic activity test was carried out using the method of Kim et al. [14], [15] with minor modifications. The test was carried out by adding 250 µL of p-nitrophenyl α-D-glucopyranoside (PNPG) 5 mM, 495 µL of 100 mM phosphate buffer into a test tube containing 5 µL of the sample (in dimethylsulfoxide (DMSO)), and then incubated at room temperature of 37ºC for 5 minutes. The reaction inhibition of enzyme activity started shortly after adding 250 µL of α-glucosidase enzyme (0.065 unit ml) (EC 3.2.1.20 from Wako Pure Chemical Industry) into the test tube and then re-incubated for 15 minutes. The reaction was stopped by adding 1 mL of 200 mM Na_2CO_3. The test sample blanks were prepared by the same preparation process, but the enzyme was replaced with 250 µL of 100 mM phosphate buffer. The activity of α-glucosidase was determined by measuring the absorption of the released *p*-nitrophenol compound using a spectrophotometer at a wavelength of 400 nm.

3. RESULTS AND DISCUSSION

Extraction of dried aerial parts of *C. asiatica* (L.) Urb. was carried out using ethanol by maceration obtained yield percentage of 9.38% (w/w). The fractionation of ethanol extracts obtained *n*-hexane, ethyl acetate, and water fractions of 6.86, 4.15, and 84.95% (w/w), respectively (Table 1).

Table 1. Yield of extraction and fractionation of *C. asiatica* (L.) Urb.

Sample	Extract weight (g)	Yield (% w/w)
Ethanol extract	253.24	9.38
n-Hexane fraction	17.16	6.86
Ethyl acetate fraction	10.38	4.15
Water fraction	212.37	84.95

Determination of total phenol content of ethanol extract, *n*-hexane fraction, ethyl acetate, and water fractions from *C. asiatica* (L.) Urb. were carried out at maximum absorption at a wavelength of 765 nm. The ethyl acetate fraction had the highest total phenol content compared to the ethanol extract and the *n*-hexane and water fractions. In current study total phenolic contents of methanol extract and different solvent (*n*-hexane, chloroform, ethyl acetate and aqueous) fractions, determined using Folin-Ciocalteu method, which is described in a different pharmacopies. The reaction is based upon the fact that phosphomolybedate and phosphotungstate in the reaction mixture react with phenolic compounds in the plant sample and generate a blue chromophere that had maximum light absorption at 760 nm. Higher the extent of phenolic compounds in the plant sample, the greater will be the intensity of blue pigment and so on.

Determination of the total flavonoid content of the ethanol extract, n-hexane, ethyl acetate, and water of Gotu kola (*Centella asiatica* (L.) urban leaf extract was carried out at the maximum absorption at a wavelength of 510 nm. By making a standard calibration curve of quercetin so that the regression equation y = 0, 0096x + 0.0191 with a correlation coefficient value (r) = 0.9817. The total flavonoid content of ethanol extract, *n*-hexane, ethyl acetate, and water fractions were 26.66; 22.66, 48.43 and 9.3 mg QE/g extract, respectively. The ethyl acetate fraction had the highest total flavonoid content compared to the ethanol extract, the *n*-hexane, and water fractions.

Table 2. Total contents of flavonoids and phenols from Gotu kola (*Centella asiatica* (L.) Urb.

Sample	Total Phenolic Content (mg GAE/g extract)	Total Flavonid Content (mg QE/g extract)
Ethanol Extract	3.21 ± 0.020	26.66 ± 0.056
n-Hexane	2.88 ± 0.059	22.66 ± 0.052

Fraction		
Ethyl acetate Fraction	14.41 ± 0.020	48.43 ± 0.052
Water Fraction	6.49 ± 0.032	9.33 ± 0.051

The results of the inhibition test on the activity of the α-glucosidase enzyme (Table 3) showed that the ethyl acetate fraction (IC_{50} 47.52 ± 0.030 ppm) had a higher inhibitory activity when compared to the ethanol extract, hexane fraction, and water fraction with inhibitory values (IC_{50}) 374.54 ± 0.040, 62.41 ± 0.031 and 507.67 ± 0.025 µg/mL. However, the anti-diabetic activity for the *n*-hexane fraction had an inhibitory value (IC_{50} 62.41 ppm) which potential to be developed.

Table 3. The results of the analysis of inhibition of antidiabetic test activity using α- Glucosidase from *Centella asiatica* (L.) Urb.

Sample	Absorbance	Concentration (µg/ mL)	Inhibition (%)	IC_{50}
Quercetin	0.5592	25	72.71	4.35 ±0.084
	0.8465	10	58.69	
	0.9177	5	55.21	
	1.1129	2.5	45.68	
	1.4386	1.25	29.78	
Ethanol extract	1.4385	200	29.79	374.54 ± 0.040
	1.6915	100	17.44	
	1.7907	50	12.60	
	1.8624	25	9.10	
n-Hexane fraction	0.6096	100	70.25	62.41 ± 0.031
	1.1112	50	45.77	
	1.3226	25	35.45	
	1.7073	10	16.67	
	1.9639	5	4.15	
Ethyl acetate fraction	0.3430	100	83.26	47.52 ± 0.030
	0.7484	50	63.47	
	1.3047	25	36.32	
	1.7001	10	17.02	
	1.7535	5	14.42	
Water fraction	1.6410	200	19.91	507.67 ± 0.025
	1.8710	100	8.68	
	1.9382	50	5.40	
	1.9993	25	2.42	
	2.0356	10	0.65	

4. CONCLUSIONS

Based on the results of the study above, it can be concluded that the fraction that has the largest yield from the fractionation of the ethanol extract of the aerial parts of *Centella asiatica* (L.) urban is the water fraction of 84.95% (w/w). The ethyl acetate fraction has a total content of flavonoids (48, 43%) and total phenol content (14.41%) which was the highest compared to ethanol extract, n-hexane, and water fractions. Ethylacetate fraction (IC_{50} 47.52 ppm) had a higher inhibition of α-glucosidase enzyme activity than inhibitory value (IC_{50}) of ethanol extract, *n*-hexane fraction, and water fraction.

The ethyl acetate fraction contains a lot of flavonoid and phenol compounds, and has the highest antidiabetic activity compared to ethanol extract, *n*-hexane fraction, and water fraction, so that the ethylacetate fraction of Gotu kola (*Centella asiatica* (L.) Urb. can be developed further to search for new drug sources anti-diabetic derived from natural ingredients.

ACKNOWLEDGMENTS

This research was funded by Indonesia Government through LPDP Grant from Financial Ministry of RI (Grant number: 97/E1/PRN/2020). The authors also thank to Dr. Teni Ernawati for her advice in this paper.

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