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Original Research Article

TLC-Densitometry Analysis Method of Asiaticoside Assay and Antioxidant Activity of *Centella asiatica* (L.) Urban Extract

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ABSTRACT

Centella asiatica (L.) is a medicinal plant with anti-inflammatory, anti-asthmatic, anti-hemorrhoidal, wound healing, and antioxidant effects. Currently, the dry extract of *Centella asiatica* is widely produced by various industries. It is necessary to determine the quality of the extracts on the market. The aims of this study are to develop the asiaticoside assay method using TLC-densitometry, identification of the compound groups, and determination of the antioxidant activity of *Centella asiatica* dry extracts and *Centella asiatica* extract on-market products. The phytochemical content of *Centella asiatica* extracts was evaluated using the TLC–densitometry method. The validation of the analysis method for asiaticoside assay was subsequently carried out, as well as the determination of antioxidant activity using the DPPH method. The method validation results showed that the TLC–densitometry method met the validation parameter criteria. Asiaticoside assay of the dry extract, simplicia, and on-market extract products were 9.07, 0.10, 0.60, and 10.53%, respectively. Furthermore, the results of the antioxidant activity of the dry extract and on-market extract products showed an IC_{50} value of 0.1166, 0.5783, and 1.9513 mg/mL, respectively. This study obtained a valid method for analyzing asiaticoside assay using the TLC-densitometry method. Asiaticoside content of dry extract was 9,07%, and extract products on-market were 0,60% and 10,53%, respectively. The IC_{50} value of the dry extract of *Centella asiatica* was in the moderate antioxidant category, while the on-market extract products were in the very weak antioxidant category.

Keywords: *Centella asiatica*, phytochemical screening, TLC densitometry, asiaticoside, antioxidant activity.

Introduction

An unhealthy lifestyle and excess air pollution could increase the body's free radicals.[1] A free radical is an unstable molecule that can attract electrons from other molecules or cells in the body. Furthermore, the cells become damaged and can develop into organ malfunction.[2] An antioxidant is a chemical compound that can donate the electron to the free radical to stabilize the molecules.[3–6] The higher number of antioxidants in the body can suppress the number of free radicals inside. Antioxidant compounds can be obtained synthetically or naturally.[7,8] *Centella asiatica* (L.) Urban is often used as a medicinal plant. Some of the activities that have been proven to be possessed by this plant are wound healing of skin scar, anti-tumour, anti-depression, inhibiting the formation of anti-fibrosis, neuroprotection, anti-inflammation, and lung protection.[9–12] In addition, *Centella asiatica* has also been shown to have activity as an antioxidant *in vitro* and *in vivo*.[12–14] Several studies show that the antioxidant activity of the methanol extract of *Centella asiatica* leaves has an IC_{50} value of 481.64 mg/mL.[13] In comparison, the 98% ethanol extract of *Centella asiatica* leaves has an IC_{50} of 125 mg/mL,[14] and the aqueous extract of *Centella asiatica* leaves has an IC_{50} value of 31.25 mg/mL.[12]

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The compounds contained in *Centella asiatica* are diverse, ranging from volatile compounds, saponins, and phenolic compounds, such as flavonoids and tannins, as well as ester compounds. Sesquiterpenes and monoterpenes, such as α-humulen, β-caryophyllene, bicyclogermacrene, germacrene-D, and myrcene 15 dominate volatile compounds belonging to the terpenoid group. The terpenoid compounds can act as antibacterial. In the saponin group, aciaticoside and madecassosside are the major compounds contained in this plant. Asiaticoside, as terpenoid glycoside, is the main component of *Centella asiatica*, which functions as an antioxidant that can eliminate free radicals, revitalize blood vessels, and improve memory.[9,15] Meanwhile, the phenolic compounds contained in *Centella asiatica* are catechin, epicatechin, kaempferol, and quercetin.[16]

In the process of developing medicinal plants, it is necessary to go through stages to meet the aspects of quality improvement, safety, formulation, and the discovery of new indications. The safety of medicinal plants also depends on the bioactive compounds content that has been proven to have certain pharmacology activities. One of the methods that can be used to observe the quality of extract products is screening for compound groups and determination of marker assay.[17] This study used TLC-Densitometry because this method has the advantages such as fast technique, high sensitivity, and low cost.[18,19] The validation of the analytical method needs to be carried out with several predefined validation parameters, such as precision, accuracy, linearity, limit of detection, limit of quantification, selectivity, and specificity. To guarantee the activity of the extract product, it is necessary to test it using the DPPH method.[20]

In this study, the phytochemical compound groups of *Centella asiatica* extract were determined. The TLC-densitometry as an analysis method for asiaticoside was validated then the asiaticoside concentration in *Centella asiatica* extract, simplicia, compared to the two on-market products named Gotu cola extracts. Further the antioxidant activity also evaluated using DPPH method.

Materials and Methods

Plant Material and Reagents
Whole herb of *Centella asiatica* purchased from Herbal Materia-Medica Batu on May 30, 2022. The identification and authentication of the plant were done by Herbal Laboratory-Materia Medica Batu, Indonesia, 074/367/102.20-A/2022), *Gotu Kola Extract White* Powder (Botanical Cube Inc, Shaanxi-China, BCL21818JXC), *Gotu Kola Extract Brown-yellow* Powder (Botanical Cube Inc, Shaanxi-China, BCL21415JXC), Standard asiaticoside (Supelco, U.S), Ethanol 70%, Chloroform (Merck, Germany), Methanol (Merck, Germany), Glacial Acetic Acid (Merck, Germany), Water (Merck, German), $FeCl_3$ (Supelco, U.S), Sulfuric Acid Anisaldehyde Reagent, Liebermann Bourchard, Dragendorff's reagent (Merck, Germany).

Method
Extraction
The extraction process was carried out by macerating 900 grams of dried herb *Centella asiatica* with 70% ethanol at a ratio of 1:10. The maceration process lasted for 24 hours at room temperature and was repeated for three times. Furthermore, the crude extract was evaporated using a rotary evaporator (Buchi, Switzerland) to obtain a concentrated extract, and the dried extract was dried using a freeze-dryer (Buchi, Switzerland).

Phytochemical screening
The phytochemical screening methods were conducted to analyze the content of the group of alkaloids, saponin, flavonoid, steroid, tannin, and triterpenoid using the standard procedures.[21,22] The phytochemical compounds of crude extract, dried extract, and on-market extract products were compared.

Determination of Asiaticoside Assay in Extract of Centella asiatica
Determination of Asiaticoside Assay was carried out using the TLC – Densitometry

Asiaticoside Standard Sample Preparation
Asiaticoside standard solutions with concentrations of 100, 200, 500, 1000, and 2000 mg/mL were dotted with 1.5 uL on the TLC plate GF_{254} (Merck, Gemrnay) to obtain total weights of 150, 300, 750, 1500, and 3000 ng, respectively. The parameters used in the method's validation are selectivity, specificity, linearity, accuracy, precision, limit of detection, and limit of quantitation.

Method Validation
Precision
10 µL extract solution with a concentration of 250 mg/mL was applied on the TLC plate and developed with the selected mobile phase. Furthermore, the developed plate was sprayed with concentrated anisaldehyde-H_2SO_4 and then scanned using TLC-densitometer (CAMAG®, Switzerland). The data taken were the value of the asiaticoside retardation factor and the peak area of asiaticoside in the extract. Precision parameter data were obtained from the %RSD retardation factor and peak area of asiaticoside.[21]

Accuracy
Asiaticoside standard solutions with 100, 125, and 150 mg/mL concentrations as the 80, 100, and 120% values were prepared for further addition to the extract. A 10 mL volumetric flask was prepared with an addition solution consisting of 5.0 mL extract and added with asiaticoside standard with the previous concentration, then extract solution without addition with concentration 4000 mg/mL, 5.0 ml extract added with 10 mL ad solvent, as well as a standard solution of asiaticoside without additions with a concentration of 125 mg/mL. Accuracy parameter data is obtained from the %recovery value.[21]

Linearity
1.5 uL Asiaticoside standard solutions with concentrations of 100, 200, 500, 1000, and 2000 mg/mL were applied on the TLC plate to obtain total weights of 150, 300, 750, 1500, and 3000 ng, respectively. Then the plate was eluted with the selected mobile phase and sprayed with concentrated anise aldehyde-H_2SO_4.[21]

Limit of Detection (LOD) and Limit of Quantification (LOQ)
Limits of detection and limits of quantitation determined from standard curve regression obtained.
LOD and LOQ data are calculated using the following formula:
LOD = 3Sy/b.....*(1)*
LOD = 10Sy/b.....*(2)*
Where Sy is the standard deviation of the function and b is the slope.[21]

Selectivity and Specificity
2 µL Asiaticoside standard solution with a concentration of 250 mg/ml were applied on the TLC plate. Value of peak purity r(s,m) and r(m,e) > 0.9900. Meanwhile, the peak identity correlation value between standard asiaticoside and asiaticoside contained in extract spectra in extracts was > 0.9900.[21]

Determination of Asiaticoside in Samples of Centella asiatica Crude Extract, Dried Extract, and Gotu kola extract brown-yellow powder and *Gotu kola extract white powder*
50 mg of dried extract dissolved in 5 mL of 70% ethanol, stirred for 10 minutes until dissolved. 200 mg of crude extract was added to 5 mL of 70% ethanol using the ultrasonic-assisted extraction method for 30 minutes at room temperature. In the test sample, 70% ethanol was added to the extract product. The extraction was carried out using the ultrasonic-assisted extraction method for 30 minutes at room temperature. Then the mixture was centrifuged (Hettich EBA-21, United Kingdom), for 10 minutes at 400 rpm, and the supernatant solution was taken for analysis with Densitometry TLC.
E. Antioxidant Activity Testing with the DPPH Method (2,2-*Diphenyl*-1-*Picrylhydrazyl)*
The antioxidant activity of the ethanol extract and the product *C.asiatica* extract compared with vitamin C was tested using the 1,1-*diphenyl*-2-*picryl hydrazyl* (DPPH) method. 40 mg of DPPH were dissolved in 100 mL of methanol to prepare a stock solution. Put 1 ml of the DPPH stock solution into a test tube, then add 3 ml of methanol, then incubated (memmert, Germany) for 30 minutes at room temperature. then the absorbance is measured at the maximum wavelength that has been obtained, namely at 517 nm using Microplate Reader (Biochrom EZ Read 2000, United Kingdom). Next, each sample and reference solution pipetted 1 mL, put into a test tube, added 1 mL of 100 mg/mL DPPH, and added 2 mL of methanol p.a, shaken until homogeneous. This solution was incubated at room temperature for 30 minutes, and its absorbance was measured at the optimum DPPH wavelength obtained. Replication was carried out three times. The IC_{50} value was calculated using the linear regression equation y = bx + a between the concentration of the test solution (x) and % Inhibition (y).[22]

Results and Discussion

Extraction of Centella asiatica
In this study, the maceration process used 70% ethanol solvent. The choice of ethanol solvent was due to its ability to extract broad polarity compounds.[23]
In this study, the maceration process was carried out for 24 hours with stirring using an overhead stirrer at 120 rpm. The stirring aims to speed up the contact between the sample and the solvent. The dried extract in obtained 241.5 grams (26.83% extract yield). According to the Indonesian Herbal Pharmacopoeia 2017, the condition for a concentrated extract is that the percentage yield value is not less than 10%.[24] The concentrated extract was then dried using a freeze dryer to obtain a dried extract of *Centella asiatica*. The dry extract weight obtained in is 161.4 grams. This decrease in weight was due to the reduced water content of the thick extract to make it dry. This is also supported by the working principle of the freeze dryer, which is to remove the water content from a material. The advantages of this method include that the resulting material will be more stable, such as no shrinkage or changes in the shape of the material structure. In addition, the rehydration power of the material will increase. This

rehydration power is very important in the pharmaceutical industry, especially for injection drug preparations. If the rehydration power increases, the solubility or solubility of the material will also be better.[25] Furthermore, the dry extract was tested for phytochemicals using TLC to determine the presence of flavonoids, alkaloids, saponins, tannins, and steroids.[26–28]

Phytochemical screening

The results of the phytochemical screening in **Table 1** show that the positive *Centella asiatica* extract contains saponins, tannins, flavonoids, triterpenoids, and steroids. According to [11], asiaticoside is a compound that belongs to the triterpenoid group.[11]

Table 1. Results of Phytochemical Screening of Crude Extract, Dried Extract and Extract Products *Centella asiatica*

The result in Table 1 shows that the extract product contains flavonoids, saponins, tannins, steroids, and terpenoids.

4. Linearity of Asiaticoside Standard Curve

The linearity of an analytical method is assessed by determining the graph of the response to the content, the correlation coefficient (r) of the regression line equation, and the residual standard deviation (Sy/x) of the regression line.[21] This residual standard deviation will calculate the regression coefficient of variation (%CV). The recommended CV value is ≤ 5%. Suppose the correlation coefficient obtained from the linear regression equation has not reached 0.999, so an analytical method can still be said to have good linearity. ICH recommends using a minimum of five concentrations in the standard curve for testing the linearity of the standard curve of an analytical method.[29] The results of measuring the asiaticoside standard curve in several samples of *Centella asiatica* extract can be seen in Table 2**.**

The linearity results are used to evaluate the response of the analyte concentration in the form of the area under the curve of the densitometer. This study obtained the standard curve equation y = 1.2571x + 5157 with r^2 of 0.9999. The standard curve formed can be seen in the following figure:

Based on the results in Figure 1. The value of the correlation coefficient r count is 0.9999, which shows a linear relationship between the total weight and the area. Besides being seen from the correlation coefficient, other parameters can also be seen, namely the coefficient of variation (Vxo) of linearity by comparing the standard deviation of the function (Sxo) and the average of the asiaticoside weight per spot used. The function's standard deviation is obtained by comparing the residual standard deviation (Sy) with the function's slope. The standard deviation formula used is as follows:

$$Sy = \sqrt{\frac{\Sigma(y-yi)^2}{N-2}} \quad \text{.....(3)}$$

Vxo calculation results are shown in the following Table 2:

Sy = 17.6998
Sxo = 10.0799
CV = 1.24%

From the results above, the CV value meets the requirements (CV <5%).

Based on the results in Table 2, it can be concluded that the linearity of the asiaticoside standard meets the good requirements so that its validation can be guaranteed. Measuring the assay of samples containing asiaticoside using the regression equation from the standard curve above can guarantee its validity when the sample levels fall within the range of the standard curve. If the assays are above or below the range of the standard curve, the validity of the measurement results using the regression equation above cannot be guaranteed.

Table 1: Results of Phytochemical Screening of Crude Extract, Dried Extract and Extract Products *Centella asiatica*

Compound Group	**Sample**			
	Crude Extract	Dried Extract	Product A	Product B
Alkaloids	-	-	-	-
Flavonoids	+	+	-	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Triterpenoid	+	+	+	+

(+) = positive; (-)= negative; Product A= Gotu kola extract brown-yellow powder; Product B= Gotu kola extract white powdeR

Table 1: Linearity of Asiaticoside Standard

Weight per Spot (ng)	**Area (AU)**	**Calculated Area (AU)**	**$(Y-Yi)^2$**
13	5340.2	5352.30	146.2890
300	5521.7	5539.69	323.6401
750	6119.5	6101.88	310.6406
1500	7045.7	7038.85	46.9225
3000	8923.4	8912.80	112.3600
Total			**939.8523**

ng=nanograms; AU=Absorbance Units; Y=Area; Yi=Calculated Area

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection is defined as the lowest concentration of analyte in a sample that can still be detected, although not always quantified. The quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the operational conditions of the method used. Limits of detection and quantification are parameters of the sensitivity of an analytical method. The smaller the value of the limits of detection and quantification indicates, the more sensitive a method is in analyzing and measuring the levels of an analyte.[21]

After obtaining the standard curve equation from the linearity of asiaticoside compounds, the standard curve equation's detection limit and quantitation limit are determined to ensure that the data obtained is above the limit and confirmed by the existing standard curve equation. Based on the results of the calculations that have been done, the LOD value is 42.2397 mg/mL, and the LOQ is 109.4244 mg/mL.

Precision

Precision measures the closeness between analytical results obtained from several measurements on the same homogeneous sample. Precision is usually expressed as the relative standard deviation of several statistically significant samples. Repeatability is accuracy under the same experimental conditions (repeated) for the analysis, the equipment, the place, and the time. At the same time, the intermediate precision is the accuracy for the experimental conditions where one is different regarding the analysis, equipment, place, and time. Precision documentation should include standard deviation, relative standard deviation (RSD), or coefficient of variation (CV). Referring to the Association of Official Analytical Chemist (AOAC) Guidelines which

are a reference in validating analytical methods, the RSD value of the acceptable repeatability precision for compounds with levels of 100 to 1000 mg/mL is no more than 4%.[30] The results of precision measurements for all components have met the requirements of AOAC, so it can be said that the method developed has met the specified criteria, and the results of precision measurements are presented in Table 3.

Determination of precision parameters is carried out to determine the closeness between the test results obtained. After measuring the %RSD value on the value of the retardation factor and the peak area of asiaticoside, the following results were obtained:

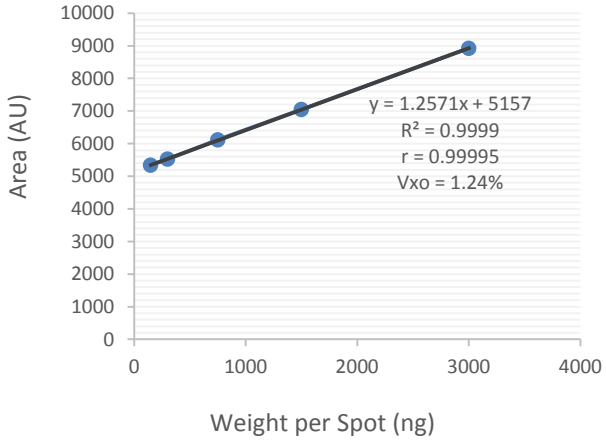

Figure 1: Linear Regression Curve of Asiaticoside

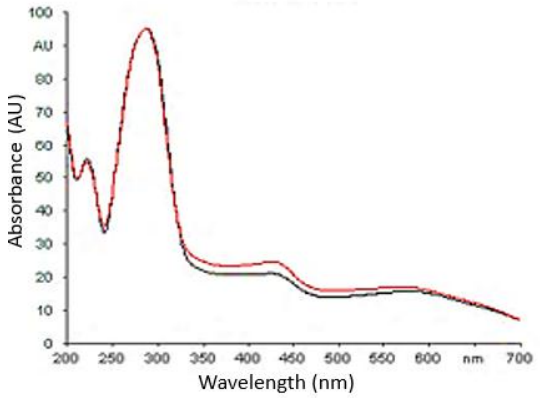

Figure 2: Specificity of Asiaticoside (—) *Centella asiatica* extract; (—) asiaticoside standard

Accuracy

Accuracy is the closeness between the measured value and the value accepted as the true value. Accuracy is expressed as the recovery percentage of the added analyte. The measurement of accuracy in this study used the standard addition method because the sample being analyzed was an extract with no known matrix, so it was impossible to make a placebo sample. The addition method is a quantitative analysis technique by adding a known amount of analyte to the sample. The recovery percentage is determined by determining what percentage of the added analyte is recovered. A practical approach to the standard addition method is to divide the sample into several equal portions and then add the standard at an increasing concentration. Referring to the accuracy value requirements stated in the AOAC, the accuracy value received for concentrations of 10 to 100 mg/mL is 80-115%, and for concentrations of 100 to 1000 mg/mL is 85-110%.[30]

Accuracy is carried out to determine the closeness of the test results obtained with the actual value to obtain precise and accurate data. Therefore, an additional solution consisting of 0.5 mL dry extract plus asiaticoside standard with a concentration of 100 mg/mL as 80% value, 125 mg/mL as 100% value, and 150 mg/mL as 120% value. Subsequently, a dried extract solution was prepared without addition with a concentration of 4,000 mg/mL, followed by 0.5 mL of dry extract plus 0.5 mL of solvent, and a standard solution of asiaticoside without addition with a concentration of 125 mg/mL. Each sample was repeated three times to see the consistency of the sample preparation and the standards used. The accuracy data obtained is also taken to calculate the %recovery and %RSD values to compare them with the data precision requirements.[21]

After development, the plate was scanned with a densitometer. The reading results are compared with the standard curve equation to obtain the test concentration and compared with the actual concentration to show the method's accuracy. After being entered into the standard curve equation, the accuracy and precision test results are obtained in the following Table 4:

Reflecting on the results of the accuracy and precision tests, it can be stated that the data meets the requirements. Those are that %recovery meets the requirements by being in the 85-110% range, and for %RSD meets the requirements (%RSD ≤ 4%).[21]

Selectivity and Specificity

After scanning the peaks of asiaticoside compounds in the sample at a wavelength of 200 – 700 nm, the peak purity correlation values are obtained as follows:

The purity test in Table 5 and Figure 2 was carried out by comparing the spectra at the peak position, namely the start position (s), the peak/maximum position (m), and the end position (e). These results indicate that the peak purity correlation value of the asiaticoside peaks in the sample has a purity value of > 0.9900. It can be stated that the asiaticoside peaks in the sample are pure (not contaminated). Then, an overlay of the spectral profile results of standard asiaticoside compounds and asiaticoside compounds in the sample is obtained. A correlation limit value of 0.99882 is obtained. This value is 0.9900, and it can be concluded that the two peaks are identical.[19]

Determination of Asiaticoside Concentrations in Crude Extract, Dried Extract, and Extract Products of Centella asiatica

The results of determining Asiaticoside assay in Crude Extract, Dried Extract, and Extract Products of *Centella asiatica* can be seen in Table 6. The sample was replicated three times to determine the assay of Asiaticoside in *Centella asiatica* extract and then calculated the average %w/w.

The Indonesian Herbal Pharmacopeia requires the asiaticoside content in the dried extract of *Centella asiatica* to be > 0.90%. Based on the results shown in Table 6, the maceration process affect the compounds from the extracted plants. The dried extract obtained contained Asiaticoside compounds of 9.07%. Small levels of Asiaticoside were is greater than in the dried plants. This might be because the dried plants contained many compounds and coarse particles. On-market product extracts containing Asiaticoside at a certain level do not guarantee that the powder is pure Asiaticoside. To produce perfect fine powders, fillers are needed, so optimization is needed to determine the solvent that can separate the fillers from the asiaticoside compounds in them. In this study, 95% ethanol solvent was used to dissolve the extract powder to facilitate the identification of asiaticoside compounds contained therein.

DPPH Method Antioxidant Activity Test

Antioxidant activity testing was carried out using the DPPH method. This method was chosen because it is a simple, easy, and fast method and only requires a small number of samples to evaluate antioxidant activity. The DPPH free radical solution has an unpaired nitrogen atom. The reaction of DPPH with hydrogen atoms contained in antioxidants can reduce the reactivity of DPPH solutions, as indicated by the fading of the purple colour to yellow.[31] Vitamin C was used as a comparison in this study. The reason for using vitamin C as a comparison was because vitamin C is a secondary antioxidant that can scavenge free radicals, prevent chain reactions, and has high antioxidant activity. Vitamin C has a hydroxyl group that acts as a free radical scavenger.[32] Antioxidant activity testing was carried out by making a DPPH solution which was then determined for the maximum wavelength. The process of determining the maximum wavelength

aims to obtain the wavelength that has the maximum absorbance. The determination of this wavelength was carried out in the range of 510-525 nm. The results for determining the maximum wavelength of DPPH obtained were 517 nm. After obtaining the maximum wavelength, the next step is measuring the absorbance of the samples for each concentration and calculating the % inhibition. The results of the absorption and % inhibition measurements obtained can be seen in Table 7 below:

Table 7. IC_{50} value of Crude Extract, Dried Extract, Extract Products of *Centella asiatica*, and Vitamin C

Antioxidant activity can be classified into five categories: very strong, strong, moderate, weak, and very weak with IC_{50} values less than 50 mg/mL, 50-100 mg/mL, 100-150 mg/mL, 150-200 mg/mL, and very weak with IC_{50} values greater than 200 mg/mL.[33] Based on these criteria, the dry extract of *Centella asiatica* is included in the criteria for strong antioxidants, and vitamin C is included in the criteria for moderate antioxidants. Based on the results of the antioxidant activity test, it can be seen that the antioxidant activity of the dry extract of *Centella asiatica* is lower when compared to vitamin C. The low antioxidant activity is thought to be caused by various factors, including the extraction method used insufficiently attracting the antioxidant chemical components in *Centella asiatica*. In addition, it is declared that vitamin C is a single substance or compound with very strong antioxidant activity, while the extract is multi-compounds.

Conclusion

Based on the study, it can be concluded that all of the extracts contain saponin, tannin, steroid, steroid, and triterpenoid. The TLC densitometry met all of the validation parameters for asiaticoside assay. The asiaticoside in the crude extract, dried extract, and the two on-marker extract products of *Centella asiatica* were 0.90%, 9.07%, 0.61%, and 10.34% respectively. Based on the antioxidant activity test, the dried extract was classified as a moderate antioxidant, while both the on-marker extract products belong to a very weak antioxidant. *Centella asiatica* dry extract has the potential to develop further as an antioxidant agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 2. Precision Result

Sample	Area (AU)	RSD Area (%)	Rf	RSD Rf (%)
Replication 1	17072.3		0.88	
Replication 2	16488.8		0.88	
Replication 3	16001.7	2.56 %	0.87	0.51%
Replication 4	16728.0		0.88	
Replication 5	16955.5		0.88	

*Rf = retention factor; RSD=Relative Standard Deviation; AU=Absorbance Units

Table 3: Accuracy Result

Sample	Area (AU)	Weight per Spot (ng)	Asiaticoside Weight (ng)	% Recovery (%)	SD (ng)	%RSD (%)
80%(1)	22145.6	576.5802	198.5184	99.26	2.7665	1.39
80%(2)	23217.9	604.4985	196.8052	98.40		
80%(3)	21829.4	568.3477	202.2181	101.11		
100%(1)	24380.4	634.7652	256.7034	102.68	7.4807	2.97
100%(2)	25008.4	651.1157	243.4225	97.37		
100%(3)	23896.2	622.1586	256.0290	102.41		
120%(1)	26088.9	679.2475	301.1857	100.40	2.2327	0.74
120%(2)	27235.5	709.1002	301.4070	100.47		
120%(3)	25783.2	671.2883	305.1587	101.72		

*SD= standard deviation; RSD=Relative Standard Deviation

Table 5. Peak Purity Result

Track	Rf	Assigned Substance	Max.signal	R (s,m)	R (m,e)	purity
Track 1	0.89	Asiaticoside	286 nm	0.998068	0.999385	Ok
Track 2	0.89	Asiaticoside	286 nm	0.999133	0.998339	Ok
Track 3	0.88	Asiaticoside	287 nm	0.999557	0.999547	Ok
Track 4	0.88	Asiaticoside	287 nm	0.998798	0.999546	Ok
Track 5	0.87	Asiaticoside	287 nm	0.997091	0.998100	Ok

*R(s,m) = Resolution start, maximun; R(m,e) = Resolution maximun, end

Table 6: Asiaticoside Assay Results

Sample	% Concentration (w/w)
Dried extract	9.07%
Dried plants	0.90%
Gotu Kola Extract White Powder	10.34%
Gotu Kola Extract Brown-yellow Powder	0.61%

*w/w= weight(gram)/weight(gram)

Table 7. IC_{50} value of Crude Extract, Dried Extract, Extract Products of *Centella asiatica*, and Vitamin C

Sample	IC_{50} Value (mg/mL)
Dried Extract	116.57
Gotu Kola Extract Brown-yellow Powder	578.34
Gotu Kola Extract White Powder	1951.29
Vitamin C	8.69

*IC_{50}= Inhibitory concentration 50

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