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# Antioxidant and Inhibition Lipase Enzyme Activity of Centella asiatica Leaf Extract

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**ABSTRACT**

Hyperlipidemia and many other metabolic diseases are related to oxidative stress. Centella asiatica is a herbal medicine with a reported antioxidant effect in vitro. Centella asiatica contains secondary metabolites asiaticoside, which are included in the terpenoid group. The study evaluated the respective antioxidant potential and lipase enzyme inhibition of Centella asiatica leaves extract (CAE). Centella asiatica were extracted in ethanol, and the extract was assayed for the measurement of asiaticoside. Ethanolic extracts of asiaticoside content were prepared for HPLC analysis. The antioxidant potential of extracts was assessed by their free radical scavenging activity, such as 2, 2-diphenyl -1-picrylhydrazyl, as well as reducing. The anti-hyperlipidemic effect was evaluated in vitro lipase inhibitory activity test carried out enzymatically using the ELISA method with simvastatin as a comparison. The results showed asiaticoside contained in CAE 1.26%; the IC50 value of the antioxidant test of CAE was 11.38 g/mL; the IC50 value of the lipase enzyme in the CAE was 26.14 g/mL. The antioxidant activity of CAE is categorized as very strong and has the potential to inhibit lipase enzymes. The study suggests that CAE has the potential to inhibit lipase activity, suppressing lipid digestion and thereby diminishing the entry of lipids into the body

**Keywords:** Antioxidant; Asiaticoside; *Centella asiatica* extract; In-vitro; Lipase

## INTRODUCTION

The use of natural ingredients is now increasing, both in medicine and for other purposes. Traditional medicines and medicinal plants are widely used by the community as an effort to preventive (prevention), promotive (improvement), and rehabilitative (recovery). Many people think that the use of traditional medicine is relatively safer than synthetic medicine. To get optimal use, people need to know adequate information about traditional medicine. Adequate information will help people to be more careful in choosing and using a traditional product in health efforts.[1]

One type of medicinal plant that can be used by the community is the Centella asiatica (CA) leaf. CA is a wild plant that can grow in plantations, fields, roadsides, and rice fields. CA leaf is widely known to the public as a medicinal plant that has benefits as a urinary laxative (diuretic), fever reducer (antipyretic), stops bleeding (haemostatics), improves blood circulation, improves memory nerves, is anti-bacterial, tonic, antispasmodic, anti-inflammatory, hypotensive, insecticide, allergy, and stimulant.[2]

Centella asiatica leaves also have benefits such as lowering cholesterol levels and increasing antioxidant content in the

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body. There is research evidence showing that CA leaf extract can reduce cholesterol in hypercholesterolemic hamsters by up to 79% and reduce triglycerides by up to 95%. CA leaves also have activity as an antioxidant.[3]

The CA plant is also often considered a weed, the leaves are shaped like a kidney with a curved base. In some communities, CA leaves are used as fresh vegetables, drinks, and traditional medicines. Centella asiatica leaves can be used for traditional medicine in the form of fresh and dry ingredients or the form of herbs. Based on several studies, CA leaves contain several active ingredients such as alkaloids, saponins, tannins, flavonoids, triterpenoid saponins, and glycosides. In addition, the most abundant compound found in CA leaves is asiaticoside.[4]

Typical and important chemical content in CA leaves is triterpene ester glycoside compounds, namely asiaticoside and madecoside, triterpene group compounds, and phenolic group compounds. The phenolic compounds contained in CA leaf have a function as a natural antioxidant. Antioxidants are compounds that can inhibit oxidation reactions by binding free radicals and reactive molecules. The antioxidant activity of phenolic compounds is due to the oxidation-reduction reaction which has an important role in neutralizing and absorbing free radicals.[1]

Based on the above study, there is an active ingredient content from CA leaves that is helpful as an antioxidant and lower cholesterol levels. This study aims to analyze the effect of CA leaves on lowering cholesterol by inhiving lipase as an antioxidant.

## METHODS

Source of plant material

The plant materials used Centella leaves obtained from Sukabumi, West Java. Dr. Hendro Wicaksono, Director of Scientific Collection Management, National Research and Innovation Agency, Cibinong, identified and authenticated the plant leaves with ID B-2314/II.6.2/DI.05.07/7/2022.

**Equipment and materials**

The types of equipment used include rotary evaporator (Buchi), moisture analyzer (Sartorius), water bath (Memmert), oven (Memmert), HPLC (Waters), ashing furnace (Thermo Scientific), grinding insert (Fritsch), ELISA, analytical balance (M*ettler toledo*).

The materials used were *Centella asiatica* leaf, Aquades, technical ethanol, asiaticoside (Sigma), DPPH (Sigma), lipase activity assay kit ( Biovision ), and vitamin C (Sigma).

**Extraction of Centella leaf**

*Centella asiatica* leaves were extracted by the maceration method. CA leaf powder as much as 100 grams in 1 liter of 70% ethanol solvent for 24 hours, with occasional stirring. After 24 hours, the extract was separated using filter paper. The filtering has been collected, then evaporation is carried out using a rotary evaporator at a temperature of 50$^{o}$C until a thick extract is obtained and weight.[5] The Centella asiatica extract (CAE) was then analyzed for yield, total flavonoids, antioxidant activity, and inhibition of lipase enzymes.

**Evaluation of extract**

Water content

Determination of water content using a moisture analyzer. The thick sample of CAE weighed as much as 3 grams. Place it in the device, and wait until the temperature is 105°C. Leave it for 5-10 minutes until the measurement is complete, the water content result will appear on the moisture analyzer monitor screen.

Total ash content

One gram of the extract was weighed carefully and then put in a silicate crucible that had previously been ignited and weighed. Then the ash is slowly ignited using a furnace (by increasing the

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temperature gradually to 600 ± 25°C for 5 hours until the ash runs out (6).

$$\% \text{ total ash content} = \frac{W2-W0}{W1} \; x \; 100\%$$

Note:
W0: weight of empty cup (g)
W1: initial extract weight (g)
W2: weight of cup + weight of extract after ashing (g)

Determination of asiaticoside levels

Determination of asiaticoside levels using the High-Performance Liquid Chromatography (HPLC) method with a Sunfire column measuring 4.6x150 mm. The detector used is a UV detector with a wavelength of 210 nm, a water rate of 1.8 mL/min, and an injection volume of 2.0 L. The gradient mobile phase used was 0.3% orthophosphoric acid (A) and acetonitrile (B).[7]

**Evaluation of the antioxidant activity of CAE**

The evaluation of the antioxidant activity of CA leaf extract was conducted by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The concentration of the bay leaf extract was 100 µg/mL for initial screening at 5, 10, 25, 50, and 100 µg/mL. Vitamin C (0.5, 1, 2, 4, and 8 µg/mL) was utilized as a standard for comparison, and 0.4 mM DPPH was used as a control. The test solution, control, and vitamin C were all incubated at 37˚C. It was then pipetted onto a UV-Vis spectrophotometer cuvette and incubated at 37 ˚C for 30 minutes. The control blank was made from ethanol and compared to vitamin C. A UV-Vis spectrophotometer with a 515 nm wavelength was also used to measure absorption. The $IC_{50}$ value, which represents the sample's ability to inhibit 50% of the oxidation process, was used to estimate antioxidant activity. It was calculated by plotting a linear relationship between the concentration of the test solution (x-axis) and the percentage antioxidant activity (y-axis).[8]

$$Antioxidant\ activity\ (\%) = \frac{\text{Ab} - \text{As}}{\text{Ab}} x\ 100\%$$

Ab is the absorbance of the blank (without sample) and As is the absorbance of the sample.

**Lipase enzyme inhibition assay**

CAE was respectively mixed with a reaction mixture containing OxiRed Probe (2 L), dimethyl-sulfoxide (DMSO), enzyme mix (2 L), lipase substrate (3 L), standard glycerol (100 mM), and assay buffer (93 L). The assay mixture contained 10 µL of one to five different concentrations (two-fold serial dilutions from 2.5 to 0.156 mg/mL) of the crude extracts, 12 µL of 20 mg/mL of PPL (type II) in 50 mM Tris–HCl pH 8.5 and 10 µL of 5.1 mM p-NPL in ethanol. The reaction was incubated at 37°C and the absorbance was measured at 570 nm after 60-90 min. Simvastatin was used a positive control and aquabidestyl as a negative control.[9,10]

The formula for the percentage of inhibition is as follows:

$$\%\text{Inhibition} = \frac{\Delta\ abs\ control - \Delta\ abs\ sample}{\Delta\ abs\ control}\ x\ 100\%$$

## RESULTS AND DISCUSSION

The extraction was carried out using the maceration method with 70% ethanol as the solvent because ethanol is safe, non-toxic, and capable of extracting more compounds from bay leaf.[11] A total of 86,1 grams of bay leaf extract was obtained from 500 grams of CA leaf powder (Figure 1). The results are consistent with previous findings by Sawatdee et al.[12] The characterization of CAE carried out in this study ensured that the extract had a particular parameter with a consistent value. Based on the characterization results (Table 1), the extract contains 1.01% water, which meets the Indonesian Herbal Pharmacopoeia II standards of less than 10%. The extract contains 7.22% total ash, as required by the Indonesian Herbal Pharmacopoeia II of less than 16.6%.[13] The ash content determination is intended to determine the physiological ash, which is the internal mineral content originating from the plant tissue itself, as well as the

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non-physiological ash that is residue from the surrounding environment such as sand and soil contained in the sample. The smaller the ash content, as well as the impurities in the CAE.[14]

The HPLC analysis of Centella asiatica showed asiaticoside peak at retention time 4.25 min which was comparable with that of standard asiaticoside. Figure 2 showed chromatogram of methanolic extract of *Centella asiatica*. Determination of the asiaticoside content of CAE in percentage are presented in Table 1. The asiaticoside content in the CAE is 1.26%. It is possible to deduce that the asiaticoside content in CAE meets the standards for total flavonoid content based on the Indonesian Herbal Pharmacopoeia II, which is not less than 0.9%.[13] This result is higher when compared to a study by Pramono et al (2014) with an asiaticosde content only 0.42%. The high content of asiaticoside in this study could be influenced by the optimal cultivation area and extraction process.[15]

The antioxidant inhibition of bay leaf extract was determined using the free radical scavenging action of DPPH. The purple color change in DPPH to yellowish and colorless indicates that atomic antioxidants neutralize DPPH free radicals. The linearity test determines whether or not the examination of the substance's concentration using the UV-Vis spectrophotometer method has a linear relationship.[16]

**Figure 1.** CA leaf extract

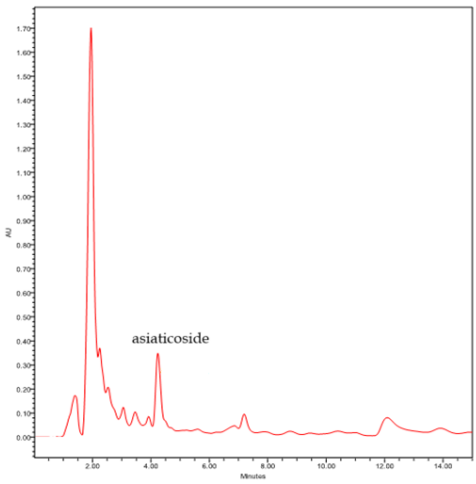


**Figure 2.** HPLC chromatogram of CA extract

**Table 1:** Characterization results of CA leaf extract

| Characterization | Result |
|---|---|
| Shape | Thick |
| Color | Dark chocolate |
| Yield | 18.7 % |
| Moisture content | 1.01 % |
| Total ash content | 7.22 % |
| Asiaticoside content | 1.26 % |

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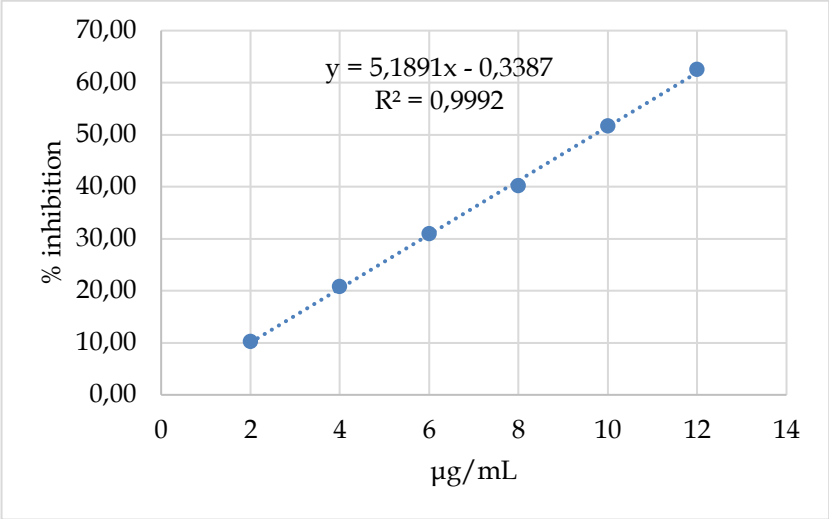


**Figure 2: Antioxidant activity linearity curve**

**Table 2.** $IC_{50}$ value of antioxidant activity of samples and vitamin C

| Sample Name | $IC_{50}$ (µg/mL) |
|---|---|
| CA leaf extract | 11.38 |
| Vitamin C | 9.70 |

**Table 3.** $IC_{50}$ value of samples and comparison of the lipase enzyme

| Sample Name | $IC_{50}$ (µg/mL) |
|---|---|
| CA leaf extract | 26.14 |
| Simvastatin | 15.16 |

The linearity parameter is used to confirm the ability of the standard to ensure that there is a directly proportional relationship between the substance concentration and the detector responses. For a good analytical procedure, the relationship coefficient is expected to be close to 1 or over 0.9900.[17] To determine the relationship between concentration and inhibition, linearity and range tests were performed by making a standard curve. The linear regression analysis result (Figure 2) yielded a linear equation, y = 5.1891x-0.3387 in a 0.9992 coefficient value suggests that the measured detector signals have a linear relationship. As a result, the confirmation of antioxidant action using UV-Visible spectrophotometry has high linearity.[18] The $IC_{50}$ value can be evaluated from the linear regression equation obtained by substituting 50 in the equation. The $IC_{50}$ indicates the concentration of the test sample (µg/mL) that inhibits 50% of the oxidation process.

The DPPH free radical method is an antioxidant assay based on hydrogen atom donation as the presence of an antioxidant molecule, which leads to a color change from purple to yellow. This is then detected at a wavelength of 515 nm. The reduced color of the DPPH solution may suggest that hydrogen atoms released by the test material react with the DPPH radical molecule to form the yellow compound 1,1-diphenyl-2-picrylhydrazil.[19] The $IC_{50}$ of a compound can be classified as "very strong" if the $IC_{50}$ is less than 50 µg/mL; "strong" if the $IC_{50}$ is between 50

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and 100 µg/mL; "moderate" if the $IC_{50}$ is between 100 and 150 µg/mL; and "weak" if the $IC_{50}$ is between 150 and 200 µg/mL.[20] Based on this classification, the $IC_{50}$ of CAE (11.38 µg/mL) could be classified as very strong. However, the value is still below that of vitamin C, with an $IC_{50}$ value of 9.70 µg/mL. The maceration process of CAE has a higher antioxidant potential. The observation is in agreement with the previous research. According to the findings, the ethanol extract obtained by the maceration method exhibits antioxidant activity that differs from the soxhlet extraction procedure (18.73%) and the infusion extraction method (40.26 µg/mL).[21]

Lipase is an enzyme that capable of catalyzing the formation of ester bonds (esterification) and the exchange of ester bonds in non-aqueous media.[22] The in-vitro assessment inhibition of lipase activity indicated catechin isolate of CAE was the most potent inhibitor of lipase ($IC_{50}$ = 26.14 µg/mL), while simvastatin showed relatively higher inhibition ($IC_{50}$ = 15.16 µg/mL). CAE showed strong inhibitioin of lipase activity. The polyphenolic-rich extracts were also shown to down-regulate some obesity-related genes, LPL, hormone sensitive lipase, fatty acid synthesis, and resistin in liver and epididymal fat.[23] This effect, based on results of the in-vitro lipase activity inhibition test, is probably due to inhibition of lipase activity. The major component of CAE was asiaticosde. Recent research showed that plant-derived polyphenols exert anti-obesity effects on preadipocyte differentiation and lipid accumulation by regulating the expression of CEBP-α and PPAR-γ, which are early transcription factors involved in preadipocyte differentiation. Considering this, it is hypothesized that polyphenols in the CAE contribute to inhibiting preadipocyte differentiation by regulating key adipogenesis genes.[24] Simvastatin was used as positive control for the hypolipidemia activities in this experiment. Simvastatin is reversible inhibitor of lipases which exerts lipases activities in the lumen and small intestine. It forms a covalent bond with the active site of gastric and pancreatic lipase, thus, the enzymes become inactive. The undigested triglycerides are not absorbed resulting in caloric deficit.[25] Catechin have activity in inhibiting the work of the lipase enzyme. In human body, oil can be hydrolyzed into saturated fatty acids by the lipase enzyme. Furthermore, fatty acids through beta oxidation can be converted into acetyl CoA which is a precursor of cholesterol.[26] Asiaticoside will suppress the precursors enhancement causing an increase in cholesterol levels in the blood. Increased free radicals make a damage to nucleic acids, proteins, and lipid membranes that could cause cancer and liver damage. The liver plays an important role in the transport and metabolism of fats, including the production of bile for the excretion of cholesterol, synthesize and oxidize fatty acids, convert fatty acids into bile acids and play a role in lipoprotein metabolism. Damage and toxic effects on the liver can interfere the metabolism and excretion of cholesterol from the body.[27]

## CONCLUSION

The study suggests that CAE has potential anti-hyperlipidemic activity and acts as an antioxidant by inhibiting the production of cholesterol. CAE may play a significant role in the prevention of diseases caused by free radicals. The research helps to understand the mechanism of action of CAE as a hyperlipidemia treatment by inhibiting the lipase enzyme.

### 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.

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