Chemical Characteristics and in vitro Antidiabetic and Antioxidant Activities of Premna serratifolia L. Leaf Infusion and Decoction

by Adelina Simamora

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Original Article

Chemical Characteristics and *in vitro* Antidiabetic and Antioxidant Activities of *Premna serratifolia* L. Leaf Infusion and Decoction

Kris Herawan Timotius[1,*], Adelina Simamora[1], Adit Widodo Santoso[2]

ABSTRACT

Introduction: Leaves of *Premna serratifolia* L. (local name: arogo) is well known as food ingredient for fish/meat based soup in Tentena, Indonesia. Evaluation of its bioactivities is needed. **Objective:** This study aimed to evaluate the α-glucosidase inhibitory and antioxidant activities of infusion and decoction of *P. serratifolia* leaves. **Methods:** The leaf samples were prepared by infusion and decoction and analysed for their α-glucosidase inhibitory and antioxidant activities, as well as total phenolic contents and LC-MS profiles. **Results:** The total phenolic content of the decoction was higher than the infusion. The UV spectra of both infusion and decoction showed peak at 325 nm and shoulder at 285 nm. Their LC-MS yielded one dominant component at 132 (100) m/z fragment, which was tentatively identified as caffeic acid. Both infusion and decoction extracts exhibited strong α-glucosidase inhibition with IC_{50} of 4.27 and 0.046 µg GAE/ml, respectively. The IC_{50} for DPPH scavenging activity of the infusion and decoction were similar; 6.82 and 7.28 µg GAE/ml, respectively. But the IC_{50} of the infusion and decoction for ferrous ion chelating activities were different; 161.66 and 28.34 µg GAE/ml, respectively. The α-glucosidase inhibitory and antioxidant activities were related to the phenolic content. **Conclusion:** The study concludes that both infusion and decoction of arogo leaves have antidiabetic effect and potential antioxidant activity. The decoction of the leaves may increase its antidiabetic capacity.

Key words: α-Glucosidase inhibitor, Antioxidant, Aqueous extract, *P. serratifolia,* Inhibition mechanism.

Kris Herawan Timotius[1,*], Adelina Simamora[1], Adit Widodo Santoso[2]

[1]*Department of Biochemistry, Faculty of Medicine, Krida Wacana Christian University, Jakarta, 11510, INDONESIA.*
[2]*Department of Herbal Medicine, Faculty of Medicine, Krida Wacana Christian University, Jakarta, 11510, INDONESIA.*

Correspondence

Kris Herawan Timotius

Department of Biochemistry, Faculty of Medicine, Krida Wacana Christian University, Jakarta, 11510, INDONESIA.

Phone no : +61-21-56942061

E-mail: kh_timotius@ukrida.ac.id

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INTRODUCTION

The leaves of *Premna serratifolia* (syn. *P.obtusifolia*), locally known as arogo or orogo, are consumed as food ingredient in Tentena, Poso, Middle Sulawesi (Celebes) especially as ingredient for fish-soup. *P. serratifolia* is a tropical shrub or a small tree (family Lamiaceae) that can grow up to one and half meters. The leaves of this plant were reported to have a strong but not disagreeable odor and are consumed by the people in Tentena along with other ingredients.[1-3] In several countries such as India and the Philippines, Premna species are used as Ayurvedic drug. There are several species of Premna, such as *Premna integrifolia* L., *P. serratifolia* L. (syn. *P.obtusifolia* R.Br.), and *Premna mucronata* Roxb. All Premna were reported to have medicinal properties, such as carminative, diuretic, spasmolytic, hypoglycaemic, expectorant and depurative for the treatment of stomach disorders, oedema, constipation, piles and cardiac diseases.[4] Most of the studies used bark, fruit, root, and stem as raw material, whereas only a few report on the bioactivities of *P. serratifolia* leaves. Recently, bioactive components have been isolated from methanol extracts of the leaves and their bioactivities have been demonstrated. Diterpens and triterpenes were separated as bioactive compounds from the leaves of *P. serratifolia*.[5-6] However, there has been very little information on bioactivities of aqueous leaf extracts, such as those prepared by infusion and decoction which are safe for human consumption and relevant for domestic environment. Therefore, it is necessary to perform a novel study to substantiate its local use as a nutraceutical leafy vegetable.[7-8]

The objectives of this study were to investigate the α-glucosidase inhibitory and antioxidant activities of infusion and decoction from leaves of *P. serratifolia*. The leaf extracts were analyzed for their total phenolic content and LC-MS profiles.

MATERIALS AND METHODS

Chemicals and plant material

All solvents and chemicals used in the experiments were of analytical grade and purchased from Sigma-Aldrich (St. Louis, USA) or Santa Cruz Biotechnology (Dallas, USA).

Premna serratifolia L. leaves were collected in September 2016 from Tentena, Poso, Middle Sulawesi, Indonesia. The plant materials have been identified and authenticated by one of the author, KHT, using

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two identification articles.[1-2] A voucher herbarium is kept in the laboratory (herbarium voucher number: KWL017).

Preparation of extracts

Leaves were sorted, dried under shadow, and powdered using a mechanical grinder. The powder was stored in an airtight container at room temperature for further analysis. Both the infusion and decoction were of aqueous leaf extracts of *P. serratifolia*. The infusion was prepared by hot water extraction *i.e.* 2 g of leaf powder was immersed in 200 ml of boiling water and allowed to cool for 15 mins. The decoction was prepared by boiling water extraction *i.e.* 2 g of leaf powder was immersed in 200 ml of boiling water and boiled until 100 ml remained.

UV-Vis spectrophotometer

The UV–VIS spectra were recorded on a UV–Vis spectrophotometer BioChrom Libra S-22 in a spectral range between 210 and 700 nm. Quartz cells were used for all absorbance measurements.

LC-MS analysis

LC-MS analysis was performed using a Mariner Biospectrometry, equipped with binary pump (Hitachi L 6200). The HPLC was interfaced with a Q-tof mass spectrometer fitted with an ESI (Electrospray Ionisation) source with positive ion mode. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. An HPLC column (Shimp-pack C8, 150 × 6 mm i.d.,) was used. Methanol with 0.3% formic acid was used as solvent and delivered at a total flow rate of 1 ml/min. A 5 µl sample was injected and eluted isocratically.

Determination of total phenolic content

The total phenolic contents of aqueous extracts of *P. serratifolia* were determined by Folin-Ciocalteu method[9] with slight modification. An aliquot (0.5 ml) of sample was mixed with 2.5 ml of Folin-Ciocalteu reagent (10% v/v). The solution was left to stand for 10 min at room temperature. The reaction was then neutralized using sodium carbonate. After incubation for 2 hours in darkness at room temperature, the absorbance was measured at 765 nm using spectrophotometer. The total phenolic content was estimated based on a linear regression equation from a standard curve of gallic acid in methanol (12.5 – 200 µg/ml, $R2 = 0.997$) and the results were expressed as µg gallic acid equivalent (µg GAE/ml).

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activities of aqueous extracts (infusion and decoction) of *P.serratifolia* leaves were evaluated according to the method previously reported[10] with some modifications. A 50 µl of sample and standard (acarbose) at various concentrations was mixed with 50 µl phosphate buffer (50 mM, pH 6.8) and 50 µl of α-glucosidase (0.5 unit/ml). After pre-incubating for 10 min at 37°C, 100 µl of substrate (1mM *p*-nitrophenyl-α-D glucopyranoside) was added to the reaction mixture and incubated for 20 min at 37°C. The reaction was terminated by the addition of 750 µl of Na_2CO_3 (100 mM). The absorbance was read at 405 nm. For the control solution, all procedures were followed except that the sample was replaced by buffer. The percentage of inhibition was calculated using the following equation:

$$\alpha - \text{Glucosidase inhibition}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where A (control): absorbance of control, A (sample): absorbance of the sample. The concentration of the samples and positive control required to inhibit 50% of the enzyme activity was defined as IC_{50} and was determined from the graph plotted against the percentage inhibition. The IC_{50} values were expressed as µg GAE/ml and compared with standard.

α-Glucosidase inhibition mechanism

The inhibition mechanism on α-glucosidase activity was determined by conducting a kinetic study of the inhibition of α-glucosidase by aqueous extract of *P. serratifolia* leaves.[11] The kinetic assay was performed using p-nitrophenyl-α-D-glucopyranoside as a substrate in increasing concentrations (0.15 – 1 mM), in the absence and presence of the extract at different concentrations (0.04, 0.13, and 0.17 µg GAE/ml). Double reciprocal plots were generated based on the Lineweaver-Burk method to analyse the mechanism of inhibition by the extract.

DPPH radical scavenging activity

The antioxidant activity of aqueous extracts from *P. serratifolia* L was determined based on procedure described in the literature[12] with minor modification. DPPH solution (0.6 mM in ethanol) was prepared and 1 ml of this solution was added to 3 ml of extract and standard solutions (BHT, ascorbic acid, and α-tocopherol) at various concentration (10 – 100 µg/ml). The reactions were incubated for 30 min in darkness at room temperature and the absorbance was read at 517 nm with spectrophotometer. Ethanol (1 ml) in place of extract was used as control. The percentage inhibition activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where A(control): absorbance of control, A(sample): absorbance of the sample. The percentage inhibition was plotted against concentration to calculate the IC_{50} which is defined as the amount of antioxidant required to decrease the inhibition of DPPH radical by 50%.[13] The IC_{50} values were expressed as µg GAE/ml and compared with the standards.

Ferrous ion chelating activity

Ferrous ion can be monitored spectrophotometrically by measuring the formation of red colour Fe(II)-Ferrozine complex.[14] However, in the presence of other chelators, complex formation is interfered, leading to a decrease in red colour. Measurement of colour reduction allows the estimation of binding ability of chelators present in test samples. The ability of aqueous extracts of *P. serratifolia* and standard (EDTA) to chelate iron (II) was estimated according to the method described previously.[15] In this method, 0.4 ml extracts and EDTA at various concentrations were added with 0.2 ml $FeSO_4$ (0.2 mM). To start the reaction, 0.2 ml ferrozine (0.5 mM) was added and the total volume was adjusted to 4 ml with water. The reaction was immediately vortexed and was left to stand in the dark at room temperature for 10 min. The absorbance was read at 562 nm using spectrophotometer. Iron chelating ability was calculated using the following equation:

$$\text{Ferrous ion chelating activity}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where A (control): absorbance of control, A(sample): absorbance of the sample. The concentration of extracts required to chelate 50% of the Fe (II) ion (IC_{50}) was calculated from the graph plotted against the percentage of inhibition. The IC_{50} value was expressed as µg GAE/mL and compared with standard.

Statistical analysis

All experiments were conducted in triplicates. Results were reported as mean ± standard deviation (SD). Regression method was used to calculate IC_{50} and enzyme kinetics.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The total phenolic content (TPC) of the leaf decoction was higher than the infusion sample, *i.e.* 539.26 ± 7.44 µg GAE/ml compared to 347.81 ± 0.21, respectively. The difference in phenolics may be due to the more concentrated sample obtained from the decoction procedure. Furthermore, the TPC of aqueous extracts was found to be dependent on the infusion time, the amount of leaves, and the aquadest temperature.

Inhibition of α-glucosidase

Both infusion and decoction extracts exhibited strong α-glucosidase activity, with IC_{50} lower than the positive standard acarbose (Table 1). The inhibitory activities of infusion and decoction samples were 200 and 18x10³ times better than acarbose. These results suggest that both extracts are more potent than acarbose in inhibiting α-glucosidase. It was observed that when compared with infusion, the capacity for α-glucosidase inhibition of leaf decoction was 90 times stronger.

Previously, many plants have been identified of having more potent inhibitors of α-glucosidase than acarbose.[16-17] Our findings provide further evidence that plant aqueous extract as demonstrated by *P. serratifolia* leaves exhibited stronger inhibition against α-glucosidase than acarbose.

The type of inhibition of the aqueous extract was analysed using Line-weaver-Burk plots. The results reveal that the inhibition was of competive mode, similar to that of acarbose (Figure 1).

Antioxidant activities

In this study, the antioxidant activities of infusion and decoction of *P. serratifolia* leaves were investigated using two different *in-vitro* assays, namely DPPH radical scavenging assay and ferrous ion chelating activity assay. DPPH radical scavenging activities of both aqueous extracts and the standard compounds are shown in Table 2. Both infusion and decoction samples showed strong radical scavenging activity. Additions of extracts induced a rapid increase in inhibition at 517 nm. The activities of leaf infusion and decoction as expressed by IC_{50} were stronger than the standards ascorbic acid and BHT, but weaker than α-tocopherol. The activities may be attributed to the hydrogen donating ability of the phenolic components in the extracts.

Table 1 : The inhibitory α-glucosidase activities of aqueous extract from *P.serratifolia* leaves.

	Concentration (µg GAE/ml)*	Inhibition (%)**	IC_{50} (µG/ml)**
Infusion	0.71	8.00 ± 3.05	
	1.42	14.69 ± 1.07	
	2.13	16.07 ± 7.63	4.27 ± 0.31
	2.84	25.85 ± 17.61	
	3.54	39.23 ± 5.71	
	5.67	67.74 ± 13.26	
Decoction	0.011	4.35 ± 0.57	
	0.022	14.57 ± 4.78	
	0.032	30.92 ± 1.52	0.046 ± 0.00
	0.043	48.36 ± 4.84	
	0.054	61.25 ± 5.03	
Acarbose			824 ± 0.06

*µg Gallic Acid Equivalent/ml.
**Values are mean ± SD (n=3).

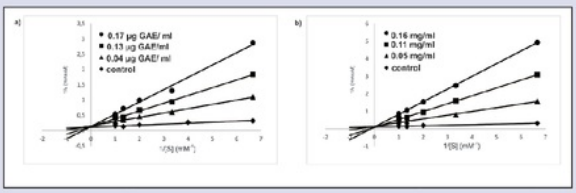

Figure 1: Line Weaver-Burk plots for the inhibition of α-glucosidase by (a) *P. serratifolia* leaves prepared by decoction (b) acarbose. p-Nitrophenyl-α-D-glucopyranoside (pNPG) was used as a substrate.

Table 2 : The DPPH radical scavenging activities of aqueous extracts from *P. serratifolia* leaves.

	Concentration (µg GAE/ml)*	Inhibition (%)**	IC_{50} (µg GAE/ml)**
Infusion	0.43	2.44 ± 0.16	
	1.74	9.65 ± 2.20	
	3.48	51.27 ± 0.26	6.82 ± 0.01
	6.96	75.12 ± 0.21	
Decoction	1.80	9.25 ± 2.6	
	3.60	24.07 ± 2.09	
	7.19	47.20 ± 1.96	7.28 ± 0.12
	10.79	79.05 ± 0.29	
	12.58	87.05 ± 0.17	
Ascorbic acid			53.24 ± 0.82
BHT			21.36 ± 0.80
α-Tocopherol			1.71 ± 0.01

*µg Gallic Acid Equivalent/ml.
**Values are mean ± SD (n=3).

Furthermore, it was found that both infusion and decoction samples demonstrated strong chelating activity for ferrous ions when compared with EDTA. As can be seen in Table 3, the percentage inhibition of the complex Fe (II)-ferrozine formation is linearly increased with increasing concentration of the aqueous extracts, indicating that both extracts readily bound ferrozine in a concentration-dependent manner. When the chelating activity was expressed in terms of its GAE content, the IC_{50} values of both extracts were lower than that of the standard EDTA, indicating stronger activities than the standard. These results suggest that the extracts may afford for protection from oxidative damage in cell.[18] It has been observed that the leaf decoction has stronger activity when compared with infusion sample. The results suggest that decocting the leaf may increase the capacity for ferrous ion chelation.

Compared with the result of antioxidant study of *P. serratifolia* leaves by Uppin *et al* (2017), the present study reported stronger antioxidant activities for both infusion and decoction. The difference could be due to the difference in solvent systems used, which were MeOH, EtOH and chloroform.

Chemical characteristics of the extracts

The UV spectra of both infusion and decoction extracts showed two major absorption bands. The first one with the absorption maximum in the spectral range between 250 and 285 nm (λ_{max} 261.8 nm) and the

Table 3 : Ferrous ion chelating activities of aqueous extract from *P. serratifolia* leaves

	Concentration (µg GAE/ml)*	Inhibition (%)**	IC_{50} (µg/ml)**
Infusion	12.88	4.21 ± 1.39	
	32.20	8.74 ± 1.84	
	64.41	21.24 ± 3.11	161.66 ± 0.01
	128.82	40.63 ± 1.50	
	161.02	50.30 ± 1.92	
	193.23	58.65 ± 2.85	
Decoction	9.99	17.20 ± 2.94	
	19.97	42.86 ± 0.07	
	29.96	55.10 ± 1.47	28.34 ± 1.24
	39.95	64.13 ± 0.47	
EDTA			332.56 ± 1.02

*µg Gallic Acid Equivalent/ml.
**Values are mean ± SD (n=3).

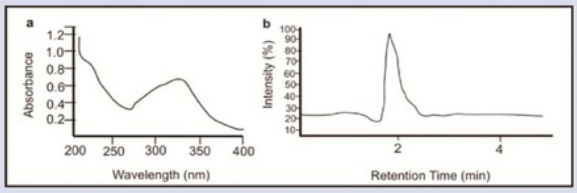

Figure 2: (a) UV-VIS scanning and (b) MS base peak chromatogram of aquaous extract of *Premna serratifolia* leaves.

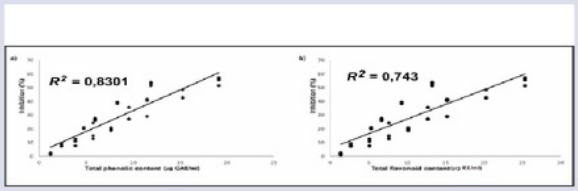

Figure 3: Correlation between total phenolic content and (a) DPPH scavenging inhibition; (b) α-glucosidase inhibition.

second one with the maximum in the range between 320 and 380 nm (λ$_{max}$ 340.9 nm). The absorption peaks in these spectral ranges indicated the presence of phenolic compounds. The LC-MS profile yielded one dominant peak of 132 (100%) m/z fragment and 170 (2.3%), and 201 (2.55%) m/z fragments (Figure 2).

Based on the MS characteristics at LC-MS analysis, the tentative compound is caffeic acid.[19] In addition, the aforementioned UV spectra are similar with that of caffeic acid spectra, with absorption maximum at 321 and a shoulder at 278 nm, which is characteristic of phenolpropenyl compounds. It has been reported by several literatures that caffeic acid has antidiabetic effect and antioxidant activity.[20-22] Moreover, the present study confirmed significant and positive correlations beween the levels of TPC and inhibition of α-glucosidase or antioxidant activities (Figure 3), which are in agreement with previous studies.[23]

In this present study, it is evidenced that aqueous extract of *P. serratifolia* L. possess antioxidant activity *in-vitro*. The antioxidant activity is evident

CONCLUSION

Our results indicated a possibly important role of the aqueous extracts from *P. serratifolia* leaves, either infusion or decoction, as inhibitor of α-glucosidase, and as source of antioxidant. Therefore, *P. serratifolia* leaves could be exploited for antidiabetic drink or food ingredient.

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CONFLICT OF INTEREST

Authors declare that there is no conflicts of interest.

ABBREVIATIONS

3,5-di-tert-butyl-4- hydoxytoluene; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl; **EDTA:** ethylene diamine tetra acetic acid; **GAE:** Gallic Acid Equivalence.

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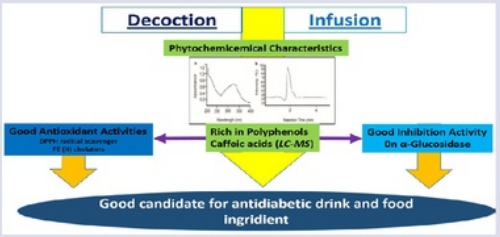
GRAPHICAL ABSTRACT



SUMMARY

- *Premna serratifolia* has been used as food condiment traditionally in Central Sulawesi/Celebes, it is not yet really understood its functional role. Our research is the first report of its capacity as an antidiabetic and antioxidant properties. The water leaf extracts of *P. serratifolia*, either prepared by infusion or decoction, have significant inhibition on α-glucosidase and antioxidant activity. Therefore, the leaves of *P. serratifolia* are recommended as good ingredient of food and beverage

ABOUT AUTHORS

Prof Dr Kris Herawan Timotius is a lecturer in microbiology and biochemistry at the Faculty of Medicine Krida Wacana Christian University. His research focus on herbal medicine including natural antibiotics, antiquorum sensing, antibiofilm, enzyme therapy, and anti enzyme activities such as alpha glucosidase and xanthine oxidase.

Adelina Simamora, M.Si, MSc (Pharm) is a researcher in the centre for herbal medicine studies and a lecturer in Biochemistry at the Faculty of Medicine Krida Wacana Christian University. At present, her research focus on the bioactive potential of medicinal plants, with special interest in various enzyme inhibition properties. Currently, her work involves various Indonesian medicinal plants and inhibition properties on α-glucosidase, xanthine oxidase, and lipase. Her interest also spans to transition and rare earth metals and their application as sensor in biological fluid.

Adit Widodo Santoso, BSc: a researcher in the centre for herbal medicine studies. His interest is mainly in the areas of microbiological activities of variety of plant species, including antibiofilm and anti-quorum sensing activities. Currently, he is focusing on bioautography involving thin layer chromatography and western blot. He is also interested in zymography studies and their applications.

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