

Phytochemical Screening and Total Antioxidant Capacity of Methanolic-Extract of Kembang Bokor Roots (*Hydrangea Macrophylla*)

Elizabeth¹, Eny Yulianti², David Limanan² and Frans Ferdinal²

¹ Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia

² Staff of Departement of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia

*Corresponding author: Email: elibebek88@gmail.com

ABSTRACT

Reactive oxygen species can cause damage to biological systems such as mutations, inhibit protein synthesis and even damage proteins. Excess free radicals will produce oxidative stress. Antioxidants are substances that play a role in preventing and repairing damage caused by oxidative stress. Antioxidants can be obtained from food and plants. One of the plants is the root of the kembang bokor (*Hydrangea Macrophylla*) which is a medicinal plant. The purpose of this study was to determine the phytochemical content and total antioxidant capacity on methanol extract of the kembang bokor roots. This is in-vitro experimental research against methanol extract of kembang bokor roots through maceration process. The in-vitro tests are qualitative phytochemical tests (Harborne) and total antioxidant capacity tests with the DPPH (Blois) method. In the qualitative test, it was found that the root extract of kembang bokor contained alkaloids, betasianin, cardioglycosides, terpenoids, steroids, glycosides, flavonoids, quinones, phenolics, saponins, tannins and coumarin. Kembang bokor roots extract has antioxidant capacity ($IC_{50} = 261.45 \mu\text{g} / \text{mL}$) that classified as very weak antioxidant. In this study we found that the antioxidant capacity which contain in kembang bokor root extract not as much as vitamin C. Although the level of antioxidant capacity of kembang bokor root extract is lower than vitamin C, it has advantages over vitamin C, kembang bokor root can treat kidney stones while high dose of vitamin C can cause kidney stones. This result proves that kembang bokor roots extract has secondary metabolites and the potential as an antioxidant.

Keywords: *Reactive oxygen species, Hydrangea Macrophylla, phytochemicals, DPPH.*

1. INTRODUCTION

Free radicals are unstable reactive molecules capable of oxidizing and converting surrounding molecules into free radicals that can start a cycle of cell destruction.¹ Reactive oxygen species (ROS) is a form of free radicals that can be produced endogenously (naturally and continuously in biological systems) and exogenously (responses to smoke, pollutants, xenobiotics, drugs, inflammatory stimuli, ischemia, and radiation).¹ A continuous increase in ROS can result in intense oxidative stress, this process being an important bridge to damage to various cellular structures, membranes, carbohydrates, proteins, lipids and DNA.^{2,3} This

process will lead to the development of inflammation, cancer, neurodegenerative diseases, cardiovascular, chronic kidney disease (CKD), macular degeneration, diabetes.^{4,5}

Antioxidants can inhibit the oxidation of other molecules and lipid peroxidation processes, also protecting the human body from free radicals and ROS.⁶ Physiologically, human body has antioxidant methods to control damage so as to minimize the activation of ROS.⁷ There are endogenous antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, or non-enzymatic compounds such as bilirubin and albumin) and exogenous (obtained from food and plants).^{6,7}

Kembang bokor or Hydrangea Macrophylla is a plant that is cultivated in various parts of the world.⁸ Kembang bokor has long been used as a traditional Chinese medicine which has antioxidant activity,⁹ contains various secondary metabolites¹⁰ and has toxicity on the leaves and flowers.^{10,11} The flower buds of the kembang bokor have secondary metabolite compounds (flavonoids, terpenoids/steroids, alkaloids and tannins), while the leaves of this plant contain secondary metabolite compounds (phenolics, terpenoids/steroids, β -sitosterols, flavonoids, polyphenols, alkaloids and anthrax).^{10,12} The roots of this plant are believed to have hepatoprotection, anti-allergic, antimicrobial, antimalarial benefits and can treat sore throats.^{10,13}

Although kembang bokor roots are known to have compounds that may act as antioxidants, research on the antioxidant ability of this plant is still very minimal. This encourages researchers to find out more about the antioxidant ability of kembang bokor roots which are expected to overcome free radicals and prevent oxidative stress that leads to a disease.

2. MATERIAL AND METHOD

In vitro experimental study of kembang bokor root extract which did in Biochemistry and Molecular Biology Laboratory, Medical Faculty, Tarumanagara University. The sample was bought in the online shop name Storez shop in West Java Province, Bogor City, Tajurhalang Bogor District, Citayam Village, Jl. Setapak No.13. First, the sample was sent to the Indonesian Institute of Science in Bogor, West of Java, Indonesia to be identified. Furthermore, kembang bokor roots were dried and made into simplicia. We used 30 grams of kembang bokor root simplicia in maceration process with methanol as solvent. Then we evaporated the result of the maceration process and we obtained 9.49 grams of paste that we would use in this study. We did phytochemicals profiling test (Harborne, 1998) and total antioxidant capacity test (Blois, 1958) by used diphenyl-2-picrylhydrazyl (DPPH) and vitamin C as the comparative standard.

2.1. Phytochemical Profiling Test

In the phytochemicals profiling test, we used several methods or reagents to determine the content of phytochemicals in kembang bokor root extract. We used Mayer and Wagner to determine the alkaloids, NaOH to specified anthocyanins and betacyanin, Keller Killani to specified the cardiac glycosides, Salkowski test to specified the content

of terpenoids, Liebermann Burchard method was used to determine the steroids, Borntrager to specified glycosides, NaOH to specified the flavonoids, H₂SO₄ was used to specified the content of quinones, follin ciocalteau to determine the phenolics, foam test to specified saponins, *ferric chloride* to specified the tannins, Amonia was used to specified the content of coumarins.

2.2. Total Antioxidant Capacity Test

In total antioxidant capacity test, we used diphenyl-2-picrylhydrazyl (DPPH) with concentration of 25 μ g/mL, then the maximum wavelength also maximum absorbance was found and used to be the control absorbance. The extract of kembang bokor root with concentration of 100 μ g/mL, 300 μ g/mL, 400 μ g/mL, and 500 μ g/mL were used in this test. Furthermore, the vitamin C with concentration of 2 μ g/mL, 4 μ g/mL, 6 μ g/mL, 8 μ g/mL, and 10 μ g/mL were used as the comparative standard. From each of the concentration, 0,5 mL was taken and reacted with 3,5 mL of DPPH. This result of reaction was obtained from the absorbance reading with GENESYS 30-Vis spectrophotometry.

After the absorbance and concentration were gotten, we calculated the percentage of inhibition (% inhibition) of kembang bokor root extract and vitamin C by used equation below:

$$\% \text{ Inhibition} = \frac{\text{control Abs.} - \text{test Abs}}{\text{control Abs.}} \times 100\%$$

When we found the % inhibition of kembang bokor root extract and vitamin C, the curves were made and the linear equation were obtained. From these linier equations, the IC₅₀ of kembang bokor root extract and vitamin C were calculated and found. Data were collected from the results of the total antioxidant capacity test using the DPPH. Furthermore, GraphPad Prism v.7.0. La Jolla, California, USA was used in this experimental research. Data was shown in table and graphic.

3. RESULT

3.1. Percentage of Yield

In this study, 30 grams of simplicia of kembang bokor root was used and the extract obtained was 9.49 grams, so the yield was 31,63% with the calculation:

$$\begin{aligned} \% \text{ Yield} &= \frac{\text{weight of extract}}{\text{weight of simplicia}} \times 100\% \\ &= \frac{9.49}{30} \times 100\% = 31.63\% \end{aligned}$$

3.2. Phytochemical Test of Kembang Bokor Root Extract

Based on the results of phytochemical tests, the extract of kembang bokor root contains alkaloids, betasianin, cardioglycosides, terpenoids, steroids, glycosides, flavonoids, quinones, phenolics, saponins, tannins and coumarin (Table 1).

Table 1 Phytochemical Content

Phytochemical	Extract	Method/reagent
Alkaloid	+	Mayer, Wagner
Anthocyanin	-	NaOH
Betacyanin	+	NaOH
Cardioglycoside	+	Keller Kiliani
Terpenoid	+	Salkowski
Steroid	+	Liebermann Burchard
Glycoside	+	Modified Borntrager
Flavonoid	+	NaOH
Quinones	+	H ₂ SO ₄
Phenolics	+	Folin Ciocalteu
Saponin	+	Foam
Tannins	+	Ferric Chloride
Coumarins	+	NH ₃ 10%

3.3. Total Antioxidant Capacity Test of Kembang Bokor Root Extract

The wavelength and maximum absorbance of DPPH were 516 nm and 0.500 nm. The absorbance value of each concentration of kembang bokor root extract was read by GENESYS 30-Vis

spectrophotometry and the % inhibition value was calculated (Table 2). Then a linear equation curve was made to get the IC₅₀ value with the X axis as the concentration of the flower root extract and the Y axis as the % inhibition (Figure 1). In this study, it was found that $Y = 0.0716X + 31.28$ and the value of $R^2 = 0.978$ and the IC₅₀ was 261.45 µg/mL.

Table 2 Data on Concentration, % Inhibition and IC₅₀ of Kembang Bokor Root Extract

Concentration of Kembang Bokor Root Extract (µg/mL)	Inhibition Percentage (%)	IC ₅₀ (µg/mL)
100	37.8	261.45
300	52.8	
400	62.4	
500	65.2	

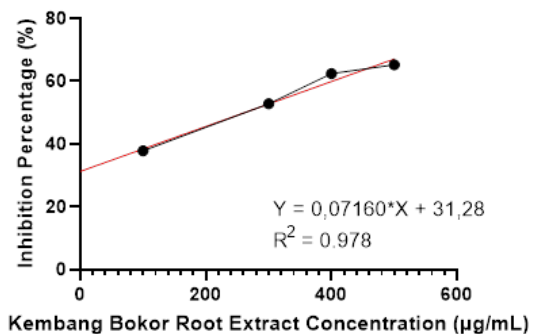


Figure 1 DPPH Test Curve of Kembang Bokor Root Extract

3.4. Standard Comparison Test of Vitamin C

The absorbance measurement of vitamin c content using GENESYS 30-Vis spectrophotometry and the % inhibition was calculated (Table 3). Then the curve of the standard linear equation of vitamin c was made (Figure 2). In this study, the equation of the linear line $Y = 6.934X + 12.52$ and $R^2 = 0.9988$ and the IC₅₀ standard of vitamin c was 5.40 µg/mL where the X axis was the concentration of vitamin c while Y was the % inhibition.

Table 3 Value of Concentration, % Inhibition and IC₅₀ Standard Vitamin C

Vitamin C Concentration (µg/mL)	Inhibition Percentage (%)	IC ₅₀ (µg/mL)
2	26.85	5.40
4	39.11	
6	54.97	
8	67.87	
10	81.81	

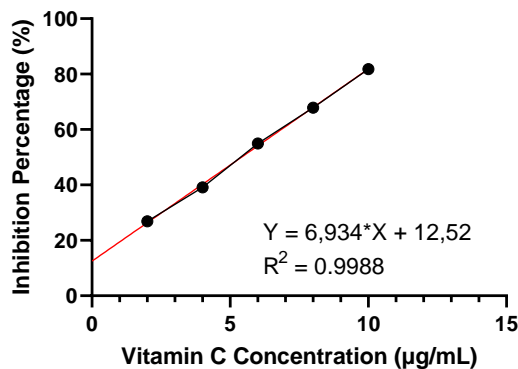


Figure 2 Vitamin C Standard Curve

4. DISCUSSION

4.1. Phytochemical Test

This study tested 12 phytochemicals contained in the extract of the roots of the kembang bokor. It is known that kembang bokor root extract contains phytochemicals in the form of alkaloids, betasianin, cardioglycosides, terpenoids, steroids, glycosides, flavonoids, quinones, phenolics, saponins, tannins and coumarin (Table 1). The results of this study are the same as Agustini et al,¹⁰ that flower buds contain glycosides, flavonoids, terpenoids/steroids, alkaloids and tannins. According to research by Agustini and Faridah et al,^{10,12} the leaves of this plant contain phenolic compounds, terpenoids/steroids, flavonoids and alkaloids. Comparison using flower buds and leaves of kembang bokor because no research has been found that examines the phytochemical content of bokor roots.

4.2. Total Antioxidant Capacity Test

Using ascorbic acid and bokor root extract to measure antioxidant capacity by calculating IC₅₀, namely the ability of the two samples to inhibit 50% of radicals in DPPH. The higher the level of antioxidant capacity of the sample is indicated by the smaller the IC₅₀ value. The standard curve of ascorbic acid obtained the value of R² = 0.9988

while the value of R² = 0.978 in the extract of the root of the kembang bokor. The IC₅₀ value for ascorbic acid was 5.40 µg/mL while the kembang bokor root extract had an IC₅₀ value of 261.45 µg/mL (Table 2 and Table 3). Meanwhile, according to Agustini et al,¹⁰ the IC₅₀ of flower buds was 1971 µg/mL and the IC₅₀ of leaves was 14.42 µg/mL. The results obtained are different from other researchers due to differences in the samples of plant parts used. Although the level of antioxidant capacity of kembang bokor root extract is lower than ascorbic acid, it has advantages over ascorbic acid. According to Pacier et al,¹⁴ vitamin c can be converted to oxalate which results in the formation of kidney stones, while Hariri et al,¹³ said that the kembang bokor root is used to treat kidney stones.

5. CONCLUSION

Based on the results and discussion of this study, it can be concluded:

1. The root extract of kembang bokor contains phytochemicals such as alkaloids, betasianin, cardioglycosides, terpenoids, steroids, glycosides, flavonoids, quinones, phenolics, saponins, tannins and coumarins.
2. The total antioxidant capacity of kembang bokor root extract within IC₅₀ is 261.45 µg/mL therefore very weak.

6. SUGGESTION

1. The total antioxidant capacity was examined by the method FRAP, ORAC
2. *In vivo* research was conducted on experimental animals to determine the antioxidant effect

ACKNOWLEDGMENTS

The author thanks to many colleagues whose research work contributed to this phytochemical and antioxidant research, and we apologized to many authors whose interesting work could not be referred to explicitly. It is an honor to acknowledge F. Ferdinal for the discussion. Generous research support is provided by Department of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University.

AUTHOR CONTRIBUTIONS

The authors contributed equally to all aspects of the article.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES

- Ahmad SI. Reactive oxygen species in biology and human health. 1st ed. New York: CRC Press; 2017. 3–12 p.
- Duan J, Kasper DL. Oxidative depolymerization of polysaccharides by reactive oxygen/nitrogen species. *Glycobiology* [Internet]. 2011 [cited 2021 Jun 10];21(4):401–9. Available from: <https://academic.oup.com/glycob/article/21/4/401/1988268>
- Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* [Internet]. 2012 [cited 2021 Jun 10];5(1):9–19. Available from: [https://www.worldallergyorganizationjournal.org/article/S1939-4551\(19\)30370-9/fulltext](https://www.worldallergyorganizationjournal.org/article/S1939-4551(19)30370-9/fulltext)
- Preiser J. Oxidative stress. *J Parenter Enter Nutr* [Internet]. 2012 [cited 2020 Dec 10];36(2):147–54. Available from: https://link.springer.com/chapter/10.1007%2F978-3-540-69361-1_27
- Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging* [Internet]. 2018 [cited 2021 Jun 10];13:757–72. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5927356/>
- Gülçin İ, Elmastaş M, Aboul-Enein HY. Antioxidant activity of clove oil—A powerful antioxidant source. *Arab J Chem* [Internet]. 2012 [cited 2020 Dec 5];5(4):489–99. Available from: <https://www.sciencedirect.com/science/article/pii/S1878535210001899?via%3Dihub>
- Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañiongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism. volume 5. Shalaby E, editor. London, united kingdom: IntechOpen London, UK; 2019. 23–24 p.
- Dilshara MG, Jayasooriya RGPT, Lee S, Jeong JB, Seo YT, Choi YH, et al. Water extract of processed *Hydrangea macrophylla* (Thunb.) Ser. leaf attenuates the expression of pro-inflammatory mediators by suppressing Akt-mediated NF-κB activation. *Environ Toxicol Pharmacol* [Internet]. 2013 [cited 2020 Jun 7];35(2):311–9. Available from: <https://www.sciencedirect.com/science/article/pii/S138266891200213X?via%3Dihub>
- Akanda MR, Tae H-J, Kim I-S, Ahn D, Tian W, Islam A, et al. Hepatoprotective role of *Hydrangea macrophylla* against sodium arsenite-induced mitochondrial-dependent oxidative stress via the inhibition of MAPK/Caspase-3 pathways. *Int J Mol Sci* [Internet]. 2017 [cited 2020 Dec 5];18(7):1482. Available from: <https://www.mdpi.com/1422-0067/18/7/1482>
- Agustini DM, Yun YF, Defara SM. Senyawa Terpenoid Dan Aktivitas Antidiabetes Ekstrak n-Heksana Dan Metanol *Hydrangea macrophylla*. In: *Prosiding Seminar Hasil Penelitian dan Pengabdian kepada Masyarakat Unjani Expo (Unex)* [Internet]. Jawa Barat, Indonesia: Universitas Jenderal Achmad Yani; 2020 [cited 2020 Jun 7]. p. 27–30. Available from: <https://mail.journal.unjani.ac.id/index.php/unex/article/view/25>
- Yang C-J, Wang Z-B, Zhu D-L, Yu Y, Lei Y-T, Liu Y. Two new cyanogenic glucosides from the leaves of *Hydrangea macrophylla*. *Molecules* [Internet]. 2012 [cited 2020 Jun 7];17(5):5396–403. Available from: <https://www.mdpi.com/1420-3049/17/5/5396>
- Faridah I. Uji praskrining aktivitas antikanker daun kembang bokor (*Hydrangea macrophylla*) dengan metode brine shrimp lethality test (Ekstrak n-Heksana dan Ekstrak Metanol) [Internet]. Malang: University of Muhammadiyah Malang; 2013 [cited 2020 Jun 7]. p. 1–5. Available from: <https://eprints.umm.ac.id/28959/>

13. Hariri MR. Kajian Pigmen Antosianin Kelopak Bunga Hortensia (*Hydrangea macrophylla*). Skripsi Jur Biol MIPA UM [Internet]. 2013 [cited 2020 Dec 10];1:1. Available from: <http://karya-ilmiah.um.ac.id/index.php/biologi/article/view/24400>
14. Pacier C, Martirosyan DM. Vitamin C: optimal dosages, supplementation and use in disease prevention. *Funct Foods Heal Dis* [Internet]. 2015 [cited 2021 Jun 10];5(3):89–107. Available from: <https://ffhdj.com/index.php/ffhd/article/view/174>