|  |  |  |
| --- | --- | --- |
| C:\Users\DR. FALODUN\AppData\Local\Microsoft\Windows\INetCache\Content.Word\IMG-20170722-WA0006 new cover.jpg | **Tropical Journal of Natural Product Research**  Available online at [https://www.tjnpr.org](https://www.tjnpr.org/)  ***Original Research Article*** | C:\Users\DR. FALODUN\AppData\Local\Microsoft\Windows\INetCache\Content.Word\IMG-20170722-WA0006 new cover.jpg |

**The Analysis of Antioxidant Activity and Capacity of Boiled and Infused Indonesian Herbals**

Christyanita P. Ekasari1\*, Sri Widyarti2, Sutiman B. Sumitro2

*1Master Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang 65145, East Java, Indonesia*

*2Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang 65145, East Java, Indonesia*

|  |  |
| --- | --- |
| ARTICLE INFO | ABSTRACT |
| Article history:  Received 13 November 2022  Revised 14 January 2023  Accepted 17 January 2023  Published online 01 February 2023 | Antioxidants from natural ingredients such as medicinal plants are crucial to improving endogenous antioxidant defense system. The aim of the study was to evaluate the antioxidant activity and capacity of boiled and infused Indonesian herbals using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The DPPH assay was conducted with triplicate repetitions to 10 herbals (*Phyllanthus niruri, Lantana camara, Piper crocatum, Curcuma xanthorrhiza, Moringa oleifera, Tamarindus indica, Kaempferia galanga, Citrus aurantifolia, Hibiscus sabdariffa*, and *Zingiber officinale*), each consisting of boiled and infused samples. The antioxidant activities of those herbals were (boiled and infused samples, respectively): 91.88 %, 91.30 % (*P. niruri*), 92.14 %, 86.60 % (*L. camara*), 53.39 %, 58.99 % (*P. crocatum*), 18.73 %, 20.86 % (*C. xanthorrhiza*), 51.35 %, 59.96 % (*M. oleifera*), 63.60 %, 16.95 % (*T. indica*), 13.14 %, 18.82 % (*K. galanga*), 20.46 %, 69.24 %. (*C. aurantifolia*), 34.49 %, 34.40 % (*H. sabdariffa*), and 27.16 %, 26.01 % (*Z. officinale*). *P. niruri* and *L. camara* reached the high R2 value. *P. niruri* and *L. camara* reached the stable antioxidant activity after 50 minutes, while the other herbals after 60 minutes. *P. niruri* and *L. camara* were the 2 herbals with the highest antioxidant activity and slightly lower antioxidant capacity. The other herbals had lower antioxidant activity and higher antioxidant capacity. There was no significant difference in antioxidant activity between boiled and infused samples of all herbals, except *T. indica* and *C. aurantifolia*.    ***Keywords*:**  antioxidant activity, antioxidant capacity, boiling, DPPH assay, herbals, infusion |
| **Copyright:** © 2023 Ekasari *et al*. This is an open-access article distributed under the terms of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/) Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. |

**Introduction**

In daily life, cells will be exposed to free radicals continuously.1 In small amounts, the function of free radicals is to maintain the cell homeostasis.2 The excessive free radicals can cause oxidative stress, which leads to DNA damage.1 If DNA damage occurs, cell death will also occur through necrosis or apoptosis, which can cause tissue damage. Tissue damage causes various diseases and contributes to the aging process of the body.3 The excessive free radicals can be countered by the antioxidant defense system.1 Antioxidants play a role in neutralizing free radical inside the body through scavenging process, so it can prevent the tissue damage (oxidative damage).3 The antioxidant molecules can deactivate the radical species through single electron transfer (hydrogen donation).2 However, the antioxidant production is often insufficient for the scavenging process against all excessive free radical, so it is necessary to supply antioxidants from outside the body, for example, from natural ingredients.3 Antioxidants from natural ingredients are very important to improve endogenous antioxidant defense system.4

Plant is one of the natural sources which has abundant antioxidant content. Several kinds of plants have abundant phytochemical content which is redox active.5 Indonesia is a country with the second highest biodiversity in the world, after the Amazon rainforest. Indonesia has high amount of native medicinal plants.6

\*Corresponding author. E mail: [christyanitaputri@gmail.com](mailto:christyanitaputri@gmail.com)

Tel: +62 857-3552-0500

**Citation:** Ekasari CP, Widyarti S, Sumitro SB. The Analysis of Antioxidant Activity and Capacity of Boiled and Infused Indonesian Herbals. Trop J Nat Prod Res. 2023; 7(1):2145-2151. http://www.doi.org/10.26538/tjnpr/v7i1.9

Official Journal of Natural Product Research Group, Faculty of Pharmacy,

University of Benin, Benin City, Nigeria.

It makes Indonesia a country that has large quantities of herbal ingredients availability from various types of medicinal plants. In this study, 10 herbals were used (with different part for each plant) i.e., *Phyllanthus niruri* (leaves), *Lantana camara* (flowers), *Piper crocatum* (leaves), *Curcuma xanthorrhiza* (rhizomes), *Moringa oleifera* (leaves), *Tamarindus indica* (leaves), *Kaempferia galanga* (rhizomes), *Citrus aurantifolia* (fruit mesocarp or albedo), *Hibiscus sabdariffa* (flowers), and *Zingiber officinale* (rhizomes). The selection of those plants parts was based on the use of medicinal plants which have been traditionally used to treat tuberculosis (TB), and also based on their antioxidant capacity and antibiotic properties.

There are 2 common herbals preparation: boiling and infusion. Boiling is one of the herbal preparation methods which is widely used in Asia. This method is conducted by heating the herbal sample in water until it boils.7,8 Meanwhile, infusion is conducted by dissolving the herbals in water. Herbal infusion is the source of biologically active natural compounds. It is easily prepared, consumed, and absorbed.9

To evaluate the potential of free radical absorption by herbal, it is necessary to analyze the activity and capacity of antioxidants. The antioxidant activity shows the overall antioxidant status in a biological sample and the calculation of the total number (concentration) of scavenged free radical. Meanwhile, the antioxidant capacity shows the reaction kinetics (rate) between antioxidant and radical, which describes the antioxidant ability in blocking the propagation stage in oxidative chain.10 There are various methods to analyze the activity and capacity of antioxidants, one of which is the DPPH assay. The main principle of DPPH assay is the scavenging reaction between antioxidants and DPPH (free radicals), and the antioxidant activity at the end of reaction will be determined colorimetrically (absorbance measurement by UV-Vis spectrophotometry). In DPPH assay, the purple chromogen radical (DPPH·) will be reduced by antioxidants (AH), so it can produce the pale-yellow hydrazine (DPPH-H).11 It is expected that the DPPH assay can provide the proper information about the antioxidant activity and capacity of 10 Indonesian herbals. No other research has ever been done to evaluate and to compare the antioxidant activity and capacity of those 10 Indonesian herbals, so this research was crucial to be conducted. The aim of the study was to evaluate the antioxidant activity and capacity of boiled and infused Indonesian herbals using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

**Materials and Methods**

*Herbal preparation*

The herbals used in this study were the 90 mesh-sized simplicial powder from 10 herbal plants with different plant parts obtained from Materia Medica Batu (Table 1). There were 2 ways of herbal preparations i.e., boiling and infusion. Each boiled herbal plant was prepared from 10 grams of powder herbals which was dissolved in 200 ml of aquadest (1:20), then was boiled to 100 °C for an hour,7,8 centrifuged at 2500 rpm for 10 minutes, and filtered using Whatman filter. Each infused herbal plant was prepared from 10 grams of powder herbals that was dissolved in 200 ml of aquadest (1:20),9 then was stirred using a magnetic stirrer for 24 hours at room temperature. Both infused and boiled herbals were then freeze-dried.

*Analysis of antioxidant activity and capacity using DPPH assay*

The DPPH stock solution was prepared first using 24 mg of DPPH powder which was dissolved in 100 ml of methanol. The DPPH working solution was then prepared using 25 ml of DPPH stock solution which was diluted in 200 ml of methanol. The absorbance of the DPPH working solution was adjusted to 0.751 Å in the wavelength of 517 nm.11 The researchers tested herbals at 4 different concentrations (0.0125 mg/ml, 0.025 mg/ml, 0.05 mg/ml, and 0.1 mg/ml). All herbals were compared to ascorbic acid. Four different concentrations of ascorbic acid (1.1 mg/ml, 4.4 mg/ml, 8.8 mg/ml, and 17.6 mg/ml) were used.

Four milliliters of DPPH working solution was added to each sample and ascorbic acid solution. The DPPH absorbance of those solutions were measured in 517 nm using a UV-Vis spectrophotometer (Thermo Scientific GENESYS 10S UV-Vis Spectrophotometer). The DPPH absorbance of each sample was then used to calculate the DPPH scavenging percentage (antioxidant activity) with the Equation (1). The absorbance was measured every 10 minutes for 60 minutes.12 The antioxidant capacity was determined based on the increase rate of antioxidant activity.

(1)

**Table 1.** The parts of the plants taken used in the study

|  |  |
| --- | --- |
| **Name of the plant** | **Plant part taken** |
| *Phyllanthus niruri* | Leaves |
| *Lantana camara* | Flowers |
| *Piper crocatum* | Leaves |
| *Curcuma xanthorrhiza* | Rhizomes |
| *Moringa oleifera* | Leaves |
| *Tamarindus indica* | Leaves |
| *Kaempferia galanga* | Rhizomes |
| *Citrus aurantifolia* | Fruit mesocarp (albedo) |
| *Hibiscus sabdariffa* | Flowers |
| *Zingiber officinale* | Rhizomes |

*Statistical analysis and data presentation*

The DPPH assay was conducted with triplicate repetitions. The data of scavenging percentage of herbals and ascorbic acid in the same concentration (25 µg/ml) were analyzed using one-way ANOVA (p < 0.05) followed by Tukey HSD tests in IBM SPSS Statistics 25. The average data of scavenging percentage of 10 herbals were presented in graphical form (line graph) using Microsoft Excel (duration (minute) as the x-axis and scavenging percentage as the y-axis), with standard deviation as error bars. The data of scavenging percentage of herbals and ascorbic acid in the same concentration (25 µg/ml) were presented as mean ± standard deviation in tabular form. The R2 value was obtained from the linear regression graph, by plotting the concentration series on the x-axis and the scavenging percentage on the y-axis, and the data was presented in tabular form. All data were analyzed descriptively.

**Results and Discussion**

The antioxidant activity shows the overall antioxidant status in a biological sample and the calculation of scavenged free radical, while the antioxidant capacity shows the reaction rate between antioxidant (from herbals) and free radical (DPPH).10 The antioxidant activity can be described through the calculation result of scavenging percentage. Meanwhile, the antioxidant capacity can be described through the time for each herbal plant to reach the steady state of the graph of the increase of scavenging percentage, marked by the black oval line in each graph. The increase rate of antioxidant activity indicates the scavenging reaction kinetics between antioxidant (from herbals) and free radical (DPPH). The scavenging percentage is referred to the percentage of antioxidant activity from herbal samples against DPPH radical which was added to the solution. The faster a sample reaches the high scavenging percentage (almost 100 % scavenging), then the lower its antioxidant capacity. The DPPH assay was conducted with triplicate repetitions. Based on the average of the scavenging percentage of 3 repetitions, the varied results were obtained. There are 3 groups of scavenging percentage i.e., high (the highest percentage reached 86% - 92%), medium (the highest percentage reached 51% - 69%), and low (the highest percentage reached 13% - 34%). In herbal samples, the concentration series of 0.1 mg/ml had the highest scavenging percentage. This indicates that the more herbal sample amount on a solution, the higher the antioxidant activity on that solution. The 10 minutes time interval only resulted in slight difference of scavenging percentages.

There were 2 herbals which had the highest scavenging percentage after 1 hour: *Phyllanthus niruri* and *Lantana camara* (both boiled and infused samples). The scavenging percentages of those herbals were 91.88 % (boiled *P. niruri*), 91.30% (infused *P. niruri*), 92.14% (boiled *L. camara*), and 86.60% (infused *L. camara*) (Figure 1). The scavenging percentages of *Phyllanthus niruri* and *Lantana camara* were close to the scavenging percentage of ascorbic acid as comparison compound (which reached 98.36% for the highest concentration of ascorbic acid solution). The boiled and infused samples of *P. niruri* and *L. camara* reached the steady state of the scavenging percentage graph faster than any other herbals (after 50 minutes) (Figure 1), which means that *P. niruri* and *L. camara* had slightly lower antioxidant capacity. The time to reach the steady state of scavenging percentage marked by the black oval line in each graph.

The phytochemicals (such as phenolics and flavonoids) are found in various plants. The phytochemicals have potential to prevent ROS-induced diseases.13 The 10 herbals in this research contain many kinds of phytochemicals which affect the antioxidant activity of herbals. Among the 10 herbals, the boiled and infused samples of *P. niruri* and *L. camara* had the highest antioxidant activity. It was presumably caused by the phytochemicals content in *P. niruri* and *L. camara* which affect the high antioxidant activity of both plants. *P. niruri* contains many phytochemicals such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins14, while *L. camara* contains flavonoid, tannins, saponins, alkaloids, cardiac glycosides, triterpenoids.15 The antioxidant activity of phenolic acids is related to the number and position of the –OH group present in the aromatic ring.2,16 The more the number of hydroxyl group (–OH), the higher the antioxidant activity of phenolic compounds.17 It was presumably that *P. niruri* and *L. camara* had high number of –OH group in the aromatic ring of their phenolic compounds, so both plants had high antioxidant activity.

On the results of the other research, it was stated that the extract of *P. niruri* had 85.54 % scavenging activity, obtained from concentration of 10 µg/ml.14 One of the flavonoids contained in *P. niruri*, quercetin, had higher scavenging activity than the extract of *P. niruri* itself from concentration 10 µg/ml (89.16%).14 This correlates with our result that *P. niruri* had the high scavenging percentage. The high scavenging activity of *P. niruri* was probably caused by its active compound content, especially quercetin (flavonoid). Along with *P. niruri, L. camara* also had the highest antioxidant activity among the 10 herbals used in this study. The DPPH free radical scavenging activity of *L. camara* flowers was 87.2 %.18 Meanwhile in the other research, the DPPH radical scavenging activities of extracts from flowers of *L. camara* were 72.30 % (80 % methanol), 67.07 % (80 % ethanol), 70.69 % (absolute methanol), and 58.46 % (absolute ethanol).19

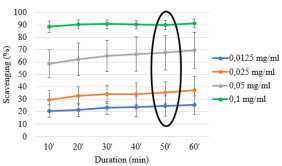
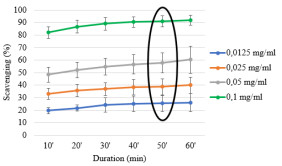
Some herbals had the medium scavenging percentages because they were not as high as the scavenging percentage of *Phyllanthus niruri* and *Lantana camara*, but also not as low as the other herbals. The scavenging percentages of this group were not more than 70% for the highest concentration of sample solution (0.1 mg/ml) after an hour. The herbals which had the medium scavenging percentage were: *Piper crocatum* (boiled and infused samples), *Moringa oleifera* (boiled and infused samples), *Tamarindus indica* (boiled sample), and *Citrus aurantifolia* (infused sample).

The boiled *Piper crocatum* sample reached 53.39 % of the scavenging percentage (Figure 2A), while the infused sample reached 58.99 % of the scavenging percentage (Figure 2B). The scavenging percentage of boiled *Moringa oleifera* sample reached 51.35 % (Figure 2C), while the infused sample reached 59.96 % (Figure 2D). The boiled and infused samples of *P. crocatum* and *M. oleifera* reached the steady state of the scavenging percentage graph after 60 minutes (Figure 2), which means that *P. crocatum* and *M. oleifera* had high antioxidant capacity. The time to reach the steady state of scavenging percentage marked by the black oval line in each graph.

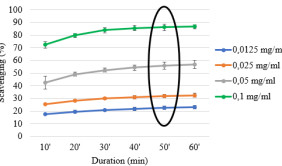
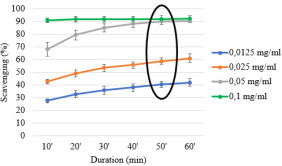
The boiled sample of *Tamarindus indica* had medium scavenging percentage, while its infused sample had the low scavenging percentage. The scavenging percentage of boiled *Tamarindus indica* reached 63.60 % (Figure 3A), while the scavenging percentage of infused *Tamarindus indica* sample reached 16.95 % (Figure 3B). On the contrary, the infused sample of *Citrus aurantifolia* had medium scavenging percentage, while its boiled sample had the low scavenging percentage. The scavenging percentage of infused *Citrus aurantifolia* reached 69.24 % (Figure 3D), while the scavenging percentage of boiled *Citrus aurantifolia* sample reached 20.46 % (Figure 3C). The boiled and infused samples of *T. indica* and *C. aurantifolia* reached the steady state of the scavenging percentage graph after 60 minutes (Figure 3), which means that *T. indica* and *C. aurantifolia* had high antioxidant capacity.

The boiling and infusion methods aimed to evaluate the difference in antioxidant activity between herbals, based on whether or not the effect of heat treatment is present. In this study, there was no significant difference between all boiled samples and infused samples, except *T. indica* and *C. aurantifolia*. The boiled sample of *T. indica* had medium antioxidant activity, while the infused sample of *T. indica* had low antioxidant activity. The increase in antioxidant activity of boiled *T. indica* was presumably caused by the release of phenolic compounds through the breakdown of cellular constituents during the heat treatment. The content of phenolic compounds would be increased, so it would also increase the antioxidant activity.8 Otherwise, the infused sample of *C. aurantifolia* had medium antioxidant activity, while the boiled sample of *C. aurantifolia* had low antioxidant activity. The decrease in antioxidant activity of boiled *C. aurantifolia* was presumably caused by the enzymatic degradation of phenolic compounds, the thermal degradation of phytochemicals, and the loss of antioxidant enzyme activity during the heat treatment.8 It also proved that the heat treatment did not affect the antioxidant activity of other herbals. This analysis result of antioxidant activity can be the serving suggestions, that *T. indica* is better consumed with boiling preparation first, while *C. aurantifolia* is better consumed by brewing it without heat treatment, so both plants will give the highest antioxidant activity that they can reach. Meanwhile, the other herbals can be served with or without heat treatment (boiling or infusion), because both methods give the similar results of antioxidant activity. This can be beneficially that all 10 herbals in this study (except *C. aurantifolia*) can get through thermal processing for consumption purpose without reducing their antioxidant ability.20

The last group of herbals was the herbals which had the low scavenging percentage. This group reached the scavenging percentages not more than 35%. The increase of scavenging percentage of this group from the lowest to the highest concentration were not significant, even some of those herbals had graphs which tend to be flat.



1. (B)



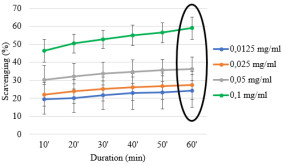
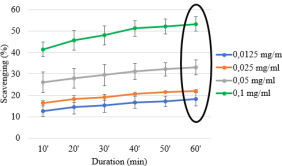
(C) (D)

**Figure 1:** The antioxidant activity based on reaction duration with DPPH radical. A: boiled *P. niruri*, B: infused *P. niruri,* C: boiled *L. camara,* D: infused *L. camara.* The black oval line marked the time for *P. niruri* and *L. camara* to reach the steady state of the graph of scavenging percentage (50 min).

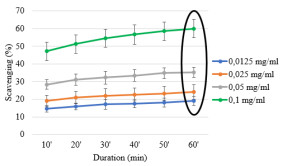
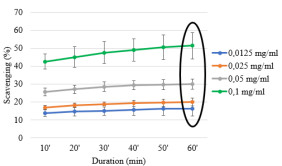
The herbals which had the low scavenging percentage were: *Curcuma xanthorrhiza* (boiled and infused samples), *Kaempferia galanga* (boiled and infused samples), *Hibiscus sabdariffa* (boiled and infused samples), *Zingiber officinale* (boiled and infused samples), *Tamarindus indica* (infused sample), and *Citrus aurantifolia* (boiled sample).

The scavenging percentage of boiled *Curcuma xanthorrhiza* sample reached 18.73 % (Figure 4A), while the infused sample reached 20.86 % (Figure 4B). The scavenging percentage of boiled *Kaempferia galanga* sample reached 13.14 % (Figure 4C), while the infused sample reached 18.82 % (Figure 4D). The boiled and infused samples of *C. xanthorrhiza* and *K. galanga* reached the steady state of the scavenging percentage graph after 60 minutes (Figure 4), which means that *C. xanthorrhiza* and *K. galanga* had high antioxidant capacity.

The scavenging percentage of boiled *Hibiscus sabdariffa* sample reached 34.49 % (Figure 5A), while the infused sample reached 34.40 % (Figure 5B). The scavenging percentage of boiled *Zingiber officinale* sample reached 27.16 % (Figure 5C), while the infused sample reached 26.01 % (Figure 5D). The boiled and infused samples of *H. sabdariffa* and *Z. officinale* reached the steady state of the scavenging percentage graph after 60 minutes (Figure 5), which means that *H. sabdariffa* and *Z. officinale* had high antioxidant capacity.

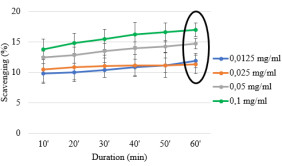
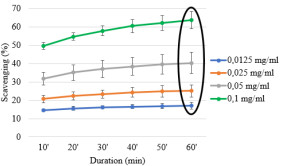


(A) (B)

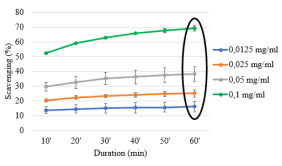
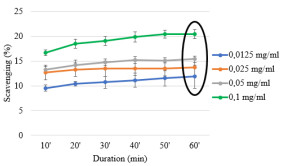


(C) (D)

**Figure 2.** The antioxidant activity based on reaction duration with DPPH radical. A: boiled *P. crocatum,* B: infused *P. crocatum,* C: boiled *M. oleifera*, D: infused *M. oleifera.* The black oval line marked the time for *P. crocatum* and *M. oleifera* to reach the steady state of the graph of scavenging percentage (60 min).



(A) (B)



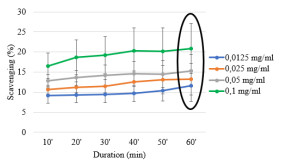
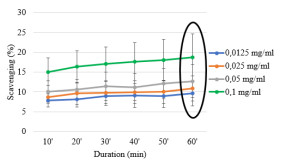
(C) (D)

**Figure 3.** The antioxidant activity based on reaction duration with DPPH radical. A: boiled *T. indica,* B: infused *T. indica,* C: boiled *C. aurantifolia,* D: infused *C. aurantifolia.* The black oval line marked the time for *T. indica* and *C. aurantifolia* to reach the steady state of the graph of scavenging percentage (60 min).

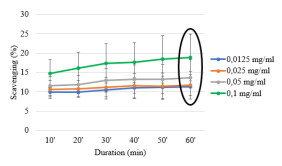
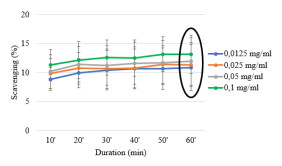
DPPH is a nitrogen radical species that has a long life and is dark in color (bluish or purplish). In organic solutions, DPPH can be detected at a wavelength of 517 nm by UV-Vis spectrophotometry. The mechanism of DPPH scavenging by antioxidants is based on the acceptance of electrons by DPPH from antioxidants (AH). When the DPPH radical meets an electron donor substrate such as an antioxidant, the radical will be scavenged and its absorbance will decrease. The purple chromogen radical (DPPH·) will be reduced by antioxidants (AH), so it will produce pale yellow hydrazine (DPPH-H).11 Phenolics from medicinal plants can donor electron to DPPH radical, so DPPH radical will lose its chromophore and its colour will turn to yellow.

The higher the concentration of phenolics or the degree of hydroxylation of phenolic compounds, the higher DPPH radical scavenging activity (antioxidant activity).19

The scavenging percentages of herbals were compared to ascorbic acid. The average of scavenging percentage of ascorbic acid in the concentrations 1.1 mg/ml to 17.6 mg/ml reached 98.18 % to 98.36 % (Figure 6). The scavenging ability of ascorbic acid is affected by its ability to donate electrons. Ascorbic acid has a high ability as an electron donor.2 Ascorbic acid is a compound which can scavenge free radicals by suppressing chain initiation or breaking chain propagation reactions of free radical. Free radicals neutralized by ascorbic acid will become harmless species.2 With the low concentration (1.1 mg/ml), ascorbic acid was able to scavenge 98.18 % of free radicals DPPH.

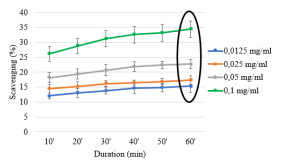
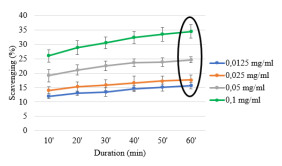


(A) (B)

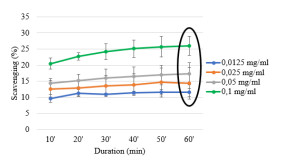
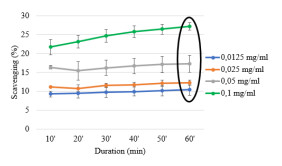


(C) (D)

**Figure 4.** The antioxidant activity based on reaction duration with DPPH radical. A: boiled *C. xanthorrhiza*, B: infused *C. xanthorrhiza***,** C: boiled *K. galanga*, D: infused *K. galanga*. The black oval line marked the time for *C. xanthorrhiza* and *K. galanga* to reach the steady state of the graph of scavenging percentage (60 min).



(A) (B)

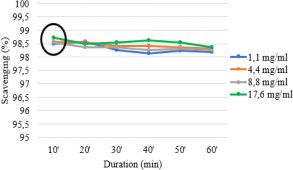


(C) (D)

**Figure 5:** The antioxidant activity based on reaction duration with DPPH radical. A: boiled *H. sabdariffa,* B: infused *H. sabdariffa*, C: boiled *Z. officinale,* D: infused *Z. officinale.* The black oval line marked the time for *H. sabdariffa* and *Z. officinale* to reach the steady state of the graph of scavenging percentage (60 min).

However, the DPPH scavenging percentages by ascorbic acid with the higher concentrations (4.4 mg/ml to 17.6 mg/ml) were not much different with concentration 1.1 mg/ml (98.27 % - 98.36 %). It was probably due to the electrons in ascorbic acid donated almost entirely to scavenge DPPH free radicals. Ascorbic acid is a compound able to scavenge the free radicals (DPPH) quickly. Ascorbic acid reached the stable antioxidant activity and high percentage scavenging (almost 100 % scavenging) in shorter time than herbals (after 10 minutes) (Figure 6), so the antioxidant capacity of ascorbic acid was much lower than herbals. The low antioxidant capacity of ascorbic acid was related to its negative impact if consumed in excess. In excess, ascorbic acid (vitamin C) can reduce the metal ions so it can promote the formation of free radical in Fenton system.2 Herbals are better than ascorbic acid, because they can donate electron to scavenge the excessive free radicals in body in a longer time (50 – 60 minutes). The

herbals with the highest antioxidant activity had slightly lower antioxidant capacity than any other herbals, because it was relatively faster in reaching a high scavenging percentage (almost 100 % scavenging).



**Figure 6.** The antioxidant activity of ascorbic acid.

**Table 2:** The antioxidant activity (%) in the same concentration (25 µg/ml) of herbal samples and ascorbic acid. The different letters indicate a significant difference *(p < 0,05)* between groups based on Tukey HSD test

|  |  |  |
| --- | --- | --- |
| **Sample** | **Boiled (%)** | **Infused (%)** |
| *Phyllanthus niruri* | 34.87 ± 4.80f | 35.90 ± 6.55f |
| *Lantana camara* | 50.17 ± 1.18g | 31.56 ± 1.22ef |
| *Piper crocatum* | 22.39 ± 1.27cd | 27.30 ± 6.22def |
| *Curcuma xanthorrhiza* | 10.77 ± 3.11a | 12.28 ± 3.23ab |
| *Moringa oleifera* | 19.97 ± 2.27bcd | 23.78 ± 1.68cde |
| *Tamarindus indica* | 24.22 ± 2.10cde | 11.73 ± 1.83ab |
| *Kaempferia galanga* | 11.03 ± 3.54ab | 12.00 ± 3.30ab |
| *Citrus aurantifolia* | 12.51 ± 0.37ab | 23.90 ± 1.18cde |
| *Hibiscus sabdariffa* | 17.63 ± 0.81abc | 17.37 ± 1.36abc |
| *Zingiber officinale* | 12.41 ± 1.09ab | 13.23 ± 2.02ab |
| Ascorbic acid | 62.58 ± 0.28h | |

Table 2 is the average comparison of the 3 repetitions of antioxidant activity between herbal samples and ascorbic acid in the same concentration, 25 µg/ml. As previously, the highest antioxidant activities were reached by *P. niruri* and *L. camara*, with scavenging percentage of 34.87 % (boiled *P. niruri*), 35.90 % (infused *P. niruri*), 50.17 % (boiled *L. camara*), and 31.56 % (infused *L. camara*). Meanwhile, the scavenging percentages of boiled and infused samples of the other herbals were ranged from 10.77 % to 27.30 %. In the same concentration as herbal samples, ascorbic acid had 62.58 % scavenging percentage.

The higher the R2 value indicates that the increase rate of the DPPH radical scavenging has a high correlation with herbal sample concentration. Ascorbic acid had the highest R2 value (0.99). Herbals had the lower R2 value than ascorbic acid. *P. niruri* and *L. camara* had high R2 value (ranged from 0.95 to 0.97) (Table 3). This indicated that the concentration series of *P. niruri* and *L. camara* had a strong correlation to the increase of DPPH radical scavenging activity. The R2 of the quercetin from *P. niruri* was 0.99, which was higher than the R2 of the extract (0.96).14 The DPPH radical scavenging activity of *L. camara* flowers had a good correlation (R2=0.81) with the total phenolics of *L. camara*. The antioxidant activity of medicinal plant is highly correlated with the total phenolics and flavonoids contained in it. The good correlation between DPPH radical scavenging activity and total phenolics indicates that phenolics have an important role as free radical scavengers, chain breakers, and electron donors.19 The other herbals with lower antioxidant activity such as *C. xanthorrhiza, K. galanga, H. sabdariffa, Z. officinale,* the infused sample of *T. indica*, and the boiled sample of *C. aurantifolia* had the lower R2 value, which ranged from 0.62 to 0.90 (Table 3). It shows that the concentration series of those herbals had lower correlation to the increase of antioxidant activity.

**Conclusion**

Among the ten herbals, *Phyllanthus niruri* and *Lantana camara* were the herbals with the highest antioxidant activity, with lower antioxidant capacity (50 minutes). Meanwhile, the other 8 herbals had lower antioxidant activity, with the higher antioxidant capacity (60 minutes). There was no significant difference in antioxidant activity between boiled and infused samples of all herbals, except *T. indica* and *C. aurantifolia*. The antioxidant activity of boiled sample of *T. indica* was significantly different and higher than the infused one. On the contrary, the antioxidant activity of boiled sample of *C. aurantifolia* was significantly different and lower than infused one. The difference was presumably due to the heat treatment in boiled samples.

**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 3:** The R2 value of herbal samples and ascorbic acid

|  |  |
| --- | --- |
| **Sample** | **R2 Value** |
| Ascorbic acid | 0.99 |
| Infused *Citrus aurantifolia* | 0.97 |
| Infused *Lantana camara* | 0.97 |
| Boiled *Tamarindus indica* | 0.96 |
| Boiled *Phyllanthus niruri* | 0.96 |
| Boiled *Lantana camara* | 0.95 |
| Boiled *Moringa oleifera* | 0.95 |
| Infused *Phyllanthus niruri* | 0.95 |
| Infused *Moringa oleifera* | 0.94 |
| Boiled *Piper crocatum* | 0.93 |
| Boiled *Zingiber officinale* | 0.90 |
| Infused *Piper crocatum* | 0.89 |
| Boiled *Hibiscus sabdariffa* | 0.87 |
| Infused *Hibiscus sabdariffa* | 0.86 |
| Infused *Zingiber officinale* | 0.86 |
| Infused *Curcuma xanthorrhiza* | 0.80 |
| Boiled *Curcuma xanthorrhiza* | 0.80 |
| Boiled *Citrus aurantifolia* | 0.78 |
| Infused *Kaempferia galanga* | 0.75 |
| Infused *Tamarindus indica* | 0.73 |
| Boiled *Kaempferia galanga* | 0.62 |

**Acknowledgements**

This research was funded by the Research Grant Hibah Penelitian Professor Universitas Brawijaya TA. 2022 No. 3084.24/UN10.F09/PN/2022.

**References**

1. Meng D, Zhang P, Zhang L, Wang H, Ho CT, Li S, Shahidi F, Zhao H. Detection of cellular redox reactions and antioxidant activity assays. J. Funct. Foods 2017; 37:467–479.
2. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. Eur. J. Med. Chem. 2021; 209(112891):1–23.
3. Kopani M, Celec P, Danixovi L, Michalka P, Biro C. Oxidative stress and electron spin resonance. Clin. Chim. Acta 2006; 364:61–66.
4. Rohn S and Kroh LW. Electron spin resonance – A spectroscopic method for determining the antioxidative activity. Mol. Nutr. Food Res. 2005; 49:898–907.
5. Wu R, Li S, Hudlikar R, Wang L, Shannar A, Peter R, Chou PJ, Kuo HCD, Liu Z, Kong AN. Redox signaling, mitochondrial metabolism, epigenetics and redox active phytochemicals. Free Radic. Biol. Med. 2020; 1–9.
6. Elfahmi, Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J. Herb. Med. 2014; 1-23.
7. Lee IH, Chung HJ, Shin JS, Ha IH, Kim MR, Koh W, Lee J. Influence of boiling duration of GCSB-5 on index compound content and antioxidative and anti-inflammatory activity. Pharmacogn. Mag. 2017; 13(51):418–424.
8. Salamatullah AM, Hayat K, Arzoo S, Alzahrani A, Ahmed MA, Yehia HM, Alsulami T, Al-Badr N, Al-Zaied BAM, Althbiti MM. Boiling technique-based food processing effects on the bioactive and antimicrobial properties of basil and rosemary. Molecules 2021; 26(7373):1–13.
9. Studzinska-Sroka E, Galanty A, Gosciniak A, Wieczorek M, Klaput M, Dudek-Makuch M, Cielecka-Piontek J. Herbal Infusions as a Valuable Functional Food. Nutrients 2021; 13 (4051):1-16.
10. Siddeeg A, AlKehayez, NM, Abu-Hiamed HA, Al-Sanea EA, Al-Farga AM. Mode of action and determination of antioxidant activity in the dietary sources: An overview. Saudi J. Biol. Sci. 2021; 28:1633-1644.
11. Gulcin I. Antioxidants and antioxidant methods: an updated overview. Arch. Toxicol. 2020; 1-65.
12. Torre MP, Cavero RY, Calvo MI, Vizmanos JL. A simple and a reliable method to quantify antioxidant activity in vivo. Antioxidants 2019; 8(142):1–11.
13. Ngamdee P, Wichai U, Jiamyangyuen S. Correlation between phytochemical and mineral contents and antioxidant activity of black glutinous rice bran, and its potential chemopreventive property. Food Technol. Biotechnol. 2016; 54:282–289.
14. Rusmana D, Wahyudianingsih R, Elisabeth M, Balqis, Maesaroh, Widowati W. Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin. Indones. Biomed. J. 2017; 9:84–90.
15. Tuyiringire N, Deyno S, Weisheit A, Tolo CU, Tusubira D, Munyampundu JP, Ogwang PE, Muvunyi CM, Heyden YV. Three promising antimycobacterial medicinal plants reviewed as potential sources of drug hit candidates against multidrug-resistant tuberculosis. Tuberculosis 2020; 124(101987):1-8.
16. Kavitha P and Sowmia C. Screening of phytochemical and in-vitro antioxidant property of a polyherbal formulation. Int. J. Pharm. Sci. Res. 2016; 7(11):4608-4614.
17. Olszowy M. What is responsible for antioxidant properties of polyphenolic compounds from plants?. Plant Physiol. Biochem. 2019; 144:135-143.
18. Anand J, Chaudhary S, Rai N. Analysis of antioxidant activity, total phenolic content and total flavonoid content of *Lantana camara* leaves and flowers. Asian J. Pharm. Clin. Res. 2018; 11(4):203-206.
19. Anwar F, Shaheen N, Shabir G, Ashraf M, Alkharfy KM, Gilani AH. Variation in antioxidant activity and phenolic and flavonoid contents in the flowers and leaves of ghaneri (*L. camara* L.) as affected by different extraction solvents. Int. J. Pharmacol. 2013; 9(7):442-453.
20. Ebhohimen EI, Edemhanrhia L, Ekozin A, Okolie PN. Effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of *Aframomum angustifolium* seed. Trop. J. Nat. Prod. Res. 2017; 1(3):125-128.