



RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT ACTIVITY OF EXTRACT COMBINATION OF GINGER AND LEMONGRASS

Faradiba¹, Rezki Amriati Syarif^{2*}, Anggi Anggrayni³, Elyana Praditha⁴, Alifiya Reski Amalia⁵

Faculty of Pharmacy, Universitas Muslim Indonesia.

Article Info:



Article History:

Received: 17 October 2024
 Reviewed: 9 November 2024
 Accepted: 18 December 2024
 Published: 15 January 2025

Cite this article:

Faradiba, Syarif RA, Anggrayni A, Praditha E, Amalia AR. Evaluation of antioxidant activity of extract combination of ginger and lemongrass. Universal Journal of Pharmaceutical Research 2024; 9(6): 1-4.

<http://doi.org/10.22270/ujpr.v9i6.1232>

*Address for Correspondence:

Rezki Amriati Syarif, Faculty of Pharmacy, Universitas Muslim Indonesia. Tel: +62-85255127325;
 E-mail: rezkiamriati.syarif@umi.ac.id

Abstract

Aim and objective: This study to determine the antioxidant activity of the combination of ginger (*Zingiber officinale*) and lemongrass (*Cymbopogon citratus*) on reducing DPPH (2,2-diphenyl-1-picrylhydrazyl).

Methods: Quantitative measurement of the antioxidant activity of ginger and lemongrass extracts, as well as a combination of both with a concentration ratio of 1:1, 2:1, 1:2 using a UV-Vis spectrophotometer at a wavelength of 516 nm to determine the IC₅₀ value.

Results: IC₅₀ value of ginger extract 10.76 µg/ml, lemongrass extract 26.69 µg/ml, IC₅₀ value 6.69 µg/ml for combination with concentration ratio 1:1, IC₅₀ value 3.32 µg/ml for combination with concentration ratio 2:1. IC₅₀ value 9.34 µg/ml for combination with concentration ratio 1:2.

Conclusions: The combination of ginger and lemongrass extracts has synergistic antioxidant activity that can increase the antioxidant effect from the strong category to very strong category.

Keywords: Combination, *Cymbopogon citratus*, DPPH assay, free radical, IC₅₀ value, *Zingiber officinale*.

INTRODUCTION

Antioxidants are compounds that can reduce, restrain and prevent the oxidation process by donating one or more electrons to free radicals so that free radicals can be muted¹. Free radicals is a compound or molecule containing one or more unpaired electrons in its outer orbital. The existence of unpaired electrons cause the highly reactive compounds looking for a partner by attacking and electron binding molecules that are in the vicinity. Antioxidants have been widely used to prevent or slow down oxidation. Natural antioxidants found in most plants are phenolic groups such as tocopherols, flavonoids, lignin and phenolic acids^{2,3}. The content of secondary metabolites or chemical content affects pharmacological activity. Ginger and lemongrass plants based on previous studies have been studied for their chemical content and antioxidant activity. Several previous studies obtained data on the antioxidant activity of white ginger leaves (*Z. officinale* Rosc. var. *Officinatum*) against DPPH free radicals using a UV-Vis spectrophotometer with a wavelength of 515 nm obtained an IC₅₀ value of 156.4827 µg/ml. Red ginger extract positively contains flavonoids,

tannins, saponins, alkaloids and terpenoids and the antioxidant activity test obtained an IC₅₀ value of 10.35 µg/mL which is classified as very strong. The antioxidant activity of red ginger (*Z. officinale* var. *Amarum*) IC₅₀ value obtained from the maceration method (ethanol 70%) of 56.58 µg/ml; remaceration (ethanol 70%) 22.1 µg/ml; maceration (ethanol 96%) 87.7 µg/ml; and remaceration (ethanol 96%) of 67.42 µg/ml^{4,5}. Lemongrass has a total phenol of 42.959 mg/kg extract, free radical scavenging activity of 64.85%, and total antioxidants of 104 µmol/L. Lemongrass extract contains tannins, alkaloids, triterpenoids/steroids, saponins, phenolics, and flavonoids. The total phenolics in the extract are 81.67 mg GAE/sample^{6,7}.

The medicinal plant used is ginger (*Z. officinale*) and lemongrass (*C. citratus*) which are often used as raw materials for medicine. Chemical content greatly affects the pharmacological activity of a plant. Various studies have shown that ginger has positive activity against various symptoms of diseases such as anti-inflammatory, antioxidant, antiemetic, antibacterial, and antidiabetic. Antioxidant compounds found in ginger are phenolic compounds in the form of

flavonoids, cinnamic acid derivatives, coumarins, and polyfunctional organic acids^{8,9}. Lemongrass contains bioactive substances such as alkaloids, flavonoids, saponins, tannins, phenolic acids, and terpenoids. In dried lemongrass, the most bioactive substances contained are phenolic acids, flavonoids and tannins which act as antioxidants that are useful in healing wounds^{10,11}. The combination of medicinal plants have produce a synergistic effect, where two or more plants work together to increase the effectiveness of treatment. The novelty of this study is determining the antioxidant activity of the combination of ginger and lemongrass.

MATERIALS AND METHODS

The samples used in this research are rhizome of *Z. Officinale* and steam of *C. citratus* collected from Makassar, South Sulawesi. Voucher specimens were prepared and deposited at the Botanical Division, Laboratory of Pharmacology-Phytochemistry Faculty of Pharmacy, Universitas Muslim Indonesia. DPPH (Sigma Aldrich), Ascorbic acid (Sigma Aldrich).

This research was conducted through an experimental procedure carried out in the laboratory using the DPPH scavenging method which was measured using a UV-Vis spectrophotometer.

Extraction

Ginger powder of 100 mg was extracted with 1000 ml of ethanol 70% using UAE (Ultrasonic Assisted Extraction) with a frequency of 42 KHz at a temperature of 50°C for 2 hours. Lemongrass powder of 100 mg was extracted with 1000 ml of ethanol 70% by maceration. Each liquid extract from the two samples was concentrated using rotary vacuum evaporator to obtain thick extract.

Qualitative test of antioxidant activity of extract samples

Preparation of DPPH

Determination of the maximum wavelength, namely the DPPH solution that has been made with a concentration of 35 µg/ml, the absorption spectrum is determined using a UV-Vis spectrophotometer at a wavelength of 400 to 700 nm.

Preparation Ascorbic acid Standard Solution

Ascorbic acid stock solution was made at a concentration of 1000 µg/ml, 5 mg of ascorbic acid was dissolved in 5 ml of methanol pro analysis. Then 5

series of concentrations of 5, 10, 15, 20, 25 µg/ml were made.

Preparation of ginger extract and lemongrass extract

Each extract sample was prepared in 5 series of concentrations of 5, 10, 15, 20, 25 µg/ml from a stock solution of 100 µg/mL.

Measurement of antioxidant activity of ascorbic acid standards and sample extracts with DPPH

Standard concentration series solutions of ascorbic acid and sample extracts of 1 ml each were put into a cuvette, 1 ml of DPPH solution was added and incubated for 30 minutes. After incubation, the absorbance was measured at a wavelength of 516 nm.

The IC₅₀ value is calculated based on the percentage of inhibition against DPPH radicals from each concentration of sample solution with the formula:

$$\% \text{ Inhibition} = \frac{\text{Abs. blank} - \text{Abs. sample}}{\text{Abs. blank}} \times 100$$

After obtaining the percentage of inhibition from each concentration, the equation $y = a + bx$ is determined by linear regression calculations where x is the concentration (µg/ml) and y is the percentage of inhibition (%). Antioxidant activity is expressed by Inhibition Concentration 50% (IC₅₀), which is the sample concentration that can reduce DPPH radicals by 50%. The IC₅₀ value is obtained from the x value after replacing $y = 50$.

RESULTS AND DISCUSSION

The extraction of each sample was selected from different extraction methods, namely ginger samples using the ultrasonic method and lemongrass samples using the maceration method. This is based on the results of previous studies (13,14) obtained the largest percentage of ginger extract soaking results of 21.29% Ultrasonic Assisted Extraction (UAE) results compared to maceration and distillation. The largest percentage of lemongrass extract soaking was 14.83 % maceration results compared to Ultrasonic Assisted Extraction (UAE). The percentage of extract soaking indicates the number of chemical compounds from the sample that can be extracted using the appropriate solvent. The number of compounds extracted in large quantities has a great opportunity for active compounds to be extracted and provide great therapeutic activity. The results of the percentage of soaking of each sample are presented in the Table 1.

Table 1: Percentage yield from extraction of ginger and lemongrass samples.

| Solvent | Sample | Extraction method | Weight of extracted sample (gm) | Weight of thick extract (gm) | Extract yield value (% v/v) |
|-------------|------------|--------------------------------------|---------------------------------|------------------------------|-----------------------------|
| Ethanol 70% | Ginger | Ultrasonic Assisted Extraction (UAE) | 100 | 10.18 | 10.18 |
| Ethanol 70% | Lemongrass | Maceration | 100 | 31.99 | 31.99 |

Each sample showed that in 100 grams of extracted sample powder, the number of chemical compounds produced was 10.18 % w/w for ginger and 31.99% w/w as the percentage of extract yield value.

Next, the extract of each sample was continued with antioxidant activity test using the DPPH radical scavenging method. The results of the comparative antioxidant activity test of ascorbic acid and each sample extract are presented in the Table 2.

Table 2: Comparative antioxidant activity test of ascorbic acid and sample extracts.

| Standard and extract samples | Concentration (µg/ml) | Sample Abs | Blank Abs | % Inhibition | Equation | IC ₅₀ (µg/ml) |
|--|-----------------------|------------|-----------|--------------|------------------------|--------------------------|
| Ascorbic acid | 5 | 0.441 | 0.896 | 50.781 | $y = 3.2924x + 46.217$ | 1.15 |
| | 10 | 0.438 | | 52.232 | | |
| | 15 | 0.395 | | 55.915 | | |
| | 20 | 0.305 | | 56.362 | | |
| | 25 | 0.312 | | 65.179 | | |
| Ginger | 5 | 0.478 | 0.896 | 46.652 | $y = 0.4531x + 45.123$ | 10.76 |
| | 10 | 0.443 | | 50.558 | | |
| | 15 | 0.427 | | 52.344 | | |
| | 20 | 0.416 | | 53.571 | | |
| | 25 | 0.390 | | 56.473 | | |
| Lemongrass | 5 | 0.476 | 0.896 | 46.875 | $y = 0.1607x + 45.871$ | 26.69 |
| | 10 | 0.471 | | 47.433 | | |
| | 15 | 0.466 | | 47.991 | | |
| | 20 | 0.457 | | 48.995 | | |
| | 25 | 0.447 | | 50.111 | | |
| Combination of ginger and lemongrass (1:1) | 5 | 0.449 | 0.896 | 49.888 | $y = 0.3281x + 47.98$ | 6.16 |
| | 10 | 0.441 | | 50.781 | | |
| | 15 | 0.423 | | 52.790 | | |
| | 20 | 0.402 | | 55.133 | | |
| | 25 | 0.395 | | 55.915 | | |
| Combination of ginger and lemongrass (2:1) | 5 | 0.439 | 0.896 | 51.004 | $y = 0.3192x + 48.94$ | 3.32 |
| | 10 | 0.436 | | 51.339 | | |
| | 15 | 0.411 | | 54.129 | | |
| | 20 | 0.403 | | 55.022 | | |
| | 25 | 0.384 | | 57.145 | | |
| Combination of ginger and lemongrass (1:2) | 5 | 0.460 | 0.896 | 48.660 | $y = 0.2879x + 47.31$ | 9.34 |
| | 10 | 0.439 | | 51.004 | | |
| | 15 | 0.438 | | 51.116 | | |
| | 20 | 0.430 | | 52.008 | | |
| | 25 | 0.400 | | 55.357 | | |

The comparative measurement results of ascorbic acid have very strong antioxidant activity with an IC₅₀ value of 1.15 µg/ml. The measurement results of ginger rhizome have strong antioxidant activity with an IC₅₀ value of 10.76 µg/ml. The measurement results of lemongrass stems have strong antioxidants with an IC₅₀ value of 26.69 µg/ml. As quoted in a previous study¹⁵, a compound is said to have very strong antioxidant activity if the IC₅₀ value is <10 µg/ml, strong if the IC₅₀ value is 10-50 µg/ml, moderate if the IC₅₀ value is 50-

100 µg/ml, weak if the IC₅₀ value is 100-250 µg/mL, and inactive if the IC₅₀ value is >250 µg/mL. The bioactive components in ginger are: gingerol, shogaol and zingiberone. Gingerol is an active compound in the group phenol which is heat resistant and has antioxidant properties⁸. Lemongrass has various pharmacological activities, one of which is antioxidants. Active compounds as antioxidants such as flavonoids, tannins, and phenols are compounds that can be found in lemongrass¹¹.

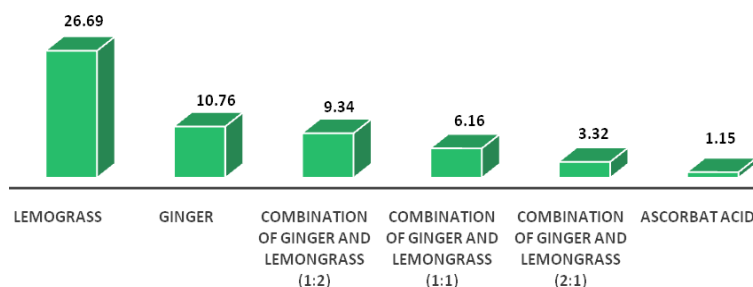


Figure 1: Comparison of IC₅₀ values between single sample extracts standards and combination of extracts.

The combination of medicinal plants can produce a synergistic effect, where two or more plants work together to increase the effectiveness of treatment. The results of measuring the combination of ginger and lemongrass with a ratio of 1:1; 2:1; 1:2 obtained IC₅₀ of 6.16 µg/ml, 3.32 µg/ml, and 9.34 µg/ml, respectively. All three values are included in the category of very strong antioxidant activity because

IC₅₀ <10 µg/ml. The combination of the two medicinal plants showed a synergistic effect because there was an increase in antioxidant activity in each plant which was initially classified as strong to more strong after being combined. The combination of ginger and lemongrass with a ratio of 2:1 showed higher antioxidant activity than the combination of 1:2. This shows that the antioxidant compounds from ginger are stronger in

influencing the rate of oxidation than antioxidant compounds from lemongrass.

Limitations of the study

The limitation of this study is discusses the antioxidant activity of ginger rhizomes, lemongrass stems and a combination of both by determining the IC₅₀ value as the observed parameter. The IC₅₀ value is used to determine the antioxidant activity of the sample including the strong and very strong categories, and to know the synergistic effect of the combination of the two sample extracts. However, this study does not have in vivo antioxidant activity data needed to determine the pharmacological dosage.

CONCLUSIONS

The conclusion of this study is that ginger rhizome and lemongrass stalks each have strong antioxidant activity with an IC₅₀ value of 10-50 µg/ml. The combination of the two sample extracts shows a synergistic effect causing the antioxidant activity to be stronger with an IC₅₀ value of <10 µg/ml.

ACKNOWLEDGEMENTS

The author would like to thanks to the Research and Development Institute of Resources (LP2S) at Universitas Muslim Indonesia (UMI) for financially supporting this study.

AUTHOR'S CONTRIBUTION

Singh RAS: writing original draft, methodology, investigation. **Singh FA:** formal analysis, data curation, conceptualization. **Sigh AA and EP:** writing, review and editing, methodology. **Sigh ARA:** formal analysis, data curation, conceptualization. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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