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Antioxidant potential effect combination of the bitter, red ginger, and turmeric extract with the DPPH method



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ABSTRACT

Introduction: Bitter (*Andrographis paniculata* or Burm.f.), red ginger (*Zingiber officinale* var *Rubrum Rhizoma*), and turmeric (*Curcuma domestica*) are medicinal herbs of Asian countries used in many traditional medicinal. This study aimed to evaluate the potential antioxidant effect combination of bitter, red ginger, and turmeric extract with the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.

Methods: This is an observational analytic study using the DPPH method to evaluate the potential antioxidant effect of four extracts, including bitter, red ginger, turmeric, and a combination of those three extracts called "SIJAKUN," which stands for Sambiloto, JAhe merah, and KUNyit in Indonesian. The antioxidant effect was evaluated by IC50 value. The comparable standard of antioxidants was Vitamin C.

Result: The IC50 value showed from the lowest to highest was 2.34, 3.75, 10.15, and 13.5 µg/mL occur in red ginger extract, "SIJAKUN" combination extract, turmeric extract, and bitter extract, respectively. The IC50 value of Vitamin C as a comparable compound was 13.2 µg/mL. The "SIJAKUN" combination extract was better than the single turmeric, bitter, and vitamin C extract, but not with red ginger extract.

Conclusion: The highest potential antioxidant effect with the DPPH method occurred in red ginger extract, even better than other extracts, such as combining the "SIJAKUN" extract based on the lowest IC50 value.

Keywords: antioxidant, bitter, red ginger, turmeric, IC50.

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INTRODUCTION

Bitter (*Andrographis paniculata* or Burm.f.) is a medicinal herb of Asian countries used as traditional medicinal. *Andrographis paniculate* in Indonesia, known as "Sambiloto," its active compounds possess favorable antiviral, anti-inflammatory, immunomodulatory, and antipyretic activities.¹⁻³ Red ginger (*Zingiber officinale* var *Rubrum Rhizoma*) was used as traditional medicine in Asia, widely used in food and beverages, also known as "Jahe Merah" in Indonesia. Red ginger has hypolipidemic, anti-emetic, antiviral, anti-motion, anti-inflammatory, and antioxidant effects.^{4,5} The rhizome of turmeric (*Curcuma domestica*), known as "Kunyit" in Indonesia, has been used as an herbal medicine, coloring agent, spice, and food additive for thousands

of years in different parts of the world, particularly in Asian countries. Turmeric widely uses because of its antioxidant, anti-inflammatory, antibacterial and anticancer effects.⁶⁻⁸

The protective effects of antioxidants against free radical damage to the body and pathological processes mediated by oxidative stress are of increasing interest and are present in daily food and pharmaceutical products.⁹ Complex antioxidant systems exist to protect human cells and organ systems from free radicals. Through various methods, diet and endogenous enzymatic and non-enzymatic antioxidants reduce the negative effects of free radicals. Natural antioxidants have been linked to improving human health, implying that they are most beneficial for preventing non-communicable cardiovascular and

metabolic diseases.^{10,11}

Screening of antioxidant properties of plants and plant-derived compounds requires appropriate methods.⁹ The DPPH method is widely used to evaluate the antioxidant properties of plant materials. It also has great advantages, such as ease of use, low cost, applicability, and automation preferences.¹² The chromatographic method was optimized, according to reaction time (slow, fast kinetics), the linearity range of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical depending on the detection conditions as well as the kind of the investigated antioxidants (reference chemicals and the ground elder prepared from fresh and dry plants). The scavenging capacity was expressed by the use of a percentage of peak inhibition and the IC50 parameters.¹³

This study aimed to evaluate the

potential antioxidant effect combination of bitter, red ginger, and turmeric extract with the DPPH method.

METHODS

This observational analytic study uses the DPPH method to evaluate the antioxidant effect. The DPPH is a stable free radical with a deep purple color and strong absorption. The presence of antioxidant substances in the media causes the DPPH radicals to transform into more stable DPPH molecule products by donating electrons or hydrogen atoms. The reduced form of DPPH undergoes a color change from purple to pale yellow, which enables spectrophotometry to measure antioxidant activity. As a measure of the outcome, the IC₅₀ value is used.¹⁴ This study was conducted in the pharmacology laboratory, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, from July to September 2021.

Materials

This study uses Bitter or sambiloto (*Andrographis paniculate*), red ginger or jahe merah (*Zingiber officinale var Rubrum Rhizoma*), and turmeric or kunyit (*Curcuma domestica*), and also, use a combination of those three extracts called "SIJAKUN" stand for Sambiloto, JAhe merah, and KUNyit extract in Indonesian. All materials from plants grow in a highland area in Batu City, Indonesia, with an altitude of ± 862 meters above sea level (masl).

Material Extraction

This study used three extracts which were divided into four groups. We use a powder derived from Sambiloto leaves, red ginger rhizome, and turmeric, as much as 2000 grams each. Extraction using the maceration method using 70% ethanol with 14,000 ml as a solvent during 6 hours evaporation process. All the extraction results were liquid, 165 ml of Sambiloto extract with 8.25% yield, 200 ml of red ginger extract with 10% yield, and 125 ml of turmeric extract with 6.25% yield. In comparison, the "SIJAKUN" combination of the three ingredients come from 20% Sambiloto, 40% red ginger and 40% turmeric.

Antioxidant analysis

The antioxidant activity of plant extracts measurement using a 2,2 diphenyl 1-picrylhydrazyl (DPPH) method.¹⁵ The level of inhibitory power (IC₅₀) indicator used to assess the antioxidant effect using the visible light spectrophotometric method. The procedure started by making several concentrations of bitter, red ginger, turmeric, and all three extracts into Ethanol solvent as intervention group, while the Vitamin C was the control group. Then 1 mL of each pipette was added to 3 mL of 40 ppm DPPH solution in Ethanol. Then wait and let the mixture at room temperature for 30 minutes. Measure the absorbance at the maximum (517 nm). This study did not perform a statistical analysis, but just compared the antioxidant effect by % inhibition result showed by IC₅₀ value which compared to each extract by µg/mL unit. Inhibition ability was evaluated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Control Absorban} - \text{Sample Absorban}}{\text{Control Absorban}} \times 100$$

RESULTS

The antioxidant activity was analyzed using the DPPH method, in which the IC₅₀ value represents the results. Four extracts were tested, including bitter, red ginger, turmeric, and "SIJAKUN" combination extracts compared with Vitamin C as the control group. The analysis was carried out twice, and then the mean value was taken, shown in Table 1. The lowest to highest results occurred in red ginger extract, "SIJAKUN" combination extract, turmeric extract, and bitter extract with IC₅₀ values of 2.34, 3.75, 10.15, and 13.5 µg/mL, respectively.

The mean IC₅₀ value of the four extracts was compared with Vitamin C as a control, shown in Figure 1. The lowest IC₅₀ value indicates a good antioxidant effect.¹⁶

DISCUSSION

The bitter extract showed the highest IC₅₀ value in this study, confirmed by another study comparing Vitamin C, *Andrographis paniculata* extract, and *andrographolide* showed the IC₅₀ values were 4.3, 220.5, and 3.2 mg/mL, respectively. The previous study also showed a lower antioxidant

effect; the IC₅₀ value of sambiloto's ethanol extract was 792.126 g/ml. From this result, the Bitter extract had the highest IC₅₀, indicating lower antioxidant properties when compared to another extract. The antioxidant activity of Bitter is due to the presence of flavonoids and andrographolide. A recent study documented that an andrographolide extract showed greater antioxidant activity due to the *Andrographis paniculata* leaf extract.¹⁷⁻²⁰

The extract of red ginger IC₅₀ result is the lowest compared to another extract. Another study showed the highest antioxidant activity in distilled water and ethanol extracts. However, the highest antioxidant activity was observed in methanol extracts. The differences in IC₅₀ values of the tested extracts were most likely due to the differences in the structure and nature of the solvents.²¹ Red ginger contains polyphenol components, including zingiberene, zingerone, shogaols, and gingerols which are antioxidant agents.²² Ginger with a high water content is very susceptible to deterioration through the growing of microorganisms carried by moisture. Drying ginger makes preserving and retaining its natural phytochemical and bioactivity content possible, including its antioxidant effect. According to a recent study, the sun-dried ginger extract had a significantly greater flavonoid and phenolic content and demonstrated the strongest antioxidant activity as assessed by the DPPH method.²³

Turmeric extract showed an IC₅₀ value lower than Vitamin C IC₅₀ value as a comparison agent. A previous study compared Turmeric extract with control (Vitamin C), which showed a similar potency in the percentage of DPPH scavenging capacity at 16 µg/mL with a slightly higher IC₅₀ value. It implied that the antioxidant activity of Turmeric extract directly interacts with a broad spectrum of harmful ROS, terminates the chain reaction initiated by free radicals via electron transfer, and is involved in regenerating other antioxidants, such as tocopherol, to their functional state.²⁴ A study evaluating turmeric with the DPPH method showed a significant free radical scavenging at the highest dose with an IC₅₀ value of 27.2 g/mL, indicating that

Table 1. Antioxidant activity results from bitter, red ginger, turmeric, and the “SIJAKUN” combination extract compared to vitamin C based on IC50 value.

	IC50 (µg/mL)		
	First replication	Secondary replication	Mean value
Bitter extract	13.71	13.28	13.5
Red ginger extract	2.29	2.38	2.34
Turmeric extract	10.18	10.11	10.15
“SIJAKUN” combination extract	3.78	3.71	3.75
Vitamin C	-	-	13.2

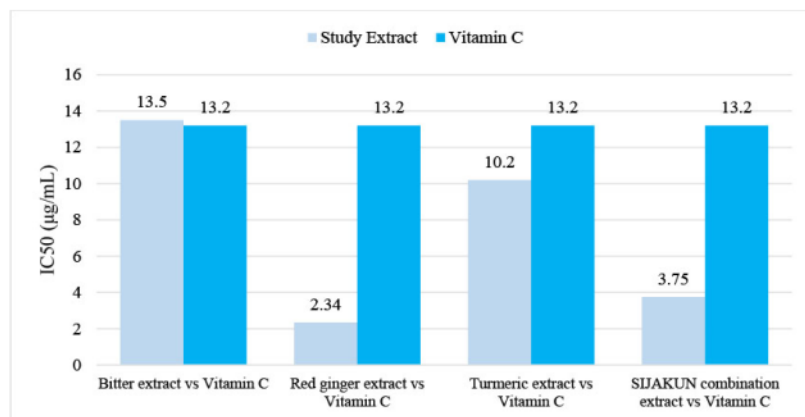


Figure 1. Diagram of IC50 (µg/mL) bitter, red ginger, turmeric, and the combination of SIJAKUN extract compared to Vitamin C.

it has a lower antioxidant effect than this study.²⁵

The combination of three, including bitter, red ginger, and turmeric extract, showed a lower IC50 value when compared to Vitamin C. This study finding states that the combination of “SIJAKUN” showed a higher antioxidant effect than a single extract of Bitter and Turmeric but not better than a red ginger extract which showed the highest antioxidant effect by its lowest IC50 value from the other extract. This study has a limitation; the IC50 value of vitamin C as the well-known antioxidant compared agent may differ from another reference.

CONCLUSION

The potential antioxidant effect of red ginger showed the highest when compared to a combination of bitter, red ginger, turmeric or “SIJAKUN” extract with the DPPH method. Although the other single extract from Bitter and turmeric also showed a good antioxidant effect. It shows that antioxidant agents can be obtained

easily because bitter, red ginger and turmeric are traditional plants familiar among Indonesians.

DISCLOSURES

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

The author was involved in developing the study concept, designing, supervising, conducting, analyzing data, and writing the manuscript. All authors prepare the manuscript and agree for this final version to be submitted to this journal.

Ethical clearance

This study protocol was approved by the Ethical Committee Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya,

with the number 089/EC/KEPK/UNUSA/2021.

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