Original Article

e-ISSN: 2581-0545 - https://journal.itera.ac.id/index.php/jsat/

Received 13th May 2026
Accepted 19th June 2026
Published 30th June 2026

Open Access

DOI: 10.35472/jsat.v10i2.2784

Phytochemical Screening and Antioxidant Activity of the Non-Oil Acetone Fraction of Red Ginger (*Zingiber officinale* var. *rubrum*) Rhizome Using the DPPH Assay

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Abstract: The growing interest in natural antioxidants has encouraged the exploration of plant-derived bioactive compounds as potential agents for mitigating oxidative stress-related disorders. This study aimed to evaluate the antioxidant activity of a non-oil acetone fraction of red ginger (*Zingiber officinale* var. *rubrum*) rhizome and to identify its major phytochemical constituents through preliminary screening. The non-oil acetone fraction was obtained after removal of n-hexane-soluble components from the crude acetone extract. The fraction was subjected to qualitative phytochemical analysis and antioxidant evaluation using the DPPH radical scavenging assay. Phytochemical screening revealed the presence of flavonoids, tannins, terpenoids, and alkaloids. The fraction exhibited concentration-dependent radical scavenging activity with an IC_{50} value of 43.61 ppm, indicating very strong antioxidant activity according to commonly used IC_{50}-based classifications. These findings suggest that the non-oil acetone fraction of red ginger represents a potential natural antioxidant source and provide a basis for further studies involving quantitative phytochemical analysis and characterization of active compounds.

Keywords: *Red ginger; Zingiber officinale var. rubrum; antioxidant activity; phytochemical screening*

Introduction

Excessive accumulation of reactive oxygen species (ROS) beyond the capacity of endogenous antioxidant defense systems can lead to oxidative stress, a condition associated with the pathogenesis of numerous chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions [1]. This has stimulated growing interest in natural antioxidants, particularly those derived from plants, as potential sources of bioactive compounds with antioxidant properties [2]. Red ginger (*Zingiber officinale* var. *rubrum*) is widely recognized as a medicinal plant rich in various bioactive compounds with diverse pharmacological properties. Previous studies have reported the presence of several classes of phytochemicals, including phenolic compounds, flavonoids, terpenoids, and alkaloids, which have been associated with antioxidant activity [3]. The antioxidant capacity of ginger has often been attributed to its phenolic and flavonoid constituents, which play important roles in free radical scavenging mechanisms [4]. Moreover, the antioxidant activity of red ginger has been extensively evaluated using the DPPH assay, a widely used method for assessing the free radical scavenging activity of plant-derived bioactive compounds [5].

The phytochemical composition of plant extracts is highly dependent on the solvent system employed. Differences in solvent polarity influence the extraction of various bioactive constituents and may consequently affect the antioxidant activity of the resulting extract. Acetone is a semi-polar solvent capable of extracting a broad range of phytochemical compounds from plant materials. In addition, the removal of n-hexane-soluble components may alter the composition of the resulting fraction and potentially influence its biological properties [6]. Although the antioxidant activity of red ginger has been extensively investigated, studies specifically focusing on the phytochemical characteristics and antioxidant activity of the non-oil acetone fraction remain limited. Therefore, this study

aimed to evaluate the antioxidant activity of a non-oil acetone fraction of red ginger (Zingiber officinale var. rubrum) rhizome using the DPPH radical scavenging assay and to identify its major phytochemical constituents through preliminary phytochemical screening. The findings of this study are expected to provide preliminary information regarding the antioxidant potential and phytochemical profile of the non-oil acetone fraction of red ginger and serve as a basis for further studies involving quantitative phytochemical analysis and characterization of active compounds.

Methods

Materials

Fresh rhizomes of red ginger (*Zingiber officinale* var. *rubrum*) were obtained from a local market in Kemiling District, Bandar Lampung, Indonesia. The chemicals used consisted of analytical-grade organic solvents, including acetone, methanol, ethanol, and n-hexane. Reagents used for phytochemical screening included Dragendorff's reagent, Mayer's reagent, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), and Liebermann–Burchard reagent. The antioxidant activity assay utilized 1,1-diphenyl-2-picrylhydrazyl (DPPH) as the free radical reagent, while ascorbic acid was used as a positive control.

The main equipment used in this study included a rotary evaporator, UV–Vis spectrophotometer, analytical balance, separatory funnel, blender, micropipettes, and standard laboratory glassware, including Erlenmeyer flasks, beakers, measuring cylinders, test tubes, and round-bottom flasks.

Sample Preparation

Fresh rhizomes of *Zingiber officinale* var. *rubrum* (200 g) were washed, cut into small pieces, air-dried at room temperature, and ground to obtain a fine powder for extraction.

Maceration

Red ginger rhizome powder (100 g) was extracted by maceration with acetone (1:10 w/v) for three 24 h cycles at room temperature. The filtrates were concentrated using a rotary evaporator at 40 °C under reduced pressure (80 rpm) to obtain the crude extract, with a yield of 1.87%.

Liquid–Liquid Extraction

The concentrated acetone extract was partitioned with n-hexane (100 mL) in a separatory funnel to remove n-hexane-soluble constituents, including essential oil components. The partitioning was performed three times, after which the non-hexane fraction was collected and concentrated under reduced pressure using a rotary evaporator. The resulting non-oil acetone fraction was obtained as a viscous extract with a yield of 1.25 g.

Phytochemical Screening

Preliminary phytochemical screening of the non-oil acetone fraction was carried out to qualitatively determine the presence of major secondary metabolite groups, including alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids using standard qualitative phytochemical procedures [7].

Flavonoid Test (Shinoda Reaction)

A 0.2 g portion of the non-oil acetone fraction was dissolved in 10 mL of ethanol and filtered. An aliquot (2 mL) of the filtrate was treated with magnesium ribbon followed by the addition of concentrated hydrochloric acid. The formation of a pink to red or orange coloration indicated the presence of flavonoid compounds [8].

Alkaloid Test

The non-oil acetone fraction (0.2 g) was dissolved in 10 mL of dilute hydrochloric acid and filtered. A 2 mL aliquot of the filtrate was treated separately with Dragendorff's and Mayer's reagents. The appearance of a reddish-brown precipitate or a cream-colored precipitate indicated the presence of alkaloids [9].

Tannin Test

The non-oil acetone fraction (0.2 g) was dissolved in 10 mL of ethanol and filtered. A 2 mL aliquot of the filtrate was treated with 10% ferric chloride ($FeCl_3$) solution. The appearance of a dark blue to greenish-black coloration indicated the presence of tannins [10].

Saponin Test (Foam Test)

The non-oil acetone fraction (0.2 g) was mixed with 10 mL of distilled water and shaken vigorously for 30 s. The formation of a stable foam layer (≥ 1 cm) that remained for at least 10 min indicated the presence of saponins [11].

Steroid/ Triterpenoid Test (Liebermann–Burchard Reaction)

The non-oil acetone fraction (0.2 g) was dissolved in 2 mL of chloroform, followed by addition of acetic

anhydride. Concentrated sulfuric acid was carefully added dropwise along the inner wall of the test tube without shaking. Color development at the interface was observed; a green to blue-green coloration indicated steroids, whereas a red, purple, or violet coloration suggested triterpenoids [10].

Antioxidant Activity

A DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution was prepared at a concentration of 160 mg/L by dissolving 4.0 mg of DPPH in methanol and diluting it to a final volume of 25 mL using a volumetric flask. The solution was protected from light by wrapping the container with aluminum foil and stored in the dark to prevent photodegradation.

The sample was prepared by dissolving 10 mg of non-oil acetone fraction of red ginger rhizome in methanol and adjusting the volume to 10 mL to obtain a stock solution with a concentration of 1000 ppm. A series of working concentrations was prepared through serial dilution of the stock solution. Ascorbic acid (vitamin C) was used as a positive control and prepared in methanol using the same concentration range as the sample.

For the antioxidant assay, equal volumes of sample or control solutions were mixed with DPPH solution in test tubes and incubated at room temperature for 30 minutes in the dark to ensure completion of the radical scavenging reaction. After incubation, absorbance was measured at 517 nm using a UV–Vis spectrophotometer. A mixture of methanol and DPPH solution was used as the blank.

The percentage of radical scavenging activity was calculated based on the reduction in absorbance relative to the control. Antioxidant activity was expressed as IC_{50} values, determined from the linear regression equation between concentration (x) and percentage inhibition (y), expressed as y = a + bx. All measurements were performed without technical replication.

Results and Discussion

Phytochemical Screening

The phytochemical screening was conducted as a qualitative approach to identify the major classes of secondary metabolites present in the non-oil acetone fraction of red ginger rhizome [7]. The analysis focused on the detection of alkaloids, flavonoids, steroids, triterpenoids, tannins, and saponins. The results of the phytochemical screening are presented in **Table 1** and **Figure 1**.

Table 1. Phytochemical screening.

Secondary Metabolite	Reagent	Result
Flavonoid	Mg+HCl	+
Tannin	$FeCl_3$	+
Terpenoid or Steroid	Liebermann–Burchard	+
Alkaloid	Mayer's	+
Alkaloid	Dragendorff's	+
Saponin	Distilled water	-

The non-oil acetone fraction of red ginger rhizome (*Zingiber officinale* var. *rubrum*) showed a positive result for flavonoids, indicated by the formation of an orange coloration following the Shinoda reaction. This indicates the presence of flavonoid compounds, which are commonly associated with antioxidant activity due to their ability to donate hydrogen atoms or electrons to stabilize free radicals.

Figure 1. The result of phytochemical screening

The tannin test yielded a positive result, as evidenced by the formation of a dark green to bluish-black coloration upon addition of ferric chloride solution. This reaction indicates the presence of phenolic compounds capable of forming complexes with Fe^{3+} ions. Terpenoid compounds were detected through the Liebermann–Burchard reaction, indicated by the development of reddish to purple coloration, confirming the presence of terpenoid constituents in the extract.

The alkaloid test was positive with both Dragendorff's and Mayer's reagents, as indicated by the formation of reddish-brown and cream precipitates, respectively, confirming the presence of alkaloid compounds. The saponin test showed no stable foam formation after vigorous shaking, indicating the absence of detectable saponin compounds under the tested conditions. The foam test remains one of the standard qualitative approaches for detecting saponins because of their surfactant properties [12]. Overall, the phytochemical screening of the non-oil acetone fraction of red ginger confirmed the presence of alkaloids, flavonoids, tannins, and triterpenoids, while saponins were not detected.

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Antioxidant Activity

The evaluation of the radical scavenging activity was conducted via the DPPH assay, where the degree of discoloration from purple to yellow reflects the sample's hydrogen-donating ability [13]. As the concentration of antioxidants increases, a corresponding decrease in the absorbance of the DPPH-H complex is observed. This spectrophotometric method was selected for its efficiency in quantifying antioxidant capacity. In this study, ascorbic acid (vitamin C) was used as a positive control to provide a reference for strong antioxidant activity. Detailed results of the non-oil acetone fraction of red ginger are presented in **Table 2** and **Figure 2**.

Table 2. The antioxidant activity test of the non-oil acetone fraction.

Concentration (ppm)	% Inhibition	IC_{50}
0	0	
20	29.58	
40	48.43	43.61
60	68.41	
80	72.78	
100	91.38	

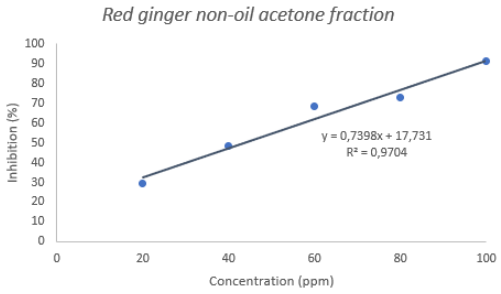

Figure 2. The antioxidant activity test of the non-oil acetone fraction.

The antioxidant activity of the non-oil acetone fraction of red ginger was evaluated based on its IC_{50} value derived from the relationship between extract concentration and DPPH inhibition (**Table 2**). The IC_{50} value of 43.61 ppm indicates potent antioxidant activity and is classified as a very strong antioxidant, according to commonly accepted criteria in which IC_{50} values below 50 ppm represent very strong radical scavenging activity [14]. For comparison, ascorbic acid, used as the positive control, exhibited an IC_{50} value of 7.74 ppm, demonstrating substantially higher radical scavenging activity than the tested fraction and confirming the reliability of the assay system. The superior antioxidant capacity of ascorbic acid is attributable to its well-known ability to readily donate electrons or hydrogen atoms to neutralize free radicals. Detailed results for ascorbic acid are presented in **Table 3** and **Figure 3**.

Table 3. The antioxidant activity test of the ascorbic acid

Concentration (ppm)	% Inhibition	IC_{50}
0	0	
2	20.44	
4	35.71	7.74
6	38.39	
8	50.49	
10	62.20	

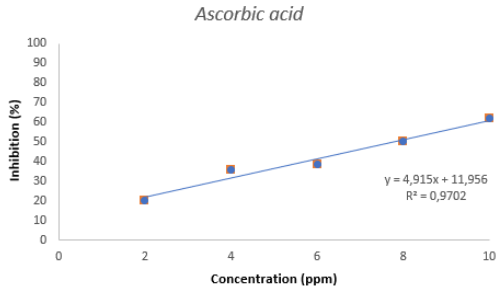

Figure 3. The antioxidant activity test of the ascorbic acid.

This strong activity of the extract is attributed to the synergistic contribution of secondary metabolites identified, including flavonoids, tannins, terpenoids, and alkaloids. Flavonoids and tannins primarily act through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, facilitated by hydroxyl groups, enabling effective stabilization of DPPH radicals. Meanwhile, other constituents such as ginger-derived phenolic and terpenoid compounds contribute to electron donation and radical stabilization, collectively enhancing antioxidant capacity.

Conclusions

This study indicates a relationship between the phytochemical composition and the antioxidant activity of the non-oil acetone fraction of red ginger. The observed radical scavenging activity may be attributed to the presence of flavonoids and tannins, with possible contributions from terpenoids and alkaloids through hydrogen atom transfer and electron transfer mechanisms.

The use of acetone as an extraction solvent may facilitate the extraction of a broad range of semi-polar bioactive compounds, which could contribute to the observed antioxidant activity. However, the specific

contribution of individual compounds cannot be conclusively determined from the present study.

These findings provide preliminary insight into the potential antioxidant properties of red ginger and its phytochemical basis. Further studies are required to isolate and characterize the active constituents and to evaluate their biological activity in more complex experimental models to better support their therapeutic potential.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors express sincere gratitude to the Institut Teknologi Sumatera for providing the research facilities.

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