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Antioxidant Potential Of Nano Spray Gel Formulation From Fermented Red Ginger Extract (*Zingiber Officinale Var. Rubrum*)

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ABSTRACT

Red ginger (Zingiber officinale var. rubrum) is an herbal plant rich in bioactive compounds such as gingerol and shogaol, which have high antioxidant activity. Fermentation with the Trichoderma harzianum fungus can increase the content of these active compounds. This study aims to determine the potential antioxidant activity of a topical nano spray gel preparation formulated using red ginger fermented extract. This study employed a laboratory experimental method with variations in the concentration of red ginger fermented extract, ranging from 0% to 1% in increments of 0.25%, 0.50%, and 0.75% in the nano spray gel preparation. Quercetin was used as a positive control. Antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results demonstrated that the formulation containing 0.75% extract exhibited an IC_{50} value of 74.84 ppm, which is categorized as a strong antioxidant. Based on these findings, the nano spray gel formulation containing fermented red ginger extract effectively enhances antioxidant activity. Further development of this herbal topical formulation may be achieved by optimizing the fermentation duration, as variations in fermentation time may significantly influence the antioxidant activity of the nanospray gel formulation.

Keywords: : red ginger, fermented, nano spray gel, antioxidant, DPPH.

ABSTRAK

Jahe merah (*Zingiber officinale* var. rubrum) merupakan tanaman herbal yang kaya akan senyawa bioaktif seperti gingerol dan shogaol yang memiliki aktivitas antioksidan tinggi. Fermentasi dengan jamur *Trichoderma harzianum* dapat meningkatkan kandungan senyawa aktif tersebut. Penelitian ini bertujuan untuk mengetahui potensi aktivitas antioksidan dari sediaan topikal nano spray gel yang diformulasikan menggunakan ekstrak fermentasi jahe merah. Penelitian ini menggunakan metode eksperimental laboratorium dengan variasi konsentrasi ekstrak fermentasi jahe merah sebesar 0%, 0,25%, 0,50%, 0,75%, dan 1% dalam sediaan nano spray gel. Kuersetin digunakan sebagai kontrol positif. Aktivitas antioksidan diuji menggunakan metode DPPH (*1,1-diphenyl-2-picrylhydrazyl*). Hasil penelitian menunjukkan bahwa sediaan dengan konsentrasi 0,75% memiliki nilai IC_{50} sebesar 74,84 ppm yang tergolong antioksidan kuat. Berdasarkan hasil tersebut, peningkatan aktivitas antioksidan pada sediaan nano spray gel dengan bahan aktif ekstrak fermentasi jahe merah mampu memberikan aktivitas antioksidan. Pengembangan sediaan topikal herbal ini dapat dilanjutkan dengan melakukan variasi lama waktu fermentasi yang mempengaruhi aktivitas antioksidan sediaan nano spray gel.

Kata kunci: jahe merah, fermentasi, nano spray gel, antioksidan, DPPH

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INTRODUCTION

Free radicals are naturally produced in the human body and, when present in excessive amounts, can lead to various diseases. To counteract the negative effects of free radicals, a sufficient intake of antioxidants is essential. Antioxidants function by inhibiting oxidation reactions and protecting body cells from damage. One of the natural sources of antioxidants commonly found in daily life is spice plants [1].

One widely used traditional medicinal plant is red ginger (*Zingiber officinale var. rubrum*), a herbal plant rich in phenolic compounds and other substances with antioxidant activity. These compounds reduce the risk of hemolysis and mitigate the body's response to oxidative stress caused by free radicals. The secondary metabolites found in ginger rhizomes generally include flavonoids, terpenoids, phenols, alkaloids, saponins [2], and essential oils. Red ginger essential oil contains active components such as gingerol and shogaol. It also contains 11.39% cineole and has the highest yield of 0.342%. Moreover, red ginger is known for its low volatility, contributing to its stability and quality in various industrial applications [3].

The advancement of natural product-based pharmaceutical technology includes fermentation processes. Research has shown that fermentation of ginger using fungi such as *Trichoderma harzianum*, which is known for its widespread distribution and rapid growth, can enhance its antioxidant properties [4]. Fermentation of ginger has been proven to increase its functional components and antioxidant activity, including elevated levels of gingerol, total flavonoids, and total polyphenols [5].

Antioxidant activity plays a critical role in neutralizing free radicals generated during the body's metabolic processes. Excessive free radicals can lead to oxidative stress, which contributes to the development of degenerative diseases such as cancer and cardiovascular disorders. In ginger fermentation studies, this process not only increases the level of zingiberene but also enhances the concentration of other antioxidant compounds. With prolonged fermentation time, chemical changes occur in ginger that potentially enhance its antioxidant activity. Phenolic and terpenoid compounds formed during fermentation have demonstrated the ability to reduce cell damage caused by free radicals [6]. Red ginger is recognized as a natural antioxidant source, containing gingerol and shogaol, which effectively inhibit oxidation processes [7]. The antioxidant activity of red ginger rhizome has been reported at 57.14 ppm [8].

The utilization of medicinal plants as raw materials in pharmaceutical formulations offers a promising approach to combat the effects of free radicals, including the use of red ginger extract [8]. Plant extracts have great potential to be developed into various dosage forms, one of which is topical preparations. Topical dosage forms are applied directly to body surfaces such as the skin [9]. One of the innovative topical formulations is nano spray gel, which combines nanoemulsion and spray gel technologies. Spray gels are modified gel preparations designed to be applied using a spray mechanism [10]. These formulations offer ease of application, a cooling sensation due to slow water evaporation, and good spreadability on the skin [11]. To enhance penetration and rapid therapeutic delivery, nano-sized particles are employed. Nanoparticles are small solid particles ranging in size from 1 to 100 nm. Their advantages include the ability to penetrate intercellular spaces and improve the solubility of poorly water-soluble drugs, thereby enhancing bioavailability and therapeutic efficacy [12]. Spray gel preparations are typically packaged in spray bottles designed for ease of use [13].

This research utilizes red ginger as the primary source due to its high content of bioactive compounds, wide pharmacological benefits, abundant availability, and ease of cultivation in Indonesia's tropical climate. In addition to its medicinal value, red ginger holds significant economic potential as one of Indonesia’s leading herbal plants. The development of red ginger-based products not only supports public health but also boosts the local economy through the cultivation and commercialization of herbal products. Traditionally, red ginger has been used to treat various ailments, such as digestive issues, muscle pain, and colds. Modern studies support further investigation of red ginger for pharmaceutical applications [14].

Based on this background, this study aims to explore the antioxidant potential of fermented red ginger (*Zingiber officinale* var. rubrum) formulated into a topical nano spray gel preparation. Given its antioxidant content, red ginger has the potential to counteract free radicals. In this study, fermented red ginger extract will be processed into nanoparticles and formulated as a nano spray gel, then evaluated for its antioxidant activity using the DPPH *(1,1-diphenyl-2-picrylhydrazyl*) method. This research is expected to optimize the antioxidant potential of red ginger through fermentation and improve its delivery effectiveness through nano spray gel formulation, ultimately providing enhanced benefits for skin health applications.

MATERIALS AND METHODS

This study employed a laboratory experimental design conducted at the Pharmaceutical Laboratory and Microbiology Laboratory of the Institute of Technology and Health Bintang Persada. The plant material used was red ginger (*Zingiber officinale* var. rubrum), collected from Nongan Village, Rendang District, Karangasem Regency, Bali. The red ginger plant was taxonomically authenticated at the Lansida Herbal Technology, Yogyakarta, Indonesia (Number. 49/LH/02/25). The antioxidant activity of the fermented red ginger extract was evaluated using the DPPH (*1,1-diphenyl-2-picrylhydrazyl*) assay.

The instruments utilized in this study included a UV-Vis spectrophotometer (Thermo Scientific Genesys), rotary evaporator (Buchi), analytical balance (Kenko), maceration jars, funnels (Iwaki), standard laboratory glassware (Iwaki), spatulas, micropipettes (Rongtai), and a hot plate (Thermo Scientific). The reagents and materials used included red ginger rhizomes (Zingiber officinale var. rubrum), 96% ethanol, distilled water (aquadest), quercetin, Dragendorff and Mayer reagents, $FeCl_3$, 10% NaOH, concentrated and 2N HCl, magnesium powder, Liebermann-Burchard reagent, chloroform, ethanol p.a., filter paper, aluminum foil, and DPPH.

Sample preparation fresh red ginger rhizomes were directly harvested from Nongan Village. The rhizomes were subjected to wet sorting to remove soil and impurities, followed by thorough washing with running water. The cleaned rhizomes were sliced into small pieces to increase surface area and then air-dried in a shaded, well-ventilated area for 6–7 days to reduce moisture content. Once dried, the rhizomes were dry-sorted to remove damaged or unsuitable parts, ground into fine powder using a blender, and passed through a mesh-40 sieve. The resulting simplicia powder was stored in sealed glass containers wrapped in aluminum foil to maintain stability.

Fermentation was carried out using Trichoderma harzianum as the fermenting microorganism. The culture medium, Potato Dextrose Broth (PDB), was sterilized in an autoclave (Yamato SM510) at 121°C for 15 minutes. Fungal inoculation was conducted aseptically under a Laminar Air Flow (LAF) cabinet. The red ginger simplicia powder was inoculated with the fungal suspension and incubated at 28°C for six days to enhance the bioactive compound content such as gingerol, flavonoids, and polyphenols [15].

To initiate the fermentation process, Potato Dextrose Agar (PDA) was prepared by dissolving 39.07 g in 100 mL distilled water and autoclaving it. After solidification, *T. harzianum* was aseptically inoculated and incubated at 28°C for 6 days. Simultaneously, 100 g of potatoes were boiled with dextrose to prepare PDB, followed by sterilization. *T. harzianum* was inoculated into the PDB and incubated for another 6 days. For the fermentation, 100 g of red ginger powder was added to the inoculated PDB and incubated in a fermentor with pH and humidity monitoring [16].

The fermented mixture was extracted using cold maceration. A total of 100 g of fermented red ginger powder was immersed in 250 mL of 96% ethanol (1:2.5 ratio) and stirred for 30 minutes. The mixture was left to stand for 72 hours, filtered using Whatman filter paper, and re-macerated twice. The combined filtrates were concentrated using a rotary evaporator at 50°C to yield a thick extract [17].

Phytochemical screening of the thick extract was performed to identify secondary metabolites qualitatively, including alkaloids, flavonoids, tannins, terpenoids, and saponins [18].

The thick extract of fermented red ginger was formulated into nano spray gel preparations with five different extract concentrations: 0% (F0), 0.25% (F1), 0.50% (F2), 0.75% (F3), and 1% (F4).

Table 1. Formulation of Nano Spray Gel Containing Fermented Red Ginger Extract (*Zingiber officinale* var. rubrum).

Material	Formulation Concentration					Function
	F0	F1	F2	F3	F4	
EFJM	0	0,25	0,5	0,75	1	Active material
Carbopol 940	0,2	0,2	0,2	0,2	0,2	Gelling agent
TEA	0,08	0,08	0,08	0,08	0,08	Emulsyfing agent
Tween 80	0,9	0,9	0,9	0,9	0,9	Surfactant
Sorbitol	0,6	0,6	0,6	0,6	0,6	Humectant
Metil paraben	0,10	0,10	0,10	0,10	0,10	Preservative
Propil paraben	0,02	0,02	0,02	0,02	0,02	Preservative
Aquadest	add 50 mL	add 50 mL	add 50 mL	add 50 mL	add 50 mL	Solvent

A total of 0.1 g of Carbopol 940 was dispersed in distilled water to form a homogenous gel. Then, 0.4 g of Triethanolamine (TEA) was gradually added to neutralize pH and increase viscosity [19].

The fermented extract was dissolved in sorbitol. Separately, methylparaben and propylparaben were dissolved in 5 mL distilled water under heating, then cooled before adding Tween 80. The sorbitol-extract mixture added slowly into the preservative-surfactant mixture while stirring. The volume was adjusted to 50 mL with distilled water, then homogenized using a magnetic stirrer at 1000 rpm for 5 hours at room temperature to form a clear, stable nanoemulsion. The prepared nanoemulsion was gradually incorporated into the gel base with continuous mixing until homogenous [19].

The antioxidant activity of each nano spray gel formula was assessed using the DPPH assay. DPPH solution, standard quercetin (1000 ppm), and sample solutions (F0–F4, each at 1000 ppm) were prepared. Serial dilutions (25, 50, 75, 100, and 125 ppm) were made from these stock solutions. Two milliliters of each dilution were mixed with 2 mL of DPPH solution in a 5 mL volumetric flask and made up to volume with ethanol p.a [20]. The mixtures were incubated for 30 minutes and the absorbance was measured at 400–600 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\%\ Inhibisi = \frac{Abs.Blanko\ DPPH - Abs.Sampel}{Abs.Blanko\ DPPH} \times 100\%$$

The IC_{50} value was determined from the linear regression equation of % inhibition versus concentration. A lower IC_{50} value indicates higher antioxidant activity.

RESULTS AND DISCUSSIONS

The fermentation process of red ginger simplicia was conducted to enhance both the quantity and quality of its bioactive compounds [21]. Extraction was performed using the maceration method with 96% ethanol as the solvent. The selection of 96% ethanol aimed to obtain an optimal yield of bioactive compounds from the fermented red ginger. The water content of ethanol is a critical factor influencing extraction efficiency; in conjunction with solvent polarity, it facilitates the extraction of a wide range of bioactive constituents [22]. In this study, 100 g of fermented red ginger simplicia produced 14.3 g of viscous extract, corresponding to a percentage yield of 14.3%. Although this value is slightly below the minimum requirement of 17% specified in the Indonesian Herbal Pharmacopoeia (2nd Edition, 2017), it can still be considered acceptable. Previous studies have reported that the yield of red ginger extract generally ranges from 8 to 15%, depending on the quality of the raw materials and the extraction method applied [23].

Phytochemical screening revealed the presence of secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and saponins. The results of the phytochemical screening of fermented red ginger extract are presented in Table 2.

Table 2. Phytochemical Screening of Fermented Red Ginger Extract

No	Compound Class	Test/Reagent	Result
1	Alkaloids	Dragendorff	+ (orange precipitate)
		Meyer	+ (white precipitate)
2	Flavonoids	Shinoda (HCl-Mg)	+ (pink)
		Bate-Smith-Metcalfe (H_2SO_4)	+ (dark reddish)
		NaOH 10%	+ (yellowish brown)
3	Tannins	FeCl3 3%	+ (greenish black)
4	Terpenoids	Liebermann-Burchard	+ (purplish brown)
5	Saponins	Hot water	+ (stable foam)

The presence of alkaloids and flavonoids in this study is consistent with the findings of Prapto *et al.* (2025), who reported that red ginger and emprit ginger extracted by maceration using 80% ethanol tested positive for both alkaloid and flavonoid compounds. The flavonoids identified in red ginger and emprit ginger belong primarily to the flavanone and anthocyanidin groups [24].

The antioxidant potential of this extract is largely attributed to the dominant presence of flavonoids and phenolics. The phenolic compounds, such as gingerol and shogaol [25]. Flavonoids function as electron donors through hydroxyl (-OH) groups, neutralizing free radicals, while phenolic compounds suppress reactive oxygen species (ROS) and reactive nitrogen species (RNS) [26]. Additionally, saponins and alkaloids contribute to antioxidant activity through superoxide scavenging and the formation of peroxyl intermediates that prevent biomolecular damage caused by oxidative stress.

Antioxidant activity of the nano spray gel formulations was assessed using the DPPH assay across five different extract concentrations (0%, 0.25%, 0.50%, 0.75%, and 1%). Among these, the formulation containing 0.75% extract (Formula 3) exhibited the strongest antioxidant activity, with an IC_{50} value of 74.84 µg/mL.

Table 3. IC_{50} Values of Fermented Red Ginger Nano Spray Gel Formulations

Formula	Extract Concentration	IC_{50} (µg/mL)
0	0%	84,43
1	0,25%	81,43
2	0,50%	80,52
3	0,75%	74,84
4	1,00%	77,64

According to Blois classification, IC_{50} values below 100 µg/mL indicate strong antioxidant activity. These findings confirm that the nano spray gel formulation containing 0.75% fermented red ginger extract has significant antioxidant potential.

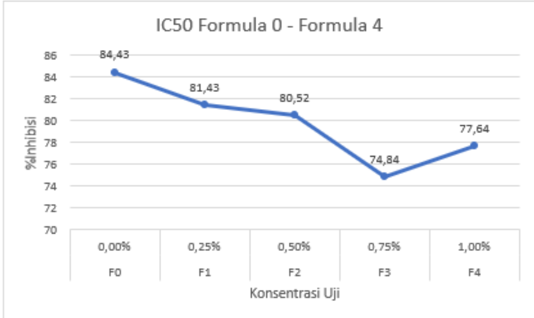

Figure 1. IC_{50} Values of Formulations F0–F4

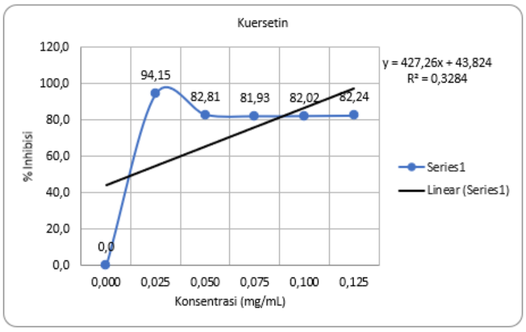

Figure 2. Inhibition Percentage vs. Quercetin Concentration

Quercetin, used as a reference antioxidant, is a potent flavonoid owing to its catechol group on the B-ring and three hydroxyl groups on the A and C rings, which enhance free radical scavenging capacity. The results demonstrate that the nano spray gel formulation, especially at 0.75% extract concentration, holds great promise as a natural antioxidant topical preparation to combat free radical-induced skin damage.

CONCLUSIONS

Based on the results of this study, the nano spray gel formulation containing 0.75% fermented red ginger extract exhibited an IC_{50} value of 74.84 ppm, indicating strong antioxidant activity. Therefore, further development of the fermented red ginger extract is warranted by investigating variations in fermentation duration to obtain more comprehensive data on the effect of the fermentation process on the antioxidant activity of the nano spray gel formulation.

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