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*Research Article*

# *In Vitro* Evaluation of Prebiotic Potential of Red Ginger (*Zingiber officinale* var. *rubrum*) Rhizome Ethanol Extract on *Lactobacillus acidophilus* and *Escherichia coli*

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## Abstract

Prebiotics, including carbohydrates and phenols, promote beneficial gut bacteria (probiotics). Red ginger (*Zingiber officinale* var. *rubrum*) rhizomes, rich in these compounds, have been traditionally used in medicine but their prebiotic potential remains unexplored. This study investigated the *in vitro* prebiotic effects of *Z. officinale* var. *rubrum* rhizomes on *Lactobacillus acidophilus* (beneficial) and *Escherichia coli* (opportunistic) bacteria. Prebiotic activity was assessed using a turbidimetric method, measuring bacterial growth via UV-Vis spectrophotometry at 600 nm. The prebiotic index and percentage inhibition were calculated to evaluate the impact on bacterial growth. Additionally, total phenol content was determined using the Folin-Ciocalteu method. Results indicate that *Z. officinale* var. *rubrum* rhizomes exhibit prebiotic properties, stimulating *L. acidophilus* growth (prebiotic index of 156.035 and percentage inhibition value of -153.128%) while inhibiting *E. coli* growth (54.343% inhibition). The rhizomes contained 31.15 mg GAE/g extract of total phenols and 23.55% carbohydrates. These findings suggest that *Z. officinale* var. *rubrum* rhizomes possess prebiotic potential, warranting further investigation for potential applications in gut health management.

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## INTRODUCTION

Prebiotics are non-digestible compounds that selectively support the growth of beneficial bacteria (probiotics) in the large intestine[1]. This enhanced probiotic population contributes to various health benefits, including suppression of pathogenic bacteria, improved intestinal motility, enhanced calcium absorption, strengthened immune function, and reduced risk of cancer[2,3]. Common prebiotic compounds include carbohydrates or dietary fiber, such as inulin, fructooligosaccharides, isomaltooligosaccharides, lactosucrose, lactulose, pyrodextrins, transgalactooligosaccharides, and xylooligosaccharides. Notably, certain non-carbohydrate compounds, like phenolic compounds, have also been identified as prebiotics[4].

Red ginger (*Zingiber officinale* var. *rubrum*) is a widely used traditional medicinal plant, with its rhizome being the primary part of interest. This rhizome is rich in biologically active secondary metabolites, including flavonoids, phenols, terpenoids, and essential oils[5]. Among these compounds, phenolic compounds like oleoresin, gingerol, and shogaol are particularly noteworthy. *Zingiber officinale* var. *rubrum* exhibits the highest oleoresin content among ginger varieties, reaching up to 3% of its dry weight[6]. Additionally, the rhizome contains approximately 2-14% gingerol and 1-2% shogaol[7].

Due to the high phenolic content in *Z. officinale* var. *rubrum* rhizomes, they might have the potential to be prebiotics[8]. However, the prebiotic effects of *Z. officinale* var. *rubrum* rhizomes have not been thoroughly investigated. This study aims

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to evaluate the prebiotic potential of *Z. officinale* var. *rubrum* rhizome extract on beneficial bacteria (*Lactobacillus acidophilus*) and opportunistic pathogens (*Escherichia coli*). Additionally, this study aimed to quantify the total phenolic and carbohydrate content of *Z. officinale* var. *rubrum* rhizome ethanol extract.

## MATERIALS AND METHODS

### *Materials*

This study utilized a range of equipment and materials for the extraction, analysis, and microbiological evaluation of *Z. officinale* var. *rubrum* rhizomes. Laboratory equipment included Pyrex glassware, a Waring blender, a Mettler Toledo analytical balance, a Memmert oven, an IKA HB 10 basic rotary evaporator, a Shimadzu UV-1800 UV-Vis spectrophotometer, an Envilife vmx-s vortex, and a Memmert water bath. Reagents and materials employed were distilled water, gallic acid (a reference standard), sulfuric acid (analytical grade, Merck), ethanol (analytical grade, Merck), phenol (analytical grade, Merck), *Z. officinale* var. *rubrum* rhizomes sourced from the Medicinal Plant and Spice Garden in Manoko, Lembang, methanol (analytical grade, Merck), Man Rogosa Sharpe Agar (MRSA), Man Rogosa Sharpe Broth (MRSB), sodium carbonate (analytical grade, Merck), Nutrient Agar (NA), Nutrient Broth (NB), Folin-Ciocalteu reagent, and glucose (reference standard, Merck). The study utilized *L. acidophilus* ATCC 4356 and *E. coli* ATCC 25922, obtained from the Microbiology Laboratory of the Faculty of Pharmacy, Universitas Islam Bandung, as bacterial strains for microbiological analysis.

### *Methods*

*Plant extraction*

*Zingiber officinale* var. *rubrum* rhizomes were obtained from local sources in Bandung. The plant material was authenticated at the Herbarium of the School of Life Sciences and Technology, Institut Teknologi Bandung (voucher specimen number: 1009/IT1.C11.2/TA.00/2023). Fresh rhizomes were sliced, dried, and subjected to maceration extraction using 96% ethanol as the solvent. The extraction process involved soaking the sliced rhizomes in ethanol for a specified duration, followed by filtration to obtain the ethanolic extract.

*Determination of total phenols and carbohydrates*

To quantify the phenol content in *Z. officinale* var. *rubrum* rhizomes, a spectrophotometric assay using Folin-Ciocalteu reagent and gallic acid as a standard was employed[9]. Similarly, the total carbohydrate content was determined using the spectrophotometric phenol-sulfuric acid method[10].

*Prebiotic activity test*

Prebiotic activity was assessed using UV-Vis spectrophotometer to measure optical density at 600 nm (OD600)[11,12]. OD600 values indicate bacterial growth in the culture medium. Additionally, prebiotic index (PI) and % inhibition were calculated using **Equations 1** and **2**. The following variables were used: $OD600_{control}$ (bacterial culture without *Z. officinale* var. *rubrum* extract), $OD600_{sample}$ (bacterial culture with *Z. officinale* var. *rubrum* extract), $P_p^0$, $P_p^{24}$, $P_G^0$, $P_G^{24}$ $P_{GF}^0$, $P_{GF}^{24}$, (OD600 values for *L. acidophilus* at time points 0 and 24 hours in different media; p: prebiotics/*Z. officinale* var. *rubrum* extract; G: glucose-containing media; GF: glucose-free media), $E_p^0$, $E_p^{24}$, $E_G^0$, $E_G^{24}$ $E_{GF}^0$, $E_{GF}^{24}$ (OD600 values for *E. coli* at time points 0 and 24 hours in different media; p: prebiotics/*Z. officinale* var. *rubrum* extract; G: glucose-containing media; GF: glucose-free media).

$$\%inhibition = \frac{(OD600_{control} - OD600_{sample})}{OD600_{control}} \text{x} 100\% \quad [1]$$

$$Prebiotic\ index = \left[\frac{(P_p^{24} - P_p^0) - (P_{GF}^{24} - P_{GF}^0)}{(P_G^{24} - P_G^0) - (P_{GF}^{24} - P_{GF}^0)}\right] - \left[\frac{(E_p^{24} - E_p^0) - (E_{GF}^{24} - E_{GF}^0)}{(E_G^{24} - E_G^0) - (E_{GF}^{24} - E_{GF}^0)}\right] \quad [2]$$

### *Data analysis*

Data collected from three experimental replicates were averaged to obtain mean values. The Kruskall-Wallis non-parametric test was employed to evaluate differences in OD600 values among the various prebiotic samples. The Mann-Whitney test was used to identify significant differences between specific pairs of samples. Statistical significance was determined at a p-value threshold of ≤0.05 using SPSS 26 software.

## RESULTS AND DISCUSSION

A total of 132.43 g of *Z. officinale* var. *rubrum* ethanol extract was obtained from 730 g of dried rhizome, yielding an 18.14% extraction efficiency. This value falls within the acceptable range of 17.0% as per Indonesian Herbal Pharmacopoeia guidelines[13]. However, it's noteworthy that previous studies reported a lower extraction yield of 7.13% for *Z. officinale* var. *rubrum*[7]. This variation can be attributed to differences in the extraction process[14].

The maceration method, employed in this study, is a simple and cost-effective technique for extracting bioactive compounds from plant materials. The efficiency of maceration can be influenced by various factors, including the sample-solvent ratio[15]. Ethanol, a commonly used solvent in phytochemical extraction due to its Generally Recognized as Safe (GRAS) status and low vapor pressure, was chosen for this study[16]. The choice of solvent and the sample-solvent ratio can significantly impact the extraction yield, with higher solvent volumes generally leading to increased extraction of bioactive compounds. Additionally, the duration of the extraction process plays a crucial role. Prolonged extraction times allow for greater penetration of the solvent into the plant material, facilitating the diffusion of compounds[14]. Phytochemical screening of the *Z. officinale* var. *rubrum* rhizome revealed the presence of various secondary metabolites, as detailed in **Table I**.

**Table I.** Phytochemisty screening of *Z. officinale* var. *rubrum* rhizome.

| Secondary metabolites | Result |
|---|---|
| Alkaloids | + |
| Polyphenols | + |
| Flavonoids | + |
| Saponins | - |
| Tannins | + |
| Monoterpenes and sesquiterpenes | + |

(+): detected; (-): not detected

Phytochemical screening of *Z. officinale* var. *rubrum* confirmed the presence of alkaloids, polyphenols, flavonoids, tannins, monoterpenes, and sesquiterpenes, aligning with previous research[17]. Polyphenols, a class of compounds found in *Z. officinale*, exhibit prebiotic properties, promoting the growth of beneficial bacteria and conferring health benefits to humans[18]. The total phenolic content in *Z. officinale* plants varies widely, ranging from 0.2 to 155.3 mg gallic acid equivalent (GAE)/g extract[19]. Notably, *Z. officinale* var. *rubrum* has been reported to possess the highest phenolic content among *Z. officinale* varieties[20]. The phenolic compounds in *Z. officinale* var. *rubrum* primarily consist of vanilloids, including gingerol, shogaol, paradol, zingerone, gingerdione, and gingerdiol[5,21].

The ethanol extract of *Z. officinale* var. *rubrum* in this study exhibited a total phenolic content of 31.15 mg GAE/g, surpassing previous reports of 21.90 mg GAE/g[22] and 12.2533 ± 0.13 mg GAE/g[23] obtained using the infusion extraction method. Variations in total phenolic content among *Z. officinale* var. *rubrum* extracts can be attributed to factors such as the extraction solvent and the intrinsic chemical properties of the plant material. The polar nature of phenolic compounds in *Z. officinale* var. *rubrum* facilitates their effective binding by ethanol[24]. Additionally, geographic variations in *Z. officinale* var. *rubrum* growth conditions, including cultivar type, soil composition, cultivation practices, and maturity, can influence the total phenolic content[25].

Besides polyphenols, carbohydrates are also prebiotic compounds[26]. Carbohydrates are the primary component of *Z. officinale* var. *rubrum* rhizomes, constituting approximately 10.1% in fresh rhizomes and a significantly higher 85% in dried rhizomes[27-29]. Starch comprises 59.29% of the carbohydrates in *Z. officinale* var. *rubrum* rhizomes[28]. In this study, the ethanol extract of *Z. officinale* var. *rubrum* contained 23.55% total carbohydrates. To assess the impact of *Z. officinale* var. *rubrum* ethanol extract on bacterial growth, we measured OD600 values of *L. acidophilus* and *E. coli* cultures. OD600 values are directly correlated with bacterial population density. As shown in **Figure 1**, the extract significantly inhibited the growth of *E. coli* while promoting the growth of *L. acidophilus*.

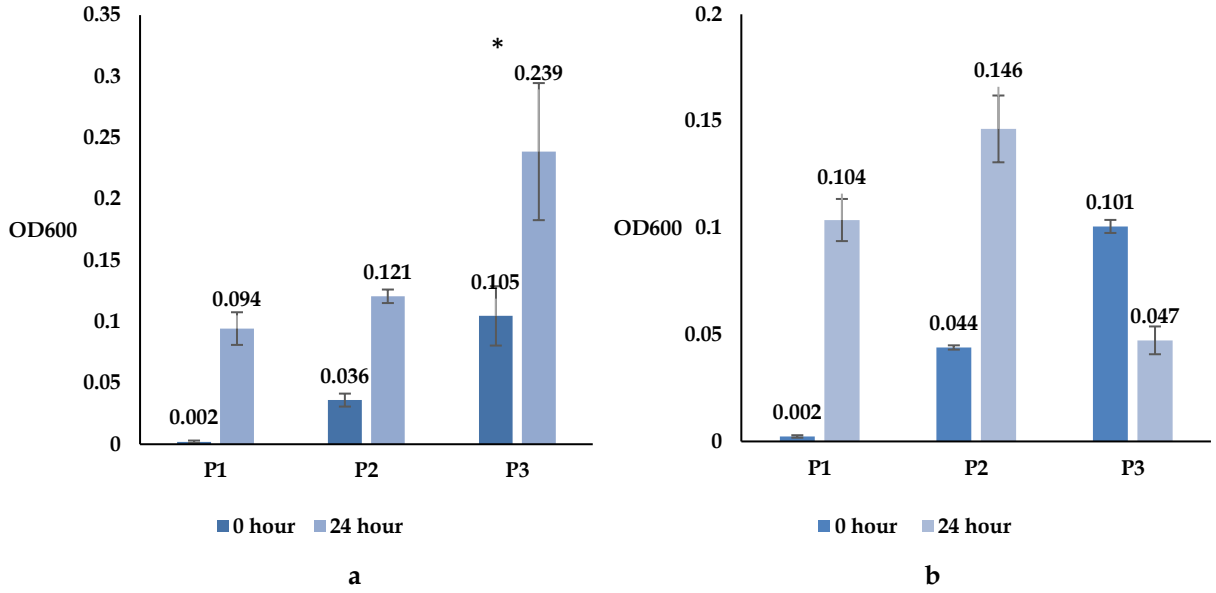


**Figure 1.** The OD600 values of *L. acidophilus* (**a**) and *E. coli* (**b**) in medium with no carbon source (P1), glucose (P2) and *Z. officinale* var. *rubrum* ethanol extract (P3). The bars are presenting the mean of OD600 values with standard deviation bar (n=3). * is significantly different compared to P1 (P=0.016) dan P2 (P=0.05).

Three experimental groups were established for this study: Group P1, cultured in a carbon-free medium; Group P2, supplemented with glucose; and Group P3, treated with *Z. officinale* var. *rubrum* ethanol extract. Glucose, a common bacterial growth medium component, served as a control[30,31]. After 24 hours of incubation, *L. acidophilus* populations exhibited significant growth in Group P3 (*Z. officinale* var. *rubrum* ethanol extract) compared to Groups P1 (no carbon source) and P2 (glucose). Conversely, *E. coli* populations decreased in Group P3, while increasing in Groups P1 and P2 (**Figure 1**).

The bacteria cultivated with glucose exhibited the highest increase in growth compared to the bacteria cultivated with *Z. officinale* var. *rubrum* ethanol extract and the control group. While glucose enhanced the growth of both *L. acidophilus* and *E. coli*, *Z. officinale* var. *rubrum* ethanol extract demonstrated a selective effect, promoting the growth of *L. acidophilus* while suppressing the growth of *E. coli*. This selective prebiotic effect is evident from the inhibition percentage values of -153.128% for *L. acidophilus* and 54.343% for *E. coli*, respectively, when cultured with *Z. officinale* var. *rubrum* ethanol extract. The phytochemical compounds present in *Z. officinale* var. *rubrum* ethanol extract may be responsible for this selective prebiotic effect by interacting with specific bacterial pathways and promoting the growth of beneficial bacteria like *L. acidophilus*[32].

The prebiotic index of *Z. officinale* var. *rubrum* ethanol extract was calculated to be 156.035. This indicates its ability to selectively stimulate the growth of beneficial bacteria compared to unfavorable bacteria, even in the presence of non-prebiotic substrates like glucose[33]. These findings suggest that *Z. officinale* var. *rubrum* ethanol extract is metabolized similarly to control prebiotics by probiotic strains. Notably, its PI was significantly higher than that of aqueous extracts of white ginger or *Zingiber officinale* Rosc. var. *officinale* (0.373-0.837)[34], emphasizing the superior prebiotic potential of *Z. officinale* var. *rubrum*.

## CONCLUSION

This study demonstrates the prebiotic potential of *Z. officinale* var. *rubrum* ethanol extract, as evidenced by its PI value of 156.035 and its selective growth promotion of *L. acidophilus* over *E. coli*. Additionally, the extract contains a significant amount of total phenols (31.15 mg GAE/g extract) and total carbohydrates (23.55%).

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## AUTHORS' CONTRIBUTION

**Conceptualization**: Bertha Rusdi
**Data curation**: Fadil Rido Gumelar, Farendina Suarantika, Bertha Rusdi
**Formal analysis**: Fadil Rido Gumelar, Farendina Suarantika, Bertha Rusdi
**Funding acquisition**: -
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**Methodology**: Fadil Rido Gumelar, Bertha Rusdi
**Project administration**: -
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**Supervision**: Farendina Suarantika, Bertha Rusdi
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**Visualization**: -
**Writing - original draft**: Fadil Rido Gumelar
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## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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