ANTIOXIDANT, ANTIBACTERIAL AND ANTI-INFLAMMATORY OF ESSENTIAL OILS FROM *Zingiber zerumbet* RHIZOMES CULTIVATED IN BUON MA THUOT CITY

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ARTICLE INFO

Received: 16/12/2024
Revised: 25/3/2025
Published: 26/3/2025

KEYWORDS

Antioxidant
Antibacterial
Anti-inflammatory
Zingiber zerumbet
Essential oils

ABSTRACT

Zingiber zerumbet (L.) Roscoe ex Sm. commonly known as bitter ginger, is used in traditional medicine to treat inflammation, rheumatism, sprains, colic pain, diarrhea, tonsillitis, and other ailments. This study aims to evaluate the essential oils extracted from mature rhizomes of *Zingiber zerumbet* cultivated in Buon Ma Thuot city for their antioxidant, antimicrobial, and anti-inflammatory properties. Using three in vitro models of antioxidant activity, it was shown that the antioxidant capacity of essential oils from *Zingiber zerumbet* rhizome was found to be independent of polyphenol content. Antibacterial testing revealed that the essential oils depends on the volatile components and effectively inhibited *Staphylococcus aureus* (IC_{50} = 28.911± 5.275 mg/mL) and *Pseudomonas aeruginosa* (IC_{50} = 32.900 ± 5.722 mg/mL) but had limited activity against *Escherichia coli*. The essential oil of *Zingiber zerumbet* has potential applications in pharmaceuticals due to its antioxidant, antibacterial, and anti-inflammatory properties, but further studies are needed to optimize extraction methods and elucidate its mechanisms of action.

KHẢ NĂNG KHÁNG OXY HÓA, KHÁNG KHUẨN VÀ KHÁNG VIÊM CỦA CÁC TINH DẦU CHƯNG CẤT TỪ CỦ GỪNG GIÓ (*Zingiber zerumbet)* ĐƯỢC TRỒNG Ở THÀNH PHỐ BUÔN MA THUỘT

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THÔNG TIN BÀI BÁO

Ngày nhận bài: 16/12/2024
Ngày hoàn thiện: 25/3/2025
Ngày đăng: 26/3/2025

TỪ KHÓA

Kháng oxy hóa
Kháng khuẩn
Kháng viêm
Zingiber zerumbet
Tinh dầu

TÓM TẮT

Zingiber zerumbet (L.) Roscoe ex Sm. thường được gọi là gừng đắng hay gừng gió, được sử dụng trong y học cổ truyền để điều trị viêm, thấp khớp, bong gân, đau bụng, tiêu chảy, viêm amidan và nhiều bệnh khác. Nghiên cứu này được thiết kế để thu tinh dầu chưng cất bằng 3 phương pháp khác nhau từ củ già của *Zingiber zerumbet* trồng tại thành phố Buôn Ma Thuột, để xác định các hoạt tính kháng oxy hóa, kháng khuẩn và kháng viêm. Sử dụng 3 mô hình thử nghiệm *in vitro* của hoạt tính kháng oxy hóa, các kết quả thu được cho thấy khả năng kháng oxy hóa của các tinh dầu từ củ gừng gió không phụ thuộc vào sự thay đổi hàm lượng hợp chất polyphenol. Trong mô hình thử nghiệm kháng khuẩn bằng dãy pha loãng nồng độ, khả năng kháng khuẩn của tinh dầu gừng gió lại phụ thuộc vào các thành phần dễ bay hơi và ức chế các vi khuẩn thử nghiệm ở IC_{50} với *Staphylococus aureus* là 28,911± 5,275 mg/mL và *Pseudomonas aeruginosa* là 32,.900 ± 5,722 mg/mL. Các tinh dầu gừng gió trong nghiên cứu này thể hiện khả năng ức chế với *Escherichia coli* thấp. Tinh dầu gừng gió có tiềm năng ứng dụng trong dược liệu nhờ hoạt tính kháng oxy hóa, kháng khuẩn và kháng viêm, nhưng cần nghiên cứu thêm để tối ưu hóa chiết xuất và xác định cơ chế tác động.

DOI: https://doi.org/10.34238/tnu-jst.11708

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1. Introduction

The *Zingiberaceae* family belongs to the *Zingiberales* order, one of the largest plant orders in the kingdom. Over a hundred years ago, ginger was first utilized as a spice and natural flavoring. Beyond its culinary applications, spices are now recognized for their potential to create innovative traditional remedies for various diseases [1]. These spices, regarded as medicinal plants, are abundant in secondary metabolites, which provide substantial sources for novel chemical compounds with therapeutic effects, including antioxidant, antibacterial, and anti-inflammatory properties [1] – [4]. For centuries, *Zingiber* species have been utilized for their antimicrobial, anti-inflammatory, and antioxidant properties [1] - [4].

Zingiber zerumbet (L.) Roscoe ex Sm. (Family: *Zingiberaceae*) is widely distributed across tropical regions, particularly in Southeast Asia, the Pacific, and Oceania. The rhizomes of the plant have been traditionally used as spices and for treating immune-inflammatory-related disorders [1], [5]. Numerous bioactive compounds have been isolated from *Z. zerumbet*, making it a potent medicinal candidate for a wide range of disorders [1] - [4], [6] - [8]. In Dak Lak province, Vietnam, this species is commonly referred to as "bitter ginger" and is abundant in Yok Don National Park, where it thrives under local climatic conditions.

This species is also cultivated in the tropics for its medicinal properties and commercial value as a spice [1]. It is traditionally used to treat stomachaches, swelling, sores, loss of appetite, ear inflammation, and diarrhea, and is a common ingredient in many traditional medicine formulations [9]. The rhizomes of *Z. zerumbet* contain essential oils rich in diverse classes of compounds, including polyphenols, alkaloids, and terpenes. These essential oils are not only employed in medicinal applications but also as raw materials in industry [6], [10].

Previous studies have indicated that the chemical composition of *Z. zerumbet* essential oil is highly variable, influenced by genetic, climatic, and soil conditions, altitude, growth phase (reproductive or vegetative), and the method of essential oil extraction [4]. However, to date, no studies have investigated the chemical constituents and pharmacological activities of the volatile oil derived from the rhizomes of *Z. zerumbet* in Dak Lak province. Furthermore, the effects of different distillation conditions on the chemical composition and pharmacological activities of *Z. zerumbet* volatile oils remain unclear [3] - [5], [9].

In order to determine the raw material standards and conditions for distilling essential oils from *Z. zerumbet* rhizome in cultivation for application in supporting the treatment of infectious and inflammatory diseases and some diseases related to metabolic disorders, in this study, we determined the antioxidant, antibacterial and anti-inflammatory properties of essential oils from *Z. zerumbet* rhizome grown in Buon Ma Thuot city, Dak Lak province.

2. Materials and methods

2.1. Plant material

Figure 1. *Zingiber zerumbet: (a) flower, (b) rhizome, (c) cross section of rhizome*

Fresh rhizomes of *Zingiber zerumbet* (Figure 1) were collected from Yok Don National Park in September 2022 (Number collected sample is TNU2024-11CB-21032022, stored at Biology department of Tay Nguyen University). These rhizomes were then cultivated in Thanh Nhat District, Buon Ma Thuot city, and harvested in December 2023.

2.2. Essential oil extraction

The rhizomes were cut into small pieces and subjected to hydrodistillation for 8 hours [3] using a Clevenger-type apparatus under three different conditions: **MT1:** Fresh *Z. zerumbet* rhizomes were distilled without the addition of NaCl, yielding essential oil labeled as ZREO 1; **MT2:** Fresh *Z. zerumbet* rhizomes were distilled with 8% NaCl (Xilong, China), yielding essential oil labeled as ZREO 2; **MT3:** *Z. zerumbet* Rhizomes were air-dried at room temperature (~25°C) for two weeks, then distilled with 8% NaCl, yielding essential oil labeled as ZREO 3. The resulting essential oils were dried over anhydrous Na_2SO_4 (Xilong, China), filtered, and stored in amber glass bottles at 4°C for further analysis.

2.3. Total phenolic and flavonoid contents

2.3.1. Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method with slight modifications. Specifically, 0.5 mL of the sample solution was mixed with 2.5 mL of Folin-Ciocalteu reagent (Merck, India) and incubated for 4 minutes. Following this, 4 mL of Na_2CO_3 solution (7.5%, Xilong, China) was added, and the mixture was incubated at room temperature for 60 minutes. The absorbance was measured at 760 nm using a UV-5200 spectrophotometer (Shanghai Metash Instruments Co., Ltd, China). The TPC was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of essential oil [6].

2.3.2. Total flavonoid content

The total flavonoid content (TFC) was quantified using the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric assay with minor adjustments. The sample solution (0.5 mL) was mixed with 0.04 mL of 5% $NaNO_2$ solution (Xilong, China) and incubated for 6 minutes. Subsequently, 0.04 mL of 10% $Al(NO_3)_3$ solution (Xilong, China) was added, followed by an additional 6-minute incubation. Afterward, 0.4 mL of 4% NaOH solution (Xilong, China) was added, and the total volume was adjusted to 1 mL with distilled water. The mixture was incubated at room temperature for 15 minutes, and the absorbance was read at 510 nm. The TFC was expressed as milligrams of quercetin equivalents (mg QE) per gram of essential oil [6].

2.3.3. Antioxidant activity

The antioxidant potential of the essential oil was evaluated using three established methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging, ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging, and ferric reducing antioxidant power (FRAP).

2.3.4. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the essential oil was determined with the reaction volume adjusted to suit laboratory condition [6]. 0.2 mL essential oil samples dissolved in methanol with different concentrations were mixed with 1 mL of 0.006% DPPH solution in methanol (prepared during the day). After mixing the sample solution with DPPH and allowing a reaction time of 30 minutes, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (6850 UV/Vis Spectrophotometer, JENWAY). A decrease in the absorbance indicated an increase in scavenging activity. The percentage of inhibition was calculated using the formula: scavenging activity % = 100*(Absorbance of blank sample - Absorbance of sample)/ Absorbance of blank sample. Here, the absorbance of blank sample represents the

DPPH solution with methanol, and the absorbance of sample corresponds to the DPPH solution with the tested oil samples. Then, a linear correlation equation (y = ax + b) is constructed between the inhibition percentage and the sample concentration, and the IC_{50} value (meant that the concentration at which 50% of DPPH free radicals are quenched) is determined. Ascorbic acid was used as the positive control. All experiments were conducted in triplicate.

2.3.5. ABTS radical scavenging activity

The ABTS radical scavenging activity was assessed based on the protocol of Reddy et al. [1], with minor adjustments. ABTS radicals were generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate, $K_2S_2O_8$, (Sigma-Aldrich) and incubating the mixture at 37°C for 16 hours in the dark. Before use, the ABTS solution was diluted with methanol to achieve absorbances of 0.70 ± 0.02 at 734 nm. The reaction was initiated by adding 2 mL of ABTS solution to 0.2 mL of the essential oil at varying concentrations, followed by incubation at 37°C for 10 minutes in the dark. The percentage of inhibition was calculated using the same formula as for the DPPH assay. The IC_{50} value was determined to express the radical scavenging efficiency of the essential oil. Vitamin E was used as the positive control. All measurements were performed in triplicate.

2.3.6. Ferric reducing antioxidant power

The Ferric reducing antioxidant power (FRAP) assay was conducted as per the method described by Ferreira et al. [9], with slight modifications. The FRAP reagent was freshly prepared by mixing 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) (Sigma-Aldrich) in 40 mM HCl (Xilong, China), 2.5 mL of 10 mM $FeCl_3 \cdot 6H_2O$ (Xilong, China), and 25 mL of 300 mM acetate buffer (pH 3.6). The reagent was pre-warmed to 37 °C. The reaction mixture consisted of 900 µL FRAP reagent, 90 µL distilled water, and 40 µL essential oil dissolved in 5% DMSO at concentrations ranging from 1 to 100 mg/mL. After incubation at 37 °C for 10 minutes, the absorbance was measured at 593 nm. Trolox was used as the positive control. The FRAP values were expressed as micrograms of $FeSO_4$ equivalents per gram of essential oil (µg $FeSO_4$/g). All experiments were performed in triplicate.

2.3.7. Antibacterial activity

The antibacterial activity of *Z. zerumbet* essential oils was evaluated against *Pseudomonas aeruginosa* ATCC®9027, *Staphylococcus aureus* ATCC®25923, and *Escherichia coli* ATCC®25922 using the broth microdilution method with slight modifications [5].

Two-fold serial dilutions of the samples were mixed with bacterial suspensions (5×10^5 CFU/mL) and incubated at 37 °C for 24 hours. MIC values were determined through macroscopic observation, spectrophotometric readings at 620 nm, and resazurin colorimetric assay. MBC values were determined by subculturing samples from non-color-changing tubes onto Mueller-Hinton agar followed by incubation at 37 °C for 24 hours. Percentage inhibition and IC50 values were calculated based on absorbance measurements. All tests were performed in triplicate.

2.3.8. Bovine serum albumin denaturation assay

The anti-inflammatory activity of *Z. zerumbet* essential oils (ZREOs) was evaluated using a modified method from Chakou et al. [8]. Essential oil samples at concentrations of 1–100 mg/mL were prepared in 0.05 M Tris buffer (pH 6.6). Each sample (500 µL) was mixed with 2% (w/v) bovine albumin fraction V (Sigma-Aldrich, USA), incubated at 37°C for 20 minutes, and heated at 70 °C for 5 minutes. After cooling, absorbance was measured at 600 nm using a UV/Vis spectrophotometer (JENWAY 6850). Diclofenac potassium (Novartis, Turkey) served as the positive control, and all assays were performed in triplicate. The percentage inhibition of albumin denaturation was calculated using the equation: Percentage inhibition = 100*(Absorbance of

control - Absorbance of sample)/ Absorbance of control. The IC_{50} value, representing the essential oil concentration required to inhibit 50% albumin denaturation, was determined by plotting percentage inhibition against sample concentration.

2.3.9. Egg albumin assay

The anti-inflammatory activity of essential oils was evaluated using the egg albumin denaturation model. Reaction mixtures consisted of 0.2 mL of 2% egg albumin solution, 2 mL of essential oil sample in 5% DMSO (or standard diclofenac potassium, Novartis), and 2.8 mL of phosphate-buffered saline (PBS, pH 7.4), making a total volume of 5 mL. Controls included 2 mL of triple-distilled water, 0.2 mL of egg albumin solution, and 2.8 mL of PBS. Samples were incubated at 37 °C for 30 minutes, followed by heating at 70°C for 15 minutes. After cooling, absorbance was measured at 280 nm using a UV/Vis spectrophotometer (JENWAY 6850). The percentage inhibition of protein denaturation was calculated as: Percentage inhibition = 100*(Absorbance of control - Absorbance of sample)/ Absorbance of control. The IC_{50} value was determined by plotting the percentage inhibition of denaturation against sample concentration.

2.3.10. Statistical analysis

All experiments were performed in triplicate, and results were expressed as means ± SD. Statistical analysis was conducted using SPSS software (version 19.0). One-way analysis of variance (ANOVA) was applied, and Duncan's multiple range test was used to identify significant differences at $p < 0.05$.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

Polyphenols, including phenolics and flavonoids, serve as natural antioxidants, modulating metabolic activity and reducing oxidative stress-related diseases such as inflammation and diabetes complications [8]. Ginger essential oil (EO) is known for its diverse polyphenol composition, which contributes to its antioxidant, antibacterial, and anti-inflammatory properties. In this study, total phenolic content and total flavonoid content of *Z. zerumbet* rhizome essential oils (ZREOs) were quantified under three distillation conditions [Table 1]. TPC averaged 15 mg GAE/g EO, while TFC was approximately 12 mg QE/g EO, consistent with the known diversity of bioactive compounds in ginger EO. Compared to other extraction methods, polyphenol levels in ZREOs were relatively low. For instance, ethanol extracts of *Z. zerumbet* rhizomes reported by Nag et al. [10] showed TPC of ~33.64 mg GAE/g and TFC of ~26.79 mg QE/g. The antioxidant potential of phenolic compounds was linked to hydroxyl groups donating hydrogen atoms via electron transfer mechanisms, thereby preventing oxidation. Variability in phenolic and flavonoid content depended on factors such as plant origin, age, climate, seasons, and nutrient availability [1]. These findings highlighted the complexity of polyphenol content and the influence of environmental and processing conditions on ZREOs' functional properties.

Table 1. *Polyphenol contents of Zingiber zerumbet rhizome essential oils*

Essential oil samples	Phenolics (mg GAE/g EO)	Flavonoids (mg QE/g EO)
ZREO 1	14.818 ± 0.822^{ab}	12.836 ± 0.508^{b}
ZREO 2	14.843 ± 0.412^{b}	11.273 ± 0.449^{a}
ZREO 3	13.620 ± 0.707^{a}	12.471 ± 0.852^{ab}

Results are expressed as mean of three replicates ± standard deviation; different letters indicate significant differences in a column according to Duncan stepwise comparisons ($p \leq 0.05$).

In general, the polyphenol content in ginger essential oil was found to be relatively low compared to other extraction methods. For example, the study by Anish Nag *et al.* [10], [11] reported a TPC of approximately 33.64 mg GAE/g and a TFC of 26.79 mg QE/g in the ethanol

extract of *Z. zerumbet* (L.) rhizomes. The antioxidant potential of phenolic compounds can be attributed to their hydroxyl groups, which act as hydrogen atom donors via electron transfer mechanisms, thereby inhibiting oxidation. The variability in total flavonoid and phenolic contents is influenced by multiple factors, including collection site, plant age, temperature, climate, radiation, seasons, herbivore activity, and nutrient availability [1]. These findings emphasize the complexity of polyphenol content and its dependence on environmental and processing conditions.

3.2. Antioxidant activity

Antioxidant potential cannot be assessed using a single assay method due to the variation in assay principles and experimental conditions. For instance, some methods, such as the DPPH assay, use organic radical producers, whereas others, like the FRAP assay, rely on metal ions for oxidation. Additionally, the time required for free radical production by oxidation reactions differs across assays. As a result, different antioxidant assays use specific control compounds, such as BHT, ascorbic acid, or trolox, depending on the rate and time of radical scavenging [1], [10]. Antioxidants can be polar, such as phenolics and flavonoids, or non-polar, such as vitamin E, and act via electron or hydrogen donation mechanisms.

In this study, the antioxidant activity of *Zingiber zerumbet* rhizome essential oils (ZREOs) was evaluated using three in vitro free radical scavenging methods: DPPH, ABTS, and FRAP assays. The results are summarized in Table 2, showcasing the IC_{50} values for radical scavenging activity of ZREOs under three distillation conditions.

Table 2. *IC_{50} values of radical scavenging activities of Zingiber zerumbet rhizome essential oils*

Name of samples	DPPH radical (mg/mL)	ABTS radical (mg/mL)	FRAP activity (FeSO4 µg/g essential oil)
ZREO 1	18.478 ± 1.569^{b}	6.807 ± 0.567^{b}	1558.686 ± 5.4297^{b}
ZREO 2	24.594 ± 2.658^{c}	11.091 ± 1.446^{c}	**999.1525 ± 4.492^{a}**
ZREO 3	**8.914 ± 2.687^{a}**	**4.607 ± 1.472^{a}**	1815.89 ± 4.492^{c}
Ascobic acid (**µg/mL**)	30.193 ± 3.297	-	-
Vitamin E (**µg/mL**)	-	28.292 ± 1.756	-
Trolox (FeSO4 **µg/mg Trolox**)	-	-	46.54 ± 1.376

Results are expressed as mean of three replicates ± standard deviation; different letters indicate significant differences in a column according to Duncan stepwise comparisons ($p \leq 0.05$); (-) mean nondetected.

3.2.1. DPPH radical scavenging activity

The DPPH assay evaluates the hydrogen-donating ability of antioxidants to neutralize free radicals. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer [10]. Among the three ZREOs, ZREO 3 showed the strongest DPPH radical scavenging activity (IC50 = 8.914 ± 2.687 mg/mL), which was significantly better than ZREO 1 and ZREO 2, suggesting that the drying process before distillation and the addition of NaCl enhanced the bioavailability of active components. The DPPH scavenging activity of ZREO 3 was also superior to the standard ascorbic acid (IC50 = 30.193 ± 3.297 µg/mL), highlighting its potential as a natural antioxidant. The relatively lower DPPH scavenging activity of ZREOs compared to conventional antioxidants like ascorbic acid suggests that non-polar compounds, such as terpenes, may play a more significant role in antioxidant activity than polyphenols. These findings suggest that the polyphenol compounds in ZREOs play a minor role in radical scavenging activity.

3.2.2. ABTS radical scavenging activity

The ABTS scavenging activity is known to be more reactive compared to the DPPH assay. While the DPPH assay involves hydrogen atom transfer, the ABTS assay operates via an electron transfer mechanism. This study also examined the ABTS radical scavenging capacity of ZREOs,

showing a concentration-dependent relationship [9], [10]. As shown in Table 2, ZREOs exhibited a stronger ability to neutralize ABTS free radicals compared to their DPPH radical scavenging ability. Among the three tested ZREOs, ZREO3 demonstrated the most potent free radical scavenging capacity, with an IC_{50} value of 4.607 ± 1.472 mg/mL, significantly outperforming tocopherol (IC50 of 28.292 ± 1.756 µg/mL).

3.2.3. Ferric reducing antioxidant power

The FRAP assay assesses antioxidant activity by measuring the reduction of ferric ions (Fe^{3+}-TPTZ) to ferrous ions (Fe^{2+}-TPTZ) at low pH, producing a blue-colored complex. This cost-effective method is widely used for screening antioxidant potential and comparing compound efficiency [4], [10]. As shown in Table 2, the antioxidant capacity of ZREOs was quantified as µg $FeSO_4$ equivalents, reflecting their ferric-reducing ability. Similar to the DPPH assay, the iron-reducing capacity of natural polyphenol-related compounds aligned with FRAP results. However, ZREO displayed weak ferric-reducing activity compared to the positive control, trolox, suggesting that its antioxidant potential is less linked to polyphenol content and more associated with terpene compounds [10].

The results indicated that the distillation conditions significantly influence the antioxidant activity of ZREOs. Essential oils distilled under MT3 conditions demonstrated the highest antioxidant capacity, potentially due to improved preparation and drying of *Z. zerumbet* rhizomes, which enhanced terpene extraction. These findings highlighted the critical role of optimizing distillation processes to maximize bioactive compound recovery in essential oils.

3.3. Quantitative evaluation of the antimicrobial effect

The antimicrobial activity of *Zingiber zerumbet* rhizome essential oils (ZREOs) was tested against *E.coli* ATCC®25922, *S.aureus* ATCC®25923, and *P.aeruginosa* ATCC®9027. The results in Table 3 revealed that antibacterial efficacy varied depending on the oil sample and bacterial strain.

Table 3. *MIC and IC_{50} values were obtained by method of serial micro dilutions*

Samples	The inhibitory concentration values of ZREO (mg/mL)					
	MIC			IC_{50}		
	***E. coli* ATCC®25922**	***S. aureus* ATCC®25923**	***P. aeruginosa* ATCC®9027**	***E.coli* ATCC®25922**	***S. aureus* ATCC®25923**	***P. aeruginosa* ATCC®9027**
ZREO 1	4.512 ± 0.886^{a}	6.197 ± 1.619^{b}	4.767 ± 1.018^{a}	82.378 ± 13.683^{c}	56.385 ± 9.334^{b}	38.835 ± 6.847^{ab}
ZREO 2	4.786 ± 1.177^{a}	5.944 ± 1.256^{ab}	5.107 ± 1.218^{ab}	67.074 ± 10.928^{b}	150.576 ± 24.383^{c}	53.464 ± 9.086^{c}
ZREO 3	9.571 ± 2.186^{b}	**4.622 ± 1.069^{a}**	**5.622 ± 1.154^{b}**	55.001 ± 8.963^{a}	**28.911 ± 5.275^{a}**	**32.900 ± 5.722^{a}**
Ampicillin (µg/mL)	0.700 ± 0.069	1.479 ± 0.684	3.510 ± 0.729	10.960 ± 1.097	13.509 ± 1.245	53.761 ± 1.264

Results are expressed as mean of three replicates ± standard deviation; different letters indicate significant differences in a column according to Duncan stepwise comparisons ($p \leq 0.05$).

ZREO3 exhibited significant activity, particularly against *S. aureus* and *P. aeruginosa*, with IC_{50} values of 28.911 ± 5.275 mg/mL and 32.900 ± 5.722 mg/mL, respectively. In contrast, its activity against *E. coli* was weaker, with an IC50 of approximately 55.001 ± 8.963 mg/mL. All ZREO samples demonstrated low activity at the minimum inhibitory concentration (MIC), ranging between 5 and 10 mg/mL.

Previous studies reported higher antimicrobial potency of *Z. zerumbet* oils from other regions. For instance, essential oils from Indonesia showed MIC values as low as 1.25 mg/mL for *E. coli* and *P. aeruginosa* and 0.625 mg/mL for *Salmonella typhi*. The antimicrobial efficacy of *Z. zerumbet* oil against *S. aureus, Bacillus cereus, P. aeruginosa,* and *E. coli* has been well-documented [6]. This activity is largely attributed to zerumbone, a primary compound with antimicrobial, antifungal, and anti-mycological properties, along with synergistic effects from

other constituents [2], [3], [12]. The differences in antimicrobial efficacy could result from variations in distillation methods, which influence the chemical composition of the oils. The superior performance of ZREO3 underscored the importance of optimizing distillation conditions to enhance the therapeutic potential of *Z. zerumbet* essential oils.

3.4. Anti-inflammatory activity

Inflammation, marked by symptoms like pain and swelling, often involves protein denaturation, a loss of structural and functional integrity caused by stressors like heat or chemicals [7]. The anti-inflammatory potential of *Z. zerumbet* rhizome essential oils (ZREOs) was evaluated based on their inhibition of heat-induced albumin denaturation.

As shown in Table 4, the data revealed that all ZREO samples exhibited moderate inhibition of both bovine serum albumin (BSA) and egg albumin denaturation, with IC50 values ranging from 52 to 64 mg/mL. For comparison, the standard diclofenac potassium exhibited significantly lower IC50 values (32 μg/mL for BSA and 18.453 μg/mL for egg albumin denaturation). Among the tested ZREOs, ZREO 2 demonstrated superior anti-inflammatory activity, with IC_{50} values of 50.423 mg/mL and 54.24 mg/mL for BSA and egg albumin, respectively. No significant difference was observed between ZREO 1 and ZREO 2, while ZREO 3 showed slightly weaker activity.

Table 4. *IC_{50} values were obtained by method of albumin denaturation*

Name of samples	IC_{50} values of denaturation by essential oils (mg/mL)	
	Bovine serum albumin	**Egg albumin**
ZREO 1	52.28 ± 1.687^{ab}	56.543 ± 3.867^{a}
ZREO 2	50.423 ± 2.758^{a}	54.24 ± 4.446^{a}
ZREO 3	54.446 ± 2.587^{bc}	60.168 ± 1.472^{b}
Diclofenac potassium (μg/mL)	32.042 ± 3.297	18.453 ± 2.327

Results are expressed as mean of three replicates ± standard deviation; different letters indicate significant differences in a column according to Duncan stepwise comparisons ($p \leq 0.05$).

Previous studies have reported anti-inflammatory activity in *Z. zerumbet* extracts and oils, including inhibition of NO and PGE2 production in LPS-induced RAW 264.7 macrophages. This activity correlates with polyphenol content and is influenced by environmental factors like soil, climate, and growth conditions [13].

4. Conclusions

To the best of our knowledge, this study is the first to compare the antioxidant, antimicrobial, and anti-inflammatory activities of Z. zerumbet fresh and dry rhizome essential oils cultivated in Buon Ma Thuot city. From the results obtained in the study, we determined the conditions for obtaining essential oils with antibacterial, antioxidant and anti-inflammatory activities from rhizomes of *Zingiber zerumbet* under cultivation conditions. The antioxidant capacity of essential oils from rhizomes of ginger obtained from the study showed that they depended on the distillation conditions and the freshness of the raw materials, specifically, the best antioxidant capacity was shown in essential oils obtained under distillation conditions with the presence of 8% NaCl and *Zingiber zerumbet* rhisome stored from two weeks after harvest. The anti-inflammatory ability of essential oil from ginger rhizome is best demonstrated in essential oil distilled from *Zingiber zerumbet* rhisome with storage time of 0-7 days and distillation conditions with 8% NaCl. In conclusion, the essential oil of *Zingiber zerumbet* rhisome obtained under the distillation condition with 8% NaCl effect of this study has antibacterial, antioxidant and anti-inflammatory potential. However, for practical applications in cosmetics, pharmaceuticals, or food preservation, further in silico and animal studies are needed to assess safety and efficacy. Additionally, limitations in sample size and experimental conditions should be addressed, and future research should focus on identifying key bioactive compounds and optimizing extraction methods.

REFERENCES

[1] D. K. R. K. S. Reddy, J. Saikrishna, K. Mahendra, and P. M. Varma, "A review on the medicinal plant *Zingiber zerumbet* phytochemical composition, traditional uses, and potential health benefits," *Journal of Medicinal Plants Studies*, vol. 12, no. 4, pp. 139-146, 2024, doi: 10.22271/plants.2024.v12.i4b.1701.

[2] M. Deng, X. Yun, S. Ren, Z. Qing, and F. Luo, "Plants of the Genus Zingiber: A Review of Their Ethnomedicine, Phytochemistry and Pharmacology," *Molecules*, vol. 27, no. 9, Apr. 2022, doi: 10.3390/molecules27092826.

[3] N. Rattanachitthawat, P. Sriamornsak, V. Puri, I. Singh, K. Huanbutta, and T. Sangnim, "Bioactivity assessment of *Zingiber zerumbet* Linn rhizome extract for topical treatment of skin diseases," *Journal of Applied Pharmaceutical Science*, vol. 13, no. 3, pp. 168-174, 2023.

[4] L. T. Huong *et al.*, "*Zingiber zerumbet* Rhizome Essential Oil: Chemical Composition, Antimicrobial and Mosquito Larvicidal Activities," *European Journal of Medicinal Plants*, vol. 30, no. 4, pp. 1-12, 2019.

[5] X. Wang *et al.*, "Antibacterial Activity and Mechanism of Ginger Essential Oil against Escherichia coli and Staphylococcus aureus," *Molecules*, vol. 25, no. 17, 2020, Art. no. 3955.

[6] A. Y. Koga, F. L. Beltrame, and A. V. Pereira, "Several aspects of Zingiber zerumbet: a review," *Revista Brasileira de Farmacognosia*, vol. 26, no. 3, pp. 385-391, 2016, doi: 10.1016/j.bjp.2016.01.006.

[7] S. R. Sarveswaran, W. J. A. B. N. Jayasuriya, and T. S. Suresh, "In vitro assays to investigate the anti-inflammatory activity of herbal extracts: A review," *World Journal of Pharmaceutical Research*, vol. 6, no. 17, pp. 131-141, 2017.

[8] H. P. Devkota *et al.*, "Bioactive Compounds from Zingiber montanum and Their Pharmacological Activities with Focus on Zerumbone," *Applied Sciences*, vol. 11, pp. 10205-10205, 2021, doi: 10.3390/app112110205.

[9] H. Ali *et al.*, "Antioxidant, cytotoxic and apoptotic activities of the rhizome of *Zingiber zerumbet* Linn. in Ehrlich ascites carcinoma bearing Swiss albino mice," *Sci. Rep.*, vol. 12, no. 1, Jul. 2022, Art. no. 12150, doi: 10.1038/s41598-022-15498-8.

[10] J. Rohmah, "Antioxidant activities using DPPH, FIC, FRAP, and ABTS methods, from ethanolic extract of lempuyang gajah rhizome (*Zingiber zerumbet* (L.) Roscoe ex Sm.)," *Sciencetific Journal of Chemical Research*, vol. 7, pp. 152-166, 2022.

[11] A. Nag, M. Bandyopadhyay, and A. Mukherjee, "ntioxidant Activities and Cytotoxicity of *Zingiber zerumbet* (L.) Smith Rhizome," *Journal of Pharmacognosy and Phytochemistry*, vol. 2, no. 3, pp. 102-108, 2013.

[12] A. A. Assiry *et al.*, "The antioxidant activity, preliminary phytochemical screening of *Zingiber zerumbet* and antimicrobial efficacy against selective endodontic bacteria," *Food Sci. Nutr.*, vol. 11, no. 8, pp. 4853-4860, Aug. 2023, doi: 10.1002/fsn3.3462.

[13] Z. A. Zakaria, A. S. Mohamad, C. T. Chear, Y. Y. Wong, D. A. Israf, and M. R. Sulaiman, "Antiinflammatory and antinociceptive activities of *Zingiber zerumbet* methanol extract in experimental model systems," *Med. Princ. Pract.*, vol. 19, no. 4, pp. 287-294, 2010, doi: 10.1159/000312715.