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RESEARCH ARTICLE

Comparative analysis of Antioxidants, Antibacterial and Anti-inflammatory Activities of Six Ginger Rhizome Extracts using Aqueous and Ethanolic Solvents

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Abstract

Ginger rhizomes are rich in a variety of bioactive compounds and have several therapeutic properties. However, the pharmacological potential of local varieties from the regions of biodiversity remains to be explored. To overcome this, the present study analyzed and systematically classified the total phenolic content (TPC), antioxidant, antibacterial and anti-inflammatory properties of twelve extract samples from six local ginger rhizomes using water and ethanol as extraction solvents. Quantitative results showed that TPC of ethanolic extract of *Curcuma aromatic* Salisb. (5.87 mg/g extract) was higher than other extracts measured by Folin-Ciocalteu method. In antioxidant assays, the ethanolic extract of *Curcuma aeruginosa* Roxb. was the most potent DPPH radical scavenger (IC_{50} of 0.66 mg/mL), whereas the aqueous extract of *Zingiber montanum* (J.Koenig) Link ex A.Dietr. showed the highest ferrous ion-chelating ability (IC_{50} of 0.14 mg/mL). Antibacterial screening using disc diffusion method revealed that only the ethanolic extract of *C. aromatica* was effective in inhibiting *Bacillus subtilis* TISTR 1248 (MIC of 25.63 mg/mL; MBC of 205.00 mg/mL). SEM analysis confirmed significant disruption of bacterial cell wall. Moreover, the anti-inflammatory evaluation on LPS-induced RAW 264.7 macrophages indicated that *Z. montanum* ethanolic extract decreased nitric oxide production by 75% without cytotoxicity, with cell viability remaining over 80%. The detailed classification analysis clearly identified the ethanolic extracts of *C. aromatica*, *Z. montanum*, and *C. aeruginosa* as the most suitable and effective candidates for targeted antibacterial, anti-inflammatory and radical-scavenging product applications, respectively. This knowledge provides an important scientific baseline for the development of standardized, high value therapeutic formulations from local traditional herbs.

Keywords: Ginger, Antioxidants, Anti-inflammatory, Antibacterial, Rhizome extracts, *Curcuma aromatica*, *Zingiber montanum*, RAW 264.7 macrophages, Nitric oxide

Introduction

The ginger plant (Zingiberaceae) has been an integral part of everyday life for centuries in a variety of cultures, as much for its medicinal properties as for its use as a foodstuff [1]. The family members are rich sources of bioactive phenolic compounds, including non-volatile pungent constituents such as gingerols, shogaols, paradols and zingerones [2]. The ginger plant is culturally and practically significant in Thai society, especially in the northeastern part. These herbs are traditionally used and cultivated by local populations along the edges of residences and rice fields for food, beauty enhancement, medicine, treatment, care, and rehabilitation [3]. Sakon Nakhon Province, which is in the center of the region, has a fertile landscape and high biodiversity, especially along the Phu Phan Mountain range. Based on these natural resources, the government has designated the province as a national prototype herbal city under the "Sakon Nakhon Herbal City Strategic" according to the National Master Plan on the Development of Thai Herbs, No.1 [4].

Although members of the ginger family are globally recognized for individual therapeutic properties such as gastrointestinal tract benefits and digestive stimulant effects [1,5,6], lipid-lowering, anti-obesity, cardioprotective actions [7-10], anti-inflammatory qualities [11,12], cancer-preventive effects [13,14], immunomodulatory activities [15] and antioxidant activity [16,17], scientific rationale increasingly calls for a multifarious evaluation of these biological activities. Chronic wounds, gastrointestinal disorders and metabolic infections are not isolated pathological conditions. Instead, it is a complex cascade in which oxidative stress damages cellular structures, leading to significant inflammatory responses that decrease the resistance of the tissues to opportunistic bacterial invasions. As a result, therapies that focus on only one target often fall short. Therefore, it is very important to investigate the herbal extracts having antioxidants, antibacterial and anti-inflammatory activities simultaneously and to develop the multi-target, synergistic therapeutic agents to break such an interconnected pathological cycle.

Given this obvious clinical and pharmacological relevance, there is still a significant gap in our knowledge of the indigenous, non-commercial ginger species found within the Phu Phan Mountain range, a biodiversity hotspot. These specific local cultivars are widely used in traditional community medicine, while the common commercial ginger varieties are well documented, without any systematic characterization of their phytochemical profiles or exact biological mechanism of action. Also, the impact of routine extraction factors such as the use of highly polar aqueous and moderately polar ethanolic solvents on the yield and dual-phase efficacy of these interconnected biological activities is yet to be fully unconfirmed.

This lack of empirical data needs to be urgently addressed. Antibiotic resistance has increased worldwide, and synthetic anti-inflammatory agents have side effects, so there is a demand for standardized natural multi-potents. The serious risk in the local context is the aggressive promotion of the commercial herbal sector by Sakon Nakhon, the transformation of traditional knowledge into marketable products without scientific validation. This could result in non-standardized, low-efficacy or potentially cytotoxic products reaching consumers, undermining public health trust and ultimately stalling the economic goals of the regional strategy. To minimize these risks, this study evaluates and compares the combined antioxidant, antimicrobial and anti-inflammatory properties of six locally grown ginger varieties extracted with water and ethanol. This extensive scientific baseline is an important step toward developing standardized high-value herbal formulations that will ensure safe consumer applications and support the long-term economic sustainability of the region.

Materials and methods

Materials

The test employed six different species of ginger plants: *Zingiber pyroglossum* Triboun & K. Larsen., *Curcuma aromatica* Salisb., *Zingiber montanum* (J.Koenig) Link ex A.Dietr., *Curcuma wanenlueanga* Saensouk, Thomudtha & Boonma, *Curcuma aeruginosa* Roxb. and *Geodorum recurvum* (Roxb.) Alston. They were gathered from Sakon Nakhon province in Thailand. The two types of bacteria used in testing are Gram-positive bacteria (*Bacillus subtilis* TISTR1248) and Gram-negative bacteria (*Escherichia coli* TISTR 527). The murine macrophage cell line (RAW 264.7) was obtained from the American Type Culture

Collection (ATCC, USA). All reagents used in the phenolic compound analysis, as well as antioxidants, antibacterial, anti-inflammatory, and cytotoxicity assays, were of analytical grade.

Sample preparation and extraction

Each ginger rhizome sample was washed, cut into small pieces, and dried at 50 °C. The dried samples were then ground into a fine powder. For extraction, 300 mL of 80% (v/v) ethanol was added to each 100 g sample. The mixture was incubated at 30 °C with continuous shaking for 24 h. After incubation, the mixture was filtered to remove the precipitate by Whatman paper no.1, and the filtrate was concentrated by evaporation. The resulting, crude extract was stored at 4 °C for further testing. For the water-based extraction, the same procedure as above was followed, except ethanol was replaced with water [18]. This procedure yielded a total of twelve extract samples, split equally between ethanolic and aqueous extractions.

Total phenolic compound content

All 12 extracts were analyzed for total phenolic content (TPC) using the Folin-Ciocalteu method, as described by Preecharram *et al*. [19]. In short, a 50 µL crude extract was mixed with Folin–Ciocalteu reagent and sodium carbonate, then incubated in the dark for 2 h. Gallic acid served as the standard, and the absorbance was measured at 765 nm. Methanol was used as a control, and TPC was expressed as gallic acid equivalents (mg/g extract). The standard curve (R^2 = 0.9950) was used for quantification, and the results were calculated using the formula.

$$C = \frac{cv}{m} \quad (1)$$

where C is total phenolic content (mg/g extract), c is gallic acid concentration from the standard curve (mg/mL), v is extracting volume (mL), and m is dry extracted weight (g).

Antioxidant activity

The DPPH method was conducted as previously described by Preecharram *et al*. [18]. In brief, 50 µL of crude extract was mixed with 0.1 mM DPPH solution in a 96-well plate and incubated in the dark at room temperature for 20 min. The absorbance was measured at 515 nm, with a DPPH solution in the extraction solvent as the negative control. The scavenging activity was calculated, and results were expressed as the half-maximal inhibitory concentration (IC_{50}).

The FIC method was carried out as described by Sutthidech *et al*. [20]. In brief, a reaction mixture containing $FeCl_2$, 70% ethanol and crude extract at different concentrations was incubated for 5 min and then ferrozine was added and incubated in dark. The absorbance of the Fe^{2+} -ferrozine complex was recorded at 562 nm using 70% ethanol as blank. The chelating ability was calculated by the difference in absorbance between samples with and without crude extract.

Antibacterial activity

The antibacterial activity of the extracts was assessed using the disc diffusion method [21]. A bacterial inoculum at a concentration of 10^8 CFU/mL was evenly distributed throughout the nutrient agar medium. After letting the surface dry for 3 to 5 min, 6 mm sterile filter paper discs were placed on each plate and impregnated with the proper concentration of the extract. The plates were then incubated at 37 °C for 16 - 20 h. The diameter of the clearance zone was measured in millimeters (mm). Ten percent dimethyl sulfoxide (DMSO) and discs containing 1 mg of tetracycline were used as negative and positive controls, respectively. The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the test samples [22]. Test compounds were first dissolved in 10% (v/v) DMSO. Each concentration (100 µL) was added to 96-well microplate containing inoculum density of 1.5×10^6 CFU/mL. The plates were mixed well and incubated at 37 °C for 24 h. Microbial growth was determined by measuring the absorbance at 600 nm after incubation. Tetracycline and 10% DMSO served as positive and negative controls, respectively, and were subjected to the same experimental conditions. MIC had the lowest concentration of extracts that could reduce bacteria by 80% compared to the control. To determine the minimum bactericidal concentrations (MBCs), aliquots from wells at or above the MIC were done by drop plate method onto nutrient agar and incubated under the same conditions. The MBC was defined as the lowest concentration at which no visible microbial colony formation was observed.

Scanning electron microscopy (SEM) analysis of bacterial morphology

The morphological characteristics of bacterial cells were examined following a modified protocol based on the method described by Jandaruang *et al*. [23]. *Bacillus subtilis* was chosen as a model organism. The bacterial cells were treated with ethanol extracts of *C. aromatica* under defined experimental conditions. After treatment, the cells were processed for the analysis of surface morphology by scanning electron microscope (SEM). The observations were performed on a JEOL JSM-IT500 IN TOUCHSCOPE™ instrument (JEOL Ltd., Japan). The samples were analyzed at the acceleration voltage of 10 kV. The micrographs were recorded at the magnification of 20,000X to observe the structural changes of the bacterial cell surface.

Nitric oxide quantification

To evaluate anti-inflammatory activity, nitric oxide (NO) production was measured in RAW 264.7 macrophage cells stimulated with lipopolysaccharide (LPS). RAW 264.7 cells were seeded in 96-well plates and incubated for 24 h at 37 °C in a CO_2 incubator to allow adherence. The cells were then treated with various concentrations (0.125, 0.250, 0.500, 1.000, and 2.000 mg/mL) of each extract, along with co-treatment with 100 ng/mL LPS, and incubated for an additional 24 h under the same conditions. Then, the culture supernatant from each well was collected and mixed with an equal volume of Griess reagent. The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 540 nm using a microplate reader (EnSight, PerkinElmer, USA). The level of NO production was expressed as a percentage relative to the control group [24].

Cell viability

The viability of RAW 264.7 cells following treatment with the extract and co-incubation with LPS was assessed using the MTT assay, following the method described by Preecharram *et al*. [19]. After the culture medium from the NO measurement was removed, the remaining adherent cells in the 96-well plates were incubated with fresh medium containing 0.5 mg/mL MTT. The plates were incubated at 37 °C in a 5% CO_2 incubator for 1 h. After incubation, the MTT-containing medium was removed, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals formed by metabolically active cells. The absorbance was measured at 570 nm using a microplate reader (EnSight, PerkinElmer, USA). Cell viability was calculated by comparing the absorbance of extract-treated cells to that of the LPS-treated control using the following equation.

$$Cell\ viability\ (\%) = [\frac{Absorbance}{AbsorbanceLPS\ control}] \times 100\% \quad (2)$$

Statistical analysis

All results are presented as mean ± standard deviation (S.D.). For statistical comparisons, a one-way ANOVA was employed, and for post hoc analysis, Duncan's multiple range test was employed. A significant threshold of $p < 0.05$ was used.

Results and discussion

Total phenolic compounds

The analysis of total phenolic compounds in 12 extract samples revealed that ethanol extracts contained phenolic compounds ranging from 1.51 to 5.87 mg/g extract, whereas water extracts had a lower range of 0.77 to 2.71 mg/g extract (**Figure 1**). These results suggest that ethanol extracts phenolic chemicals more efficiently than water. This is likely due to ethanol's moderate polarity, compared to water, which is highly polar. Phenolic compounds contain both polar hydroxyl (-OH) groups and non-polar aromatic rings, making them more soluble in moderately polar solvents like ethanol [25]. Additionally, ethanol can dissolve low-polarity organic compounds, facilitating the extraction of a broader range of phenolic compounds with varying solubility.

Among the ethanol extracts, *C. aromatica* had the highest phenolic content (5.87 mg/g extract), followed by *Z. montanum* (4.39 mg/g extract) and *C. aeruginosa* (4.18 mg/g extract). After examining the literature, Adebooye *et al*. [26], have reported a TPC of 0.70 mg GAE/g fresh weight of *Solanum nigrum* in a water extract [26]. According to Uddin *et al* [27], the TPC of *Portulaca oleracea*'s methanol extract is 3.60 mg GAE/g dry weight. The present study's phenolic content values differed somewhat from those found in previous research. However, the plant species, the presence of varying amounts of sugars, and geographic variance all affect the phenolic component composition [28].

Moreover, the extraction method plays a crucial role in yielding. Studies have shown that using high-frequency sound waves during extraction can increase the yield by 28% compared to conventional methods [29].

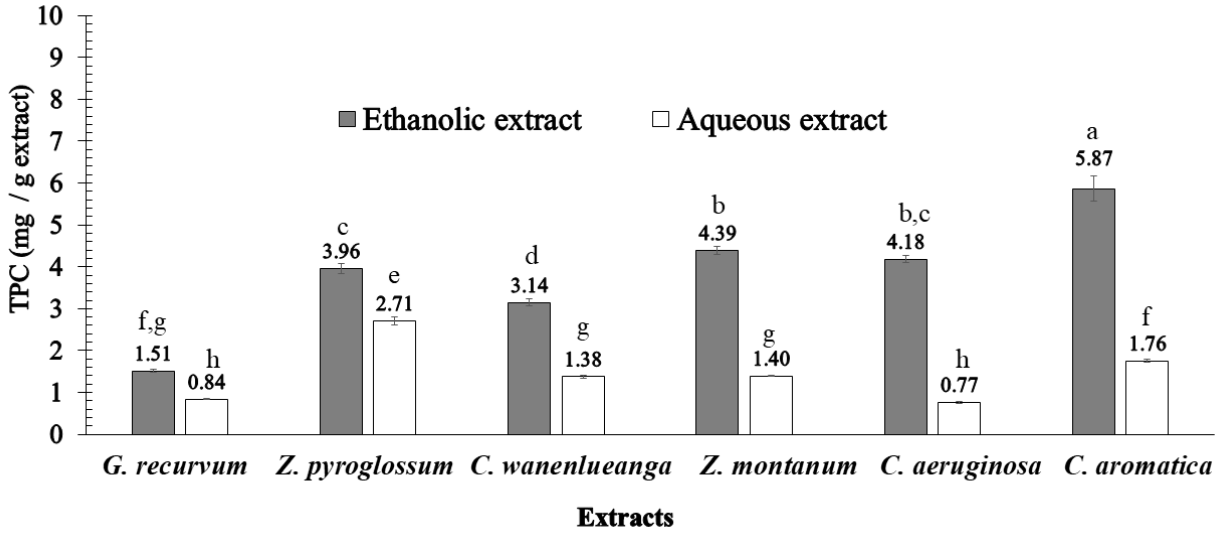


Figure 1 The total phenolic contents of six ginger that extracted with aqueous and ethanolic solvents. Significant differences at $p < 0.05$ are indicated by the letters above the bars. The error bars show ± S.D. (n = 3).

Antioxidant activity

The analysis of DPPH antioxidant capacity in 12 extracts demonstrated that ethanol extracts exhibited remarkably lower IC_{50} values, ranging from 0.66 to 5.03 mg/mL, compared to the significantly higher IC_{50} values of water extracts, which ranged from 6.17 to 91.33 mg/mL (**Figure 2**). This stark contrast underscores the superior DPPH antioxidant capacity of ethanol extracts, which can be attributed to their higher phenolic compound content. Phenolic compounds are renowned for their potent antioxidant properties [2,30]. This makes ethanol extracts a more powerful option for antioxidant applications.

Among the ethanol extracts, *C. aeruginosa* exhibited the highest antioxidant capacity (IC_{50}; 0.66 mg/mL), followed by *Z. montanum* (IC_{50}; 1.35 mg/mL) and *Z. pyroglossum* (IC_{50}; 2.00 mg/mL). In contrast, among the water extracts, *Z. montanum* demonstrated the highest antioxidant activity (IC_{50}; 6.17 mg/mL). Members of the ginger family are known to contain potent antioxidant compounds such as 6-gingerol and shogaol [2].

Metal ions are a common source of free radicals; therefore, metal ion binding is an effective strategy for reducing free radical formation. Analyzing the iron ion-binding ability of 12 extract samples revealed that the ethanol extracts had IC_{50} values ranging from 0.16 to 1.55 mg/mL, while the water extracts exhibited IC_{50} values between 0.14 and 1.35 mg/mL. According to these findings, the water extracts showed a better capacity to bind iron ions. This is probably because they were more effective at extracting phenolic compounds with ortho structures. Phenolic compounds with an ortho-structure, where hydroxyl (-OH) groups are attached to adjacent carbon atoms on aromatic ring (e.g., positions 1 and 2, 2 and 3, or 3 and 4), have a strong ability to bind iron ions effectively [31].

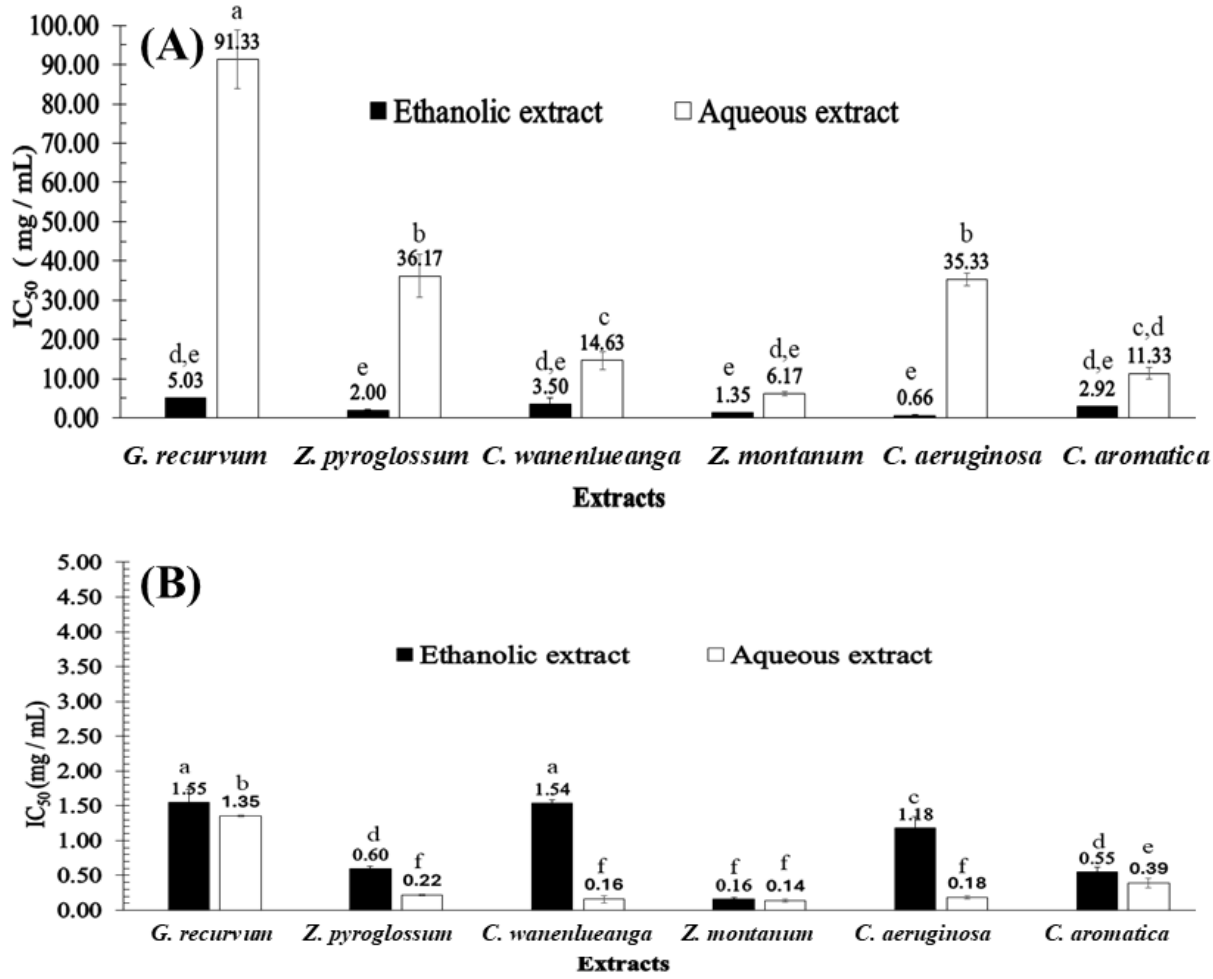


Figure 2 Antioxidant activity by DPPH assay (A) and FIC assay (B) of six ginger that extracted with aqueous and ethanolic solvents. The letters above the bars indicate significant differences at $p < 0.05$. Error bars display ± S.D. (n = 3).

Among the tested extracts, the three with the highest iron ion-binding ability were the water extract of *Z. montanum* (0.14 mg/mL) and *C. wanenlueanga* (0.16 mg/mL), and the ethanol extract of *Z. montanum* (0.16 mg/mL). Nonetheless, the aqueous and ethanolic extracts of *Z. montanum*, together with the aqueous extracts of *Z. pyroglossum*, *C. wanenlueanga*, and *C. aeruginosa*, showed noticeably better ferrous ion chelating activity than the other extracts at a 95% confidence level. The existence of polar phytochemical elements, such flavonoids and phenolics, which are better extracted in aqueous and ethanolic solvents, may be the cause of this increased chelation capacity. These substances are well-known for their high metal-binding abilities, which support their possible medicinal and antioxidant benefits.

The total phenolic content and antioxidant capacities (DPPH and FIC assays) of the twelve ginger rhizome extracts are summarized in **Table 1**, to provide a clear comparative view of the quantitative results in all stages of the experiment. There are three main observations that can be pointed out that help to clarify these results. Firstly, there is a clear trend of solvent dependency in which the ethanol extracts consistently yielded higher TPC ranging between 1.51 and 5.87 mg/g extract than the aqueous extracts with values ranging from 0.77 to 2.71 mg/g extract. In direct correlation with the better DPPH radical scavenging capacity in the ethanolic group, the TPC is higher. Secondly, an interesting difference was observed in the FIC assay: the aqueous extracts, despite having lower TPC, generally showed higher iron-chelating capacities (lower IC_{50} values) than the ethanolic extracts for most species. This

agrees with the hypothesis that the aqueous extraction method may be highly selective for some polar phenolics or flavonoids with high metal-binding affinities. Finally, the relationship between TPC and antioxidant capacity is not strictly proportional between different species. For example, although the ethanol extract of *C. aromatica* has the highest overall TPC (5.87 mg/g extract), the strongest DPPH activity was recorded for *C. aeruginosa* (IC_{50} = 0.66 mg/mL). Thus, the structural profile and composition of phenolic compounds in each ginger species are more important for their antioxidant efficacy than the absolute amount alone.

Table 1 Overall summary of total phenolic content and antioxidant activities of the twelve ginger rhizome extracts.

	TPC (mg/g extract)		DPPH IC_{50} (mg/mL)		FIC IC_{50} (mg/mL)	
	Ethanol	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**
G. recurvum	1.51	0.84	5.03	91.33	1.55	1.35
Z. pyroglossum	3.96	2.71	2.00	36.17	0.60	0.22
C. wanenlueanga	3.14	1.38	3.50	14.63	1.54	0.16
Z. montanum	4.39	1.40	1.35	6.17	0.16	0.14
C.aeruginosa	4.18	0.77	0.66	35.33	1.18	0.18
C. aromatica	5.87	1.76	2.92	11.33	0.55	0.39

Antibacterial activity

A study on the antibacterial activity of all 12 crude extracts revealed that most extracts did not inhibit bacterial growth. However, the ethanol extract of *C. aromatica* effectively inhibited against *B. subtilis*, with a clear zone diameter of 24.54 mm (**Figure 3**), comparable to the antibiotic tetracycline at a dose of 1 mg. The MIC and MBC of ethanolic extract of *C. aromatica* is 25.63 and 205.00 mg/mL, respectively (**Table 2**).

The lack of antibacterial activity in most extracts may be due to the low concentration of active compounds. However, these ginger plants are rich in essential oils, which have known antibacterial properties. The antibacterial activity of these plants may be attributed to various essential oil components, including citral, sabinene, β-phellandrene, β-pinene, α-pinene, γ-elemene, germacrene B, β-caryophyllene, δ-elemene, and γ-caryophyllene [32,33].

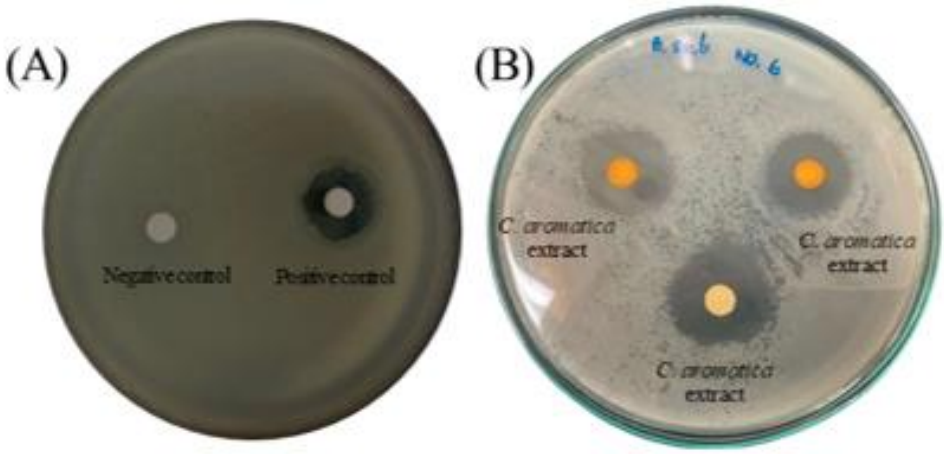


Figure 3 Inhibition zone of *C. aromatica* ethanolic extract against *B. subtilis*: Negative control (10% DMSO) and Positive control (1 mg Tetracycline) (A), *C. aromatica* extract (410 mg/mL) (B).

Table 2 Antibacterial activity in ethanolic extract of *C. aromatica* against *B. subtilis* and *E. coli*.

Bacteria	Diameter of clear area (mm)			MIC (mg/mL)	MBC (mg/mL)
	C. aromatica	Tetracyclines	10% DMSO		
B. subtilis	24.54	22.47	0.00	25.63	205.00
E. coli	0.00	17.81	0.00	-	-

Morphology of bacteria by SEM

The results of this study demonstrate the effects of the ethanol extract of *C. aromatica* on the morphology of *B. subtilis*. In the untreated control group, the bacterial cells maintained a typical rod shape with smooth, intact cell walls. They were arranged in short chains and exhibited a relatively uniform size and structure (**Figure 4(A)**), which is typical for this Gram-positive bacterium. In contrast, exposure to the ethanol extract of *C. aromatica* led to significant changes in cell shape. The treated cells showed major abnormalities, including changes from the usual rod shape to irregular or twisted forms, cell shrinkage, structural collapse, and cell wall damage that caused cell lysis (**Figure 4(B)**). These findings align with earlier reports that rhizome extracts of *C. aromatica* have strong antibacterial effects against Gram-positive bacteria [34]. The observed changes in cell shape indicate that the ethanol extract directly affects both the cell wall and the cytoplasmic membrane of *B. subtilis*. This effect may be attributed to the presence of bioactive compounds like phenolic constituents, which are 6-gingerol and 6-shogaol [35]. They can penetrate and disrupt lipid structures in the cell membrane. This disruption increases membrane permeability, causing leakage of vital biomolecules and ions, which ultimately leads to a loss of cellular balance [36]. Additionally, the presence of lysed cells and cellular debris shows that the extract has bactericidal activity, consistent with the MBC values presented in **Table 2**. The underlying mechanism may involve breaking down peptidoglycan, a major structural component of Gram-positive bacterial cell walls, as well as creating oxidative stress in the cells (**Figure 5**). The antibacterial action of this extract may involve breaking down membranes in a way like the barrel-stave, carpet, or toroidal pore models, which describe the insertion and interaction of antimicrobial peptides with bacterial cell membranes [37]. This results in irreversible damage to cell structure and function.

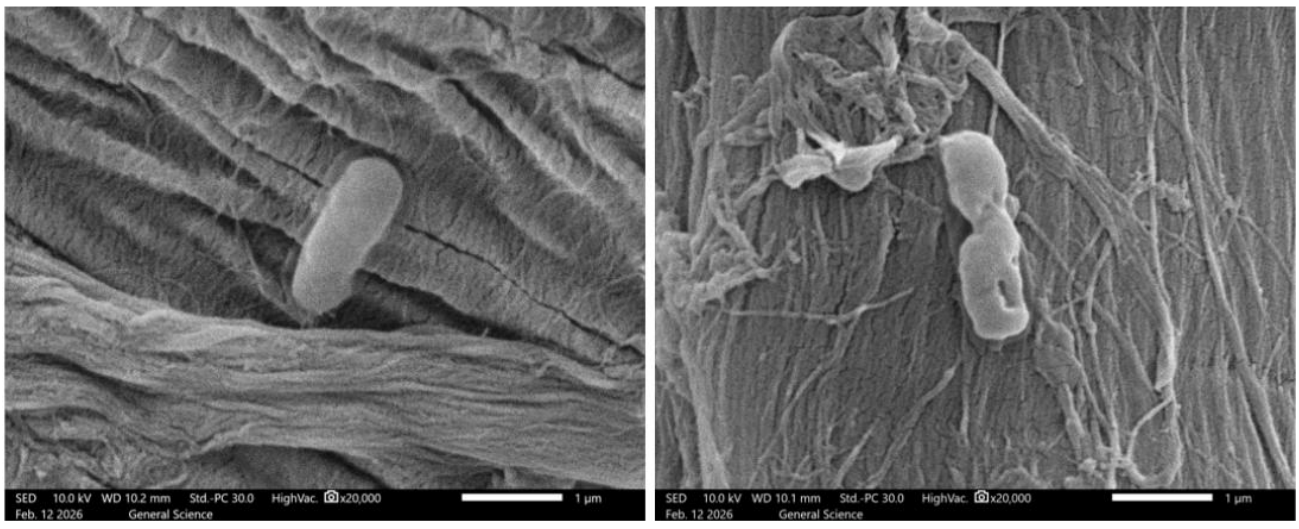


Figure 4 SEM images of *B. subtilis* after incubation with (A) PBS (control), (B) *C. aromatica* extract.

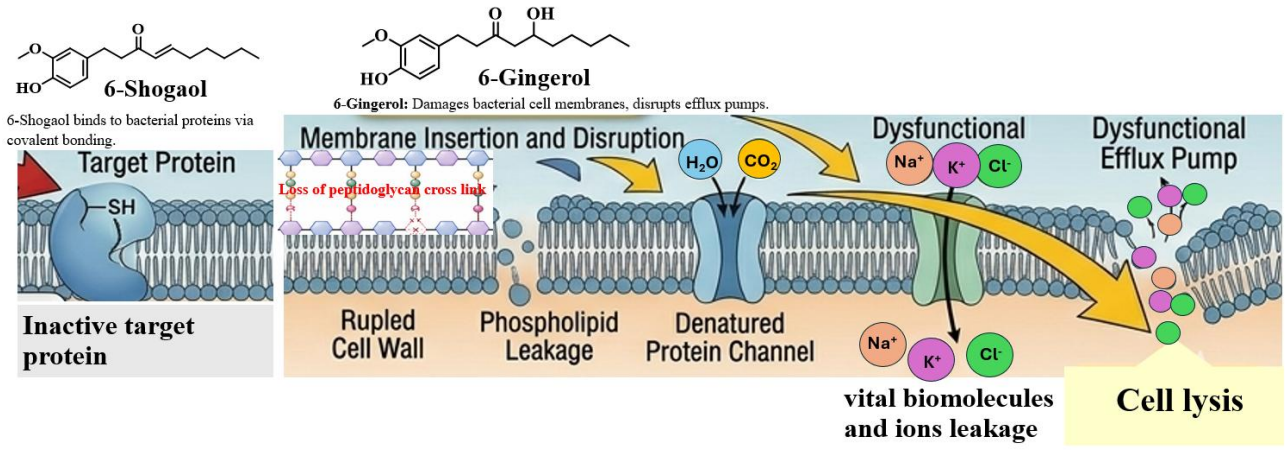


Figure 5 Schematic representation of the proposed mechanism of action by which the ethanol extract of *C. aromatica* disrupts *B. subtilis* cells.

Effect of extracts on NO production

The anti-inflammatory activity of six ethanol extracts, tested at concentrations of 0.125 - 2.000 mg/mL, is shown in **Figure 6**. After inducing inflammation in RAW 264.7 cells with LPS for 24 h, the extracts of *G. recurvum*, *C. wanenlueanga*, *Z. montanum* and *C. aeruginosa* significantly reduced NO levels. At a concentration of 2.000 mg/mL, the extracts of *G. recurvum*, *C. wanenlueanga*, *Z. montanum* and *C. aeruginosa* reduced NO levels by 45%, 59%, 75% and 63%, respectively. The NO reduction was concentration-dependent, with *Z. montanum* extract at 2.000 mg/mL showing the highest inhibition (75%). In contrast, *Z. pyroglossum* extract reduced NO levels at 0.125 - 0.500 mg/mL, but increasing the concentration to 1.000 - 2.000 mg/mL had no further effect. *C. aromatica* extract did not reduce NO levels. For the aqueous extracts, only the extract of *Z. pyroglossum* reduced nitric oxide levels. At concentrations of 0.125 - 1.000 mg/mL, it decreased NO levels by 15% - 38%, while at 2.000 mg/mL, it achieved a 79% reduction. However, at this concentration, cell viability was low (49%) (**Figure 7**). Based on these findings, the ethanolic extract of *Z. montanum* was the most promising candidate for the treatment of inflammatory-related diseases, since it showed the highest potency to suppress inflammatory mediators without any compromise to cell viability.

These results indicated that the ethanolic extract demonstrated notable anti-inflammatory activity, which correlates with its high phenolic content. Notably, 6-gingerol and 6-paradol, major phenolic constituents in the *Zingiberaceae* family, are well-documented for their anti-inflammatory properties [38]. Previous studies have reported that phenolic compounds isolated from the roots and rhizomes of *Notopterygium incisum*, particularly Notoflavinols A and B, can significantly inhibit NO production [39]. Furthermore, phenolic compounds such as rutin and quercetin have been shown to reduce NO levels without exerting cytotoxic effects [40].

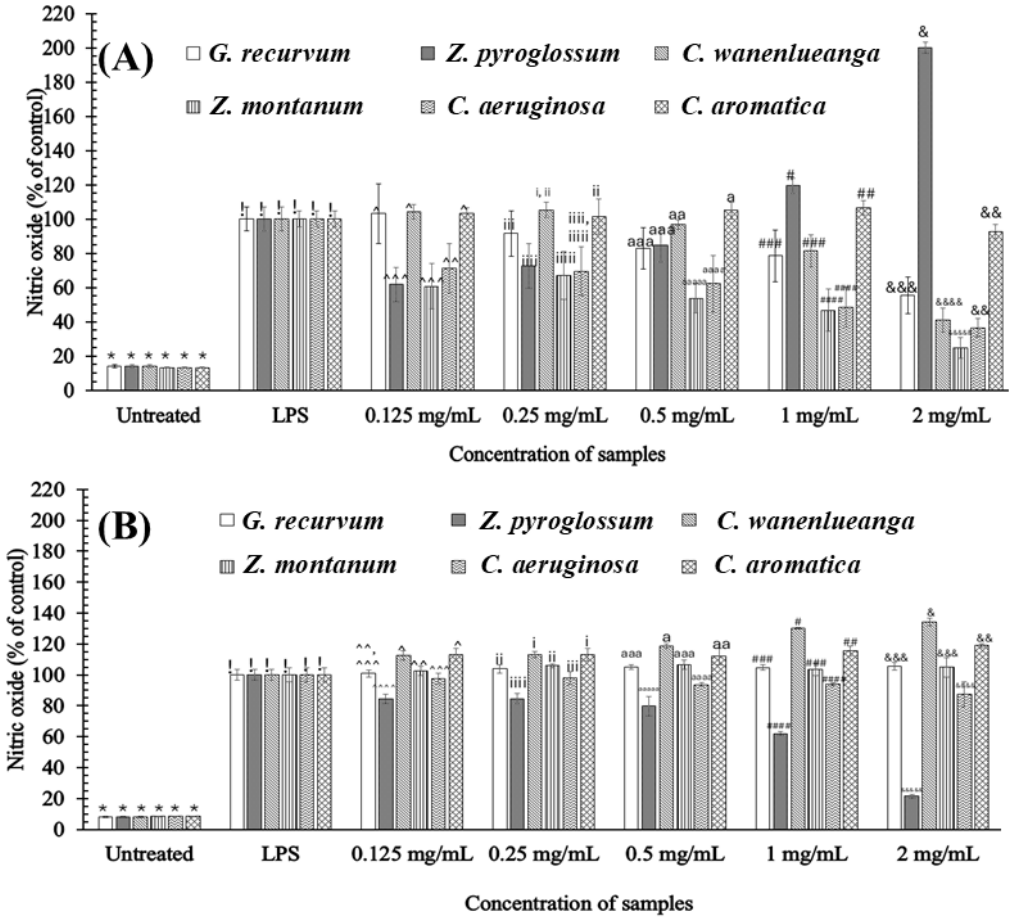


Figure 6 Nitric oxide production by ethanolic extracted (A) and aqueous extracted (B) of six gingers. In each graph, significant differences at $p < 0.05$ are indicated by the symbols above the bars. The error bars show ± S.D. (n = 3).

Effect of extracts on cell viability

To evaluate the potential cytotoxic effects of the extracts, cell viability was assessed in RAW 264.7 cells following LPS stimulation. The viability of LPS-treated cells was designated as 100%. The results indicated that all 12 extracts did not significantly affect cell viability, with all treatments maintaining viability above 80%, except for the aqueous extract of *Z. pyroglossum* at 2.000 mg/mL, which reduced cell viability to 49% (**Figure 7**).

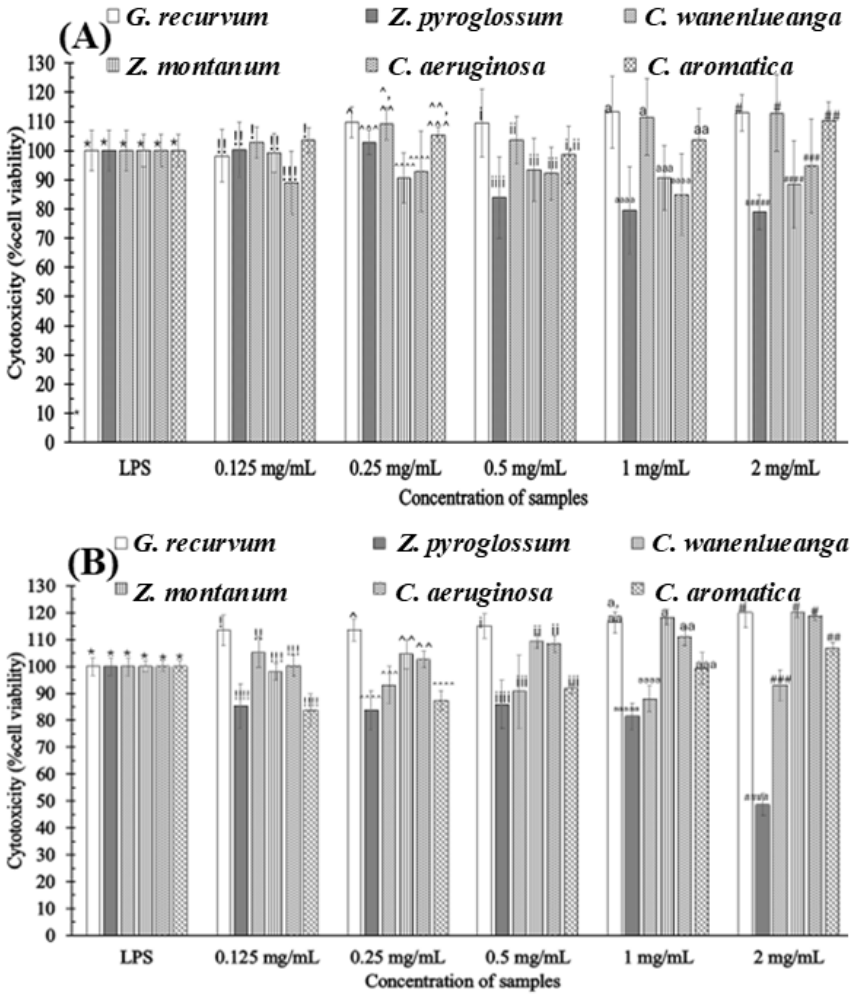


Figure 7 Effect of ethanolic extracted (A) and aqueous extracted (B) of six gingers on cell viability. In each graph, the symbols above the bars indicate significant differences at $p < 0.05$. Error bars display ± S.D. (n = 3).

Table 3 Classification of the most acceptable ginger rhizome extracts based on their predominant bioactivities.

Primary bioactivity application	Recommended extract (Solvent)	Key finding supporting the classification
Antibacterial	*C. aromatica* (Ethanolic)	Highest TPC (5.87 mg/g extract) and exclusively exhibited strong inhibition against *B. subtilis* (MIC 25.63 mg/mL).
Anti-inflammatory	*Z. montanum* (Ethanolic)	Highest NO reduction (75%) in RAW 264.7 cells while maintaining high cell viability (>80%).
Antioxidant (Radical Scavenging)	*C. aeruginosa* (Ethanolic)	Strongest DPPH free radical scavenging capacity (IC_{50} = 0.66 mg/mL).
Antioxidant (Metal Chelating)	*Z. montanum* (Aqueous & Ethanolic)	Highest ferrous ion-binding ability (IC_{50} = 0.14 - 0.16 mg/mL).

Therefore, the apparent NO reduction observed with the 2.000 mg/mL *Z. pyroglossum* aqueous extract may be primarily attributed to cytotoxicity rather than a true anti-inflammatory effect. In contrast, the NO inhibition observed with the other extracts is likely due to their genuine inhibitory effects on NO production, as supported by the high cell viability values maintained across all tested concentrations.

Conclusions

To have a broad outlook and to increase the practical utility of plants studied, a classification analysis was made using the different bioactivity results (**Table 3**). The twelve extracts were then categorized according to their predominant therapeutic potential and safety profiles from this analysis. The ethanolic extract of *C. aromatica* was the best acceptable candidate for antibacterial applications, which was supported by its high total phenolic content and exclusive inhibition zones against *Bacillus subtilis*. The ethanolic extract of *Z. montanum* is highly recommended for anti-inflammatory activity due to its remarkable ability to inhibit nitric oxide production without cytotoxicity in RAW 264.7 macrophages. Ethanolic extract of *C. aeruginosa* is classified as the best free radical scavenger with the lowest IC_{50} value in DPPH assay. On the other hand, some aqueous extracts like *Z. pyroglossum* showed evident anti-inflammatory activity, but were excluded from the recommended categories due to pronounced cytotoxicity. This systematic classification provides a scientific baseline of the local ginger species from Sakon Nakhon for specific herbal product development.

In accordance with this systematic classification, the experimental results indicated that the total phenolic content, as determined by the Folin-Ciocalteu method, was the highest in the ethanol extract of *C. aromatica* (5.87 mg /g extract). Ethanol extract of *C. aeruginosa* exhibited the highest antioxidant activity (IC_{50} is 0.66 mg/mL) by DPPH assay. The water extract of *Z. montanum* showed the highest iron-binding ability (IC_{50}, 0.14 mg/mL). The ethanolic extract of *C. aromatica* exhibited the highest antibacterial activity against the *Bacillus subtilis* TISTR 1248 with MIC is 25.63 mg/mL and MBC is 205.00 mg/mL among the tested extracts. Scanning electron microscopy (SEM) imaging of cell surface revealed clear evidence of damage of bacterial cells. In RAW 264.7 macrophage, anti-inflammatory analysis of ethanol extract of *Z. montanum* showed a reduction of 75% in nitric oxide production. Cell viability assay indicated that the survival rate of RAW 264.7 macrophages treated with aqueous and ethanolic ginger extracts was more than 80%. These results showed the potential of ginger rhizome extracts in the development of herbal products for antioxidants, anti-inflammatory and antibacterial applications.

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Declaration of generative AI in scientific writing

The authors employed Microsoft 365, ChatGPT and Copilot only for grammar and spelling correction when preparing this manuscript. The tool did not aid the creation of scientific content, data analysis, or interpretation. The authors are solely responsible for the final version of the manuscript, having thoroughly reviewed and edited all outputs.

CRediT author statement

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