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ARTICLE

In Vitro Anti-Bacterial and Anti-Fungal Activities of Extracts from Different Parts of 7 Zingiberaceae Plants

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ABSTRACT

This study aimed to explore the anti-bacterial and anti-fungal activities of extracts from different parts of plants in the Zingiberaceae family. The inhibitory rate, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of leaf and stem, and root and rhizome extracts from *Alpinia katsumadai* Hayata, *Alpinia oxyphylla* Miq × *Alpinia henryi* K. Schumann, *Alpinia oblongifolia* Hayata, *Alpinia nigra* (Gaertn.) Burtt, *Amomum villosum* Lour, *Alpinia zerumbet* (Pers.) Burtt. et Smith and *Alpinia oxyphylla* Miq were determined using the fungus cake method and double dilution method. The seven Zingiberaceae plants exhibited characteristic antibacterial activities against pathogenic bacteria and fungi. At a 1.5 mg mL^{-1}, *A. zerumbet* root and rhizome extracts exhibited strong inhibitory activity against *S. aureus* and *E. coli*, with 83.23% and 79.62%, respectively. In addition, *A. zerumbet* leaf and stem extracts had an inhibitory rate of 90.85% against *P. aeruginosa*. At the same concentration, the leaf and stem, root and rhizome extracts of *A. katsumadai* had the best antibacterial effect against *F. oxysporum*, with inhibition rates of 84.46% and 84.73%, respectively. Moreover, *A. katsumadai* and *A. zerumbet* leaf and stem extracts had the most significant antibacterial effect against *S. aureus*, with a MIC of 0.063 mg mL^{-1}. Thus, both *A. katsumadai* and *A. zerumbet* extracts had significant antibacterial activity. In addition, by comparing the inhibitory effect of extracts from different parts, it was found that the inhibitory rate and average inhibitory rate of extracts from leaf and stem were higher than those from root and rhizome. The chemical constituents of *A. katsumadai* and *A. zerumbet*, determined by the high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), revealed that citric acid (CA), alpinetin, and pinocembrin (PNCB) were the functional constituents yielding the antibacterial activity. Overall, *A. katsumadai* and *A. zerumbet* have the potential to be developed as new plant fungicides and bactericides.

KEYWORDS

Zingiberaceae; pathogens; *in vitro* antibacterial activity; minimum inhibitory concentration (MIC); minimum bactericidal concentration (MBC); high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS)

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Nomenclature

CA	citric acid
PNCB	pinocembrin
UPLC-MS/MS	high-performance liquid chromatography-tandem mass spectrometry

1 Introduction

Zingiberaceae consists of perennial herbaceous plants with a characteristic fragrance, and some plants are used as raw materials for a few Chinese herbal medicines [1,2]. But mostly, it has prostrate and horizontal rhizomes, massively expanding at the root ends. Zingiberaceae is mainly distributed in the tropical and subtropical regions and mostly in Asia, Africa, and tropical America, with about 57 genera and 1600 species identified worldwide [3,4]. The Zingiberaceae family is commonly used as medicinal materials, food, spices, cosmetics, or ornamental plants [5,6]. Specifically, it is rich in pharmacological activities, including antibacterial [7], anti-tumor [8], anti-oxidation [9], anti-allergy [10] and anti-inflammatory [11]. At present, many studies in China, Brazil and Japan have focused on the bacteriostasis of Zingiberaceae with respect to food hygiene [12], human health [13], animal and plant disease prevention [14,15]. Through research report shows that many Zingiberaceae plants on Gram-positive bacteria and Gram-negative bacteria have obvious growth inhibition. Among them, *Alpinia galanga* [16] and *Kaempferia galanga* [17] ethanol extracts, *Hedychium spicatum* [18] and *Amomum subulatum* [19] methanol extracts on *Staphylococcus aureus* have the obvious bacteriostatic action. At the same time, *Hedychium spicatum* [18] and *Amomum subulatum* [19] methanol extracts, *Zingiber officinale* [20] ethanol extracts also had a good growth inhibition effect on Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella*. In addition, the methanol extracts, ar-turmerone, and curcumin isolated from the *Curcuma longa* roots have antibacterial activities against *Phytophthora infestans*, *Fusarium solani*, and *Alternaria alternata* [21]. At present, it is known that Zingiberaceae plants contain chemical components with antibacterial effect such as linalool [22], beta pinene [22], kaempferol, gingerol [23], alpinetin [24], etc. These components may make Zingiberaceae plant extracts have an antibacterial effect, which is of development value as antibacterial agents.

Since Zingiberaceae plants contain flavonoids, alkaloids, phenolic acids, diarylheptanoids and other complex bioactive ingredients [9], many researchers have carried out various chemical and biological studies on Zingiberaceae. The roots, leaves, leaf stems, fruits, and flowers of plants in the Zingiberaceae family have different chemical components and pharmacological activities [11]. However, there are no reports that evaluated the antibacterial activity among Zingiberaceae plants' different parts. In the present study, the antibacterial activities of *Alpinia katsumadai* Hayata, *Alpinia oxyphylla* Miq × *Alpinia henryi* K. Schumann, *Alpinia oblongifolia* Hayata, *Alpinia nigra* (Gaertn.) Burtt, *Amomum villosum* Lour, *Alpinia zerumbet* (Pers.) Burtt. et Smith, *Alpinia oxyphylla* Miq were screened against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Fusarium oxysporum*, and *Pseudomonas solanacearum in vitro*. In addition, chemical constituents of Zingiberaceae were determined by UPLC-MS/MS. The findings in this study will provide data to support and a theoretical basis for developing natural plant bacteriostatic agents in the food industry to enhance their application value.

2 Materials and Methods

2.1 Test Plant Materials and Pathogen

The whole plant with rhizome of *A. katsumadai*, *A. oxyphylla* × *A. henryi*, *A. oblongifolia*, *A. nigra*, *A. villosum*, *A. zerumbet*, *A. oxyphylla* were obtained from the plant Germplasm Resources Base of Zhongkai University of Agriculture and Engineering. The samples were identified by Associate Professor Hu Xiu of Zhongkai University of Agriculture and Engineering. Then, the voucher specimens were deposited in the

ZKYDL herbarium with the following herbarium numbers; ZKYDL 041401 for *A. katsumadai*, ZKYDL 041402 for *A. oxyphylla* × *A. henryi*, ZKYDL 041403 for *A. oblongifolia*, ZKYDL 041404 for *A. nigra*, ZKYDL 041405 for *A. villosum*, ZKYDL 041406 for *A. zerumbet*, ZKYDL 041407 for *A. oxyphylla* for future references; We procured stocks of *S. aureus*, *E. coli*, *P. aeruginosa*, *F. oxysporum*, and *P. solanacearum* were purchased from Microbial Species Preservation Center.

2.2 Extraction Procedure

The whole plants with rhizome were washed and dried at room temperature (24°C) for five days, and further oven-dried at 40°C. The dried plants were divided into two parts. One part was leaves and stems, and the other part was roots and rhizomes. Materials were independently crushed, and passed through a 30-mesh sieve (Lvruo screen mesh). Next, refer to the Yakubu et al. [25] extraction method slightly modified, 0.05 kg of crushed leaves and stems, and roots and rhizomes were weighed and soaked in three changes of 70% ethanol in the ratio of 1:10 (m:V) at room temperature for 1 h each time. During the first extraction, we used ultrasonic-assisted extraction for 5 min. After extraction, we used rotate evaporation to paste the shape, and store in refrigerator at 4°C for future studies as a reserve.

2.3 Determination of Antibacterial and Antifungal Activity

According to the method of El-Tarabily [26], the growth inhibition activity of the plant extracts against the five pathogens was tested using the fungus cake method. Specifically, 50, 100, and 150 mg of extracts were dissolved in 1 mL dimethyl sulfoxide (DMSO), filtered using a 0.22 μm microporous membrane, then mixed in 99 mL medium without extracts to prepare medium containing extracts concentration of 0.5, 1.0, 1.5 mg mL^{-1}, poured into a petri dish with a diameter of 8.5 cm and waited for it to cool and solidify.

After 5 days incubation, AGAR plugs with a diameter of 5 mm were cut from the edge of the colony and inoculated in the medium containing extracts for 48 h (bacteria cultured at 37°C and fungi cultured at 28°C). We used medium without extracts as control. Then measure the diameter of the pathogen on the culture medium. Each treatment was replicated thrice. The inhibition rate was calculated using the formula below.

Colony diameter (cm) = measurement diameter – fungus/bacteria cake diameter (0.5 cm)

Inhibition rate = [(control colony diameter – treatment colony diameter)/control colony diameter] × 100%.

2.4 Preparation of Pathogens Suspension

A total of 3 mL of sterile water was added to the solid cultured media inoculated with the bacterial and fungal pathogens, and the colonies were scraped off using an inoculation ring. The scraped bacterial and fungal colonies were transferred into test tubes. An appropriate amount of sterile water was added to each test tube. The concentrations were adjusted to 1×10^8 CFU/mL using a McFarland turbidimetric tube after uniformly shaking the test tubes [27].

2.5 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC were determined using the double dilution method [28]. Precisely, 100 μL medium and 100 μL extracts (1.0 mg mL^{-1}) were added to 96 well plates. The concentration gradient was adjusted to 1.0, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0.008, 0.004, 0.002 and 0.001 mg mL^{-1} per well plate. Next, 20 μL bacterial/fungal solution was added to each well. In addition, 100 μL bacterial solution was added to the 12th well plate as the control. Finally, 15 μL of 0.5% triphenyl tetrazole chloride (TTC) was added to each well and incubated at a constant temperature for 24 h. The MIC for each extract was determined at the point when no red color was displayed in the well with the minimum concentration. From each well where no bacterial/fungal growth was observed, 100 μL of the well content was inoculated into an AGAR medium

and incubated 24 h (bacteria cultured at 37°C and fungi cultured at 28°C). The MBC for each extract was defined as the colony growth of less than 5.

2.6 Phytochemical Analysis of Extract by UPLC-MS/MS

Extraction of plant components: Extracts from leaves, stems, roots and rhizomes were freeze-dried in vacuum (ScientZ-100F) and ground (30 Hz, 1.5 min) with a grinder (MM 400, Retsch) to powder form. Powder (100 mg) was put in 1.2 ml 70% methanol to extract with 30 s vortex every 30 min for 6 times. The samples were put in the refrigerator at 4°C overnight. The supernatant was obtained after centrifuge (12000 RPM, 10 min), then filtered with microporous membrane for UPLC-MS/MS analysis. The effluent after UPLC was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

UPLC Conditions: The sample extracts were analyzed using a UPLC-ESI-MS/MS system (UPLC, SHIMADZU Nexera X2, MS, Applied Biosystems 6500 Q TRAP). The analytical conditions were used chromatographic column (Agilent SB-C18 1.8 µm, 2.1 mm * 100 mm) with mobile phase (A phase was ultrapure water (0.1% formic acid added), B phase was acetonitrile (0.1% formic acid added)). Elution gradient: the proportion of B phase was 5% (0.00 min), the proportion of B phase increased linearly to 95% (9.00 min), and remained at 95% (1 min), the proportion of B phase decreased to 5% (10.00–11.10 min), and balanced at 5% (14 min). The flow rate was 0.35 mL/min at 40°C of the column temperature with 2 µL of the injection volume.

The mass spectrum conditions: LIT and triple quadrupole (QQQ) scans were used on a triple quadrupole linear ion trap mass spectrometer (Q TRAP). AB6500 Q TRAP UPLC/MS/MS system was performed with ESI Turbo Ion Spray interface. Both positive and negative ion modes can be executed by Analyst 1.6.3 software (AB Sciex). Operating parameters of ESI source: ion source, turbine spray; source temperature 550°C; ion spray voltage (IS) 5500 V (positive ion mode)/−4500 V (negative ion mode); the ion source gas I (GSI), gas II (GSII) and curtain gas (CUR) (50 psi, 60 psi and 25.0 psi, respectively), and the collision-induced ionization parameter (high). Instrument tuning and quality calibration were executed with 10 and 100 µmol/L polypropylene glycol solution in QQQ and LIT mode, respectively. QQQ scan employs MRM mode and uses collision gas (nitrogen) as medium. DP and CE of each MRM ion pair were achieved by further DP and CE optimization. A specific set of MRM ion pairs was monitored at each period based on the eluted metabolites in each period.

2.7 Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Science (SPSS) software. Data were statistically described as frequencies and percentages. Origin 2022 was used for data graphing and mapping.

3 Results and Discussion

3.1 Inhibition Effects of Zingiberaceae Extracts to Pathogens

As can be seen from Figs. 1 and 2, the tested plant extracts had different antibacterial activities against the tested pathogens. On the one hand, the plant extracts had a higher inhibitory ability against food-borne pathogens than against plant-derived pathogens. The plant extracts had better antibacterial activities against *P. solanacearum* than the other four pathogens. Through data analysis, it also showed that plant extracts had certain inhibitory ability on *S. aureus*, *E. coli* and *P. aeruginosa*, indicating that the tested plant extracts had certain inhibitory effect on Gram-positive bacteria and Gram-negative bacteria. Moreover, the inhibitory ability of extracts on bacteria was stronger than that of fungi. Among them, plant extracts had the best inhibitory effect on *P. aeruginosa*, with an average inhibitory rate of 63.47% (1.0 mg mL^{-1}), and the inhibitory rate of leaf and stem extracts of *A. zerumbet* on *P. aeruginosa* was as high as 90.5%. In

addition, the growth inhibition effect of *A. zerumbet* root and rhizome extracts on *S. aureus* and *E. coli* was also significant, 83.23% and 79.62%, respectively. However, most of the extracts showed poor inhibitory effect on *P. solanacearum*, and some even showed no inhibitory activity. The inhibitory degrees of plant extracts on the tested pathogens were *P. aeruginosa* > *S. aureus* > *E. coli* > *F. oxysporum* > *P. solanacearum*.

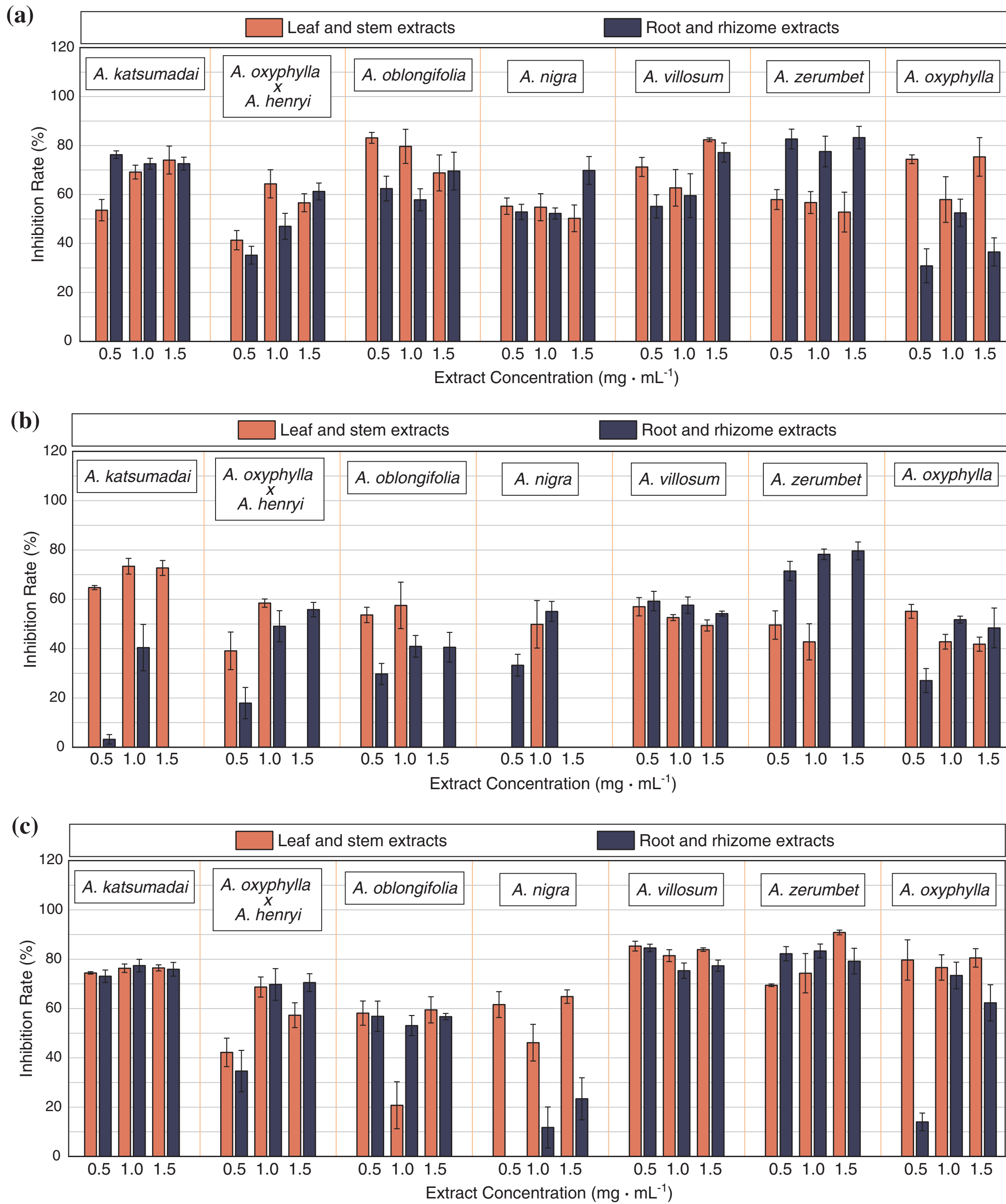

Figure 1: (Continued)

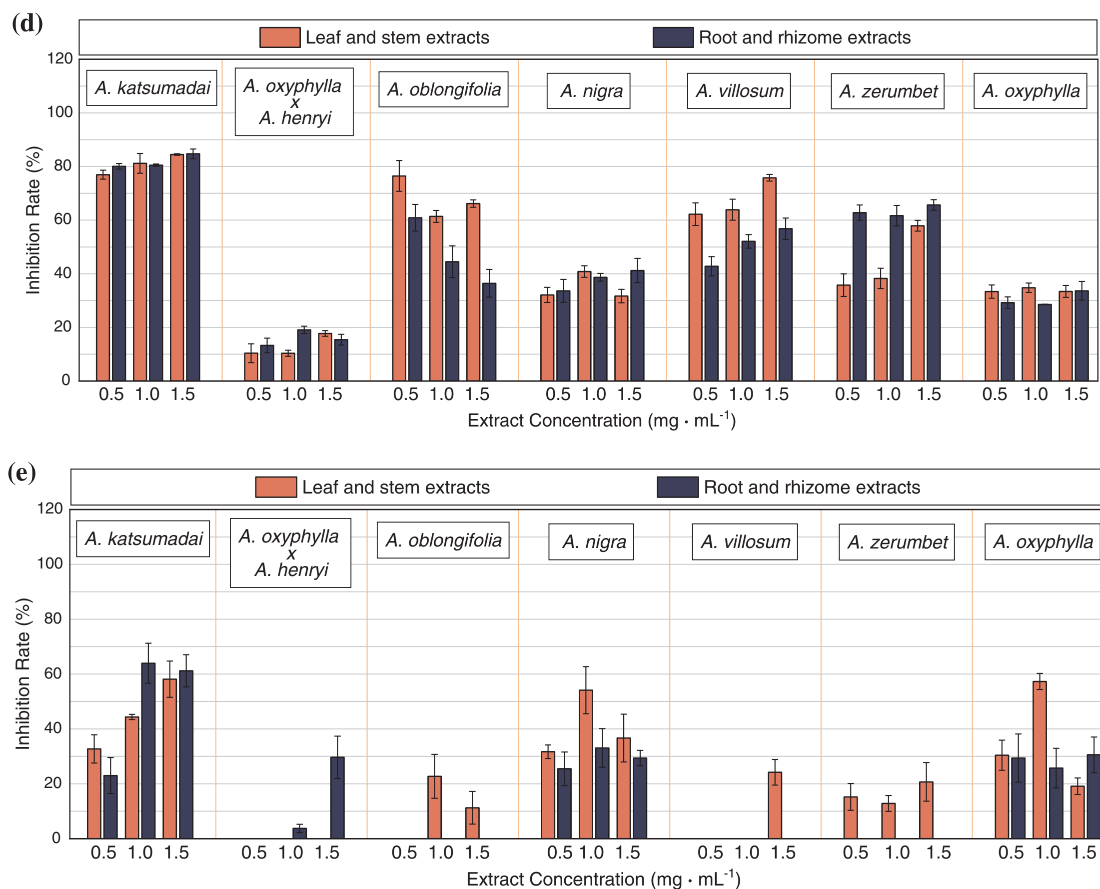

Figure 1: (a): Inhibitory effect of Zingiberaceae extracts against *S. aureus*, (b): Inhibition effect of Zingiberaceae extracts against *E. coli*, (c): Inhibition effect of Zingiberaceae extracts against *P. aeruginosa*, (d): Inhibition effect of Zingiberaceae extracts against *F. oxysporum*, (e): Inhibition effect of Zingiberaceae extracts against *P. solanacearum*

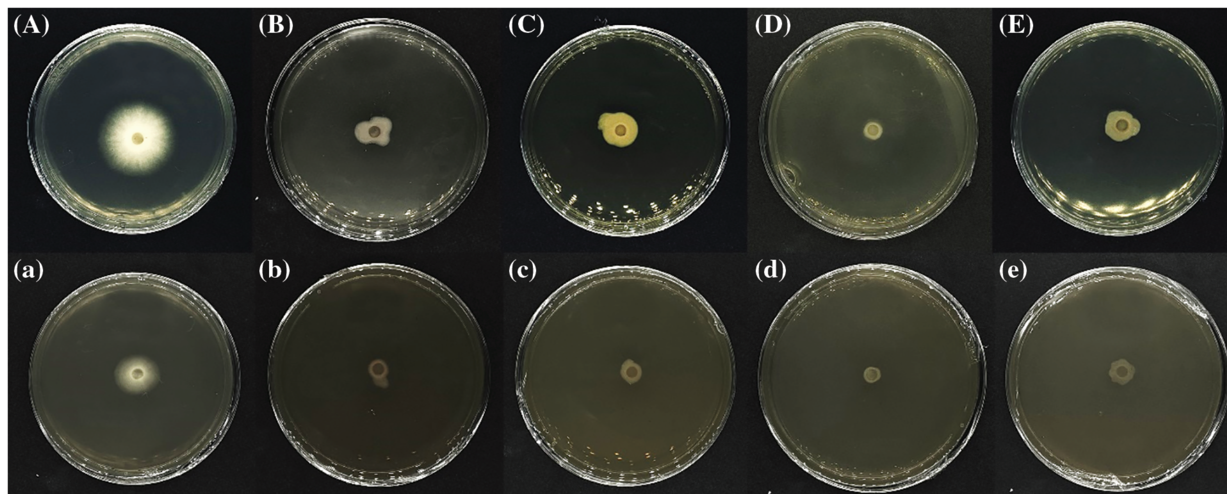

Figure 2: Effect on antimicrobial activity of *A. katsumadai* leaf and stem extracts. (A,a): *F. oxysporum*, (B, b): *S. aureus*, (C,c): *E. coli*, (D,d): *P. aeruginosa*, (E,e): *P. solanacearum*, (A~E): Culture medium without added extracts, (a~e): Culture medium supplemented with 1.5 mg mL^{-1} *A. katsumadai* leaf and stem extracts

On the other hand, the same extract also had different antibacterial activities against different pathogens, and there was no similarity in antibacterial spectrum and antibacterial activities of the same genus of plants. According to the comparison, the antibacterial effects of *A. katsumadai*, *A. villosum* and *A. zerumbet* were more outstanding, and their inhibition rate on *S. aureus* and *P. aeruginosa* can reach more than 80%, and the overall inhibitory effect was relatively average. Under the three concentration gradients, only a few extracts showed gradient changes in inhibition rate. For example, the inhibitory effect of extracts from leaf and stem, root and rhizome of *A. katsumadai* reached the best when the concentration was 1.5 mg mL^{-1}, and the inhibitory rate was 84.46% and 84.73%, respectively. The inhibitory rates of the extracts from leaf and stem, root and rhizome of *A. villosum* on *E. coli* and *F. oxysporum* also varied with the concentration. The inhibitory rates of *E. coli* were 57.01% and 59.27%, respectively, when the concentration was 0.5 mg mL^{-1}. But the inhibitory rates on *F. oxysporum* were the most significant when the concentration was 1.5 mg mL^{-1}. The inhibition rates were 75.79% and 77.35%, respectively. This indicated that it was not the higher the concentration of the extracts, the better the inhibitory effect of the pathogen, which may be related to the gradient span was not obvious. Furthermore, there were some differences in antibacterial effect between extracts from different parts of the plant (Fig. 3). The inhibitory rates of leaf and stem were higher than those of root and rhizome, and the inhibitory rates of leaf and stem extracts on *S. aureus* and *P. aeruginosa* were relatively concentrated, while the inhibitory rates of root and rhizome extracts were relatively dispersed.

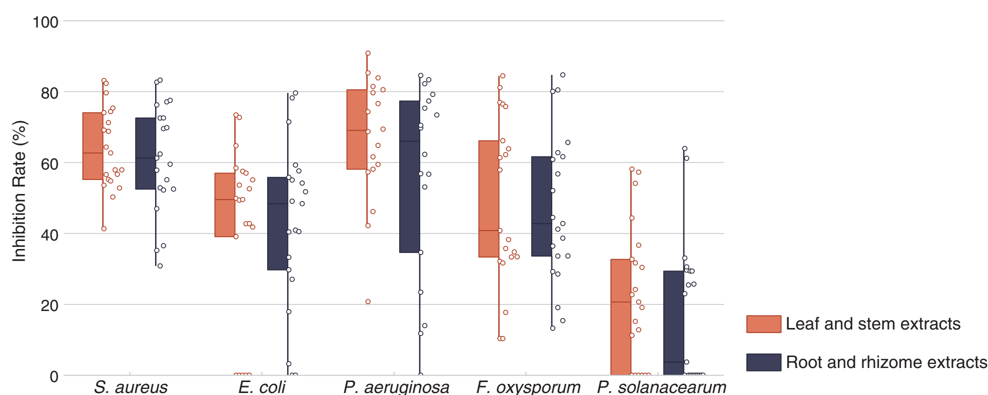

Figure 3: Comparison of antibacterial effects between leaf and stem, and root and rhizome extracts

3.2 The MIC and MBC of Zingiberaceae Extracts

The MIC of the 14 Zingiberaceae plant extracts differed across the different extracts (Table 1). The *A. katsumadai* leaf and stem extracts and *A. oxyphylla* × *A. henryi* root and rhizome extracts had the best antibacterial effect on *S. aureus*, the MIC and MBC of the two extracts were 0.063 and 0.5 mg mL^{-1}, respectively. Not only that, *E. coli* was sensitive to the leaf and stem extracts of *A. katsumadai*, with MIC of 0.25 mg mL^{-1} and MBC of 0.25 mg mL^{-1}. The root and rhizome extracts of *A. katsumadai* had the best antibacterial effect against *P. aeruginosa*, with MIC of 0.125 mg mL^{-1} and MBC of 0.25 mg mL^{-1}. However, the extracts from the leaf and stem of *A. oxyphylla* × *A. henryi* had a better antibacterial effect on *F. oxysporum*, with MIC and MBC of 0.125 and 0.5 mg mL^{-1}, respectively. The best antibacterial effect on *P. solanacearum* was expressed by the leaf and stem extracts of *A. zerumbet*, with MIC of 0.125 mg mL^{-1} and MBC of 0.5 mg mL^{-1}. Overall, a comprehensive analysis of MIC and MBC revealed that *A. katsumadai*, *A. oxyphylla* × *A. henryi*, and *A. zerumbet* leaf and stem extracts had a good antibacterial effect *in vitro*.

Table 1: The MICs and MBCs of extracts from 7 plants in the Zingiberaceae family

Plant extracts		*S. aureus*		*E. coli*		*P. aeruginosa*		*F. oxysporum*		*P. solanacearum*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Leaf and stem extracts	*A. katsumadai*	0.063	0.5	0.25	0.25	0.125	0.5	0.25	0.5	0.25	>1
	A. oxyphylla × *A. henryi*	0.125	1	0.25	0.5	0.125	0.5	0.125	>1	0.125	>1
	A. nigra	0.125	0.5	0.25	>1	0.125	>1	0.25	>1	0.125	>1
	A. oblongifolia	0.125	0.5	0.5	0.5	0.25	0.5	0.25	>1	0.125	1
	A. villosum	0.125	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	>1
	A. zerumbet	0.063	1	0.25	1	0.25	>1	0.25	>1	0.125	0.5
	A. oxyphylla	0.25	0.5	0.25	0.5	0.25	0.5	0.25	1	0.25	1
Root and stem extracts	*A. katsumadai*	0.125	0.5	0.25	0.5	0.125	0.25	0.25	0.5	0.25	>1
	A. oxyphylla × *A. henryi*	0.063	0.5	0.25	0.5	0.25	0.5	0.25	1	0.125	>1
	A. nigra	0.25	1	0.5	0.5	0.25	0.5	0.25	0.5	0.5	>1
	A. oblongifolia	0.063	1	0.25	0.5	0.25	0.5	0.25	0.5	0.125	>1
	A. villosum	0.125	1	0.25	1	0.25	0.5	0.25	1	0.125	>1
	A. zerumbet	0.25	>1	0.5	0.5	0.5	0.5	0.5	1	0.25	>1
	A. oxyphylla	0.125	0	0.25	0.5	0.25	0.5	0.25	0.5	0.25	>1

3.3 Phytochemical Composition of A. katsumadai and A. zerumbet Extracts by UPLC-MS/MS

The results of 3.1 and 3.2 showed that *A. katsumadai* and *A. zerumbet* had significant antibacterial effect, analysis of their chemical components using UPLC-MS/MS technology were presented in Table 2. Their total ions current were shown in Figs. 4 and 5.

Table 2: Chemical composition of *A. katsumadai* and *A. zerumbet* extracts

Serial number	*A. katsumadai* leaf and stem extracts	Relative content	*A. katsumadai* root and rhizome extracts	Relative content	*A. zerumbet* leaf and stem extracts	Relative content	*A. zerumbet* root and rhizome extracts	Relative content
1	L-Leucine	3.70%	4-Guanidinobutanal	3.26%	Citric Acid	3.34%	4-Guanidinobutanal	3.91%
2	L-Isoleucine	3.57%	Cycloleucine	3.26%	L-Isoleucine	3.14%	Cycloleucine	3.91%
3	Methylmalonic acid	2.15%	L-Pipecolic Acid	3.26%	L-Leucine	1.76%	L-Pipecolic Acid	3.91%
4	Succinic Acid	2.11%	L-Leucine	2.69%	L-Norleucine	1.65%	Citric Acid	3.23%
5	LysoPC 16:0	2.08%	L-Norleucine	2.66%	Quercetin-4′-O-glucuronide	1.64%	L-Isoleucine	2.65%
6	L-Valine	1.75%	L-Isoleucine	2.64%	LysoPC 18:2	1.62%	Methylmalonic acid	2.32%
7	LysoPC 18:1	1.74%	Citric Acid	2.51%	Quercetin-5-O-glucuronide	1.53%	3-Hydroxy-3-methylpentane^{-1},5-dioic acid	2.25%
8	LysoPC 18:2	1.71%	Methylmalonic acid	2.47%	LysoPC 16:0	1.46%	LysoPC 18:2	2.21%

(Continued)

Table 2 (continued)

Serial number	*A. katsumadai* leaf and stem extracts	Relative content	*A. katsumadai* root and rhizome extracts	Relative content	*A. zerumbet* leaf and stem extracts	Relative content	*A. zerumbet* root and rhizome extracts	Relative content
9	Citric Acid	1.69%	Succinic acid	2.42%	Sinapoylglucuronic acid	1.43%	LysoPC 18:1	2.08%
10	Alpinetin	1.55%	LysoPC 18:1	2.27%	Succinic acid	1.36%	LysoPC 18:2(2n isomer)	1.88%
11	1,7-diphenyl-4,6-diene-3-heptanone	1.54%	Pinocembrin (Dihydrochrysin)	2.14%	naphthisoxazol A	1.34%	LysoPC 16:0	1.81%
12	Pinocembrin (Dihydrochrysin)	1.49%	Alpinetin	2.09%	Kaempferol-3-O-(6″-acetyl)glucoside	1.33%	L-Norleucine	1.61%
13	3-Indoleacrylic acid	1.43%	LysoPC 18:2	1.96%	Methylmalonic acid	1.33%	Alpinetin	1.52%
14	Naphthisoxazol A	1.43%	L-Arginine	1.92%	LysoPC 18:2(2n isomer)	1.32%	Adenosine	1.46%
15	L-Phenylalanine	1.41%	1,7-diphenyl-4,6-diene-3-heptanone	1.71%	3-Indoleacrylic acid	1.32%	Pinostrobin Chalcone	1.46%

Note: The table lists displayed the top 15 phytochemical compounds in each extract.

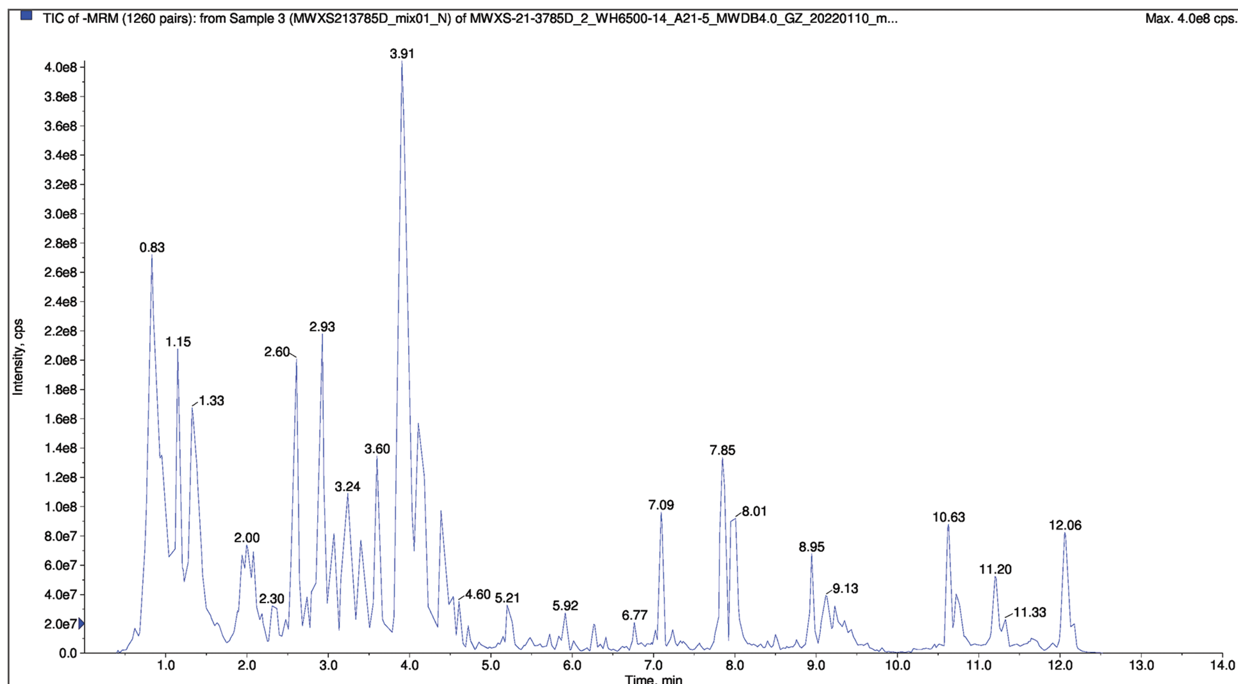

Figure 4: Total ions current (Anion mode)

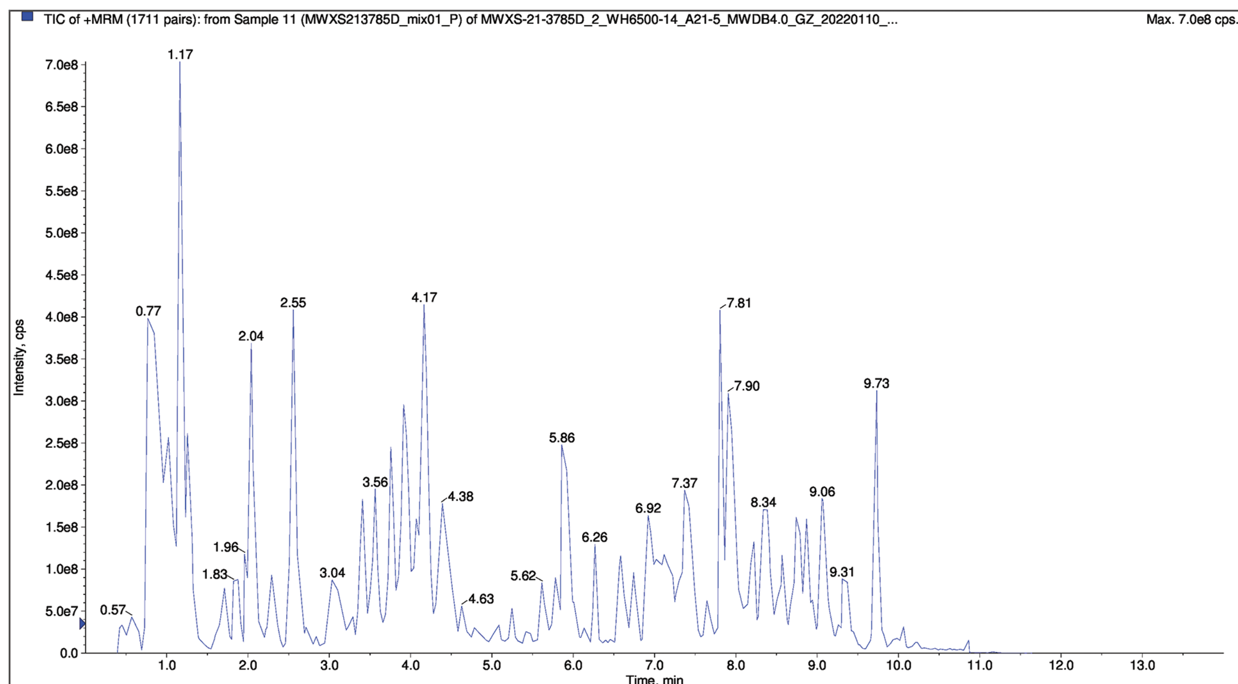

Figure 5: Total ions current (Positive ion mode)

The results showed that flavonoids, organic acids, lipids, amino acids and derivatives were the main components of the plant extracts (Fig. 6). Among them, flavonoids and organic acids have been proved to have significant antibacterial effects by most reports [29,30]. They were present in all kinds of plants, with high concentrations in vegetables and fruits, where they existed as secondary metabolites. In addition, they were highly valuable bioactive compounds with antioxidant, anti-inflammatory, neuroprotective, anti-cancer, and anti-diabetes effects [31,32].

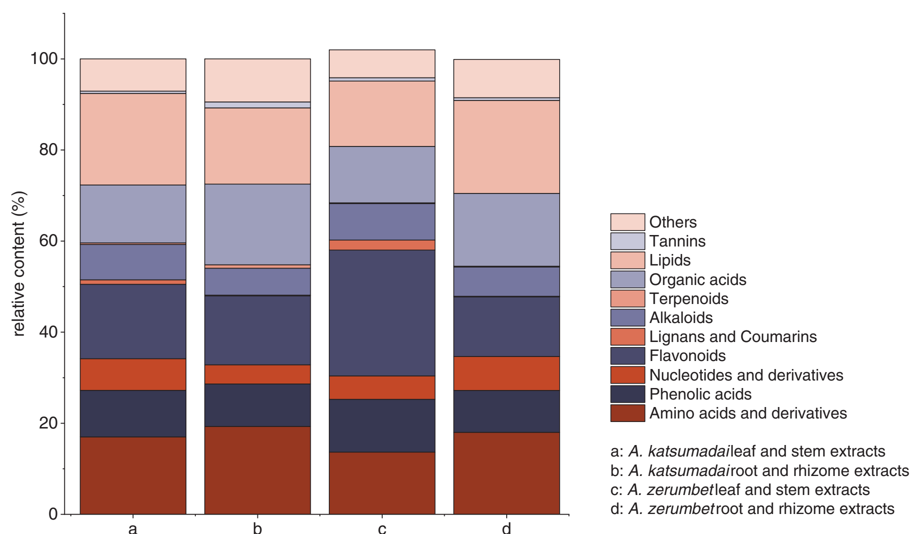

Figure 6: The proportion of each component in the extracts

Among the chemicals tested, CA, Alpinetin (Fig. 7) and PNCB (Fig. 8) were the ones with antibacterial activity. CA is an important organic acid, mostly existing in citrus plants, and has an antibacterial effect against *E. coli*, *Salmonella enterica*, *Shigella flexneri*, and *Enterococcus faecalis* [33–35]. Zhao et al. [36] observations under a scanning electron microscopy (SEM) revealed that *S. aureus* cells treated with CA lost their original shape, resulting in distorted and irregular shapes with holes on the surface, accounting for 29.2% of the damage to the cells. In addition, the microbial membrane structure was damaged by the natural CA compounds; a typical weak acid used as a food preservative by lowering the pH and killing or inhibiting the microbial growth. In addition, CA is used as a bivalent ions isolation agent for calcium and magnesium ions. However, the outer membrane of Gram-negative bacteria has an interference effect. PNCB has also been reported to have anti-*Staphylococcus aureus* effects *in vitro* and *in vivo* [37]. It inhibits cell damage mediated by *S. aureus* α-toxin, a porogen toxin expressed by most Staphylococci [38]. Similarly, alpinetin has broad-spectrum antibacterial activity, exhibiting inhibitory effects against most Gram-negative bacteria such as *E. coli* [24]. In addition, Chen et al. [39] found *in vitro* that alpinetin had good antibacterial activity against drug-resistant *Aeromonas Hydrophila*. Alpinetin could cause *Aeromonas Hydrophila* to shrink, damage cell wall and cell membrane, and cause loss of cytoplasm and internal cavitation. So it can be speculated that the antibacterial effect of alpinetin may be achieved by damaging bacterial cell walls and promoting cell membrane permeability.

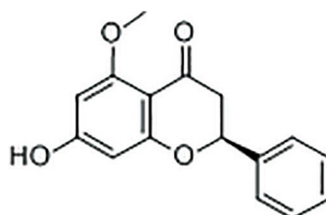

Figure 7: Chemical formula for alpinetin

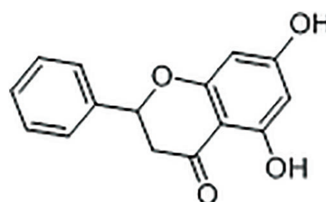

Figure 8: Chemical formula for PNCB

4 Conclusion

In recent advances, studies on the antibacterial activity of plants in the Zingiberaceae family have mostly focused on essential oils and single plant parts. However, in the present study, several Zingiberaceae plants' parts were studied for the first time, and their antibacterial activities were compared. In addition, the plant extracts with better antibacterial activity were screened to determine their phytochemical composition. Seven Zingiberaceae plants exhibited different degrees of antibacterial activity against five pathogens. In the case of pathogens, the inhibitory effect of all the extracts against bacterial pathogens was higher than that of the fungal pathogen. Among the tested plants, *A. zerumbet* extracts had the best inhibitory effect on bacteria(*P. aeruginosa*), with an inhibition rate of 90.85%. The *A. katsumadai* extracts had the best inhibitory effect on fungi (*F. oxysporum*), with an inhibition rate of 84.73%. Moreover, The MIC of bacteria (0.063 mg mL^{-1}) was lower than that of fungi (0.25 mg mL^{-1}).

There was no obvious gradient deviation in the inhibition rate of plant extracts on the pathogens at the three concentration gradients, which might be due to the insufficient gradient span. Further study could be conducted by increasing the concentration gradient. Antibacterial activity of extracts from leaf and stem was somewhat higher than that of extracts from root and rhizome in studies on different portions of Zingiberaceae plants, which should be paid more attention to in the future. At the same time, the pathogen inhibition rate and the matching MIC value are not always negatively correlated due to the varied activities of the components in the extracts.

According to the experimental results of inhibition rate and MIC, *A. katsumadai* and *A. zerumbet* were screened out to have prominent antibacterial activities. Specifically, the inhibitory effect of *A. katsumadai* and *A. zerumbet* extracts against *E. coli* was stronger than that of *Alpinia purpurta* extracts (MIC 21.12 mg mL^{-1}) [40]. In addition to Zingiberaceae plants, compared with plant extracts with a strong inhibitory effect on pathogens such as cinnamon (MIC 55 mg mL^{-1}) [41] and garlic (MIC 65 mg mL^{-1}) [41], the MIC of *E. coli* is also higher than that of *A. katsumadai* and *A. zerumbet* leaf and stem extracts. It can be seen that the antibacterial effect of *A. katsumadai* and *A. zerumbet* extracts is more significant. Through the determination of chemical composition, the extract can be identified as rich in flavonoids and organic acids. Among them, CA, Alpinetin and PNCB may be functional components with antibacterial effects. Hence, it is important to further investigate how the mixture of these chemicals is special for awarding antibacterial activities in these plants. However, the effects of different extraction conditions, including different solvents, solvent ratio, and extraction temperature, on compound yield necessitate further investigations [42]. In addition, the thermal stability and antibacterial activity of CA, alpinetin, and PNCB against pathogens should be explored to understand their antibacterial mechanism for the exploitation of plant resources [43].

Overall, more attention should be paid to the utilization of leaves and stems of Zingiaceae in the future, which can not only prevent the waste of resources on leaves and stems, but also promote the research and development of a new, safer and more natural antibacterial agent [44,45]. They have the potential to replace chemical synthetic antibacterial agents and have high application value.

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