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Chemical Composition and Effect of Aqueous Extract of Alpinia Oxyphylla Miq. on Inhibition of Salmonella Typhi

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Abstract: Objective: To investigate the separation and extraction process of the primary chemical constituents of Alpinia oxyphylla Miq. against Salmonella typhi, and its antibacterial efficacy, providing theoretical basis for novel gastrointestinal epidemic drugs and interventions against pathogen transmission. **Methods:** Alpinia oxyphylla Miq.: purified water-to-material ratio 1:5, refluxed at 100°C for 6 hours, repeated three times. Alpinia oxyphylla Miq. extract was concentrated by freeze-drying at a 30:1 solid-to-liquid ratio to obtain the final extract. The efficacy of the extraction method and antibacterial activity were assessed using the disk diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to establish the minimum therapeutic thresholds. Gas chromatography-mass spectrometry (GCMS) was employed to identify the primary bioactive constituents and their relative concentrations in the Alpinia oxyphylla Miq. aqueous extract. **Results:** The water extract of Alpinia oxyphylla Miq. Miq. demonstrated potent antibacterial activity against Salmonella typhi ($P < 0.001$), with an inhibition zone diameter of 8.43 ± 0.11 mm, MIC of 0.25 g/ml, and MBC of 0.5 g/ml. The extraction process was established as follows: Alpinia oxyphylla Miq. : purified water ratio 1:5, reflux heating at 100°C for 6 hours, repeated three times, followed by freeze-drying at 30:1 concentration. This yielded an extract rich in , primarily comprising octadecanoic acid (44.27%), palmitic acid (26.18%), caproic acid (8.15%), 10-hydroxydecanoic acid (7.83%) as the main components, supplemented by 3-hydroxy-dodecanoic acid (1.78%), cis-13-eicosenoic acid (1.58%), valeric acid (1.21%), heptanoic acid (1.13%), nonanoic acid (1.06%), oleic acid (0.87%), and butyric acid (0.47%). **Conclusion:** This extraction process for novel antimicrobial compounds from Alpinia oxyphylla Miq. demonstrates high stability, operational feasibility, and precise composition and efficacy. It provides a practical theoretical foundation for developing novel natural medicines against infectious diseases caused by Salmonella typhi, while offering further insights for antimicrobial drug development.

Keywords: Alpinia Oxyphylla Miq; Salmonella Typhi; Extraction; Antibacterial Activity

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1.Introduction

1.1 Research Background and Importance

Alpinia oxyphylla Miq., also known as Yizhizi or Yizhi, is the dried mature fruit of Alpinia oxyphylla Miq. (Zingiberaceae), primarily produced in Guangxi, Yunnan, Hainan, Guangdong, and Fujian provinces. It ranks among the Four Great Southern Medicinal Herbs. It possesses a warm nature and pungent flavour, containing active constituents such as zingerone, kaempferol, euryzine A, euryzine B, and euryzine alcohol. It exhibits effects of warming the spleen to arrest diarrhoea and consolidating essence to reduce urination. It is frequently employed for spleen-cold diarrhoea, abdominal cold pain, excessive salivation, kidney deficiency with enuresis, frequent urination, and seminal emission with turbid discharge. Modern applications include clinical use and fundamental research in diarrhoea, Alzheimer's disease, cancer, kidney disease, and bone disorders [1-5]. Salmonella typhi is one of the most prevalent serotypes within the Salmonella genus. As a zoonotic, foodborne pathogen affecting both humans and animals, it evades host immune clearance through immune escape mechanisms, leading to persistent infections that pose a severe threat to human health and public safety [6-10]. While extensive research exists on chemically synthesised drugs targeting Salmonella typhi, these present safety concerns and high production costs. Furthermore, research into natural medicines and bioactive compounds against this pathogen remains underdeveloped, significantly limiting its prevention, control, and treatment. Consequently, exploring novel natural medicines against Salmonella typhi infection holds considerable significance.

1.2 Research Objectives

This study employed a common and straightforward liquid solvent heating extraction process to isolate the active components of Alpinia oxyphylla Miq. against Salmonella Typhi, followed by high-fold concentration. Chemical composition and content analysis were conducted using GCMS. Bacteriostatic activity was evaluated using the zone of inhibition as an efficacy indicator, while MIC and MBC values were determined to establish dose-response relationships. This provides a scientific basis for the development and clinical application of new drugs based on the antimicrobial efficacy of natural products.

2.Materials and Methods

2.1 Materials

Salmonella Typhi CMCC (B) 50071 (Shanghai Luwai), Alpinia oxyphylla Miq. (Beijing Tongrentang), 0.22 μm needle filter, McFarland turbidity tubes (Hunan Bickman), Nutrient agar medium, Nutrient broth medium (Jinan Baibo), 204178 - Digital Vernier Caliper (SYNTEK, Germany), SPX-80B Biochemical Incubator (Tianjin Saidelisi), THZ-100 Constant Temperature Shaker Incubator (Shanghai Yetuo), Agilent 7890 B-5977 Gas Chromatography-Mass Spectrometry System (Agilent Technologies, USA), RV 10 auto pro V rotary evaporator (Germany, Eka), 6 mm × 0.7 mm sterile blank antimicrobial susceptibility discs (UK, Oxoid), WP-UPS-10/20 fully automatic water purifier (Sichuan, Watpu).

2.2 Methods

2.2.1 Separation and extraction process for active constituents from Alpinia Oxyphylla Miq. Aqueous Extract

Alpinia oxyphylla Miq. was pulverised and sieved through an 80-mesh screen. The powder-to-purified water ratio was 1:5. The mixture was soaked at room temperature for 2 hours, Reflux heated at 100°C for 6 hours, repeated three times. Centrifuged at 10,000 rpm for 1 hour; combine supernatants and freeze-dry at a 30:1 ratio to solidify the test drug. For use, dissolve in purified water to a concentration of 1 g/ml for subsequent experiments.

2.2.2 GCMS Analysis of Bioactive Components in Alpinia Oxyphylla Miq. Aqueous Extract

Gas chromatography employed an HP-5MS column (30 m × 0.25 mm × 0.25 μm) with a constant flow rate of 1 mL/min using helium as carrier gas. Column temperature followed the programmed ramping schedule in Table 1. Injection port temperature was 280°C, with a 1 μL injection volume and 2:1 split ratio. Mass spectrometry employed an electron impact ionisation source with an electron energy of 70 eV, an ion source temperature of 220°C, a transfer line temperature of 280°C, and a solvent delay of 3 minutes. Data were acquired in full scan mode over the range of 10–650 amu. GCMS data were analysed using Mass Hunter (VB.07.00) software for compound identification via integration. Search parameters were: SNR (signal-to-noise ratio) 2; Sharpness threshold 25%; Absolute height threshold 500 points; Relative height 0.1%. Automatic library searching utilised the NIST 17 database with a score threshold of 60; absolute height threshold set to 100 points; relative height threshold set to 0.5%.

Table 1: Temperature-programmed conditions

Temperature ramp rate	Temperature	Hold time/min	Hold time
	40	0	0
5	100	3	15
20	300	3	28

2.2.3 Efficacy of Alpinia Oxyphylla Miq. Aqueous Extract in inhibiting Salmonella typhi

Sterile solution (concentration 1 g/ml) was obtained by passing the solid-state final product aqueous solution through a 0.22 μm needle filter twice. Salmonella Typhi was diluted with sterile water to a concentration of 1.5×10^{7} cfu. Using a pipette, 20 μL of the bacterial suspension was dropped onto 9 cm nutrient agar plates and spread evenly. A sterile blank antibiotic susceptibility disc (6 mm diameter) was then placed on each plate. A 20 μL pipette volume of sterile drug solution was applied to fully saturate the disc for the treatment group. A purified water group was established as a control using the same method. Both groups were incubated in a 37°C incubator for 24 hours, after which the diameter of the inhibition zone was measured using calipers.

2.2.4 Dose-response relationship of Alpinia Oxyphylla Miq. Aqueous Extract in inhibiting Salmonella typhi

Dissolve 2 g of the solid final product in 2 mL sterile broth. Filter twice through a 0.22 μm needle filter. Prepare ten dilutions using a half-serial dilution method (initial drug concentration: 1 g/mL). Dilute Salmonella typhi in sterile water to a concentration of 1.5×10^{8} cfu using sterile water. Pipette 100 μl (neglecting this minute volume) into each group to achieve a bacterial concentration of 1.5×10^{7} cfu. Incubate at 37°C for 24 hours, then determine the MIC by observing turbidity. For all clear groups, mix thoroughly, then pipette 20 μl of the suspension onto 9 cm nutrient agar plates, spreading evenly. Incubate the plates at 37°C for 24 hours, then observe colony growth to determine the minimum bacterial concentration (MBC).

2.2.5 Statistical Methods

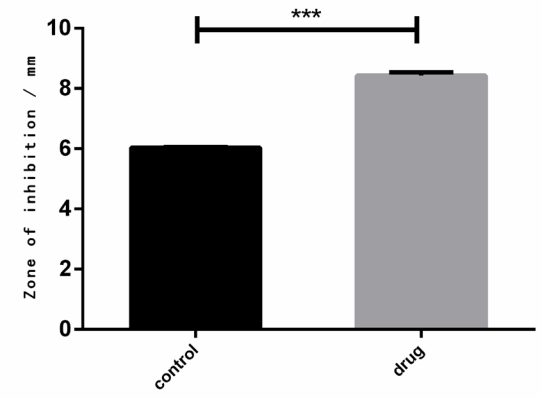
Statistical analysis was performed using GraphPad Prism 6.01 software. $P < 0.05$ was considered statistically significant.

3.Results

3.1 Inhibitory Effect of Alpinia Oxyphylla Miq. Aqueous Extract on Salmonella Typhi

The inhibition zone diameter in the treatment group was 8.43 ± 0.11 mm, while that in the control group was 6.04 ± 0.01 mm, demonstrating a significant difference ($P < 0.001$). This indicates that the water extract of Alpinia oxyphylla Miq. effectively inhibits Salmonella Typhi, as shown in Figure 1.

*Figure 1: Statistical diagram of inhibition zones（note: ***P < 0.001）*



3.2 GCMS Analysis of Bioactive Components in Aqueous Extract of Alpinia Oxyphylla Miq.

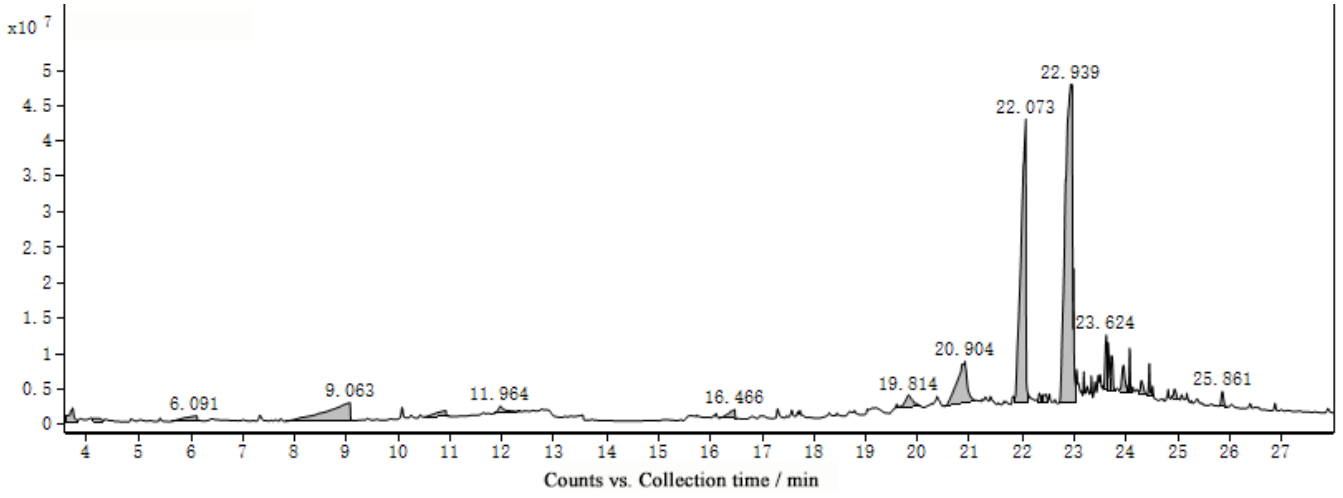
Analysis revealed four principal fatty acids: octadecanoic acid (44.27%), palmitic acid (26.18%), caproic acid (8.15%), 10-hydroxydecanoic acid (7.83%) as the four principal fatty acids, each constituting over 5% of the total. Additionally, seven minor fatty acids were identified: 3-hydroxy-dodecanoic acid (1.78%), cis-13-eicosenoic acid (1.58%), pentanoic acid (1.21%), heptanoic acid (1.13%), nonanoic acid (1.06%), oleic acid (0.87%), and butanoic acid (0.47%) as seven secondary representative organic acid components. These collectively constitute the anti-Salmonella typhimurium system of the water

extract of Alpinia oxyphylla Miq., as shown in Table 2 and Figure 2.

Table 2 : GCMS Analysis Results

Name	Molecular Formula	Retention time/min	Peak Area Percentage/%
cis-Vaccenic acid	C18H34O2	22.939	44.27
n-Hexadecanoic acid	C16H32O2	22.073	26.18
Hexanoic acid	C6H12O2	9.063	8.15
10-Hydroxydecanoic acid	C10H20O3	20.904	7.83
3-hydroxy-Dodecanoic acid	C12H24O3	19.814	1.78
cis-13-Eicosenoic acid	C20H38O2	23.66	1.58
Pentanoic acid	C5H10O2	6.091	1.21
Heptanoic acid	C7H14O2	10.903	1.13
Nonanoic acid	C9H18O2	16.466	1.06
Oleic Acid	C18H34O2	24.071	0.87
Butanoic acid	C4H8O2	4.215	0.47

Figure 2 : GCMS base peak chromatogram



3.3 Dose-response Relationship between Aqueous Extract of Alpinia Oxyphylla Miq. and Salmonella Typhi

MIC and MBC assays yielded MBC at 0.5 g/ml and MIC at 0.25 g/ml, indicating that higher concentrations of Alpinia oxyphylla Miq. extract are required to inhibit Salmonella typhi, demonstrating a clear dose-response relationship.

Conclusion

Digestive system infections severely impact human health, not only through direct tissue damage caused by pathogens but also through associated complications or comorbidities. The persistent emergence of drug-resistant pathogens and frequent outbreaks place a continuous burden on society and public finances, making the pursuit of more effective, low-toxicity, and economical therapeutic agents crucial [11-16]. Natural products and their derivatives, already successfully applied clinically, offer inherent advantages such as safety, accessibility, high efficacy, and low toxicity, making them ideally suited to address this pressing issue. Therefore, the effective isolation and extraction of antibacterial active components from these sources, coupled with the evaluation of their antimicrobial effects, can lay the theoretical foundation for traditional Chinese medicine in combating digestive system infections.

This study employed a straightforward technique of reflux extraction with water to isolate water-soluble components from Alpinia oxyphylla Miq.. Utilising GC-MS omics analysis, it identified a diverse array of antibacterial active constituents, primarily comprising organic acids such as octadecanoic acid, palmitic acid, hexanoic acid, and 10-hydroxydecanoic acid. Organic acid compounds, being common substances with broad sources and high safety profiles, exhibit well-established antibacterial efficacy. Their effectiveness has been demonstrated in studies on digestive system infections such as Helicobacter pylori and Salmonella [17-19]. The extraction of numerous organic acid compounds in this study provides technical assurance for the practical application of this process route. Furthermore, significant antibacterial activity against Salmonella typhi was observed ($P < 0.001$), with an inhibition zone diameter of 8.43 ± 0.11 mm. The minimum inhibitory concentration (MIC) was determined to be 0.25 g/ml, with MBC at 0.5 g/ml. This indicates a positive correlation between inhibitory strength and drug concentration, demonstrating the feasibility of applying this extraction process to typhoid Salmonella drug development and providing reference for its rational clinical application.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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