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Phytochemical screening and antibacterial activity of Zingiber officinale rhizome extracts from Sierra Leone

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Abstract

Background: Antimicrobial resistance (AMR) threatens effective treatment of common infections, particularly in low- and middle-income countries, and has renewed interest in plant-derived therapeutics (Ventola, 2015; Hughes and Andersson, 2017; Yousfi *et al.*, 2021) [18, 9, 21]. Ginger, *Zingiber officinale*, has a long history in traditional medicine and contains phenolic and terpenoid constituents with reported antimicrobial activity (Ali *et al.*, 2008; Grzanna *et al.*, 2005) [2, 7].

Aim: To evaluate the phytochemical composition and *in vitro* antibacterial activity of solvent extracts of *Zingiber officinale* rhizomes cultivated in Sierra Leone.

Methods: A laboratory-based study was conducted from February to May 2025. Fresh rhizomes were authenticated and processed; powdered material was Soxhlet-extracted using ethanol, petroleum ether, and distilled water (400 g per solvent). Crude extracts were concentrated and screened qualitatively for major phytochemical classes. Antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella spp.* was assessed by agar-well diffusion on Mueller-Hinton agar using ciprofloxacin (5 micrograms) as positive control and DMSO as negative control (CLSI, 2020) ^[5].

Results: From 1,200 g of powdered rhizome, 15.58 g of crude extract was obtained (overall yield 1.30% w/w). Per-solvent yields were ethanol 9.29 g (2.32%), petroleum ether 3.43 g (0.85%), and water 2.86 g (0.71%). Phytochemical screening indicated tannins, flavonoids, phenols, carbohydrates, anthraquinones, alkaloids, and terpenoids, with ethanol extract showing the richest profile. Zones of inhibition (mm) for ethanol extract were 23.0 (*S. aureus*), 16.0 (*Klebsiella spp.*), and 14.0 (*E. coli*); petroleum ether: 12.0, 17.0, 9.0; aqueous: 10.0, 15.0, 8.0, respectively. Ciprofloxacin produced 27-29 mm zones; DMSO showed no inhibition.

Conclusion: Solvent selection strongly influenced both extraction efficiency and antibacterial activity, with the ethanol extract of *Z. officinale* demonstrating the greatest chemical diversity and inhibitory effects, particularly against *S. aureus*. Findings support ginger as a promising source of antibacterial agents and justify further quantitative and MIC/MBC-guided investigations (Ali *et al.*, 2008; Semwal *et al.*, 2015; Mao *et al.*, 2019) ^[2, 16, 12].

Keywords: Zingiber officinale, phytochemical screening, Soxhlet extraction, antibacterial activity, agar well diffusion, ciprofloxacin, Sierra Leone, Staphylococcus aureus, Escherichia coli, Klebsiella spp.

Introduction

The global burden of infectious diseases remains substantial and is compounded by the rapid rise of antimicrobial resistance (AMR), jeopardizing public health and socioeconomic development (Ventola, 2015; Hughes and Andersson, 2017) [18, 9]. Drivers of AMR include antibiotic misuse/overuse, inadequate infection prevention, and a shortage of new antibiotics, prompting urgent exploration of alternative strategies (Yousfi *et al.*, 2021) [21].

Medicinal plants provide structurally diverse bioactive compounds. Ginger (*Zingiber officinale*) has been used across Chinese, Ayurvedic, and Greek medicine for more than two millennia and features in historical records from Egypt and Europe (Awe *et al.*, 2013; Gigon, 2012; Kochhar, 1986; Alakali *et al.*, 2009) [3, 6, 17]. It exhibits anti-inflammatory, antioxidant, antimicrobial, antifungal, and antiviral properties (Grzanna *et al.*, 2005; Wang and Ng, 2005; Ali *et al.*, 2008; Mahboubi, 2019) [7, 19, 13]. Despite its extensive ethnomedical use, the antibacterial properties of Sierra Leonean ginger remain under-characterized, warranting a focused evaluation.

This study investigated the phytochemical composition and antibacterial activity of Z. officinale extracts prepared with solvents of differing polarity and compared there *in vitro* activities against clinically relevant bacteria (Sebiomo *et al.*, 2011; Karuppiah and Rajaram, 2012) [15, 10].

Materials and Methods

Study design and sites

A laboratory-based experimental study was conducted from February to May 2025 at the Department of Pharmaceutical Sciences Laboratory, College of Medicine and Allied Health Sciences (COMAHS), University of Sierra Leone, and the Microbiology Laboratory at the Pharmacy Board of Sierra Leone (PBSL).

Plant material and authentication

Fresh rhizomes were purchased at a local market in Freetown and authenticated at the Department of Botany, Fourah Bay College. A voucher specimen was deposited (FWTA, ed. 2, 3:70; UPWTA, ed. 1, 474). Rhizomes were washed, sliced, shade-dried for two weeks, pulverized, and stored airtight.

Processing and Soxhlet extraction

Approximately 1,440 g powdered rhizome was obtained from ~ 4 kg fresh material. For extraction, 400 g was apportioned per solvent (ethanol, petroleum ether, distilled water). Powders were packed into cellulose thimbles; Soxhlet extraction was performed using a 250 mL round-bottom flask and condenser. Typical durations were ~ 3.5 h (petroleum ether), 4-6 h (ethanol), and ~ 6 h (water), continuing until siphon solvent clarified. Solvents were removed by rotary evaporation (aqueous concentrated on a 40-50 degrees °C water bath). Extracts were weighed and stored at 4 degrees C in amber bottles.

Qualitative phytochemical screening

Standard tests were employed: Molisch's, Fehling's, and

Benedict's (carbohydrates/reducing sugars); Mayer's (alkaloids); froth test (saponins); ferric chloride (tannins); magnesium/HCl (flavonoids); Borntrager's (anthraquinones); and Salkowski/chloroform-H2SO4 interface (terpenoids) (Trease and Evans, 1989; Harborne, 1973). Presence was recorded as strongly present (++), present (+), or absent (-).

Bacterial isolates and standardization

Reference/clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella spp*. were obtained from PBSL. Viability was confirmed on nutrient agar. Saline suspensions were adjusted to 0.5 McFarland standard prior to testing.

Antibacterial assay

Mueller-Hinton agar was prepared per manufacturer's directions. Plates were inoculated using sterile swabs. Agar wells (7 mm) were cut, specified volumes of each extract and controls were applied. Plates were incubated at 37 degrees C for 24 h. Ciprofloxacin (5 micrograms) served as positive control and DMSO as negative control. Zones of inhibition were measured in mm (CLSI, 2020) [5].

Data handling

Extraction yields were computed as (crude mass / initial plant mass) x 100. Zone diameters were summarized by organism and solvent.

Results

From 1,200 g of powdered rhizome, 15.58 g of crude extract was obtained (overall yield 1.30% w/w).

Table 1: Percentage	e yield of <i>Zingib</i>	er officinale ex	tracts by solvent
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Solvent	Crude extract mass (g)	Percentage yield (%)
Ethanol	9.29	2.32
Petroleum ether	3.43	0.85
Distilled water	2.86	0.71

Table 2: Phytochemical composition of Zingiber officinale extracts

Phytochemical component	Ethanol extract	Petroleum ether extract	Aqueous extract
Tannin	++	+	+
Flavonoid	++	++	-
Phenol	++	++	+
Saponin	-	+	=
Carbohydrate	++	++	++
Anthraquinones	++	+	++
Alkaloids	++	+	+
Terpenoids	+	++	+

Table 3: Zones of inhibition (mm) for Zingiber officinale extracts and controls

Isolate	Ciprofloxacin (5 micrograms)	DMSO	Ethanol extract	Petroleum ether extract	Aqueous extract
Staphylococcus aureus	29	0	23	12	10
Klebsiella spp.	28	0	16	17	15
Escherichia coli	27	0	14	9	8

Discussion

Solvent polarity governed both extraction yields and chemical profiles. Ethanol afforded the highest yield and the richest phenolic/flavonoid pattern, consistent with reports that alcohols better solubilize gingerols, shogaols, and other phenolics than water, whereas non-polar solvents enrich terpenoids (Ali *et al.*, 2008; Semwal *et al.*, 2015; Mao *et al.*, 2019; Ravindran *et al.*, 2016) ^[2, 16, 12, 14].

The ethanol extract exhibited the largest inhibition zones overall, notably against *Staphylococcus aureus*, while Gramnegative bacteria showed comparatively smaller zones aligned with outer-membrane permeability barriers. The relatively strong activity of the petroleum ether extract against *Klebsiella spp.* suggests contributions from sesquiterpene-rich fractions (Ravindran *et al.*, 2016) [14]. Ciprofloxacin outperformed all extracts as expected; DMSO had no effect,

validating that activity derived from plant constituents (CLSI, 2020) [5].

Putative mechanisms for ginger-derived antibacterials include membrane disruption, leakage of intracellular contents, and interference with energy metabolism (Semwal *et al.*, 2015; Wang *et al.*, 2020) [16, 20].

Limitations

- Qualitative phytochemical screening may yield operatordependent calls, chromatographic quantitation (e.g., HPLC/LC-MS/GC-MS) was not performed.
- MIC/MBC determinations were not conducted, agar well diffusion underestimates poorly diffusing/non-polar actives.
- Extract dosing/viscosity across wells was not standardized in the record; this may influence zone diameters.
- Dryness to constant mass was not documented; trace solvent could slightly inflate crude mass.

Conclusion

Ethanol, petroleum ether, and aqueous extracts of *Zingiber officinale* rhizome demonstrated solvent-dependent differences in composition and antibacterial activity. Ethanol yielded the greatest extract mass and phytochemical breadth and showed the strongest inhibition, particularly against *Staphylococcus aureus*. These findings substantiate ginger's potential as a source of antibacterial agents and motivate further, standardized potency testing and quantitative chemistry to guide development.

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