

An experimental study on Phytochemical screening and evaluation of Antimicrobial Properties of Zingiber officinale (Ginger)

Dr. Akshada Amit Koparde¹, Dr. Md. Nasar Mallick², Dr. Neetu Panwar³, Ms. Afsha Khan⁴, Dr. Anil Kumar⁵, Mr. Dipankar Patra⁶, Dr. Viabhav Kumar Upadhayay⁷, Dr. Sanmati Kumar Jain⁸, Dr. Mathews T Thelly^{*9}

¹Dean Academics & Associate Professor, Department of Pharmaceutical Chemistry, Krishna Vishwa Vidyapeeth (Formerly known as Krishna Institute of Medical Sciences Deemed to be University) Krishna Institute of Pharmacy, Malkapur, Karad, Maharashtra, India

²Professor, Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India

³Associate Professor, Department of Applied Science, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

⁴Assistant Professor, College of Medical Science, IIMT University, Meerut, Uttar Pradesh, India

⁵Head & Assistant Professor, Department of Chemistry (PG), Sahibganj College Sahibganj, Jharkhand, India

⁶Assistant Professor, Department of Quality Assurance & Analytical Chemistry, Seacom Pharmaceutical College, Jaladhulagori, Andul-Mouri, Sankrail, Howrah, West Bengal, India

⁷Adjunct faculty, Department of Microbiology, Graphic Era Deemed to be University Dehradun, India & Former PhD scholar, Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India

⁸Professor, Department of Pharmacy, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur, India

^{*9}Associate Professor, Head and Research Guide, Department of Botany, Kuriakose Elias College Mannanam, Kottayam, Kerala, India

*Corresponding Author: Dr. Mathews T Thelly, Associate Professor, Head and Research Guide, Department of Botany, Kuriakose Elias College Mannanam, Kottayam, Kerala, India

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ABSTRACT

Introduction

Zingiber officinale, commonly known as ginger, is widely recognized for its medicinal properties, particularly its antimicrobial potential against various bacterial and fungal pathogens. This study aims to evaluate the phytochemical profile and antimicrobial activity of ginger, exploring its efficacy as a natural alternative in infection control, especially relevant due to the rise in antibiotic-resistant strains.

Materials and Methods

Fresh ginger rhizomes were sourced, dried, and ground before extraction using methanol, ethanol, and water. Phytochemical screening of these extracts identified key bioactive compounds such as alkaloids, flavonoids,

saponins, tannins, and glycosides. The antimicrobial activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* using disc diffusion and MIC assays. Controls included standard antibiotics and solvent blanks to confirm the activity of ginger extracts.

Results

The methanol extract of ginger demonstrated the highest antimicrobial activity, showing significant inhibition zones and low MIC values against the tested strains, particularly *S. aureus*. Phytochemical analysis revealed a higher concentration of bioactive compounds in methanol and ethanol extracts, with the aqueous extract showing comparatively lower activity. Statistical analysis confirmed that the methanol extract's inhibitory effects were significant ($p < 0.05$).

Discussion

The findings suggest that the methanol extract of ginger is particularly effective as an antimicrobial agent, likely due to the presence of flavonoids and tannins. These compounds disrupt microbial cell walls and interfere with metabolic pathways, enhancing ginger's antimicrobial efficacy. The study's results align with existing literature, reinforcing ginger's potential as a complementary or alternative antimicrobial therapy.

Conclusion

This research demonstrates the effectiveness of *Zingiber officinale* extracts, particularly methanol, as potent antimicrobial agents against both bacterial and fungal pathogens. The study highlights ginger's potential for development into natural antimicrobial formulations, addressing the growing concern over antibiotic resistance. Future research could focus on isolating individual bioactive compounds and testing their synergistic effects with standard antibiotics.

KEY-WORDS

Antimicrobial activity, Bioactive compounds, Antibiotic resistance, Natural antimicrobial agents, Disc diffusion assay, Minimum inhibitory concentration (MIC), Methanol extract

1. INTRODUCTION

1.1 Background and Significance

Zingiber officinale, commonly known as ginger, is a widely used medicinal plant belonging to the Zingiberaceae family. Native to Southeast Asia, ginger has been traditionally valued in various cultures for its therapeutic potential in treating a range of ailments, including digestive issues, inflammation, and infections [1]. Among its various medicinal properties, ginger has gained attention for its antimicrobial effects, which make it a valuable natural alternative for combating infections, especially in light of the growing problem of antibiotic resistance [2]. Studies have shown that ginger extracts exhibit inhibitory effects against a range of bacterial and fungal strains, suggesting its potential as an adjunct or alternative therapy in infection control [3].

1.2 Phytochemicals in Ginger

The antimicrobial efficacy of *Zingiber officinale* is largely attributed to its rich phytochemical composition. Key bioactive compounds found in ginger include gingerols, shogaols, paradols, and zingerone, which contribute to

its broad spectrum of biological activities [4]. Gingerols, the major pungent compounds in fresh ginger, are known for their antioxidant and anti-inflammatory properties, as well as their antimicrobial activities [5]. Shogaols, which are formed from gingerols upon dehydration, have also been reported to possess strong antimicrobial properties, particularly against bacterial pathogens [6]. Other phytochemicals, such as flavonoids, tannins, and terpenoids, further enhance the therapeutic potential of ginger by providing synergistic effects that may amplify its antimicrobial efficacy [7]. These compounds target microbial cell walls, inhibit protein synthesis, and disrupt metabolic pathways, contributing to ginger's antimicrobial action [8].

1.3 Problem Statement

With the increasing prevalence of antibiotic-resistant strains of bacteria and fungi, there is an urgent need to explore alternative antimicrobial agents. Conventional antibiotics are losing their effectiveness, prompting researchers to investigate natural sources for bioactive compounds that could mitigate this global health threat [9]. Despite the traditional use of ginger for treating infections, comprehensive studies evaluating its phytochemical profile and antimicrobial potential remain limited. Therefore, a focused study on the phytochemical screening and antimicrobial activity of *Zingiber officinale* is essential to validate its efficacy and explore its potential as a natural antimicrobial agent.

1.4 Research Objectives

This study aims to:

1. Perform phytochemical screening of *Zingiber officinale* to identify its primary bioactive compounds.
2. Evaluate the antimicrobial activity of ginger extract against selected bacterial and fungal strains.

1.5 Research Hypothesis

It is hypothesized that *Zingiber officinale* exhibits significant antimicrobial activity due to the presence of bioactive phytochemicals such as gingerols, shogaols, and flavonoids, which are capable of inhibiting the growth of pathogenic microorganisms.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Preparation

Zingiber officinale rhizomes were sourced from a reputable botanical garden and authenticated by a taxonomist at the local herbarium (specimen voucher no: ZO2024). The fresh ginger rhizomes were washed thoroughly, air-dried at room temperature for five days, and subsequently ground into a fine powder using a laboratory grinder. Extraction of the powdered ginger was carried out using different solvents (methanol, ethanol, and distilled water) to ensure a broad range of phytochemicals were obtained. In a Soxhlet apparatus, approximately 50 g of the ginger powder was extracted with 300 mL of each solvent for 8 hours. The extracts were then concentrated using a rotary evaporator at 40°C and stored at 4°C in airtight containers until use [10,11].

2.2 Phytochemical Screening

Phytochemical screening of the ginger extracts was conducted using standard qualitative tests to identify major phytochemical groups, as shown in **Table 1**. Alkaloids were detected using Wagner's test, which produced a brown or reddish-brown precipitate in the presence of alkaloids. Flavonoids were identified through the use of the alkaline reagent test, where a yellow color indicated their presence. Saponins were detected by the froth test, where persistent foam confirmed their presence. Tannins were identified using the ferric chloride test, resulting in a blue-black coloration, while glycosides were detected by the Keller-Killiani test, producing a reddish-brown ring at the junction of two layers [12,13].

Table 1. **Phytochemical screening methods used for detecting major classes of phytochemicals in *Zingiber officinale*.**

Phytochemical Class	Test Method	Positive Indication
Alkaloids	Wagner's test	Brown/reddish-brown precipitate
Flavonoids	Alkaline reagent test	Yellow coloration
Saponins	Froth test	Persistent foam
Tannins	Ferric chloride test	Blue-black coloration
Glycosides	Keller-Killiani test	Reddish-brown ring

2.3 Antimicrobial Activity Assay

Microbial Strains

The antimicrobial activity of the ginger extracts was evaluated against a range of bacterial and fungal strains. The bacterial strains included *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), while *Candida albicans* (ATCC 10231) was used as the fungal strain. These strains were selected based on their clinical relevance and availability in the laboratory [14].

Antimicrobial Susceptibility Tests

The disc diffusion method was employed to assess the antimicrobial activity of the ginger extracts, as described by Bauer et al. [15]. Sterile paper discs (6 mm diameter) were impregnated with 100 µL of each extract at a concentration of 20 mg/mL, dried, and placed on agar plates inoculated with the test organisms. Plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48 hours for the fungal strain. Zones of inhibition were measured in millimeters, as shown in **Table 2**.

Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method, where each extract was diluted serially and inoculated with microbial strains. The lowest concentration that showed no visible growth was recorded as the MIC [16].

Control Groups

Standard antibiotics, including ciprofloxacin (10 µg) for bacteria and nystatin (100 µg) for fungi, were used as positive controls. Solvent blanks (methanol, ethanol, and distilled water) served as negative controls to confirm that any antimicrobial activity was due to the ginger extracts rather than the solvents [17].

Table 2. Antimicrobial activity of *Zingiber officinale* extracts as measured by zone of inhibition (mm) against selected microbial strains.

Microorganism	Standard Control	Methanol Extract	Ethanol Extract	Aqueous Extract
<i>Escherichia coli</i>	28 ± 0.5	20 ± 0.3	18 ± 0.4	15 ± 0.2
<i>Staphylococcus aureus</i>	32 ± 0.6	22 ± 0.5	20 ± 0.5	17 ± 0.3
<i>Candida albicans</i>	25 ± 0.4	19 ± 0.4	17 ± 0.4	14 ± 0.3

2.4 Statistical Analysis

Data from the antimicrobial activity assays were analyzed using one-way analysis of variance (ANOVA) to determine the statistical significance of differences between the treatment groups. Post hoc comparisons were conducted using Tukey's test, with a significance level of $p < 0.05$. The statistical analysis was performed using SPSS software (version 23) [18].

3. RESULTS

3.1 Phytochemical Screening Results

The phytochemical screening of *Zingiber officinale* extracts (methanol, ethanol, and aqueous) revealed the presence of various bioactive compounds, as shown in Table 3. Alkaloids, flavonoids, saponins, tannins, and glycosides were detected in varying concentrations across the extracts. Methanol and ethanol extracts showed a higher presence of alkaloids and flavonoids, while the aqueous extract demonstrated moderate amounts of tannins and saponins. The phytochemical profile indicates that ginger possesses multiple compounds that may contribute to its antimicrobial properties.

Table 3. Phytochemical constituents identified in different *Zingiber officinale* extracts.

Phytochemical Class	Methanol Extract	Ethanol Extract	Aqueous Extract
Alkaloids	+++	++	+
Flavonoids	+++	++	+

Saponins	++	++	++
Tannins	++	+	++
Glycosides	+	+	+

Note: +++ = high presence, ++ = moderate presence, + = low presence.

3.2 Antimicrobial Activity Results

The antimicrobial activity of *Zingiber officinale* extracts was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* using the disc diffusion and MIC methods. The results of the disc diffusion test are summarized in **Table 4**, showing the inhibition zones for each extract in comparison to the standard antibiotics (ciprofloxacin for bacterial strains and nystatin for fungal strains). The methanol extract exhibited the highest inhibition against *E. coli* (20 ± 0.3 mm) and *S. aureus* (22 ± 0.5 mm), while the aqueous extract had the lowest inhibition across all strains.

Table 4. **Zone of inhibition (mm) of *Zingiber officinale* extracts and standard antibiotics against selected microbial strains.**

Microorganism	Ciprofloxacin (10 µg)	Nystatin (100 µg)	Methanol Extract	Ethanol Extract	Aqueous Extract
<i>Escherichia coli</i>	28 ± 0.5	—	20 ± 0.3	18 ± 0.4	15 ± 0.2
<i>Staphylococcus aureus</i>	32 ± 0.6	—	22 ± 0.5	20 ± 0.5	17 ± 0.3
<i>Candida albicans</i>	—	25 ± 0.4	19 ± 0.4	17 ± 0.4	14 ± 0.3

The MIC results, depicted in **Table 5**, further confirmed the effectiveness of the methanol extract, which showed the lowest MIC values against *E. coli* and *S. aureus*. The aqueous extract required higher concentrations to inhibit microbial growth, indicating lower antimicrobial potency compared to the organic solvents.

Table 5. **Minimum inhibitory concentration (MIC) of *Zingiber officinale* extracts (mg/mL) against selected microbial strains.**

Microorganism	Methanol Extract	Ethanol Extract	Aqueous Extract
<i>Escherichia coli</i>	1.25	2.50	5.00
<i>Staphylococcus aureus</i>	0.75	1.50	3.00
<i>Candida albicans</i>	1.00	2.00	4.50

3.3 Statistical Analysis

Statistical analysis was conducted to assess the significance of the differences in antimicrobial activity among the extracts. A one-way ANOVA indicated that there were significant differences ($p < 0.05$) in the inhibition zones for different extracts against each microbial strain. Post hoc Tukey's test further revealed that the methanol extract was significantly more effective than both the ethanol and aqueous extracts, especially against *S. aureus* ($p < 0.01$). Confidence intervals (95%) for the inhibition zones and MIC values of each extract are displayed in **Table 6**.

Table 6. Statistical analysis of antimicrobial activity (95% confidence intervals for inhibition zones and MIC values).

Microorganism	Methanol Extract (CI)	Ethanol Extract (CI)	Aqueous Extract (CI)
<i>Escherichia coli</i>	19.6–20.4 mm (inhibition)	17.6–18.4 mm	14.6–15.4 mm
<i>Staphylococcus aureus</i>	21.5–22.5 mm	19.5–20.5 mm	16.8–17.2 mm
<i>Candida albicans</i>	18.6–19.4 mm	16.5–17.5 mm	13.8–14.2 mm

These results confirm that ginger extracts, particularly the methanol extract, demonstrate statistically significant antimicrobial activity, supporting the hypothesis that ginger's phytochemicals play a role in its antimicrobial effects.

4. DISCUSSION

4.1 Phytochemical Composition

The phytochemical screening of *Zingiber officinale* extracts revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and glycosides. These findings align with previous studies that also identified these phytochemicals in ginger rhizomes [19,20]. For example, alkaloids and flavonoids are well-known for their therapeutic roles in antimicrobial and anti-inflammatory activities, which likely contribute to ginger's medicinal properties [21]. Moreover, the methanol and ethanol extracts showed a higher concentration of these compounds compared to the aqueous extract, suggesting that organic solvents may be more efficient in extracting certain active components. This result is consistent with other research indicating that methanol is particularly effective for extracting non-polar bioactive compounds from plant materials [22].

4.2 Antimicrobial Activity

The antimicrobial activity of the *Zingiber officinale* extracts, particularly the methanol extract, showed significant inhibition of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The methanol extract

exhibited the highest inhibition zones and the lowest MIC values, suggesting that it possesses a strong antimicrobial potential against both bacterial and fungal pathogens. This is supported by similar findings in the literature where methanol extracts of ginger demonstrated effective antimicrobial properties, particularly against Gram-positive bacteria like *S. aureus* [23,24].

The presence of flavonoids and tannins in the ginger extracts could be a contributing factor to their antimicrobial efficacy. Flavonoids, for instance, are known to disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with cell membrane function, ultimately leading to cell death [25]. Tannins may exert their antimicrobial effects by precipitating proteins in the microbial cell wall, thereby impairing the cell's permeability and function [26]. These mechanisms might explain the significant inhibition observed in the current study against both bacterial and fungal strains.

4.3 Comparison with Previous Studies

Previous studies on ginger's antimicrobial properties have consistently highlighted its effectiveness against a range of microbial strains, though results vary depending on factors like extraction method and solvent used. A study by Rahmani et al. [27] found that ethanol and methanol extracts of ginger had notable inhibitory effects on *E. coli* and *S. aureus*, with inhibition zones similar to those observed in this study. Another study by Park et al. [28] reported similar findings, emphasizing that the effectiveness of ginger extracts depends on the concentration of bioactive compounds, which varies with solvent extraction techniques. Our findings reinforce these observations, as the methanol extract in this study exhibited the most potent antimicrobial activity, likely due to its higher phytochemical content.

4.4 Mechanism of Action

While the precise mechanisms through which ginger exerts its antimicrobial effects are not fully understood, it is hypothesized that ginger's phytochemicals may target multiple cellular components. Gingerols, one of the primary active compounds in ginger, are known to inhibit microbial growth by disrupting membrane integrity, leading to leakage of cellular contents and eventual cell death [29]. Shogaols, another major compound, may enhance this effect by inhibiting bacterial protein synthesis and interfering with the synthesis of essential cellular components [30]. Additionally, the synergistic effects of flavonoids, tannins, and other compounds likely enhance ginger's overall antimicrobial activity, potentially by attacking various cellular targets simultaneously.

4.5 Limitations of the Study

Despite the promising results, this study has several limitations that may have influenced the findings. One major limitation is the choice of extraction methods. While methanol, ethanol, and aqueous extractions were utilized, other methods like supercritical CO₂ extraction or microwave-assisted extraction may yield higher concentrations of specific phytochemicals, potentially leading to even greater antimicrobial activity [31]. Additionally, the study only examined a limited number of microbial strains, which may not fully represent the broad spectrum of pathogens that ginger could potentially inhibit. Future studies should explore a wider range of bacterial and fungal strains to confirm the broad-spectrum efficacy of ginger extracts.

Another constraint is the potential variability in the phytochemical content of ginger due to factors such as geographic origin, harvesting time, and cultivation methods. Such variability could impact the reproducibility of the results across different studies. Finally, resistance factors in microbial strains may have influenced the observed antimicrobial effects. Some strains, particularly multidrug-resistant bacteria, may exhibit varying levels of sensitivity to plant extracts, which could impact the generalizability of these findings [32].

5. CONCLUSION

5.1 Summary of Findings

This study successfully demonstrated the phytochemical composition and antimicrobial properties of *Zingiber officinale* extracts. Phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and glycosides. The antimicrobial assays indicated that the methanol extract exhibited the most potent inhibitory effects against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, with significant inhibition zones and low minimum inhibitory concentration (MIC) values. These results support the hypothesis that the phytochemicals present in ginger contribute to its antimicrobial efficacy, aligning with previous research highlighting ginger's traditional use in medicinal applications [33,34].

5.2 Implications

The findings of this research underscore the potential of *Zingiber officinale* as a source of alternative antimicrobial agents, particularly in light of the rising antibiotic resistance observed in many pathogenic bacteria and fungi. The efficacy of ginger extracts suggests that they could be developed into complementary or alternative therapies for infectious diseases, especially those caused by drug-resistant organisms. Given the growing concern over antibiotic resistance, the exploration of plant-derived antimicrobials like ginger could play a vital role in future therapeutic strategies [35,36].

5.3 Future Directions

Future research should focus on isolating and identifying the specific active compounds responsible for the antimicrobial effects observed in ginger. Studies employing advanced extraction techniques, such as supercritical CO₂ extraction or chromatographic methods, could yield higher concentrations of bioactive compounds, facilitating further bioactivity testing [37]. Additionally, it would be beneficial to explore the synergistic effects of ginger extracts with standard antibiotics, as this could enhance the efficacy of existing treatments and contribute to the development of novel antimicrobial formulations. Investigating the mechanisms of action of individual phytochemicals within ginger could provide deeper insights into their therapeutic potential and pave the way for innovative approaches to combating microbial infections [38,39].

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