Investigating the Antibacterial and Antioxidant Properties of *Zingiber officinale* (Ginger) Extracts

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Abstract

Ginger (*Zingiber officinale*), a well-known medicinal plant used in traditional medicine, has been studied for its potential antibacterial and antioxidant properties. This study evaluated the phytochemical profile, antioxidant activity, and antibacterial efficacy of methanol and aqueous ginger rhizome extracts. Phytochemical screening confirmed the presence of vital bioactive compounds such as flavonoids, alkaloids, glycosides, phenols, and saponins, with methanol extracts demonstrating higher bioactive content. Antioxidant capabilities were measured using DPPH radical scavenging assay, revealing that methanol extracts exhibited strong antioxidant activity (98.60% at 400 µg/mL) with an IC_{50} of 9.06 µg/mL, while aqueous extracts showed 96.15% scavenging activity and an IC_{50} of 6.22 µg/mL. Antibacterial activity was evaluated using the Minimum Inhibitory Concentration (MIC) method against four bacterial strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Streptococcus pneumonia*. The methanol extract exhibited the strongest activity against *Streptococcus pneumonia* with an MIC of 2.44 mg/mL, while higher concentrations were required for *E. coli* and *K. pneumoniae*. These findings suggest that ginger extracts hold significant potential as natural antibacterial and antioxidant agents, supporting their traditional use in treating various infections and oxidative stress-related diseases. The results provide a scientific basis for the therapeutic potential of ginger in modern medicine.

Keywords: Ginger (*Zingiber officinale*), methanol extract, DPPH radical, HPLC, Medicinal plant, Spice, rhizome.

INTRODUCTION

Medicinal plants have long been the cornerstone for curing various pathological conditions across diverse cultures worldwide. According to the World Health Organization, approximately 80% of the global population relies on traditional medicine, which prominently includes plant extracts and their bioactive constituents [1]. These traditional healthcare systems utilize a wide variety of plant-based treatments, owing to their natural bioactive compounds, often referred to as phytochemicals, secondary metabolites, or bio-nutrients. These compounds are crucial for their therapeutic effects due to their clear physiological impacts on the human body [3]. Research has identified a broad spectrum of these phytochemicals—including alkaloids, chlorogenic acid, gallic acid, hydroquinone, tannins, terpenoids, flavonoids, among others—in various parts of plants such as the bark, leaves, flowers, and fruits, highlighting their diverse medicinal potential [1].

Ginger (*Zingiber officinale* Roscoe), a prominent member of the Zingiberaceae family, is believed to have originated in China before spreading to other parts of Southeast Asia and subsequently across various continents [11]. This plant is not only fundamental in culinary applications but also possesses a multitude of therapeutic, folk medicinal and nutritional benefits [5]. Traditionally, ginger has been utilized in both Chinese and Ayurvedic medicine systems to address a range of ailments such as nausea, diarrhea, stomach discomfort, and cardiac conditions [23]. It is also valued for its ability to enhance the palatability of medicinal preparations, stimulate bile production, alleviate joint pain associated with arthritis, and provide relief from respiratory and throat infections [17-18].

Pharmacologically, ginger's efficacy as an anti-inflammatory agent is well-documented through its ability to inhibit key enzymes like cyclooxygenase-1 and cyclooxygenase-2, leading to a reduction in prostaglandin synthesis and consequent inflammation [27]. Additionally, traditional literature and some empirical studies suggest that ginger possesses potent antioxidant and antibacterial properties, which are attributed to its unique phytochemical profile [1]. However, despite these traditional claims, there remains a gap in comprehensive scientific evidence, necessitating further investigation into ginger's antioxidative capabilities using methodologies like the DPPH assay. This study seeks to explore the presence and activity of phytochemicals in ginger extracts and to detail their specific antioxidant mechanisms.

Purified rhizome extracts of ginger have been consistently shown to possess significant antioxidant activity. These extracts contain a rich blend of phytochemicals such as gingerols,

shogaols, and zingerone, which are primarily responsible for combating oxidative stress [22-26]. The antioxidant capacity of these extracts is typically assessed through various established assays, including DPPH, FRAP, and ABTS, which measure their effectiveness in scavenging free radicals [16]. Beyond their antioxidant properties, these extracts also contain a variety of other beneficial phytochemicals like terpenoids, flavonoids, and phenolic compounds. These constituents are linked to a range of health benefits, including anti-inflammatory, anti-cancer, and antimicrobial effects [2].

The current research is directed at an in-depth antibacterial, antioxidant, and phytochemical evaluation of *Zingiber officinale's* bioactive purified rhizome. The study aims to determine the IC_{50} activities of ginger extracts (both aqueous and methanol) and identify the active constituents responsible for these bioactivities. Through this investigation, the study hopes to solidify the biomedical value of ginger, contributing to a deeper understanding of its health benefits and potential therapeutic applications.

METHODOLOGY

Collection and Preparation of Ginger Rhizome Extracts

Fresh ginger rhizomes were sourced from local markets in Noida, U.P., India. Upon collection, the rhizomes were thoroughly washed under tap water, air-dried, and subsequently homogenized into a fine powder using mechanical grinders. The powdered rhizomes were stored in airtight containers to prevent degradation of active compounds. For extraction, 10 grams of this powdered material was mixed with 25 mL each of methanol and water in separate conical flasks. These mixtures were subjected to Soxhlet extraction for a continuous period of 48 hours, facilitating comprehensive phytochemical leaching. Post-extraction, the resultant solutions were filtered through Whatman No. 1 filter paper to remove particulate residues. The clear filtrates were then concentrated using a water bath under reduced pressure until dryness, yielding semi-solid extracts. These extracts were sealed in containers and stored at 4°C for further phytochemical and antioxidant evaluations.

Solvent Extraction Preparation

The previously mentioned powdered ginger material was combined with methanol and distilled water. This mixture was filtered through Whatman No. 1 filter paper to ensure removal of all solid particles. The filtrate was then placed on an orbital shaker at 100 rpm and left overnight to enhance the extraction process. The resultant dry extracts were weighed and stored at 4°C in a refrigerator to preserve their chemical integrity for future tests.

Comprehensive Phytochemical Screening

1. Alkaloids Detection: To determine alkaloid content, different sections of the methanol extract were treated with Mayer's, Dragondorff's, and Wagner's reagents. The formation of cream, orange, and brown precipitates respectively indicated the presence of different types of alkaloids [21].

2. Flavonoids Evaluation: Flavonoid content was assessed using the Shinoda test. Pieces of magnesium ribbon and a few drops of concentrated hydrochloric acid (HCl) were added to the extract. A change to pink or tomato-red color indicated the presence of flavonoids [1].

3. Tannins Identification: Tannins were detected by adding a few drops of alcoholic ferric chloride ($FeCl_3$) solution to the extract. A resultant blue color confirmed the presence of tannins [21].

4. Cardiac Glycosides Testing: The Keller-Kilian test was used for cardiac glycosides. To the dry extract powder, 1 mL of $FeCl_3$ reagent (a mix of 5% $FeCl_3$ and glacial acetic acid) was added followed by a few drops of concentrated sulfuric acid. A greenish-blue color development indicated the presence of cardiac glycosides [2].

5. Steroids Analysis: For steroids, 1 gram of the extract dissolved in 10 mL of chloroform was layered with strong sulfuric acid. A yellow layer with green fluorescence and a red supernatant indicated the presence of steroids [24].

6. Saponins Determination: The Frothing test involved vigorously shaking the extract with distilled water. The formation of a stable foam higher than 1.5 cm indicated saponins [21].

7. Phenols Test: Phenol presence was confirmed by adding 5 drops of 10% ferric chloride solution to the extract. The appearance of blue or green color indicated phenols [20].

DPPH Antioxidant Activity Assay

Antioxidant activity of the ginger extracts was quantified using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. For this assay, 5 mL of 0.004% DPPH solution was mixed with 50 µL of the extract at concentrations ranging from 100 to 400 µg/mL. The mixture was vortexed and incubated for 30 minutes in a dark environment at room temperature. Absorbance was measured at 520 nm using a spectrophotometer, with 80% methanol serving as the blank and ascorbic acid as a positive control [16-28]. This assay provides a measure of the extract's ability to act as a free radical scavenger, indicative of its antioxidant potential. There will be three copies of each measurement. The following equation is used to determine the DPPH scavenging effect: DPPH radical scavenging activity (%) =

$$= \frac{\text{(Absorbance of control - Absorbance of the sample)}}{\text{Absorbance of control}} \times 100$$

(Absorbance of control)

Where:

The absorbance of control = Absorbance of DPPH radical + methanol

The absorbance of sample = Absorbance of DPPH radical + sample extract /standard.

Isolation of Bioactive Components

Bioactive compounds were isolated using column chromatography with a silica gel column as the stationary phase. The silica gel used (mesh size 60–120, E. Merck, 0.063–0.200 mm) was packed into a glass column of appropriate dimensions, typically chosen based on the sample quantity. Before loading the sample, the silica gel was activated by heating at 105°C for 2 hours to remove moisture, which could affect the separation process. The column was equilibrated with a suitable solvent system based on the isolated compounds' polarity.

The sample containing the bioactive compounds was dissolved in a minimal amount of the starting solvent and gently loaded onto the silica column. Elution was carried out using a gradient of solvents that increased polarity with methanol. Fractions were collected regularly and monitored for bioactivity or specific chemical properties through thin-layer chromatography (TLC).

The collected fractions that showed similar retention times or spots on TLC plates were pooled and concentrated using a rotary evaporator. These pooled fractions were further purified using High-Performance Liquid Chromatography (HPLC). In HPLC, a reverse-phase C18 column was typically used, with a mobile phase consisting of methanol.

The HPLC process involved monitoring at specific wavelengths, generally based on UV absorbance or fluorescence, to detect the bioactive compounds. The retention time and peak shape provided initial indications of compound purity. Final pure fractions were collected, evaporated, and stored at 4°C.

Antibacterial Activity

Test for Microorganisms

The microorganisms used in this study for antimicrobial testing were clinical isolates obtained from the National Centre for Microbial Resource (NCMR). The strains included *Escherichia coli* (MCC 3094), *Klebsiella pneumoniae* (MCC 3094), *Bacillus subtilis* (MCC 2010), and *Streptococcus pneumonia* (MCC 2425). These bacteria were chosen for their relevance in both clinical infections and research concerning drug resistance. The strains were maintained on Luria Bertani (LB) media at the laboratory of Allele Life Sciences, Noida, Uttar Pradesh, India, following standard protocols for culture maintenance

Preparation of Culture Media

To prepare Luria Bertani (LB) agar, 35 g of agar powder was dissolved in 500 ml of distilled water, adjusted to 1 litre, and autoclaved at 121°C for 15 minutes at 15 psi. After cooling to 40°C, the medium was poured into sterile petri dishes and incubated overnight at 37°C to ensure sterility. The plates were stored at 4°C. For LB broth, 20 g of powder was dissolved in distilled water, sterilized similarly, incubated overnight at 37°C in a shaker, and stored at 4°C. Brain Heart Infusion (BHI) agar and broth were prepared by dissolving 52 g of powder in 1 litre of distilled water, autoclaved at 121°C for 15 minutes, and handled similarly to ensure sterility before storage at 4°C

Bacterial Culture

The bacterial strains used in this study, including *Escherichia coli* (MCC 3099), *Klebsiella pneumoniae* (MCC 3094), *Bacillus subtilis* (MCC 2010), and *Streptococcus pneumonia* (MCC 2425), were selected as standard control strains for quality assurance. These strains were obtained from the National Centre for Microbial Resource (NCMR) and were maintained on Luria Bertani (LB) agar to ensure the viability and purity of the bacterial cells. The initial bacterial cultures were prepared by streaking each strain onto fresh LB agar plates and incubating at 37°C. Fresh subcultures were prepared on the day of the experiment by inoculating 100 µL of the primary culture into 50 ml of LB broth. These cultures were incubated in a shaker at 200 rpm overnight (approximately 16-18 hours) at 37°C, allowing bacterial growth until visible turbidity developed. The turbidity was measured spectrophotometrically at 595 nm, ensuring the absorbance was maintained between 0.08–0.13, corresponding to a bacterial concentration equivalent to 0.5 McFarland standards (approximately 1×10^8 CFU/ml). The bacterial suspension was then diluted to a working concentration of 1×10^6 CFU/ml using sterile LB broth, ready for further experimental use in antimicrobial assays. This process ensured consistency in bacterial density across experiments and facilitated reliable data collection.

ELISA Technique

The Minimum Inhibitory Concentrations (MICs) of bacterial strains were determined using the standard micro broth dilution method in a sterile 96-well microplate format. Bacterial strains, including *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Streptococcus pneumonia*, were first cultured on Luria Bertani (LB) agar plates and incubated at 37°C for 16 to 24 hours to ensure optimal growth. After incubation, individual colonies were selected from the plates and suspended in LB broth, with the bacterial concentration

adjusted to match the 0.5 McFarland standards (approximately 1×10^8 CFU/ml). This standard ensures consistency in the number of bacterial cells used across all experiments.

In the micro broth dilution procedure, 100 µL of the bacterial suspension was inoculated into each well of a 96-well microtiter plate. The antimicrobial agents or plant extract preparations were serially diluted in LB broth, with 100 µL of each dilution added to the bacterial suspension wells. This created a final volume of 200 µL per well. The concentrations of the antimicrobial agents or extracts typically ranged from high to low to establish a gradient, allowing the determination of the lowest concentration capable of inhibiting bacterial growth.

To ensure accuracy and reproducibility, proper controls were maintained:

- a negative control (containing only broth without bacteria)
- a positive control (containing bacteria without the antimicrobial agent)
- a sterility control (containing broth alone to verify sterility)

After adding all components, the microtiter plates were incubated at 37°C for 16-24 hours without shaking. After incubation, bacterial growth in each well was assessed by measuring the optical density at 700 nm using a microplate reader. Wells without visible turbidity (i.e., those showing no bacterial growth) were recorded as the MIC, representing the lowest concentration of the antimicrobial agent that inhibited bacterial growth. The data were further analyzed to calculate MIC values for each bacterial strain tested. This method provides a reliable and reproducible approach to evaluating the efficacy of antimicrobial agents against specific bacterial strains.

Biofilm Formation

For biofilm formation, 200 µL of the bacterial suspension was carefully inoculated onto sterile coverslips placed inside sterile Petri dishes. The coverslips were used as a solid surface to promote the attachment and growth of biofilm-forming planktonic cells. The inoculated coverslips were spread evenly to ensure uniform distribution of the bacterial cells. The petri dishes were then sealed with parafilm to prevent contamination and minimize evaporation of the culture medium during incubation. The plates were incubated at 37 °C for 48 to 72 hours for sufficient biofilm formation. This incubation period facilitates the development of mature biofilms, which were subsequently used for further analysis. The biofilm growth was monitored visually throughout the incubation to ensure proper formation. Post-incubation, the coverslips were gently washed with sterile phosphate-buffered saline (PBS) to remove any non-adherent planktonic cells, leaving behind the established biofilm. The biofilms formed on

the coverslips were subjected to subsequent analyses, including microscopic examination and quantification assays.

RESULTS AND DISCUSSION

The extraction process of *Zingiber officinale* (ginger) rhizomes using methanol and water resulted in yields of 1.5% and 1.2%, respectively. These yields indicate the effectiveness of methanol as a solvent in extracting a higher amount of bioactive compounds from the ginger rhizome compared to water. Typically, the choice of solvent in the extraction process can significantly influence the quantity and types of phytochemicals extracted due to differences in solvent polarity (**Table 1**). Methanol, being more polar than water, can dissolve a wide range of polar compounds, hence a slightly higher yield. This finding is consistent with previous research, which suggests that methanol often extracts a higher quantity of phytochemicals due to its effectiveness in solubilizing various polar secondary metabolites [7-8].

Table 1: The percentage yield of solvent extracts of *Zingiber officinale* (Rhizome)

S.No.	Solvent	Weight of dried extract (g)	Yield (%)	Colour	Consistency
1	Methanol	15	1.5	Dark brown	Paste
2	Water	12	1.2	Light brown	Paste

The phytochemical analysis of the ginger extracts revealed varying presence and concentrations of key bioactive components such as alkaloids, glycosides, flavonoids, saponins, steroids, tannins, and phenols were found in both methanol and water extracts, indicating their broad solubility and significant role in plant defense mechanisms against microorganisms and insects. Glycosides were detected in methanol extracts but not in water extracts, suggesting that their extraction is more favorable in non-polar to moderately polar solvents (**Table 2**). Flavonoids, noted for their antioxidant properties, were more prevalent in methanol extracts, aligning with the solvent's ability to extract phenolic compounds effectively. Saponins showed a strong presence in methanol extracts, which is crucial given their role in plant defense and their potential therapeutic activities including antimicrobial and antioxidant properties. Phenols were highly present in both extracts, particularly in methanol, highlighting its efficiency in extracting these compounds known for their strong antioxidant capabilities.

These findings are crucial as they align with previous studies indicating that phenolic compounds and flavonoids exhibit significant antioxidant and anti-inflammatory activities

[5]. The presence of these compounds in ginger extracts supports the traditional use of ginger in treating inflammatory conditions and other oxidative stress-related diseases.

The presence of these diverse phytochemicals in ginger extracts underlines the plant's potential as a source of natural antioxidants, which could mitigate oxidative stress and inflammation, a common pathway in many chronic diseases [12]. The confirmation of these bioactive compounds in ginger supports its traditional uses and provides a scientific basis for its efficacy. This also aligns with the WHO's emphasis on the importance of traditional medicine, particularly in developing countries where modern healthcare is less accessible. Furthermore, the extraction yields and phytochemical content are in line with findings from other studies that have explored the chemical composition of ginger and its functionality as a medicinal plant. For example, the detection of saponins and phenols at significant levels correlates with their roles in antimicrobial and antioxidant activities, which are well documented in the literature [8-5].

Table 2: Phytochemical Screening of Methanol and Water Extracts

S/No.	Phytochemical Components	Names of Reagents Used	Name of Extracts	
			Methanol	Water
		Mayers test	++	++
1	Alkaloids	Dragondroff' s test	+	+
		Wagner's test	+	+
2	Glycosides	Keller-kiliani 's test	++	-
3	Flavonoids	Shinoda test	++	+
4	Saponins	H_2SO_4test	+++	+
5	Steroids	Frothing test	+	+
6	Tannins	Ferric chloride test	+	+
7	Phenols	$FeCl_3$	+++	++

Note (+++): Strongly positive, (++): positive, (+): Trace, and (–): Not detected

The DPPH radical scavenging assay conducted on ginger rhizome extracts using different solvents demonstrated that both methanol and water extracts exhibit significant antioxidant activity, with methanol extracts showing slightly higher activity. Specifically, at a concentration of 400µg/ml, the methanol extract exhibited a scavenging activity of 98.602±0.26%, while the water extract showed 96.148±0.117%. These activities were just

shy of the activity shown by the standard antioxidant, ascorbic acid, which was 99.472±0.026%. This pattern was consistent across different concentrations, indicating a dose-dependent increase in scavenging activity (**Table 3**).

The higher antioxidant activity observed in the methanol extract compared to the water extract can be attributed to the solvent's efficacy in extracting a broader range of phenolic and non-phenolic phytochemicals, which are well-known for their antioxidant properties. Methanol, being a polar solvent, is particularly effective in solubilizing phenolic compounds, which are responsible for scavenging free radicals [13].

The IC_{50} values, which indicate the concentration required to inhibit 50% of the DPPH free radicals, further underscore the antioxidant potency of the extracts. Lower IC_{50} values correspond to higher antioxidant potential, with the water extract showing an IC_{50} of 6.220±0.091 µg/ml and the methanol extract showing 9.065±0.128 µg/ml. This suggests that while methanol extracts demonstrated higher percentage inhibition at higher concentrations, water extracts were more potent on a per unit basis, likely due to the concentrated presence of specific water-soluble antioxidants (**Table 4**).

These findings imply that ginger extracts are a potent source of natural antioxidants, which could play a crucial role in combating oxidative stress-related diseases such as cardiovascular diseases, cancers, and neurodegenerative disorders. The ability of these extracts to scavenge free radicals can be leveraged to develop functional foods or nutraceuticals that provide health benefits beyond basic nutrition.

Previous studies have consistently shown that ginger contains various antioxidant compounds, primarily gingerols, shogaols, and zingerone. These compounds are known to contribute significantly to the antioxidant activity observed in ginger extracts. For instance, a study reported that ginger extracts exhibited high antioxidant activities, attributed primarily to these compounds. The current study's findings align with these observations, confirming the robust antioxidant potential of ginger extracts as reported in earlier research.

Furthermore, the detailed phytochemical profile provided in this study, including the presence of glycosides, flavonoids, and saponins, complements the understanding of ginger's pharmacological activities, supporting its traditional use in herbal medicine. This is consistent with the literature that suggests a complex interplay of various phytochemicals contributes to the overall health benefits offered by ginger [12-40].

Table 3: DPPH Radical Scavenging Capacity of Rhyzome of *Zingiber officinale*

Concentration of	Inhibition (%)

S/No.	extract (µg/ml)	Methanol extracts	Water extracts	Ascorbic acid (std)
1	100	85.135±0.093	86.331±0.117	95.880±0.142
2	200	89.795±0.046	91.141±0.188	97.741±0.261
3	300	92.498±0.026	95.589±0.163	98.157±0.142
4	400	98.602±0.026	96.148±0.117	99.472±0.026

Note: Values are given as Mean ± SD of three replicates

Table 4: Antioxidant Potential (IC_{50}) of Rhizome Extract of *Zingiber officinale*

S/No.	Extracts/Standard	Methanol extracts	Water extracts
1	Ginger (rhizome)	9.065±0.128	6.220±0.091
2	Ascorbic acid (std)	1.131±0.091	1.131±0.091

Note: Values are given as Mean ± SD of three replicates

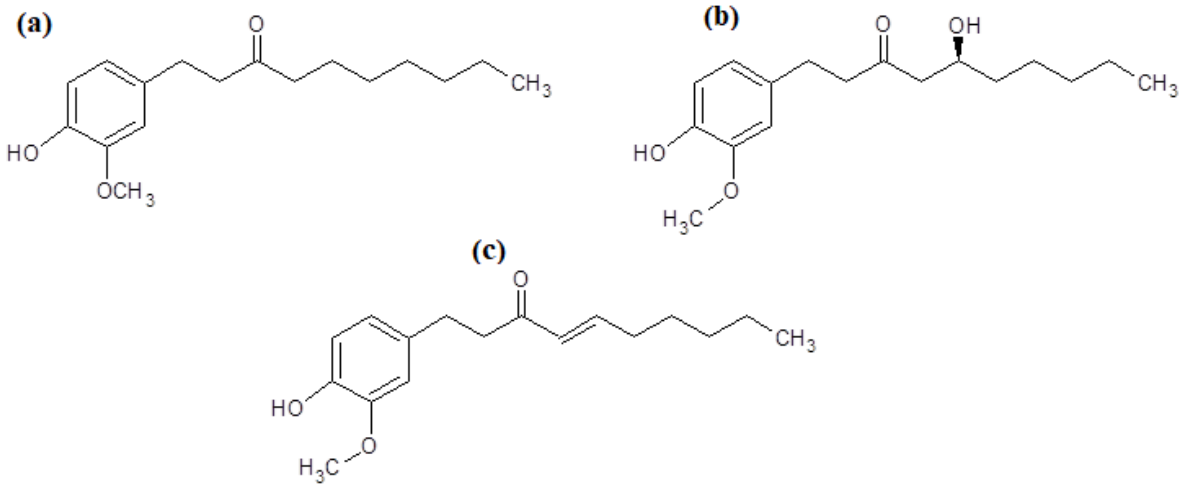

Fig. 1: Chemical components of ginger (a) Paradol (b) Gingerol (c) Shogoal

HPLC Analysis

High-Performance Liquid Chromatography (HPLC) analysis successfully identified and quantified three bioactive phytochemical compounds in the methanol fractions of *Zingiber*

officinale (ginger). The identified compounds were confirmed by comparing their retention times (RT) and peak areas with those of pure standard compounds. The method of external standards was employed to construct a calibration curve, utilizing a series of concentrations ranging from 0.2 to 100 µL. This analysis was conducted in collaboration with Allele Life Sciences, Noida, India, using their proprietary phytochemical database to ensure accurate identification.

The HPLC chromatogram (Fig. 2) explicitly highlights the presence of gingerols, one of the major bioactive components in *Z. officinale*. Gingerols have been widely reported in the literature for their potent pharmacological activities, including anti-inflammatory, antioxidant, and anticancer properties [6-14]. The quantification of these gingerol compounds in the methanol fraction suggests a high concentration of bioactive constituents, confirming the therapeutic potential of *Z. officinale* extracts.

Constructing a standard curve for gingerol acid (Fig. 2) allowed precise quantification of these compounds in the methanol extracts. The calibration curve demonstrated strong linearity, which suggests that the HPLC method used is highly reliable and reproducible for determining gingerol concentrations.

The identification and quantification of gingerols in the methanol fractions of *Z. officinale* hold significant implications for both traditional and modern medicinal applications. Gingerols have been extensively studied for their therapeutic effects, including their ability to reduce inflammation, modulate the immune system, and act as potent antioxidants. The findings from this HPLC analysis reinforce the therapeutic claims associated with *Z. officinale* in Ayurvedic and other traditional medicine systems. Precisely, ginger's use as a remedy for respiratory ailments, gastrointestinal discomfort, and inflammatory conditions aligns with the presence of gingerols, which are known to exert such effects.

Moreover, these results support the potential of ginger-based products in developing nutraceuticals or pharmaceuticals to manage various health conditions, including inflammatory diseases and certain cancers. The high concentration of gingerols detected in the methanol fraction of ginger suggests that these bioactive compounds could be effectively extracted and utilized for therapeutic purposes.

Previous studies have also highlighted the presence of gingerols as the primary active compound in *Z. officinale*. In a study by [28], gingerol and its derivatives were identified as critical contributors to ginger's anti-inflammatory and analgesic properties. Additionally, [4] reported similar findings, with gingerols being present in high concentrations in ethanol and

methanol extracts of ginger rhizomes. The retention times and peak profiles observed in our study are consistent with those reported in the literature, further validating the accuracy of our HPLC method.

Comparing the current findings to previous studies, the concentrations of gingerols identified in this study are in line with those reported by [41], who found that ginger extracts, particularly those rich in [6]-gingerol, exhibited significant anticancer activity against colorectal cancer cells. This highlights the pharmacological relevance of gingerol-rich extracts in disease management.

Furthermore, ginger in traditional medicine, particularly in Ayurvedic practices, is supported by numerous studies documenting its efficacy in treating respiratory issues such as cough, asthma, and bronchitis. In a review by [25], ginger juice mixed with honey was reported to be highly effective in managing cough and cold symptoms, findings that are consistent with the use of ginger in traditional Ayurvedic formulations. Our findings of high gingerol content further substantiate these traditional claims, suggesting that the bioactive compounds identified in our study are likely responsible for the therapeutic benefits observed in clinical and traditional settings.

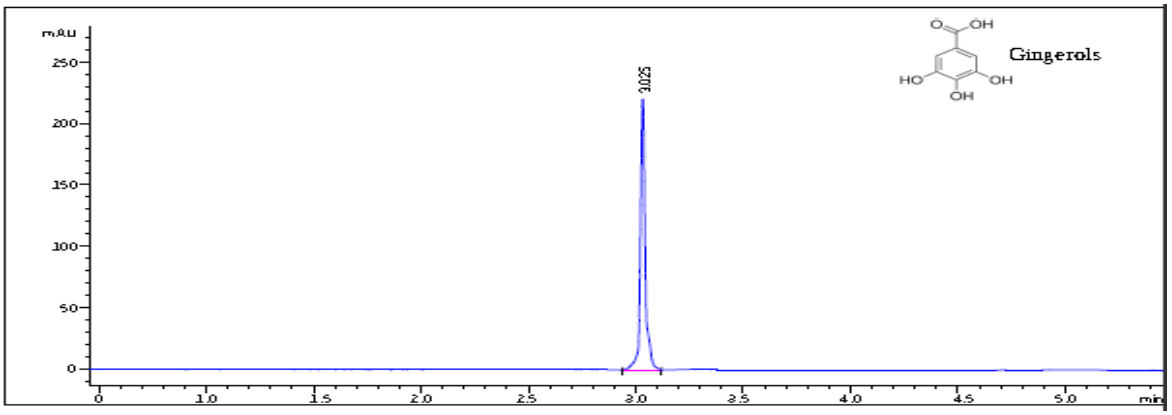

Fig. 2a: HPLC Chromatogram of Gingerols

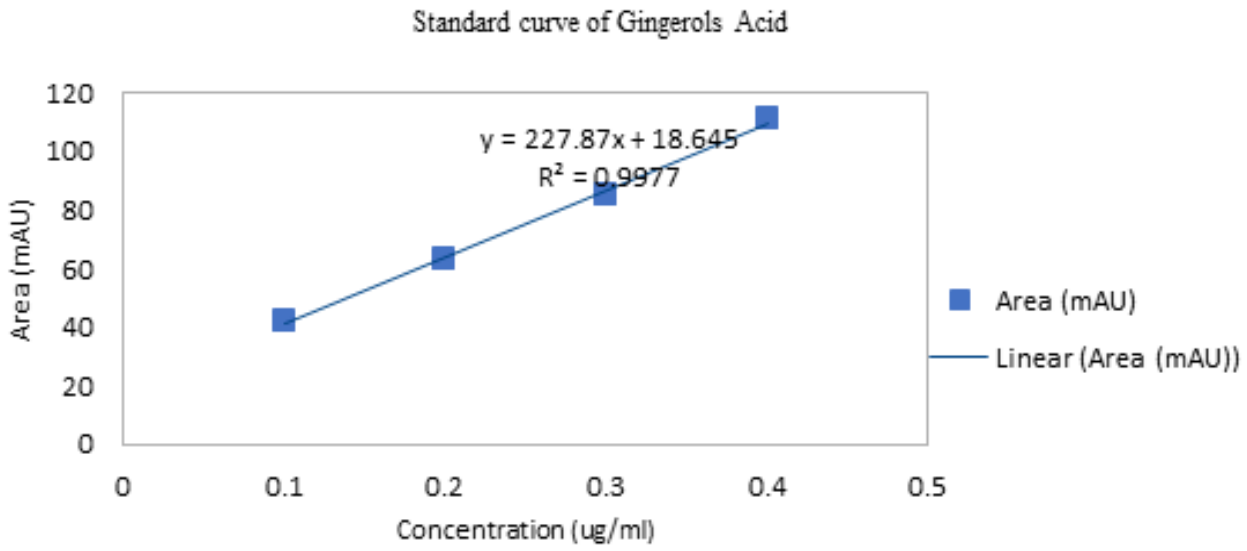

Fig. 2b: Gingerol Acid Standard Curve

Screening of Antibacterial Activities:

The antibacterial activity of the methanol rhizome extract of *Zingiber officinale* (ginger) was evaluated by determining the Minimum Inhibitory Concentration (MIC) against four bacterial strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Streptococcus pneumoniae*. The MIC represents the lowest concentration of the plant extract that inhibits the visible growth of bacteria after 16 hours of incubation at 37°C. The MIC values were obtained using an ELISA plate reader, which also allowed for the determination of zones of inhibition.

Table 5: Minimum Inhibitory Concentration (MIC) of Rhizome Extract

Rhizome extracts	***E. coli* (mg/ml)**	***K. pneumoniae* (mg/ml)**	***B. subtilis* (mg/ml)**	***S. pneumonia* (mg/ml)**
Z. officinale	642.6	730.7	55.03	2.442

The results showed that the MIC values ranged from 2.442 to 730.7 mg/mL, depending on the bacterial strain. Specifically, the ginger extract exhibited the strongest antibacterial activity against *Streptococcus pneumonia*, with an MIC of 2.442 mg/mL, followed by moderate

activity against *Bacillus subtilis* (55.03 mg/mL). However, the extract required much higher concentrations to inhibit the growth of *Escherichia coli* (642.6 mg/mL) and *Klebsiella pneumoniae* (730.7 mg/mL), indicating weaker antibacterial efficacy against these strains (Figure 3a-d) (Table 5).

The differential antibacterial activity of *Z. officinale* extract can be attributed to the varying cell wall structures of the bacterial strains. *S. pneumoniae* is a Gram-positive bacterium with a relatively simpler cell wall structure, primarily composed of peptidoglycan, which makes it more susceptible to bioactive compounds in the ginger extract. Similarly, *B. subtilis*, another Gram-positive bacterium, exhibited moderate sensitivity to the ginger extract, likely due to the disruption of its peptidoglycan layer.

In contrast, *E. coli* and *K. pneumoniae*, both Gram-negative bacteria, possess an outer membrane rich in lipopolysaccharides, which acts as a barrier to many antimicrobial agents. The higher MIC values observed for these strains indicate that the bioactive compounds in *Z. officinale* may have limited permeability through the outer membrane, reducing their antibacterial efficacy. The presence of efflux pumps and other resistance mechanisms in Gram-negative bacteria may further explain the reduced sensitivity of these strains.

The findings suggest that *Z. officinale* rhizome extract could be a promising candidate for the development of natural antibacterial agents, particularly against Gram-positive bacteria such as *S. pneumonia* and *B. subtilis*. Given the lower MIC values for these strains, the extract may serve as a valuable alternative or complement to conventional antibiotics, especially in cases where antibiotic resistance is an issue.

The potent activity against *S. pneumonia* at a concentration of 2.442 mg/mL is particularly significant, as this bacterium is responsible for serious infections such as pneumonia and meningitis. The relatively low concentration required to inhibit its growth highlights the therapeutic potential of ginger-derived compounds in treating such infections.

However, the higher MIC values for *E. coli* and *K. pneumoniae* suggest that while the ginger extract exhibits some antibacterial activity against Gram-negative bacteria, it may require higher doses or the use of synergistic agents to enhance its efficacy. This could be explored in future studies to improve the antibacterial spectrum of ginger-based treatments.

The antibacterial properties of *Z. officinale* have been well-documented in previous studies. For instance, a study by [9] found that ginger extracts exhibited stronger antibacterial activity against Gram-positive bacteria compared to Gram-negative strains, consistent with the results of the present study. Similarly, [2] reported that *Z. officinale* was highly effective against *S.*

pneumonia, with MIC values comparable to those observed in this study, further validating the potency of ginger against this pathogen.

In contrast, studies by [10-32] demonstrated the relative resistance of Gram-negative bacteria such as *E. coli* and *K. pneumoniae* to various plant extracts, including ginger. These findings align with the higher MIC values reported in our study, confirming that Gram-negative bacteria generally require higher concentrations of plant-derived compounds for growth inhibition due to their more complex cell envelope structures.

Moreover, the use of ELISA in this study to assess antibacterial activity represents a novel approach, as previous studies predominantly relied on agar diffusion methods. The use of ELISA allowed for more precise quantification of antibacterial effects, improving the reproducibility and reliability of the results.

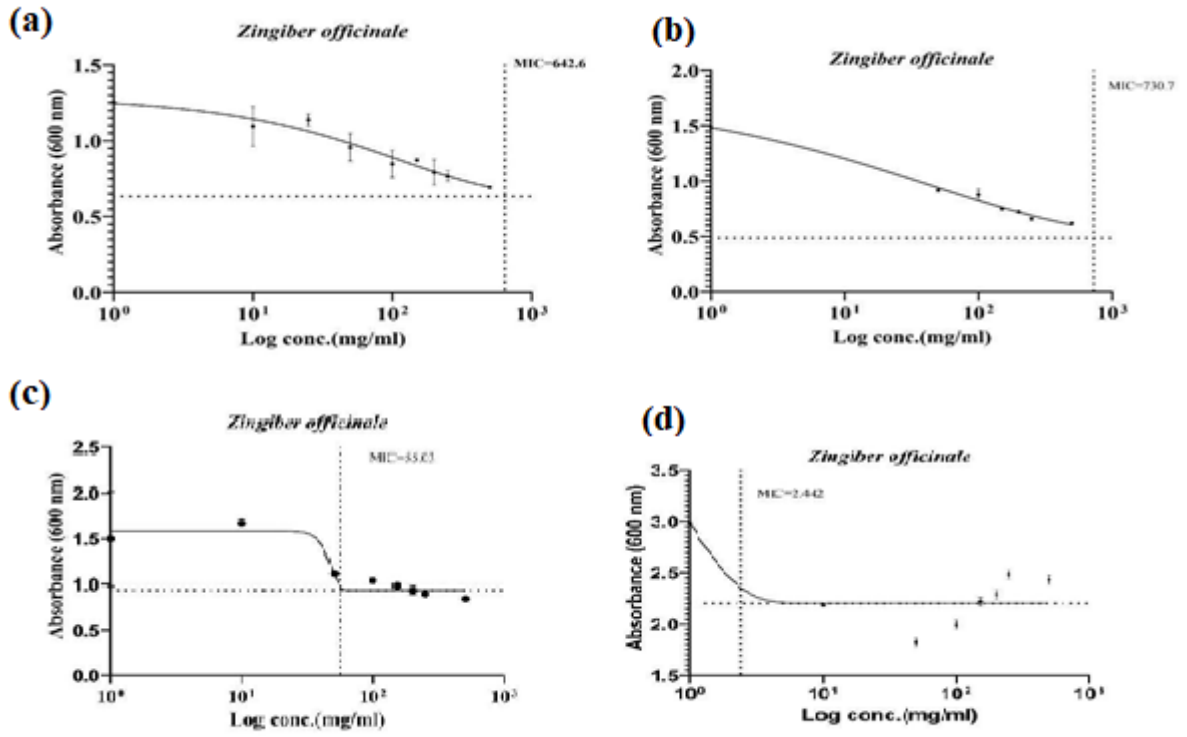

Fig: 3: Line graphs showing the MIC of ginger against (a) *Escherichia coli* (b) *Klebsiella pneumoniae* (c) *Bacillus subtilis* (d) *Streptococcus pneumonia*

Bacteria Biofilm Formation

Biofilm formation is a complex and highly organized process where bacteria adhere to a surface and form a protective slimy matrix that enables them to proliferate and survive under hostile conditions. Biofilms can form on both living (e.g., tissue surfaces) and nonliving

surfaces (e.g., medical devices or industrial equipment). Once formed, biofilm-producing bacteria exhibit a remarkable increase in antibiotic resistance, with some studies showing resistance levels as much as 5,000 times higher than those of their planktonic counterparts. This resistance is largely due to the protective extracellular matrix (ECM), which physically impedes the penetration of antibiotics and facilitates genetic exchange among the bacterial population, including the transfer of resistance genes.

In the present study, the ability of *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Streptococcus pneumonia* to form biofilms was assessed. Crystal violet staining was used to reveal the established biofilms, and the presence of biofilm was confirmed via optical microscopy. All tested bacterial strains demonstrated biofilm formation, though to varying degrees, as summarized in Table 5.

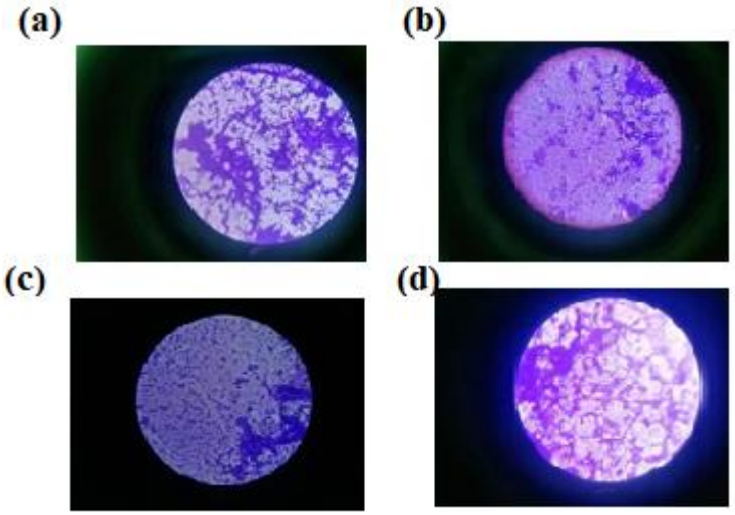

Fig: 4: The Formation of Biofilm by Gram-negative and Gram-positive Bacteria (a) *E. coli* (b) *B. subtilis* (c) *K. pneumoniae* (d) *S. pneumonia*

Table 5: Bacterial Biofilm Formation

S/No.	Bacterial Strains	Biofilm Formation
1	*Escherichia coli*	Biofilm formed
2	*Bacillus subtilis*	Biofilm formed
3	*Klebsiella pneumoniae*	Biofilm formed
4	*Streptococcus pneumonia*	Biofilm formed

The results revealed that *Escherichia coli* and *Streptococcus pneumonia* exhibited the strongest biofilm formation, while *Bacillus subtilis* and *Klebsiella pneumoniae* formed biofilms to a lesser extent.

The variation in biofilm formation among the bacterial strains can be attributed to differences in their genetic regulatory systems, extracellular matrix composition, and environmental

adaptability. Gram-negative bacteria like *E. coli* and *K. pneumoniae* are known for forming dense biofilms, largely due to their production of a robust extracellular matrix composed of polysaccharides, proteins, and extracellular DNA (eDNA). This matrix not only provides structural support but also acts as a shield against antibiotic penetration.

On the other hand, Gram-positive bacteria like *S. pneumonia* and *B. subtilis* form biofilms using slightly different mechanisms. *S. pneumonia* produces a biofilm that is rich in teichoic acids and peptidoglycan, which may explain its strong biofilm formation observed in this study. *B. subtilis*, while capable of forming biofilms, typically shows weaker biofilm structures compared to *S. pneumonia* due to the differences in matrix composition and the signalling pathways that regulate biofilm formation.

The stronger biofilm formation observed in *E. coli* and *S. pneumonia* suggests a higher level of antibiotic resistance, making them more resilient to conventional treatment strategies. Biofilms contribute to chronic infections, as their matrix can block immune responses and increase bacterial persistence in host tissues or on medical devices, complicating treatment regimens.

These findings highlight the challenges associated with treating biofilm-associated infections, particularly for bacteria like *E. coli* and *S. pneumonia*. The enhanced biofilm-forming ability of these bacteria suggests that infections caused by these strains could be more difficult to manage using standard antibiotic therapies. Biofilms not only provide a physical barrier but also create a microenvironment that facilitates bacterial communication and genetic exchange, potentially increasing the spread of antibiotic resistance.

From a clinical perspective, the strong biofilm formation observed in this study underscores the importance of developing novel strategies to combat biofilm-related infections. Potential approaches could include the use of biofilm-disrupting agents, the development of drugs that target the extracellular matrix, or combination therapies that enhance antibiotic penetration into biofilms. Additionally, preventive measures, such as the use of surface coatings that inhibit bacterial adhesion, could be explored to reduce biofilm formation on medical devices.

The biofilm-forming capacity of *E. coli* observed in this study is consistent with findings from previous studies. According to [21], *E. coli* is known for its robust biofilm formation, particularly in urinary tract infections, where biofilm-associated resistance often leads to recurrent infections. Similarly, [35] reported that *E. coli* biofilms are highly resistant to antibiotics, largely due to the thick polysaccharide matrix that inhibits drug penetration.

The strong biofilm formation by *S. pneumonia* observed here aligns with earlier studies by [18], who demonstrated that *S. pneumonia* biofilms play a significant role in respiratory infections, such as pneumonia and otitis media. These biofilms not only protect the bacteria from antibiotic treatment but also contribute to the chronic nature of these infections.

In contrast, the moderate biofilm formation observed in *K. pneumoniae* and *B. subtilis* is supported by research from [38], who found that while *K. pneumoniae* can form biofilms, their structure is often less dense than that of other Gram-negative bacteria, leading to a slightly lower level of resistance. Similarly, *B. subtilis*, a well-known soil bacterium, forms biofilms primarily for survival in harsh environments, but its biofilms are less robust compared to those of pathogens like *S. pneumonia*.

Conclusion

This study confirmed the significant antibacterial and antioxidant properties of *Zingiber officinale* extracts, reinforcing its long-standing use in traditional medicine. Phytochemical analysis identified several bioactive compounds responsible for these activities, with methanol extracts showing higher bioactive content and stronger antioxidant and antibacterial effects compared to aqueous extracts. The antioxidant assay demonstrated potent free radical scavenging abilities, particularly in methanol extracts, which exhibited near-comparable activity to standard ascorbic acid. Moreover, the antibacterial efficacy of the ginger extracts, particularly against *Streptococcus pneumonia*, underscores its potential as a natural remedy for bacterial infections, especially for Gram-positive strains. These findings highlight ginger's dual role as an effective antimicrobial and antioxidant agent, suggesting that it can serve as an alternative or complementary treatment in managing infections and oxidative stress-related conditions. Future research should focus on the isolation and characterization of individual bioactive compounds and further clinical investigations to explore the full therapeutic potential of ginger. While ginger is safe for consumption, it is important to assess its interactions with medications and possible adverse effects when used in larger doses.

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