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Gc-Ms-based phytochemical profiling and antibacterial efficacy of *terminalia chebula* extract against gastrointestinal pathogens resistant to β-lactam antibiotics

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ABSTRACT

Introduction:
The increasing prevalence of antibiotic-resistant bacteria, particularly β-lactam-resistant strains, has become a serious global health concern, limiting the effectiveness of conventional antimicrobial therapies. Gastrointestinal pathogens such as *Bacillus cereus*, *Salmonella typhimurium*, and *Escherichia coli* are among the common organisms associated with resistant infections. In response to this challenge, medicinal plants like *Terminalia chebula*, known for their broad-spectrum bioactivity in traditional medicine, are being explored as promising natural sources of antimicrobial agents. This study aimed to evaluate the antibacterial activity of ethanolic and propanolic extracts of *Terminalia chebula* against gastrointestinal bacteria resistant to selected β-lactam antibiotics.

Materials and Methods:
Ethanolic and propanolic extracts were prepared by cold maceration and analyzed using gas chromatography–mass spectrometry (GC-MS) to identify major bioactive constituents. Antibacterial activity was assessed using agar well diffusion assays, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined.

Results:
GC-MS analysis revealed that 1,2,3-benzenetriol (pyrogallol) was the most abundant and the most important antimicrobial compound in the ethanolic extract. In the propanolic extract, propanoic acid was identified as the most abundant constituent; however, D-limonene was determined to be the most potent antimicrobial compound. Both extracts exhibited considerable antibacterial activity against all tested strains, with *B. cereus* being the most sensitive, followed by *S. typhimurium* and *E. coli*.

Conclusion:
The antibacterial effects observed can be attributed to key phytochemicals such as pyrogallol and propanoic acid, which may act by disrupting bacterial cell walls and interfering with metabolic functions. These findings highlight the potential of *Terminalia chebula* as a natural therapeutic agent against drug-resistant infections. Further in vivo studies and clinical evaluations are warranted to explore its clinical applicability.

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Introduction

The growing emergence of antibiotic-resistant pathogenic bacteria, particularly strains resistant to β-lactam antibiotics, has become a critical threat to clinical care quality and patient safety worldwide. According to the World Health Organization (WHO), antimicrobial resistance (AMR) is estimated to cause at least 700,000 deaths annually, and this figure could rise to 10 million per year by 2050 if no effective action is taken (1,2). Resistance to β-lactam antibiotics-including carbapenems, cephalosporins, and penicillins-has significantly reduced the efficacy of frontline antibiotics, thereby limiting treatment options and leading to increased morbidity, mortality, and healthcare costs.

Controlling antibiotic-resistant infections is now a top priority in global public health and poses a formidable challenge to hospitals and healthcare systems around the world. In response to this crisis, researchers are increasingly exploring alternative therapeutic strategies, including the use of plant-based compounds with antimicrobial properties. Notably, the WHO estimates that around 80% of the global population relies on herbal medicine for some aspect of their primary healthcare needs (3). This growing reliance reflects both the cultural heritage and the potential efficacy of traditional medicinal plants in managing infections, including those caused by multidrug-resistant organisms.

Given this pressing issue, the exploration of natural products, especially medicinal plants, has gained significant attention as a promising approach to discover novel antibacterial agents. *Terminalia chebula*, a well-known plant in traditional Ayurvedic medicine, contains diverse bioactive compounds such as phenols and tannins, which have demonstrated anti-inflammatory, antioxidant, digestive, respiratory, and antibacterial properties. Identifying, isolating, and characterizing these natural compounds offers great potential for developing effective alternatives to combat resistant bacterial strains, thereby contributing to improved patient safety and a reduced burden of antimicrobial resistance (2,3). Therefore, this study aims to evaluate the antibacterial activity of *Terminalia chebula* extract against clinically important gastrointestinal bacterial pathogens, especially strains resistant to β-lactam antibiotics. Additionally, gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the bioactive phytochemical constituents responsible for the observed antibacterial effects. The ultimate goal is to support the development of natural therapeutic agents that can aid in controlling resistant infections and enhancing the quality and safety of clinical care. This study aims to support these strategies by exploring natural alternatives with antibacterial potential. Specifically, it research to identify bioactive compounds from *Terminalia chebula* extract that can inhibit resistant bacterial strains. By doing so, the study intends to contribute to improving hospital infection control measures and ultimately enhance patient safety and the overall quality of healthcare services.

Materials and Methods

Introduction to the geographical area of plant collection

The plant samples were collected from Minab County, located in Hormozgan Province, southeastern Iran. Minab is approximately 90 kilometers from the provincial capital, Bandar Abbas, and lies at geographical coordinates of around 27°06'N latitude and 57°08'E longitude. With an average elevation of 200 meters above sea level, the area is characterized by a hot and dry climate, featuring very hot summers and mild winters. Annual precipitation ranges between 100 and 200 millimeters, primarily occurring during the winter months. Given that *Terminalia chebula* is naturally found in tropical regions such as India and China, and considering that Minab possesses similar climatic and ecological conditions, this area provides a favorable environment for the growth of this plant, making it a suitable site for studying its phytochemical and biological properties.

Plant Identification and Collection

Over the course of one year, from **2**024, field operations were conducted to identify and collect the natural habitats of medicinal plant species in the Minab region. These operations included visiting various areas and collecting plant samples from their natural habitats. The samples were

accurately identified and confirmed by experts from the Herbarium of Islamic Azad University, Gorgan Branch. then, the required plant for experiments and extract preparation were stored under appropriate conditions. These conditions included a dark, cool, and dry environment to ensure complete removal of moisture from the plants. After drying, the fruits of *Terminalia chebula* were carefully ground using a Panasonic MJ-J176P device from Japan and uniformly powdered to prepare them for the extraction stage. The powder was stored in airtight containers under cool and dry conditions to maintain its quality and prevent potential contamination.

Preparation of the Terminalia chebula Extracts

The extraction was carried out using the cold maceration method. Initially, 5 g of *Terminalia chebula* fruit powder was mixed with 50 mL of 70% ethanol (Merck, Germany) and kept at 4°C for two weeks. After the maceration period, the mixture was centrifuged (Behdad BH-1200, Iran) at 4000 rpm for 20 minutes, and the supernatant was collected. The ethanolic extract was concentrated using a rotary evaporator under vacuum and then dried completely at room temperature for 48 hours. A total of 1.50 g of ethanolic extract was obtained from the 5 g of plant powder. After removal of the ethanolic extract, the upper remaining plant material was subjected to a second extraction with 50 mL of 96% propanol (Merck, Germany) under the same cold maceration conditions (4°C, two weeks). Following centrifugation, solvent removal, and drying, approximately 0.07 g of propanolic extract was obtained. Both extracts were stored in tightly sealed containers at 4°C until further analysis.

GC-MS Analysis of Ethanolic and Propanolic Extracts of Terminalia chebula

The chemical constituents of the ethanolic and propanolic extracts of *Terminalia chebula* were analyzed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass selective detector. A 2 µL aliquot of each concentrated extract was injected in split mode (1:5) into an HP-5MS capillary column (30 m × 0.25 mm, 1 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min.

The oven temperature was programmed from an initial 60°C (held for 2 min) to 280°C at a rate of 5°C/min, with a final hold time of 20 minutes. Mass spectrometry was performed using electron impact ionization at 70 eV, scanning a mass range of m/z 40–500. Compound identification was achieved by comparing retention times and mass spectra to those in NIST and Wiley spectral libraries.

This analysis revealed the major bioactive compounds present in both extracts, highlighting differences in their phytochemical profiles (4).

Bacterial Strain

The bacterial strains used in this study were *Salmonella typhimurium* (PTCC 1596), *Bacillus cereus* (PTCC 1154), and *Escherichia coli* (PTCC 1338), all obtained in lyophilized form from the Iranian Research Organization for Science and Technology (IROST). The strains were revived in the microbiology laboratory at Islamic Azad University, Gorgan Branch, by inoculating each into Brain Heart Infusion (BHI) medium and incubating for 24 hours at 37°C. Subsequently, several isolated colonies from the 24-hour culture of each bacterial strain were transferred to Nutrient Broth (Himedia, India), medium and incubated at 37°C for 1 to 2 hours until the turbidity reached 0.5 McFarland (equivalent to 1.5 × 10^8 CFU/ml) (5,6).

Screening of Pathogenic Bacteria for Sensitivity and Resistance to Selected Beta-Lactam Antibiotics

The disk diffusion method and protocol provided by CLSI was used to assess the sensitivity and resistance of the tested pathogenic bacteria to beta-lactam antibiotics. A bacterial suspension equivalent to 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL) was prepared and uniformly spread over the surface of Mueller-Hinton Agar (Ibresco, Italy). Standard antibiotic disks including Penicillin G (10 U), Ampicillin (10 µg),

Penicillin (10 U), Ceftriaxone (30 µg), and Cefixime (10 µg) (Padtan Teb Company, Iran) were placed on the agar surface. The plates were then incubated at 37°C for 24 to 48 hours. After incubation, the inhibition zones (mm) were measured, and bacterial sensitivity or resistance to each antibiotic was determined accordingly (5,6).

Following incubation, bacterial sensitivity or resistance was assessed by measuring the diameter of the inhibition zones surrounding each disk. The findings were then interpreted in accordance with the recommendations provided by the Clinical and Laboratory Standards Institute 2021 (CLSI).

Antibacterial Activity by Well Diffusion Method

The antibacterial activity of the plant extracts was evaluated using the based on diffusion in agar and by the well method A bacterial suspension equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml) was prepared and evenly spread on the surface of Mueller-Hinton Agar (Ibresco, Italy) plates using a sterile swab. A sterile cork borer was used to aseptically punch wells into the agar that were 7 mm in diameter. 100 µl of various plant extracts were then cautiously pipetted into every well. After that, the plates were incubated for 24 to 48 hours at 37°C. To evaluate the antibacterial activity following incubation, the diameter of the inhibition zones surrounding the wells was mm. To guarantee reproducibility, each test was run in triplicate. The following was the interpretation of the results: Resistance was defined as inhibition zones < 9 mm, moderate sensitivity as 9–12 mm, and sensitivity as > 12 mm (7,8).

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

A stock solution of the pure *Terminalia chebula* extract was prepared at a concentration of 100 mg/mL in dimethyl sulfoxide (DMSO,10%) (Sigma-Aldrich, Germany). Serial dilutions of this stock solution were then prepared in nutrient broth to achieve various working concentrations of the extract. A volume of 100 µL from each dilution was added to the wells of sterile 96-well microplates. Subsequently, bacterial suspensions were added to each well to reach a final inoculum density of approximately 5×10^5 CFU/mL (5,6,9). The microplates were incubated at 37°C for 24 hours. Each assay included positive control wells (containing bacteria without extract) and negative control wells (containing broth only). After incubation, wells were inspected for visible turbidity. The lowest concentration of the extract at which no turbidity was observed was recorded as the MIC (5,6,9). To determine the MBC, aliquots of 10 µL from wells showing no turbidity (i.e., MIC and higher concentrations) were subcultured onto Mueller-Hinton agar plates. Following incubation at 37°C for 24 hours, the lowest concentration at which no bacterial colonies grew was recorded as the MBC. All experiments were performed in triplicate, and the results were reported as mean values (5,6,9).

Statistical Analysis

Statistical analyses were performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA). Data were expressed as mean ± Standard Error (SE) from three independent experiments. To determine the significance of differences among multiple groups, one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test for pairwise comparisons. In addition, independent t-tests were applied where appropriate. A p-value less than 0.05 was considered statistically significant Graphs were drawn using Microsoft Excel 2022.

Results

Antibiotic Susceptibility Testing of Bacterial Strain Antibiotic susceptibility tests were performed using the disk diffusion method for *E.* coli, B. cereus, and S. typhimurium. None of the tested antibiotics showed any inhibition zones against any of these bacterial strains, indicating complete resistance of all three bacteria to all antibiotics tested (Table 1).

Table 1: Results of the antibiotic disk diffusion test. In the table, the symbol "R" indicates resistance, meaning no detectable inhibition zone was observed for the respective antibiotic against the tested bacterium.

Antibiotics	E. coli	S. typhimurium	B. cereus
Ampicilin	R	R	R
ceftriaxon	R	R	R
Penicillin G	R	R	R
Penicillin	R	R	R
Cefixime	R	R	R

Results of antibacterial activity of ethanolic extract of *Terminalia chebula* using well method

The extract had a dose-dependent inhibitory activity against *B. cereus*, as indicated by the table, with inhibition zone Diameter dropping from 23.66 ± 1.76 mm at 100 mg/ml to 8.33 ± 1.33 mm at 6.25 mg/ml. As indicated by the various letters (a–d) in the statistical grouping column, statistical analysis using one-way ANOVA and Tukey's post hoc test showed significant differences ($p<0.05$) between the majority of concentration groups. These findings demonstrate that the extract had a strong effect on *B. cereus*, especially at higher concentrations.

There was no statistically significant difference between the inhibition zones for S. typhimurium at 100 and 50 mg/ml, which were 17.50 ± 0.29 mm and 9.00 ± 2.88 mm, respectively, and allocated to group "a" ($p > 0.05$). The absence of numerous concentration comparisons for *E. coli* may be the reason for the lack of statistical grouping, despite the bacterium's inhibitory zone measuring 17.00 ± 0.58 mm at 100 mg/ml. Overall, the evidence points to *Terminalia chebula* extract's concentration-dependent efficacy and higher antibacterial activity against *B. cereus* than against *E.* coli and S. typhimurium (Tabel 2).

Tabel 2. Average diameter of the growth inhibition zone of the tested pathogenic bacteria in the presence of different concentrations of ethanolic extract

Bacterial Strain	Extract Concentration (mg/ml)	Inhibition Zone Diameter (mm)
B. cereus	100	23.6667 ± 1.76383 [a]
B. cereus	50	19.3333 ± 0.33333 [ab]
B. cereus	25	15.0000 ± 0.57735 [bc]
B. cereus	12.5	9.6667 ± 2.18581 [cd]
B. cereus	6.25	8.3333 ± 1.33333 [d]
E. coli	100	17.0000 ± 0.57735
S. typhimurium	100	17.5000 ± 0.28868 [a]
S. typhimurium	50	9.0000 ± 2.88675 [a]

Tabel 2: Mean ± SE from three independent replicates is used to express values. ANOVA, Tukey's post hoc test, and the independent t-test were used for statistical analysis. Shared letters signify no significant difference, while different letters imply statistically significant differences ($p < 0.05$). The results related to the mean inhibition zone diameters of the tested pathogenic bacteria exposed to 100 mg/mL of the ethanolic extract of *Terminalia chebula* are presented in Figure 1. These findings highlight the notable sensitivity of *B. cereus*, a Gram-positive bacterium, compared to the other strains. Interestingly, no statistically significant difference was observed between the mean inhibition zones of the two Gram-negative bacteria, *S. typhimurium* and *E. coli*, indicating a relatively similar response to the extract (Figure 1).

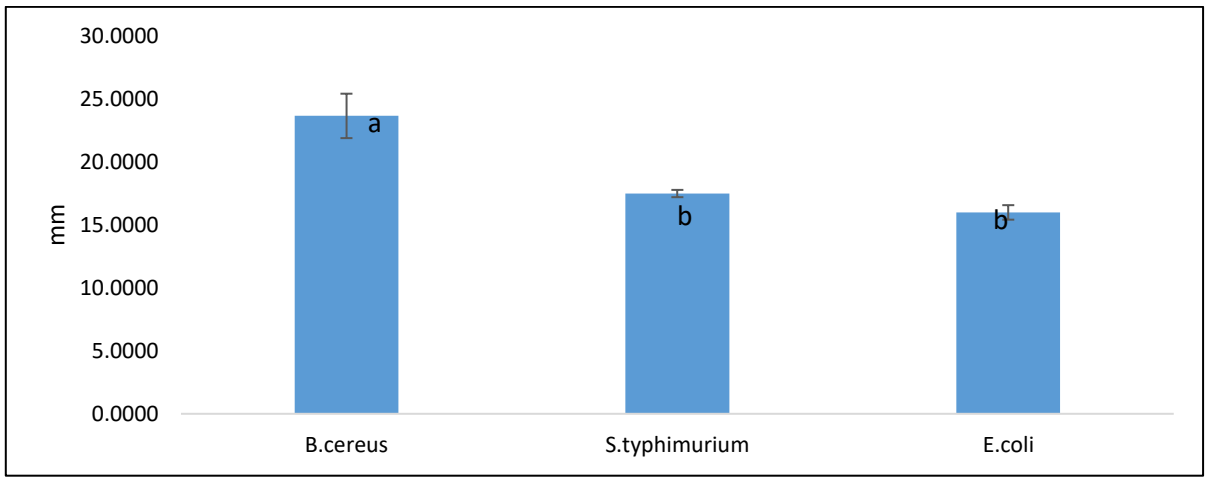

Figure 1: Mean diameter of the inhibition zone of the tested pathogenic bacteria exposed to 100 mg/mL of *Terminalia chebula* ethanolic extract

In Figure 1 The mean diameter of the inhibition zone of the tested pathogenic bacteria exposed to 100 mg/mL of *Terminalia chebula* ethanolic extract is presented. Values are expressed as mean ± SE from three independent replicates. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Shared letters indicate no significant difference, while different letters denote statistically significant differences at $p < 0.05$.

Results of MIC and MBC of Ethanolic and Propanolic Extracts

The results of the determination of MIC and MBC for the ethanolic and propanolic extracts of *Terminalia chebula* demonstrated similar antibacterial activity against *E. coli*, with both extracts showing an MIC of 12.5 mg/mL and an MBC of 25 mg/mL. These values indicate the extract's ability to inhibit growth and completely kill the bacteria at these concentrations. In contrast, for *S. typhimurium*, differences were observed between the two extracts: the propanolic extract exhibited equal MIC and MBC values of 25 mg/mL, suggesting that bacterial growth inhibition and killing were achieved at the same concentration. However, the ethanolic extract had an MIC of 25 mg/mL but required a higher MBC of 50 mg/mL, indicating that a greater concentration was necessary for complete bactericidal effect. Regarding *B. cereus*, the ethanolic extract showed higher sensitivity with an MIC of 6.25 mg/mL and an MBC of 12.5 mg/mL, whereas the propanolic extract required higher concentrations, with an MIC of 12.5 mg/mL and an MBC of 25 mg/mL. These findings reflect variations in the antibacterial potency of the two extracts against different bacterial strains, suggesting that the type of solvent used for extraction influences the antibacterial efficacy. Similar to the agar well diffusion method, this method also demonstrated greater sensitivity of *B. cereus* compared to the other two bacteria, as lower concentrations of *Terminalia chebula* extract were sufficient to inhibit growth and kill this bacterium relative to the others. Overall, the antibacterial activity of both extracts improved with increasing concentration and decreased at lower dilutions.

Analysis of Essential Phytoconstituents in *Terminalia chebula* by GC-MS Technique

GC-MS analysis of the ethanol extract of *Terminalia chebula* identified major bioactive compounds such as 1,2,3-Benzenetriol, Phenol, and Benzoic acid, collectively accounting for 86.89% of the total components, indicating a profile rich in phenolics, aldehydes, fatty acids, and alkaloids (Figure 2).

Similarly, the propanol extract revealed compounds like Propanoic acid, Hexadecanoic acid, and D-limonene, making up 83.35% of the extract. This extract showed a diverse profile including fatty acids, terpenes, alkynes, heterocycles, and sulfur-containing compounds (Figure 3) (Tabel 3).

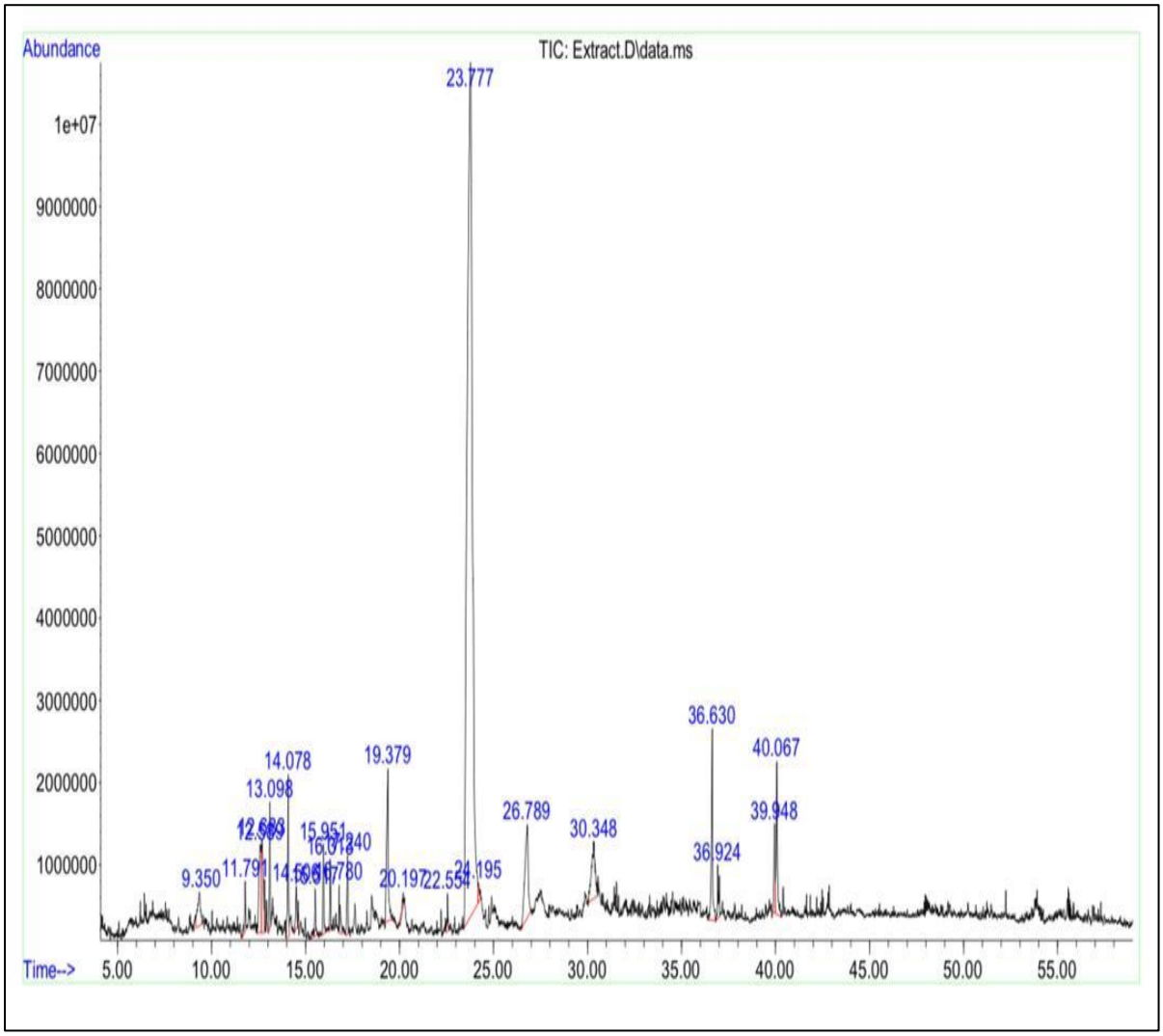

Figure 2: GC-MS chromatogram showing retention times and chemical profiles of major bioactive compounds in the ethanol extract of Terminalia chebula

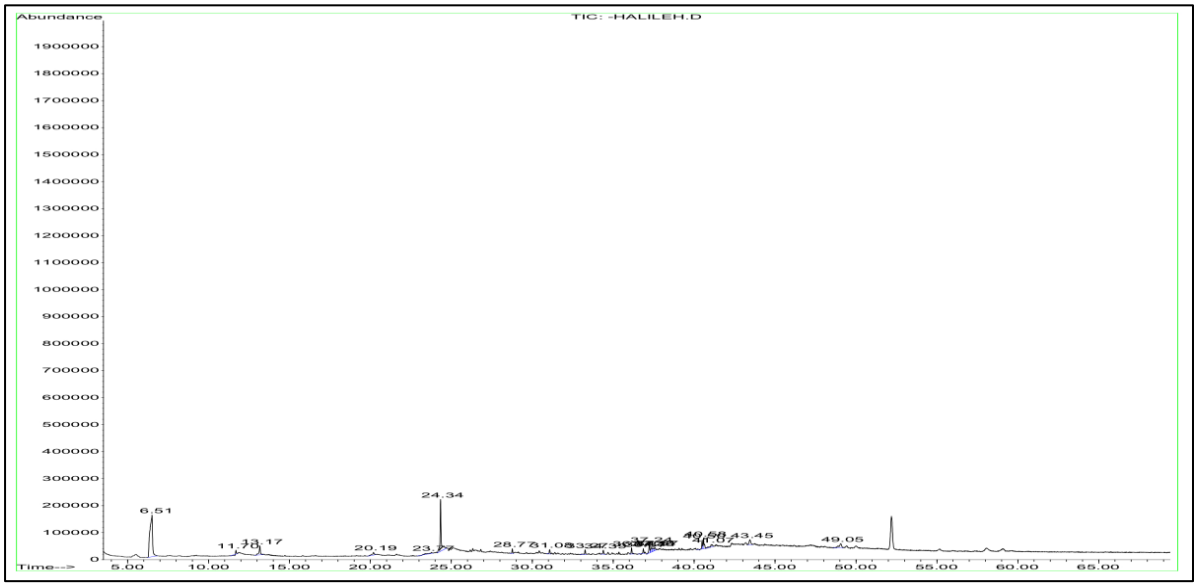

Figure 3: GC-MS chromatogram showing retention times and chemical profiles of major bioactive compounds in the propanol extract of *Terminalia chebula*

Table 3: GC-MS identified compounds in ethanolic and propanolic extracts of *Terminalia chebula*, showing relative abundance (Area %), CAS numbers, and identification quality scores.

Extract Type	Area (%)	CAS No	Qual	Compound Name
Ethanolic Extract	64.19	000087-66-1	95	1,2,3-Benzenetriol
	5.15	000108-95-2	91	Phenol
	4.43	000099-06-9	97	Benzoic acid
	3.84	000067-47-0	87	2-Furancarboxaldehyde, 5-
	3.53	000057-10-3	99	Hexadecanoic acid
	2.88	056554-35-0	95	9,17-Octadecadienal
	2.87	999206-18-6	74	IMPERIALINE
Propanolic Extract	40.46	000106-36-5	78	Propanoic acid
	20.61	128608-98-0	83	4,4-Dimethyl-2-(3-phenyl-2-thienyl)oxazoline
	4.8	000628-97-7	89	Hexadecanoic acid
	4.28	005989-27-5	97	D-limonene
	3.85	000593-39-5	95	6-Octadecenoic acid
	3.16	002937-53-3	50	Thiosulfuric acid (S-(2-aminoethyl) ester)
	2.87	999206-18-6	93	7-Pentadecyne
	1.93	036126-09-7	78	9,10-dihydro-7-methoxy-4a-methyl-2(4aH)-phenanthrenone
	1.39	099027-75-5	78	6,8-dimethylbenzocyclooctene

Discussion

The biological activities of plant extracts are largely due to their chemically diverse and naturally occurring bioactive constituents, which can act individually or synergistically to exert pharmacological effects (9). Terminalia chebula was selected for this study due to its notably high tannin content, estimated at approximately 30–40%, as well as its rich phytochemical profile. Tannins have been reported to possess potent antibacterial properties, primarily through their ability to penetrate bacterial cell walls, disrupt key metabolic processes, and ultimately damage internal cellular structures (9,10).

Besides tannins, the extract contains several other major constituents including various forms of chebulic acid, gallic acid, ellagic acid, amino acids, and flavonoids, all of which have been linked to diverse pharmacological activities such as antioxidant, antimicrobial, and anticancer effects (11,12).

In this study, Terminalia chebula extracts demonstrated significant antibacterial activity against important gastrointestinal pathogens, including B .cereus, E .coli, and S. typhimurium. These results are consistent with previous studies highlighting the broad-spectrum antimicrobial efficacy of Terminalia chebula , largely attributed to its phenolic compounds and hydrolyzable tannins. Interestingly, Gram-positive bacteria (B. cereus) were more susceptible to the extracts compared to Gram-negative bacteria (E. coli and S. typhimurium). This observation aligns with the well-documented differences in bacterial cell wall architecture: Gram-positive bacteria possess a thick peptidoglycan layer that, despite its rigidity, is more accessible to hydrophilic and hydrophobic compounds, while Gram-negative bacteria have an additional outer membrane containing lipopolysaccharides that acts as a permeability barrier, reducing the penetration of many antimicrobial agents (13).

Our GC-MS analysis identified 1,2,3-Benzenetriol (pyrogallol) as the predominant compound, comprising over 64% of the Ethanolic extract. This concentration is notably higher than the 21–43% reported by Thoithoisana Devi et al. (2023) in extracts from the Manipur region of India. Their research linked 1,2,3-Benzenetriol to strong antioxidant activity and caspase-dependent apoptosis induction

in HCT-116 colon cancer cells, indicating its potential anticancer properties (14). The higher concentration observed in our extract may therefore suggest enhanced therapeutic efficacy, particularly in anticancer applications. The multifunctional bioactivity of 1,2,3-Benzenetriol is further supported by findings from Jie Wang et al. (2016), who demonstrated its role in inducing oxidative stress, upregulating DNA repair genes, and enhancing antioxidant enzyme activities in *Microcystis aeruginosa* (15).

Additionally, Patel et al. (2015) showed that chebulinic acid, a key compound in *Terminalia chebula* , exhibits strong affinity for the DNA gyrase enzyme of *Mycobacterium tuberculosis*, including quinolone-resistant strains, by displacing the Tyr129 residue and inhibiting DNA binding. This mechanism underlines the importance of *Terminalia chebula* phytochemicals in addressing antimicrobial resistance (16).

Our GC-MS analysis revealed that D-limonene is the predominant compound, constituting over 4.28% of the propanol extract. Reported by Yingjie Han et al (2019) Limonene, a monoterpenoid from many plant essential oils, exhibits strong antimicrobial activity, especially against food-borne pathogens. It damages the cell membrane and wall of Listeria monocytogenes, increasing membrane permeability and causing leakage of vital cell components. Additionally, limonene disrupts bacterial energy metabolism by inhibiting ATP synthesis and respiratory enzymes, making it an effective natural antimicrobial agent (17).

Conclusion

The extract of *Terminalia chebula* contains significant bioactive compounds, with 1,2,3-benzenetriol (pyrogallol) being the predominant and most abundant compound identified in the ethanolic extract, and also responsible for its main antibacterial activity. In contrast, the major compound in the propanolic extract is D-limonene, which plays a key role in its antibacterial effects, while propanoic acid constitutes the highest proportion of the compounds in this extract. Other important constituents across both extracts include phenol, benzoic acid, and hexadecanoic acid, together accounting for over 80% of the identified compounds. This extract exhibited notable antibacterial activity, particularly against β-lactam antibiotic-resistant bacteria, with the strongest effect observed on *B. cereus*. Therefore, *Terminalia chebula* represents a promising natural source for combating β-lactam-resistant bacterial infections and holds potential as an alternative or complementary agent to conventional antibiotics.

Importantly, the findings of this study may contribute to the development of more effective clinical care strategies and inform quality management and patient safety policies. Future research should focus on the practical application of these natural compounds in clinical settings, including in vivo studies and clinical trials, to fully realize their potential in combating antibiotic-resistant infections.

References

1. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. Antimicrobial resistance: a growing serious threat for global public health. Healthcare. 2023;11(13):1946.
2. Selvarajan R, Obize C, Sibanda T, Abia ALK, Long H. Evolution and emergence of antibiotic resistance in given ecosystems: possible strategies for addressing the challenge of antibiotic resistance. Antibiotics (Basel). 2022;12(1):28.
3. Chopra M, Deswal G, Chopra B, Kriplani P, Dass R, Grewal AS, et al. Therapeutic and health promoting potential of Terminalia chebula: an exploratory literature review. Curr Tradit Med. 2024;10(7):65-77.
4. Jokar E, Sateei A, Ebadi M, Ahmadi Golsefidi M. Changes in the medicinal and antimicrobial compounds, methyl palmitate, methyl stearate, and bis (2-ethylhexyl) phthalate in American agave (Agave americana L. cv Marginata) under urea treatment. Iran J Plant Physiol. 2023;13(3):4637-43.
5. Notashfard A, Nojavan Asghari M. Inhibitory and bactericidal effect of aqueous pepper extract (Capsicum annum L.), capsaicin, and capsaicin combination with amoxicillin against Streptococcus pyogenes. J Med Microbiol Infect Dis. 2024;12(4):259-69.
6. Yazdani A, Koohsari H, Sadegh Shesh Poli M. Antibacterial activity of honey and bee pollen collected from bee hives from three climatic regions of Golestan Province against some

clinical isolates with high antibiotic resistance. Res Innov Food Sci Technol. 2025;14(1):19-28.
7. Sajjadi SS, Koohsari H, Sadegh Shesh Poli M. Antibacterial activity of soy milk fermented by kefir grain against a number of pathogenic bacteria. Food Res J. 2024;34(2):61-80.
8. Rashti Z, Koohsari H. Antibacterial effects of supernatant of lactic acid bacteria isolated from different dough's in Gorgan city in north of Iran. Integr Food Nutr Metab. 2015;2(3):193-6.
9. Al-Musawi MH, Ibrahim KM, Albukhaty S. In vitro study of antioxidant, antibacterial, and cytotoxicity properties of Cordia myxa fruit extract. Iran J Microbiol. 2022;14(1):97-103.
10. Afzal M, Khan AS, Zeshan B, Riaz M, Ejaz U, Saleem A, et al. Characterization of bioactive compounds and novel proteins derived from promising source Citrullus colocynthis along with in-vitro and in-vivo activities. Molecules. 2023;28(4):1743.
11. Nayak SS, Ankola AV, Metgud SC, Bolmal UK. An in vitro study to determine the effect of Terminalia chebula extract and its formulation on Streptococcus mutans. J Contemp Dent Pract. 2014;15(3):278-82.
12. Raval A, Yadav S, Narwani S, Somkuwar K, Verma V, Almubarak H, et al. Antibacterial efficacy and surface characteristics of boron nitride coated dental implant: an in-vitro study. J Funct Biomater. 2023;14(4):201.
13. Norajit K, Laohakunjit N, Kerdchoechuen O. Antibacterial effect of five Zingiberaceae essential oils. Molecules. 2007;12(8):2047-60. doi:10.3390/12082047. Retraction in: Molecules. 2008;13(3):488-9.
14. Thoithoisana Devi S, Devika Chanu K, Singh NB, Chaudhary SK, Keithellakpam OS, Singh KB, et al. Chemical profiling and therapeutic evaluation of standardized hydroalcoholic extracts of Terminalia chebula fruits collected from different locations in Manipur against colorectal cancer. Molecules. 2023;28(7):2901.
15. Wang J, Liu Q, Feng J, Lv JP, Xie SL. Effect of high-doses pyrogallol on oxidative damage, transcriptional responses and microcystins synthesis in Microcystis aeruginosa TY001 (Cyanobacteria). Ecotoxicol Environ Saf. 2016;134:273-9.
16. Patel K, Tyagi C, Goyal S, Jamal S, Wahi D, Jain R, et al. Identification of chebulinic acid as potent natural inhibitor of M. tuberculosis DNA gyrase and molecular insights into its binding mode of action. Comput Biol Chem. 2015;59:37-47.
17. Han Y, Sun Z, Chen W. Antimicrobial susceptibility and antibacterial mechanism of limonene against Listeria monocytogenes. Molecules. 2019;25(1):33.