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Research Article

Evaluation of Phytochemical Screening, GC-MS Analysis and Anti-Bacterial Potentiality of *Terminalia chebula* Fruit

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Abstract

India is renowned for its native plant *Terminalia chebula* (Combretaceae), known for its extensive therapeutic and ethnomedicinal properties. This study aimed to evaluate the phytochemical composition and antibacterial efficacy of its fruit extracts using the agar well diffusion method. The crude fruit extract and its fractions exhibited notable antibacterial activity, particularly the methanol extracts, prompting GC-MS analysis. This analysis revealed ten different phyto-compounds, with 1, 2, 3-Benzenetriol (44.52% area percentage) and Quinic acid (17.47% area percentage) being the predominant compounds. These compounds are associated with diverse medicinal properties including anti-diabetic, antioxidant, anti-inflammatory, anti-cancer, and antimicrobial effects. These findings underscore the considerable antibacterial potential of *T. chebula* and its rich chemical composition, offering insights into its phytochemical profile for potential pharmaceutical applications.

Keywords: *Ethnomedicinal, Phytochemical, Agar well diffusion, GC-MS, Pharmaceutical.*

Introduction

The rise and dissemination of drug-resistant microbes pose a significant threat to the efficacy of existing medications and stand as a primary cause of treatment ineffectiveness. The burden of morbidity and mortality has disproportionately affected developing nations due to heightened risk factors associated with economic transitions. Antibiotics, previously hailed as miraculous remedies, now struggle to combat resistant bacteria. Many multidrug-resistant microbes have emerged, posing severe threats. In recent years, the approval rate of new antimicrobial medicines has sharply declined, and the availability of effective antimicrobials is projected to diminish rapidly (Dubale, *et al*., 2023).

Medicinal plants offer a complementary or alternative approach to modern medical treatments, harnessing a vast array of structural and biological diversity. Within plants lie phytochemicals, compounds with potent inhibitory effects on pathogen growth. A pressing challenge in global healthcare revolves around the necessity for new, efficacious, and affordable drugs, particularly in developing regions. Some strains of Staphylococcus and Streptococcus are implicated in respiratory and skin infections, while Pseudomonas and various members of Enterobacteriaceae contribute to gastrointestinal and urogenital ailments and wound contamination. These microorganisms have developed substantial resistance to older antibiotics (Wasihun, *et al*., 2023). Hence, there is a critical need to prioritize the discovery of antimicrobial drugs sourced from medicinal plants, especially those traditionally

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employed by healers to combat infectious diseases.

Terminalia chebula, widely recognized as "Harard" in the Indian sub-continent, holds prime medicinal importance. In China, the ripe fruit of *T. chebula* is referred to as "Hezi," while the unripe fruit is known as "Xiqingguo" (Bhatt, *et al*., 2017). It is extensively utilized across various systems of medicine in India, including Ayurveda, Siddha, Unani, and Homeopathy. It holds a prominent position in Unani Materica Medica, being highly regarded for its therapeutic effects in treating a diverse range of ailments such as asthma, hemorrhoids, sore throat, gastric disorders (including vomiting, anorexia, and flatulence), diarrhea, dysentery, splenomegaly, epilepsy, leprosy, skin disorders, melancholia, gout, and joint pain (Kolla, *et al*., 2017). *T. chebula* holds the esteemed title of the "King of Plants" in Ayurveda (Cock, 2015) due to its widespread inclusion in numerous Ayurvedic medicines. Its usage spans various ailments, attributed to its detoxifying and regenerative properties. Additionally, its therapeutic efficacy is attributed to the presence of phenolics, tannins, and flavonoids (Eshwarappa, *et al*., 2015). The main objective of this study was to evaluate the GC-MS analysis and investigate the antibacterial potential of various solvent extracts derived from plants.

Materials and Methods

Sample Collection

The plant *T. chebula* was collected from the natural forest areas in Paderu (18°04'57.10"N, 82°37'30.32"E, elevation 970m) Andhra Pradesh, India in January 2023 and authenticated by Prof. S.B. Padal, Andhra University, Botany Department, Visakhapatnam, Andhra Pradesh, India, with accession number AUV: 25503

Extraction of plant material

The fruit of the plant was harvested, dried in the shade at 32°C, and then finely ground into a powder. This powder underwent sieving through a 0.5 mm sieve to ensure uniform particle size and was stored in sterile containers until needed. Acetone, methanol, and water were chosen as solvents for fruit extraction, in increasing order of polarity. Using a Soxhlet extractor (Harborne, 1984), phytochemicals from the plant material were isolated with the selected solvents, and subsequently stored for 72 hours in sealed jars. Concentrated extracts were obtained by collecting the extracts in a rotary evaporator at 45°C under reduced pressure. The crude extracts were then stored in a refrigerator at 4°C for future use.

Preliminary Phytochemical Screening

The preliminary phytochemical analysis using the crude extracts was conducted following standard protocols (Vishnu, *et al*., 2019).

The extraction values were calculated using the formula shown below:

Yield (%) = W_1/ W_2 x 100

Wherein, W_1 is the extract's weight after the solvent has evaporated and W_2 is the dry weight of the plant sample.

Collection of test pathogens

Four test bacteria were utilized in this study, including two gram-negative strains, *Salmonella enterica* (MTCC 98) and *Pseudomonas aeruginosa* (MTCC 129), as well as two gram-positive strains, *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497). These bacterial cultures were obtained from the Microbial Type Culture Collection (MTCC) at the CSIR-Institute of Microbial Technology (IMTECH) in Chandigarh, India.

Antibacterial activity assay

The antibacterial potential of plant extracts on Nutrient Broth (NB) agar medium was evaluated using the agar well diffusion technique. After inoculating the prepared media into Petri plates and allowing it to solidify, a sterile L-shaped bent rod was used to evenly spread 100 µL of bacterial culture. Wells with a diameter of 5 mm was created on the plates using a sterile cork borer and filled with various solvent extracts (10 mg, 5 mg, and 2.5 mg in 10% aqueous Dimethyl sulfoxide (DMSO)), as well as positive and negative controls of 20 µL each. Following 24 hours of incubation at 37°C, the zones of

inhibition (mm) for the different extracts were measured. Streptomycin (100 µg/mL) served as the positive control, while DMSO (10%) served as the negative control. Results from each test were averaged after three repetitions, and strict aseptic conditions were maintained throughout the microbiological experiment (Murray, *et al*., 1995).

GC-MS analysis

The extracts were analyzed using Agilent Technologies GC-MS (GC-8890, GC/MS 5977 MSD) with an injection mode comprising an 18 mL/min split flow and a 3 mL/min purge flow. The oven temperature was programmed to increase from 75°C to a maximum of 360°C. The column utilized was a Polar Columns (DB-WAX) & HP-5 MS UI with a flow rate of 1.21 mL/min, and helium gas (99.99%) served as the carrier gas. The column temperature ranged from 60°C to 325°C. The GC-MS analysis lasted for a total of 53.5 minutes. Sample components were ionized using the EI mode (70 eV). Spectra of unknown components were compared to those of known components using the Spectral Library and Database (licensed NIST 2017 Library; Software: Open Lab CDS 2.5 version).

Results and Discussion

Soluble compound percentages of different solvent extracts

Table 1 summarizes the percentages of soluble compounds extracted from *Terminalia chebula* fruit using three different solvents: methanol, acetone, and water (aqueous extraction). The extraction process was carried out with a consistent weight of powdered fruit material (100 grams) for each solvent. Methanol extraction yielded 10.5 grams of soluble compounds from 100 grams of powdered *T. chebula* fruit, indicating that methanol is fairly effective in extracting soluble compounds from the fruit. Using acetone as the solvent resulted in 9.3 grams of soluble extract from the same amount of powdered fruit, giving a slightly lower percentage of 9.3%. This shows that acetone is also an effective solvent, though marginally less effective than methanol in this context. Water extraction yielded the highest amount of soluble compounds, with 11.6 grams obtained from 100 grams of powdered material. This represents 11.6% of the initial weight, indicating that water is the most effective solvent among the three for extracting soluble compounds from *T. chebula* fruit. The differences in extraction efficiency can be attributed to the varying polarities of the solvents, which influence their ability to dissolve different types of compounds present in the *T. chebula* fruit. The choice of solvent significantly impacts the yield of soluble compounds. For practical applications, such as in pharmaceutical or nutraceutical formulations, selecting the appropriate solvent based on the desired extract composition and yield is crucial.

Table 1: Percentages of the soluble compounds in *T. chebula* fruit extracts

The solvent used for extraction	Weight of the powered material	Weight of the soluble extract	Percentage of the extract
Methanol	100 gms	10.5	10.5
Acetone	100 gms	29.3	29.3
Aqueous	100 gms	11.6	11.6

Preliminary qualitative phytochemical evaluation

The qualitative screening conducted on the different *T. chebula* extracts indicated the presence of various compounds including carbohydrates, proteins, fixed oils and fats, alkaloids, phenols, tannins, flavonoids, terpenoids, glycosides, and saponins, as shown in Table 2. Methanol and acetone extracts exhibited a broad spectrum of secondary metabolites, followed by the aqueous extract. However, phenols, tannins, terpenoids, and glycosides were consistently detected in all three solvent extracts. Methanol as a solvent is highly effective in extracting a wide range of phytochemicals, including carbohydrates, proteins, alkaloids, phenols, tannins, flavonoids, terpenoids, and

glycosides. Water is effective for phenols and tannins, moderately effective for terpenoids, glycosides, and saponins, but not effective for carbohydrates, proteins, alkaloids, or flavonoids. Acetone is effective for carbohydrates, phenols, tannins, flavonoids, terpenoids, and glycosides, but not for proteins, alkaloids, or saponins.

Table 2: Preliminary phytochemical analysis of different solvent extracts of *T. chebula* fruit

Plant Constituents	Methanol extract	Aqueous extract	Acetone extract
Carbohydrates	++	--	+
Proteins	+	-	-
Fixed oils and fats	-	-	-
Alkaloids	+	-	-
Phenols	++	++	+
Tannins	++	++	+
Flavanoids	+	-	+
Terpenoids	++	+	++
Glycosides	++	+	+
Saponins	-	+	-

Previous research has indicated that the tannin content in *T. chebula* fruits is substantial, ranging from approximately 32% to 34%, with variations observed based on geographical distribution (Jayaramkumar, 2009; Kumar, *et al*., 2003). A team of researchers identified 14 hydrolyzable tannin components from *T. chebula* fruits, including gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl- β-D-glucose, 1,6-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose, and terchebulin (Juang, *et al*., 2004). In a study conducted by Praveen, *et al*. in 2019, it was revealed that the leaf extract of *T. chebula* comprises various phytocompounds. Proteins, carbohydrates, and tannins were identified in both aqueous and methanolic extracts, while alkaloids were found in both aqueous and methanolic extracts of the fruit. Flavonoids were present in both types of extracts from all parts except the root. Saponins and glycosides were detected in all extracts. Furthermore, volatile oil was solely detected in the methanolic and aqueous extracts (Vemuri, *et al*., 2019). According to Baliah, *et al*. in 2014, the fruit extracts of *T. chebula* Retz. were found to contain a variety of phytochemicals, including glycosides, alkaloids, flavonoids, phenolic compounds, saponins, steroids, quinine, and tannins (Baliah & Astalakshmi, 2014). In a subsequent study by Tariq, *et al*. in 2013, it was observed that alkaloids, flavonoids, and terpenoids were present in the methanolic extract of the leaf, fruit, seed, stem, and root of *T. chebula* (Tariq & Reyaz 2013).

Antibacterial activity

The antibacterial bioassay results against pathogens using crude extracts of the *T. chebula* plant (10 mg, 5 mg, and 2.5 mg of each) were presented in terms of the diameter of the zone of inhibition. The antibacterial activity outcomes for gram-negative and gram-positive bacteria are depicted in Tables 3 and 4, and Figures 1 and 2, respectively.

Against gram-negative bacterial strain

The methanol extract, at a dosage of 10 mg, exhibits a zone of inhibition measuring 21.3±1.2 mm and 14.3±0.57 mm against *Salmonella enterica* and *Pseudomonas aeruginosa*, respectively. Decreased dosages result in diminished inhibitory effects, with the lowest dosage (1.25 mg) showing the least impact. Notably, the methanol extract displays significant inhibition against *P. aeruginosa* across all dosages, indicating its effectiveness against this bacterium. While the inhibitory effect against *S. enterica* is notable, it decreases with lower dosages. The acetone extract demonstrates a zone of inhibition against both bacteria at all dosages, albeit with

diminishing effectiveness at lower dosages. Interestingly, at a dosage of 10 mg, the zone of inhibition is higher for *S. enterica* (19±1.3 mm) compared to *P. aeruginosa* (13.6±0.57 mm). The acetone extract consistently exhibits inhibitory effects against both bacteria. On the other hand, the aqueous extract shows a notable zone of inhibition against *S. enterica* at all dosages but remains inactive against *P. aeruginosa* across all tested dosages. However, the inhibitory effect is generally lower compared to methanol and acetone extracts. As a positive control, streptomycin demonstrates a zone of inhibition measuring 32±1.23 mm and 31±0.95 mm against *S. enterica* and *P. aeruginosa*, respectively.

Against gram-positive bacterial strain

The methanol extract demonstrates a significant inhibitory effect against *Staphylococcus aureus* at all dosages. However, there is no inhibition against *Streptococcus mutans* at dosages of 2.5 mg and 1.25 mg, and minimal inhibition is observed at dosages of 5 mg and 10 mg. The highest zone of inhibition (23.3±1.2 mm) is observed when methanol extract is applied to *S. aureus* at a dosage of 10 mg. The acetone extract consistently exhibits inhibitory effects against both bacteria at all dosages, with a more pronounced effect against *S. aureus* compared to *S. mutans*. The highest zone of inhibition (21.6±0.57 mm) is observed against *S. aureus* at a dosage of 10 mg. On the other hand, the aqueous extract displays a zone of inhibition against *S. aureus* but is inactive against *S. mutans*. The inhibitory effect is moderate compared to methanol and acetone extracts. As a positive control, streptomycin exhibits a zone of inhibition measuring 30±1 mm and 32.3±0.57 mm against *S. aureus* and *S. mutans*, respectively.

Table 3: Zone of Inhibition of different extracts (Methanol, Acetone and Aqueous) of *T. chebula* fruit in millimeters at the dosages of 10mg, 5mg, 2.5mg and 1.25 mg against gram-negative bacteria

Extract	*Salmonella enterica*				*Pseudomonas aureginosa*			
	10mg	5 mg	2.5mg	1.25 mg	10mg	5 mg	2.5mg	1.25 mg
Me	21.3 ± 1.2	20.6± 0.75	20 ±1	15.3 ±0.57	14.3±0.57	12±1	-	-
Ac	19 ±1.3	14 ± 1.2	12.3 ± 0.57	11.6 ± 0.57	13.6±0.57	10.3±0.57	-	-
Aq	15.3± 0.57	11±1	10.3±0.57	-	-	-	-	
+ Ve	32 ± 1.23 mm				31± 0.95			

Data showing zone of inhibition in mm. me: methanol extract, Ac: acetone extract and Aq: aqueous extract. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

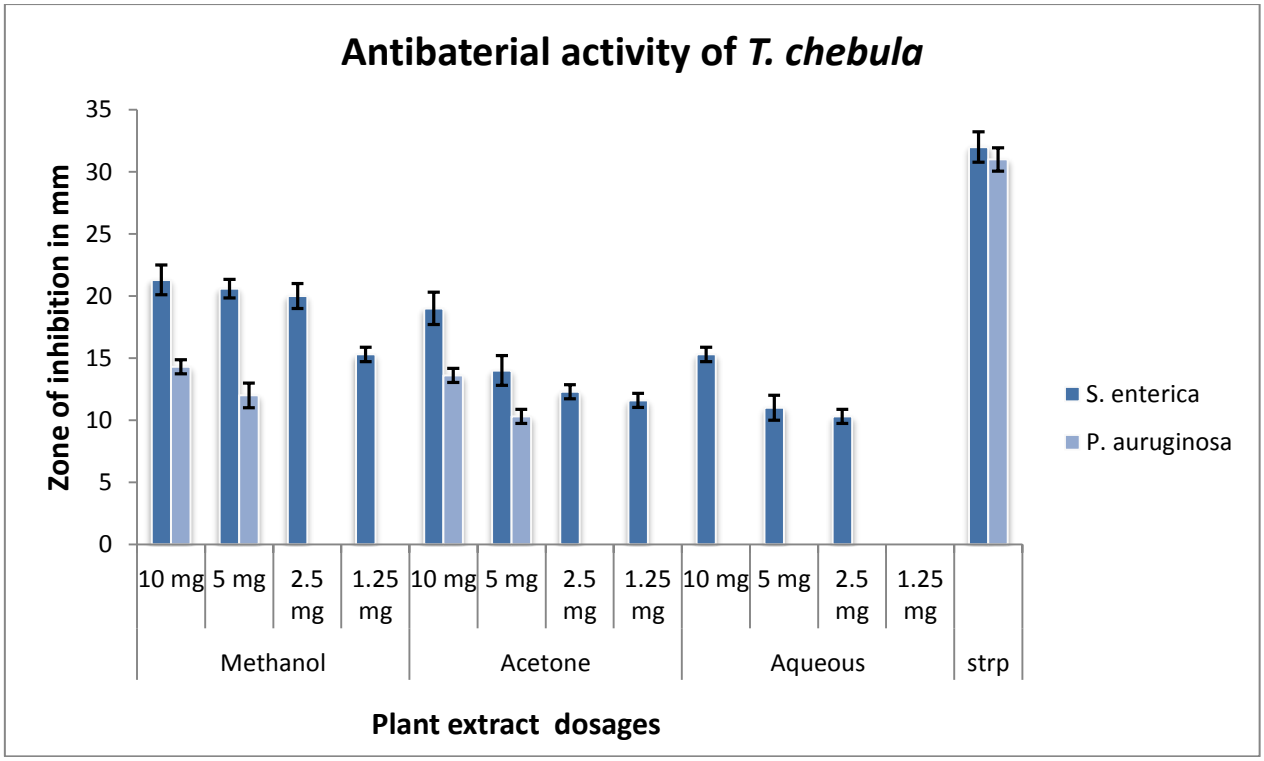

Fig 1: Antibacterial activity (% of inhibition) of *T. chebula* against gram-negative bacteria

Table 4: Zone of Inhibition of different extracts (Methanol, Acetone and Aqueous) of *T. chebula* fruit in millimeters at the dosages of 10mg, 5mg, 2.5mg and 1.25 mg against gram-positive bacteria

Extract	*Staphylococcus aureus*				*Streptococcus mutans*			
	10mg	5 mg	2.5mg	1.25 mg	10mg	5 mg	2.5mg	1.25 mg
Me	23.3 ± 1.2	16.5± 0.75	-	-	21.3 ±1.3	16 ± 1.2	13.3 ± 0.57	9.6 ± 0.57
Ac	21 ±1	16.3 ± 0.57	11.3 ± 0.57	9.6 ± 0.57	20.6±0.57	17.3±0.57	12±0.57	10±0.57
Aq	18.3± 0.57	11±1	-	-	22±0.75	17±1	13±0.67	12±0.57
+ Ve	30 ± 1mm				32.3± 0.57			

Data showing zone of inhibition in mm. me: methanol extract, Ac: acetone extract and Aq: aqueous extract. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

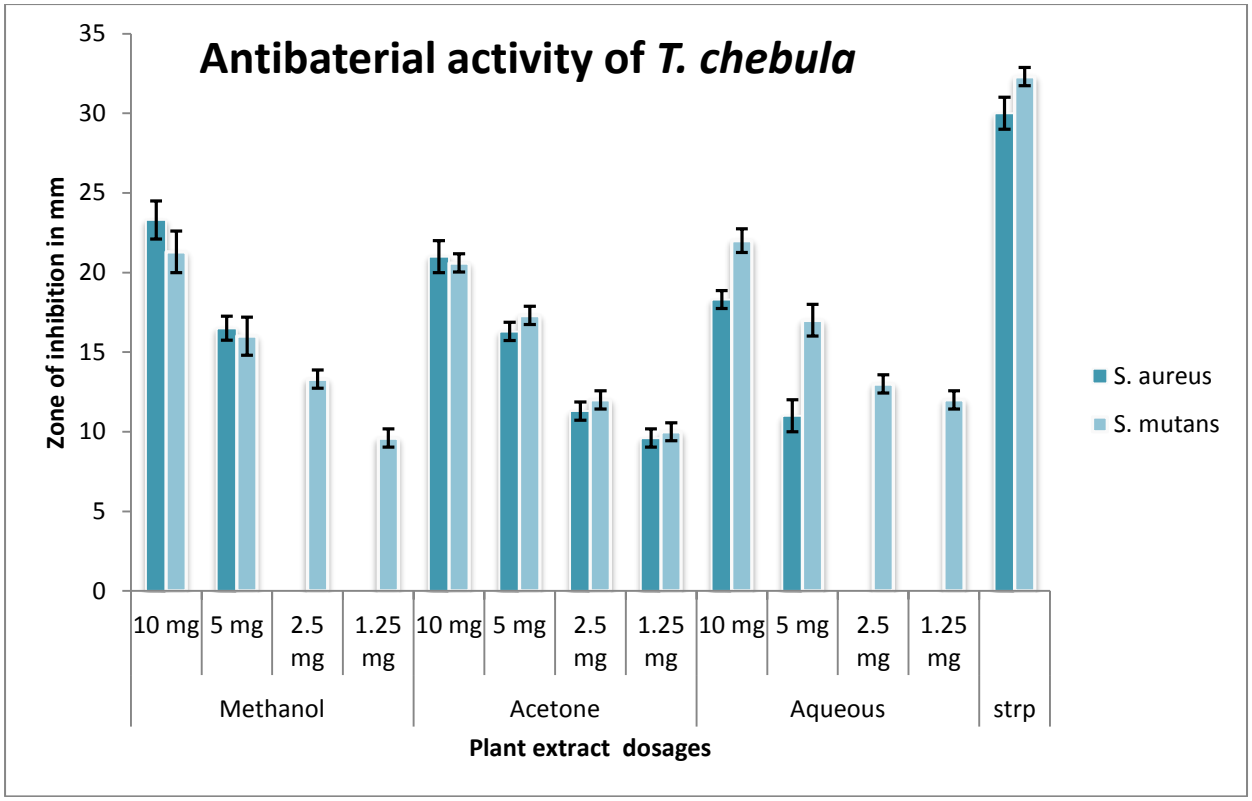

Fig 2: Antibacterial activity (% of inhibition) of *T. chebula* against gram-positive bacteria

As per Kannan, *et al*., (2009), the fruit extract of *T. chebula* exhibited notable effectiveness against a range of bacteria, including *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Mary and Archana (2013) discovered that the aqueous extract of *Terminalia chebula* Retz. Displayed potent antimicrobial activity, particularly notable against *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. Mostafa, *et al*. (2011) demonstrated that both methanol and aqueous extracts of *Terminalia chebula* were efficacious against *Escherichia coli*, *Salmonella* species, *Shigella* species, and *Vibrio cholerae*. Ghosh, *et al*. (2007) documented notable antibacterial potential in the methanolic leaf extract against *Enterobacter aerogenes* and *Proteus vulgaris*, whereas the aqueous leaf extract exhibited efficacy against *Proteus vulgaris* and *Enterobacter aerogenes.*

Furthermore, the leaf extract of *T. chebula* demonstrated comparable potency to antibiotics such as ciprofloxacin, gentamycin, kanamycin, ofloxacin, and cephalexin. Shankara, *et al*. (2012) revealed that the ethanol extract of *T. chebula* leaf gall exhibited superior antibacterial activity compared to the aqueous extract, with *Staphylococcus aureus* being the most susceptible organism. However, *Serratia marcescens* and *Proteus mirabilis* showed less susceptibility to ethanol and aqueous extracts, respectively. Jagtap and Karkera (1999) determined that the aqueous extract of *T. chebula* had antibacterial activity against *S. mutans*, with a minimum inhibitory concentration (MIC) of 6%. Kim, *et al*. (2006) reported potent inhibitory effects of ellagic acid derived from *T. chebula* fruits against *Clostridium perfringens* and *Escherichia coli*. Moreover, Suguna, *et al*. (2002) identified antimicrobial activity in alcohol extract of *T. chebula* leaves, particularly against *Staphylococcus aureus* and *Klebsiella*.

The present study findings align with previous literature, indicating that *T. chebula* extracts exhibit robust antibacterial activity against the tested bacterial strains. Methanol and acetone extracts tend to demonstrate higher inhibitory effects compared to the aqueous extract. Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, show greater susceptibility to the extracts compared to gram-positive bacteria like *Streptococcus*

mutans. Dosage is a critical factor, with higher dosages generally resulting in larger zones of inhibition. Overall, the study underscores the potential antibacterial properties of *T. chebula* fruit extracts, with methanol and acetone extracts showing enhanced effectiveness against the tested bacteria.

GC-MS chemical profiling

The methanol extract was selected for Gas Chromatography-Mass Spectrometry (GC-MS) analysis from the three plant specimens due to its efficient extraction capacity for a diverse array of phytochemical constituents, including phenolic compounds, alkaloids, flavonoids, and various other compounds. This superior phytochemical extraction capability can be attributed to the polarity of methanol and its ability to disrupt the cellular membranes of plants. Notably, the methanol extract exhibited the highest degree of inhibition against microbial growth compared to other solvent extracts explored in this study. Therefore, we utilized GC-MS to conduct a comprehensive phytochemical profiling of the methanolic fruit extracts derived from *T. chebula*, thereby enabling a detailed chemical characterization of the plant specimen.

The experimental process outlined in this research led to the detection of several peaks in the Gas Chromatography-Mass Spectrometry (GC-MS) data obtained from the methanol extract of *T. chebula*, as depicted in Fig 3. These peaks indicated the presence of 10 different chemical compounds. Subsequently, all these compounds were meticulously recorded in Table 5, which contains information such as percentages of peak area, molecular formulas, molecular weights, and their sequence based on retention times. Moreover, an extensive review of existing literature was conducted to explore the biological activities associated with these compounds. The analysis revealed that most of these compounds possess various pharmacological and therapeutic properties. Particularly noteworthy is the diverse array of bioactive effects exhibited by the compounds identified through GC-MS analysis, including anti-cancer, antimicrobial, anti-inflammatory, analgesic, antioxidant, and pain-relieving properties.

The compounds identified in the GC-MS spectrum were analyzed, revealing the prominence of certain components. Particularly noteworthy are 1,2,3-Benzenetriol, with a peak area percentage of 44.52%, and Quinic acid, with 17.47% area percentage, as the major compounds in this fruit methanol extract. Additionally, 2-Propenoic acid, 3-phenyl, contributed 10.28%, while Shikimic acid and Tridecanoic acid exhibited area percentages of 7.37% and 6.74%, respectively, indicating their presence in relatively higher amounts. Diclosulam and Benzoic acid hydrazide also played significant roles, with area percentages of 4.39% and 3.24%, respectively. Other noteworthy compounds include 2,2-Dimethyl-4-pentenoic acid (2.80%), D-(+)-Galactose (1.92%), and Diglycolamine (1.26%).

Table 5: Phytochemical compounds reported in methanol extract of *T. chebula* fruit

S. No	Compound name	Rt minute	Mol. weight g/mol	Mol. Formula	Area%
1.	Diclosulam	8.770	406.2	$C_{13}H_{10}Cl_2FN_5O_3S$	4.39
2.	1,2,3-Benzenetriol	12.396	126.11	$C_6H_6O_3$	44.52
3.	2-Propenoic acid, 3-phenyl	13.473	148.16	$C_9H_8O_2$	10.28
4.	D-(+)-Galactose	16.494	180.16	$C_6H_{12}O_6$	1.92
5.	Quinic acid	17.554	192.17	$C_7H_{12}O_6$	17.47
6.	Benzoic acid, hydrazide	19.328	136.15	$C_7H_8N_2O$	3.24
7.	Shikimic acid	21.013	174.15	$C_7H_{10}O_5$	7.37
8.	Diglycolamine	25.338	105.14	$C_4H_{11}NO_2$	1.26
9.	Tridecanoic acid	26.236	214.34	$C_{13}H_{26}O_2$	6.74
10.	2,2-Dimethyl-4-pentenoic acid	30.979	128.169	$C_7H_{12}O_2$	2.80

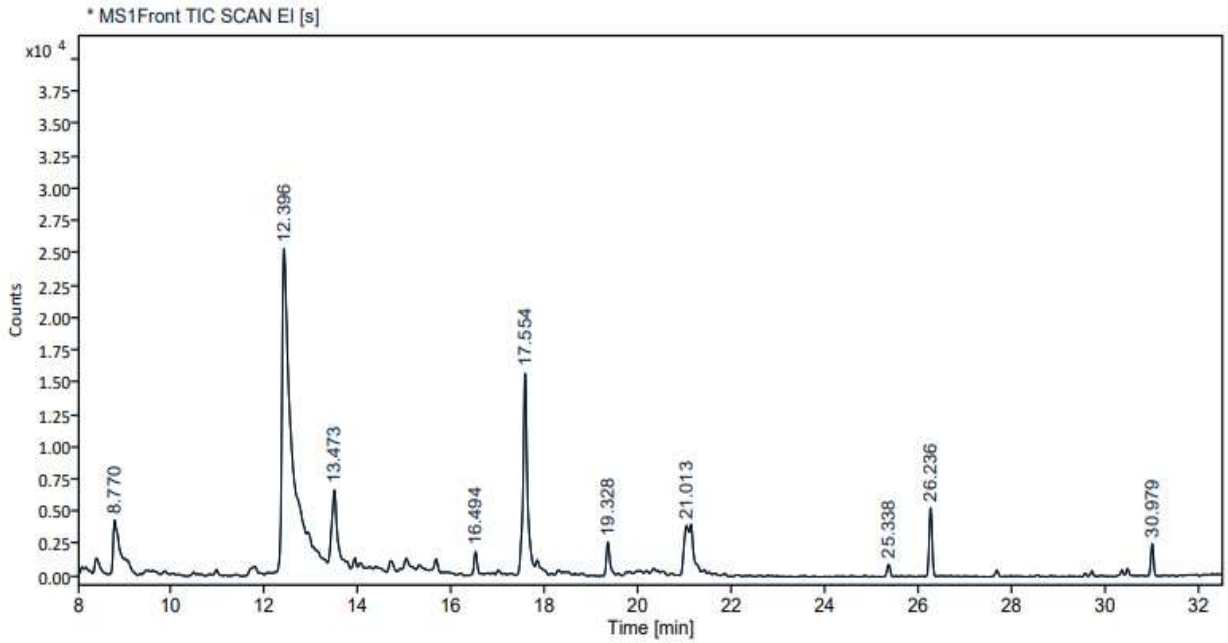

Fig 3: GC-MS chromatogram of methanolic fruit extract of *T. chebula*

Diclosulam is a synthetic compound with herbicidal properties, belonging to the sulfonylurea class, utilized for weed control in various crops (Grichar, *et al*., 2004). 1,2,3-Benzenetriol is a natural phenolic compound recognized for its antioxidant properties. It finds potential applications in pharmaceuticals, food additives, and cosmetics due to its antioxidative activity (Choi, *et al*., 2012). 2-Propenoic acid, 3-phenyl, also known as cinnamic acid, possesses antioxidant, anti-inflammatory, and anticancer properties. Classified as a phenylpropanoid, it is employed in cosmetics, food additives, and pharmaceuticals owing to its antioxidative and anti-inflammatory effects (Pontiki, *et al*., 2014).

Galactose is a monosaccharide commonly occurring in nature and serves as an essential component of glycoproteins and glycolipids. Belonging to the aldohexose group, it is utilized in cell culture media, pharmaceuticals, and the food industry (Xu, *et al*., 2020). Quinic acid is a cyclic polyol compound present in various plants, renowned for its antioxidant and anti-inflammatory properties. Widely employed in pharmaceuticals and cosmetics, it is valued for its antioxidative and anti-inflammatory effects (Zhang, *et al*., 2013). Benzoic acid hydrazide is a hydrazide derivative of benzoic acid, recognized for its antibacterial and antifungal activities. It holds promise in pharmaceuticals and agriculture as antimicrobial agents (Maslat, *et al*., 2002).

Shikimic acid serves as a crucial intermediate in the biosynthesis of aromatic amino acids and exhibits antiviral properties. This cyclohexene derivative finds utility in the pharmaceutical industry for the synthesis of oseltamivir (Tamiflu), an antiviral drug (Bochkov, *et al*., 2011). Diglycolamine, derived from ethylene glycol, is primarily utilized as an absorbent in gas purification processes. With wide-ranging applications in industries, it is employed in gas treatment processes for CO2 capture and the removal of acidic gases (Moore, *et al*., 1984). Tridecanoic acid, a saturated fatty acid, demonstrates antimicrobial and antifungal activities. It holds promise in pharmaceuticals and cosmetics as an antimicrobial agent (Kabara, *et al*., 1972).

In a study by Singh and Kumar (2013), *Terminalia chebula* fruits were found to contain sixty-four constituents, including the first

detection of kaempferol-3-O-rutinoside flavonoid and Vitamin E. Pyrogallol, constituting 46.26% of the extract, was isolated and tested for antimicrobial activity, showing significant potential against selected pathogens via Disc Diffusion Assay. Notably, the crude ethyl acetate fraction of the fruits exhibited comparable antimicrobial activity to pure pyrogallol, highlighting its efficacy as a major component. According to Devi, *et al*. (2023), 1,2,3-Benzenetriol emerged as the predominant chemical constituent across all four extracts of *T. chebula*, constituting a range of 20.95% to 43.56%. Additionally, 2-Cyclopenten-1-one, 5-hydroxymethylfurfural, and catechol were consistently found in all extracts. These findings align with the current investigation, which also identified 1,2,3-Benzenetriol as the most abundant chemical constituent in the methanol extract of *T. chebula* fruit. 1,2,3-Benzenetriol and pyrogallol are recognized for their diverse biological properties, such as antibacterial, antioxidant, antifungal, antiviral, antiseptic, antidermatitic, cardioprotective, antimutagenic, and pesticidal activities **(Hirasawa & Takada, 2004; Biskup, *et al.*, 2013; Cynthia, *et al.*, 2018)**. In contrast to earlier findings, our study presents the most recent analysis of the GC-MS profile of the methanolic extracts of the fruit.

Conclusion

The preliminary screening of *T. chebula* fruit extracts, including methanol, acetone, and aqueous extracts, hinted at the potential for deriving antibacterial medicinal compounds. These extracts contain a variety of phytochemicals such as carbohydrates, proteins, oils, alkaloids, phenols, tannins, flavonoids, terpenoids, glycosides, and saponins. Notably, the methanol extracts demonstrated efficacy against *S. aureus* and moderate effectiveness against other bacterial strains. A comparison with standard extracts revealed *S. aureus* as the most susceptible pathogen to *T. chebula*, while *P. aeruginosa* showed greater resistance.

These findings offer a solid scientific rationale for employing *T. chebula* in traditional medicine for bacterial infections. GC-MS analysis identified ten phytochemical components possessing antifungal, antibacterial, antioxidant, anti-inflammatory, and anticancer properties. However, further investigation is warranted to isolate and characterize the active principles for potential pharmaceutical use. The chemical analysis highlighted robust antibacterial properties, emphasizing the importance of isolating pure compounds, elucidating their structures, and subjecting them to antimicrobial tests. The researchers anticipate that continued studies in this realm will contribute to the development of novel antimicrobial and anticancer drugs.

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