# Investigating Antibacterial Activities of Phytochemicals from *Terminalia chebula* and *Terminalia bellirica* fruit extract: An *in vitro* and *in silico* Study

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***Abstract***

*This study aims to investigate the antibacterial activities of phytochemicals derived from Terminalia chebula (T. Chebula) and Terminalia bellirica (T. bellirica) through a combined approach of in vitro and in silico analyses. T. chebula and T. bellirica are medicinal plants widely recognized for their therapeutic properties and have been traditionally used to treat various infectious diseases. The potential antibacterial properties of these plants have drawn significant interest in recent years. In the in vitro phase of the study, crude extracts of dried fruit of T. chebula and T. bellirica are prepared and subjected to various antibacterial assays against a panel of clinically significant bacterial strains. It has been found that both extracts were effective against Staphylococcus aureus but had no inhibitory effect on Escherichia coli and Salmonella typhi at the tested concentrations.*

*Furthermore, the study incorporated an in silico analysis to predict the interactions between the active phytochemical constituents and Staphylococcus aureus targets. Molecular docking was employed to examine the binding affinity and mode of interaction of these phytochemicals with the active site of protein DNA Gyrase of Staphylococcus aureus (3U2D). Most of the phytochemicals posed the highest binding affinity than inbuilt ligands. The protein interacted effectively with phytochemicals through various types of interactions including hydrogen bonding, electrostatic interactions, carbon-hydrogen bonding, metal-ion coordination and hydrophobic interactions. These interactions suggest potential binding mechanisms and provide important information about the potential biological activities of these phytochemicals. From ADME and toxicity analysis, phytochemicals "Syringic acid," "Ellagic acid" and "Punicalagin" from Terminalia chebula and "Ellagic acid," "Chebulagic acid" and "Terchebin" from Terminalia bellirica emerged as potential druggable candidates with relatively low toxicity concerns.*

**Keywords:** *Terminalia chebula (T. chebula), Terminalia bellirica* (*T. bellirica),* Antibacterial activity, *Staphylococcus aureus*, DNA-Gyrase, Molecular docking, ADME-toxicity analysis.

## Introduction

*T. chebula* and *T. bellirica* are renowned for their therapeutic properties in traditional Ayurvedic medicine[1]. Both fruits have been utilized extensively in various formulations to treat a wide range of ailments due to diverse phytochemical composition. *T. chebula*, commonly known as Haritaki and *T. bellirica*, or Bahera are two of the three key ingredients in Triphala, the traditional herbal formulation in Ayurveda. Each of these fruits has unique properties and contributes to the overall health benefits of Triphala when combined with Amla (*Emblica officinalis*). When Haritaki, Bibhitaki and Amla are combined to form Triphala, their individual properties synergize, creating a powerful and well-balanced herbal remedy. Triphala is highly regarded in Ayurveda for its ability to support overall well-being and to promote longevity[9]. Its comprehensive benefits make it a popular choice for a wide range of health concerns.

Individually, *T. chebula*, is rich in bioactive compounds such as tannins, flavonoids (e.g. quercetin, kaempferol), phenolic compounds, gallic acid, chebulinic acid and chebulagic acid (Table 1). These compounds have demonstrated significant antioxidant activity, helping to neutralize harmful free radicals and reduce oxidative stress in the body. Additionally, chebulagic acid has exhibited anti-inflammatory properties by inhibiting various inflammatory mediators, making *T. chebula* an attractive candidate for combating inflammation-related conditions[12].

On the other hand, *T. bellirica*, is also packed with various bioactive components. It contains alkaloids (e.g. berberine), tannins, flavonoids (e.g. quercetin, rutin), ellagic acid and gallic acid (Table 1). These compounds contribute to its potential antimicrobial properties and provide a range of health benefits[7]. Bahera has shown antimicrobial activity against various bacterial strains, making it a promising natural agent in the fight against antibiotic-resistant bacteria[7]. The combination of flavonoids, alkaloids and terpenoids found in both *T. chebula* and *T. bellirica* contributes to their potent medicinal effects, especially antibacterial properties. These phytochemicals work synergistically to enhance their therapeutic potential, making them valuable candidates for addressing modern health challenges such as antibiotic resistance.

In the modern era, antibiotic resistance has become a serious global health threat. The World Health Organization estimates that if left unaddressed, antibiotic resistance could cause 10 million deaths annually by 2050[14]. Consequently, there is an urgent need for novel and effective antimicrobial

agents. This study's relevance stems from the exploration of potential antibacterial properties in phytochemicals derived from *T. chebula* and *T. bellirica* fruits, harnessing their traditional therapeutic potential to address modern health challenges.

The utilization of *in silico* techniques along with *in vitro* analysis, specifically molecular docking, provides an efficient, cost-effective and predictive approach for investigating potential interactions between these phytochemicals and bacterial proteins[8]. Molecular docking studies can shed light on the mechanisms of action of these compounds and can help to predict their efficacy, providing valuable preliminary data before conducting *in vitro* and *in vivo* studies[8]. ADME toxicity analysis has also been conducted in the study.

This is a comprehensive process that provides critical information about a drug candidate's behavior in the body, its potential for toxicity and its overall safety profile. This analysis is an essential step to ensure the selection of safe and effective drug candidates for further clinical evaluation[4].

This study, therefore, aims to explore the *in vitro* and *in silico* antibacterial activity of phytochemicals extracted from *T. chebula* and *T. bellirica* fruit. The outcomes of this research would provide valuable insights into the development of potent, natural and non-toxic solutions against antibiotic-resistant bacterial strains.

## Material and Methods

**Chemicals and Reagents:** *T. Bellirica* fruit extract and *T. Chebula* fruit extract, Muller Hinton agar, Tryptone Soya agar and double distilled deionized water were used.

**Collection of Fruit:** The fruits of *T. bellirica* were collected during December 2022 from Malappuram, Kerala. The fruits were washed thoroughly under running tap water for 2-3 times to remove dirt and then shade dried at room temperature.

**Preparation of Fruit Extract:** Approximately, 100g of *T. chebula* fruit was dried, crushed and ground into a fine powder after removing the seeds. The powdered fruit (30g) was then subjected to extraction using 50ml of water and shaken for 3 hours using a magnetic stirrer. After allowing it to stand for half an hour, a funnel was set on a tripod stand, lined with a Whatmann no. 41 filter paper, with a beaker placed underneath. Following the half-hour waiting period, the extract was filtered into the beaker using a sterilized Whatmann no. 40 filter paper and the resulting extract was stored in sterile bottles for antimicrobial analysis.

The methodology was carried out meticulously to ensure the purity and sterility of the obtained extract, making it suitable for subsequent antimicrobial studies. The same procedure was repeated for *T. bellirica.* The steps for preparation for *T. bellirica* are shown in figure 1.

**Table 1**
**Phytochemicals present in *T. Chebula and T. Bellirica***

| | ***T. Chebula*** | | ***T. Bellirica*** |
|---|---|---|---|
| 1 | Arjungenin | 1 | Arjungenin |
| 2 | Beta-Sitosterol | 2 | Beta-Sitosterol |
| 3 | Chebulagic Acid | 3 | Chebulagic Acid |
| 4 | Ellagic Acid | 4 | Ellagic Acid |
| 5 | Ethyl Gallate | 5 | Ethyl Gallate |
| 6 | Gallic Acid | 6 | Gallic Acid |
| 7 | Tannic Acid | 7 | Tannic Acid |
| 8 | Arjunolic Acid | 8 | Anolignan B |
| 9 | Caffeic Acid | 9 | Belleric Acid |
| 10 | Chebulic Acid | 10 | Bellericagenin A |
| 11 | Chebulinic Acid | 11 | Bellericagenin B |
| 12 | Daucosterol | 12 | Bellericaside A |
| 13 | Ellagitannin | 13 | Bellericaside B |
| 14 | Ferulic Acid | 14 | Beta-Glucogallin |
| 15 | Maslinic Acid | 15 | Corilagin |
| 16 | Punicalagin | 16 | Methylenedioxyflavan |
| 17 | Sennoside A | 17 | Termilignan |
| 18 | Shikimic Acid | | |
| 19 | Syringic Acid | | |
| 20 | Terchebin | | |
| 21 | Terchebulin | | |
| 22 | Terflavin A | | |
| 23 | Vanillic Acid | | |

**Fig. 1: Preparation steps of fruit extract of *T. bellirica***

***In vitro* Antibacterial Analysis:** In this antimicrobial activity study, three multidrug-resistant bacterial strains namely *Escherichia coli*, *Salmonella Typhi* and *Staphylococcus aureus* were utilized for the investigation. The disc-diffusion method, a widely recognized culture-based microbiology assay, was employed to evaluate the antimicrobial properties of *T. chebula* and *T. bellirica* fruit extract. To prepare the necessary media, tryptone soya agar (TSA) and tryptone soya broth (TSB) were autoclaved, poured into sterile Petri dishes and test tubes respectively while Muller Hinton agar (MHA) was solidified in sterile Petri dishes. For bacterial culture preparation, pure culture plates of each bacterial strain were used and fresh TSB tubes were inoculated and incubated. Following that, the MHA plates were inoculated with the bacterial cultures using sterile cotton swab sticks. The *T. bellirica* fruit extract was then placed on the MHA plate and a penicillin antibiotic disk was added at a distance from the sample.

Later, the plates were incubated at a specific temperature for 24 hours. The antibacterial activity was assessed by measuring the diameter of the inhibition zone (IZ) surrounding the discs. The assay was repeated three times and the mean zone of inhibition diameters (mm) produced by each fruit extract was used to express the antibacterial activity.

**Molecular Docking Methodology:** Molecular docking was employed to investigate the inhibitory mechanism of phytochemicals from *T. chebula* and *T. bellirica* against *Staphylococcus aureus*. The crystal structure of the target protein, DNA Gyrase (Protein Data Bank ID: 3U2D)[6] was obtained from the Protein Data Bank (PDB)[9] and prepared using Discovery Studio. Ligands, representing the phytochemicals, were sourced from the IMPPAT database in pdbqt format and underwent preparation. The binding site on the protein was identified based on literature and defined using Discovery Studio tools. AutoDock Vina[13] was utilized for molecular docking, with docking parameters specified as follows: center_x = 16.1616, center_y = -19.3633, center_z = 6.6837, size_x = 40, size_y = 40, size_z = 40 and exhaustiveness = 8. These parameters determined the search space or grid box coordinates and the extent of the docking search, allowing for the exploration of various conformations and orientations of the ligand within the binding site. AutoDock Vina's scoring function ranked the docking poses based on their predicted binding affinity. Docking results were analyzed and interactions with key residues were identified using Discovery Studio[2].

**Drug Likeness and ADME Toxicity Prediction:** Drug-likeness and ADME (Absorption, Distribution, Metabolism and Excretion) prediction play crucial roles in the process of drug discovery and development. They help in assessing the viability of a potential drug candidate in terms of its behavior in the human body and its resemblance to existing approved drugs. Toxicity prediction helps in determining the potential safety concerns associated with the use of the drug. The online software SwissADME[5] was used for ADME analysis and DataWarrior[11] for toxicity study.

## Results and Discussion

***In vitro* Antibacterial Activity of *T. bellirica*:** The antibacterial activity of aqueous extract of *T. bellirica* was determined against *Escherichia coli* , *Salmonella typhi* and *Staphylococcus aureus* by using disc diffusion method. The positive control used was penicillin. The bacterial inhibition of fruit extract was examined on the basis of zone of inhibition described in table 2.

The highest zone of inhibition was obtained for *Staphylococcus aureus* and no inhibition zone was obtained for others. The zone of inhibition for *Staphylococcus aureus* was obtained in the range of 1.4mm to 2.2mm. Here the zone of inhibition is used to express the measurement of the susceptibility of the bacteria towards the aqueous extract of *T. bellirica* fruit.

***In vitro* Antibacterial Activity of *T. Chebula*:** The antibacterial activity of aqueous extract was examined against pathogenic bacteria using agar well diffusion method. The positive control used was pencillin. The antibacterial tendency of the fruit extracts was determined

based on the zone of inhibition as shown in table 3. The inhibition zones of *Staphylococcus aureus* were in the range of 1.9 to 2.5mm. The highest zone of inhibition was obtained against *Staphylococcus aureus* with no inhibition against others. The aqueous extract could not inhibit the growth of *Escherichia coli* and *Salmonella typhi*. The permeability of the compound and the resistance mechanisms displayed by the microbes could be the reason for the variable zones of inhibition exhibited by the pathogens.

**Molecular Docking:** Molecular docking was carried out to predict the binding affinities of phytochemicals of *T. bellirica* and *T. chebula* at the active sites of protein DNA Gyrase of *Staphylococcus aureus*. The docking scores in terms of binding affinity of different phytochemicals obtained were tabulated and given in table 4. The docking scores provided an estimate of the binding affinity of each phytochemical to DNA gyrase.

**Table 2**
**Zone of inhibition of fruit extract of *T. bellirica***

| S.N. | Organism | Zone of inhibition 60% (mm) | Zone of inhibition 40% (mm) | Zone of inhibition 20% (mm) |
|---|---|---|---|---|
| 1 | *Escherichia coli* | NIL | NIL | NIL |
| 2 | *Salmonella typhi* | NIL | NIL | NIL |
| 3 | *Staphylococcus aureus* | 2.2 | 2 | 1.4 |

**Table 3**
**Zone of inhibition of fruit extract of *T. Chebula***

| S.N. | Organism | Zone of inhibition 60% (mm) | Zone of inhibition 40% (mm) | Zone of inhibition 20% (mm) |
|---|---|---|---|---|
| 1 | *Escherichia coli* | NIL | NIL | NIL |
| 2 | *Salmonella typhi* | NIL | NIL | NIL |
| 3 | *Staphylococcus aureus* | 2.5 | 2 | 1.9 |

**Table 4**
**Molecular docking score of various phytochemicals**

| ***T. Bellirica*** | | ***T. Chebula*** | |
|---|---|---|---|
| **Compounds** | **Binding Affinity (Kcal/Mol)** | **Compounds** | **Binding Affinity (Kcal/Mol)** |
| Chebulagic Acid | -9.1 | Terflavin A | -10.1 |
| Corilagin | -8.9 | Terchebulin | -9.9 |
| Ellagic Acid | -8.7 | Chebulinic Acid | -9.2 |
| Methylenedioxyflavan | -8.2 | Chebulagic Acid | -9.1 |
| Bellericagenin A | -8.0 | Ellagitannin | -9.0 |
| Bellericaside A | -8.0 | Terchebin | -8.8 |
| Termilignan | -7.8 | Ellagic Acid | -8.7 |
| Anolignan B | -7.6 | Punicalagin | -8.7 |
| Arjungenin | -7.4 | Sennoside A | -8.5 |
| Beta-Glucogallin | -7.3 | Daucosterol | -8.0 |
| Beta-Sitosterol | -7.3 | Maslinic Acid | -7.7 |
| Belleric Acid | -7.3 | Arjunolic Acid | -7.6 |
| Bellericagenin B | -7.2 | Arjungenin | -7.4 |
| Bellericaside B | -6.7 | Beta-Sitosterol | -7.2 |
| Ethyl Gallate | -6.6 | Chebulic Acid | -7.2 |
| Gallic Acid | -6.3 | Caffeic Acid | -7.1 |
| D-Galactose | -6.0 | Ferulic Acid | -6.7 |
| D-Glucose | -5.7 | Ethyl Gallate | -6.6 |
| D-Fructose | -5.6 | Gallic Acid | -6.3 |
| L-Rhamnose | -5.6 | Syringic Acid | -5.9 |
| Mannitol | -5.4 | Shikimic Acid | -5.8 |
| Tritriacontan-9-One | -5.3 | Vanillic Acid | -5.8 |
| Tetratriacontane | -4.6 | Co-crystallized ligand | -7.5 |

Among the phytochemical constituents of *T. bellirica*, chebulagic acid has high docking score against DNA Gyrase protein. Chebulagic acid demonstrates the highest predicted binding affinity with a score of -9.1, suggesting that it may have a strong interaction with DNA gyrase. Several compounds including Corilagin, Ellagic Acid, Methylenedioxyflavan, Bellericagenin A, Bellericaside A, Termilignan, Anolignan B, also exhibit relatively high docking scores as compared to the co-crystallized ligand, indicating favorable binding interactions with DNA gyrase. D-Galactose, D-Glucose, D-Fructose, L-Rhamnose, Mannitol, Tritriacontan-9-one and Tetratriacontane show lower docking scores, suggesting weaker binding affinity towards DNA gyrase compared to the other phytochemicals.

The study also investigated the interactions between high affinity phytochemicals from *T. bellirica* and the DNA gyrase protein of *Staphylococcus aureus*. DNA gyrase is a crucial enzyme involved in DNA replication and is an attractive target for antimicrobial drug development. The phytochemicals under scrutiny were chebulagic acid, corilagin, ellagic acid and methylenedioxyflavan.

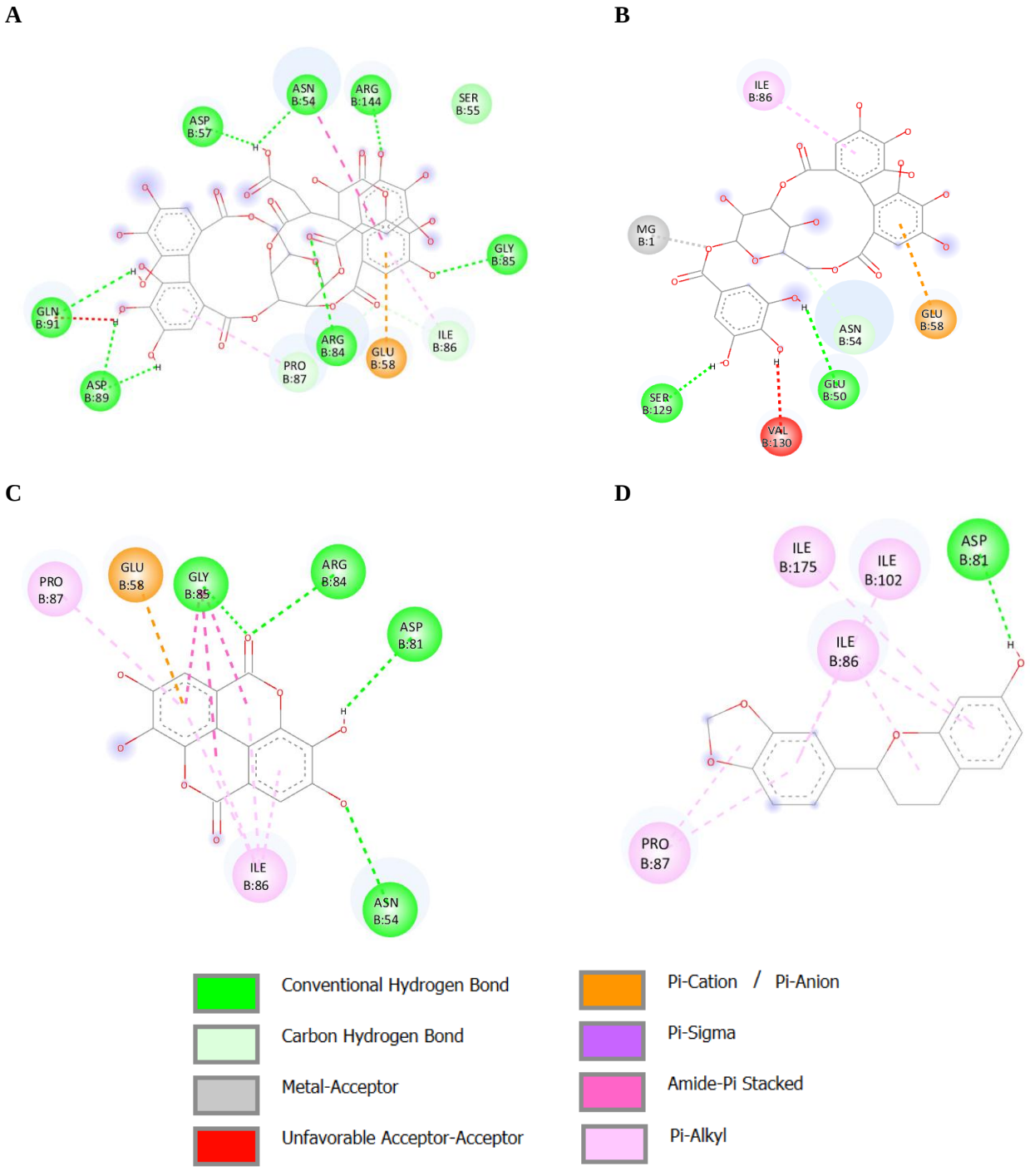


**Fig. 2: The docking interactions between the protein DNA gyrase and the four phytochemicals from *T. bellirica* A) Chebulagic Acid, B) Corilagin, C) Ellagic Acid and D) Methylenedioxyflavan.**

These phytochemicals exhibit a diverse range of binding interactions with the DNA gyrase protein (Figure 2). These interactions include conventional hydrogen bonds, electrostatic Pi-Anion interactions and hydrophobic Pi-Pi stacked and Pi-Alkyl interactions. This diversity suggests that these phytochemicals may have multiple modes of binding to the protein which could contribute to their potential antimicrobial activities.

The interactions were analysed in detail and several notable findings emerged. First, hydrogen bond interactions were observed between the phytochemicals and specific amino acid residues of the DNA gyrase protein. For instance, chebulagic acid formed conventional hydrogen bonds with the protein's amino acids ARG84, GLY85 and ARG144. Similarly, corilagin exhibited hydrogen bond interactions with residues SER129, GLU50 and ASN54, among others. Ellagic acid also formed hydrogen bonds with ASN54, ARG84 and GLY85. Methylenedioxyflavan showed hydrogen bonding with ASP81.

The presence of hydrogen bond interactions between the phytochemicals and specific amino acid residues of the DNA gyrase protein indicates that these compounds may be able to interfere with the enzyme's function. DNA gyrase is critical for DNA replication in bacteria and inhibiting its activity can lead to bacterial cell death. Thus, the observed interactions suggest that these phytochemicals may have potential antimicrobial properties against *Staphylococcus aureus*.

In addition to hydrogen bonds, electrostatic interactions were observed in the case of corilagin which exhibited a Pi-anion interaction with GLU58 of the DNA gyrase protein. Moreover, hydrophobic interactions were prominent in the ligand where Pi-Pi stacked interactions occurred with itself. Hydrophobic interactions, such as Pi-Pi stacked and Pi-Alkyl interactions, are known to play a significant role in ligand-protein binding. Ellagic acid demonstrated hydrophobic Pi-Alkyl interactions with the protein residues ILE86 and PRO87. Similarly, methylenedioxyflavan exhibited hydrophobic Pi-Alkyl interactions with ILE86, ILE175, ILE102 and PRO87. The presence of these interactions further supports the potential of these phytochemicals as antimicrobial agents. Hydrophobic interactions can stabilize ligand-protein complexes and enhance the specificity and affinity of binding.

In conclusion, the interactions between phytochemicals from *T. bellirica* and the DNA gyrase protein of *Staphylococcus aureus* reveal promising structural features and potential antimicrobial properties. These findings provide a foundation for further research and development of these phytochemicals as potential antimicrobial drugs against *Staphylococcus aureus* infections.

The binding affinity between selected phytochemicals derived from *T. chebula* (Table 2), was investigated. Based on the binding scores, it can be inferred that Terflavin A exhibited the highest binding affinity with a score of -10.1 followed by terchebulin (-9.9) and chebulinic acid (-9.2). These phytochemicals have a strong potential to interact with the DNA gyrase protein of *S. aureus*, indicating that they may interfere with its function and potentially inhibit the growth of the bacteria. Ellagic acid and punicalagin both showed docking scores of -8.7, indicating significant binding affinity as well. Daucosterol (-8), arjungenin (-7.4), beta-sitosterol (-7.2) and chebulic acid (-7.2) also demonstrated considerable binding interactions with the DNA gyrase protein. Ferulic acid (-6.7) and gallic acid (-6.3) exhibited relatively weaker binding affinities compared to the other phytochemicals tested. However, it is important to note that even weaker binding affinities can still have biological significance as they may contribute to overall antimicrobial activity.

The interactions between phytochemicals from *T. chebula* and the DNA gyrase protein of *Staphylococcus aureus* are of significant interest due to the potential antimicrobial properties of these natural compounds. In this ligand-protein docking analysis, three phytochemicals having highest binding affinity, namely, terflavin A, terchebulin and chebulinic acid were studied for their interactions with the DNA gyrase protein.

Terflavin A exhibited several crucial interactions with the DNA gyrase protein. The ligand formed conventional hydrogen bonds with GLY85, ASP81, ASN54 and with SER128. These hydrogen bonds play an essential role in stabilizing the ligand within the protein's active site, potentially contributing to its binding affinity. Additionally, terflavin A is engaged in electrostatic interactions including Pi-Cation interactions with ARG84 and Pi-Anion interactions with ASP57 and GLU58. These electrostatic interactions are known to enhance ligand binding through charge-charge interactions. Furthermore, terflavin A participates in hydrophobic interactions with ILE86 and VAL130, further stabilizing the ligand-protein complex.

Similarly, terchebulin displayed key interactions with the DNA gyrase protein. The ligand formed hydrogen bonds with ARG84, VAL130, GLU50 and SER129. These hydrogen bonds contribute to the specific binding of terchebulin to the protein. Additionally, terchebulin engaged in Pi-Anion interactions with GLU50 and GLU58 enhances its binding affinity. Hydrophobic interactions with ILE86 further stabilized the ligand-protein complex.

Chebulinic acid also displayed significant interactions with the DNA gyrase protein. The ligand formed hydrogen bonds with SER55, VAL130, GLU50, SER128 and ASP53. These hydrogen bonds probably play a critical role in the specific binding of chebulinic acid to the protein. Furthermore, the ligand formed Pi-cation interactions with Mg and engaged in Pi-Anion interactions with GLU50 and GLU58, furthering its binding affinity.

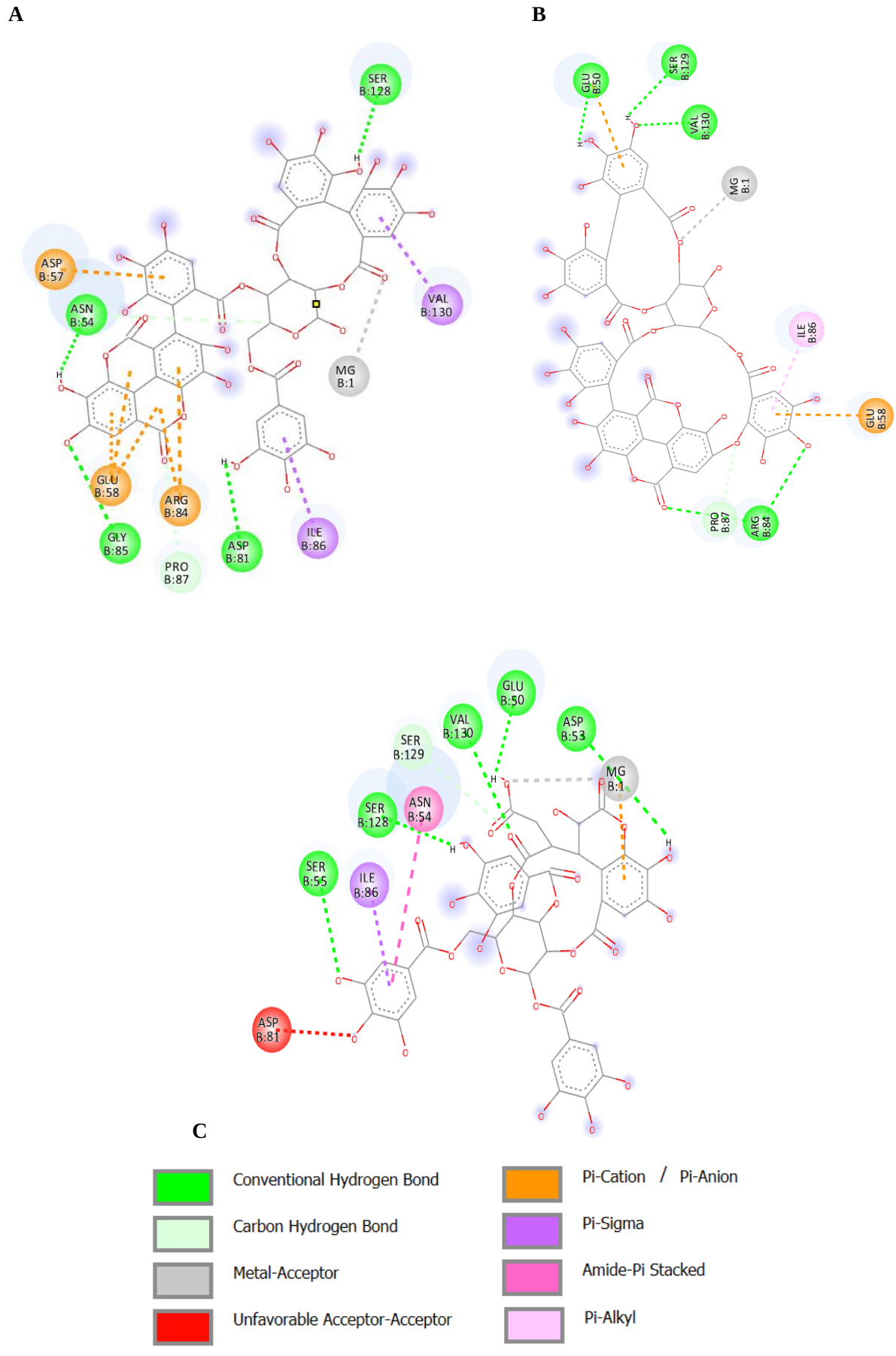


**Fig. 3: The docking interactions between the protein DNA gyrase and the three phytochemicals from *T.chebula* A) Terflavin A, B) Terchebulin and C) Chebulinic Acid**

Additionally, chebulinic acid formed hydrophobic interactions with ILE86, ASN54 and SER55, contributing to the stability of the ligand-protein complex.

In conclusion, the ligand-protein docking analysis of phytochemicals from *T. chebula* with the DNA gyrase protein of *Staphylococcus aureus* revealed specific and crucial interactions between the ligands and the protein. Terflavin A, terchebulin and chebulinic acid exhibited hydrogen bonding, electrostatic and hydrophobic interactions which play vital roles in their antimicrobial activities. These interactions suggest that these phytochemicals have the potential to inhibit the activity of DNA gyrase, an essential enzyme for bacterial replication.

**ADME-and Toxicity Analysis:** The identification and evaluation of drug candidates with optimal ADME (Absorption, Distribution, Metabolism and Excretion) and toxicity properties are crucial steps in drug discovery and development. In this analysis, we assessed the ADME of various phytochemical compounds obtained from *T. chebula* and *T. bellirica* using in silico tools SwissADME. These tools predict key ADME parameters and toxicity risks, helping researchers to identify promising drug candidates early in the drug discovery process. The information provided mainly focuses on physicochemical properties, solubility and potential interactions with biological targets.

Phytochemicals are naturally occurring compounds found in plants and have been of interest for their potential health benefits. However, some phytochemicals may also exhibit toxic or adverse effects on human health[10]. Toxicity studies of the phytochemical compounds obtained from *T. chebula* and *T. bellirica* have been carried out using the software DataWarrior.

**ADME and Toxicity of Phytochemicals from *T. chebula*:** ADME phytochemicals from *T. chebula* are deduced as follows. 'Solubility' is a critical factor that affects drug absorption and bioavailability. Compounds with good solubility are more likely to dissolve in physiological fluids and be absorbed effectively, increasing the chances of successful drug delivery. From the data, compounds like "Syringic acid," "Ellagic acid," and "Vanillic acid" showed good solubility, indicating that they might have a higher likelihood of being orally bioavailable.

**GI Absorption:** GI absorption is essential for orally administered drugs. Compounds with high GI absorption are more likely to be absorbed from the gastrointestinal tract into systemic circulation. In our dataset, "Sennoside A," "Terchebin," and "Chebulinic acid" demonstrated relatively high GI absorption, suggesting that they have the potential for effective oral delivery.

**Plasma Protein Binding:** Plasma protein binding influences the distribution and pharmacokinetics of drugs in the body. Compounds with high protein binding may have lower free drug concentrations, limiting their therapeutic effects. Among the compounds analyzed, "Chebulagic acid" and "Maslinic acid" exhibited moderate plasma protein binding which might be advantageous for maintaining higher free drug levels.

**Cytochrome P450 Inhibition:** Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism. Compounds that inhibit CYP enzymes may cause drug-drug interactions and affect the metabolism of co-administered drugs. In the dataset, none of the compounds were predicted to be CYP inhibitors, suggesting a lower risk of significant drug interactions.

**Pgp Substrate Potential:** P-glycoprotein (Pgp) is an efflux transporter that pumps drugs out of cells, reducing their intracellular concentration. Compounds that are substrates for Pgp may face reduced cellular uptake. None of the compounds were identified as Pgp substrates, promising for drug delivery purposes.

**Lipinski Violations:** The Lipinski's rule of five is a widely used guideline to assess drug-likeness. Compounds with more than two Lipinski violations may have reduced oral bioavailability. "Ellagic acid," "Chebulagic acid," and "Chebulinic acid" showed fewer Lipinski violations, suggesting they possess better drug-like properties.

The analysis of the toxicity data for the compounds has revealed important insights into their potential safety profiles (Table 5). Toxicity is a critical factor in drug development, as it determines the risk of adverse effects and the overall safety of the compound for human use. Among the analysed compounds, some show low toxicity levels, making them more favorable candidates for further investigation, while others exhibit higher toxicity, raising concerns about their safety.

Compounds such as ellagitannin, terchebulin, ellagic acid, punicalagin, shikimic acid, vanillic acid, chebulic acid, arjungenin, sennoside A, beta-sitosterol and daucosterol demonstrate low toxicity, as they do not exhibit mutagenic, tumorigenic, reproductive toxic, or irritant effects. These compounds may have a better chance of being developed into safe and effective drugs. On the other hand, compounds like syringic acid, ethyl gallate, chebulinic acid, arjunolic acid and terflavin A show high toxicity levels with potential mutagenic, tumorigenic and reproductive toxic effects. These compounds raise red flags on safety and suggest that further investigations should be conducted before considering them for drug development.

Based on the *in-silico* analysis of ADME and toxicity properties using SwissADME, "Syringic acid," "Ellagic acid," and "Punicalagin" appear to be promising phytochemical compounds from *T. chebula* for drug development. These compounds demonstrated favorable characteristics such as good solubility, moderate GI

absorption, low plasma protein binding and a reduced number of Lipinski violations and toxicity alerts.

**ADME-and Toxicity of Phytochemicals from *T. bellirica*:** From the solubility analysis, "Sennoside A," "Syringic acid," "Ellagic acid," and "beta-Glucogallin" were among the compounds showing good to very good soluble properties. These compounds have the potential to be effectively delivered and absorbed in the body. "Sennoside A," "Terchebin," and "Chebulinic acid" showed relatively high GI absorption, suggesting that these compounds could be efficiently absorbed through the gastrointestinal tract. Plasma protein binding influences drug distribution and impacts the availability of the free, active form of the drug.

**Table 5**
**Toxicity results of Phytochemicals from *T. Chebula***

| Molecule Name | Mutagenic | Tumorigenic | Reproductive Effective | Irritant |
|---|---|---|---|---|
| Syringic Acid | high | none | none | none |
| Ellagitannin | none | none | none | none |
| Terchebin | none | none | none | high |
| Chebulagic Acid | none | none | none | none |
| Terchebulin | none | none | none | none |
| Ellagic Acid | none | none | none | none |
| Punicalagin | none | none | none | none |
| Shikimic Acid | none | none | none | none |
| Vanillic Acid | none | none | none | none |
| Ethyl Gallate | none | none | none | none |
| Chebulinic Acid | high | high | high | none |
| Chebulic Acid | none | none | none | none |
| Arjunolic Acid | none | none | none | high |
| Ferulic Acid | none | none | none | none |
| Caffeic Acid | none | none | none | none |
| Maslinic Acid | none | none | none | none |
| Gallic Acid | none | none | none | none |
| Arjungenin | none | none | none | none |
| Terflavin A | high | high | high | none |
| Sennoside A | none | none | none | none |
| Beta-Sitosterol | none | none | none | none |
| Daucosterol | none | none | none | none |

**Table 6**
**Toxicity results of phytochemicals from *T. bellirica***

| Molecule Name | Mutagenic | Tumorigenic | Reproductive Effective | Irritant |
|---|---|---|---|---|
| Bellericaside B | high | none | none | none |
| Methylenedioxyflavan | none | none | none | none |
| Termilignan | none | none | high | none |
| Belleric Acid | none | none | none | none |
| Chebulagic Acid | none | none | none | none |
| Bellericagenin B | none | none | none | none |
| Bellericagenin A | none | none | none | none |
| Bellericaside A | none | none | none | none |
| Ellagic Acid | none | none | none | none |
| Anolignan B | none | none | none | none |
| Ethyl Gallate | none | none | none | none |
| Beta-Glucogallin | none | none | none | none |
| Corilagin | none | none | none | none |
| Gallic Acid | none | none | none | none |
| Arjungenin | none | none | none | none |
| Beta-Sitosterol | none | none | none | none |

Compounds with moderate plasma protein binding are desirable, as they can maintain higher free drug levels in circulation. "Chebulagic acid" and "Maslinic acid" demonstrated moderate plasma protein binding, indicating their potential as drug candidates with good distribution properties. In this analysis, none of the compounds was predicted to be CYP inhibitors suggesting a lower risk of CYP-related drug interactions. Additionally, none of the compounds was identified as Pgp substrate which is beneficial for drug delivery as Pgp can efflux drugs and can limit their absorption. "Ellagic acid," "Chebulagic acid," and "Chebulinic acid" exhibited fewer Lipinski violations, implying that they possess favorable drug-like characteristics.

Table 6 presents the toxicity results of various phytochemicals from *T. bellirica*. Among the compounds listed, bellericaside B is the notable one with mutagenic potential. Mutagens are substances that can induce mutations in DNA, potentially leading to adverse health effects including the development of cancer. Bellericaside B also shows high mutagenicity, indicating a potentially higher risk. Termilignan appears to have high reproductive effectiveness which might raise concerns about its impact on fertility or reproductive health. However, it is essential to interpret these results cautiously, as reproductive effects could vary based on specific dosages and exposure levels.

Most of the listed phytochemicals show no mutagenic, tumorigenic, reproductive effectiveness, or irritant properties. This is generally reassuring, suggesting that these compounds may not pose significant health risks in the evaluated categories. Fewer toxicity alerts suggest a lower likelihood of safety concerns. "Ellagic acid," "Chebulagic acid," and "Terchebin" had relatively fewer toxicity alerts indicating that these compounds may have a reduced risk of adverse effects.

In conclusion, based on the *in silico* analysis of ADME and toxicity properties, "Ellagic acid," "Chebulagic acid," and "Terchebin" stand out as potential candidates from *T. bellirica*. These compounds demonstrated favorable solubility, GI absorption and distribution properties with no predicted CYP inhibition or Pgp substrate potential. They also showed a low number of Lipinski violations and toxicity alerts, suggesting promising drug-like properties and potential safety. These compounds have shown low toxicity levels and do not exhibit mutagenic, tumorigenic, reproductive toxic, or irritant effects in the toxicity analysis.

## Conclusion

In conclusion, the study explored the potential antibacterial properties of phytochemicals present in the crude extracts of the dried fruits of *T. chebula* and *T. bellirica* against clinically significant bacterial strains. The results indicated that both extracts were effective against *Staphylococcus aureus* but had no inhibitory effect on *Escherichia coli* and *Salmonella typhi* at the tested concentrations.

The study also investigated the interactions of docked phytochemicals from *T. bellirica* and *T. chebula* with the DNA gyrase protein of *Staphylococcus aureus*. Phytochemicals such as chebulagic acid, corilagin, ellagic acid and methylenedioxyflavan from *T. bellirica* were identified as potential candidates, exhibiting diverse modes of binding to the protein which could contribute to their potential antimicrobial activities. Furthermore, the ligand-protein docking analysis of phytochemicals from *T. chebula* with the DNA gyrase protein revealed specific and crucial interactions. Compounds like terflavin A, terchebulin and chebulinic acid demonstrated hydrogen bonding, electrostatic and hydrophobic interactions, suggesting their potential to inhibit the activity of DNA gyrase, a vital enzyme for bacterial replication.

Based on *in silico* analysis of ADME and toxicity properties, "Syringic acid," "Ellagic acid," and "Punicalagin" from *T. chebula* appear to be promising phytochemical compounds for drug development, demonstrating favorable characteristics and potential safety profiles. Similarly, "Ellagic acid," "Chebulagic acid," and "Terchebin" from *T. bellirica* exhibited favorable properties including good solubility, GI absorption and distribution without predicted CYP inhibition or Pgp substrate potential and low toxicity levels.

Overall, these findings suggest that phytochemicals from *T. chebula* and *T. bellirica* show potential as antimicrobial agents, particularly against *Staphylococcus aureus* and may have valuable drug development properties.

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