

ISOLATION OF KEY PHYTOCHEMICALS FROM *TERMINALIA CHEBULA* LEAVES AND EVALUATION OF ITS IMMUNOMODULATORY POTENTIAL

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ABSTRACT

This research work aims to identify and analyze bioactive constituents from the extract made from the leaves of *Terminalia chebula* using both computational and experimental approaches, with possible immune boosting and antiviral properties. For its broad pharmacological properties, including antibacterial, antiviral, antioxidant, anti-inflammatory, and immunomodulatory activities, *Terminalia chebula* is highly regarded and recognized. These medicinal potentials are ascribed to its abundant content of bioactive phytochemicals, including tannins, phenolics, flavonoids, glycosides, and sterpenoids. Due to the worldwide health crisis caused by infectious diseases like COVID-19, scientists are once again interested in studying plant-based substances to see if they might boost the immune system and fight viruses. In this light, *T. chebula* emerges as an interesting candidate for research owing to its historic medicinal significance and pharmacological diversity. The study uses Gas Chromatography–Mass Spectrometry (GC-MS) to identify compounds in detail and ADME (Absorption, Distribution, Metabolism, and Excretion) profiling with Swiss ADME to check how well the identified molecules work as drugs and how similar they are to drugs. Then, molecular docking experiments are done on selected drugs with good ADME profiles. These studies focus on important immunological and viral proteins, like Interleukin-6 and a protein linked to COVID-19, to see how well they attach and how biologically important they might be.



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I. INTRODUCTION

Protection of the human body from infections and inflammatory diseases through innate and adaptive responses involves the immune system playing a crucial role with cytokine signalling pathways like interleukin-6 (IL-6) being essential for inflammation control. Chronic inflammation and severe health issues during viral infections, can be a result of dysregulation of IL-6. Phytochemicals derived from medicinal plants, especially *Terminalia Chebula*, have garnered interest for their ability to modify immune responses and influence cytokine pathways, offering alternatives to conventional medicines associated with adverse consequences [1-4]. *T. chebula* has bioactive substances such tannins, phenolics, and flavonoids, and it has showed potential in treating immune-related illnesses by affecting cytokines like IL-6. This intention behind this study is to focus on the immunomodulatory potential of specific bioactive compounds from *T. chebula* with the help of an integrated in silico methodology, including phytochemical profiling, ADME parameter screening, and molecular docking with IL-6 and TMPRSS2, with the goal of proving that they may be used as medicines to treat inflammatory and infectious disorders, notably new viral infections like COVID-19 [5].

II. MATERIALS AND METHODS

II.1 SAMPLE COLLECTION AND SEQUENTIAL SOLVENT EXTRACTION

Leaves of *Terminalia chebula* were freshly collected and washed with distilled water to eradicate surface level contaminants and then shade-dried at room temperature and using a mechanical grinder, finely powdered. To obtain phytochemical fractions of varying polarity, sequential solvent extraction was employed which enabled broad-spectrum compound recovery.

II.1.1 Cold Percolation Extraction

To preserve thermolabile constituents, cold percolation was initially performed as shown in Fig. 1(a,c). Powdered leaf material of 100g approximately was soaked in 375 ml hexane, which was followed by incubation at room temperature for 24 h while agitating it from time to time. The mixture was filtered after the incubation and for further extraction, the residual plant material was air-dried. The process was repeated as the same using ethyl acetate by incubating the dry residue in 500ml of solvent for 24h. The filtrated were separately collected, respectively.

II.1.2 Hot Percolation Extraction (Soxhlet Method)

To enhance the extraction efficiency of less soluble compounds, hot percolation was subsequently carried out [6], [7]. Dried plant powder, about 75 g was placed in a Soxhlet thimble and extracted with hexane using 250–300 mL of solvent for seven reflux cycles. Then, the plant material that was residual was subjected to Soxhlet extraction with ethyl acetate for four cycles. The obtained extracts, by solvent evaporation, were then filtered and concentrated at room temperature (Fig. 1(b,d)). Once dried, the extract residues were scraped and weighed for the determination of and stored in sterile Eppendorf tubes for subsequent analysis.



Figure 1: (a) Cold percolation with hexane.

Source: Authors, (2026).



Figure 1: (b) Hot percolation with hexane.

Source: Authors, (2026).



Figure 1: (c) Cold percolation with ethyl acetate.

Source: Authors, (2026).



Figure 1: (d) Hot percolation with ethyl acetate.

Source: Authors, (2026).

II.2 QUALITATIVE PHYTOCHEMICAL SCREENING

To identify major classes of secondary metabolites present in the sequential extracts, qualitative screening was performed. Standard chemical tests were employed to detect anthraquinones, carbohydrates, phlobatannins, tannins, steroids, saponins, coumarins, flavonoids, phenols, alkaloids, terpenoids, quinones, glycosides, cardiac glycosides based on precipitate formation and characteristic calorimetric changes [8], [9]. The presence of phytoconstituents was determined by observing visual changes such as colour development, precipitate formation, or the appearance of coloured rings at phase interfaces, after the addition of specific chemical reagents to the extracts. Using Molisch's test, carbohydrates were determined; by the foam test, saponins; flavonoids by the alkaline reagent test; and alkaloids using Mayer's reagent and using Folin-Denis test, tannins and phenols were determined. Sulfuric acid treatment was used to find quinones, while chloroform–ammonia and Keller–Kiliani tests were used to find glycosides and cardiac glycosides, respectively. NaOH was used to; steroids and phytosterols by chloroform–concentrated sulfuric acid reaction; phlobatannins by hydrochloric acid; and anthraquinones by ammonia treatment. Positive and negative results were recorded in tabular form, with '+' indicating presence and '–' indicating absence of specific phytochemical groups. The detailed results are presented in the Results section.

II.3 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was employed to quickly and effectively check the qualitative chemical makeup of the extracts before doing more extensive profiling. We made silica-gel TLC plates and put samples from several extracts 1 cm above the baseline. Hexane and ethyl acetate were mixed in a 70:30 ratio to make the mobile phase. The plates were put in the mobile phase until the solvent got close to the top. Then they were dried in the air and looked at under UV light at 254 nm and 365 nm. Band patterns were documented as representative of unique phytochemical components.

Even though Rf values weren't measured, comparing band intensities at different wavelengths gave us a way to judge how complex a compound is, which is in keeping with recent TLC research that looked at plant chemical profiles.

II.4 GCMS ANALYSIS

Gas-Chromatography-Mass Spectroscopy was employed to perform the chemical profiling of the selected extract to identify components that are semi-volatile and volatile. Vaporization of the sample was done in the injector port and was carried by an inert gas through a capillary column. In the mass detector, fragment patterns of the eluted molecules were ionized and were compared with spectral libraries for compound identification. The peak, molecular formula and retention time for each detected metabolite were detected [10].

II.5 ADME SCREENING

The compounds obtained from GC-MS were analysed for drug-likeness using in-silico ADME filters. Non-BBB permeability, High gastrointestinal absorption, bioavailability score ≥ 0.55 , CYP inhibition profile, topological polar surface area, P-glycoprotein substrate status, Lipinski's rule of five, acceptable logP range, number of rotatable bonds and topological polar surface area were included in the screening parameters. The molecules that satisfied majority of these criteria were considered as suitable candidates for docking studies [11], [12].

II.6 MOLECULAR DOCKING

Docking at molecular level was carried out to investigate the binding interactions of selected phytochemicals with two biologically relevant human targets, Interleukin-6 (IL-6) and Transmembrane Protease Serine 2 (TMPRSS2). IL-6 was selected due to its central role in inflammatory signalling and cytokine-mediated immune dysregulation, particularly in pathological cytokine release syndromes observed in infections like COVID-19 [12]. TMPRSS2 was chosen because it is a key host serine protease in priming viral spike proteins and facilitating SARS-CoV-2 entry into host cells; thus, docking against this target provides a preliminary in silico screening of phytochemicals for potential interaction with a critical host factor associated with COVID-19 pathogenesis, making it a relevant molecular target in the COVID-19 infection pathway [13], [14]. Three-dimensional crystal structures of the target proteins, human IL-6 (PDB ID: 1ALU) and TMPRSS2 (PDB ID: 7NMEQ), were retrieved from the Protein Data Bank (PDB) and are shown in Fig. 2 and 3, respectively. Ligand structures for gallic acid and aminoacethydrazide were obtained from the PubChem database as shown in Figures 4 and 5. The water molecules and non-essential heteroatoms were removed and the proteins were prepared using PyMOL post polar hydrogens were included.

Further preparation was completed in AutoDock Tools (ADT) 1.5.7 by assigning Kollman charges, repairing missing atoms, and converting the structures into PDBQT format with AutoDock 4 (AD4) atom types. Ligand preparation involved defining the torsion tree and root to maintain conformational flexibility, and the ligands were subsequently exported as PDBQT files. Grid boxes were defined around functionally relevant regions identified from co-crystallized ligands and reported binding residues in the literature, enabling focused docking toward biologically meaningful interaction sites as established in previous molecular docking studies targeting IL-6 and SARS-CoV-2-associated host proteins [6], [12]. The Grid Parameter File (GPF) was made using these grid parameters, and AutoGrid (part of the AutoDock package) was used to make affinity maps. The Lamarckian Genetic Algorithm (LGA) was used to run docking simulations, as described in the Docking Parameter File (DPF). After the docking was done, we looked at the docking log (DLG) files and prioritized the binding conformations based on the lowest anticipated binding energy and cluster analysis. These kinds of grid definition methodologies are already well-known in molecular docking operations to improve physiologically relevant sampling and forecasting accuracy.

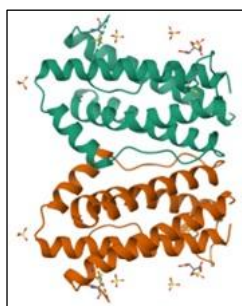


Figure 2: 3D structure of human interleukin-6
Source: Authors, (2026).

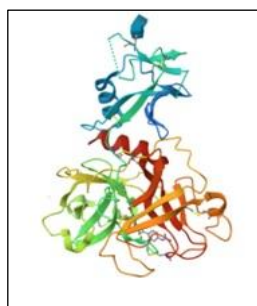


Figure 3: 3D structure of human TMPRSS2 in complex with Nafamostat.
Source: Authors, (2026).

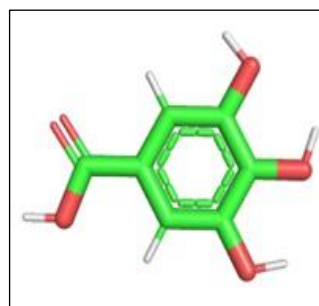


Figure 4: 3D structure of gallic acid.
Source: Authors, (2026).

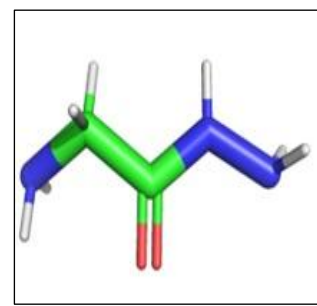


Figure 5: 3D structure of aminoacethydrazide.
Source: Authors, (2026).

III. RESULTS AND DISCUSSIONS

III.1 SEQUENTIAL SOLVENT EXTRACTION YIELD

By employing cold and hot percolation methods with hexane and ethyl acetate, sequential solvent extraction of *Terminalia chebula* leaves was carried out and the extraction yields are summarized in Table 1. Higher extraction yields were given by cold percolation when compared to hot percolation for both the solvents. Ethyl acetate yielded the highest extract recovery (1.96%), which was followed by cold hexane extraction (1.64%) in cold percolation.

On the other hand, hot percolation yielded comparatively lower recoveries, with values ranging from 0.41% to 0.77%. The increased effectiveness of cold percolation implies that mild extraction conditions are more conducive to the recovery and preservation of moderately polar and thermolabile phytoconstituents. The preponderance of semi-polar compounds in *Terminalia chebula* leaves was demonstrated by the consistently higher yields obtained from ethyl acetate compared to hexane. These results support the choice of cold percolation extracts for further computational and phytochemical examinations. The dried extract residues collected after solvent evaporation formation are shown in Fig. 6.

Table 1: Yield of sequential solvent extraction of *Terminalia chebula* leaves.

Extract (From sequential solvent extraction)	Dry weight (g)	Yield in grams	Yield in percentage
Cold percolation with hexane	100	1.64	1.64%
Cold percolation with ethyl acetate	100	1.96	1.96%
Hot percolation with hexane	75	0.58	0.41%
Hot percolation with ethyl acetate	75	0.41	0.77%

Source: Authors, (2026).



Figure 6: Dried extracts residues collected in Eppendorf tubes after solvent evaporation & powder formation.

Source: Authors, (2026).

III.2 RESULTS OF QUALITATIVE SCREENING OF PHYTOCHEMICAL

Qualitative phytochemical screening revealed the presence of phenols, tannins, carbohydrates, quinones, terpenoids, steroids, and phytosterols in the extracts, whereas saponins, flavonoids, alkaloids, glycosides, cardiac glycosides, coumarins, phlobatannins, and anthraquinones were absent as shown in Fig. 7 (Table 2). Finding phenols, tannins, terpenoids, and steroids is proper with the already known chemical profile of *Terminalia* species and supports their reported anti-inflammatory, antioxidant and immunomodulatory properties. Certain phytochemical group such as flavonoids and alkaloids were absent and may be attributed to variations in plant part, geographical origin, solvent polarity, and extraction conditions. These findings offered a biochemical justification for prioritizing phenolic-rich compounds for subsequent ADME screening and molecular docking investigations.

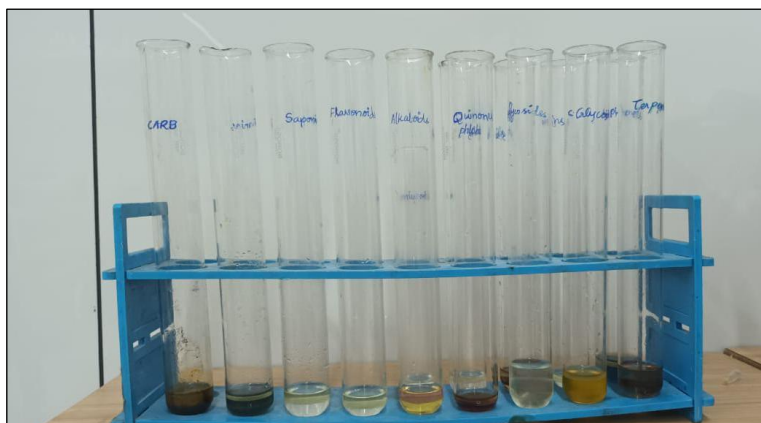


Figure 7: Phytochemical analysis results.

Source: Authors, (2026).

III.3 THIN LAYER CHROMATOGRAPHY PROFILE

We used TLC profiling to find out how complicated the phytochemicals were and how well the extracts separated. Only a few clear bands could be seen with both short- and long-wavelength UV light as represented in Fig. 8 and 9. There wasn't much migration because most of these bands were close to the baseline. The low resolution means that the mobile phase and the polar silica stationary phase don't match up, which makes compounds attach to the surface strongly. Dark quenching areas under short-wave UV suggest that there are conjugated aromatic systems, while reddish or orange fluorescence under long-wave UV means that there are naturally fluorescent metabolites, including phenolics or pigments. Even though TLC didn't do a great job of separating things, it was a decent initial step in fingerprinting because it revealed that the extract was complicated and supported the presence of phenolic chemicals observed during phytochemical screening.

Table 2: Results of phytochemical analysis.

Test	Result
Presence of Carbohydrates	Positive
Presence of Tannins	Positive
Presence of Saponins	Negative
Presence of Flavonoids	Negative
Presence of Alkaloids	Negative
Presence of Quinones	Positive
Presence of Glycosides	Negative
Presence of Cardiac Glycosides	Negative
Presence of Terpenoids	Positive
Presence of Phenols	Positive
Presence of Coumarins	Negative
Presence of Steroids and Phytosteroids	Positive
Presence of Phlobatannins	Negative
Presence of Anthraquinones	Negative

Source: Authors, (2026).

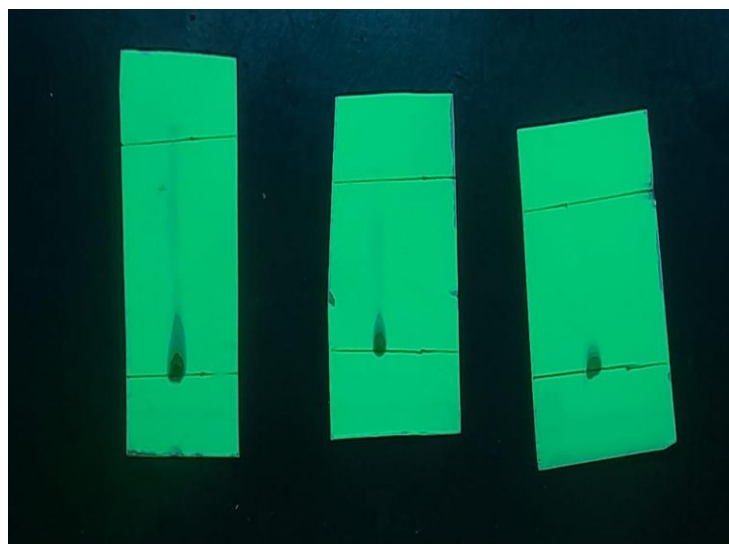


Figure 8: Visualization Under Short-Wavelength UV.
Source: Authors, (2026).

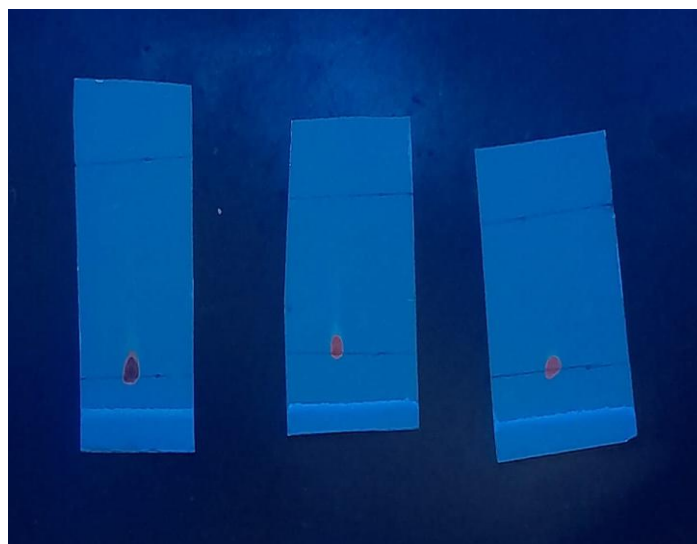


Figure : 9: Visualization Under Long-Wavelength UV Light.
Source: Authors, (2026).

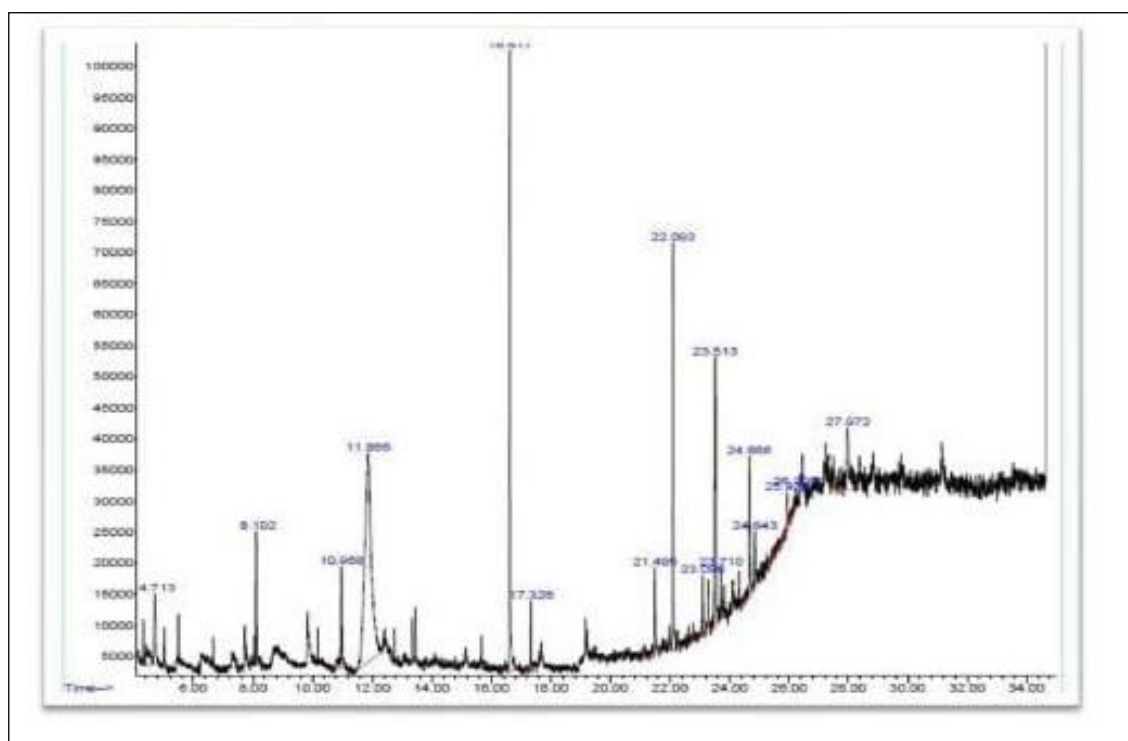
III.4 GCMS ANALYSIS AND ADME SCREENING RESULTS

A profile of multiple phytoconstituents with retention times that were defined along with molecular characteristics were granted by GC-MS analysis and is given in Table. 3. Prioritization of all the compounds based on pharmacokinetic sustainability detected through subsequent ADME evaluation was enabled based on pharmacokinetic sustainability. Through application of the selected filters, gallic acid and aminoacethydrazide were identified as the most compliant molecules, demonstrating favourable drug-likeness, acceptable metabolic properties and high predicted absorption. These candidates were further advanced for molecular docking against IL-6 and TMPRSS2 targets. All spectral analysis of the solvent extraction of *Termnalia chebula* leaves is given in Figure 10.

Table 3: Compounds identified through GCMS Analysis of *Terminalia chebula* Leave.

S. NO	NAME OF THE COMPONENTS	FORMULA	RETENTION TIME
1.	Hydrazine,methyl-	CH ₆ N ₂	1.548
2.	Oxirane-2-carboxylic acid,ethyl ester	C ₅ H ₈ O ₃	2.996
3	Benzene,(2-azido-1-propenyl)-,(E)-	C ₉ H ₉ N ₃	3.039
4	Aminoacethydrazide	C ₂ H ₇ N ₃ O	3.094
5	Dipropyloxydiacetate	C ₁₀ H ₁₈ O ₅	3.21
6	Platinum,tetrakis(triethylphosphine)-,(t-4)-	C ₂ H ₆₀ P ₄ Pt	4.153
7	Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	C ₂₀ H ₂₂ N ₆ O ₆	4.517
8	Acetic acid	C ₂ H ₄ O ₂	4.517
9	Propanoic acid, ethyl ester	C ₅ H ₁₀ O ₂	5.136
10	n-Propyl acetate	C ₅ H ₁₀ O ₂	5.27
11	Toluene	C ₇ H ₈	6.336
12	N-[5-(1-Cyano-2-furan-2-yl-vinyl)-[1,3,4]thiadiazol-2-yl]-benzamide	C ₁₆ H ₁₀ N ₄ O ₂ S	14.304
13	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	C ₁₄ H ₄₄ O ₆ Si ₇	15.302
14	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	16.097
15	Oleic acid,3-(octadecyloxy)propyl ester	C ₃₉ H ₇₆ O ₃	16.535
16	Propanoicacid,2-methyl-,3-hydroxy-2,4,4-trimethylpentyl ester	C ₁₂ H ₂₄ O ₃	16.673
17	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	C ₁₂ H ₃₈ O ₅ Si ₆	16.966
18	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	17.046
19	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	C ₁₂ H ₃₈ O ₅ Si ₆	18.02

Source: Authors, (2026).

Figure 10: All spectral Analysis of solvent extraction of *Terminalia chebula* Leaves.

Source: Authors, (2026).

III.5 MOLECULAR DOCKING ANALYSIS

III.5.1 Gallic Acid–HumanTMPRSS2 Interaction

Binding energies ranging from -3.01 to -3.71 kcal/mol were yielded by docking of gallic acid with human TMPRSS2, with predicted inhibition constants in the millimolar range (Table. 4). The best-scoring pose (Model 7) exhibited a binding energy of -3.71 kcal/mol, as shown in Fig. 11 a and b. The docked complex revealed weak, non-covalent interactions dominated by hydrogen bonding and van der Waals forces. Transient interaction is indicated by the low binding affinity and it suggests that gallic acid is unlikely to act as a potent TMPRSS2 inhibitor. But its steady presence in the binding area backs up its possible use as a reference scaffold for future structural improvements.

Table 4: Gallic acid–Human TMPRSS2 docking results.

Docking Run	Binding Energy (kcal/mol)	Predicted Ki (mM)
1	-3.53	2.58
2	-3.24	4.24
3	-3.29	3.90
4	-3.04	5.95
5	-3.56	2.46
6	-3.37	3.40
7	-3.71	1.92
8	-3.34	3.54
9	-3.01	6.19
10	-3.37	3.39

Source: Authors, (2026).

III.5.2 Aminoacethydrazide–Human TMPRSS2 Interaction

Binding energies between -2.27 and -4.63 kcal/mol were displayed by Aminoacethydrazide. a binding energy of -4.63 kcal/mol was showed by the most favourable pose (Run 4) and a predicted Ki of 0.20 mM, as shown in Fig. 12 a, b. Several poses crowded around -3.9 to -4.1 kcal/mol, which indicates reproducible binding behavior (Table.5). Even though the affinity was the modest, consistent docking poses suggest stable interaction tendencies. The ligand's tiny size and low hydrophobic surface area make it such that the interaction strength is limited. This makes it hard for the ligand to connect to the TMPRSS2 pocket.

Table 5: Aminoacethydrazide–Human TMPRSS2 docking results.

Docking Run	Binding Energy (kcal/mol)	Predicted Ki (mM)
1	-2.82	8.50
2	-4.08	1.02
3	-3.22	4.35
4	-4.63	0.20
5	-3.90	1.38
6	-3.99	1.19
7	-2.46	15.79
8	-3.62	2.23
9	-2.27	21.79
10	-4.12	0.96

Source: Authors, (2026).

III.5.3 Gallic Acid–Interleukin-6 Interaction

When gallic acid and interleukin-6 (IL-6) were docked together, the binding energies ranged from -4.32 to -4.89 kcal/mol, and the inhibition constants were in the micromolar range (Table. 6). The best pose has a binding energy of -4.89 kcal/mol and a Ki of 258.59 μ M (Fig. 13 a, b). The fact that many poses are grouped together around -4.8 kcal/mol shows that the interaction patterns are constant. IL-6 doesn't have a deep catalytic pocket, therefore these interactions probably happen on the surface, which suggests that IL-6 may have a modulatory role instead of an inhibitory one.

Table 6: Gallic Acid–Interleukin-6 docking results.

Docking Run	Binding Energy (kcal/mol)	Predicted Ki
1	-4.88	266.29
2	-4.36	636.09
3	-4.89	262.52
4	-4.89	258.59
5	-4.77	319.01
6	-4.83	286.85
7	-4.87	270.79
8	-4.82	291.11
9	-4.88	265.85
10	-4.32	679.04

Source: Authors, (2026).

III.5.4 Aminoacethydrazide–Interleukin-6 Interaction

The binding energies for aminoacethydrazide were between -2.94 and -4.23 kcal/mol, and the best posture had a Ki of 798.16 μ M as represented in Fig. 14 a, b (Table 7). The narrow range of binding energies shows that the binding orientations can be repeated, even when a highly polar ligand is present. These interactions are similar to temporary connections at IL-6 surface interfaces, which is in keeping with the structure of cytokine proteins and gives us a mechanism to change contacts. General Interpretation: The study gives a scientifically sound reason for future structure-guided optimization and experimental verification of chemicals made from *Terminalia chebula*.

Table 7: Aminoacethydrazide–Interleukin-6 docking results.

Docking Run	Binding Energy (kcal/mol)	Predicted Ki (mM)
1	-3.77	1.74
2	-3.09	5.45
3	-3.29	3.30
4	-4.23	798.16
5	-3.29	3.90
6	-2.94	7.01
7	-3.72	1.87
8	-3.78	1.69
9	-3.58	2.37
10	-3.78	1.71

Source: Authors, (2026).



Figure 11: (a) Overall docked pose and (b) Hydrogen bond interactions of gallic acid within TMPRSS2.
Source: Authors, (2026).



Figure 12: (a) Overall docked pose and (b) Hydrogen bond interactions of Aminoacethydrazide with TMPRSS2 active site residues.
Source: Authors, (2026).



Figure 13: (a) Overall docked pose and (b) Hydrogen bond interactions of gallic acid with Interlukein6 active site residues.
Source: Authors, (2026).



Figure 14: (a) Overall docked pose and (b) Hydrogen bond interactions of aminoacethydrazide with Interlukein6 active site residues.
Source: Authors, (2026).

IV. CONCLUSIONS

This study employed a comprehensive experimental-computational methodology to identify phytochemicals from *Terminalia chebula* and elucidate their effects on host-associated targets critical for inflammatory pathways. Sequential solvent extraction and phytochemical screening confirmed the presence of bioactive classes, whereas ADME filtering made it easier to rank compounds in a systematic method. Among the shortlisted compounds, aminoacethydrazide, a relatively underexplored constituent of *T. chebula*, demonstrated consistent but moderate binding interactions with TMPRSS2 and interleukin-6 in molecular docking analyses. Although the observed binding energies indicate weak to moderate affinity rather than strong inhibition, the reproducibility of docking poses, acceptable pharmacokinetic properties, and limited prior investigation highlight aminoacethydrazide as a potential candidate for further in-depth computational optimization and experimental validation. The results show that using integrated phytochemical and in silico screening procedures to locate exploratory bioactive scaffolds is useful without making claims about their medicinal effectiveness that are too strong.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Evelyn, Sai Prathiksha, Rithik Sam Abraham, J. Sandhya.

Methodology: Evelyn, Sai Prathiksha, J. Sandhya.

Investigation: Evelyn, Sai Prathiksha, Rithik Sam Abraham.

Discussion of results: Evelyn, Sai Prathiksha, Rithik Sam Abraham, J. Sandhya.

Writing – Original Draft: Evelyn, Sai Prathiksha, Rithik Sam Abraham.

Writing – Review and Editing: Evelyn, Sai Prathiksha, Rithik Sam Abraham, J. Sandhya.

Resources: Evelyn, Sai Prathiksha, J. Sandhya.

Supervision: J. Sandhya.

Approval of the final text: J. Sandhya.

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