

Unveiling the dual anti-viral and anti-bacterial potential of *Tridax procumbens*: integrating system biology and molecular modeling for therapeutic insights

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Abstract

This study evaluates the dual anti-viral and anti-bacterial activity of *Tridax procumbens* using system biology and molecular docking targeting the tumor necrosis factor (TNF) signaling pathway. The bioactive potential of *Tridax procumbens* was studied using phytochemical databases, and the essential biomolecules were selected for the study. The principle of geneVenn diagrams and protein target prediction was used to identify overlapping molecular targets with viral and bacterial diseases, which was later validated using various database mining tools like GeneCards and OMIM. Cytoscape software was applied to construct protein-protein interaction networks, which showed that TNF, AKT1, EGFR, SRC, and ESR1 are the hub genes of the networks. The gene ontology (GO) enrichment analysis also recapitulated the modulated biological processes, cellular components, and molecular functions of *Tridax procumbens* compounds, including their action on the TNF signaling in inflammation and immune responses. Molecular docking studies showed strong binding affinities of compounds such as cynaroside and 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside to TNF, and molecular dynamics simulations confirmed these interactions' stability. The findings suggest that *Tridax procumbens* exerts its therapeutic effects by modulating the TNF signaling pathway, offering significant anti-viral and anti-bacterial properties. This integrative approach provides insights into the *Tridax procumbens*'s mechanisms of action, supporting its potential as a source of novel therapeutic agents against infectious and bacterial diseases. There is still a need for further experimental research to define the full spectrum of *Tridax procumbens*' therapeutic application.

Keywords

Tridax procumbens, tumor necrosis factor, system biology, anti-viral, anti-bacterial, molecular modeling

Introduction

The global health landscape faces continuous challenges due to the emergence of infectious diseases caused by viral and bacterial pathogens. The need for therapeutics is of particular significance, considering the emerging trend of antibiotic resistance and the high rate of mutations exhibited by viruses. Medicinal plants traditionally provide a wealth of valuable secondary metabolites that have been historically used to treat many diseases. *Tridax procumbens*, called coat buttons, is a plant that has recently been predominantly studied based on the value it holds for its medicinal use (Lakhera et al. 2021; Ingole et al. 2022). The current research emphasizes investigating the two important aspects of *Tridax procumbens* as an anti-viral activity and an anti-bacterial activity by using systems biology and molecular modeling analysis, mainly in TNF signaling pathways. *Tridax procumbens* is quite common in tropical and subtropical regions and has been used by traditional healers to treat wounds and inflammation and against certain diseases through its antimicrobial properties (Ingole et al. 2022). *Tridax procumbens* is endowed with a host of potential constituents, including flavonoids, alkaloids, tannins, saponins, and several other phytochemicals with therapeutic benefits (Ikewuchi et al. 2016; Syed et al. 2020). Although there are many traditional applications of this plant to treat anti-viral and anti-bacterial diseases, there are few scientific investigations to elucidate the molecular mechanisms of this plant. This study has been designed to address the shortcomings as mentioned above by adopting modern bioinformatics and computational biology methodologies to understand the mechanism of action of *Tridax procumbens* compounds with key components of the TNF signaling pathway. TNF is a signaling molecule that plays a significant role in various immune reactions, inflammation mediation, and cell survival. TNF is a crucially important cytokine responsible for the inducement of defense mechanisms against infections (Holbrook et al. 2019; Syed et al. 2020). Upon its binding to the TNFR1 and TNFR2 receptors, TNF induces its mechanisms of bioaction, which firstly include activation of MAP kinase and NF-KB signaling paths responsible for the formation of the transcriptional and post-transcription orders of the genes that regulate inflammation, immunity, and apoptosis (Ruiz et al. 2021; Chédotal et al. 2023). Abnormal TNF signaling has been correlated to chronic inflammatory conditions and cancers. Considering the significance of TNF in the immune response, modulating its signaling would be a good way to address the absence of prophylactics and therapeutics for anti-viral and anti-bacterial treatments (Webster and Vucic 2020; Andretto et al. 2023; Preedy et al. 2024).

The combination of systems biology and molecular modeling can be considered an effective method for understanding the interrelationships between components of biological systems. Systems biology enables the identification of systems-level properties for biological path-

ways and networks, thereby shedding light on how their component molecules interact. Molecular modeling, on the other hand, is a process through which the interactions among the molecules can be portrayed and simulated in a more realistic and precise manner at the atomic level (Manaihiya et al. 2024; Peluso and Chankvetadze 2024). Combining these approaches, this study aims to identify key bioactive compounds in *Tridax procumbens* that modulate the TNF signaling pathway, thereby uncovering potential therapeutic agents with dual anti-viral and anti-bacterial properties¹. In the current study, we identified most components and interactions in the TNF signaling pathway using the KEGG database and highlighted the essential nodes and interactions regulated by TNF. We used molecular docking and simulation to determine the binding energy and pose of TPC to the TNF pathway-associated proteins. In-vitro assays would further do this computational approach to validate the anti-viral and anti-bacterial efficacy of the identified compounds. Through this integrative methodology, we aim to provide a detailed mechanistic understanding of how *Tridax procumbens* exerts its therapeutic effects and to highlight its potential as a source of novel anti-viral and antibacterial agents. The study outcomes will be used to explain the therapeutic value of *T. procumbens* and derive new approaches to its use. Thus, this study reveals new information on the direct or indirect modulatory effect of specific compounds in *T. procumbens* on the TNF signaling pathway; these compounds might serve as the foundation for further investigations of viral and bacterial infections. Afterward, there are plans to test these bioactive compounds *in vivo* further to determine whether they are therapeutic and safe.

Materials and methods

Screening active compounds

The active compounds found in *Tridax procumbens* were identified through the Indian Medicinal Plants, Phytochemistry and Therapeutics, and KNApSACk databases (Afendi et al. 2012) (Fig. 1 for compound structures). We conducted an extensive literature search via Google Scholar and PubChem using the search term “*Tridax procumbens*”.

Finding common targets

A GeneVenn diagram was constructed to highlight the common molecular targets between the active compound's gene and target genes for viral and bacterial diseases. This tool helps visualize the overlap and relevance of these compounds in combating various pathogens.

Identifying protein targets

We specifically used the Swiss Target Prediction server to identify the proteins with which our compounds interact.

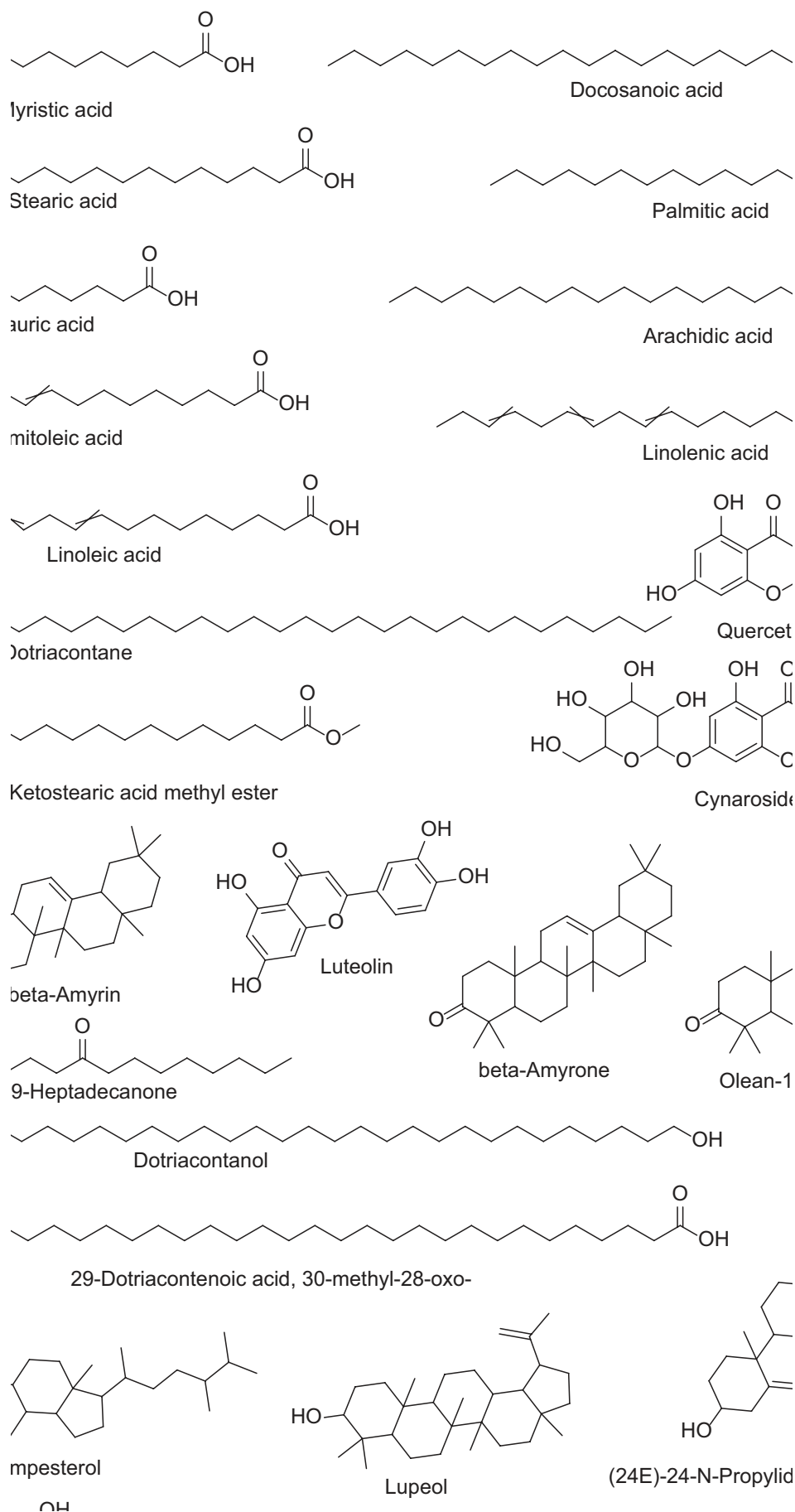


Figure 1. Structure of *Tridax procumbens* containing phytochemicals.

This server compares 2D and 3D chemical structures to predict the possible targets for the used compounds. Furthermore, we relied on the GeneCards and the Online Mendelian Inheritance in Man database (OMIM) (Amberger et al. 2015) to identify target proteins associated with diseases. We used the Ven Plot Diagram method to look for common genes between our compounds and target disease genes to understand potential impacts better.

Constructing protein-protein interaction networks

To identify the targets, we mapped protein-protein interaction: Gene interaction on the STRING database: solid circles represent the genes, and the interconnected structures represent the proteins, as illustrated in Fig. 5. Interaction by lines of different colors indicates biological processes. We used Cytoscape and its CytoHubba plug-in to view the interaction network easily. In this, compounds and protein interactions are represented by nodes and edges.

Gene ontology (GO) analysis

We used FunRich 3.1.3 to perform functional enrichment analysis of gene ontology, which includes studying biological pathways (BPA), cellular components (CC), and biological processes (BPR). This factor assists us in determining the role of genes in various contexts. Furthermore, we explored the Kyoto Encyclopedia of Genes and Genomes pathways for our chosen common gene. A p-value of less than 0.05 was used to determine statistical significance, indicating the importance of our gene ontology findings.

Molecular docking

To evaluate how well the top target identified through the described topological analysis interacts with 29 selected bioactive compounds. As mentioned, three-dimensional structures of these compounds were retrieved from the PubChem database and then imported into Maestro, where they underwent energy minimization. As a result, a molecular database in.sdf format was created. Finally, the 3D structure of the TNF target protein was retrieved from the RCSB using PDB ID 2AZ5 (He et al. 2005), and preparation was conducted with the help of Maestro's protein preparation wizard (Madhavi Sastry et al. 2013). The preparation process included the removal of irrelevant molecules and water molecules. Thereafter, with the Dock protocol, we docked compounds into the protein's ligand area to determine the compounds' interaction with the target protein.

Molecular dynamics

We ran molecular dynamics simulations of this most promising molecule to know how it would behave under hypothesized biological conditions. In other words, these simulations show how the molecule behaves in the

solvent environment. We simulated an orthorhombic box with a dimension of 12 Å on each side; the volume was optimized via the buffer size method. We used TIP3P and force-filed OPLS3e from Schrodinger Inc. standards for simulating proteins and ions16 for the standard water model. To mimic the physiological environment, we added sodium chloride (NaCl) to the system in a concentration of 0.15 M; we used sodium (Na+) and chloride (Cl-) ions (Harrach and Drossel 2014; Padhi et al. 2022). These simulations were run for 150 nanoseconds based on the Desmond molecular dynamics module, resulting in around 1500 snapshots showing the model behavior in the system. These were run under the NPT ensemble at a constant temperature of 300 K and 1 bar of pressure, ensuring the system was equilibrated and in the proper position during the simulation (Yu and Dalby 2020).

Result

Compound and disease targets

A total of 309 compound targets of *Tridax procumbens* containing phytochemicals were obtained from the Swiss Target Prediction server. The additional targets linked to bacterial and viral infectious diseases were sourced from the Genecard and OMIM databases. As shown in Fig. 2, the Venn diagram of viral infectious disease targets and bacterial disease targets depicts the overlap between 14,388 and 14,893 targets. The three datasets have 126 common gene targets of the compounds and the selected target diseases.

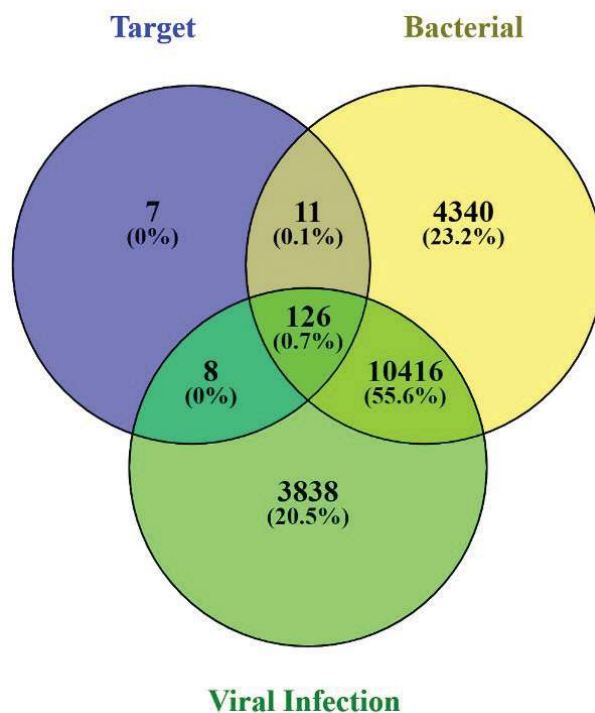


Figure 2. A Venn plot diagram highlighting overlapping targets between the potential compounds and disease-related genes.

Insight from gene ontology and KEGG pathway

To determine the biological mechanisms of *Tridax procumbens* phytochemicals against viral and bacterial disease, we performed GO enrichment analysis on 126 potential therapeutic targets of *Tridax procumbens* phytochemicals used in the treatment of using the DAVID database (Fig. 3). The study encompassed various GO categories, including cellular components, molecular function, biological pathways, and biological processes. We obtained the top 10 GO terms that were visualized in the bar plot based on their probability and percentage of genes involved in their terms. The graph represents the percentage of genes associated with each term and their enrichment's statistical significance (p-value). The GO terms for cellular components highlighted several key locations where modulated genes were significantly enriched. The cytoplasm had the highest proportion of genes (60.3%), indicating a predominant localization and potential cytoplasmic functions. The plasma membrane also showed significant enrichment (42.9%), suggesting that *Tridax pro-*

cumbens may influence membrane-associated activities. Additionally, nucleoplasm (11.1%) and microsomes (6.3%) showed notable enrichment, though to a lesser extent. Other components, such as the extracellular space (9.5%), endoplasmic reticulum (16.7%), and membrane (7.9%), displayed moderate gene associations, indicating diverse cellular localizations affected by *Tridax procumbens* (Fig. 3). The molecular function GO terms identified several critical activities modulated by the treatment. Catalytic activity (17.5%) was significantly enriched, underscoring the influence of *Tridax procumbens* on enzymatic functions. Notably, protein serine/threonine kinase activity (6.3%) showed strong modulation ($p < 0.001$), indicating significant impacts on kinase activities crucial for various signaling pathways. Transmembrane receptor protein tyrosine kinase activity (6.3%) was also significant, suggesting an effect on receptor-mediated signaling mechanisms. Other activities, such as oxidoreductase (6.3%), DNA topoisomerase (1.6%), and hydrolase (4.8%) activities, showed lesser but notable enrichments. The pathway analysis revealed several significant pathways. The TRAIL signaling pathway recorded the highest enrich-

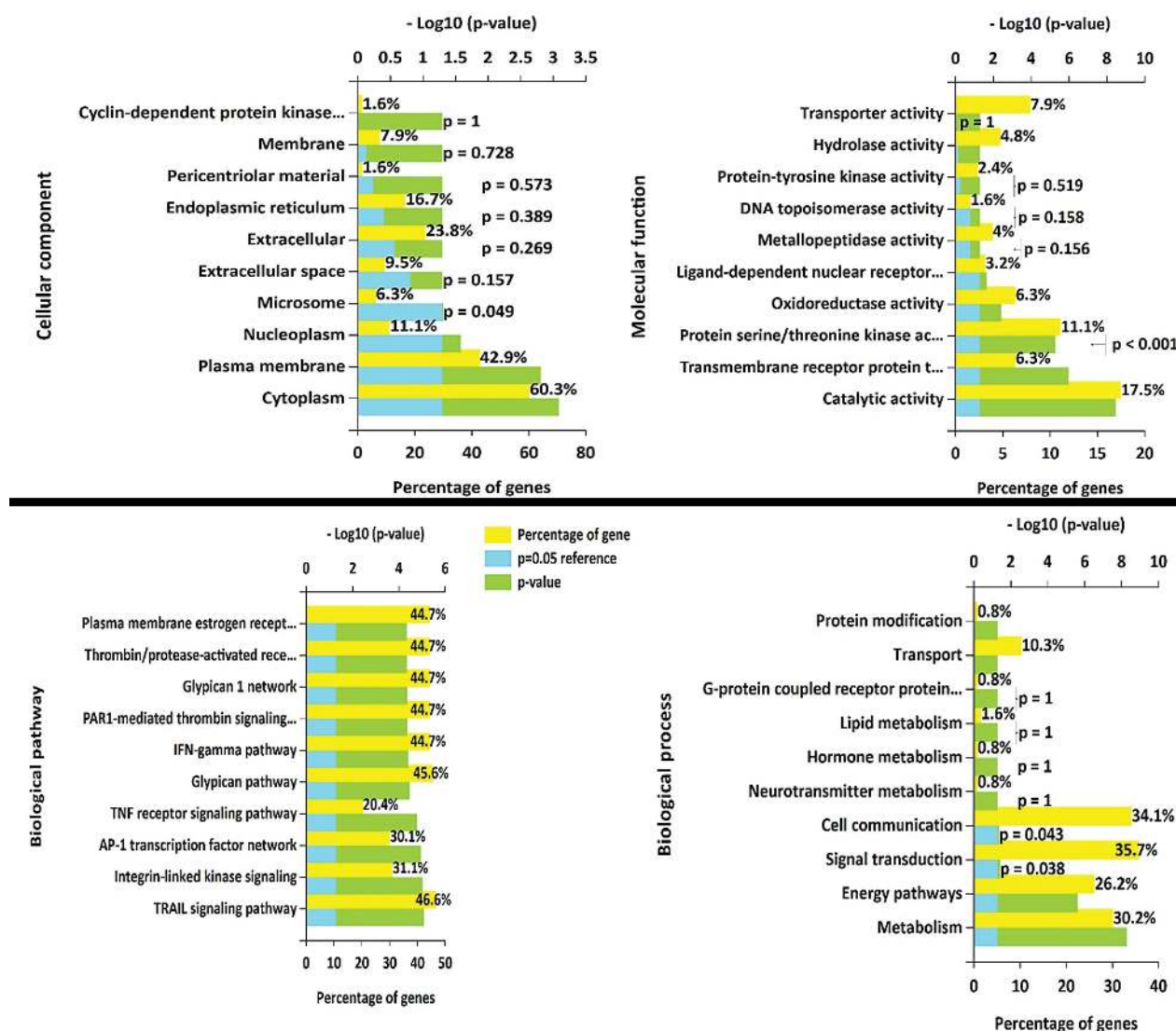


Figure 3. Gene ontology categorization and pathway enrichment bar chart.

ment, 46.8%, which indicated a possible role of *Tridax procumbens* in apoptotic signaling. The glypican pathway was 45.6% enriched, thus showing the possible role in cellular communication and signaling. 45.6% enrichment was also recorded for the IFN-gamma pathway, PAR1-mediated thrombin signaling, or thrombin/protease-activated receptor pathways, showing the possible immunomodulatory and thrombotic response of *Tridax procumbens*. The TNF receptor signaling pathway contributed 30.1% enrichment, showing the major inflammatory responses. Other highly enriched biological processes by *Tridax procumbens* include cell communication, 34.1%, the most significant process, demonstrating its effects on intercellular signaling (Fig. 3). At 26.2%, signal transduction was also highly significant among cell cellular signaling mechanisms. Other processes, such as energy pathways (30.2%); metabolism (34.1%); or protein modification (0.8%), demonstrate how the metabolic and functional configurations change under treatment. The G-protein coupled receptor protein signaling pathway at 10.3% shows the possible effects on GPCR-mediated signaling. However, the hormone metabolism, 1.8%, and the metabolism of numerous neurotransmitters, 1.6%, indicate additional regulatory roles in hormonal and neurotransmitter pathways.

In conclusion, the results of the gene ontology analysis provide valuable information on the dual anti-viral and anti-bacterial activity of *Tridax procumbens*. The overwhelming contribution of the processes that occurred in the cytoplasm and plasma membrane indicates that the action of this plant is primarily aimed at the cytoplasmic process and the functions associated with the membrane. The localization of the processes corresponds to the potential ways of the plant's influence on the cell, likely through membrane integrity and intracellular signaling pathways.

Insight from protein-protein interaction analysis

In protein-protein interaction analysis, we performed a network-based analysis to determine the main factors involved in the dual anti-viral and anti-bacterial activity of *Tridax procumbens*. We analyzed which metrics could be used, such as out-degree, in-degree, clustering coefficient, average clustering coefficient, and protein-protein interaction visualization. We also viewed several statistics on the number of nodes in the network, network density, and shortest path length. The out-degree distribution is presented in the top left corner, and it resembles the in-degree distribution shown in the top right corner. Both charts depict a right-skewed pattern, meaning that most genes have fewer connections than a few genes with a high number of connections. This enables us to suppose that it has a scale-free network property with a small number of hub genes that have a lot of interactions with other genes (Fig. 4). The highest out-degree is dashed in red and equal to 60, meaning it has the most outgoing degree of interaction. The highest in-degree is also dashed in red and amounts to 60 (Fig. 4). In the scatter plot in the bottom left corner, we plot the clustering coefficient as a function of the number of neighbors. As we can see,

a clustering coefficient is more likely to increase as neighbors increase. This means that genes with more connections are more prone to clustering in densely knit groups. Hence, highly connected genes might be central to some subnetworks and be vital in keeping them fully functional.

Similarly, the average clustering coefficient on the bottom right chart decreases as neighbors increase. This means that although the highly connected nodes form a dense cluster, the overall network becomes less densely connected as it grows. It is possible that as more genes are included in the network, fewer new clusters of highly interconnected nodes are expected to form. The PPI network shows interaction between the top-ranked genes. The TNF node is the most top-ranked, with a score of 69. Other top hubs in the network include AKT1 with 64 and EGFR with 56, while SRC and ESR1 are equally ranked with a score of 55. The hub genes in the network have high connectedness points, making them at the center of the network. These hub genes are potential targets that *Tridax Procumbens* act upon to affect the anti-viral and anti-bacterial processes. The network statistics further expound on the structure and characteristics of the gene interaction network. The network has 125 nodes and 6 in diameter, with a density of 0.02 (Fig. 5A). The clustering coefficient, 0.275, reveals moderate clustering throughout the network. There are 3,537 shortest paths, and the characteristic path length is 2.206, suggesting a relatively shorter path between nodes, characteristic of small networks. The average number of neighbors per node is 14.624, revealing modest connectivity. TNF Tumor Necrosis Factor The high score indicates that the *Tridax Procumbens* act on the inflammatory response. AKT1, Protein Kinase B The high connectivity shows more centrality on the drug's effect on the cellular system (Fig. 5B).

Another is epidermal growth factor receptor (EGFR), which is highly involved in regulating cell growth, survival, proliferation, and differentiation. The high centrality of this node within the network structure indicates that *Tridax procumbens* may affect cell-signaling pathways related to growth and proliferation. SRC and ESR1 are similarly highly expressed genes (Wee and Wang 2017; Patnaik et al. 2022). Proto-oncogene tyrosine-protein kinase Src and estrogen receptor 1 (ESR1) participate in signaling pathways regulating cell division, survival, and differentiation (Roskoski 2015). Among other important genes whose inhibition could be targeted by the drug, I can mention PPARG, PTGS2, MMP9, GSK3B, and PARP1 (Fig. 5B). PPARG is PPAR-gamma involved in regulating the body's energy balance and metabolism, whereas PTGS2 is normally expressed by the immune system cells in normal tissues and is induced to the intercellular space by bacterial lipopolysaccharides and cytokines (Ahmadian et al. 2013). Matrix metalloproteinase 9 (MMP9) is an enzyme from the matrix metalloproteinase family; GSK3B is a glycogen synthase kinase-3 beta involved in energy compromise between the metabolism of athletes and mental health in opioid addiction and schizophrenia (Pramanik et al. 2018). The final high-expressed gene is PARP1—Poly [ADP-ribose] polymerase 1, which removes loose strands of nucleotides within the DNA double helix before the individual strands are copied (Wang et al. 2019b).

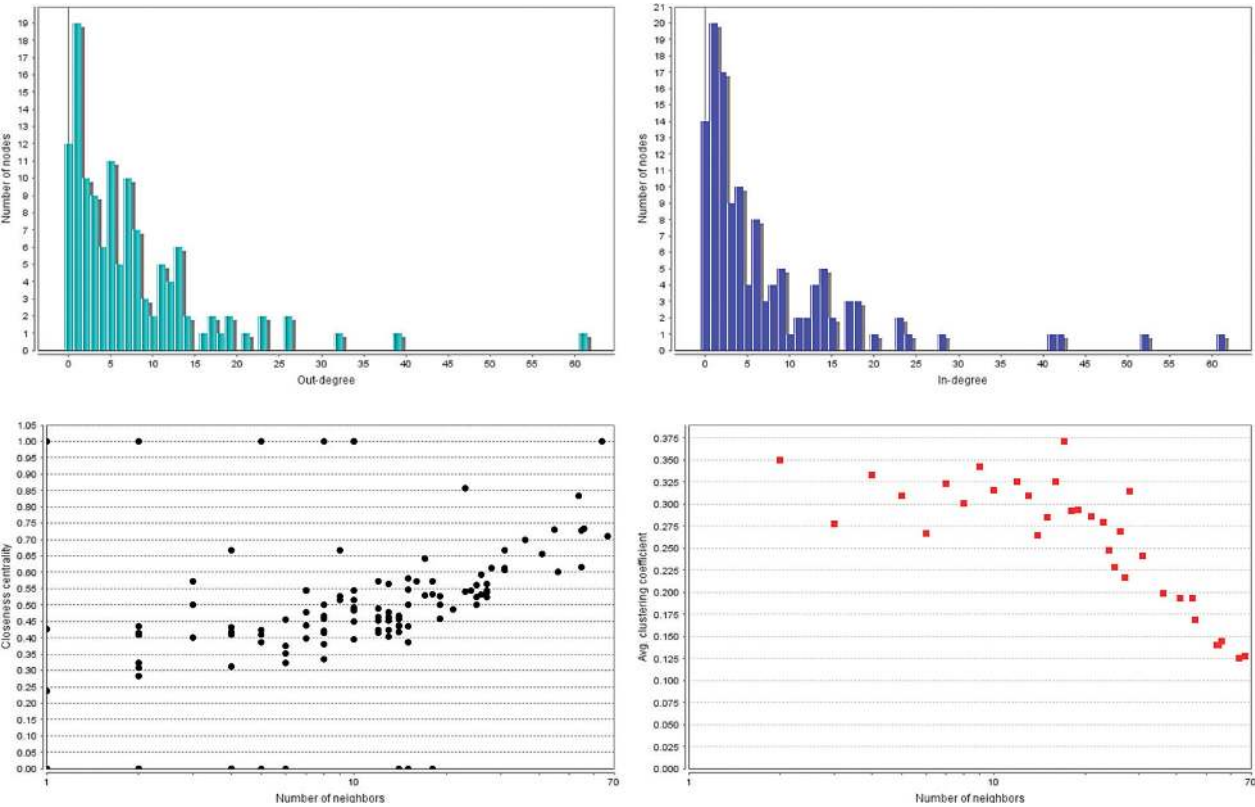


Figure 4. Metric analysis from protein-protein interaction analysis.

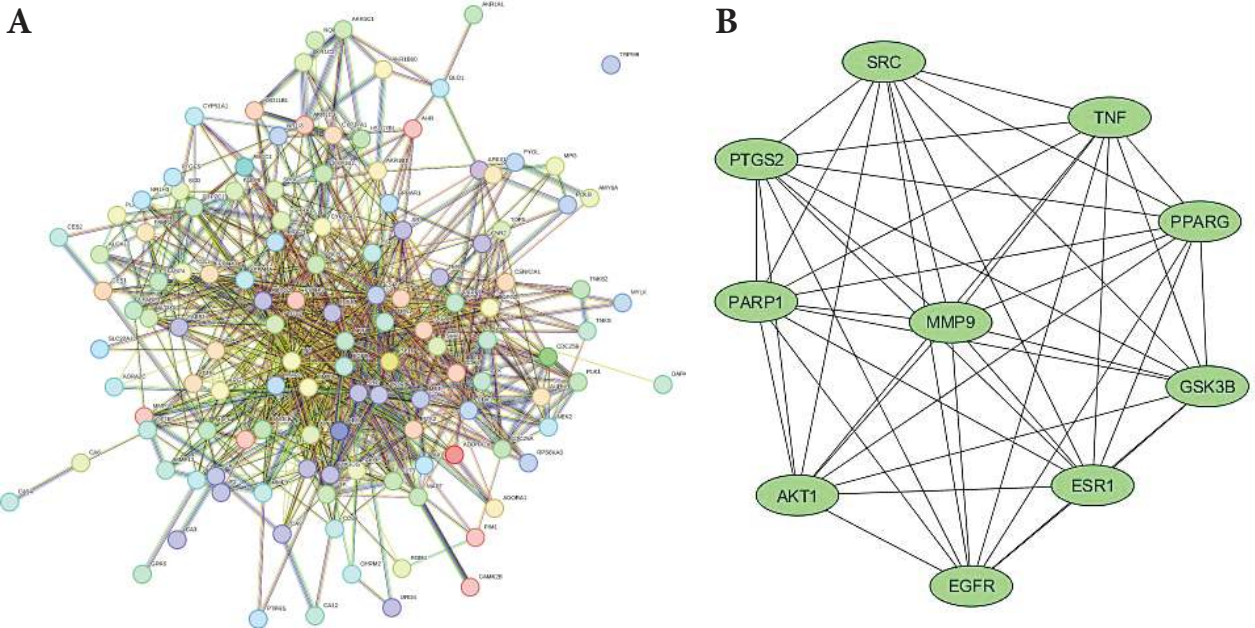


Figure 5. A. Interactions of gene targets were visualized using Cytoscape and network analysis; **B.** Top 10 Gene Target Interactions that was Visualized through Cytoscape.

Insight from KEGG analysis

The KEGG pathway analysis of the TNF signaling pathway depicts numerous critical components and interactions influenced by the tumor necrosis factor. It plays a crucial role in several biological processes, such as inflammation, immune response, and apoptosis, and is associated with several key nodes and molecular interactions. TNF binds to its receptors, TNFR1 and TNFR2, and by recruiting adaptor

proteins like TRADD, TRAF2, and RIP1, triggers multiple downstream signaling events. The activation of the MAPK and the NF-kappa B signaling pathways is triggered. In the MAPK pathway, the intermediaries from the top are TAK1, MEKK1, and MEK3/6, which activate JNK and p38 MAP kinases, which in turn activate transcription factors like AP-1, modulating the expression of pro-inflammatory genes (Wang et al. 2019a) (Fig. 6). In the NF-kappa B pathway, the IKK complex is activated, leading to the

phosphorylation and degradation of I κ B α . This phenomenon allows NF-kappa B to translocate into the nucleus and promote transcription, thus increasing inflammatory cytokines, chemokines, and survival genes (Guo et al. 2024).

The TNF signaling pathway shows its bifurcation capacity to induce apoptotic or survival signals. TNF can stimulate apoptotic cell death when it agitates complex formation with FADD and caspase-8, while in contrast, it can also agitate activation of NF-kappa B, producing anti-apoptotic proteins to enhance cell survival (van Loo and Bertrand 2022). Moreover, this pathway links with other vital pathways, such as the PI3K-Akt pathway, which affects cell survival, and the JNK pathway, which affects stress responses. The pathway for TNF connects with inflammation and immune response, signaling since TNF executes a vast range of immune functions. TNF also boosts immune response by upturning inflammatory mediators such as IL-1 and IL-6, and subsequently, it upregulates infection or injury sites through the recruitment of immune cells (Alam et al. 2021) (Fig. 6). The TNF pathway is crucial as it facilitates the activation of NF-kappa B, which is necessary to express genes that participate in immune cell activation, differentiation, and survival. These genes include transmembrane proteins such as the adhesion molecules and cytokines necessary for leukocyte recruitment and activation (Webster and Vucic 2020). The pathway of TNF signaling has been studied with the aid of systems biology and molecular modeling to describe its potential

in dual anti-viral and anti-bacterial agents. This signaling pathway has also been noted to promote the generation of anti-viral protein production while enabling immune cells to easily eliminate viral infection. It has been identified that the pathway enhances the phagocytic activity by promoting the responsive production of ROS and antimicrobial peptides targeting selective anti-viral infection and bacteria, allowing systems to obliterate disease-causing microorganisms such as bacteria by macrophages and neutrophils (Chen et al. 2023; Liu et al. 2023).

In conclusion, *Tridax procumbens* has immense potential as a medicinal plant to modulate the TNF signaling pathway, offering therapeutic potential. The compounds derived from *T. procumbens* probably inhibit TNF signaling from excessively overactivating, preventing excessive inflammation and subsequent tissue damage. Some bioactive compounds from *T. procumbens* could enhance certain aspects of TNF signaling, allowing the body to respond effectively against pathogens. Understanding the interaction of *Tridax procumbens* with the TNF pathway can also guide the development of new biomimetics that could have dual anti-bacterial and anti-viral potential in a balanced manner to avoid side effects. More so, the findings show an interplay of several signaling pathways and molecular functions, which might interconnect to enable *T. procumbens* therapies. The network analysis suggests TNF, AKT1, EGFR, SRC, and ESRI's centrality, the probable target, and interaction for the *T. procumbens* dual anti-viral and anti-bacterial potential.

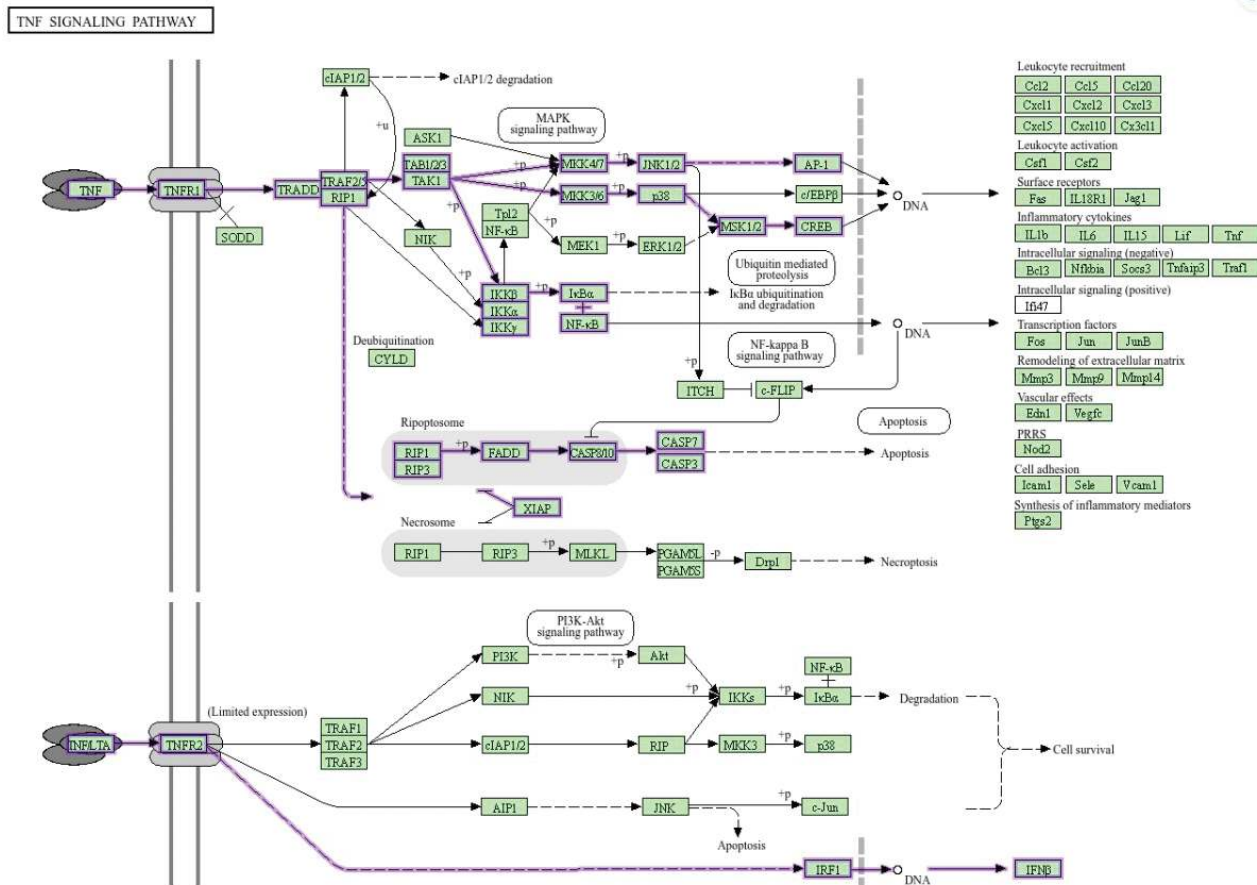


Figure 6. KEGG pathway analysis of TNF signaling pathway.

Molecular docking

The molecular docking study was conducted to examine the anti-bacterial and anti-viral action of the *Tridax procumbens* derivatives. The study primarily included target proteins, and TNF was the particular focus. Ten phytochemicals from *Tridax procumbens* were used in the study. The results confirmed that these phytochemicals had better binding affinities over others on the target genes. The reported 5,7,2,3,4-pentahydroxy-3,6-dimethoxyflavone 7-glucoside compound showed a binding affinity value close to the co-crystal ligand on the TNF gene. The docking score of the same was found to be -2.308. Cynaroside was observed to have a highly remarkable binding affinity value of -8.157 in docking, whereas the other compound 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside also showed the highly prominent binding affinity value of -8.371. Moreover, isoquercitrin also had a significant docking value of -7.016 in the binding site (Table 1).

Table 1. Docking score of *Tridax procumbens* containing phytochemicals.

Compound	Dock Score	Compound	Dock Score
5,7,2,3,4'-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside	-8.371	Lupeol	-1.821
Cynaroside	-8.157	14-Ketostearic acid methyl ester	-1.661
Isoquercitrin	-7.016	Olean-12-en-3-one	-1.612
Tridaxidone	-6.768	beta-Amyrone	-1.612
Quercetin	-5.505	Docosanoic acid	-1.553
6-Hydroxyluteolin	-5.412	beta-Amyrin	-1.148
6,3'-dimethyl ether 5-rhamnoside			
Luteolin	-5.129	Arachidic acid	-0.674
Stigmasterol	-3.095	Myristic acid	-0.521
beta-Sitosterol	-2.913	Dotriacontanol	-0.102
Campesterol	-2.659	Linoleic acid	0.117
(24E)-2	-2.466	Stearic acid	0.297
4-N-Propylidenecholesterol			
Coc-crystal ligand	-2.308	Dotriacontane	0.425
9-Heptadecanone	1.261	29-Dotriacontenoic acid, 30-methyl-8-oxo-	0.569
Linolenic acid	1.432	Palmitoleic acid	0.676
Lauric acid	3.017	Palmitic acid	0.82

A more detailed picture of these interactions was obtained. Thus, three hydrogen bonds were observed for compound 5,7,2,3,4'-pentahydroxy-3,6-dimethoxyflavone 7-glucoside with the TNF target gene: one bond with Gly148 and Gln149 through 4th and 5th OH groups on the tetrahydro-2H-pyran moiety and Leu120 with the OH group of the benzene ring (Fig. 7). Furthermore, Tyr59 forms a pi-pi hydrophobic interaction with the octahydro-2H-chromene moiety. Cynaroside forms four hydrogen bonds with the TNF target gene: three between the 3rd, 4th, and 5th OH groups of the tetrahydro-2H-pyran moiety with Tyr119 and Gly121 and one between the OH atom of the benzene ring and Tyr151 (Fig. 7). Furthermore, non-bonded hydrophobic interactions bond with Tyr59, Ala96, Leu94, Ile118, and Ile155. In contrast,

the co-crystal ligand forms only one hydrogen bond between the OH group of the octahydro-2H-chromene moiety and Leu120 but also has non-bonded hydrophobic interactions with Tyr59, Ala96, Leu94, Pro117, Ile118, Tyr119, and Ile155, as well as polar interactions with Ser60, Gln61, and Ser95 (Fig. 7). The visualization of these data is presented in fig. 11 and shows the areas and types of interactions between these compounds and the TNF target gene.

Molecular dynamics

In order to better understand the interaction of the compounds with the target protein, we generated a 150 ns molecular dynamics simulation using DESMOND software. The compounds studied here are TNF-bound with 5,7,2,3,4'-pentahydroxy-3,6-dimethoxyflavone 7-glucoside, TNF/cynaroside, and TNF in a co-crystal ligand. The parameters followed in this study are RMSD, root-mean-square fluctuation, and protein-compound interactions.

For compound cynaroside, the RMSD values of the C-alpha atoms within the protein complex stabilized at about 80 nanoseconds and became stable at 0.8 Å throughout the rest of the simulation (Fig. 8A). Compound 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside first took about 80 nanoseconds to equilibrate, remaining steady in the binding pocket until the end of 150 nanoseconds. It started stabilizing after 50 nanoseconds when the values fell below 1.2 Å, indicating a solid bond to the protein site. On the other hand, the RMSF values of 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside and cynaroside indicated few fluctuations, but those present were minor and within the range of 3.0 Å. The low values at the binding site revealed that the protein had a stable interaction with cynaroside and 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside. The secondary structure of the protein indicated that the alpha-helices and beta-strands constituted about 17.88% of the protein. The high proportion of these structures in the protein contributed to its stability and function (Fig. 8B).

Regarding interactions, 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside formed three hydrogen bonds with TNF protein residues, including Tyr119, Gly121, and Tyr151. The role in hydrophobic interactions involved residues such as Tyr59, Ala96, Leu94, Ile118, and Ile155 (Fig. 8B). As for cynaroside, its simulation demonstrated the stability of RMSD values after five nanoseconds and continued until 80 nanoseconds. The values were stable at around 0.3 Å. In this case, the cynaroside compound was quickly stabilized within 80 nanoseconds. Next, it experienced fluctuations within 2.7 Å but stabilized at 1.2 Å after 130 nanoseconds. RMSF value for this C-compound was minimal, and enhancement in the protein loop and terminal shows 3.0 Å. The lesser RMSF at the binding site was between the compound and protein. The secondary structure analysis shows that alpha helices and beta strands are counted in the 17.88% structure of the protein (Fig. 8A).

The interaction analysis for cynaroside showed three hydrogen bonds with residues Tyr119, Gly121, and Ser60, with the latter interacting through a water bridge with the OH group of the octahydro-2H-chromene moiety, as shown in

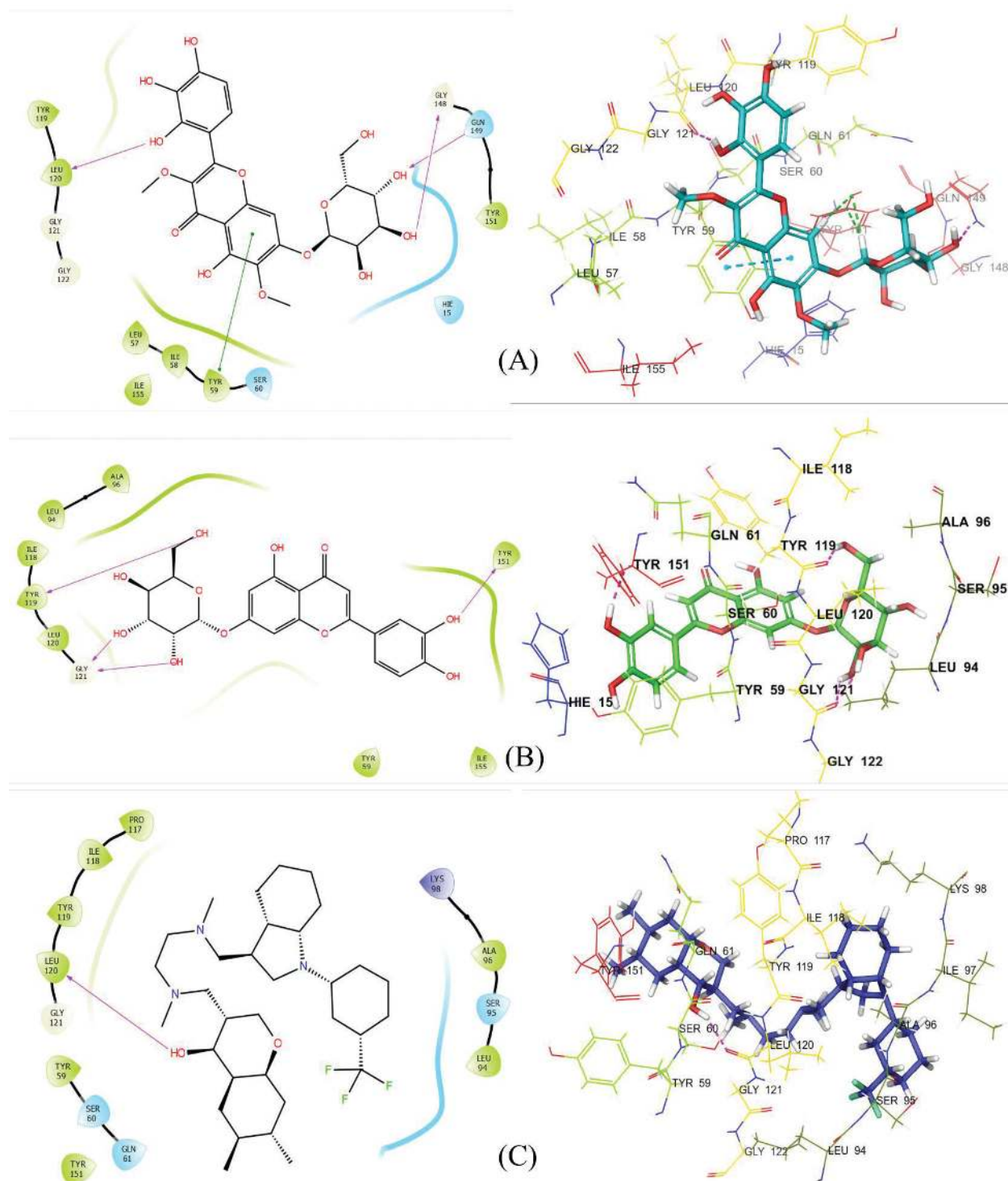


Figure 7. 2D and 3D visualization of compound-protein interactions for selected compounds (A. Image shows compound for 5,7,2',3',4'-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside; B. Image shows compound for cynaroside, and C. Image shows compound for co-crystal ligand) with the protein.

figures (Fig. 8A). The co-crystal ligand stabilized in RMSD values after around 30 nanoseconds and maintained around 2.4 Å, as illustrated in figure. The ligand equilibrated in around ten nanoseconds and stayed stable in the binding pocket until 50 nanoseconds. After 50 nanoseconds, it fluctuated but remained within 2.4 Å until the end of the simulation. The RMSF values for the co-crystal ligand indicated minimal fluctuation in the protein's loop and terminal regions within 3.0 Å, which was good, and the lower

RMSF values in the binding site showed stable interaction. The secondary structure analysis showed that the alpha helix and beta strand were 17.95% of the protein. The interaction analysis for the co-crystal ligand showed one hydrogen bond with Tyr151, and the OH group of the octahydro-2H-chromene moiety contributed 30%, as shown in figure. The histogram plot in figure showed different types of interaction in the co-crystal ligand, such as hydrogen bonding, water bridges, and hydrophobic interaction. (Fig. 8C).

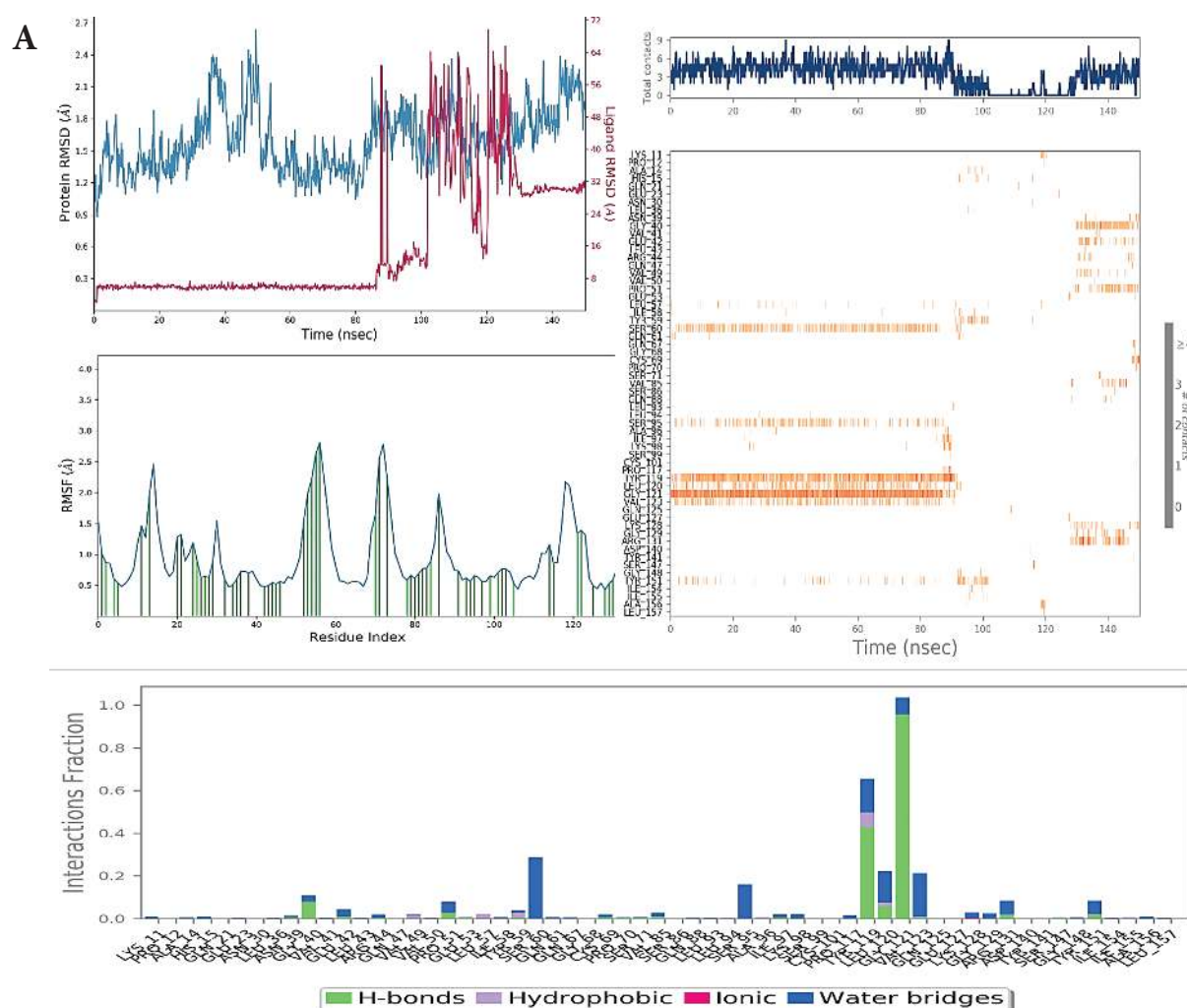


Figure 8. A. The hit compound cynaroside targeted the TNF protein complex using molecular dynamics (MD) simulation evaluation. The top left image shows the root mean square deviation (RMSD), with the RMSD of proteins in blue and the compound in red. The up-right image presents a heat map analysis for protein-ligand complexes. The middle-left image represents individual amino acids' root mean square fluctuation (RMSF) within proteins. The bottom image has a two-dimensional (2D) interaction diagram that maps interactions between the compound and the protein; **B.** The hit compound 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside targeted the TNF protein complex using molecular dynamics (MD) simulation evaluation. The top left image shows the root mean square deviation (RMSD), with the RMSD of proteins in blue and the compound in red. The up-right image presents a heat map analysis for protein-ligand complexes. The middle-left image represents individual amino acids' root mean square fluctuation (RMSF) within proteins. The bottom image has a two-dimensional (2D) interaction diagram that maps interactions between the compound and the protein; **C.** Co-crystal ligand analysis of the TNF protein complex through molecular dynamics (MD) simulation. In the top left picture, the root mean square deviation (RMSD) is blue for the protein and red for the ligand. The top right image shows a heatmap analysis of the protein-ligand complex. The middle left figure demonstrates root mean square fluctuation (RMSF) for amino acids in proteins individually. The lower image is a two-dimensional (2D) interaction diagram illustrating interactions between ligands and proteins.

Discussion

Integrating systems biology and molecular modeling in this study has provided valuable insights into the dual anti-viral and anti-bacterial potential of *Tridax procumbens*, particularly through its interaction with the Tumor Necrosis Factor (TNF) signaling pathway. We adopted a multi-pronged strategy involving bioinformatics, molecular docking, and molecular dynamics simulations focusing on bioactive compounds from the plant *Tridax procumbens*. It is important to understand the role of the TNF signaling pathway in regulating immune responses, inflammation, and cell survival. TNF activates the signaling pathways through its receptors,

TNFR1 and TNFR2, and activates several downstream signaling cascades, such as the MAPK and the NF- κ B pathway. These pathways are responsible for the functional expression of genes associated with inflammation, immunity, and apoptosis. We reported molecular docking studies, which indicated that TNF binds to compounds like cynaroside and 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside with docking scores of -8.157 and -8.371, respectively. These scores favor their ability to regulate TNF activity.

The protein-protein interaction (PPI) network analysis identified nodes like TNF, AKT1, EGFR, SRC, and ESR1 as key components of the anti-viral and anti-bacterial mechanisms of *Tridax procumbens*. The further GO enrichment

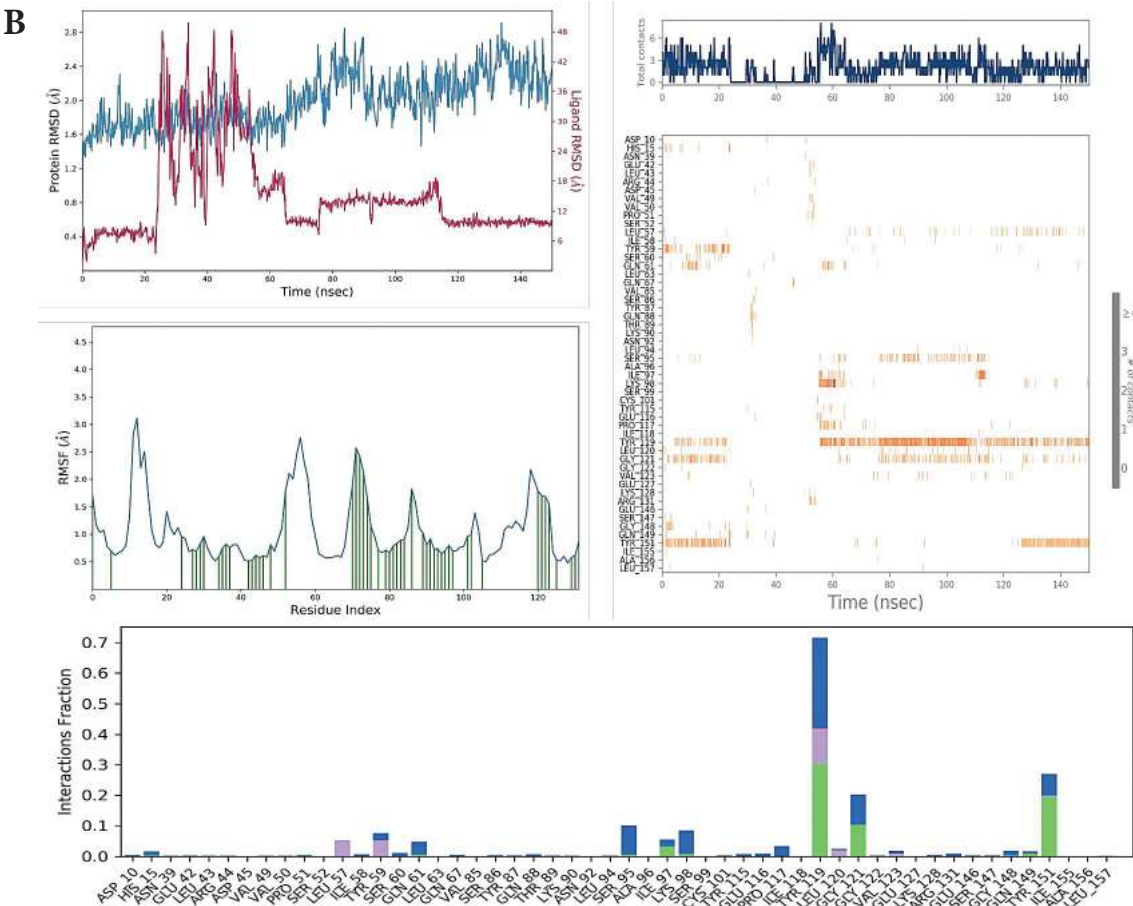


Figure 8. Continued.

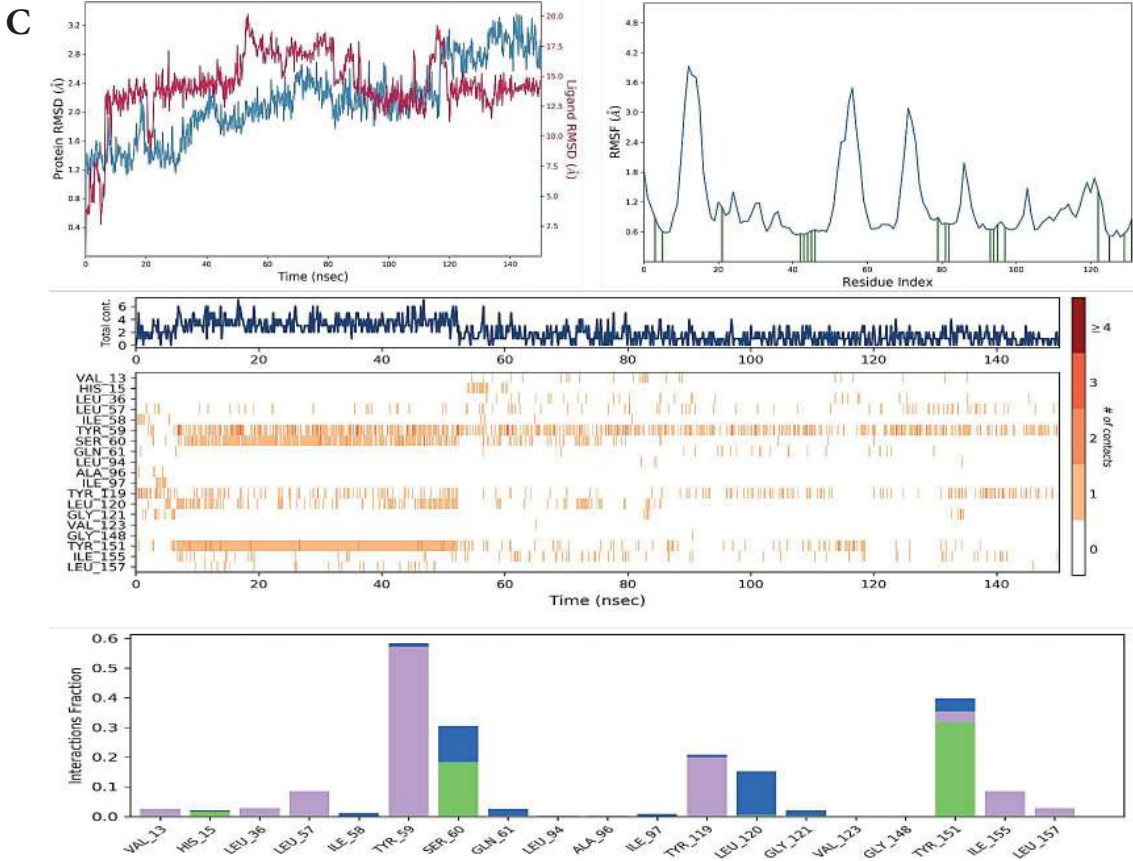


Figure 8. Continued.

analysis also showed the molecules involved in the biological processes, cellular components, and molecular functions influenced by these compounds. Interestingly, the significantly enriched genes regulated by *T. procumbens* are mainly engaged in the cytoplasm and plasma membrane, which can support the potential mechanisms by which *T. procumbens* may act at the cellular level by altering the membrane integrity and signal transduction. The insights offered by the molecular dynamics simulations also enabled a greater understanding of the stability and behavior of these compounds within the TNF binding pocket when conditions are closer to the physiological conditions. For example, cynaroside kept the same RMSD values and had consistent hydrogen contact with some residues of TNF protein, which showed stable and robust interaction. Likewise, 5,7,2,3,4-Pentahydroxy-3,6-dimethoxyflavone-7-glucoside established stable interactions and showed slower fluctuations than other compounds with therapeutic potential.

Although our findings are promising, the study has limitations. The first drawback is that computational prediction is done without in vitro testing. Though these methods give preliminary solid data, future research will focus on in vitro and in vivo research to establish the effectiveness and safety of these bioactive compounds. The bioavailability and toxicity of these compounds will be important considerations for formulating them as potential therapeutic agents. Further studies examining potential mechanisms of action and possible synergistic combinations with anti-viral and anti-bacterial drugs may be beneficial in generating additional therapeutic opportunities to improve treatment outcomes.

Conclusion

This study shows that *Tridax procumbens* could be an essential plant source of new bioactive compounds with dual anti-viral and anti-bacterial activity. Using molecular modeling and systems biology, we explored several vital molecules targeting the TNF signal transduction cascade, an essential immunological mediator in inflammation. The findings provide a foundation for further research into the therapeutic applications of *Tridax procumbens*, highlighting its promise in addressing the global challenge of infectious diseases. Additional research will include toxicological studies and bioassays in vitro and in vivo to

assess these bioactive compounds' possible therapeutic efficacy and toxicity. The findings of this study not only offer to explore the potential of *Tridax procumbens* containing phytochemicals and open new opportunities for exploring new molecules for anti-viral and anti-bacterial infections. We intend to perform the in vitro validation in our upcoming study. Further research and validation will lead to recognizing *Tridax procumbens* containing phytochemicals as essential bioactives in managing infectious diseases.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

Muthanna Saadi Farhan: Conceptualized the study, performed the system biology analysis, and contributed to writing and editing the manuscript AND, and supervised the entire project.

Farrah Rasool Jaafar: Conducted the molecular modeling experiments, analyzed the data, and drafted the initial manuscript.

Amjad I. Oraibi: Provided critical feedback, revised the manuscript for important intellectual content.

All authors have read and approved the final manuscript.

Data availability

All of the data that support the findings of this study are available in the main text.

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