Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2026; 12 (1): 48 – 54
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2026.v12.i1.17780

Predicting the Anti-Tuberculosis Mechanism of *Vitex cofassus* Methanolic Extracts through Network Pharmacology Analysis

(*Prediksi Mekanisme Antituberkulosis Ekstrak Metanol Vitex cofassus melalui Analisis Farmakologi Jejaring*)

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Article Info:
Received: 26 July 2025
in revised form: 12 September 2025
Accepted: 27 March 2026
Available Online: 30 March 2026

Keywords:
Tuberculosis
Vitex cofassus
Network pharmacology
IL-6
TNF-α

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ABSTRACT

Background: *Vitex cofassus* is a medicinal plant with potential anti-tuberculosis (TB) properties that remain poorly understood at the molecular level. **Objectives:** This study aimed to investigate the anti-TB potential of *V. cofassus* leaf extract through phytochemical profiling, target identification, and molecular interaction analysis. **Material and Methods:** The methanolic leaf extract was analyzed using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). Network pharmacology was employed to identify potential TB targets and core proteins, while molecular docking was performed to evaluate the binding affinities of lead compounds against critical inflammatory targets. **Results:** LC-HRMS revealed 223 compounds, with 98 meeting drug-likeness criteria. Network pharmacology identified 140 potential TB targets, highlighting IL-6, TNF-α, IL-1β, TLR4, STAT3, and TP53 as core proteins. The extract's effects are suggested to act through immune defense responses at the cell membrane related to inflammation. Molecular docking demonstrated that Compounds 169, 112, 53, and 37 exhibited stronger binding affinity toward IL-6 compared to the native ligand, while Compound 123 showed superior binding to TNF-α. **Conclusions:** These results suggest that *V. cofassus* extract could inhibit TB by targeting critical inflammatory and immune pathways, particularly through the modulation of IL-6 and TNF-α.

How to cite (APA 6th Style):
Omir, S. W. A., Ruslin, R., & Arba, M. (2026). Predicting the anti-tuberculosis mechanism of *Vitex cofassus* methanolic extracts through network pharmacology analysis. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 12*(1), 48-54. doi:10.22487/j24428744. 2026.v12.i1.17780

INTRODUCTION

Tuberculosis (TB), caused primarily by *Mycobacterium tuberculosis*, is the second deadliest infectious disease after COVID-19 in 2022, causing 1.3 million deaths. It affects around 10.6 million people annually, with the majority of cases found in low- and middle-income countries including those in Southeast Asia (45%), Africa (23%), and Western Pacific (17%) (WHO, 2024). Individuals infected with TB may experience symptoms such as coughing, fever, night sweats, weight loss, and reduced appetite. A person with untreated TB can transmit the disease to approximately 10–15 individuals per year (Bagcchi, 2023; Emery et al., 2021; Houben & Dodd, 2016). Moreover, data showed that approximately 25% (2 billion) of the global population harbors *Mycobacterium tuberculosis* antigens without showing any signs or symptoms of active TB, a condition referred to as tuberculosis infection (TBI) (Bagcchi, 2023; Houben & Dodd, 2016). Without treatment, it is estimated that around five to ten percent of individuals with TBI will go on to develop active TB (WHO, 2024).

The primary approach to treating TB involves the use of isoniazid and rifampicin antibiotics. These drugs are crucial due to their ability to kill both actively dividing and dormant *Mycobacterium tuberculosis* bacteria (Genestet et al., 2025; Peloquin & Davies, 2021). In addition, rifapentine and rifabutin serve as important alternatives because of their distinct pharmacokinetic (PK) and pharmacodynamic (PD) characteristics (Kendall et al., 2021; Rifat et al., 2018). However, multidrug-resistant tuberculosis has emerged as a major global health concern due to resistance to first-line drugs such as rifampicin and isoniazid.

On the other hand, approximately 80% of the global population utilizes some form of traditional and complementary medicine alongside conventional biomedical approaches. The World Health Organization (WHO) has repeatedly urged the integration of traditional and complementary into healthcare systems, partly to enhance the consistency and quality of traditional medicine. Numerous ethnobotanical studies have documented the use of medicinal plants to treat various infections (Yeung et al., 2020). One of the popular medicinal plants with recognized beneficial effects on human health is *Vitex* genus, belonging to the Lamiaceae family (formerly included in the Verbenaceae family) (Souto et al., 2020). The *Vitex* genus contains various compounds, including sesquiterpenes, iridoids, flavonoids, lignans, steroids, and diterpenes. Several of these compounds have demonstrated activities such as anticancer, antiviral, hepatoprotective, antimicrobial, and antipesticidal activities (Islam et al., 2024; Mahmoud et al., 2025; Mottaghipisheh et al., 2024; Nyamweya et al., 2023; Pan et al., 2014; Rasyid et al., 2017; Singh & Bharadvaja, 2025; Sirotkin, 2025; Summaya et al., 2023; Yanfei et al., 2020). In addition, previous research has demonstrated that leaf and bark extracts of *Vitex* species exhibit antimicrobial activity against a wide range of microorganisms including *Bacillus aureus*, *Micrococcus*

luteus, *Staphylococcus aureus*, *Salmonella aureus*, and *Mycobacterium smegmatis* (Devipriya et al., 2023).

In this study, we investigated the potential of the methanolic extract of *V. cofassus* as an anti-tuberculosis agent. We employed network pharmacology study based on liquid chromatography-high resolution mass spectrometry (LC-HRMS) data to elucidate the multi-target effects of bioactive compounds contained in the methanolic extract of *V. cofassus* on various targets and pathways related to TB. Molecular docking was also performed to provide mechanistic insights on the interactions of single compound against a particular receptor target.

MATERIAL AND METHODS

Collection and preparation of plant material, chemical profiling via LC-HRMS, and in silico drug-likeness evaluation

Leaves of *V. cofassus* were collected in June 2024 from the Forest Management Unit (FMU) Gantara, located in Liwu Metingki Village, Muna District, Southeast Sulawesi, Indonesia. The plant species was identified by the Pharmacy Laboratory at Universitas Halu Oleo, Indonesia. After collection, the leaves were sorted while still moist, washed, thinly sliced, and air-dried. The resulting dried material (simplisia) was then macerated using methanol as the extraction solvent, and the solvent was evaporated to obtain a crude extract. This crude methanolic extract of *V. cofassus* was subjected to analysis using liquid chromatography–high-resolution mass spectrometry (LC-HRMS) to determine its chemical composition (Arba et al., 2025b; Zubair et al., 2021).

Chromatographic separation was performed on a Thermo Scientific™ Vanquish™ UHPLC Binary Pump system coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer, providing detailed metabolic profiling of the extract. A Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm ID × 2.6 µm) was used for the analysis. The specific parameters and analytical procedures such as mass error tolerance of -5 to 5 ppm, confidence score > 80%, the use of ChemSpider and mzCloud databases followed previous report (Windarsih et al., 2022).

The identified compounds were further evaluated through LC-HRMS analysis for drug-likeness using the SwissADME web server (http://www.swissadme.ch/) (Daina et al., 2017). Screening was based on the Lipinski Rule of Five, which includes criteria such as molecular weight ≤ 500 Da, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a log P value ≤ 5. Additional filters included a bioavailability score greater than 0.55 and high gastrointestinal absorption.

Compounds that violated more than one of the Lipinski rules, had a bioavailability score below 0.55, or exhibited low gastrointestinal absorption were excluded from subsequent analysis.

Target screening

Target prediction for the methanolic extract of *V. cofassus* was conducted using the SwissTargetPrediction and SEA (Similarity Ensemble Approach) databases (Daina et al., 2019; Keiser et al., 2007). Meanwhile, tuberculosis (TB)-related genes were identified through searches in the OMIM (https://www.omim.org) and GeneCards (https://www.genecards.org) databases (Amberger et al., 2015; Arba et al., 2025a; Rebhan et al., 1998). These disease-associated genes were further refined by selecting the top 500 targets (Jiang et al., 2021). Finally, the overlapping targets between the predicted compound targets and TB-related genes were determined and visualized using a Venn diagram generated with the Bioinformatics and Systems Biology online tool (https://bioinformatics.psb.ugent.be/webtools/Venn).

Protein-Protein Interaction (PPI) Network

Protein-protein interaction (PPI) networks were constructed using the STRING database (https://string-db.org), with common target proteins used as input. The analysis was restricted to Homo sapiens and set to a high confidence score threshold of 0.700. The resulting PPI network was visualized and analyzed using Cytoscape software (version 3.10.2) (Shannon et al., 2003).

Gene ontology (GO) analysis and KEGG path

To explore the biological significance of the predicted targets, Gene Ontology (GO) enrichment analysis was performed using the Metascape (https://www.metascape.org) and shinyGO 0.80 platforms [27–29], covering aspects such as biological processes, cellular components, and molecular functions (Ge et al., 2020; Kanehisa et al., 2017; Zhou et al., 2019).

Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted to identify the key metabolic and signaling pathways affected by the bioactive compounds and target proteins associated with *V. cofassus* and tuberculosis. These pathways were considered as potential mechanisms underlying the therapeutic action of the compounds.

Molecular Docking

Molecular docking was carried out to assess the binding orientation and affinity of the compounds toward the selected three most relevant receptor targets, specifically interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1 beta (IL-1β). The 3D structures (PDB files) of these target proteins were obtained from the Protein Data Bank (https://www.rcsb.org).

The compounds identified from the LC-HRMS analysis of the methanolic extract of *V. cofassus* were converted from 2D to 3D structures using the LigPrep module in Maestro, employing the OPLS_2005 force field (Arba et al., 2017; Citra et al., 2023). Protein and ligand preparations were conducted according to standard docking protocols (Arba et al., 2022) using Maestro Schrödinger software version 11.1.012, release 2017-1 (Schrödinger, New York, NY, USA) (Sastry et al., 2013). Docking grids were generated based on the binding sites of each protein's native ligand (Vulpetti et al., 2024).

RESULTS AND DISCUSSION

Identification of active compounds and target prediction

A total of 223 bioactive compounds were detected in the methanolic extract of *V. cofassus* using LC-HRMS (Table S1). Drug-likeness evaluation based on Lipinski's Rule of Five, bioavailability score, and gastrointestinal absorption criteria revealed that 125 compounds did not meet the requirements. These include Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 8, Compound 9, Compound 10, Compound 11 and Compound 12. (Table S2).

As a result, only 98 compounds were selected for further analysis. Using the SwissTargetPrediction and SEA databases, 1,177 and 501 potential targets of *V. cofassus* were predicted, respectively. Meanwhile, 1,253 and 500 gene targets associated with TB were identified through the OMIM and GeneCards databases. A total of 140 overlapping targets were found at the intersection of *V. cofassus* targets and TB-related genes, which were considered hub genes, as illustrated in the Venn diagram (Figure 1).

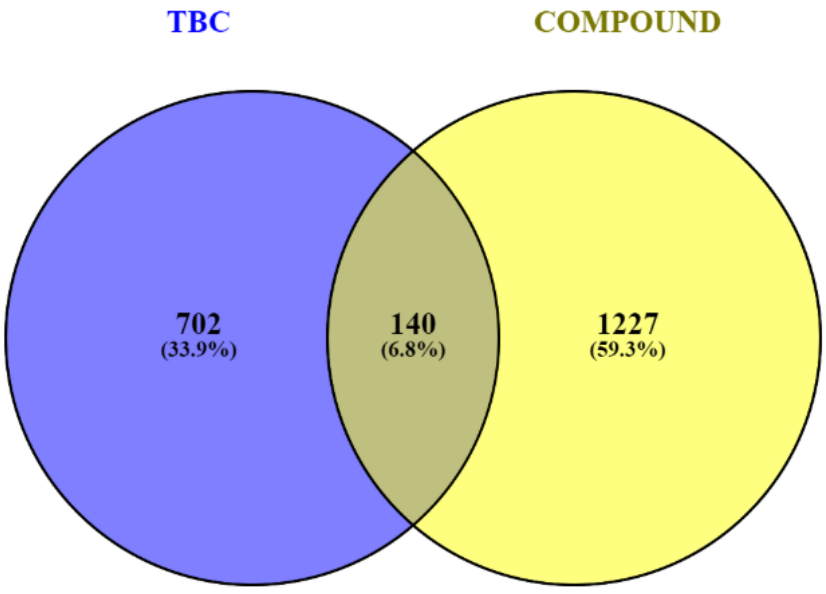


Figure 1. Venn diagram illustrating common targets of tuberculosis (TB) and *V. cofassus* compounds. Blue represents 702 TB genes, yellow indicates 1227 *V. cofassus* targets, and yellow-green highlights the overlapping 140 genes between the two.

Construction of compound-target network analysis

From the initial 140 identified targets, the association between bioactive compounds in the methanolic extract of *V. cofassus* and TB therapeutic targets was established. These targets were analyzed using the STRING database to generate protein-protein interaction (PPI) network data, which was subsequently visualized in Cytoscape. The resulting compound-target network consisted of 139 nodes (representing target genes and their corresponding compounds) and 1018 edges (depicting interactions between nodes), as shown in Figure 2.

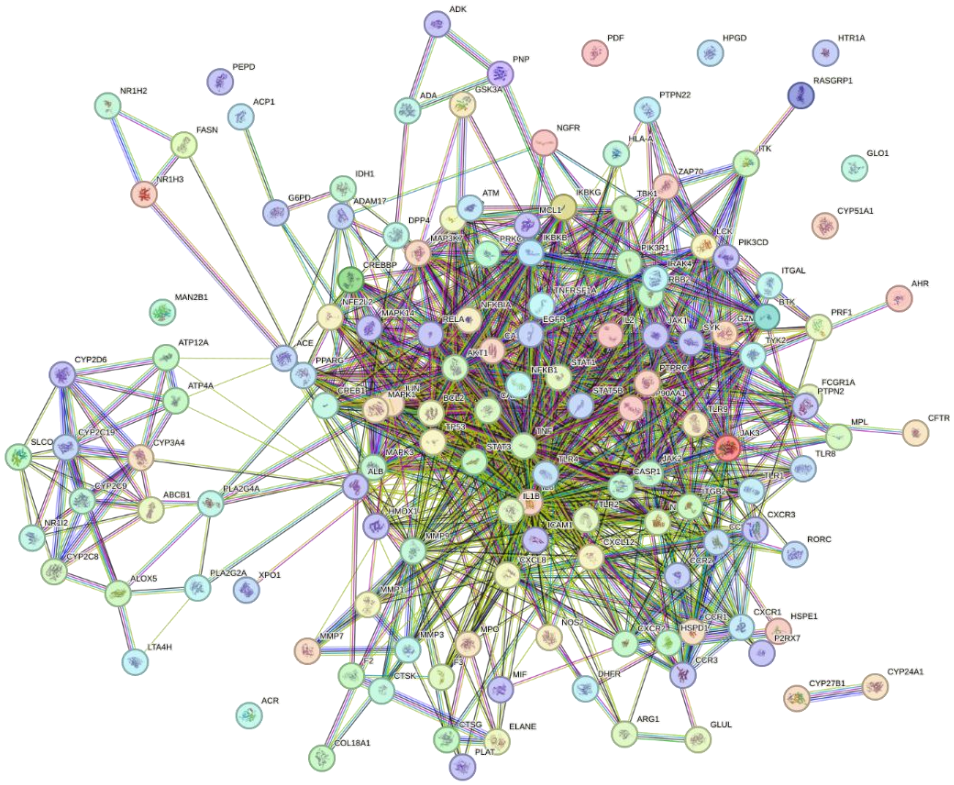


Figure 2. The protein-protein interaction (PPI) network was constructed using the STRING database, comprising 139 nodes (representing potential targets) and 1018 edges (denoting protein-protein interactions).

The top 10 hub targets were identified from the 139 nodes based on highest degree values, suggesting their pivotal role in the PPI network for potential anti-TB activity. Table 1 presents these key nodes along with their closeness centrality and betweenness centrality metrics.

Table 1. The top ten targets ranked by network centrality metrics.

Name	Degree	Closeness centrality	Betweenness centrality
Interleukin-6 (IL-6)	65	0.627451	0.098452
Tumor necrosis factor-α (TNF-α)	63	0.61244	0.087746
Interleukin-1 beta (IL-1β)	56	0.600939	0.053768
Toll-like receptor 4 (TLR4)	55	0.589862	0.06649

Name	Degree	Closeness centrality	Betweenness centrality
Signal transducer and activator of transcription 3 (STAT3)	50	0.589862	0.093674
Tumour suppressor p53 (TP53)	46	0.573991	0.085809
AKT serine/threonine kinase 1 (AKT1)	44	0.561404	0.030143
Epidermal growth factor receptor (EGFR)	43	0.561404	0.048309
Signal transducer and activator of transcription 1 (STAT1)	43	0.556522	0.024056
Nuclear factor kappa-B 1 (NFKB1)	40	0.540084	0.014515

The degree value indicates the number of predicted interactions between a component and its targets, with higher values signifying greater biological importance. Our analysis identified several high-degree targets, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), toll-like receptor-4 (TLR4), signal transducer and activator of transcription-3 (STAT3), tumour suppressor p53 (TP53), AKT serine/threonine kinase-1 (AKT1), epidermal growth factor receptor (EGFR), signal transducer and activator of transcription-1 (STAT1), and nuclear factor kappa-B 1 (NFKB1), which were consequently designated as hub targets.

Two additional centrality measures further characterized network topology: closeness centrality (measuring a node's proximity to all other nodes in the network) and betweenness centrality (identifying nodes that function as critical bridges in network connectivity) (Zhou et al., 2016).

The top 10 proteins from each centrality measure (degree, betweenness, closeness) were cross-analyzed using a Venn diagram, revealing six consensus targets: IL-6, TNF-α, IL-1β, TLR4, STAT3, and TP53 (Figure 3). These overlapping proteins were identified as the most promising therapeutic targets, with Figure 3 panels a-c displaying the top-ranked proteins for each centrality measure, and panel d illustrating their intersection.

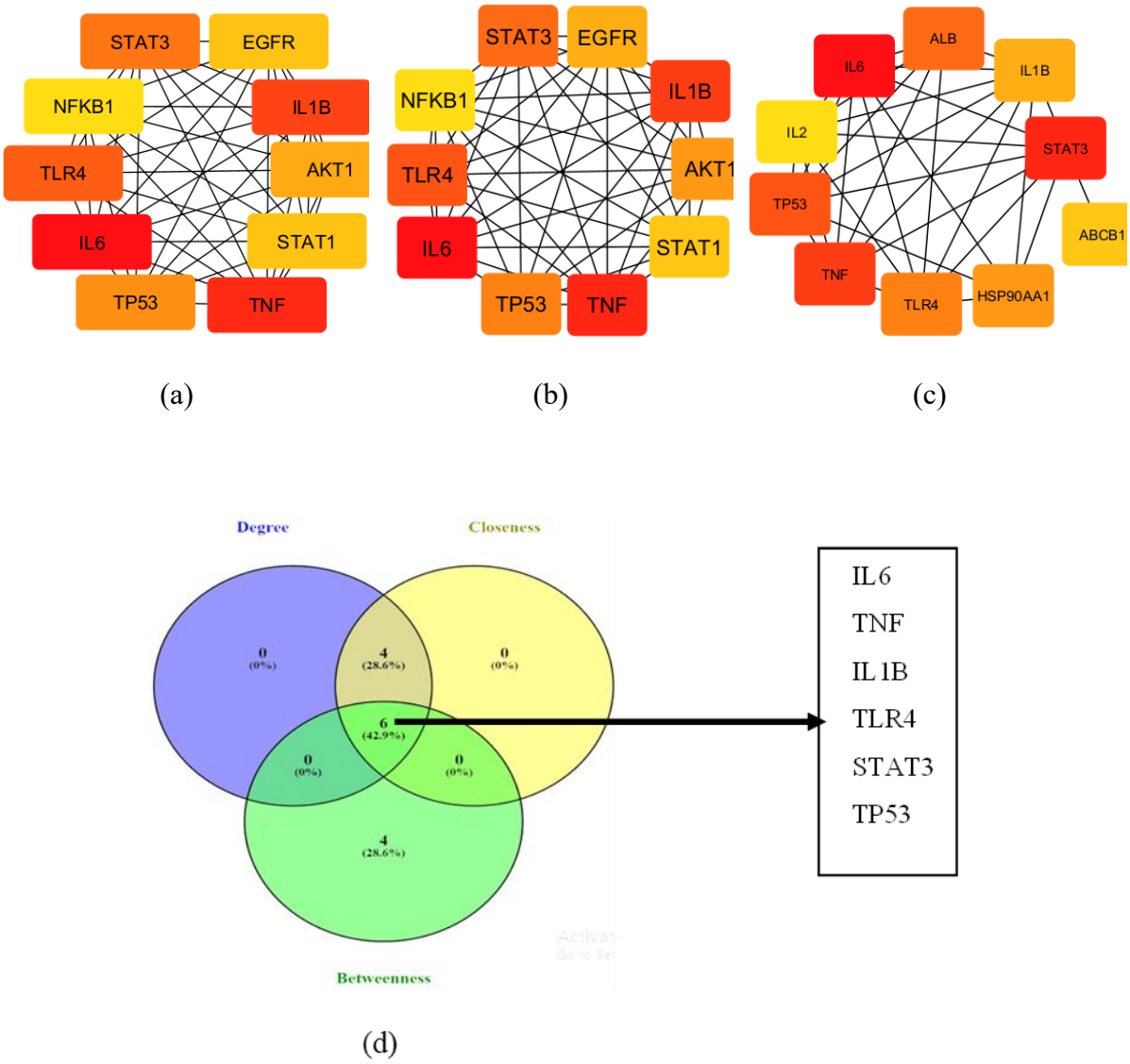


Figure 3: The top 10 potential target proteins ranked by: (a) degree centrality, (b) betweenness centrality, and (c) closeness centrality. The Venn diagram analysis (d) reveals six consensus targets that appear across all three centrality measures: IL-6, TNF-α, IL-1β, TLR4, STAT3, and TP53.

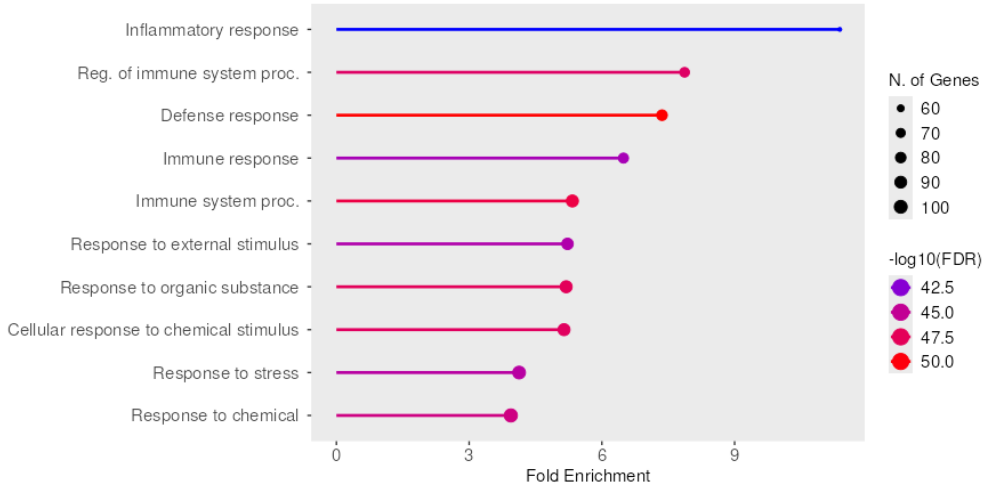
Functional enrichment analysis using Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

To investigate the anti-TB mechanisms of the 140 overlapping targets, we performed functional enrichment analysis using Gene Ontology (GO) and KEGG pathway databases via ShinyGO 0.80. The analysis covered biological processes (BP), cellular components (CC), and molecular functions (MF), revealing significant enrichment of 1001 BP terms, 129 CC terms, 254 MF terms, and 186 KEGG pathways associated with TB pathogenesis."

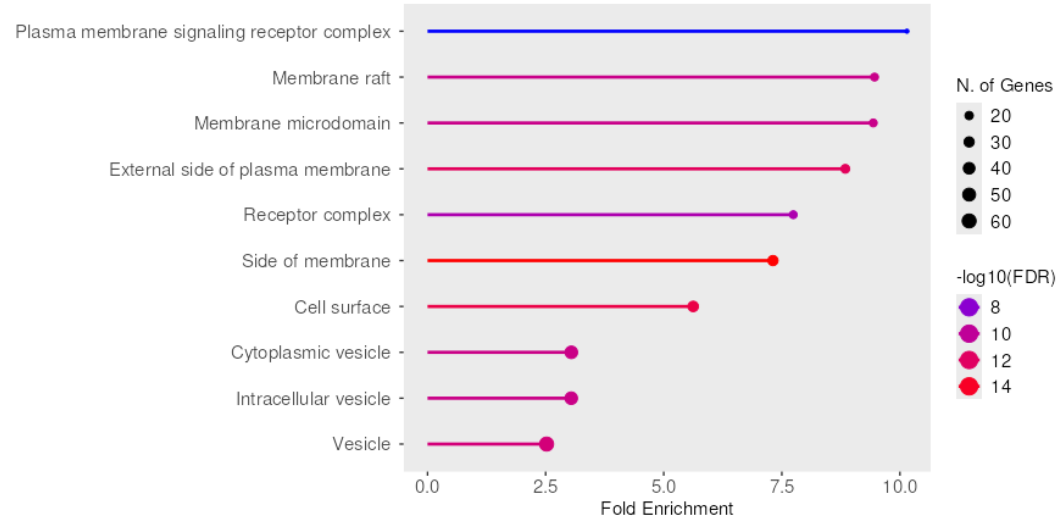
The GO biological process analysis revealed the top ten enriched pathways, demonstrating that the target genes of *V. cofassus* methanolic extract were predominantly associated with defense response, immune system processes, response to organic substance, cellular response to chemical stimulus, regulation of immune system processes and response to chemical. Cellular component analysis localized these target genes primarily to the side of membrane, cell surface, external side of plasma membrane, vesicle, cytoplasmic vesicle. Molecular function characterization indicated significant enrichment in enzyme binding, identical protein binding, signaling receptor binding, phosphatase binding, protein serine/threonine/tyrosine kinase activity, and so on.

KEGG pathway analysis identified several TB-relevant signaling pathways associated with *V. cofassus*'s therapeutic effects, including: lipid and atherosclerosis, hepatitis B, coronavirus disease-COVID-19, Kaposi sarcoma-associated herpesvirus infection and toll-like receptor signaling pathway. Figure 4 presents a bubble map illustrating the most significant GO terms and KEGG pathways.

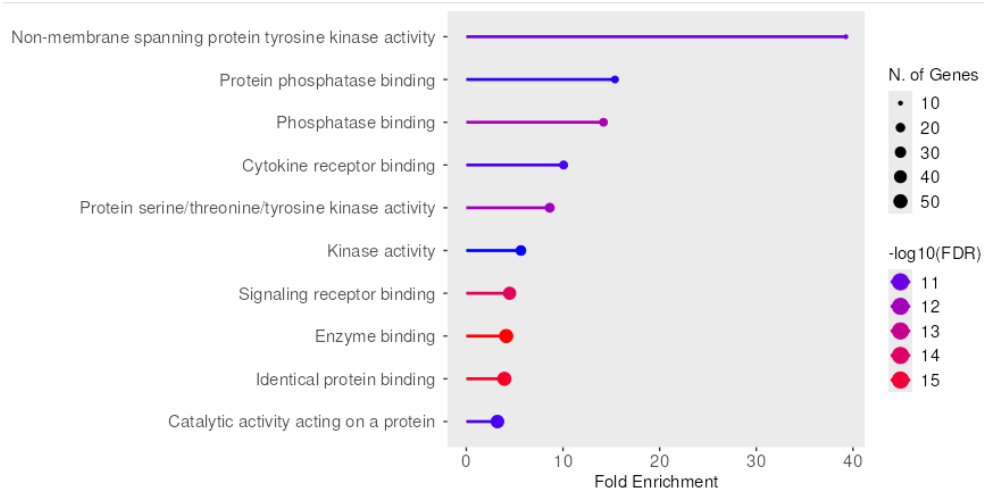
a.



b.



c.



d.

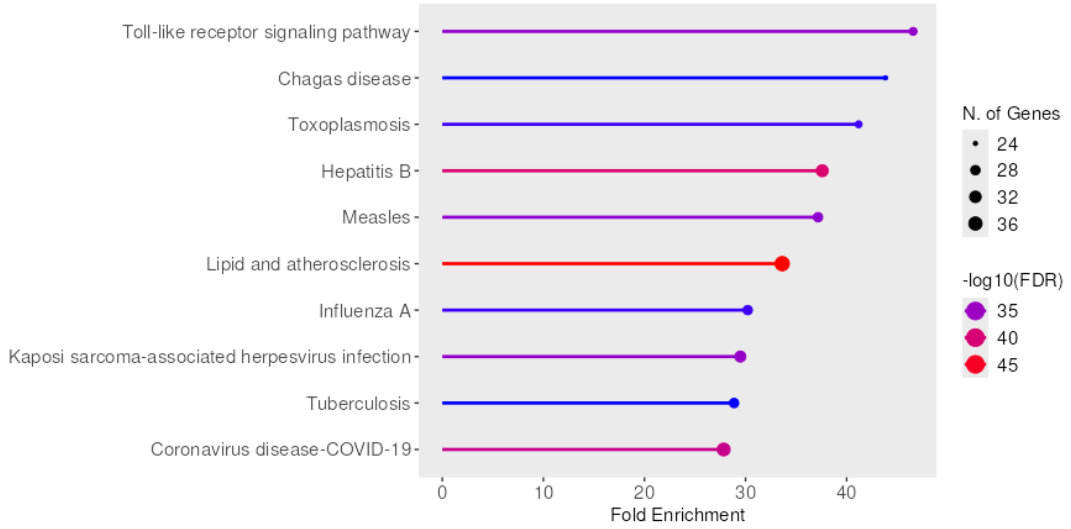


Figure 4. A comprehensive bubble map analyzing the bioactive compounds from *V. cofassus* methanolic leaf extract through: (a) GO biological processes (b) GO cellular components (c) GO molecular functions (d) KEGG pathways.

Molecular Docking Analysis of Key Targets

Through integrated network analysis (compound-target, PPI, and compound-target-disease), we identified IL-6, TNF-α, and IL-1β as top-ranked proteins for molecular docking studies. Selected compounds were docked as follows: IL-6: 5 compounds (Compound 37, Compound 53, Compound 89, Compound 112, Compound 169), TNF-α: 7 compounds (Compound 16, Compound 37, Compound 53, Compound 72, Compound 122, Compound 123, Compound 140), and IL-1β: 1 compound (Compound 208).

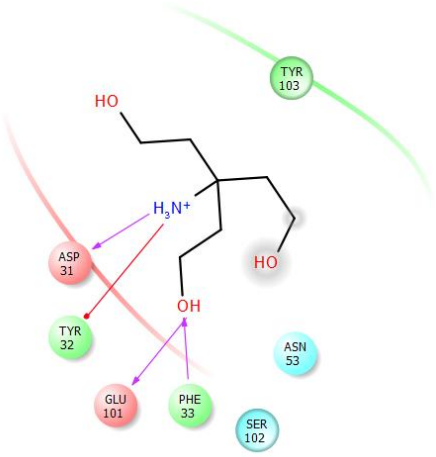
Binding affinity results (Table 2) showed that IL-6 complexes exhibited binding energies ranging from -1.917 kcal/mol (Compound 89) to -6.131 kcal/mol (Compound 169), compared to its native ligand (-2.939 kcal/mol), TNF-α complexes showed energies between -1.064 kcal/mol (Compound 72) and -5.033 kcal/mol (Compound 123), versus the native ligand (-4.059 kcal/mol), and IL-1β

complex (Compound 208) demonstrated weaker binding (-2.781 kcal/mol) than its native ligand (-6.746 kcal/mol).

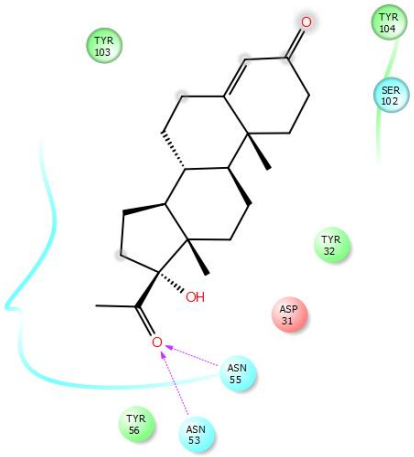
Table 2: The binding energy (kcal/mol) of compound docked to IL-6, TNF-α, and IL-1β.

Compound	Binding energy (kcal/mol)		
	IL-6 (PDB ID :4CNI)	TNF-α **(PDB ID :2AZ5)**	**IL-1B (PDB ID :8RYS)**
Native ligand	-2.939 (TAM)	-4.059 (307)	
Compound 16		-2.021	
Compound 37	-3.443	-3.443	
Compound 53	-3.636	-3.438	
Compound 72		-1.064	
Compound 89	-1.917		
Compound 112	-4.897		
Compound 122		-3.044	
Compound 123		-5.033	
Compound 140		-3.982	
Compound 169	-6.131		
Compound 208			-2.781

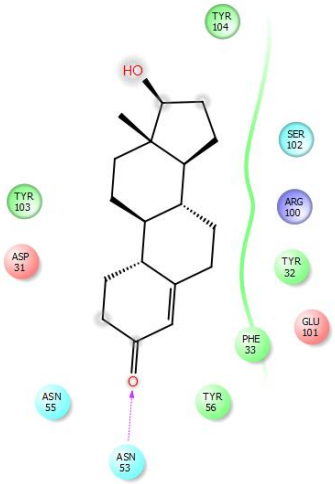
Figure 5 and Figure S1 illustrate the molecular structures and binding conformations of compounds docked to IL-6. The analysis revealed that strong binding affinities for Compounds 37 (-3.443 kcal/mol), Compound 53 (-3.636 kcal/mol), Compound 112 (-4.897 kcal/mol), and particularly Compound 169 (-6.131 kcal/mol). Relatively weaker interaction was observed for Compound 89 (-1.917 kcal/mol). Compound 169 was identified as andrographolide, which is a terpenoid. Previous studies have shown that terpenoids exhibit antitubercular activity (dos Reis et al., 2016; Kataev et al., 2018; Sansinenea & Ortiz, 2014). Compound 112, on the other hand, is embelin, which is a quinone, which have broad pharmacological properties including antimycobacterial activity (Das & Kalyani, 2025; Halicki et al., 2018; Tran et al., 2004). Meanwhile Compound 37 and Compound 53 are hydroxyprogesterone and nortestosterone, respectively, both of which are steroid, which are also known to exhibit antitubercular activity (Copp & Pearce, 2007).



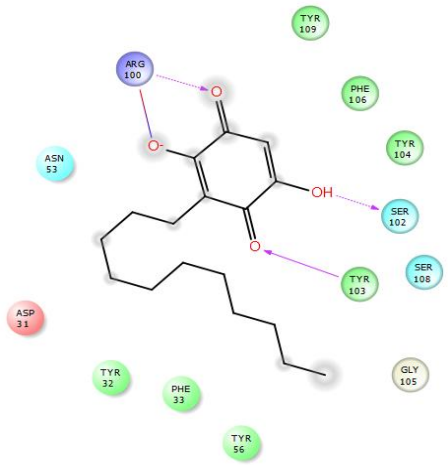
Native ligand: -2.939 kcal/mol



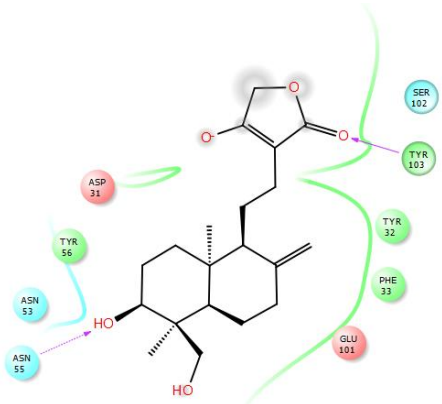
Compound 37: -3.443 kcal/mol



Compound 53: -3.636 kcal/mol



Compound 112: -4.897 kcal/mol

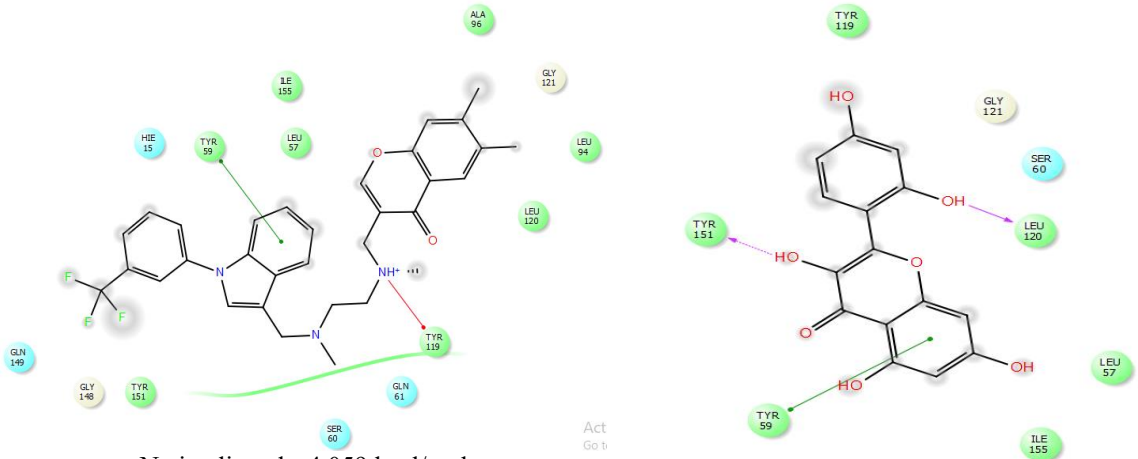


Compound 169: -6.131 kcal/mol

Figure 5. The favorable binding conformations to IL-6, with the following binding affinities: Compound 169 demonstrated the strongest interaction (-6.131 kcal/mol), Compound 112 showed substantial binding (-4.897 kcal/mol), Compounds 53 (-3.636 kcal/mol) and Compound 37 (-3.443 kcal/mol) exhibited moderate affinity.

The binding conformations of compounds docked to TNF-α are presented in Figure 6 and Figure S2. Binding affinity analysis revealed that Compound 123 exhibited the lowest binding energy (-5.033

kcal/mol), indicating high affinity, moderate to weak interactions as represented by Compound 140 (-3.982 kcal/mol), Compounds 37 (-3.443 kcal/mol) and Compound 53 (-3.438 kcal/mol), Compound 122 (-3.044 kcal/mol), Compound 16 (-2.021 kcal/mol), and Compound 72 (-1.064 kcal/mol). Compound 208 docked to IL-1β revealed the binding energy of -2.781 kcal/mol (Table S3). Compound 123 is a quercetin, which is a flavonoid, they are well known for antitubercular activity (Cao et al., 2019; Dong et al., 2015; Omokhua-Uyi et al., 2023). However, as per our understanding, this is the first report on anti-TB potential of the Compound 169, Compound 112, Compound 37, Compound 53, and Compound 123.



Native ligand: -4.059 kcal/mol

Compound 123: -5.033 kcal/mol

Figure 6. Molecular docking analysis revealed Compound 123 showed the strongest binding affinity to TNF-α (-5.033 kcal/mol).

Subsequent molecular docking studies focused on the three highest-priority targets - IL-6, TNF-α, and IL-1β- to evaluate binding interactions. Previous studies have shown that innate immunity represents the host's primary defense against invading pathogens. Two central pathways, NF-κB and MAPK, regulate innate immune responses by controlling the synthesis of cytokines including TNF-α and IL-6. In *M. tuberculosis* infection, both NF-κB and MAPK signaling contribute to cytokine production in macrophages (Li et al., 2015). TNF-α is highly expressed in TB granulomas. In human cells, an excess of TNF-α can increase *M. tuberculosis* growth (Dawa et al., 2021; Tebruegge et al., 2015; Tezera et al., 2020).

CONCLUSION

This study demonstrates that the methanolic extract of *Vitex cofassus* may represent a promising candidate for tuberculosis therapy. Through LC-HRMS-based network pharmacology and molecular docking approaches, we identified 140 key targets shared between *V. cofassus* and TB-related genes. Among these, six stood out as most important: interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), toll-like receptor 4 (TLR4), signal transducer and activator of transcription 3 (STAT3), and tumour suppressor p53 (TP53). The findings suggest that the extract exerts its potential anti-tuberculosis activity by modulating immune and inflammatory pathways. When tested against major TB-linked proteins (IL-6, TNF-α, and IL-1β), several plant compounds showed strong binding, especially Compound 37, Compound 53, Compound 112, and Compound 169 (for IL-6), and Compound 123 (for TNF-α). Considering the global challenge of extensively drug-resistant TB, the identification of *V. cofassus* as a potential anti-TB agent in the current study provides a valuable lead for the development of alternative therapeutic options. Nevertheless, as the present study is entirely in silico, further validation through in vitro and in vivo studies is required to confirm its therapeutic potential.

ACKNOWLEDGEMENT

This study was supported by the Penelitian Tesis Magister Grant from Direktorat Penelitian dan Pengabdian kepada Masyarakat, Direktorat Jenderal Riset dan Pengembangan, Ministry of Higher Education, Science, and Technology Republic of Indonesia, under contract number 068/C3/DT.05.00/PL/2025.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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