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RESEARCH ARTICLES

Eksplorasi Mekanisme Kardioprotektif Fraksi Etil Asetat Vitex cofassus melalui Farmakologi Jejaring

Exploring the Cardioprotective Mechanisms of the Ethyl Acetate Fraction of *Vitex cofassus* Through Network Pharmacology

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Abstract

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide, with an increasing prevalence each year. The complexity of CVD pathophysiology, which involves multiple biological pathways, required a multitarget therapeutic approach. This study aimed to investigate the potential anti-cardiovascular activity of the ethyl acetate fraction of Vitex cofassus leaves through integrated phytochemical analysis, network pharmacology and molecular docking approaches. The ethyl acetate fraction of *Vitex cofassus* leaves was analyzed using liquid chromatography-high-resolution mass spectrometry (LC-HRMS), identifying 232 compounds, of which 104 met drug-likeness criteria. Network pharmacology screening revealed 274 potential cardiovascular-related targets. Gene Ontology (GO) analysis highlighted enriched biological processes including responses to oxygen-containing compounds, while cellular component analysis localized target genes primarily to plasma membrane structures, neuron projections, and synapses. Molecular functions were significantly associated with signaling receptor activity and kinase activity, while KEGG pathway analysis further linked *Vitex cofassus* to key cardiovascular pathways including neuroactive ligand-receptor interactions. The results of Network pharmacology analysis identified SRC, HSP90AA1 and TNF-a proteins as potential targets associated with cardiovascular diseases. Molecular docking studies demonstrated strong binding affinities of selected compounds to HSP90AA1, particularly Compound 74 (-9.703 kcal/mol) and Compound 199 (-9.471 kcal/mol), surpassing the HSP90AA1 native ligand. Similarly, TNF-α binding analysis revealed potent interactions, with Compound 20 (-4.483) and Compound 74 (-4.444) exhibiting superior affinity compared to the native ligand. In conclusion, these findings suggest that *Vitex cofassus* ethyl acetate fraction contains bioactive compounds with multi-target cardiovascular protective effects, supporting its potential as a therapeutic candidate for cardiovascular diseases.

Keywords: cardiovascular, network pharmacology, src, Vitex cofassus

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Abstrak

Penyakit kardiovaskular (CVD) merupakan penyebab utama kematian di seluruh dunia, dengan prevalensi yang terus meningkat setiap tahunnya. Kompleksitas patofisiologi CVD yang melibatkan berbagai jalur biologis memerlukan pendekatan terapi multitarget. Penelitian ini bertujuan untuk mengevaluasi aktivitas antikardiovaskular fraksi etil asetat daun Vitex cofassus melalui pendekatan fitokimia, farmakologi jaringan, dan molecular docking. Fraksi etil asetat daun Vitex cofassus dianalisis menggunakan liquid menggunakan LC-HRMS sehingga teridentifikasi 232 senyawa, di mana 104 senyawa di antaranya memenuhi kriteria sifat mirip obat. Analisis jejaring farmakologi menemukan 274 target kardiovaskular potensial. Analisis Gene Ontology (GO) menemukan proses biologis terkait Vitex cofassus di antaranya respon terhadap senyawa kimia, sedangkan analisis komponen seluler mengindikasikan bahwa target gen terutama berada di struktur membran sel. Analisis fungsi molekuler terkait dengan aktivitas pengikatan enzim, sedangkan analisis jalur KEGG menunjukkan keterkaitan Vitex cofassus dengan jalur kardiovaskular termasuk interaksi ligan reseptor neuroaktif. Hasil analisis jejaring farmakologi mengidentifikasi protein SRC, HSP90AA1 dan TNF-α sebagai target potensial yang berhubungan dengan penyakit kardiovaskular. Hasil penambatan molekul menunjukkan bahwa senyawa-senyawa terpilih memiliki afinitas ikatan yang kuat terhadap HSP90AA1, terutama senyawa 74 (-9,703 kcal/mol) dan senyawa 199 (-9,471 kcal/mol), yang bahkan melampaui ligan alami HSP90AA1. Demikian pula, analisis ikatan terhadap TNF-α menunjukkan interaksi yang kuat, di mana senyawa 20 (-4,483 kcal/mol) dan senyawa 74 (-4,444 kcal/mol) memiliki afinitas yang lebih baik dibandingkan ligan alaminya. Kesimpulannya, temuan ini menunjukkan bahwa fraksi etil asetat dari Vitex cofassus mengandung senyawa bioaktif dengan efek perlindungan kardiovaskular multi-target, sehingga mendukung potensinya sebagai kandidat terapeutik untuk penyakit kardiovaskular.

Kata Kunci: *farmakologi jejaring, kardiovaskular, src, Vitex cofassus*

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Highlight:

- The study successfully identified over a hundred drug-like candidate compounds from *Vitex cofassus* leaves that target hundreds of cardiovascular-related proteins.
- Through network pharmacology and KEGG pathway analyses, the plant extract was shown to work via a multi-target mechanism to provide cardioprotective effects, primarily by modulating biological processes such as responses to chemical substances/oxygen and suppressing inflammatory and atherosclerosis pathways.
- Molecular docking analysis confirmed that several key bioactive compounds (such as Compound 74 and Compound 20) demonstrated better binding affinity toward key target proteins (HSP90AA1 and TNF-a) than their natural ligands.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality and disability worldwide, accounting for approximately 19 million deaths annually and 32.9% of deaths from non-communicable diseases (Qureshi et al., 2025; Jurado et al., 2025; Goh et al., 2025; Tsao et al., 2022). Globally, CVD-related deaths increased by nearly 19% between 2010 and 2020, with the highest mortality rates reported in Eastern Europe, Central Asia, the Middle East, and parts of Africa. Most new CVD cases occur in individuals aged 50 years and older (Meeks and Agyemang, 2025). In response to this growing burden, the United Nations has targeted a 25% reduction in premature CVD mortality through improved prevention and early diagnosis strategies.

Cardiovascular disease (CVD) encompasses a group of disorders affecting the heart and blood vessels, including coronary artery disease, stroke, heart failure, and peripheral artery disease (Qureshi et al., 2025). These conditions are commonly associated with multiple risk factors, such as hypertension, elevated LDL cholesterol, diabetes, obesity, smoking, physical inactivity, impaired kidney function, atherosclerosis, and genetic predisposition to heart disease (Parsa et al., 2025; Goh et al., 2025).

Due to the shortcomings of conventional approaches in delivering precision medicine for CVDs, there is a growing need for innovative strategies that can provide deeper insight into the complex regulatory processes involved, ultimately improving the ability to predict and understand disease progression. Among the most cost-effective strategies to address these metabolic risk factors are pharmaceutical treatments.

Ethyl acetate is a semi-polar solvent known to effectively extract bioactive secondary metabolites, particularly flavonoids, phenolic compounds, and terpenoids, which possess strong antioxidant and anti-inflammatory activities associated with cardiovascular protection (Jiang et al., 2015). Previous studies have demonstrated that ethyl acetate fractions generally exhibit higher phenolic and flavonoid contents compared to other solvent fractions, contributing to their significant antioxidant, anti-inflammatory, and cardioprotective properties. Oxidative stress and inflammation are major contributors to the development of cardiovascular disease. Therefore, the ethyl acetate fraction was considered a promising source of bioactive compounds for cardiovascular therapeutic investigation. The genus Vitex comprises medicinal plants with recognized beneficial effects on human health such as antimalarial, antioxidant, and antibacterial activities (Rasamison et al., 2009). Prasad et al. (2017) reported cardioprotective effect of *Vitex negundo*.

This study investigated the ethyl acetate fraction of *Vitex cofassus* for its potential as a cardiovascular protective agent. However, little is known about the molecular mechanisms underlying the cardioprotective effects of *Vitex cofassus*, which this study aims to elucidate. Leveraging network pharmacology and liquid chromatography-high resolution mass spectrometry (LC-HRMS) data, we sought to uncover how its bioactive compounds interact with multiple cardiovascular targets and pathways. Furthermore, molecular docking analysis provided deeper insights into the binding mechanisms between key compounds and specific receptor targets.

METHODS

Vitex cofassus leaves were gathered in June 2024, from the Forest Management Unit (FMU) Gantara in Liwu Metingki Village, Muna District, Southeast Sulawesi,

Indonesia. The species was authenticated by the Pharmacy Laboratory of Universitas Halu Oleo, Indonesia. The freshly harvested leaves were cleaned, thinly sliced, and air-dried to produce dried simplicia. The dried material was then subjected to methanol maceration, followed by solvent evaporation to yield a crude extract. Subsequent fractionation with ethyl acetate and purification via column chromatography was performed. Finally, the ethyl acetate fraction was analyzed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS) to characterize its chemical constituents (Zubair et al., 2021, Arba et al., 2025).

Chromatographic analysis was conducted using a Thermo Scientific™ Vanquish™ UHPLC Binary Pump system interfaced with a Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer for comprehensive metabolic profiling. Separation was achieved on a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm ID, 2.6 µm particle size), with analytical conditions consistent with established methodologies (Windarsih et al., 2022).

The LC-HRMS-identified compounds were assessed for drug-like properties via the SwissADME web tool (http://www.swissadme.ch/). Initial screening applied Lipinski's Rule of Five criteria: molecular mass (≤500 Da), hydrogen bond donors (≤5), hydrogen bond acceptors (≤10), and LogP (≤5). Further selection required compounds to demonstrate a bioavailability score >0.55 and high gastrointestinal absorption predicted by SwissADME. Substances failing more than one Lipinski criterion, showing bioavailability scores below 0.55, or exhibiting poor intestinal absorption were eliminated from subsequent investigation.

Potential biological targets of the ethyl acetate fraction from *Vitex cofassus* were predicted using SwissTargetPrediction and SEA (Similarity Ensemble Approach) databases. SwissTargetPrediction was selected because of its reliable target prediction performance based on chemical similarity analysis and its extensive validated bioactivity database. Only Homo sapiens targets with probability scores >0 were included for further analysis (Keiser et al., 2007). Cardiovascular-related genes were retrieved from OMIM (https://www.omim.org) and GeneCards (https://www.genecards.org) databases (Rebhan et al., 1998; Amberger et al., 2015; Arba et al., 2025). With focus on the top 500 most relevant targets (Jiang et al., 2021). Common targets between the predicted compound targets and cardiovascular-related targets were identified and visualized through a Venn diagram created using the Bioinformatics and Systems Biology online platform (https://bioinformatics.psb.ugent.be/webtools/Venn).

The protein-protein interaction (PPI) network was generated using the STRING database (https://string-db.org) with the common target proteins as queries. The analysis was conducted specifically for Homo sapiens, applying a stringent confidence cutoff score of 0.700. Nodes represented proteins, while edges represented protein–protein interactions. Hub genes were identified in Cytoscape based on degree, betweenness centrality, and closeness centrality analyses.The obtained PPI network was subsequently visualized and examined using Cytoscape software (version 3.10.2) (Shannon et al., 2003).

Gene Ontology (GO) enrichment analysis was conducted using Metascape (https://www.metascape.org) and shinyGO 0.80 to elucidate the biological relevance of the predicted targets, examining biological processes, cellular components, and molecular functions (Kanehisa et al., 2017; Zhou et al., 2019; Ge et al., 2020).

Statistical significance was determined using a *p-value* cutoff of <0.05, and multiple testing correction was conducted using the false discovery rate (FDR) method

to reduce false-positive results. Pathways and GO terms with adjusted FDR values <0.05 were considered significantly enriched. Furthermore, KEGG pathway analysis was employed to identify crucial metabolic and signaling pathways modulated by the bioactive compounds and target proteins linked to both *Vitex cofassus* and cardiovascular function. These pathways were interpreted as potential therapeutic mechanisms of action.

Molecular docking was performed using Maestro Schrödinger software (v11.1.012). Docking grids were generated based on the native ligand binding sites of each target protein. Docking validation was conducted by redocking the native ligand, and an RMSD value < 2.0 Å was considered acceptable. Molecular docking simulations were performed to evaluate the binding conformations and affinities of the identified compounds against three key receptor targets: sarcoma (SRC), heat shock protein 90 alpha family class A member 1 (HSP90AA1), and tumor necrosis factor-α (TNF-α). The three-dimensional crystal structures of these proteins were retrieved from the Protein Data Bank (https://www.rcsb.org).

The LC-HRMS-characterized compounds from *Vitex cofassus* ethyl acetate fraction were structurally optimized using the LigPrep module in Maestro (Schrödinger Suite), with energy minimization performed under the OPLS_2005 force field (Citra et al., 2025). Protein and ligand preparations followed established docking protocols (Arba et al., 2022) in Maestro Schrödinger software (v11.1.012, 2017-1 release) (Sastry et al., 2013). For each target protein, docking grids were defined based on the crystallographic coordinates of their respective native ligand binding sites (Vulpetti et al., 2024).

RESULTS AND DISCUSSIONS

Identification of bioactive compounds and target prediction

LC-HRMS analysis identified 232 bioactive compounds in the ethyl acetate fraction of *Vitex cofassus* (Supplementary Table S1). Initial screening using Lipinski's Rule of Five, combined with bioavailability score (>0.55) and gastrointestinal absorption criteria, excluded 128 compounds (including Compound 1, Compound 2, Compound 3, Compound 5, and so forth as shown in Supplementary Table S2), leaving 104 compounds for subsequent investigation.

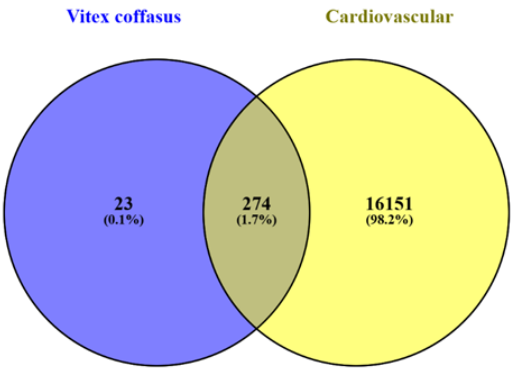


Figure 1. A Venn diagram showing shared targets between cardiovascular-related genes and compounds from *Vitex cofassus*. The yellow section represents cardiovascular genes, the blue section denotes *Vitex cofassus* targets, and the light yellow overlapping area highlights the common genes between both groups

Target prediction analysis yielded 300 and 204 potential protein targets from TargetNet and SEA databases, respectively. Comparative analysis with cardiovascular-related genes from OMIM (1,158 targets) and GeneCards (16,056 targets) databases identified 274 overlapping targets (Figure 1), which were designated as hub genes for further study.

Compound-target network analysis

The 274 potential targets were used to construct a network mapping the relationships between *Vitex cofassus* bioactive compounds and cardiovascular therapeutic targets. Protein-protein interaction (PPI) analysis was performed using the STRING database, with the resulting network visualized in Cytoscape. The final network comprised 270 nodes (representing target genes and their corresponding compounds) connected by 2,966 interaction edges (Figure 2).

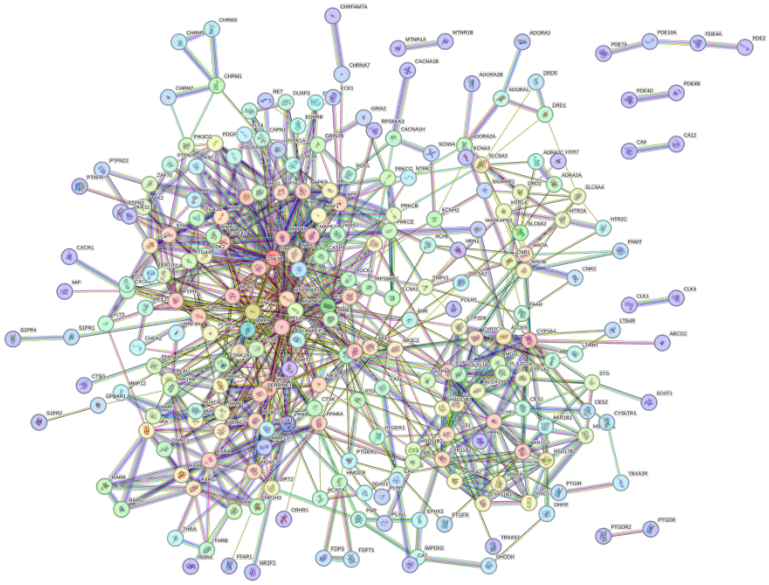


Figure 2. The protein-protein interaction (PPI) network, generated via STRING database analysis, consisted of 270 target proteins connected by 2,966 functional interactions

From this network, we identified the top 10 hub targets exhibiting the highest degree values, indicating their central regulatory roles in potential anti-cardiovascular mechanisms. Their network topological properties including degree, closeness centrality (node accessibility within the network), and betweenness centrality (bridging influence) are detailed in Table 1.

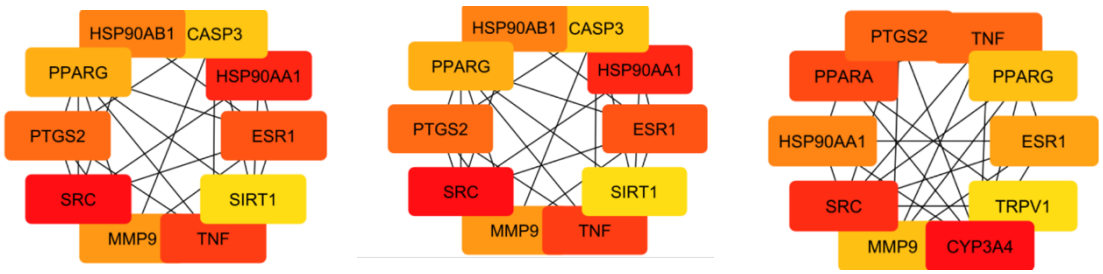
Table 1. Top 10 hub targets characterized by network centrality parameters: Degree: Number of direct protein interactions; Closeness centrality: Measure of node proximity to all others; Betweenness centrality: Indicator of a node's control over network information flow

Name	Degree	Closeness	Betweenness
Sarcoma (SRC)	42	0.4359464627151052	0.17058244993514118
Heat shock protein 90 alpha family class A	39	0.41081081081081083	0.08156490221643325

Name	Degree	Closeness	Betweenness
member 1 (HSP90AA1)			
Tumor necrosis factor-α (TNF-α)	33	0.4137931034482759	0.09284413725741601
Heat shock protein 90 alpha family class A member 1 (HSP90AA1)	29	0.3931034482758621	0.03841012576015561
Estrogen receptor (ESR1)	27	0.41834862385321103	0.09782415656097705
Prostaglandin endoperoxide synthase 2 (PTGS2)	26	0.4049733570159858	0.12413030108904753
Matrix metalloproteinase-9 (MMP9)	25	0.4021164021164021	0.058624272070281674
Caspase 3 (CASP3)	24	0.38841567291311757	0.04730096701988529
Peroxisome proliferator-activated receptor gamma (PPARG)	24	0.4	0.08082664924813539
sirtuin 1 (SIRT1)	23	0.38578680203045684	0.048330417885924105

Network analysis of key targets

The degree metric, representing the number of direct interactions between a target protein and other network components, serves as a key indicator of biological relevance - higher values denote greater functional significance. Our investigation revealed several high-degree targets, most notably sarcoma (SRC), heat shock protein 90 alpha family class A member 1 (HSP90AA1), tumor necrosis factor-α (TNF-α), heat shock protein 90 alpha family class B member 1 (HSP90AB1), estrogen receptor (ESR1), prostaglandin endoperoxide synthase 2 (PTGS2), matrix metalloproteinase-9 (MMP9), caspase 3 (CASP3), peroxisome proliferator-activated receptor gamma (PPARG), and sirtuin 1 (SIRT1), as principal hub targets. Network analysis employed two additional metrics: (1) closeness centrality, quantifying a node's average path length to all others, and (2) betweenness centrality, measuring a protein's bridging function in network communication (Zhou et al., 2016).



(a) (b) (c)

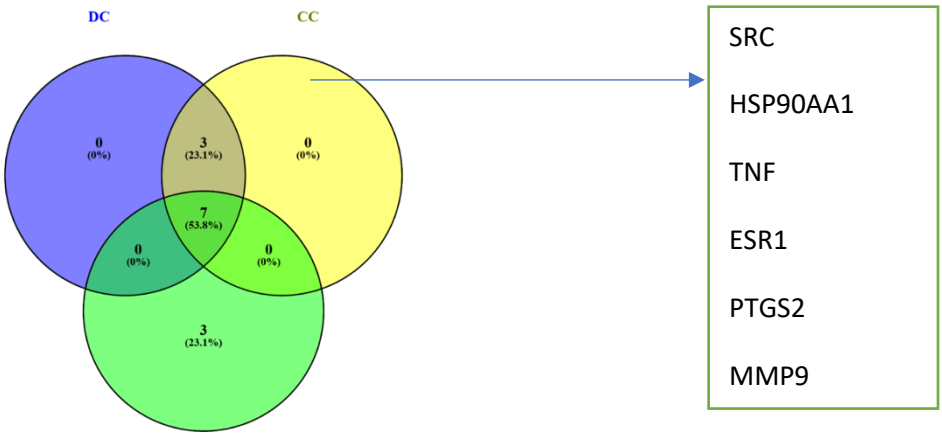


Figure 3. Network centrality analysis of potential target proteins: (a) degree centrality, (b) betweenness centrality, and (c) closeness centrality. Venn diagram integration (d) identified seven consensus therapeutic targets present across all centrality measures: SRC, HSP90AA1, TNF-*a*, ESR1, PTGS2, MMP9, and PPARG

Intersection analysis of the top 10 targets from each centrality measure revealed seven consensus proteins: SRC, HSP90AA1, TNF-*a*, ESR1, PTGS2, MMP9, and PPARG (Figure 3). These overlapping targets, visualized through Venn analysis (Figure 3d), represent the most promising candidates for therapeutic intervention, with panels a-c displaying the top-ranked proteins for each respective centrality measure.

Functional enrichment analysis of potential targets

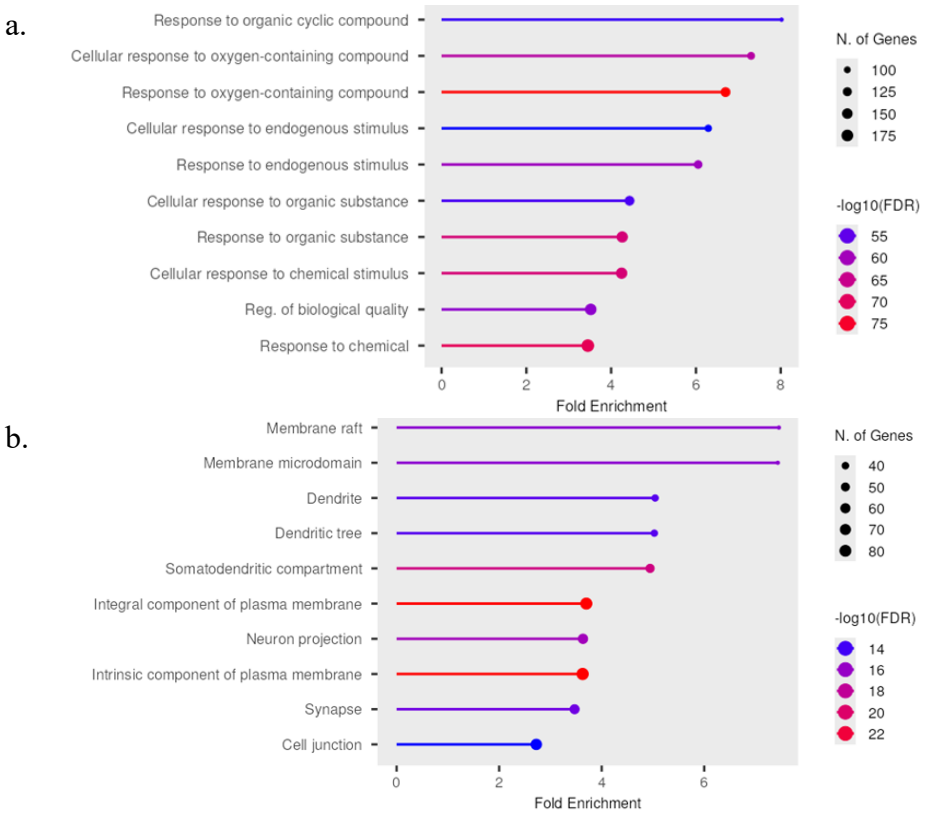
To elucidate the anti-cardiovascular mechanisms of the 274 overlapping targets, we conducted comprehensive functional enrichment analysis using Gene Ontology (GO) and KEGG pathway databases through ShinyGO 0.80. The analysis revealed that the GO terms consisted of 749 significantly enriched biological processes (BP), 88 cellular components (CC), and 247 molecular functions (MF).

The gene ontology (GO) biological process analysis identified the top ten enriched pathways, indicating that the target genes of the ethyl acetate fraction of *Vitex cofassus* were mainly linked to response to oxygen-containing compound, response to chemical, cellular response to chemical stimulus, response to organic substance, cellular response to oxygen-containing compound, response to endogenous stimulus, regulation of biological quality, cellular response to organic substance, response to organic cyclic compound, and cellular response to endogenous stimulus.

The GO cellular component analysis revealed that the target genes were predominantly localized to the intrinsic component of plasma membrane, somatodendritic compartment, neuron projection, membrane microdomain, membrane raft, synapse, dendritic tree, dendrite, and cell junction. The GO molecular function analysis showed significant enrichment in signaling receptor activity, molecular transducer activity, nuclear receptor activity, ligand-activated transcription factor activity, transition metal ion binding, transmembrane signaling receptor activity, g protein-coupled receptor activity, cation binding, zinc ion binding, and protein serine/threonine/tyrosine kinase activity.

KEGG pathway analysis revealed multiple cardiovascular-related signaling pathways linked to the therapeutic effects of *Vitex cofassus* ethyl acetate fraction, such

as neuroactive ligand-receptor interaction, pathways in cancer, calcium signaling pathway, CAMP signaling pathway, metabolic pathways, sphingolipid signaling pathway, serotonergic synapse, microRNAs in cancer, lipid and atherosclerosis, steroid hormone biosynthesis, and so forth. Figure 4 displays a bubble map highlighting the most significant GO terms and KEGG pathways. Based on KEGG pathway analysis, SRC, TNF-*a*, and HSP90AA1 are key targets in the atherosclerosis pathway that play roles in inflammation, oxidative stress, and plaque formation; therefore, their inhibition may provide potential cardioprotective effects. The development of cardiovascular diseases, particularly atherosclerosis, is a complex process involving the interaction of lipid accumulation, inflammatory responses, cell proliferation, and apoptotic mechanisms. The VLDLR and LOX-1 receptors play important roles in the early stages of atherogenesis through increased lipoprotein accumulation. Activation of LOX-1 induces oxidative stress and endothelial dysfunction, which trigger the initiation of atherosclerotic lesions (Libby et al., 2002). Oxidative stress and DNA damage activate the P53 protein, which subsequently induces protein expression. An imbalance among these pathways accelerates the progression of cardiovascular disease and increases the risk of acute events such as myocardial infarction and stroke. Therefore, proteins involved in these pathways have potential as therapeutic targets to prevent disease progression and cardiovascular complications.



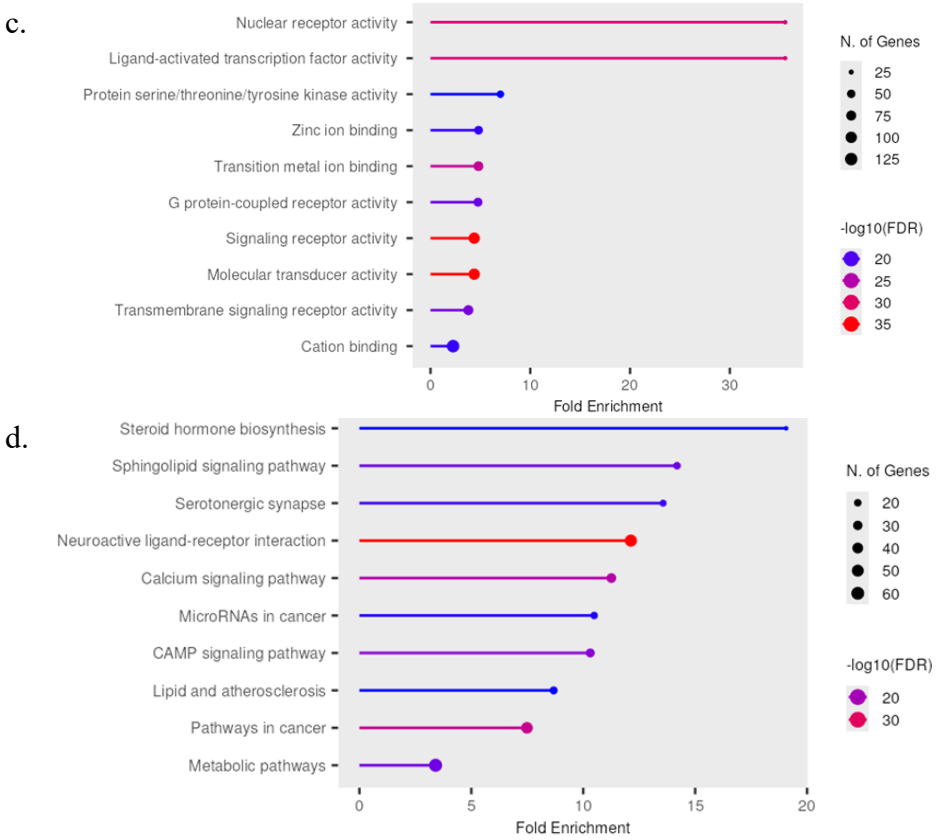


Figure 4. A detailed bubble map evaluating the bioactive compounds from the ethyl acetate fraction of *Vitex cofassus* leaves across: (a) Gene Ontology (GO) biological processes, (b) GO cellular components, (c) GO molecular functions, and (d) KEGG pathways

In conclusion, GO enrichment analysis indicates that the target genes of the ethyl acetate fraction of *Vitex cofassus* are mainly involved in cellular responses to chemical and oxygen-related stimuli, with prominent localization in neuronal and membrane-related structures and functions associated with signal transduction, receptor activity, and kinase regulation. In addition, KEGG pathway analysis highlights their involvement in several cardiovascular and disease-related signaling pathways, including lipid and atherosclerosis, calcium signaling, and neuroactive ligand–receptor interaction. Overall, these findings suggest that the ethyl acetate fraction of *Vitex cofassus* may exert therapeutic effects through multi-target modulation of key biological processes and signaling pathways relevant to cardiovascular diseases. Based on KEGG pathway analysis, SRC, TNF-*a*, and HSP90AA1 are key targets in the atherosclerosis pathway that play roles in inflammation, oxidative stress, and plaque formation; therefore, their inhibition may provide potential cardioprotective effects. The development of cardiovascular diseases, particularly atherosclerosis, is a complex process involving the interaction of lipid accumulation, inflammatory responses, cell proliferation, and apoptotic mechanisms.

Molecular docking analysis of key targets

Using an integrated network approach (combining compound-target, protein-protein interaction, and compound-target-disease analyses), we identified SRC,

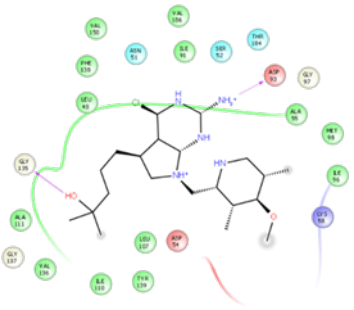
HSP90AA1, and TNF-*a* as the highest-priority proteins for molecular docking. The selected compounds for docking were as follows SRC: 1 compound (Compound 216), HSP90AA1: 11 compounds (Compound 4, Compound 6, Compound 29, Compound 31, Compound 74, Compound 87, Compound 123, Compound 131, Compound 133, Compound 199, Compound 225), and TNF-*a*: 7 compounds (Compound 4, Compound 19, Compound 20, Compound 24, Compound 74, Compound 147, Compound 173).

Further, binding affinity results (Table 2) revealed that HSP90AA1-ligand complexes had binding energies ranging from -5.855 kcal/mol (Compound 131) to -9.703 kcal/mol (Compound 74), outperforming its native ligand (-7.244 kcal/mol). TNF-*a*-ligand complexes exhibited binding energies between -3.128 kcal/mol (Compound 19) and -4.483 kcal/mol (Compound 20), compared to its native ligand (-3.647 kcal/mol). The SRC complex (Compound 216) showed weaker binding (-5.024 kcal/mol) than its native ligand (-7.694 kcal/mol).

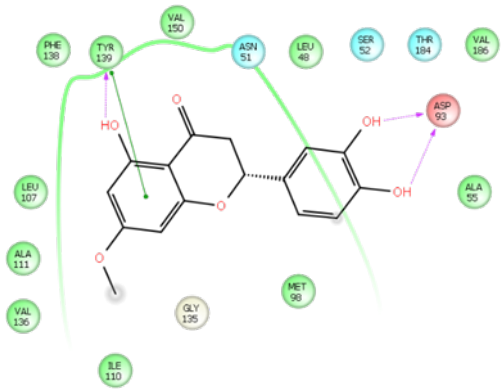
Table 2. Computed binding energies (kcal/mol) for molecular docking of bioactive compounds against key targets (SRC, HSP90AA1, TNF-*a*)

Compound	Binding energy (kcal/mol)		
	SRC (PDB ID :2SRC)	HSP90AA1 (PDB ID :7S9H)	TNF-*a* (PDB ID :2AZ5)
Native ligand	-7.694	-7.244	-3.647
Compound 4		-8.847	-4.115
Compound 6		-9.020	
Compound 19			-3.128
Compound 20			-4.483
Compound 24			-4.004
Compound 74		-9.703	-4.444
Compound 87		-8.395	
Compound 123		-6.387	
Compound 131		-5.855	
Compound 133		-7.874	
Compound 147			-3.976
Compound 199		-9.471	
Compound 216	-5.024		

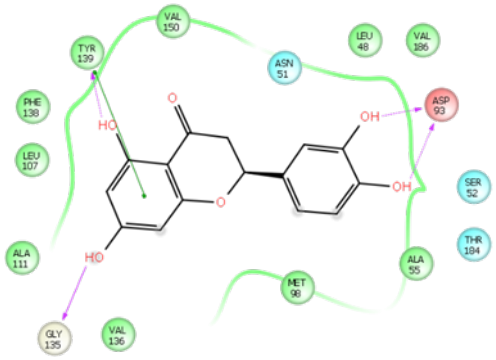
The molecular structures and binding conformations of HSP90AA1-docked compounds are depicted in Figure 5 Binding affinity analysis demonstrated particularly strong interactions for Compound 74 (-9.703 kcal/mol) and Compound 199 (-9.471 kcal/mol), followed by Compound 6 (-9.020 kcal/mol), Compound 4 (-8.847 kcal/mol), Compound 87 (-8.395 kcal/mol), and Compound 133 (-7.874 kcal/mol). Moderately weaker binding was observed for Compound 123 (-6.387 kcal/mol), and Compound 131 (-5.855 kcal/mol). The binding energy results indicate that the compound exhibits better binding affinity compared to the native ligand of the HSP90AA1 protein. This suggests that lower binding energy values correspond to more stable interactions and, consequently, stronger binding affinity (Issa et al., 2019).



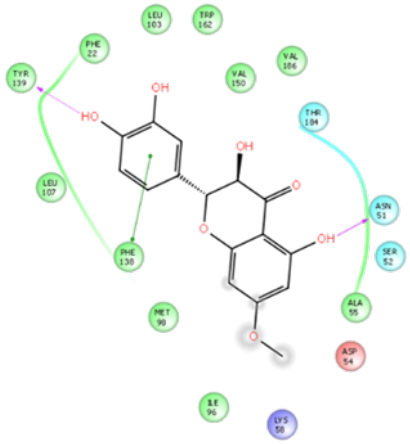
Native ligand: -7.244 kcal/mol



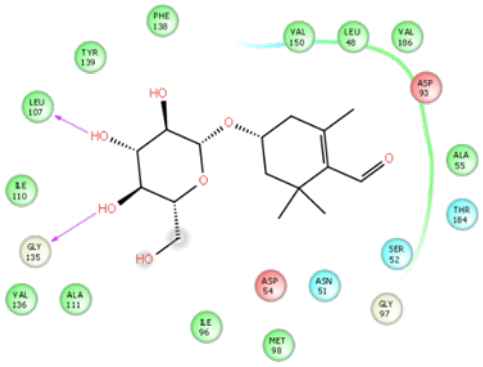
Compounds 4: -8.847 kcal/mol



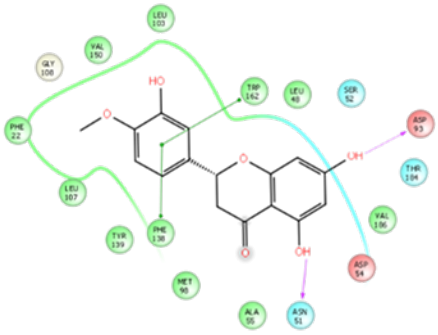
Compound 6: -9.020 kcal/mol



Compound 74: -9.703 kcal/mol
kcal/mol



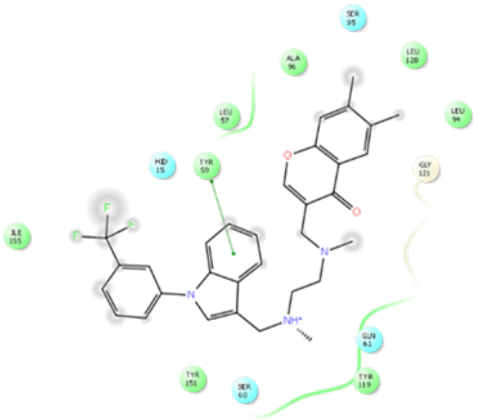
Compound 87: -8.395 kcal/mol



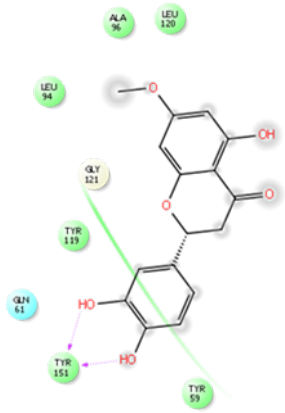
Compound 199: -9.471 kcal/mol
kcal/mol

Figure 5. Optimal binding poses of selected compounds with HSP90AA1, displaying the following binding affinities: compound 4 (-8.847 kcal/mol), Compound 6 (-9.020 kcal/mol), compound 74 (-9.703 kcal/mol), compound 87 (-8.395 kcal/mol), and compound 199 (-9.471 kcal/mol)

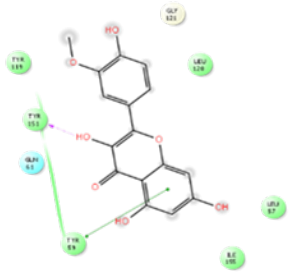
Figures 6 illustrate the binding conformations of compounds docked to TNF-*a*. Binding affinity analysis showed favorable interactions for several compounds, with Compound 20 exhibiting the strongest binding (-4.483 kcal/mol), followed by Compound 74 (-4.444 kcal/mol), Compound 4 (-4.115 kcal/mol), Compound 24 (-4.004 kcal/mol), and Compound 147 (-3.976 kcal/mol). Additionally, docking studies with SRC revealed that Compound 216 had a binding energy of -5.024 kcal/mol (Table S3).



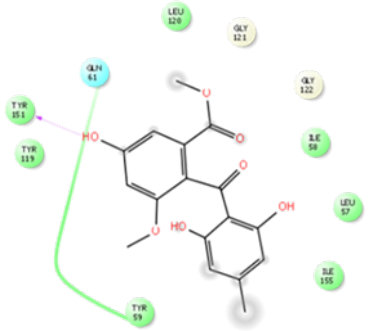
Native ligand: -4.059 kcal/mol



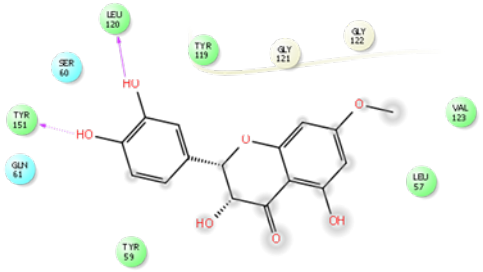
Compound 4: -4.115 kcal/mol



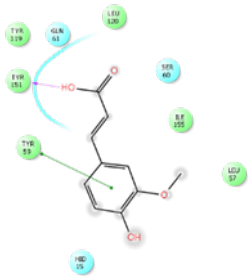
Compound 20 (-4.483 kcal/mol)



Compound 24 (-4.004 kcal/mol),



Compound 74 (-4.444 kcal/mol)



Compound 147 (-3.976 kcal/mol).

Figure 6. The most stable binding conformations of selected compounds with TNF-α and their corresponding binding energies: compound 4 (-4.115 kcal/mol), compound 20 (-4.483 kcal/mol), compound 24 (-4.004 kcal/mol), compound 74 (-4.444 kcal/mol), and compound 147 (-3.976 kcal/mol)

CONCLUSIONS

This study demonstrates the ethyl acetate fraction of *Vitex cofassus* leaves possesses significant potential as a source of multi-target cardiovascular therapeutics. Through an integrated phytochemical and network pharmacology approach, we identified 104 drug-like compounds in its ethyl acetate fraction, with 274 putative cardiovascular-related targets. Key proteins, including SRC, HSP90AA1, TNF-α, ESR1, PTGS2, MMP9, and PPARG, were identified as central mediators of its pharmacological effects. Functional enrichment analyses revealed critical roles in oxidative stress response, membrane signaling, nuclear receptor regulation, and cardiovascular-relevant pathways such as calcium signaling, lipid metabolism, and atherosclerosis. Notably, molecular docking confirmed strong interactions between bioactive Compound 74-HSP90AA1 (-9.703) and Compound 20-TNF-*a* (-4.483), with binding affinities exceeding those of native ligands. These findings not only validate the traditional use of *V. cofassus* but also provide a scientific foundation for its development as a multi-target phytotherapeutic agent against cardiovascular diseases. Further *in vitro* and *in vivo* studies are warranted to explore its efficacy and safety for clinical applications.

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