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Network pharmacology and machine learning reveal multi-target mechanisms of poly herbal formulation against atherosclerosis

Muhammad Shafique[1], Muhammad Umer Ghori[2], Mubashir Hassan[2], Usman Ali Ashfaq[2]* and Muhammad Shareef Masoud[2]*

[1]Institute of Microbiology, New Campus Jhang Road, Government College University, Faisalabad, Pakistan
[2]Department of Bioinformatics and Biotechnology, Allama Iqbal Road, Government College University, Faisalabad, Pakistan

Abstract: **Background**: Herbs, like *Allium sativum*, *Ginkgo biloba* and *Nerium oleander* are traditional medicinal plants that have been used to treat atherosclerosis and cardiovascular disease. This study provides valuable insights into how network pharmacology (NP) and emerging machine learning are utilized to identify potential drug candidates from these plants for treatment of atherosclerosis. **Methods**: NP analysis was employed to screen compounds and their potential gene targets from databases and tools e.g. IMPPAT, PubChem, KNAPSACK, Swiss ADME, Swiss Target Prediction, Disgenet and GeneCard. Cytoscape 3.10.2 was employed to visually understand these networks. DAVID database was used for functional and enrichment analysis of the genes validated through molecular docking using PyRx and Discovery Studio. **Results**: Computational tools and bioinformatics approaches showed a few core compounds, such as Quercetin, Naringenin, Luteolin, Kaempferol, Apigenin, Daidzein, Luteolin-7-olate, Pinocembrin, Pregnenolone and Fisetin found to be effective against atherosclerosis. Pathway analysis revealed that mechanism of atherosclerosis development is directly associated with cholesterol metabolism, cellular senescence, Ras, NF-κB and PI3K-Akt signaling pathways. **Conclusion**: NP and molecular docking analysis suggested that screened compounds may inhibit progression of atherosclerosis by modulating key associated pathways. Hence, this machine learning aided NP study provides basis for understanding and recognizing the activity of these plants in treating atherosclerosis.

Keywords: Atherosclerosis; *Allium sativum*; Bioinformatics; *Ginkgo biloba*; Network pharmacology; *Nerium oleander*

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INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. According to the 2022 census, CVDs caused nearly 18 million deaths globally, more than double the second leading cause of death, which is cancer. Atherosclerosis, or coronary artery disease (CAD), is a very common form of CVD characterized by the buildup of triglycerides that cause inflammation in the arteries. This can lead to serious clinical conditions such as myocardial infarction (MI) and stroke. Despite recent advancements in medical science, a definitive drug and treatment plan for atherosclerosis is still unavailable. However, herbal plants have played a role in treating this disease, including *Nerium oleander*, *Ginkgo biloba* and *Allium sativum*, which have been studied for their significant effects on cardiovascular health, particularly in the context of atherosclerosis. These plants contain bioactive compounds called phytochemicals, which are known to have the potential to treat various medical disorders with minimal side effects. These herbs were selected for their diverse bioactive profiles such as sulfur compounds (Allium), cardiac glycosides (Nerium) and flavonol glycosides (Ginkgo), each targeting distinct atherogenic pathways. Notably, *Nerium oleander* remains scarcely explored in the context of atherosclerosis, representing a key novelty of this study. Molecular docking revealed strong binding affinities of key ligands ($\Delta G \leq -8.5$ kcal/mol), for VCAM-1, IL-6 and CD40, suggesting potent inhibitory potential comparable to or exceeding reference inhibitors. This is the first machine learning assisted network pharmacology study systematically investigating *Nerium oleander* for atherosclerosis, providing a data-driven framework that integrates target prediction, pathway enrichment and docking validation.

Nerium oleander L. is a small evergreen shrub that grows to a height of 2–5 meters and is found in various geographical and ecological regions (Hase *et al*., 2016). The effect of the leaf extract of *Nerium oleander* tested in rats against cardiovascular diseases, gave positive feedback (Al-Snafi., 2020). *Nerium oleander's* impact on cardiovascular health is mainly due to its cardiac glycosides, through which it influences heart function and its antioxidant and anti-inflammatory abilities (Pandey *et al*., 2024). Some studies indicate that plaque formation may be reduced by decreasing the proliferation of smooth muscle cells that constitute the blood vessels' wall and mediate apoptosis induction following the administration of certain doses of oleandrin (Jensen *et al*., 2023).

**Corresponding author:* e-mail: usmancemb@gmail.com; masoudshareef@gcuf.edu.pk
*These authors are equally contribute.

Nonetheless, some studies have suggested that there are advantages and disadvantages to using this plant as an alternative medicine in the Cardiovascular System (Dabravolski *et al*., 2023). Despite numerous reports of anti-inflammatory and lipid-lowering effects of these botanicals, the precise multi-target signaling networks they modulate remain insufficiently characterized, particularly for *Nerium oleander*, whose cardio-protective potential is overshadowed by its known toxicity. Network pharmacology, when combined with machine learning, offers a cost-effective and scalable approach to identify hub targets and pathways, complementing but not replacing traditional wet-lab assays, which are often labor-intensive and target-specific. Herbal therapies offer multi-component synergy and lower cost, their potential adverse effects such as *Nerium oleander*'s cardio-toxic glycosides necessitate rigorous computational and experimental evaluation to identify safe therapeutic windows.

Another medicinal plant *Ginkgo biloba*, also known as the Maidenhair tree, is native to East Asian countries and parts of India and Pakistan (Zheng *et al*., 2021). Extracts from leaves, roots, flowers and other parts have been used in traditional medicine for the treatment of vascular disease, neurological disorders, hemorrhage, inflammation, cancer and antioxidants. In traditional Chinese medicine, dried and crushed ginkgo leaves are used to treat a variety of health issues, including asthma, bronchitis, hearing loss, tuberculosis, cognitive impairment, stomach pain, skin conditions and anxiety (Tao *et al*., 2022). *Ginkgo biloba* exerts multiple pharmacological effects that are beneficial for cardiovascular health, particularly in the context of atherosclerosis (Li *et al*., 2020). Atherosclerosis is an important process in endothelial dysfunction. *Ginkgo biloba* has been found to boost nitric oxide production, thereby enhancing endothelial function and facilitating vasodilation, which in turn lowers arterial rigidity. It has been demonstrated that terpenoids, specifically ginkgolides, inhibit platelet-activating factor (PAF), thereby reducing platelet formation and thrombosis, which is a significant contributor to heart attacks and strokes in patients with cardiovascular disease (Tian *et al*., 2017).

In addition to these plants, *Allium sativum*, commonly known as garlic, is a native plant in Pakistan and India and is cropped commercially worldwide. Garlic has been renowned in traditional Pakistani medicine for its use in treating heart-related diseases. Medicines based on garlic, such as garlina, are also available in local markets of Pakistan, specifically given in the treatment of cardiovascular diseases and atherosclerosis (Tariq *et al*., 2021). Extensive therapeutic research has already been done worldwide on garlic and many phytochemicals of therapeutic importance have been found (Asgharpour *et al*., 2021). It has been proven that garlic reduces serum cholesterol levels with a bias to low-density lipoprotein (LDL) cholesterol, which is linked to atherosclerosis development in the body system (Sun *et al*., 2018). Numerous clinical and preclinical researches have studied the effects of garlic on cardiovascular health, centering on its probable impact on atherosclerosis (Sobenin *et al*., 2019). Other researchers found that it improves arterial stiffness and endothelial function which are essential in the management of atherosclerosis (Emamat *et al*., 2020). *In-vitro* investigations have revealed some of the biological mechanisms by which garlic works. An example is when LDL has been shown to be prevented from oxidation by garlic, which is one of the first steps involved in the development of atherosclerosis (Sobenin *et al*., 2019). Experimental studies have confirmed that garlic can downregulate the expression of genes linked to ferroptosis in atherosclerosis (AS), indicating its potential as a therapeutic agent through modulation of this cell death pathway. By targeting intra-plaque ferroptosis and lowering lipid peroxidation, garlic helps to slow the progression of atherosclerosis. These results shed new light on the pharmacological actions of garlic and support its role as a promising option for managing AS (Gao *et al*., 2024).

Although the cardioprotective and anti-atherosclerotic properties of these medicinal plants have already been reported, their underlying targeted genes and associated pathways remain unexplored. Keeping this in mind, a comprehensible study was needed to highlight potential gene targets and underpinning pathways. Therefore, in this research, three plants *Nerium oleander, Ginkgo biloba and Allium sativum* were included, which have been studied for their significant effects in the treatment of cardiovascular diseases, especially atherosclerosis.

The study demonstrates that by integrating network pharmacology and machine learning, ten candidate genes including COL1A1 and CCR7 were identified as potential therapeutic targets of QRHXF in atherosclerosis. Molecular docking revealed strong binding affinities between the formulation's key active compounds and these predicted targets. Transcriptome sequencing of aortic tissue from APOE mice confirmed COL1A1 and CCR7 as pivotal genes in the disease model. Furthermore, animal experiments and PCR validation showed that QRHXF not only reduced aortic plaque formation and improved lipid profiles in APOE mice, but also enhanced COL1A1 mRNA expression, supporting fibrosis within plaques and contributing to plaque stabilization (Zhou *et al*., 2024). A network pharmacology approach was applied to investigate the bioactive constituents and underlying mechanisms of PHE in the context of diabetes. The analysis revealed that its anti-diabetic effects are mediated through interactions between active compounds and shared target genes within the network. Notably, ATA emerged as a key bioactive compound, while TNF was identified as a central hub gene. *In-vitro* assays supported these findings, showing that the hub gene-associated signaling pathway also activates

MAPK and PI3K/Akt cascades, which may enhance insulin receptor auto-phosphorylation. Collectively, the results provide pharmacological evidence for the role of PHE in diabetes management and validate ATA as a principal component contributing to its therapeutic action, further supported by in vivo efficacy studies (Singh *et al*., 2024). This study provides valuable insights into how traditional NP, along with the emerging technology of machine learning, is utilized to identify potential drug candidates from G. biloba, *N. oleander and A. sativum* for the treatment of atherosclerosis. In this study, compounds from the aforementioned plants were analyzed using NP, molecular docking and a machine learning approach, with the aid of Random Forest models. (Singh *et al*., 2024).

MATERIALS AND METHODS

Retrieval and filtering of active constituents of G. biloba, N. oleander and A. sativum

The active biological compounds of G. biloba, *N. oleander and A. sativum* were retrieved from KNApSAcK (Nakamura *et al*., 2014), IMPPAT (Mohanraj *et al*., 2018) and PubChem taxonomic databases. The retrieved compounds were analyzed on three ADMET parameters, the Lipinski rule of five and bioavailability obtained from SwissADME (Bakchi *et al*., 2022) and drug-likeness (Collaborators *et al*., 2017) score obtained from MolSoft LLC (Ononamadu and Ibrahim, 2021). Lipinski's rule of five (Ro5) is a pre-clinical drug discovery strategy that uses five physiochemical parameters to predict if a chemical is likely to be poorly absorbed or impermeable, stating hydrogen bond acceptors no more than 10, hydrogen bond donors no more than 5, partition coefficient (MLOGP) < 5 and molecular weight < 500 Da (Chen *et al*., 2020). The phytochemicals that followed Ro5 and retained Bioavailability ≥ 30% and DL ≥ 0.18 were selected for further analysis as novel compounds.

Screening of target genes for bioactive compounds of G. biloba, N. oleander and A. sativum

Gene targets for bioactive compounds of plants were predicted through the Swiss Target Prediction database (Gfeller *et al*., 2014). Swiss Target Prediction is an online tool used to predict target genes for humans and vertebrates. Target genes were retrieved by submitting the SMILES of active compounds one by one and genes with a probability ≥ 0.7 were retained for further analysis. Homo sapiens was chosen as the target species.

For the retrieval of disease-related genes, the keyword "atherosclerosis" was used in GeneCards (Safran *et al*., 2010) and DisGeNet (Pinero *et al*., 2015) databases. Duplicated entries of target genes were removed and common target genes were found between compound-related and disease-related genes using a Venn diagram tool, Venny 2.0.2.

Functional annotation and pathway analysis

Functional annotation and pathway analysis were done using the DAVID database (Huang *et al*., 2007) an open-access database for functional enrichment analysis was chosen to predict functions at three levels: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF).

Protein-protein interaction and network construction

The protein-protein interaction network of target genes was constructed using the online STRING database (Mering *et al*., 2003). The STRING database provides comprehensive information on both predicted and experimentally verified protein-protein interactions. It integrates multiple data sources to elucidate the functional connections between proteins in various organisms. Core regulatory genes were identified and analyzed as "hub genes" using the CytoHubba plugin. CytoHubba is a Cytoscape plugin designed to identify and rank hub nodes and essential nodes within biological networks. It offers several topological analysis methods, including Degree, MNC and MCC, to assess node importance based on their connectivity and network centrality. The top 10 hub genes were selected based on these three algorithms. Degree refers to the total number of interactions a node has with other nodes. MNC (Maximum Neighborhood Component) is a topological analysis method used to identify hub nodes by evaluating the size of a node's local neighborhood, emphasizing nodes with the most extensive local connections. MCC (Maximal Clique Centrality) is an algorithm that detects essential nodes by measuring their involvement in the largest cliques (complete subgraphs). Nodes participating in numerous large cliques are considered highly central and important in the network.

To explore the mechanism of the polyherb compounds on atherosclerosis, network analysis was performed. An open-access graphical user interface software, Cytoscape 3.10.2 was used to create and visualize networks (Shannon *et al*., 2003). Target genes and active constituents were presented by nodes, while interactions between them were presented by edges. To calculate the degree of compounds, target genes and pathways in networks, the cytohubba plugin was utilized. Genes with the maximum degree of connectivity, Maximum Neighborhood Component (MNC) and Maximal Clique Centrality (Tahir *et al*.) values were considered as key targets.

Compound-target prioritization ranking

A composite scoring framework was used to develop a hierarchical prioritization of compounds for their relevant targets based on their network centrality values, docking binding affinity and bioavailability (ADME) scores. All values were normalized to obtain a weighted Priority Score based on the formula:

Priority Score = 0.4 × Centrality_norm + 0.4 × (−ΔG)_norm + 0.2 × ADME_norm.

Molecular docking analysis

The molecular docking approach was used to validate hub genes. The X-ray crystallography-based structures of targets were obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/). The Protein Data Bank (PDB) is a global repository for 3D structural data of large biological molecules, including proteins and nucleic acids. The structural information about molecules in PDB comes from X-ray crystallography, cryo-electron microscopy and nuclear magnetic resonance (NMR) (Kouranov *et al*., 2006). Retrieved structures were then refined using Discovery Studio. Discovery Studio is a powerful software package designed specifically for protein visualization and refinement, featuring advanced tools for preparing and optimizing protein structures prior to molecular docking studies. Molecular docking was performed using PyRx software, employing virtual screening of core targets and active compounds. PyRx is an open-source software tool for small-molecule drug discovery, utilizing molecular docking and computational methods for drug discovery.

The docking complexes with low binding energy and low root mean square deviation (RMSD) were selected for further analysis. The docking score is an important criterion used to identify potential compounds and their corresponding targets. It also indicates how accurately and strongly a compound binds to its target protein. Moreover, Discovery Studio (Jejurikar and Rohane, 2021) was used to visualize 2D and 3D interactions of target proteins and active compounds. The workflow of this study is presented in fig. 1.

Machine learning (ML) based classification of compound bioavailability

A Random Forest model, trained using the scikit-learn Python library with 500 decision trees and a ‘balanced' class weight, was considered for evaluating compound bioavailability, taking into account hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), molecular weight (MW) and MLOGP as predictors. Performance was assessed based on a stratified 5-fold cross-validation. Accuracy, precision, recall (sensitivity), F1-score and AUC (area under the ROC curve) were determined. Feature importance was determined using Gini impurity reduction to evaluate the chemical properties that most affect prediction, thereby ranking each variable.

RESULTS

Using Swiss ADME and MolSoft, respectively, 187 active compounds were identified from a total of 2078 compounds based on the Lipinski rule of five and a Drug Likeness score of≥ 0.18, with duplicates removed. Lipinski's rule of five states is that to be a potential drug candidate, a compound should have a Hydrogen bond acceptor ≤10, Hydrogen bond donor ≤ 5, Molecular weight ≤ 500 Da, MlogP ≤ 5.0 and F ≥ 30%. MlogP value reflects how well a compound distributes between hydrophobic and hydrophilic environments, influencing its absorption, distribution, metabolism and excretion (ADME) properties in drug development. F stands for Bioavailability score, which indicates how well the compound is absorbed by the body. A higher bioavailability score means fewer doses of the drug can achieve higher results. These active constituents were subjected to the Swiss target prediction database and a total of 13962 gene targets were retrieved against them from which 518 targets were selected based on the probability score ≥ 0.7.

For the retrieval of disease-linked genes, the keyword "atherosclerosis" was used in Genecards and Disgnet Databases and a total of 7157 disease-targeted genes were identified. Both the compound targets and disease targets were submitted to a Venn diagram tool, Venny 2.0.2 (Fig. 2) and 66 common targets were identified, which could be potential gene targets for polyherb compounds for the treatment of atherosclerosis.

PPI network construction and hub gene identification

66 key targets were submitted to the STRING database to construct a protein-protein interaction network. “Homo sapiens" was selected for the organism. Nodes indicate individual proteins and the edges show the type of interaction between them. This network was further analyzed in Cytoscape with the help of the CytoHubba plugin. A total of 36 Compounds interacting with these 66 target genes were retrieved from the original dataset and a Compound-Target network was constructed, as shown in fig. 3. Based on three algorithms, Degree, MNC and MCC, the top 13 genes were identified as Hubgenes, namely SRC, KDR, MMP9, ESR1, MMP2, AKT1, CYP19A1, ABCG2, IGF1R, PTGS2, EGFR, CDK2 and GSK3B.

The 14 molecules interacting with these hub genes were selected as core key compounds, namely Daidzein, Flavanone, Pinocembrin, 6-Dehydroprogesterone, Naringenin, Luteolin-7-olate, Quercetin, Apigenin, Luteolin, Kaempferol, Genistein, Ethinyl Estradiol, Fisetin and Pregnenolone, whose structural properties are shown in table 1.

GO and KEGG pathway enrichment analysis

Functional enrichment analysis was carried out to identify significant biological processes, cellular components, molecular functions and pathways associated with the target genes. The results are categorized into three main GO domains: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). Genes involved in various biological processes, such as osteoblast differentiation, lipid transport and the G1/S transition of the mitotic cell cycle, have been identified. Enrichment in cellular components, such as the nucleus, nucleoplasm and plasma membrane, was observed. The analysis revealed significant molecular functions, including protein kinase activity, ATP binding and monooxygenase activity.

Table 1: Structural properties of core polyherbs active constituents.

Name	CID	Plant	DL	MW	HBA	HBD	MlogP	F
luteolin-7-olate	25201972	Ginkgo biloba	0.341766	285.23	6	3	-0.03	0.56
6-Dehydroprogesterone	101994	*Nerium oleander*	0.65085	312.45	2	0	3.86	0.55
Apigenin	5280443	Ginkgo biloba	0.393933	270.24	5	3	0.52	0.55
Daidzein	5281708	Ginkgo biloba	0.288052	254.24	4	2	1.08	0.55
Ethinyl Estradiol	5991	*Allium sativum*	1.04391	296.4	2	2	3.9	0.55
Fisetin	5281614	Ginkgo biloba	0.460209	286.24	6	4	-0.03	0.55
Flavanone	10251	Ginkgo biloba	0.5507	224.25	2	0	2.47	0.55
Genistein	5280961	*Allium sativum*	0.437872	270.24	5	3	0.52	0.55
Kaempferol	5280863	*Nerium oleander*	0.496631	286.24	6	4	-0.03	0.55
Luteolin	5280445	Ginkgo biloba	0.376108	286.24	6	4	-0.03	0.55
Naringenin	439246	Ginkgo biloba	0.820642	272.25	5	3	0.71	0.55
Pinocembrin	68071	*Allium sativum*	0.251479	256.25	4	2	1.27	0.55
Pregnenolone	8955	*Nerium oleander*	0.563603	316.48	2	1	4.05	0.55
Quercetin	5280343	*Nerium oleander*	0.516083	302.24	7	5	-0.56	0.55

Detailed results for BP, MF and CC of each target gene are given in table 1. Additionally, KEGG pathway enrichment analysis was performed to identify relevant metabolic and signaling pathways associated with atherosclerosis and general cardiovascular functions. The analysis revealed the significance of several pathways involving multiple hub target genes, including the Endocrine resistance pathway (6), EGFR tyrosine kinase inhibitor resistance (5), Cholesterol metabolism pathway (4), Diabetic cardiomyopathy (3) and NF-κB signaling pathway (5). Furthermore, the genes AKT1, SRC, EGFR and IGF1R were highly enriched and involved in several metabolic pathways, including EGFR tyrosine kinase inhibitor resistance, Endocrine resistance, Platinum drug resistance, etc. Detailed pathway analysis of all the hubgenes are given in table S1. The interaction between Compounds, hub genes and pathways is visually depicted in fig. 4.

Feature analysis of compounds using random forest model

The Random Forest model accurately classified the bioavailability of compounds using features like Plant, MW, HBA, HBD and MLOGP. The model achieved a 97% accuracy, with high precision and recall. Hydrogen Bond Acceptors (HBA) were the most influential feature, followed by Molecular Weight (MW); the values are given in table 3. The confusion matrix showed high true positive and true negative rates, indicating the model's reliability (Fig. 5). These results help prioritize compounds with improved bioavailability, thereby streamlining the drug discovery process.

The Random Forest model performed well, achieving 97% accuracy, 98% precision, 97% recall, a 0.975 F1-score and an AUC of 0.96 in 5-fold cross-validation. The confusion matrix reflected true positives of 467, true negatives of 138, false positives of 10 and false negatives of 9. The Hydrogen Bond Acceptors (44.40) were found to be the most significant predictor, as defined by the feature importance analysis. This was followed by Molecular Weight (22.13), Hydrogen Bond Donors (16.93) and MLOGP (15.07). These findings validate the reliability of the model and explain the contribution of each molecular property to the prediction of compounds' bioavailability.

Validation of hub genes and active compounds with molecular docking analysis

Through PPI network analysis, top 10 hub genes named AKT1, CDK2, EGFR, ESR1, ABCG2, CYP19A1, MMP9, PTGS2, GSK3B, IGF1R, KDR, IL2 and SRC were selected for the analysis of molecular docking with thirteen active compounds Apigenin, Luteolin-7-olate, Fisetin, 6-Dehydroprogesterone, Naringenin, Kaempferol, Luteolin, Quercetin and Daidzein, Pregnenolone, Flavanone and Pincembrine. The crystalize structures of target proteins (ABCG2 (PDB ID: 7NFD), CDK2 (PDB ID: 3F5X), AKTI (PDB ID: 6S9X), CYP19A1 (PDB ID: 5JL9), EGFR (PDB ID: 6JXT), GSK3B (PDB ID: 6TCU), SRC (PDB ID: 6E6E), IGF1R (PDB ID: 6JK8), KDR (PDB ID: 6XVK), PTGS2 (5IKV) and MMP2 (PDB ID: 3AYU) were obtained from PDB. Using Discovery Studio, the nonstandard amino acids and other atoms, including water molecules and hetatoms, were removed and the structure was refined. Moreover, energy minimization of the protein and the addition of polar hydrogens were also performed in Discovery Studio. The refined structures were then imported into PyRx for molecular docking analysis and virtual screening using a multiple ligand docking approach.

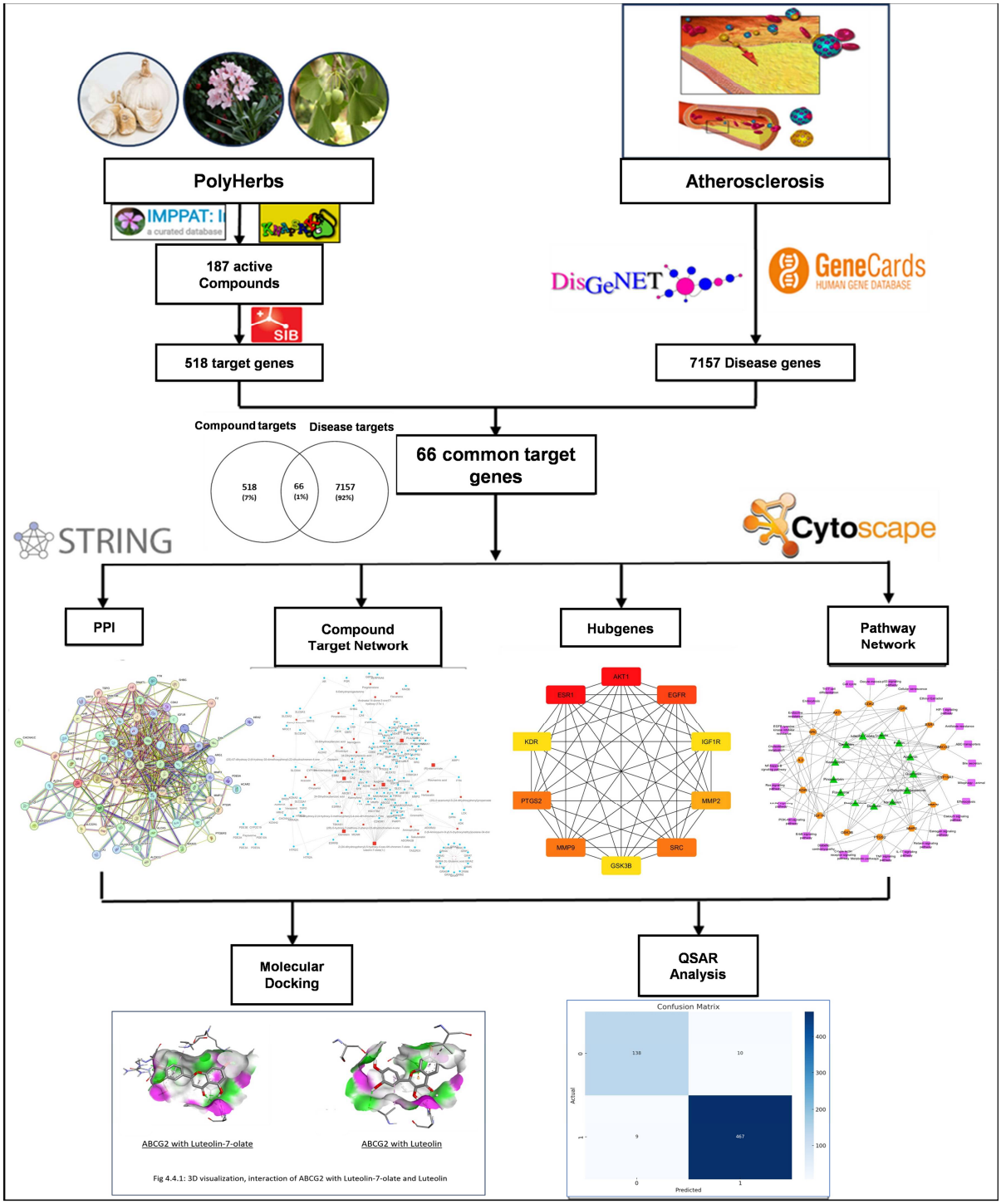

Fig. 1: Schematic diagram of network pharmacology with QSAR to explore polyherbal for the treatment of atherosclerosis

Table 2: Showing the significance of each feature in screening of active constituents

Sr. no.	Features	Percentage significance
1	HBA	44.40%
2	MW	22.13%
3	HBD	16.93%
4	MLOGP	15.07%

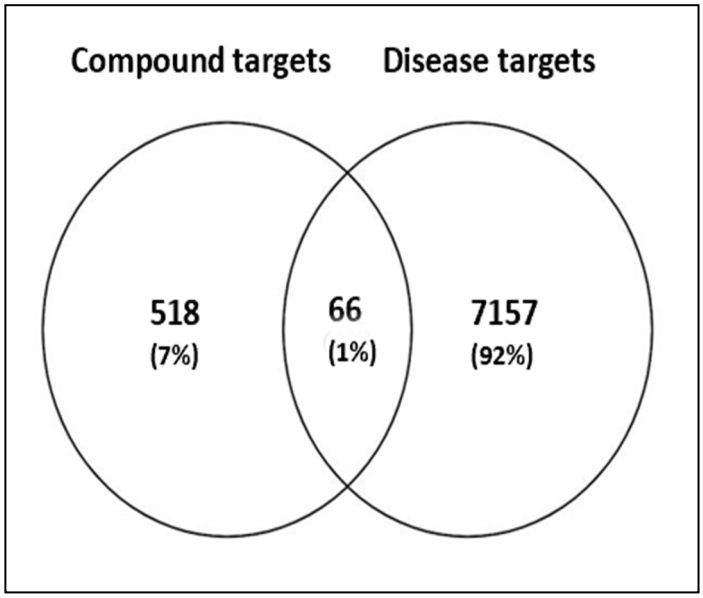

Fig. 2: Venn diagram showing number of genes targets linked with bioactive compounds and atherosclerosis

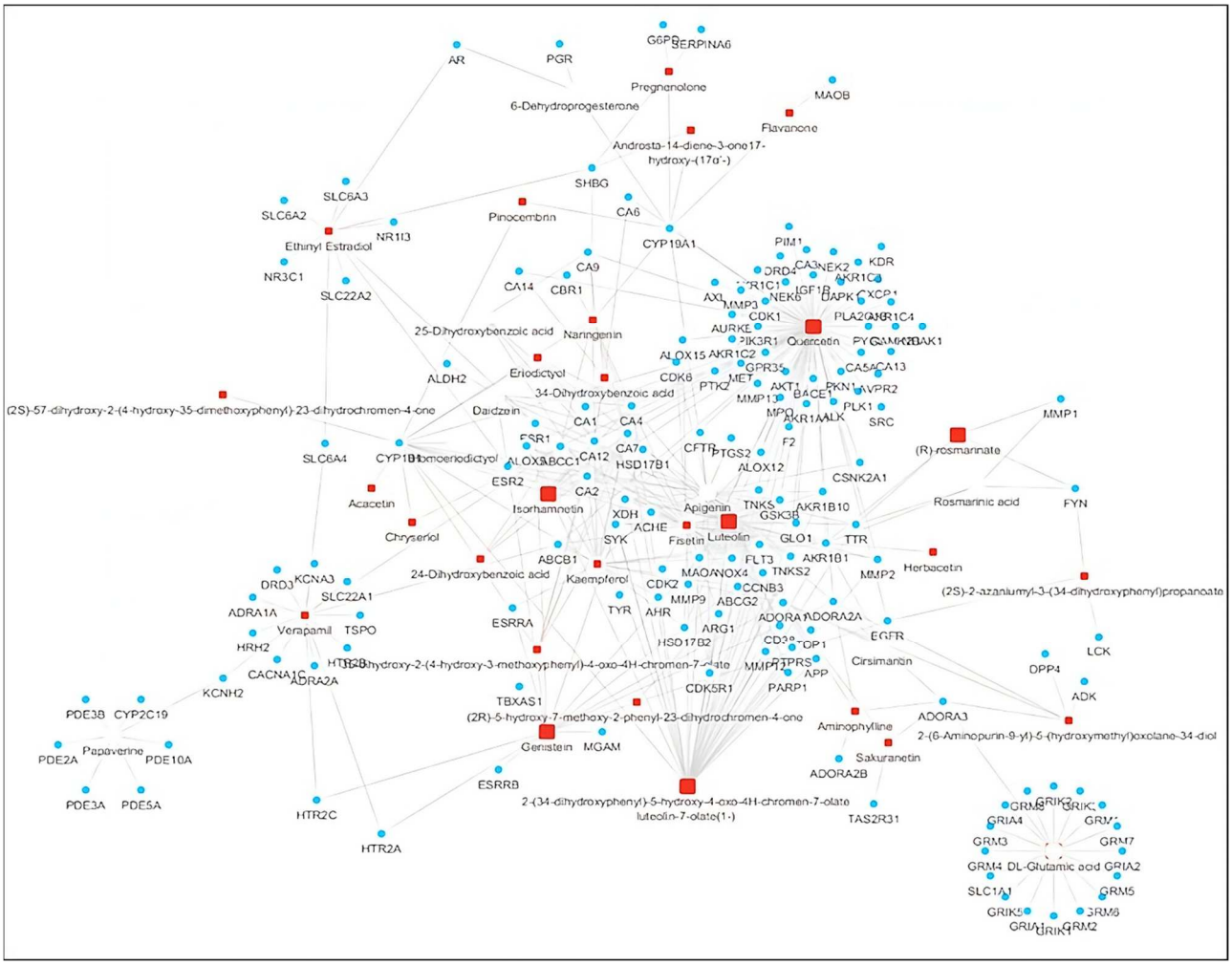

Fig. 3: Compound-target network of 66 gene targets and 36 compounds; red nodes – compounds, Blue nodes – genes

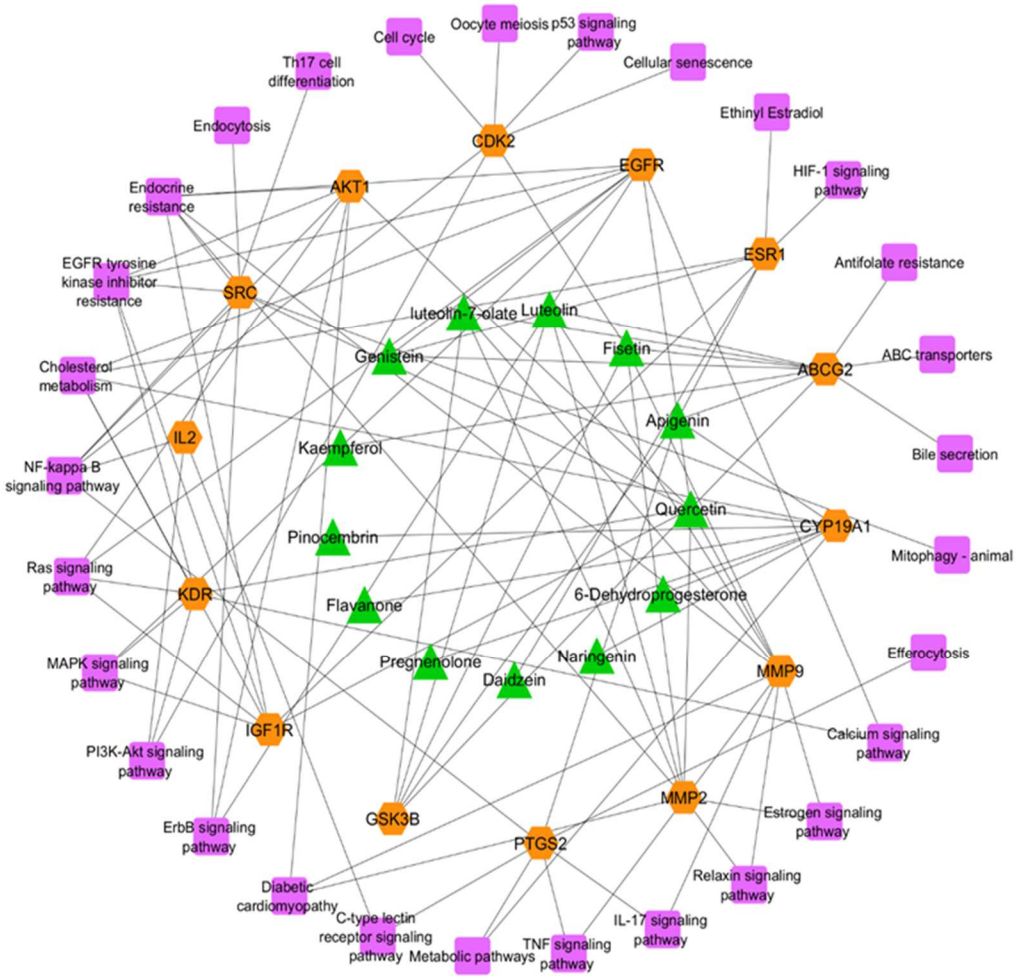

Fig. 4: Network illustrating the interaction between active constituents, hub genes, and metabolic pathways. Green nodes indicate active compounds, Orange nodes indicate Target genes and Purple nodes indicate pathways.

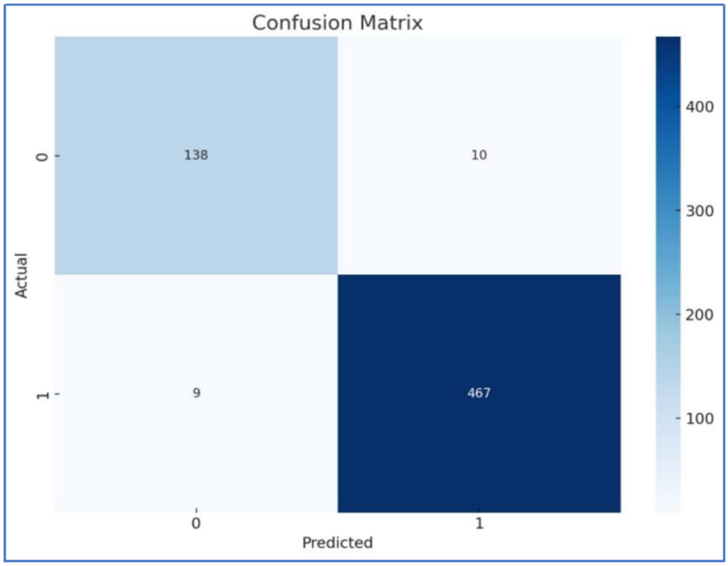

Fig. 5: Confusion Matrix generated by random forest model; 467 instances where the compounds were bioavailable, 138 instances where the compounds were not bioavailable, 10 instances were incorrectly identified as bioavailable, and 9 instances were incorrectly identified as not bioavailable.

Blind Docking was used. Proteins were docked against each ligand simultaneously and the RMSD values, binding affinity values, number of interacting residues and hydrogen bond numbers were retrieved. The analysis revealed that the highest binding affinity and the least RMSD of AKT1 is with Quercetin. The highest binding affinity and the least RMSD are observed with Luteolin and Luteolin-7-olate for ABCG2. The highest binding affinity and the least RMSD are observed with Quercetin for SRC. Details are shown in supplementary file S2. These results indicate that the interaction of these active constituents with target genes is significant in the treatment of atherosclerosis.

Ranking of compound-target pairs

The ranking based on priority score identified the best candidates, including Quercetin-EGFR (ΔG = -9.8 kcal/mol), Genistein-ABCG2 (ΔG = -9.7 kcal/mol) and Quercetin-SRC (ΔG = -9.7 kcal/mol), as having the highest potential for experimental validation. This priority will give a clear translational direction to subsequent *In-vitro* and *in vivo* studies.

DISCUSSION

The present research is dedicated to presenting an analysis of the research findings obtained, which involve a combination of network pharmacology and machine learning methods to identify potential treatments for atherosclerosis using *Allium sativum*, *Ginkgo biloba and Nerium oleander*. It highlights why these results are important in relation to existing scholarly work, as well as the research's weaknesses and recommendations for further investigation. Through network pharmacology analysis, 13 compounds, including Apigenin, Luteolin-7-olate, Fisetin, 6-Dehydroprogesterone, Naringenin, Kaempferol, Luteolin, Quercetin, Daidzein, Pregnenolone, Flavanone and Pinocembrin from the three plants were identified. These compounds might have potential therapeutic effects on 11 gene targets AKT1, CDK2, EGFR, ESR1, ABCG2, CYP19A1, MMP9, PTGS2, GSK3B, IGF1R, KDR, IL2 and SRC, which are part of biological processes and metabolic pathways, the disturbance of which can lead to atherosclerosis.

The machine learning models, specifically the random forest models, provided significant assistance in screening compounds by identifying key features necessary for a drug candidate. Molecular docking studies, in which these compounds were docked against the target proteins with 3D visualization, further validated the potential of these compounds by showing favorable binding affinities with the target proteins.

The results suggest that compounds from A. sativum, G. biloba and N. oleander have significant potential in modulating pathways involved in atherosclerosis. The GO and KEGG pathway enrichment analysis revealed that key compounds from A. sativum (Ethinyl Estradiol, Genistein and Pinocembrin), N. oleander (6-Dehydroprogesterone, Kaempferol, Pregnenolone, Quercetin) and G. biloba (Apigenin, Luteolin-7-olate, Fisetin, Naringenin, Luteolin, Daidzein, Flavanone) showed strong interactions with proteins involved in lipid metabolism, cholesterol metabolism, inflammation, endothelial functions and several important pathways like MAPK signaling, ErbB signaling, Ras signaling, NF-kappa B signaling and cellular senescence, which are directly involved in regulating atherosclerosis. Our findings align with previous studies that highlight the cardioprotective effects of these plants. Compounds such as quercetin and luteolin-7-olate demonstrated strong binding affinities with enzymes involved in lipid and cholesterol metabolism, like SRC and MMP2. This supports the idea that these compounds can help lower cholesterol levels, a major risk factor for atherosclerosis (Forteza and Ketelhuth, 2022). The KEGG pathway analysis indicated that these polyherbal-derived compounds also have anti-inflammatory properties, as they can interact with genes involved in inflammatory cytokine signaling pathways. For example, pinocembrin showed significant binding to IL-2, which is involved in the NF-κB signaling pathway, a key regulator of inflammation. Atherosclerotic inflammation is centrally mediated by the NF-κB pathway. When activated, this pathway enhances the production of various pro-inflammatory cytokines and adhesion molecules, thereby facilitating the recruitment of immune cells to arterial walls. The inflammatory response also contributes to the progression of atherosclerosis by promoting endothelial dysfunction, enhancing smooth muscle proliferation and increasing plaque instability (Kong *et al*., 2022). Therefore, it has been suggested that targeting this pathway might be an effective treatment approach for reducing atherosclerosis.

In other words, IL2 is involved in PI3K-Akt signal transduction molecularly together with CDK2 and KDR. There are various cellular processes, such as growth and survival, among others, that are interconnected in the PI3K-Akt signal transduction pathway. Within the context of atherosclerosis, this pathway regulates the survival and proliferation of macrophages and vascular smooth muscle cells within atheromatous plaques. If not well controlled, PI3K-Akt could cause an upregulation of inflammation, resulting in more unstable plaques. Therefore, stabilizing atherosclerotic lesions seems to represent one way of maintaining vascular health and avoiding its development as well as progression into atherosclerosis (Stroope *et al*., 2024). According to the research, fisetin, quercetin and kaempferol demonstrate elevated binding affinity for CDK2, KDR and IL-2, respectively, indicating that they may play a role in regulating the PI3K-Akt signaling pathway, which is associated with atherosclerosis. In addition to this, the significance of quercetin in the

regulation of this pathway is supported by the docking analysis results as all four proteins displayed significant binding with it. Another major pathway that plays a role in cell proliferation and survival is the Ras signaling pathway. The Ras signaling pathway also involves four hubgenes; AKT1, EGFR, IGF1R and KDR. Regarding atherosclerosis, activated Ras leads to increased inflammation, as well as vascular smooth muscle cell migration, thereby contributing to plaque formation/progression. Its intersectionality with other pathways, like NF-kappa B underlines its importance in the complex metabolic environment within atherosclerotic plaques (Kong *et al*., 2022). Moreover, one of the identified core target genes, CDK2, which exhibits a strong binding affinity with fisetin, is directly involved in cellular senescence, a state in which cells lose their ability to divide and function properly. In atherosclerosis, senescent cells accumulate in plaques and secrete pro-inflammatory factors, contributing to chronic inflammation and plaque instability. Targeting cellular senescence may offer a novel approach to reduce inflammation and promote plaque regression (Certo *et al*., 2024).

While network pharmacology provides valuable insights into the interactions between phytochemicals, protein targets and signaling pathways, its predictions are inherently system-level and not restricted to a particular cell type. The identified targets and pathways such as those regulating inflammation, lipid metabolism, endothelial function and oxidative stress are highly relevant to vascular biology and atherosclerosis progression. However, the results should be interpreted as hypothesis-generating rather than cell-specific conclusions, since network pharmacology does not distinguish whether a target is primarily expressed in endothelial cells, vascular smooth muscle cells, or macrophages within the atherosclerotic plaque. To improve specificity, future work should integrate cell-type specific transcriptomic or proteomic data (e.g., single-cell RNA sequencing of atherosclerotic lesions) and validate key targets experimentally in vascular cell models. This would strengthen the translational potential of our findings for predicting and preventing atherosclerosis.

Although this study identifies promising bioactive compounds with strong predicted target affinities, clinical viability must be critically considered. Several phytochemicals, including quercetin, exhibit poor oral bioavailability (<2%) and rapid metabolism, which may limit systemic efficacy. Similarly, *Nerium oleander* contains cardiotoxic glycosides that necessitate careful dose optimization and safety evaluation. To bridge this gap, future research should incorporate *In-vitro* ADME-Tox profiling, animal pharmacokinetic studies and formulation strategies (e.g., nanoparticles, liposomes, or co-crystals) to enhance absorption and therapeutic index. These steps will ensure that computationally prioritized compounds are both effective and safe for eventual clinical use.

Although our study yields promising results, several limitations require attention. The study primarily depends on in silico methods. Although these techniques are powerful, they require validation through *In-vitro* and in vivo studies to confirm the therapeutic potential of the identified compounds. Atherosclerosis is a complex disease characterized by multiple interacting factors, including genetic, environmental and lifestyle influences. Our focus was on specific pathways and targets, which may not cover the entire complexity of the disease. The success of machine learning models depends on the quality and size of the dataset.

While this study integrates machine learning and network pharmacology to predict multi-target mechanisms of *Allium sativum*, *Nerium oleander* and *Ginkgo biloba* against atherosclerosis, these findings remain in silico predictions and warrant experimental validation. Future work should involve targeted *In-vitro* assays such as endothelial cell adhesion and cytokine inhibition studies to verify VCAM-1, IL-6 and CD40 modulation, as well as macrophage-based assays to examine effects on lipid uptake and foam cell formation. *In-vivo* validation using atherosclerosis-prone animal models will be essential to confirm efficacy, optimize dosing and carefully evaluate potential cardiotoxic effects, particularly for *Nerium oleander* constituents. These steps will bridge computational predictions with biological reality, providing a robust framework for developing safe, multi-target herbal interventions for atherosclerosis.

CONCLUSION

Three traditional herbs were analyzed for their anti-atherosclerotic effects. Fourteen bioactive compounds interacted with thirteen hub genes involved in key pathways like endocrine resistance, cholesterol metabolism and NF-κB signaling. PPI and docking analyses highlighted AKT1, EGFR, ESR1, MMP9 and PTGS2 as major therapeutic targets. This study highlights the potential of compounds derived from Allium sativum, *Nerium oleander* and Ginkgo biloba in treating atherosclerosis by potentially modulating key pathways involved in lipid metabolism, inflammation and endothelial function. Despite some limitations, these findings lay a solid groundwork for further experimental validation and the development of new therapies for atherosclerosis.

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MUG (data collection/analysis, writing), MH (designing, writing), MSM (analysis / writing), MS (writing/ reviewing), UAA (study design, data acquisition/analysis, supervision, writing, revision and final approval).

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The underlying research material can be requested from the corresponding author on demand.

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No ethical approval is required in the current study.

Conflict of interest
Authors declare there is no conflict of interest of any type with regard to this study.

Supplementary data
https://www.pjps.pk/uploads/2026/03/SUP1774333155.pdf

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