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Original Article

Network pharmacology and molecular docking approach to identify the mechanisms and molecular targets of *Allium ascalonicum* L. extract against atherosclerosis

[Farmacología de redes y enfoque de acoplamiento molecular para identificar los mecanismos y objetivos moleculares del extracto de *Allium ascalonicum* L. contra la aterosclerosis]

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Abstract

Context: Atherosclerosis remains a leading cause of mortality globally, and current therapeutic strategies are often insufficient. *Allium ascalonicum* L. (shallot) has demonstrated cardioprotective effects, but its molecular mechanisms remain inadequately defined.

Aims: To elucidate the molecular mechanisms and potential targets of *A. ascalonicum* extract in the prevention and treatment of atherosclerosis.

Methods: A network pharmacology and molecular docking approach was employed to investigate the interactions between key bioactive compounds (kaempferol, quercetin, quercetin 3-O-alpha-L-rhamnoside, and allicin) and atherosclerosis-associated proteins.

Results: Target prediction identified 1,644 atherosclerosis-related proteins interacting with *A. ascalonicum* compounds. Among these, Nrf2 was highlighted as a primary target involved in lipid metabolism, oxidative stress, and inflammation. Molecular docking revealed strong binding affinities between Nrf2 regulators (KEAP1) and bioactive compounds, particularly quercetin 3-O-alpha-L-rhamnoside.

Conclusions: The findings suggest that *A. ascalonicum* may serve as a potential anti-atherosclerotic agent through modulation of the KEAP1-Nrf2 pathway. These results warrant further experimental validation.

Keywords: *Allium ascalonicum* L.; atherosclerosis; molecular docking; network pharmacology.

Resumen

Contexto: La aterosclerosis sigue siendo una de las principales causas de mortalidad a nivel mundial, y las estrategias terapéuticas actuales suelen ser insuficientes. *Allium ascalonicum* L. (chalota) ha demostrado efectos cardioprotectores, pero sus mecanismos moleculares aún no están completamente definidos.

Objetivos: Dilucidar los mecanismos moleculares y las posibles dianas del extracto de *A. ascalonicum* en la prevención y el tratamiento de la aterosclerosis.

Métodos: Se empleó un enfoque de farmacología de redes y acoplamiento molecular para investigar las interacciones entre compuestos bioactivos clave (kaempferol, quercetina, quercetina 3-O-alfa-L-ramnósido y alicina) y proteínas asociadas a la aterosclerosis.

Resultados: La predicción de dianas identificó 1644 proteínas relacionadas con la aterosclerosis que interactúan con los compuestos de *A. ascalonicum*. Entre ellas, Nrf2 se destacó como una diana principal implicada en el metabolismo lipídico, el estrés oxidativo y la inflamación. El acoplamiento molecular reveló fuertes afinidades de unión entre los reguladores de Nrf2 (KEAP1) y compuestos bioactivos, en particular la quercetina 3-O-alfa-L-ramnósido.

Conclusiones: Los hallazgos sugieren que *A. ascalonicum* podría actuar como un potencial agente anti-aterosclerótico mediante la modulación de la vía KEAP1-Nrf2. Estos resultados requieren una mayor validación experimental.

Palabras Clave: acoplamiento molecular; *Allium ascalonicum* L.; aterosclerosis; farmacología de redes.

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INTRODUCTION

Atherosclerosis is associated with high global morbidity and mortality. Globally, cardiovascular disease s the leading cause of death, with approximately 17.9 million deaths each year (Poznyak et al., 2020; Qiao et al., 2022). According to the 2018 Indonesian Basic Health Research (Riskesdas), the prevalence of cardiovascular disease in Indonesia reached 1.5%, up from 0.5% in 2013 (Riskesdas Kementrian Kesehatan RI, 2018). Rewrite for clarity: "Atherosclerosis prevalence is projected to increase globally due to persistent risk factors." Hyperlipidemia, inflammation, and oxidative stress are the primary risk factors contributing to the development of coronary arterial atherosclerosis, which can lead to coronary heart disease and other cardiovascular complications (de Mello Barros Pimentel et al., 2023).

The standard therapy for atherosclerosis using statins has not been sufficiently effective in preventing and treating it, because statins act only on antihyperlipidemic and anti-inflammatory pathways, not on antioxidant pathways, prompting the development of herbal remedies (Badacz et al., 2022). *Allium ascalonicum* L. is a widely used herbal plant for the prevention and treatment of various diseases (Elkanawati et al., 2025). *A. ascalonicum* variety Bima originates from Brebes, Indonesia, and has advantages over other types of onions, higher flavonoid and phenolic content, which act as powerful antioxidants. It also has smaller bulbs but is rich in bioactive compounds, making it more efficient in therapeutic applications (Nurcahyo et al., 2020). *A. ascalonicum* extract contains various bioactive compounds with therapeutic potential in managing hyperlipidemia, inflammation, and oxidative stress. Among these compounds, quercetin, kaempferol, and allicin are the primary focus due to their significant pharmacological properties. Quercetin is a flavonoid commonly found in *A. ascalonicum* and is known for its strong antioxidant activity. Quercetin exists in three forms: quercetin-3,4'-in-O-glucoside, quercetin-4'-O-glucoside (quercetin), and aglycone. Approximately 98% of the flavonoids in the ethanol extract of *A. ascalonicum* consist of quercetin-3,4'-di-O-glucoside and quercetin, while quercetin aglycone is less available in its natural form but can be formed through metabolic processes. Quercetin glycosides have potential as an anti-atherosclerotic agent but require enzymatic conversion for optimal activity. Quercetin aglycone exhibits better cellular penetration due to its lipophilic nature, allowing it to cross the enterocyte membrane via simple diffusion, thereby increasing its bioavailability (Adeyemo et al., 2022).

Many studies have shown that quercetin, a flavonoid abundant in *A. ascalonicum* extract, has demonstrated lipid-lowering and anti-inflammatory properties (Adeyemo et al., 2022; Tran et al., 2020). Its glycosidic and aglycone forms differ in bioavailability, with the latter offering better cellular penetration (Salehi et al., 2020). A study by Huwait et al. (2021) reported that quercetin did not cause cytotoxic effects at various concentrations tested in an *in vitro* study. Similarly, kaempferol and allicin contribute to antioxidative and antihypertensive effects, respectively (Yao et al., 2020). The combination of quercetin and kaempferol in *A. ascalonicum* extract may provide a stronger synergistic effect in addressing cardiovascular disease (Jan et al., 2022). Allicin has significant therapeutic effects, can lower hypertension, and improve heart health by reducing inflammation and oxidative stress. Allicin also has antimicrobial properties that often contribute to chronic inflammation (Gao et al., 2024; Iwar et al., 2024). However, the underlying molecular mechanisms through which these compounds act against atherosclerosis remain underexplored.

An *in silico* study is important for better understanding the molecular mechanisms of action of bioactive compounds in *A. ascalonicum* extract. These methods enable researchers to identify bioactive components, biological targets, and the pharmacological mechanisms of complex compounds in herbal formulations. Researchers identified interactions between bioactive compounds in *A. ascalonicum* extract and biological pathways involved in the reduction of hyperlipidemia, inflammation, and oxidative stress. Network pharmacology is well-suited to herbal extracts, as they contain many active compounds that act on multiple biological targets simultaneously. The network pharmacology approach can map the complex relationships among multiple compounds, multiple targets, and multiple pathways, enabling the identification of synergistic or complementary effects among compounds. Atherosclerosis involves many biological pathways, so the network pharmacology approach can help us understand how combinations of herbal compounds modulate various pathways simultaneously. Network pharmacology is more realistic than the single-target approach, as it is consistent with the natural, holistic, and pleiotropic mechanisms of phytopharmaceuticals. Network pharmacology provides insight into how active compounds in *A. ascalonicum* can interact with various targets in the body. This approach helps identify the potential therapeutic benefits of *A. ascalonicum* extract, enabling the design of more effective treatment strategies for treating atherosclerosis.

MATERIAL AND METHODS

A. ascalonicum search profile

The SMILES structures of the major bioactive compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The compounds analyzed were bioactive compounds abundant in *A. ascalonicum* extract, as reported in previous studies, including kaempferol, quercetin, quercetin 3-O-alpha-L-rhamnoside, and allicin (Adeyemo et al., 2022; Wenli et al., 2019).

Prediction of druglikeness compounds

The SWISSADME (http://www.swissadme.ch/) database was used to predict the intestinal absorption potential of compounds based on the Lipinski rule of five (Rule of five/RO5/Druglikeness) parameters. The Lipinski rule of five was used to predict the physicochemical properties of a compound when administered orally. The parameters of RO5 included a molecular weight less than 500 Da, a number of hydrogen-bond donors less than 5, a number of hydrogen-bond acceptors less than 10, and a log P coefficient less than 5. The selected compounds were those that met the five Lipinski criteria.

Prediction of toxicity profile

Toxicity profiles of the main bioactive compounds in *A. ascalonicum* extract were predicted using ADMETLab 3.0 (https://admetlab3.scbdd.com/). The canonical SMILES of kaempferol, quercetin, quercetin 3-O-α-L-rhamnoside, and allicin were analyzed to assess parameters such as human hepatotoxicity, Ames mutagenicity, hERG inhibition, LD_{50}, and carcinogenicity. The predictions, based on integrated QSAR models, provided an overview of potential toxicological risks, supporting the selection of safe bioactive candidates for further pharmacological evaluation.

Prediction of bioactive compound activity with the structure analysis relationship (SAR) approach

The potential of compounds in *A. ascalonicum* extract was analyzed using WAY2DRUG PASS prediction (http://www.pharmaexpert.ru/passonline/predict.php) as an anti-cholesterol, anti-oxidant, and anti-inflammatory treatment. WAY2DRUG Pass Prediction uses SAR analysis to compare input compounds and compounds that were known to have certain potentials (Fig. 1). The more similar the structure of the compound, the higher the prediction value was obtained. Compounds that have similar structures can be predicted to have similar potentials. The probability of being active (Pa) was the predicted output of WAY2DRUG PASS, which described the potential of a compound being tested. If the Pa value was greater than 0.7, it indicated that the compound was predicted to have fairly good bioactivity because it had a high resemblance to compounds in the database. The Pa value indicates the accuracy of the obtained prediction function; the higher the Pa value, the better the accuracy (Filimonov et al., 2014; Szklarczyk et al., 2021).

Target protein prediction

The target analysis of *A. ascalonicum* extract was obtained from the SEA Target (https://sea.bkslab.org/) (Keiser et al., 2007). The target prediction was obtained by including the SMILE identified in stage 1. Cut off p-value < 0.05 and Max Tanimoto Coefficient SEA Target score > 0.4 (Range 0-1). Proteins associated with atherosclerosis were obtained from the DisGeNET (https://disgenet.com/) and GeneCards (https://www.genecards.org/) databases. The target related to the disease and the target database (DB) were then mapped using a Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) to determine the intersection of targets. Database-derived targets annotation was carried out using the DAVID web server (https://david.ncifcrf.gov/) (Sherman et al., 2022) with biological process terms and KEGG pathways ($p<0.05$).

Network pharmacology analysis

The interaction between the target protein of *A. ascalonicum* and atherosclerosis was analyzed using the STRING DB V.12.0 database (Szklarczyk et al., 2021). The inputs provided are as follows:

Submit: Protein target *Allium ascalonicum* L. and atherosclerosis

Organism: *Homo sapiens*

Required score: Highest confidence (0.9)

The TSV-formatted string analysis was then processed in Cytoscape v. 3.10 for further analysis. CytoScape was used to perform network analysis to determine the topological score.

Verification with molecular docking

To analyze the relationship between the primary target of *A. ascalonicum* extract and its core compounds, a molecular docking approach was used. The 3D structure of the selected target protein was obtained from the RCSB PDB database (https://www.rcsb.org/), namely the 5CGJ crystal structure of murine Keap1 in complex with RA839, a non-covalent small-molecule binder to KEAP1 and selective activator of Nrf2 signalling (Winkel et al., 2015). The previously reported 3D structure of the bioactive ligand from *A. ascalonicum*

extract was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) with the ID listed in Table 1.

High-resolution protein structures were selected from the RCSB PDB database, prioritizing complete sequences with resolution <2.5 Å. The source organism was "*Homo sapiens*"; the refinement resolution was 2.5 Å; and the protein structure sequence was complete, with potential as a small-molecule ligand. In addition, water molecules and native ligands from the obtained protein were removed using PyMOL software. Protein preparation was performed using PyMOL 2.5.2, while ligand energy minimization was performed using the universal force field in Open Babel within PyRx V.1.0 (Trott & Olson, 2010). Docking was performed using Autodock Vina integrated within PyRx V.1.0. Docking was performed using the target docking method (specific docking). The gridbox size was adjusted to the position of the amino acid residues bound to the RA839 control from the previous study (Winkel et al., 2015).

Molecular docking results were obtained as binding affinity or affinity energy from the interaction between the compound and the protein, and then visualized using BioVia Discovery Studio 2024 software. Molecular docking results was verified based on binding affinity and binding pose.

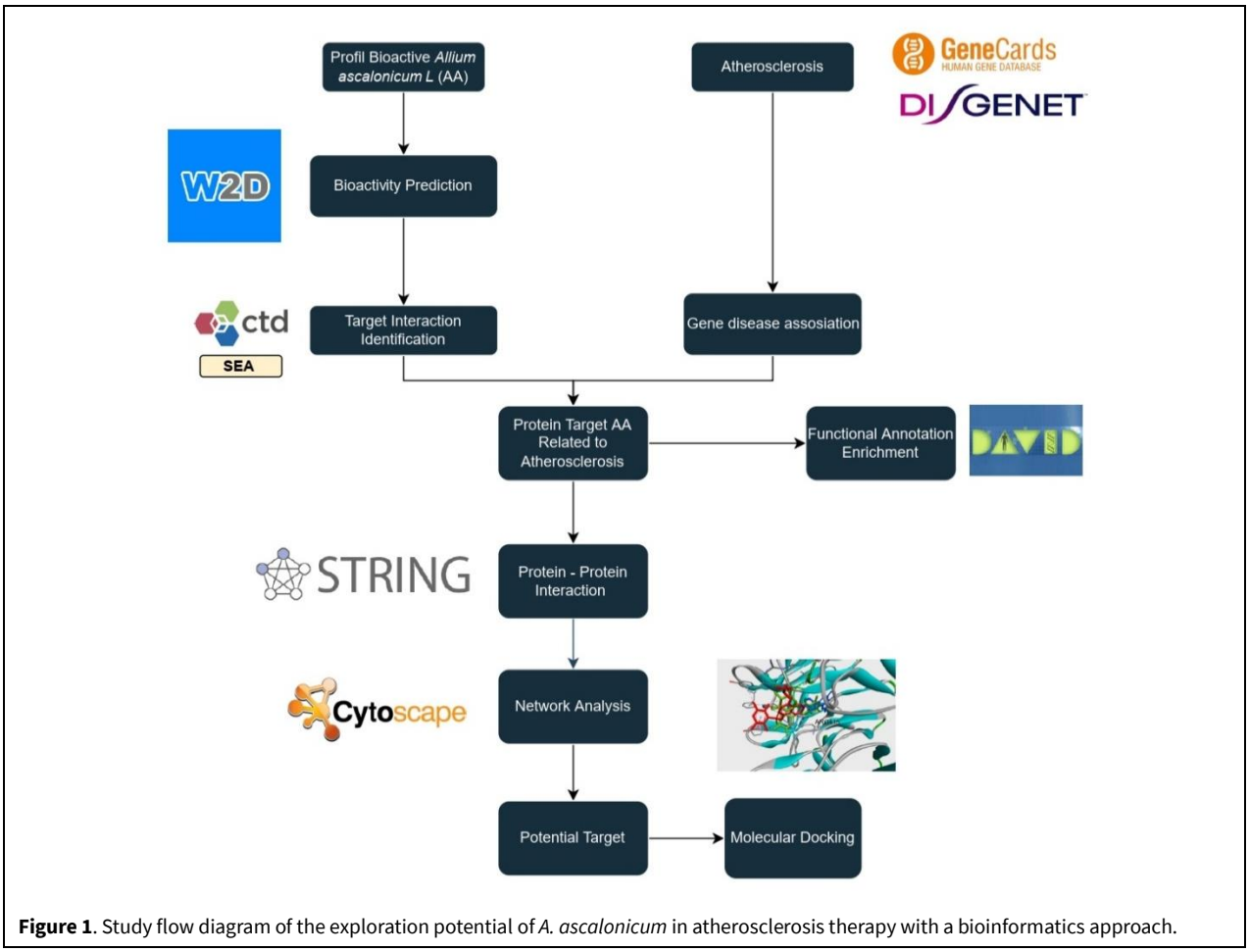


Figure 1. Study flow diagram of the exploration potential of *A. ascalonicum* in atherosclerosis therapy with a bioinformatics approach.

Table 1. Bioactive Profile *A. ascalonicum* extract.

Compound name	CID	Isomeric_SMILES
Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Quercetin 3-O-alpha-L-rhamnoside	5280459	C[C@H]1[C@@H]([C@H]([C@H]([C@@H](O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)O)O)O)O
Allicin	65036	C=CCSS(=O)CC=C

Table 2. Druglikeness profile of *A. ascalonicum* compounds (Lipinski's criteria).

Bioactive	Kaempferol	Quercetin	Quercetin 3-O-alpha-L-rhamnoside	Allicin	Statin	RA839	Sterile water
Formula	$C_{15}H_{10}O_6$	$C_{15}H_{10}O_7$	$C_{21}H_{20}O_{11}$	$C_6H_{10}OS_2$	$C_{25}H_{38}O_5$	$C_{25}H_{28}N_2O_4S$	H_2O
MW	286.24	302.24	448.38	162.27	418.57	452.57	18.02
#H-bond acceptors	6	7	11	1	7	5	4
#H-bond donors	4	5	7	0	5	4	2
Consensus Log P	1.58	1.23	0.22	1.61	4.11	4.11	4.11
Lipinski	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
GI absorption	High	High	Low	High	High	High	High
P-gp substrate	No	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	Yes	Yes
CYP2C9 inhibitor	No	No	No	No	Yes	Yes	Yes
CYP2D6 inhibitor	Yes	Yes	No	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	No	No	Yes	Yes	Yes
H-HT	0.386179209	0.337382317	0.437905908	0.185488388	0.687154233	0.967311	0.346075

RESULTS AND DISCUSSION

Search profile of *A. ascalonicum*

The results of the search for the bioactive profile of *A. ascalonicum* extract reported in the previous study are shown in Table 1.

Prediction of druglikeness compounds

Based on SWISSADME tracing, kaempferol, quercetin, quercetin 3-O-alpha-L-rhamnoside, and allicin are readily absorbed when administered orally because they meet Lipinski's 5 rules (Table 2). Simple empirical rules and predictive models based on molecular descriptors and physicochemical properties aid in classifying the oral activity rankings of molecules from a chemical compound library. The Lipinski 'rule-of-5' is the first qualitative rule developed as a tool for chemists to design orally active compounds and is now widely used in the selection of drug molecules for further development (Varma et al., 2006). Knowledge of compounds that function as substrates or non-substrates of the protein permeability glycoprotein (P-gp, proposed as the most important member among ATP-binding transporters or ABC transporters) is key in evaluating active efflux through biological membranes, for example, from the gastrointestinal wall to the lumen or from the brain. One of the main roles of P-gp is to protect the central nervous system (CNS) from xenobiotics. Knowledge of molecular interactions with cytochrome P450 (CYP) is also very important. This superfamily of isoenzymes plays a key role in drug elimination through metabolic biotransformation. It has been proposed that CYP and P-gp may act synergistically to process small molecules, thereby enhancing the protection of tissues and the organism. Inhibition of isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) is one of the main causes of pharmacokinetic drug interactions, leading to toxic or other undesirable effects due to decreased clearance and accumulation of drugs or their metabolites. Therefore, in drug discovery, it is crucial to predict the tendency of molecules to cause significant drug interactions through CYP inhibition, as well as to determine which isoenzymes are affected (Daina et al., 2017). Kaempferol and quercetin are compounds to consider due to their potential for drug interactions through inhibition of CYP1A2, CYP2D6, and CYP3A4. Meanwhile, quercetin 3-O-alpha and allicin exhibit a much safer pharmacokinetic profile with respect to potential drug interactions.

Drug-induced liver injury (DILI) (Human Hepatotoxicity, H-HT) is a serious issue for patient safety and a major cause of drug withdrawals from the market. Adverse liver effects in clinical trials often lead to late and costly discontinuation of drug development programs. In this database, samples classified as non-hepatotoxic are compounds not associated with hepatotoxic keywords or adverse liver reactions. The remaining compounds are categorized as compounds that show adverse liver reactions. Category 0: non-hepatotoxic; Category 1: hepatotoxic. The output value is the probability of hepatotoxicity, ranging from 0 to

1. None of the compounds analyzed in this discussion were detected to have high human hepatotoxicity.

Based on drug suitability analysis using Lipinski's Rule-of-5 parameters, all bioactive compounds of *A. ascalonicum* tested (kaempferol, quercetin, quercetin 3-O-alpha-L-rhamnoside, and allicin) met the criteria for good oral bioavailability, as shown in Table 2. This indicates that these compounds possess physicochemical characteristics that enable good oral absorption. Specifically, kaempferol and quercetin, which have molecular weights below 500 Da and moderate log P values, suggest that these compounds exhibit a good balance between water solubility and membrane permeability. Good oral bioavailability is an important consideration in drug development, as it relates to ease of administration and patient compliance (Varma et al., 2006).

Prediction of bioactive compound activity with structure analysis relationship (SAR) approach

Based on a search using the PASS Online database, the bioactive ingredient *A. ascalonicum* extract had predictive scores such as antihyperlipidemia (0.51), anti-inflammatory (0.60), lipid metabolism regulator (0.51), antioxidant (0.74), NF-E2 related factor 2 stimulant (0.32), and free radical scavenger (0.68) (Fig. 2).

The results of SAR prediction using the PASS WAY2DRUG showed that the bioactive compound *A. ascalonicum* extract has the potential to be an antihyperlipidemic, with a significant probability value (Pa > 0.5). Quercetin was identified as the compound with the highest antihyperlipidemic potential, consistent with several previous experimental studies reporting quercetin's ability to lower cholesterol and triglyceride levels, as shown in Fig. 3 (Deng et al., 2020; Tan et al., 2021). The bioactive compound *A. ascalonicum* extract is also predicted to have anti-inflammatory (Pa = 0.60), antioxidant (Pa = 0.74), and free radical scavenger activity (Pa = 0.68), all of which are important mechanisms in the prevention of atherosclerosis. Despite a lower predictive score, its role in antioxidant pathways warrants further investigation, and is still relevant for further investigation given the important role of Nrf2 in cellular antioxidant defense.

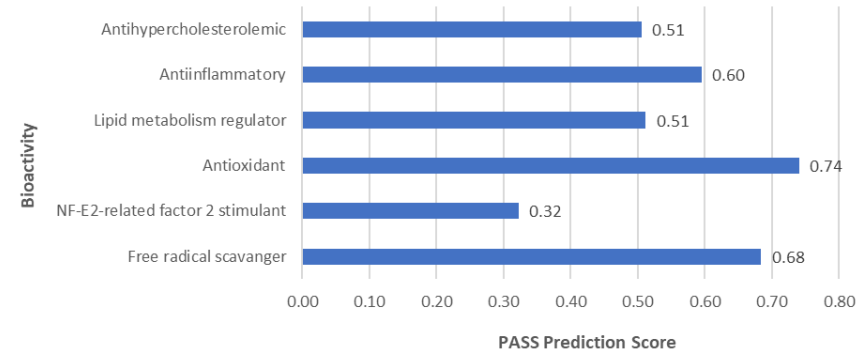


Figure 2. Prediction of bioactive potential of *A. ascalonicum* extract with Pass Online.

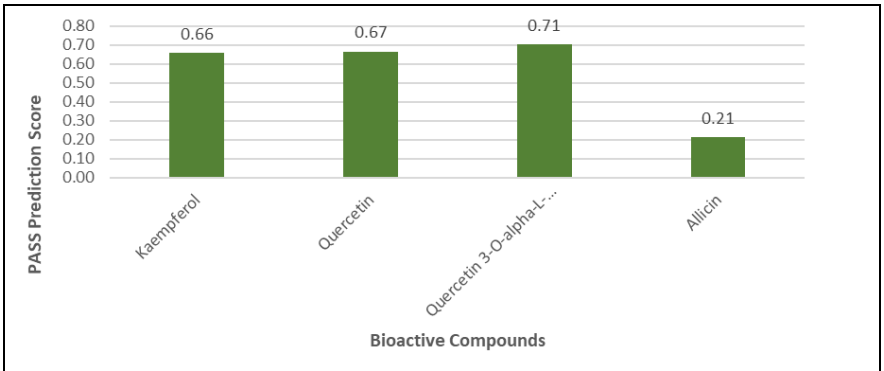


Figure 3. Prediction of bioactive compound of *A. ascalonicum* extract with Pass Online.

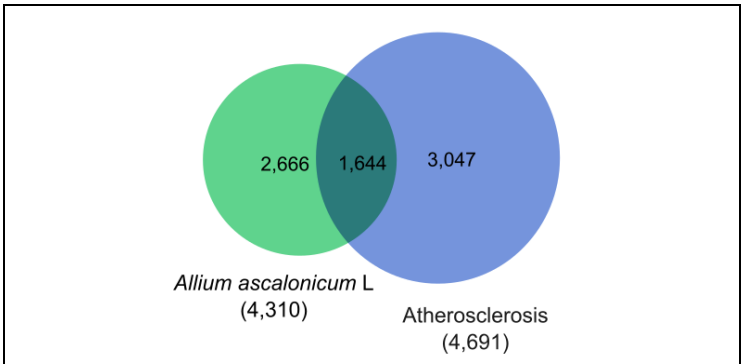


Figure 4. Venn diagram atherosclerosis linkage and target *Allium ascalonicum*.

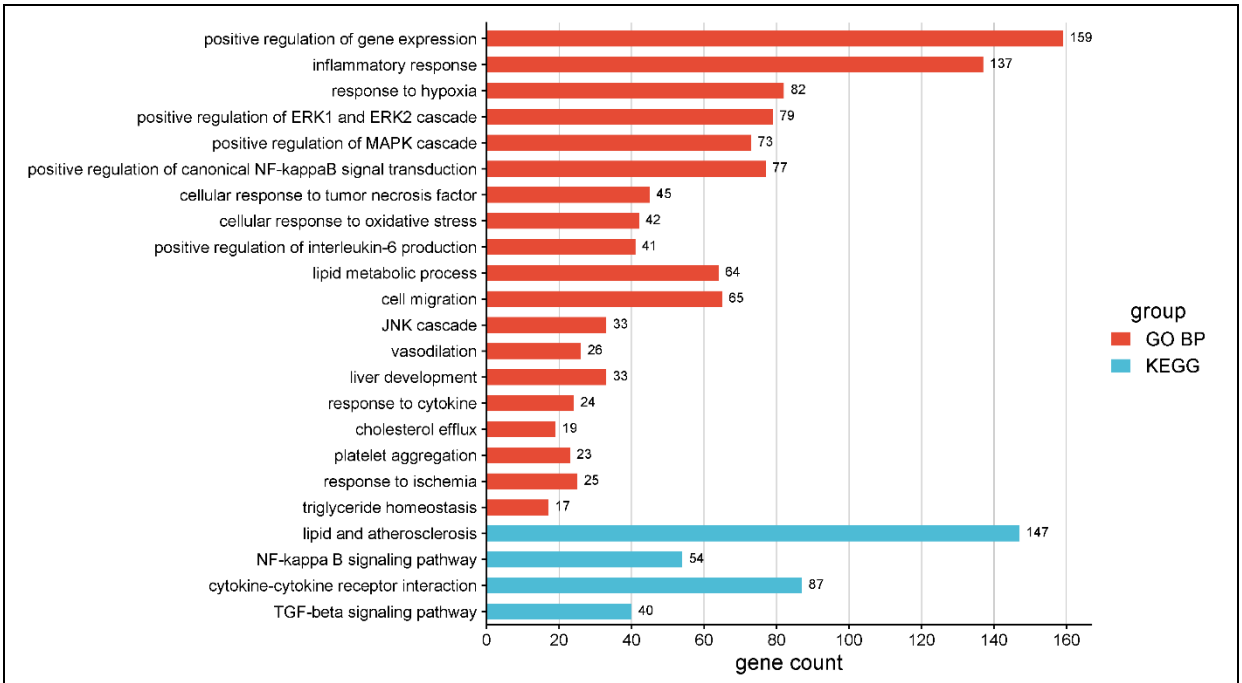


Figure 5. Functional analysis of *A. ascalonicum* target.

Target protein prediction

Based on tracing the target protein of *A. ascalonicum*, 4,309 target proteins were found to interact with *A. ascalonicum*. There are 1,644 proteins associated with atherosclerosis (Fig. 4). Proteins that can interact with the active ingredients of *A. ascalonicum* and are associated with atherosclerosis can be viewed in Excel. The target proteins of *A. ascalonicum* are involved in inflammatory pathways, proliferation, migration, and responses to oxidative stress. The active compounds of *A. ascalonicum* play a central role in inflammatory pathways (Fig. 5).

Network pharmacology analysis indicates that the bioactive compounds in *A. ascalonicum* can interact with numerous proteins implicated in atherosclerosis pathogenesis. Interestingly, Nrf2 was identified as a target protein involved in all pathways relevant to atherosclerosis, namely lipid metabolism, inflammatory response, and response to oxidative stress. This suggests that the Nrf2 pathway may be a central mechanism in the anti-atherosclerotic effects of the bioactive compound extract of *A. ascalonicum*.

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the expression of antioxidant and phase II detoxification genes (Yagishita et al., 2020). Under normal conditions, Nrf2 is bound to the KEAP1 protein in the cytoplasm and undergoes proteolytic degradation. However, under oxidative stress or in the presence of Nrf2 inducers, the KEAP1-Nrf2 complex is disrupted, leading to Nrf2 accumulation and nuclear translocation, thereby activating target gene expression (Dinkova-Kostova & Copple, 2023). Activation of the Nrf2 pathway has been reported to protect against atherosclerosis by reducing

oxidative stress, inflammation, and endothelial dysfunction (Barbosa et al., 2019).

Network pharmacology analysis

The targets related to *A. ascalonicum* extract and atherosclerosis are numerous, so an attempt was made to identify the top 100 proteins with the best degree value. The degree value indicates the number of interactors (edges) associated with the protein (Ashtiani et al., 2018). The higher the degree value, the more proteins interact (Fig. 6). Functional enrichment analysis (GO, pathway clustering) with FDR, all significant (FDR/p<0.05) the redder the more significant (Fig. 7). NFE2L2 is a protein involved in all the pathways to be studied, namely lipid and atherosclerosis, inflammation, and response to oxidative stress (Fig. 8).

Molecular docking

Based on the results of the molecular docking search, the rmsd control redock value is 0.343 Angstrom, indicating that the docking results are consistent with the experimental research conducted by Winkel et al. (2015). The RA839 control value (-11 kcal/mol) is the highest bond affinity among the compounds. The more negative the docking value, the stronger the interaction between the protein and the ligand compound. Although the value is not as good as the interaction between the control and KEAP1, the interaction of the *A. ascalonicum* extract compound is still classified as a strong interaction. Generally, bond energies below -7.0 kcal/mol indicate strong bond energies (Liu et al., 2021). Quercetin 3-O-alpha-L-rhamnoside (-9.3 kcal/mol) is a bioactive compound in shallots with a bond affinity value close to the control (Table 3).

The most abundant compound in *A. ascalonicum* extract can interact with KEAP1 and NRF2. By disrupting the interaction between KEAP1 and NRF2, it is hoped that NRF2 can be activated. Activation of the NF-E2-related transcription factor 2 (Nrf2) maintains cellular homeostasis in response to oxidative stress through the regulation of several cytoprotective genes. In the absence of stress factors, Nrf2 activity is inhibited by its interaction with Keap. RA839 acutely induces the expression of Nrf2 target genes. RA839 is a selective inhibitor of the Keap1/Nrf2 interaction and a useful tool compound for studying Nrf2 biology. Arg415 is the amino acid that mediates the control interaction with KEAP1, according to research by Winkel et al. (2015). Interaction with ARG415 was also found in the redocking results we obtained. Thus, the bioactive compound *A. ascalonicum* can interact at a similar position to the control when interacting with KEAP1. Red: native control ligand, while green is the docking control performed, with coordinates similar to a score of 0.343 (Fig. 9).

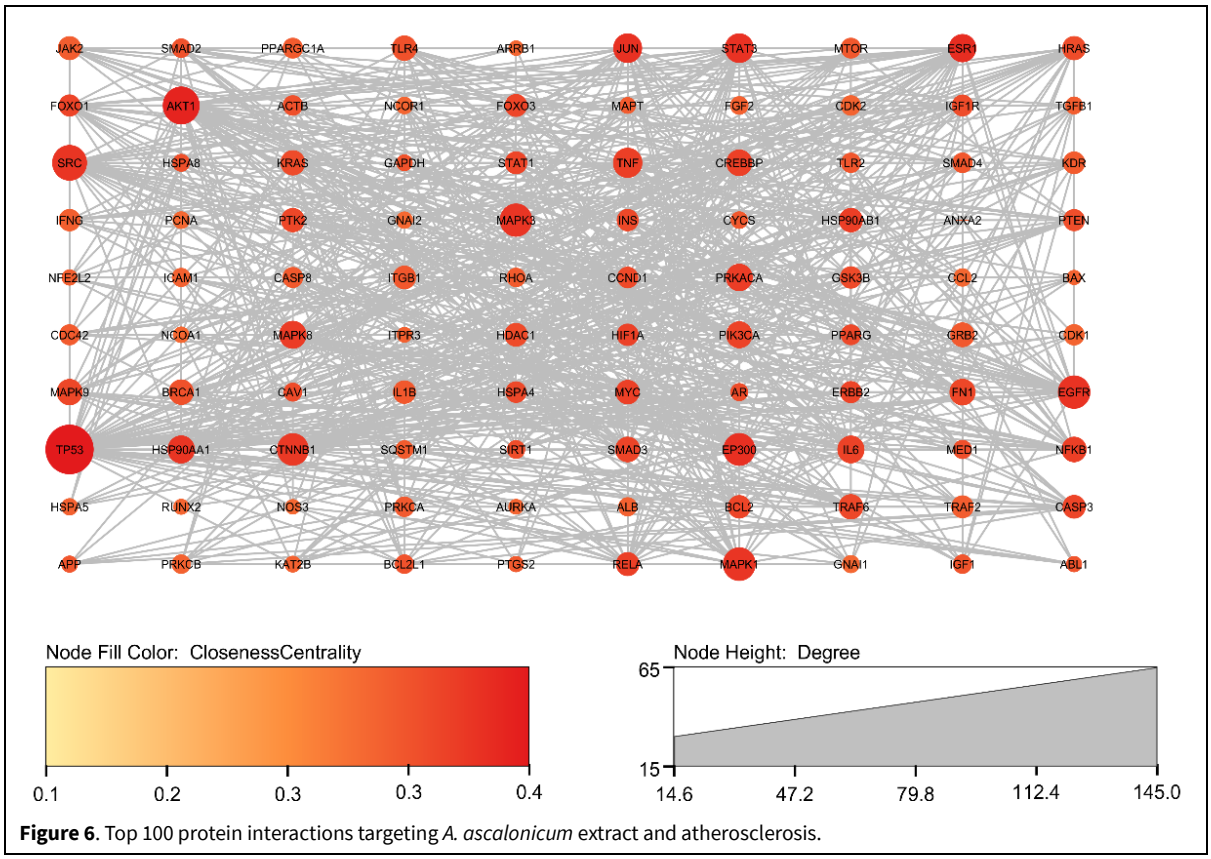


Figure 6. Top 100 protein interactions targeting *A. ascalonicum* extract and atherosclerosis.

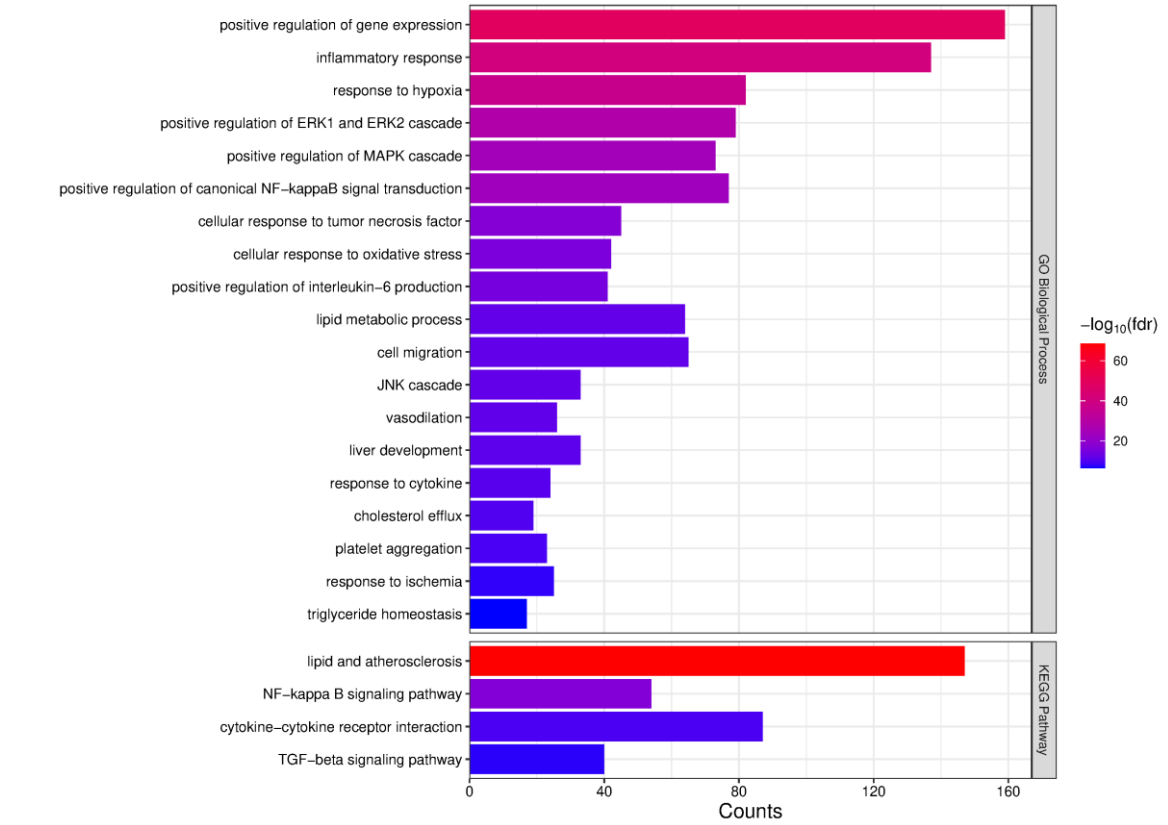


Figure 7. Functional enrichment analysis (GO, pathway clustering) with FDR.

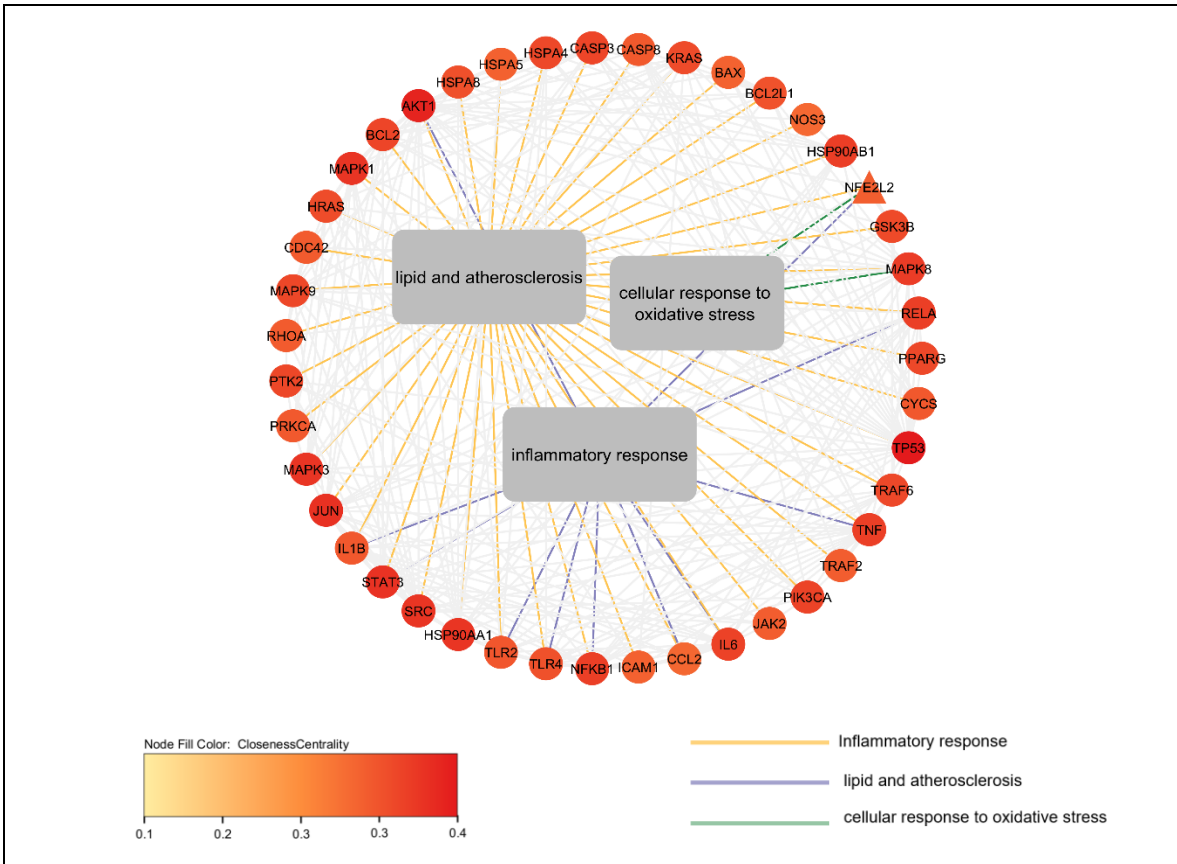


Figure 8. Interaction of *A. ascalonicum* target proteins with lipids, atherosclerosis, the inflammatory response, and cellular responses to oxidative stress.

Table 3. Molecular docking of bioactive *A. ascalonicum* extract and KEAP1 inhibitor (NFE2L2 activator).

Compound name	CID	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrofobic bond interaction
Kaempferol	5280863	-9	GLY367 VAL606 VAL512 VAL463 GLY464	***ARG415*** **ALA556**
Quercetin 3-O-alpha-L-rhamnoside	5280459	-9.3	VAL463 GLY462 ***ARG415*** SER363 GLY364 GLY603 GLY509	**ALA556**
Control 51M	5CGJ	-11	SER508 ARG483 ARG415	TYR334 ALA556 PHE577 TYR572

Red: amino acids similar to the control.

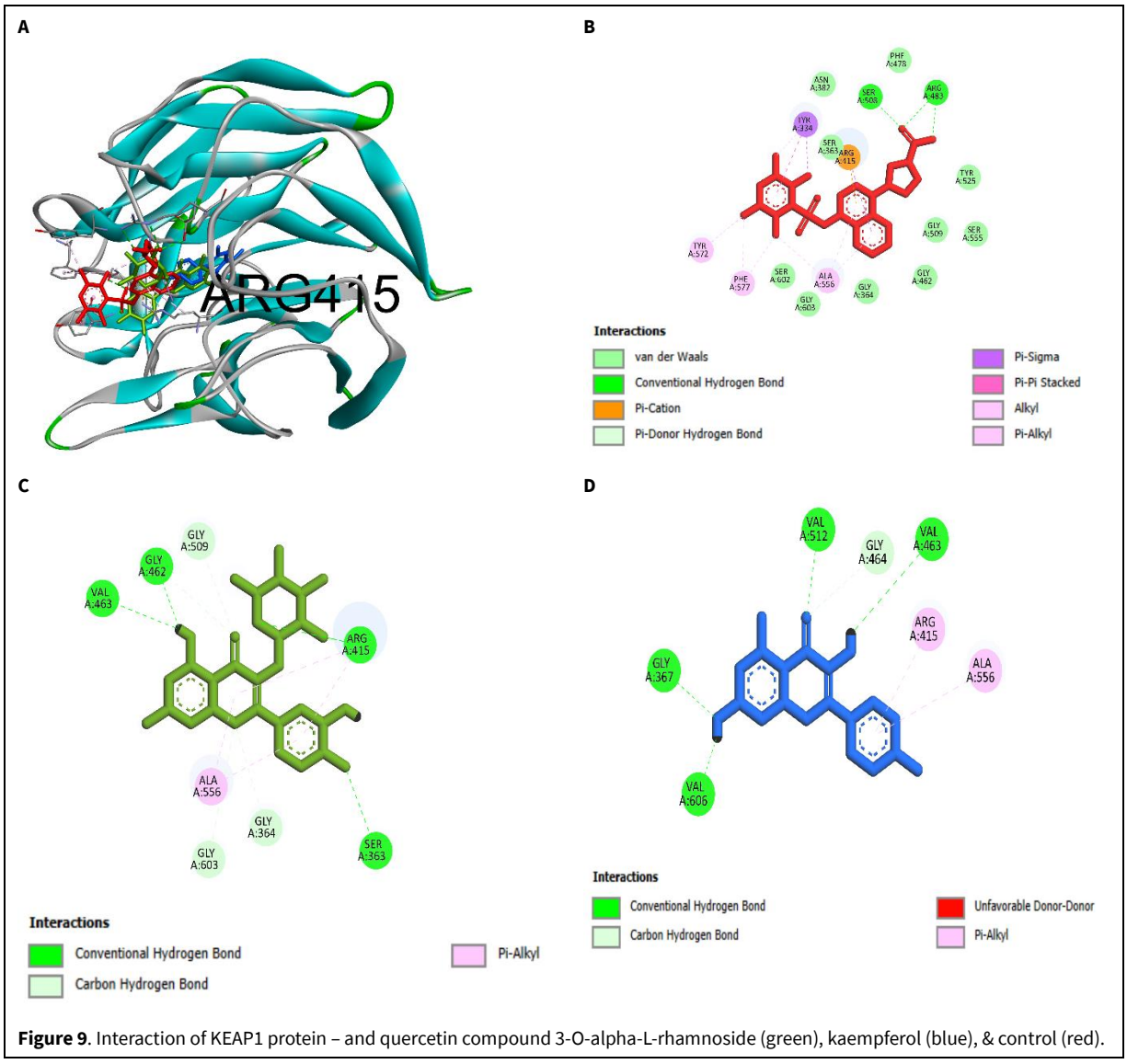


Figure 9. Interaction of KEAP1 protein – and quercetin compound 3-O-alpha-L-rhamnoside (green), kaempferol (blue), & control (red).

Molecular docking results show that kaempferol and quercetin 3-O-alpha-L-rhamnoside have strong binding affinities with KEAP1, with binding energies of -9.0 kcal/mol and -9.3 kcal/mol, respectively. Although these values are not as good as the control RA839 (-11 kcal/mol), they still indicate strong interactions (< -7.0 kcal/mol). Quercetin 3-O-alpha-L-rhamnoside has the closest binding affinity value to the control, indicating its potential as a promising KEAP1 inhibitor (Table 4).

Molecular interaction analysis shows that kaempferol and quercetin 3-O-alpha-L-rhamnoside interact with the key residue ARG415 on KEAP1, similar to the interaction shown by the control KEAP1 inhibitor (RA839). ARG415 is an important residue that

mediates the KEAP1-NRF2 interaction (Winkel et al., 2015). Interactions with these residues suggest that the bioactive compounds in *A. ascalonicum* extract may disrupt KEAP1-NRF2 interactions, thereby releasing NRF2 to activate antioxidant and anti-inflammatory genes. The ability to interact with the same molecular targets as the control inhibitor reinforces the potential of onion bioactive compounds as NRF2 activators.

Implications on atherosclerosis

Based on the above findings, the bioactive compounds in *A. ascalonicum* extract, particularly quercetin 3-O-alpha-L-rhamnoside and kaempferol, have potential as therapeutic agents in the treatment of atherosclerosis through several mechanisms:

1. **Activation of the Nrf2 pathway**: By inhibiting KEAP1, the bioactive compounds in *A. ascalonicum* can activate Nrf2, which in turn stimulates the expression of antioxidant and detoxification enzymes, inhibits the formation of reactive oxygen species, and reduces oxidative stress—a key factor in the onset and progression of atherosclerosis.
2. **Anti-inflammatory effects**: Nrf2 also plays a role in inhibiting NF-κB activation and the production of pro-inflammatory cytokines. These anti-inflammatory effects can reduce the infiltration of inflammatory cells into blood vessel walls and inhibit the formation of atherosclerotic plaques (Jiang et al., 2020).
3. **Lipid metabolism regulation**: The bioactive compounds in *A. ascalonicum* have the potential to regulate lipid metabolism, which can lower blood lipid levels and reduce lipid accumulation in blood vessel walls.
4. **Direct antioxidant effects**: In addition to activating Nrf2, the bioactive compounds in *A. ascalonicum* also have direct antioxidant effects as free radical scavengers, which can protect LDL from oxidation and reduce foam cell formation, an early step in the pathogenesis of atherosclerosis.

These findings are consistent with experimental and epidemiological studies reporting the protective effects of *A. ascalonicum* against cardiovascular disease (Luo et al., 2022; Terao, 2023). The results of this study provide a molecular basis for these cardioprotective effects. This study is consistent with *in vitro* and *in silico* studies conducted by Huwait et al. (2021), which showed that quercetin inhibits the migration of THP-1 monocytes, MCP-1, and ICAM-1, and increases cholesterol flow, which may be mediated through the LXR/RXR signaling pathway. This study is also consistent with network pharmacology and molecular docking studies conducted by Tan et al. (2021), which showed that quercetin can alleviate pathological changes in heart failure by regulating the AKT1-eNOS-MMP9 pathway and inhibiting cell apoptosis. Therefore, quercetin 3-O-alpha-L-rhamnoside, quercetin, kaempferol, and allicin in *A. ascalonicum* may help prevent cell infiltration in atherosclerotic plaques and reduce the risk of stroke or brain damage.

Limitations and directions for further research

Although this *in silico* study provides valuable insights into the potential anti-atherosclerotic mechanisms of the bioactive compound extract of *A. ascalonicum*, several limitations should be noted:

1. *In silico* prediction results need to be further verified through *in vitro* and *in vivo* studies in atherosclerosis disease models.
2. The bioavailability and pharmacokinetics of bioactive compounds in *A. ascalonicum* extract *in vivo* may differ from *in silico* predictions, particularly due to the complexity of absorption, distribution, metabolism, and excretion processes in the body.

Table 4. Interaction of amino acid residues KEAP1 and ligand compounds.

Compound name	CID	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrophobic bond interaction
Kaempferol	5280863	-9	GLY367 VAL606 VAL512 VAL463 GLY464	***ARG415*** **ALA556**
Quercetin 3-O-alpha-L-rhamnoside	5280459	-9.3	VAL463 GLY462 ***ARG415*** SER363 GLY364 GLY603 GLY509	**ALA556**
Control 51M	5CGJ	-11	SER508 ARG483 ARG415	TYR334 ALA556 PHE577 TYR572

Red: Amino acids similar to control.

3. Interactions between bioactive compounds in the *A. ascalonicum* extract have not been considered in this study. For further studies, it is recommended to validate the effects of bioactive compounds in *A. ascalonicum* extract on the activation of the KEAP1-Nrf2 pathway using an atherosclerosis cell culture model, and to evaluate the effects of *A. ascalonicum* bioactive compounds in an atherosclerosis animal model to confirm the predicted anti-atherosclerotic effects predicted, developing pharmaceutical formulations that can enhance the bioavailability of bioactive compounds in *A. ascalonicum* extract, particularly quercetin 3-O-alpha-L-rhamnoside, which showed the best potential in this study, and exploring the potential synergistic effects between bioactive compounds in *A. ascalonicum* extract in anti-atherosclerotic effects.
4. It is recommended to conduct further *in vitro* and *in vivo* tests, e.g., HEK293 or macrophage-based cell tests, luciferase reporter assays for Nrf2, or Western blots for HO-1 expression in macrophage/endothelial cell models, LDL oxidation assay, transwell migration assay, HPLC analysis of extract, and *in vivo* study using a mouse atherosclerosis model (ApoE−/− mice).
5. Assess the combinatorial effect of kaempferol, allicin, and quercetin on inflammatory biomarkers.
6. Despite strong docking affinities, *in vivo* efficacy is contingent on compound stability, absorption, and metabolic degradation, which were not evaluated in this study.

CONCLUSION

The bioactive compounds in *A. ascalonicum* extract, particularly quercetin 3-O-alpha-L-rhamnoside, quercetin, kaempferol, and allicin, have potential as therapeutic agents for the treatment of atherosclerosis through modulation of the KEAP1-Nrf2 pathway. Network Pharmacological analysis identified Nrf2 as the primary target protein involved in all pathways relevant to atherosclerosis. Bioactive compounds from *A. ascalonicum* extract also meet the Lipinski Rule of Five criteria for good oral bioavailability and are predicted to exhibit antihyperlipidemic, anti-inflammatory, and antioxidant activities, all of which play important roles in the prevention and therapy of atherosclerosis. Future *in vitro* and *in vivo* validations are necessary to confirm these findings.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, or editing of this manuscript. All content was developed solely by the authors, who take full responsibility for its accuracy and integrity.

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Concepts or ideas	x						
Design		x			x		
Definition of intellectual content	x			x			
Literature search	x		x				x
Experimental studies	x				x		
Data acquisition			x	x			x
Data analysis	x	x	x				
Statistical analysis							
Manuscript preparation	x					x	
Manuscript editing				x	x	x	
Manuscript review	x	x	x	x	x	x	x

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