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Original Article

DECODING THE THERAPEUTIC POTENTIAL OF *EMPON-EMPON*: A BIOINFORMATICS EXPEDITION UNRAVELING MECHANISMS AGAINST COVID-19 AND ATHEROSCLEROSIS

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ABSTRACT

Objective: This study aims to elucidate the main compounds and mechanisms of action of *Empon-empon* (EE), a traditional Indonesian herb used for treating COVID-19 and atherosclerosis, utilizing an integrated network pharmacology and molecular docking approach.

Methods: Active compounds in EE were obtained through the KNApSAcK, screening active compounds using parameters: oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. Compound-related target genes were collected from GeneCard, ChemBL, and Traditional Chinese Medicine Systems Pharmacology (TCMSP). Disease targets were obtained from the GeneCard database. The protein-protein interaction (PPI) network was built using STRING and visualized using Cytoscape. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis using ShinyGO. Molecular docking analysis using Autodock Vina in PyRx.

Results: We identified 18 main compounds in EE. PPI analysis obtained 5 central EE targets involved in treating COVID-19 and atherosclerosis, namely E1A Binding Protein P300 (EP300), Heat Shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1), SRC Proto-Oncogene (SRC), Estrogen Receptor 1 (ESR1), and RELA Proto-Oncogene (RELA). GO and KEGG analysis illustrated EE's pharmacological effects through pathways in cancer, lipid and atherosclerosis, and PI3K-Akt signaling, including Coronavirus disease. Catechin and quercetin exhibited the strongest binding affinity to EP300; licarin B and delphinidin to HSP90AA1; epicatechin and delphinidin to SRC; galangin and ellagic acid to ESR1; and guaiacin and licarin B to RELA.

Conclusion: This research provides a strong foundation regarding the main compound and mechanism action of EE in treating atherosclerosis and COVID-19, suggesting potential as a novel therapeutic agent.

Keywords: Atherosclerosis, COVID-19, Empon-empon, Networkpharmacology, Molecular docking

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INTRODUCTION

The global pandemic has resulted in more than 700 million cases and 7 million deaths worldwide [1]. The clinical manifestations of COVID-19 disease are characterized by high levels of inflammation mediated by proinflammatory cytokines [2-4]. This condition can last six months or more, leading to a condition known as long COVID-19 [5, 6]. Clinical studies have found that SARSCOV-2 and other respiratory viruses, such as influenza, can trigger cardiovascular damage [7, 8] and exacerbate pre-existing cardiovascular disorders, including hypertension and coronary heart disease [9–11].

Atherosclerosis, a chronic inflammatory vascular disease, serves as a primary precursor to diverse cardiovascular disorders, such as myocardial infarction, stroke, and peripheral artery disease [12, 13]. The disease is characterized by chronic inflammation and disruption of endothelial hemostasis, fostering conditions that elicit excessive inflammatory responses, hypertrophy, and ventricular fibrosis [14, 15]. Beyond the health implications, atherosclerosis poses a substantial economic burden on society [16].

Conventional atherosclerosis therapies, including statins, have been associated with adverse effects that may exacerbate the disease and compromise immune function, thereby increasing susceptibility to viral infections, including COVID-19 [17–19]. Amid the pandemic's peak, the empirical and safe utilization of the traditional herb "*Empon-empon*" emerged, demonstrating swift efficacy in treating atherosclerosis and COVID-19 disorders [20]. EE is a traditional Indonesian concoction made from a combination of turmeric (*Kunyit/Curcuma longa Linn*), aromatic ginger (*Kencur/Kaempferia galangal*), red ginger (*Jahe merah/Zingiber officinale var rubrum*), galangal (*Lengkuas/Alpinia galanga),* cinnamon (*Kayumanis/Cinnamomum cassia*), nutmeg (*Pala/Myristica fragrans*), cloves (*Cengkeh/Syzygium aromaticum),* lemongrass (*Serai wangi/Cymbopogon citratus*), and Bay leaf (*Daun salam/Syzygium polyanthum Wight Walp*).

While preclinical evidence supports the herb's effectiveness, a comprehensive investigation employing computational approaches is essential to unveil active compounds with the best affinity and elucidate the signaling pathway mechanisms integral to treating COVID-19 and atherosclerosis; this represents a pivotal stride toward the development of innovative drugs for the treatment of both conditions. The present study utilized network pharmacology molecular docking to unravel active compounds and their molecular mechanisms in the treatment of COVID-19 and atherosclerosis, leveraging the robust capabilities of network pharmacology to enhance the scientific interpretation of drug mechanisms, complemented by molecular docking to validate their interactions.

MATERIALS AND METHODS

Identification in EE decoction and Screening of EE bioactive compounds

Information regarding active compounds in plants collected from the KNApSAcK database (http://www.knapsackfamily.com/knapsack_core/top.php) (accessed on September 27, 2023) and the Herbal Database (https://herbaldb.farmasi.ui.ac.id) [21]. To identify the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile of active compounds present in EE, crucial parameters for evaluation encompass OB ≥ 30%, DL ≥ 0.18, and Lethal Dose Fifty (LD_{50})>150 mg/kg. To assess OB and DL parameters, we

used the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php)(accessed on September 30, 2023). The LD_{50} value for toxicity information was taken from the Prediction of Toxicity of Chemicals (ProTox-II) web server (https://tox-new.charite.de/protox_II), and a screening process was carried out for compounds that meet the ADMET criteria. Compounds identified as meeting the specified criteria will become the main compounds in EE, which will be analyzed further.

Collection of compound target and disease target

To identify target-associated main compounds in EE, we utilized GeneCards (https://www.genecards.org/), ChemBL (https://www.ebi.ac.uk/chembl/), and the TCMSP database (https://lsp.nwu.edu.cn/tcmsp.php/), by inputting the chemical name. Targets related to COVID-19 and atherosclerosis were obtained from the GeneCards database (accessed on October 1, 2023. The target genes of EE, COVID-19 and atherosclerosis were subjected to analysis using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) (accessed on October 1, 2023, to assess the target genes of EE that have related to COVID-19 and atherosclerosis The outcomes of this investigation were visually depicted in a Venn diagram, illustrating the intersecting target genes shared between EE, COVID-19, and atherosclerosis.

Construction of a PPI network

The genes identified from the intersecting region of the Venn diagram plot were uploaded to the STRING database (https://string-db.org/, accessed on November 7, 2023), with *homo sapiens* selected as the species. The PPI network comprising 878 genes was constructed with a high confidence level (interaction score>0.9), and non-relevant targets were concealed. The results obtained from the STRING database were downloaded and imported into Cytoscape 3.10.1. Subsequently, statistical analysis was conducted based on the topology parameters.

Core target and central target screening

To identify core targets of EE relevant to COVID-19 and atherosclerosis, we employed topology parameters, including degree, betweenness centrality (BC), and closeness centrality (CC) within Cytocluster in Cytoscape. Nodes exceeding the median for all three topological parameters were selected to construct a subnetwork. This method is considered acceptable for core target screening [22]. Concurrently, utilizing the cytoHubba plugin in Cytoscape, we considered betweenness, closeness, degree, maximum neighborhood component (MNC), and bottleneck parameters to identify the top 15 targets. The intersection of the obtained targets represents the central targets [23].

GO functional annotation and KEGG enrichment

To predict the mechanisms underlying the effectiveness of EE in treating COVID-19 and atherosclerosis, GO and KEGG analyses were conducted using the ShinyGO 0.77 database (http://bioinformatics.sdstate.edu/go/) (accessed on Oct 1, 2023). The organism selected for analysis was "Human," and a False Discovery Rate (FDR) cutoff limit value of>0.05 was set. The x-axis of the analysis was scaled based on the number of genes.

This analytical approach aims to elucidate the biological processes, molecular functions, and cellular components associated with the identified genes and uncover relevant pathways through KEGG analysis. The parameters, including the chosen FDR cutoff value, ensure a stringent and reliable analysis of the functional annotations and pathways related to EE's efficacy against COVID-19 and atherosclerosis.

Construction of compound-target-pathway interaction network

The compound-target-pathway interaction network was constructed by establishing connections between main compounds and core EE targets involved in treating COVID-19 and atherosclerosis, along with the top 10 KEGG pathways. The resulting network was visualized using Cytoscape 3.10.1.

This network comprehensively represents the relationships between main compounds, their target genes, and the associated pathways. Cytoscape visualization capabilities facilitate a clear understanding of the complex interactions within the compound-target-pathway network, aiding in the interpretation and analysis of the molecular mechanisms underlying the therapeutic effects of EE on COVID-19 and atherosclerosis.

Molecular docking prediction

The molecular docking analysis aimed to validate the results of the compound-target-pathway network construction by providing binding affinity values. Specifically, main compounds with a score level above 5 were selected for analysis. The structures of the main compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (accessed on November 10, 2023). Central targets were chosen, and their structures were obtained from the RCSB database (https://www.rcsb.org) (accessed on November 14, 2023) and saved in PDB format files. The crystal structures of the main targets used were as follows: EP300 (PDB ID: 8GZC), HSP90AA1 (PDB ID: 1UY6), SRC (PDB ID: 2C01), ESR1 (PDB ID: 1UOM), and RELA (PDB ID: 1VJ7).

The main compounds served as ligands in the molecular docking process, while central targets acted as receptors. The receptor preparation involved removing water molecules and existing ligands using Biovia Discovery Studio 2021. The binding conformation between the ligand and receptor was predicted using Autodock Vina in PyRx [24] version 0.98. The best binding energy was utilized to assess the potential for ligand-receptor binding [25]. The molecular interactions between proteins and ligands were visualized using Discovery Visualization Studio 2021.

Table 1: Information on the main compounds in EE

MOL ID	Compound	Type	OB (%)	DL	MW	Herb	LD_{50} (mg/kg)
MOL006824	Alpha amyrin	Terpenoids	39.51	0.76	426	*Jahe merah*	70000
MOL007985	Austrobailignan	Terpenoids	42.54	0.42	342	*Pala, Serai*	1500
MOL000940	Bisdemethoxycurcumin	Terpenoids	77.38	0.26	308	*Jahe merah, Kunyit*	2560
MOL000096	Catechin	Flavonoids	49.68	0.24	290	*Kayumanis*	10000
MOL004798	Delphinidin	Flavonoids	40.63	0.24	303	*Jahe merah*	5000
MOL001002	Ellagic Acid	Phenolic	43.06	0.43	302	*Cengkeh*	2991
MOL000073	Epicatechin	Flavonoids	48.96	0.24	290	*Jahe merah, Kayumanis*	10000
MOL002563	Galangin	Flavonoids	45.55	0.21	270	*Kencur, Lengkuas*	3919
MOL009243	Guaiacin	Phenolic	48.78	0.31	328	*Kayumanis*	5000
MOL001942	Isoimperatorin	Phenolic	45.46	0.23	270	*Cengkeh*	3800
MOL000354	Isorhamnetin	Flavonoids	49.60	0.31	316	*Serai*	5000
MOL004564	Kaempferide	Flavonoids	73.41	0.27	300	*Kayumanis*	3919
MOL000422	Kaempferol	Flavonoids	41.88	0.24	286	*Cengkeh, Serai*	3919
MOL000260	Licarin B	Flavonoids	65.55	0.40	324	*Pala*	720
MOL000006	Luteolin	Flavonoids	36.16	0.25	286	*Serai*	3919
MOL000098	Quercetin	Flavonoids	46.43	0.28	302	*Jahe merah, Serai*	159
MOL001558	Sesamin	Flavonoids	56.55	0.83	354	*Kayumanis*	1500
MOL001506	Squalene	Terpenoids	33.55	0.42	285	*Daun salam, Kayumanis*	5000

RESULTS AND DISCUSSION

Active compounds in EE

Database searches identified 960 bioactive compounds in EE; after filtering, 18 compounds that meet OB>30% requirements, DL>0.18, $LD_{50>}$150 mg/kg were obtained. 18 Compounds that meet the screening criteria are the main compounds in EE, which consist of alpha amyrin, austrobailignan, bisdemethoxycurcumin, catechin, delphinidin, ellagic acid, epicatechin, galangin, guaiacin, iso imperatorin, isorhamnetin, kaempferide, kaempferol, licarin B, luteolin, quercetin, sesamin, and squalene. Information on the main compounds in EE is presented in table 1. The research results show that terpenoids, flavonoids, and phenolics are the main compound types.

Screening of COVID-19 and atherosclerosis target from main compounds

We obtained 2045 targets connected to 18 main compounds, 5013 targets related to atherosclerosis, and 14606 targets associated with COVID-19. Potential targets in EE for treating COVID-19 and atherosclerosis result from the intersection between main compound targets, atherosclerosis targets, and COVID-19 targets, represented in a Venn diagram. This analysis identified 878 potential targets in EE that exhibit promise for treating COVID-19 and atherosclerosis (fig. 1A).

PPI network analysis

PPI network analysis was performed to identify groups of proteins interacting in complexes and functional pathways related to the observed disease. PPI network analysis shows that there are 878 nodes and 3526 edges (fig. 1B), which are then filtered to get the target core.

Target screening core and central target selection

Core target screening reflects the involvement of potential targets of EE in treating COVID-19 and atherosclerosis. A total of 106 core targets were obtained; these targets were plotted in an interaction network, and 106 nodes and 844 edges were obtained (fig. 2A). Furthermore, to filter central targets, we use the CytoHuba plugin. The central target is the intersection of targets obtained from the five topology parameters (degree, betweenness, closeness, MNC, and bottleneck) (fig. 2B-2F).

Five central targets were obtained: (EP300, HSP90AA1, ESR1, SRC, and RELA) (fig. 2G). These central targets are pivotal components in the network, indicating their potential significance in the therapeutic modulation of COVID-19 and atherosclerosis.

Details regarding the topological characteristics of the 26 core targets are presented in table 2.

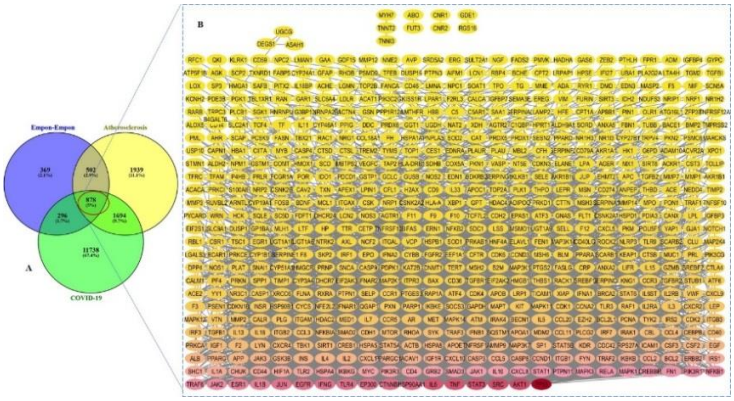


Fig. 1: Potential targets of COVID-19 and atherosclerosis in EE and the PPI network. (A) The Venn diagram shows 878 potential targets of COVID-19 and atherosclerosis in EE. (B) PPI network 878 potential targets of COVID-19 and atherosclerosis in EE. The color change from yellow to red indicates the degree value is increasing

Table 2: Information on the topological characteristics of 26 core targets

Target	Degree	Betweenness centrality	Closenness centrality	Clustering coefficient	Topological coefficient
AKT1	43	0.0714	0.6287	0.2359	0.1836
SRC	42	0.0654	0.6213	0.2706	0.1891
TP53	41	0.0597	0.5899	0.2878	0.1936
STAT3	40	0.0613	0.6105	0.2859	0.2090
EGFR	35	0.0426	0.5966	0.2824	0.1898
ESR1	35	0.0326	0.5899	0.3412	0.2171
TNF	33	0.0481	0.5866	0.3068	0.1856
IL6	32	0.0474	0.5707	0.3024	0.1837
JUN	32	0.0300	0.5738	0.3871	0.2134
HSP90AA1	29	0.0371	0.5615	0.2635	0.1961
NFKB1	28	0.0203	0.5440	0.3836	0.2295
RELA	27	0.0193	0.5412	0.3732	0.2266
CTNNB1	27	0.0191	0.5469	0.3390	0.2238
TLR4	26	0.0315	0.5440	0.2831	0.1871
EP300	26	0.0265	0.5440	0.4154	0.2186
PIK3R1	25	0.0130	0.5330	0.4267	0.2245
MAPK1	25	0.0159	0.5556	0.3833	0.2218
TRAF6	24	0.0220	0.5357	0.2899	0.2057
ERBB2	24	0.0131	0.5440	0.4058	0.2296
FN1	20	0.0302	0.5250	0.3000	0.2068
INS	16	0.0257	0.5122	0.2750	0.1995
PPARG	16	0.0097	0.4976	0.3500	0.2112
CD44	15	0.0143	0.4884	0.2762	0.2008
ACTB	13	0.0065	0.4773	0.2179	0.2222
CASP3	15	0.0106	0.5122	0.3143	0.2049
GAPDH	10	0.0092	0.4646	0.2889	0.2494

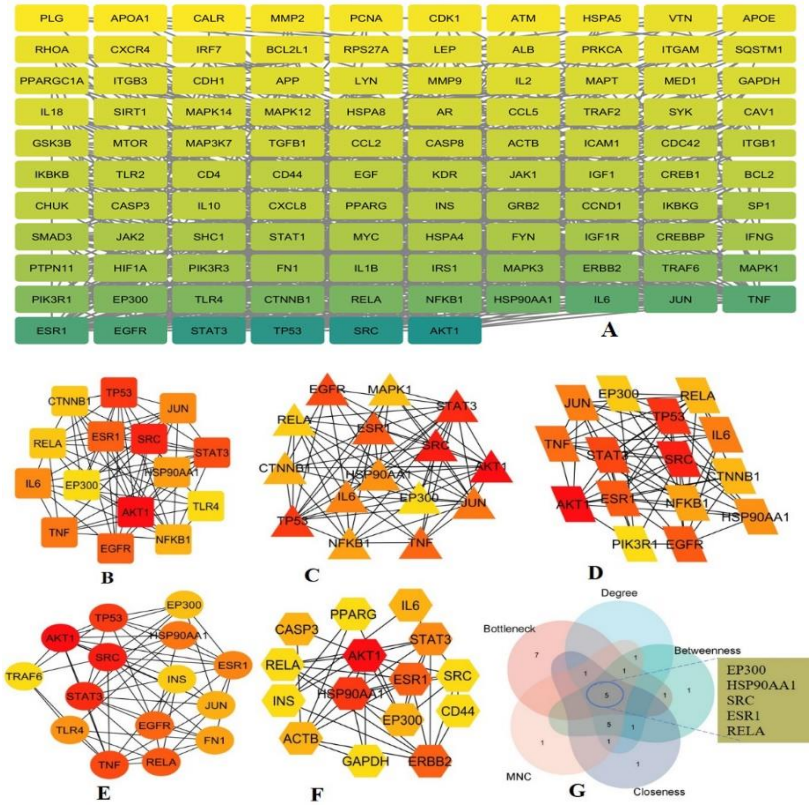


Fig. 2: The network of core and central COVID-19 and atherosclerosis targets in EE. (A) The network of 106 core targets. The degree value is more significant the more saturated the node's color. The network of the top 15 core targets was screened using (B) Degree, (C) CC (D) MNC, (E) BC, and (F) Bottleneck. (G) The Venn diagram shows five central targets

GO functional and KEGG enrichment analysis

KEGG enrichment analysis and GO functional analysis were performed to investigate the biological processes and metabolic pathways involving EE in regulating COVID-19 and atherosclerosis. Research showed that 2521 functional GO were generated, 1000 genes involved in Biological Process (BP), 794 genes involved in Molecular Function (MF), 469 genes involved in Cellular Component (CC), and 258 genes involved in KEGG pathways were annotated. The top 20 items based on the number of annotations to functional areas are represented in fig. 3.

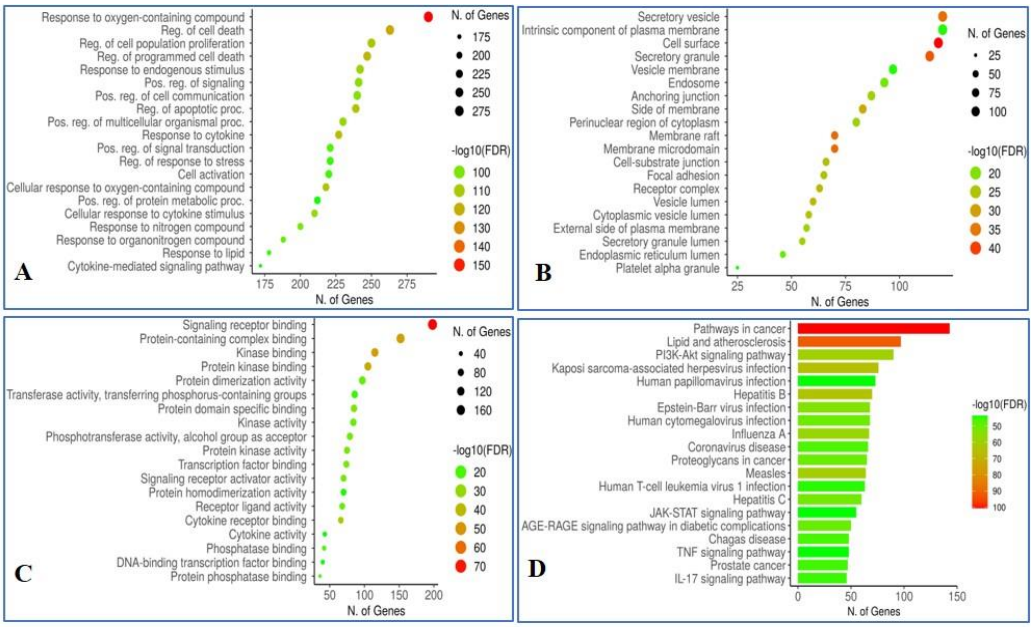


Fig. 3: GO and KEGG enrichment analyses in EE treating COVID-19 and Atherosclerosis. GO using (A) Biological Process, (B) Cellular Component, (C) Molecular Function; and (D) KEGG enrichment analyses in EE. The combined score was used to sort the pathway term results (based on-log FDR)

The most regulated BP is a response to oxygen-containing compounds, regulation of cell death, and regulation of cell proliferation (fig. 3A). In addition, most of the CC are regulated through secretory vesicles, intrinsic components of the plasma membrane, and cell surface (fig. 3B). MF is most regulated through signaling receptor binding, protein-containing complex binding, and signal receptor activity (fig. 3C). Top 20 KEGG enrichment pathways through pathways in cancer, lipid and atherosclerosis, PI3K-Akt signaling pathway including Coronavirus disease (fig. 3D).

Compound–target-pathway interaction network

Integrating 18 main compounds, 106 core targets, and the top 10 KEGG pathways resulted in a network comprising 134 nodes and 861 edges (fig. **6**). Information topology of the compounds and KEGG pathways is shown in table 3. Compounds and metabolic pathways were sorted based on degree values, where a higher degree value indicates more excellent connectivity with the targets.

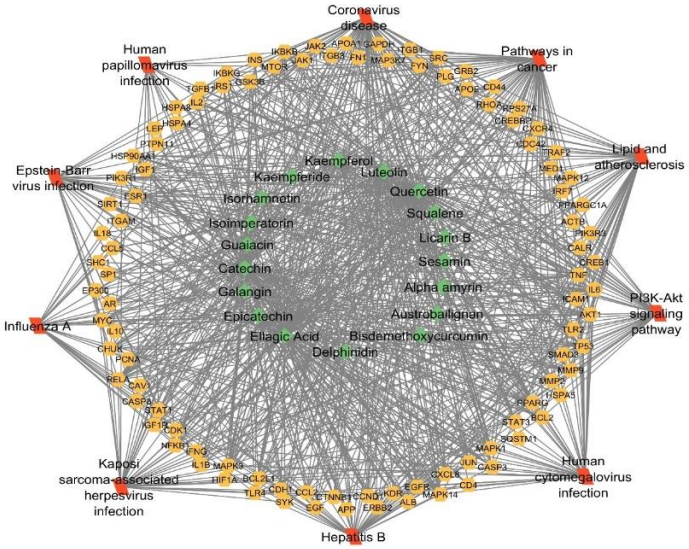


Fig. 4: Compounds–Core target–pathway interaction network. **The orange circle represents the core targets, the green diamond represents the main compounds, and the red rectangle represents the KEGG pathways**

Table 3: Topology of the compounds and pathways

Node	Degree	BC	CC
Catechin	81	0.1737	0.6598
Quercetin	79	0.1580	0.6465
Ellagic Acid	72	0.1490	0.6066
Luteolin	43	0.0307	0.4741
Epicatechin	40	0.0313	0.4638
Kaempferol	38	0.0261	0.4571
Galangin	19	0.0068	0.4025
Squalene	16	0.0049	0.3902
Isorhamnetin	14	0.0031	0.3902
Delphinidin	15	0.0036	0.3902
Isoimperatorin	8	0.0014	0.4655
Bisdemethoxycurcumin	8	0.0009	0.3636
Guaiacin	8	0.0009	0.3721
Kaempferide	7	0.0006	0.3721
Licarin B	6	0.0006	0.3657
Alpha amyrin	6	0.0004	0.3596
Sesamin	5	0.0004	0.3678
Austrobailignan	2	0.0000	0.3575
Coronavirus disease	65	0.0605	0.5161
Pathways in cancer	54	0.0556	0.5161
Lipid and atherosclerosis	44	0.0388	0.4776
PI3K-Akt signaling pathway	38	0.0249	0.4571
Human cytomegalovirus infection	38	0.0242	0.4507
Hepatitis B	38	0.0228	0.4539
Kaposi sarcoma-associated herpesvirus infection	37	0.0205	0.4476
Influenza A	30	0.0156	0.4211
Epstein-Barr virus infection	30	0.0149	0.4267
Human papillomavirus infection	17	0.0031	0.3902

The results of the compound-target-pathway interaction reveal that the main compounds in EE have many connections with core targets that mediate the Coronavirus disease and atherosclerosis pathways. These findings are quantified through degree values, betweenness centrality (BC), and closeness centrality (CC) in topology. Furthermore, compound-target-pathway interaction highlights three principal pathways influenced by the main compounds in EE, specifically Coronavirus disease (degree: 64), Pathways in Cancer

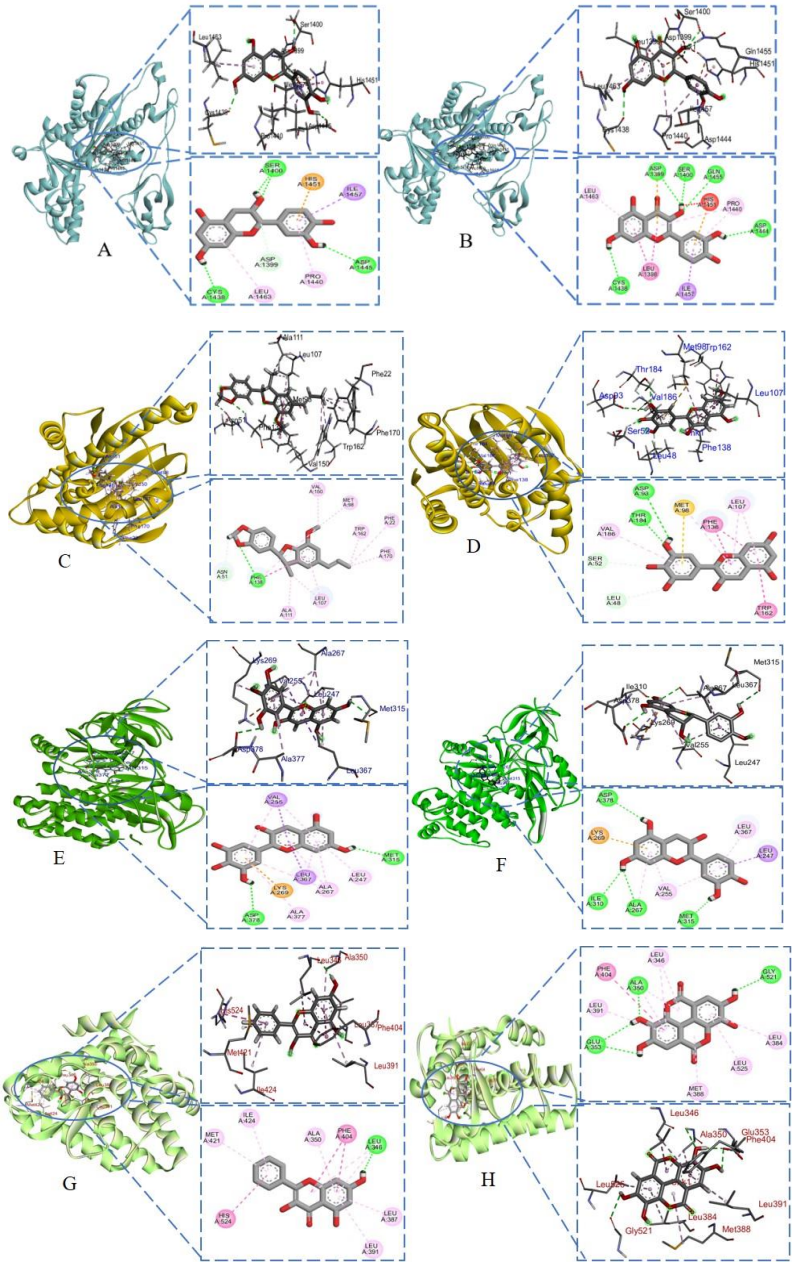
(degree: 54), and Lipid and Atherosclerosis (degree: 44)

Molecular docking

We selected main compounds with degree values above five based on the compound-target-pathway interaction for further validation through molecular docking analysis. Selected main compounds include alpha amyrin, bisdemethoxycurcumin, catechin, delphinidin, ellagic acid, epicatechin, galangin, guaiacin, isoimperatorin, isorhamnetin, kaempferide, kaempferol, licarin B, luteolin, quercetin, sesamin, and squalene. The central targets involved in the interaction analysis were EP300 (PDB ID: 8GZC), HSP90AA1 (PDB ID: 1UY6), SRC (PDB ID: 2C01), RELA (PDB ID: 1VJ7), and ESR1 (PDB ID: 1UOM). The main compound and central target interaction results were quantified based on their binding affinity, as presented in table 4**.** The compound with the best binding affinity was selected for detailed interaction visualization, as depicted in fig. 5.

A lower score of binding affinity indicates a more substantial binding ability. The binding affinity score is significant the more saturated the node's color. The reference drug is highlighted in bold text.

The results of molecular docking analysis revealed that all main compounds in EE that interacted with the central target showed solid binding affinity, each having a score below -7.0 kcal/mol. Catechin, ellagic acid, and quercetin, when bound to the EP300, showed comparable binding affinity to the reference drug (-9.4 kcal/mol). HSP90AA1 displays the highest connectivity to EE's main compound. Molecular docking analysis reveals a robust bond with a binding affinity of<-8.0 kcal/mol for all compounds in EE when bound to HSP90AA1. Epicatechin, isoimperatorin, and delphinidin are the compounds that exhibit the most robust binding to the SRC complex, surpassing the reference drug (Dasatinib: -8.5 kcal/mol). Galangin, ellagic acid, bisdemethoxycurcumin, and squalene are the main compounds that bind strongly to the ESR1 complex, equivalent to reference drugs (Fulvestrant: -8.6 kcal/mol). Guaiacin, licarin B, and sesamin bind robustly more than other compounds to the RELA, surpassing the reference drug (Sulfasalazine: -7.9 kcal/mol). To visualize the type of interaction and the position of the ligand on the receptor site, two compounds with the best binding affinity were selected to represent each target (fig. 5).



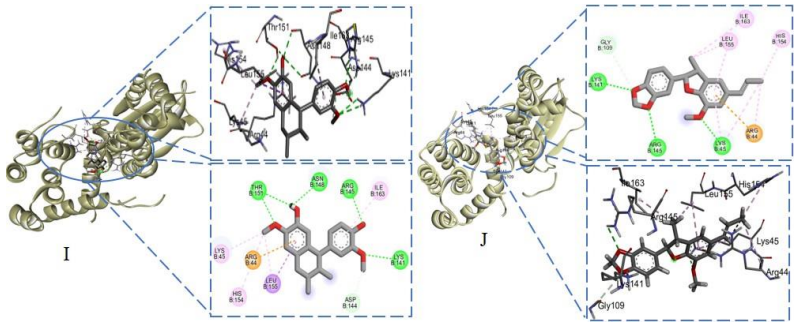


Fig. 5: Molecular docking visualization compounds in EE treating atherosclerosis and COVID-19. (A) EP300-Catechin (B) EP300-Quercetin, (C) HSP90AA1-Licarin B, (D) HSP90AA1-Delphinidin, (E) SRC-Delphinidin, (F) SRC-Epicatechin, (G) ESR1-Galangin, (H) ESR1-Ellagic acid, (I) RELA-Guaiacin, (J) RELA-Licarin B

Table 4: Molecular docking analysis result of main compounds with central targets

Compounds	Binding affinity (kcal/mol)				
	EP300	HSP90AA1	SRC	RELA	ESR1
Bisdemethoxycurcumin					-8.4
Catechin	-9.3	-8.6	-8.5	-7.7	-8.2
Delphinidin		-9.0	-8.6		
Ellagic acid	-9.2	-8.5	-8.2	-8.0	-8.8
Epicatechin		-8.5	-8.6	-7.3	-8.4
Galangin		-8.5		-7.2	-8.6
Guaiacin		-8.8		-8.2	
Isoimperatorin			-8.6		-7.7
Isorhamnetin		-8.1	-8.5	-7.4	-7.9
Kaempferide		-8.5	-8.5		-7.1
Kaempferol		-8.5		-7.1	-8.4
Licarin B		-9.9		-8.0	-7.0
Luteolin		-9.1		-7.7	-7.9
Quercetin	-10.0	-8.9	-7.6	-7.6	-8.2
Sesamin				-8.0	
Squalene			-7.6		-8.6
A-485	-9.6				
Atorvastatin		-9.0			
Dasatinib			-8.5		
Sulfasalazine				-7.9	
Fulvestrant					-8.6

The visualization results depict the binding of the central target complex with the main compounds EE, which play a crucial role in the treatment of both COVID-19 and atherosclerosis. This binding occurs within the designated pocket and involves hydrogen bonds interacting with the protein surface. Additionally, stabilization is achieved through diverse types of carbon-hydrogen bonds, specifically pi-sigma, pi-sulfur, pi-alkyl, and pi-anion interactions, as illustrated in fig. 5. These findings elucidate the potential of EE in treating COVID-19 and atherosclerosis by inhibiting the signaling pathways EP300, HSP90AA1, SRC, ESR1, and RELA.

DISCUSSION

Atherosclerosis is characterized by the accumulation of plaque in arteries, elevating the risk of cardiovascular disease. Viral infections exacerbate this condition, leading to inflammatory disorders and severe complications, including heart attacks, strokes, and mortality [26]. Emerging evidence indicates the efficacy of EE in individuals with atherosclerosis infected with COVID-19 [20]. This therapeutic effect is attributed to EE's terpenoids, flavonoids, and phenolic compounds. However, a comprehensive understanding of the most potent compounds, their target genes, and mechanisms of action remains a research gap, constraining the application of EE.

The utilization of network pharmacology to delineate compound-target interactions and analyze target effects on disease signaling pathways is instrumental in elucidating traditional medicine's pharmacological mechanisms and developing novel drugs.

This study systematically investigated EE's pharmacological mechanisms in treating COVID-19 and atherosclerosis. In the initial step, screening was conducted using the TCMSP database and ProTox II web server based on the compounds' physicochemical and pharmacokinetic properties. We analyzed active compounds that meet the ADMET screening criteria, focusing on parameters such as OB, DL, and LD_{50}. This screening aimed to ensure that the compounds could be effectively absorbed, reach systemic circulation after oral administration and possess a structure analogous to known drugs. The main compounds in EE that meet these stringent criteria include alpha amyrin, austrobailignan, bisdemethoxycurcumin, catechin, delphinidin, ellagic acid, epicatechin, galangin, guaiacin, isoimperatorin, isorhamnetin, kaempferide, kaempferol, licarin B, luteolin, quercetin, sesamin, and squalene. Toxicity predictions indicate that these compounds exhibit a high LD_{50} value (>150 mg/kg), suggesting a lack of toxicity.

In selecting the central target in PPI analysis, apart from using topology parameters, degree, BC, and CC, we also use MNC and bottleneck parameters. The bottleneck parameter serves as a metric to assess the significance of a protein within a network. A protein with a high bottleneck score indicates a more robust connection with proteins related to essential pathways; in terms of regulation in a network, the bottleneck is a much more significant indicator of essentiality than degree [27].

We also used MNC to identify groups of proteins tightly connected to proteins with the same biological function in the network [28, 29] so that the selected proteins genuinely play a central role in the network. Our findings show that the main compounds in EE can potentially treat COVID-19 and atherosclerosis by affecting five central targets: EP300, HSP90AA1, SRC, ESR1, and RELA.

The EP300 protein has been reported to play a pivotal role in upregulating the expression of profibrotic genes, including alpha-smooth muscle actin and collagen, leading to heightened scar tissue formation in arteries. Additionally, dysregulation of EP300 contributes to increased production of proinflammatory cytokines and VEGFA [30]. Increased expression of VEGFA plays an essential role in forming new blood vessels and plaque formation in arteries, which can accelerate the development of atherosclerosis [31]. Inhibition targeting EP300 contributes as a novel therapy against fibrosis. Molecular docking results show that catechin, ellagic acid, isorhamnetin, and quercetin can bind strongly to EP300 (binding affinity<-9.0 kcal/mol), so they have the potential to inhibit EP300 expression. The compounds in EE that bind more strongly to EP300 are catechin and quercetin.

Although HSP90AA1 is a protective protein and maintains hemostatic conditions, its dysregulation can have an impact on undesirable conditions, including its role in inducing inflammation by activating NF-kB and STAT3 transcription, which promotes proinflammatory cytokines including IL-6 and IL-8 [32]. HSP90AA1 also stimulates vascular inflammation and endothelial dysfunction, triggering atherosclerotic plaque development [33]. Current studies indicate elevated levels of HSP90AA1 in individuals with atherosclerosis, and the use of HSP90AA1 inhibitors has shown promise in reducing inflammation associated with atherosclerosis [34]. HSP90AA1 is also known to facilitate the entry of viruses into cells and plays a role in viral gene expression at the transcriptional and translational levels [35]. HSP90AA1 inhibition has potential in therapeutics for atherosclerosis and COVID-19. Our investigation shows that all the main EE compounds that correlate with HSP90AA1 can bind strongly to the binding site (binding affinity<-8.0 kcal/mol); these results indicate that EE has the potential to act as an inhibitor of HSP90AA1. The compounds in EE that bind more strongly to the HSP90AA1 complex are delphinidin and licarin B.

SRC plays a vital role in regulating macrophage functions, including foam cell formation, induction of cellular migration, expression of proinflammatory cytokines, and the development of lesions that trigger plaque growth in arteries [36]. Macrophage activation is involved in the severity of inflammatory diseases, including rheumatoid arthritis, atherosclerosis, diabetes, obesity, cancer, and osteoporosis [37]. Preclinical studies show that SRC inhibition can prevent the development of atherosclerosis by decreasing endothelial ICAM-1 expression, inhibiting macrophage recruitment [38]. Our research reveals that the main compound in EE correlated with SRC can bind strongly to the SRC binding site (binding affinity<-7.0 kcal/mol). Compounds that can bind superiorly to the SRC complex are epicatechin and galangin.

The role of ESR1 has been reported to be involved in the pathological process of Hepatitis B virus (HBV) infection and facilitates viral replication [39]. Clinical studies show that the administration of ESR1 antagonists can reduce VEGFA mRNA and protein [40]. VEGFA is a marker of inflammation because it can stimulate the production of proinflammatory cytokines, thereby expanding inflammation, a condition that can worsen patients with atherosclerosis. Molecular docking analysis showed that the main compounds of EE can bind strongly to the ESR1 complex; the compounds that can bind more strongly are guaiacin and licarin B.

RELA is an NfKB family also known as p65. The role of RELA has been reported to have a significant influence on tumor development and poor prognosis in NSCLS (non-small cell lung cancer) patients [41]. It plays an essential role in the NfKB, pathogenesis of vascular proliferative diseases, inflammation [42], and atherosclerosis [43], so agents targeting RElA gene inhibition are promising therapeutics for curing atherosclerosis and inflammation caused by COVID-19. Our study revealed that the main compounds in EE can bind strongly to the RELA complex (binding affinity<-7.0 kcal/mol), and the main compounds that can bind very strongly are guaiacin, licarin B, sesamin, and ellagic acid.

In this study, network pharmacology analysis and molecular docking technology revealed the mechanism of EE in treating COVID-19 and atherosclerosis through the pathway in cancer, lipid and atherosclerosis, and coronavirus disease signaling pathways through inhibition of central targets: EP300, HSP90AA1, SRC, ESR1, and RELA, these results form the basis of which robust for further research related to COVID-19 and atherosclerosis.

CONCLUSION

In this study, 18 main compounds in EE were obtained, namely alpha amyrin, austrobailignan, bisdemethoxycurcumin, catechin, delphinidin, ellagic acid, epicatechin, galangin, guaiacin, isoimperatorin, isorhamnetin, kaempferide, kaempferol, licarin B, luteolin, quercetin, sesamin and squalene. PPI analysis followed by GO and KEGG analysis revealed that the pharmacological actions of EE in treating COVID-19 and atherosclerosis through the central signaling targets EP300, HSP90AA1, SRC, ESR1, and RELA. Molecular docking results showed that catechin and quercetin showed the highest potential in inhibiting EP300; licarin B and delphinidin inhibit HSP90AA1; epicatechin and delphinidin inhibit SRC; galangin and ellagic acid inhibit ESR1; and guaiacin and licarin B inhibit RELA. Overall, this research provides a strong foundation regarding the main compound of EE and its mechanism of action in treating atherosclerosis and COVID-19, suggesting its potential as a novel therapeutic agent.

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AUTHORS CONTRIBUTIONS

The manuscript was written through the contributions of all authors, and all authors have approved the final version.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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