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RESEARCH ARTICLES

Analisis *Network Pharmacology-based* terhadap Senyawa Aktif Tanaman Rosela *(Hibiscus sabdariffa)* dan Manggis (*Garcinia mangostana L.)* sebagai Anti-Hiperkolesterolemia

Network Pharmacology-based Analysis of Chemical Compounds from Roselle (Hibiscus sabdariffa) and Garcinia mangostana L. for Anti-hypercholesterolemic Activity

Diah Dwi Darma[1], Habiburrahman Zulfikri[2,3], Heri Setiawan[1*]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia
[2]Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Indonesia
[3]Biological Systems Engineering Lab, Institute for Biosystems and Bioengineering, Universitas Indonesia, Depok, Indonesia

Abstract

Roselle tea *(Hibiscus sabdariffa)* is widely used as a traditional remedy for hypertension and hyperlipidemia, while mangosteen (Garcinia mangostana L.) is known to contain secondary metabolites with potential efficacy in the management of hypercholesterolemia. This study aimed to investigate the activity and mechanism of action of the combination of both plants as anti-hypercholesterolemic agents through a Network Pharmacology-based analysis. Active compound identification was performed using the KNApSAcK Family database, ADMET analysis was conducted using the pkCSM and ProTox servers, and protein-protein interactions were analyzed using Cytoscape and STRING. Mechanisms of action were further elucidated through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathwayy analyses using ShinyGO 0.77. The results indicated that β-sitosterol from mangosteen occupied a central role in the anti-hypercholesterolemic interaction network, as indicated by the highest degree value (12.6658), while β-sitosterol, 3,4-dihydroxybenzoic acid, and quercetin from roselle were found to regulate key target genes including ABCA1, APOA1, BAX, BCL2, CASP3, and CD36. These genes were involved in lipid metabolism and the pathophysiology of atherosclerosis, which represents a primary complication of hypercholesterolemia. Further research is warranted to validate the efficacy of the combination of extracts or active compounds from both plants, both in vitro and in vivo.

Keywords: *Hibiscus sabdariffa,* hypercholesterolemia, network pharmacology

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Email:
info@salnesia.id, jika@salnesia.id
Phone:
+62 85255155883

Abstrak

Teh Rosella (Hibiscus sabdariffa) digunakan secara luas sebagai pengobatan tradisional untuk hipertensi dan hiperlipidemia, sedangkan manggis (Garcinia mangostana L.) diketahui mengandung metabolit sekunder yang berpotensi dalam penanganan hiperkolesterolemia. Penelitian ini bertujuan untuk mengetahui aktivitas dan mekanisme kerja kombinasi kedua tanaman tersebut sebagai agen anti-hiperkolesterolemia melalui analisis berbasis network pharmacology. Penelusuran senyawa aktif dilakukan melalui basis data KNApSAcK Family, analisis ADMET menggunakan pkCSM dan ProTox, serta interaksi antarprotein dianalisis menggunakan Cytoscape dan STRING. Mekanisme kerja ditelusuri melalui analisis Gene Ontology (GO) dan Kyoto Encyclopedia of Genes and Genomes (KEGG) menggunakan ShinyGO 0.77. Hasil menunjukkan bahwa β-sitosterol dari manggis memiliki peran sentral dengan nilai degree tertinggi (12,6658), sementara β-sitosterol, asam 3,4-dihidroksibenzoat, dan quercetin dari Rosella meregulasi gen ABCA1, APOA1, BAX, BCL2, CASP3, dan CD36. Gen-gen tersebut terlibat dalam metabolisme lipid dan patofisiologi aterosklerosis yang merupakan komplikasi utama dari hiperkolesterolemia. Penelitian lebih lanjut diperlukan untuk membuktikan efektivitas kombinasi ekstrak atau senyawa aktif dari kedua tanaman ini, baik secara in vitro maupun in vivo.

Kata Kunci: *Hibiscus sabdariffa, hiperkolesterolemia, network pharmacology*

*Correspondence Author:
Heri Setiawan, email: heri.setiawan@farmasi.ui.ac.id

Highlight:

- The combination of roselle and mangosteen produces numerous potential active compounds that work together simultaneously to lower bad cholesterol levels in the body.
- The active compounds from both plants are proven to effectively interact directly with key proteins that regulate fat metabolism and cholesterol formation.
- Analysis shows that this combination works by regulating lipid (fat) metabolism pathways and vitamin absorption pathways, making it highly potential for development as a functional herbal therapy for hypercholesterolemia.

INTRODUCTION

Hypercholesterolemia is a prominent modifiable risk factor for cardiovascular disease (CVDs), typically characterized by a plasma low-density lipoprotein cholesterol (LDL-C) level of ≥ 130 mg/dL (Bosco et al., 2024). CVDs represent a major contributor to global mortality among chronic non-communicable diseases (NCDs). According to the World Health Organization (WHO), NCDs remain the leading cause of mortality worldwide, accounting for approximately 75% of premature deaths (before the age of 70), particularly in low- and middle-income countries (WHO, 2022). The use of pharmacological agents, dietary modifications, and herbal preparations to manage CVDs, particularly in controlling hyperlipidemia can reduce risk factors associated with

hypertension, obesity, diabetes, and coronary heart disease. While pharmacological agents such as statins are widely used to manage hypercholesterolemia, their long-term use may be associated with adverse effects, prompting increasing interest in plant-derived bioactive compounds as complementary or alternative therapeutic options. *Hibiscus sabdariffa* (HS), commonly referred to as Rosella, Jamaica sorrel, red sorrel, Indian red sorrel, wonjo, and karkade, is native to India and Malaysia (Solangi et al., 2017). *Hibiscus sabdariffa* L. is an annual dicotyledonous plant belonging to the Malvaceae family. Phytochemical investigations have identified the presence of phytosterols, flavonoids, saponins, and various glycosides, as well as carbohydrates, ascorbic acid, citric and malic acids, and pigments including hibiscin, gossypetin, quercetin, myricetin, hibiscetin, and sabdaretin. The primary pigments are anthocyanins namely cyanidin-3-glycoside and delphinidin-3-glycoside which possess antioxidant properties and do not exhibit toxic or mutagenic effects (Aguirre-García et al., 2019). Roselle tea is widely consumed globally both as a beverage and as a traditional remedy for hypertension and hyperlipidemia (Hopkins et al., 2013). Sabzghabaee et al. (2013) reported that HS calyx powder produced a significant decrease in serum total cholesterol, LDL, and triglyceride levels in 72 participants. Supplementation with HS has been shown to reduce total cholesterol and LDL cholesterol, though its effect on triglyceride reduction remains inconsistent across studies. Despite evidence of beneficial effects on the lipid profile in animal and human studies, Aziz et al. (2013) found that HS did not produce a statistically significant effect compared to placebo, black tea, or dietary intervention alone, suggesting that the therapeutic potential of HS may be enhanced through combination with other bioactive plant sources.

Garcinia mangostana L., commonly known as mangosteen, is utilized across various parts of the plant as an alternative medicine for a broad range of diseases, with documented pharmacological effects including antidiabetic, antihyperlipidemic, antiobesity, antioxidant, analgesic, anti-inflammatory, anticancer, antidepressant, antibacterial, renoprotective, and neuroprotective properties. This plant is widely available in Southeast Asian countries including Indonesia, Malaysia, Myanmar, Thailand, Cambodia, and Vietnam (Mahmudah et al., 2020). The mangosteen pericarp and roselle flower petals have been used in Indonesia as antiproliferative, anti-inflammatory, and antimicrobial agents.

Despite the individual therapeutic promise of both *H. sabdariffa* and *G. mangostana*, research specifically investigating their combination particularly in the context of anti-hypercholesterolemic activity remains scarce, representing a critical gap in the current literature. To address this gap, the present study aimed to investigate the bioactive compounds of *H. sabdariffa* and *G. mangostana* and their mechanisms of action against hypercholesterolemia using a Network Pharmacology-based approach. Network pharmacology integrates drug and disease targets within biological information networks by capitalizing on the systematic interplay between pharmacological agents and their disease-related targets (Li et al., 2022). This approach enables comprehensive and systematic screening of secondary metabolites through the predicted interaction of disease-related targets and active compound components, thereby providing a scientific basis for the development of herbal-based combination therapies for hypercholesterolemia.

METHODS

Identification of active compounds from *Hibiscus sabdariffa* and *Garcinia*

mangostana L. plants was carried out based on the KNApSAcK family database (Afendi et al., 2012). Furthermore, the identification of physicochemical and pharmacokinetic properties from pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) (Pires et al., 2015) and toxicity data were obtained from the ProTox prediction server (https://tox-new.charite.de/protox_II/) (Banerjee et al., 2024). Compounds meeting the acceptance criteria based on Lipinski's rule of five that have good ADME and low toxicity are then identified as protein targets via the GeneCards website (https://www.genecards.org/). The top 25 gene targets were selected for each active compound search.

Disease target identification was performed using the GeneCards database. The top 200 targets were selected from the protein target search using the keyword "hypercholesterolemia." Overlapping gene targets between hypercholesterolemia and the test compounds were then identified using a Venn diagram generated via the web tool Venny.

Compound data from the KNApSAcK Family database and protein target data were subsequently integrated, and the relationships among plants and their constituent compounds, compounds and target proteins, and target proteins associated with anti-hypercholesterolemic activity were visualized using the Cytoscape 3.9.1 bioinformatics platform. Within Cytoscape, the identified active compounds and their corresponding targets were imported into the software to construct a regulatory network diagram depicting the "active compound–potential therapeutic target" interactions, with results visualized according to the distinct properties of each node (Li et al., 2022).

The identified potential therapeutic targets were imported into the STRING network platform (https://string-db.org/), with the research species set to "Homo sapiens." Targets with the highest reliability (score > 0.9) were selected through a comprehensive assessment, while discrete targets were hidden (Zhao et al., 2022). A protein-protein interaction (PPI) network diagram was then constructed, and the data was exported in TSV format. This TSV file was imported into Cytoscape, where topology analysis was conducted using the CytoNCA Plug-in. By evaluating the values of betweenness, closeness, degree, eigenvector, and local average connectivity, the core gene network was established, focusing on networks with values exceeding their median. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed using the ShinyGO 0.7 website (http://bioinformatics.sdstate.edu/go/). This analysis was performed to see the GO function and KEGG Pathwayy enrichment of the active compound target for antihyperlipidemic. Then, the Human species was selected, a list of therapeutic targets was entered, and 30 Pathwayys were selected to be shown in the analysis results. Analysis with GO has three types of analysis, namely biological process (BP), cellular component (CC), and molecular function (MF). The results of each analysis can be filtered based on the enrichment value FDR (False discovery rate) <0.05. Hits with a P-value of 0.05 were selected and utilized to generate GO histograms and bubble maps for KEGG signaling Pathwayys (Ge et al., 2020). Ultimately, Cytoscape software was employed to construct a network diagram of common target signaling Pathwayys.

RESULTS AND DISCUSSIONS

Based on the search results from the KNApSAcK Family database, sixteen active compounds were identified from *H. sabdariffa*, namely eugenol, myricetin, trigonelline, 3,4-dihydroxybenzoic acid, cholesterol, ergosterol, spinasterol, quercetin, hibiscetin,

hibiscetin 3-glucoside, delphinidin 3-sambubioside, citric acid, D-galactose, alpha-terpineol, daphniphylline, and malic acid. Some of these compounds belong to the flavonoid class, including myricetin, delphinidin 3-sambubioside, and quercetin, while others belong to the terpenoid group, such as spinasterol. The active compounds identified in *H. sabdariffa* extract may be further expanded through literature searches and access to additional databases. A Network Pharmacology-based study reported approximately 50 active compounds associated with anti-hyperglycemic effects in *H. sabdariffa* (Niu et al., 2022). Herranz-Lopez et al. (2017) reported that approximately 21 flavonoid compounds in *H. sabdariffa* extract had been structurally identified by mass spectrometry.

A search for active compounds using the keyword "*Garcinia mangostana* L." on the KNApSAcK database yielded three metabolites: (−) -β-sitosterol, talbotaflavone, and cyanidin 3-(2G-glucosylrutinoside). The literature indicates that the primary metabolites in mangosteen are xanthones, particularly α-mangostin, β-mangostin, and γ-mangostin (Obolskiy et al., 2009), which were not found in this database. Of the three active compounds identified from *G. mangostana*, only β-sitosterol could be mapped to gene targets via the GeneCards database, resulting in a total of seventeen active compounds carried forward for further analysis. The KNApSAcK Family database was selected in this study as it constitutes one of the most comprehensive and well-curated repositories of plant-derived secondary metabolites, providing reliable and species-specific metabolite relational data that is particularly suited for natural product-based pharmacological investigations (Afendi et al., 2012).

All seventeen active compounds were subsequently analyzed for their physicochemical, pharmacokinetic, and toxicity properties using the pkCSM web server and the ProTox prediction server. The pkCSM platform was selected for its validated, graph-based machine learning models that generate reliable predictions of ADMET properties, while ProTox prediction server was employed for toxicity profiling owing to its capacity to predict oral toxicity, toxicity classification, and multiple toxicological endpoints based on experimentally verified toxicity data (Pires et al., 2015; Banerjee et al., 2024). The results yielded eight compounds meeting the criteria of Lipinski's rule of five with acceptable toxicity profiles, as presented in Table 1. Lipinski's rule of five states that orally bioavailable drugs must have a molecular weight of ≤ 500 Da, a hydrogen bond donor (HBD) count of ≤ 5, a hydrogen bond acceptor (HBA) count of ≤ 10, and a log P (octanol-water partition coefficient) value of ≤ 5 (Lipinski, 2004). These parameters serve as essential criteria for oral therapeutic compounds to ensure maximal absorption across biological membranes. The remaining seven compounds trigonelline, 3,4-dihydroxybenzoic acid, quercetin, citric acid, D-galactose, alpha-terpineol, and malic acid demonstrated log *p-values* of < 5 and molecular weights of < 500 Da, satisfying the Lipinski criteria, as detailed in Table 2.

Assessment of ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties was conducted to select compounds with potential for drug development that demonstrate acceptable safety profiles. Human intestinal absorption described the ability of a compound to be absorbed across the intestinal membrane, while skin permeability reflected the degree of compound penetration through the skin. Among the identified compounds, alkaloids such as trigonelline and terpenoids such as alpha-terpineol and β-sitosterol demonstrated favorable intestinal absorption. The volume of distribution at steady state (VD_{ss}) described the extent to which a drug is distributed throughout the body relative to its plasma concentration, with an acceptable threshold value of ≥ −0.15 L/kg. Quercetin exhibited the highest VD_{ss} value (1.559),

followed by the two terpenoid compounds, alpha-terpineol (0.207) and β-sitosterol (0.193). Terpenoid compounds also demonstrated the ability to penetrate the blood-brain barrier (BBB).

Metabolic characteristics were evaluated through microsomal enzyme analysis, specifically focusing on inhibitory and substrate interactions with cytochrome P450 2D6 (CYP2D6), which catalyzes reactions involved in drug metabolism. CYP2D6 is an enzyme encoded by the *CYP2D6* gene in humans that plays a crucial role in metabolism and elimination of approximately 25% of clinically used medications. Consequently, for drugs metabolized by CYP2D6, individuals with ultrarapid metabolizer phenotypes eliminate these drugs rapidly, thereby reducing therapeutic efficacy, while those with poor metabolizer phenotypes exhibit slow elimination, leading to elevated plasma drug levels and an increased likelihood of adverse effects (Wang and Skolnik, 2009). Notably, all eight selected compounds were found to be neither substrates nor inhibitors of the CYP2D6 enzyme, suggesting a low potential for CYP2D6-mediated drug interactions.

Drug excretion parameters were assessed through organic cation transporter 2 (OCT2) and total renal clearance. Total renal clearance indicates the rate of compound elimination from the body through the kidneys, expressed in units of mL/min/kg body weight. OCT2 is the primary renal uptake transporter that is essential for the disposition and renal clearance of most cationic drugs and endogenous substances. Current guidelines from the FDA and EMA advise assessing OCT2 liability for drugs that have significant renal elimination or are likely to be co-administered with OCT2 substrates (Koepsell, 2004). The eight selected compounds were not OCT2 substrates and had a total clearance ranging from 0.407 to 1.219 mL/min/kg. Toxicity profiling was subsequently conducted based on the median lethal dose (LD_{50}). The lowest toxicity was found in trigonelline, with an LD_{50} value of 5,000 mg/kg, classified under toxicity class 5, while the highest toxicity was found in citric acid, with an LD_{50} value of 89 mg/kg, classified under toxicity class 3.

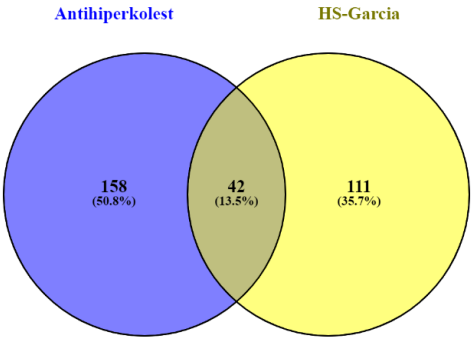


Figure 1. Venn diagram of active compound targets (yellow) and disease targets (blue)

The eight selected compounds were subsequently subjected to protein target identification using the GeneCards database. For two other compounds found in mangosteen, namely talbotaflavone and cyanidin 3-(2G-glucosylrutinoside), no data was found on the genecard, presumably due to limited research on these compounds within current databases. The top 25 proteins were retrieved from each compound found on the genecards. The total protein targets from the 8 compounds were 153 protein targets. Identification of protein targets generated 2390 candidate targets, from which

the top 200 targets were selected based on their relevance scores. Furthermore, the intersection between the disease target and the active compound was selected and 42 target proteins were obtained (Figure 1).

In qualitative analysis with Cytoscape 3.9.1, there were 7 active compounds connected to 42 anti-hypercholesterolemia protein targets and 49 nodes (7 active compound nodes and 42 protein target nodes). Furthermore, quantitative analysis was carried out using the Analyze Network menu to determine the total value of Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC) at each node. These parameters describe the important role of a node or in this case the active compound in a network. The results showed the highest value with a total degree of 12.6658 for the active compound beta-sitosterol, followed by 12.6554 for quercetin, and 10.9982 for 3,4-dihydroxybenzoic acid (Table 3). The highest degree value recorded for β-sitosterol, derived from G. mangostana, suggests that this compound occupies a central position within the anti-hypercholesterolemic interaction network.

The 42 target proteins were analyzed for protein-protein interactions using STRING-db. A total of 38 nodes and 371 edges were identified, where nodes represent target proteins and edges represent their interactions. The PPI enrichment p-value was <1.0e-16, indicating that these proteins interact more frequently than expected for a random set of similar size and degree distribution. This enrichment suggests that the proteins are at least partially biologically related as a group (Figure 3). Protein interactions were further visualized using Cytoscape 3.9.1 and quantified based on Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC). STRING-db data in TSV format were imported into Cytoscape. The results showed that AKT1 had the highest degree value (66.9814), followed by ALB (60.8909) and TNF (60.8886).

AKT1 is a serine/threonine kinase directly involved in the pathogenesis of malignancy. Previous network pharmacology studies have identified AKT1 as a core target protein with critical roles in hyperlipidemia. It is also involved in insulin signaling, endothelial function, and metabolic regulation (Cheng et al., 2024). Tumor necrosis factor (TNF) is a cytokine that acts as a major regulator of inflammatory responses. It is upregulated in hypercholesterolemia, contributing to vascular inflammation and endothelial dysfunction, and promotes atherosclerosis by enhancing adhesion molecule expression and immune cell recruitment (Prasad and Mishra, 2022). Furthermore, ALB, which encodes albumin, does not have a direct role in hypercholesterolemia or atherosclerosis pathophysiology. However, albumin is essential for lipid transport and metabolism. As a result, patients develop hypercholesterolemia as a compensatory increase in circulating lipoproteins, making it a key biochemical feature of the condition (Suppressa et al., 2019; Varughese et al., 2022).

In the lipid and atherosclerosis Pathwayy (Figure 6) the targets marked in red are the target genes of several active compounds tested. Some of these are ABCA1, APOA1, BAX, BCL2, and FAS that regulated by β-sitosterol in mangosteen. Quercetin in Rosella plants has BAX, BCL2, CASP3 and MAPK1 targets. 3,4-Dihydroxybenzoic acid in Rosella plants regulates BAX and CD36 proteins, while Trigonelline has AKT1 and TNF, and Malic acid in PIK3CA, PPARG, and TNF. These genes not only play a role in lipid metabolism and the formation of atherosclerosis, namely FAS, CD38, PPARG, ABCA1 (Feng et al., 2018).

ShinyGO analysis on 42 genes can be seen in Figure 5. The top 20 items were visualized and analyzed to produce histogram and lollipop graphs against BP, CC, and MF. The results obtained by the GO BP value showed that Neuron apoptotic process

had the highest FDR value with 15 genes involved in 253 Pathwayys. The highest FDR value in GO CC was in Phosphatidylinositol 3-kinase complex, class IA with 2 genes involved in 3 Pathwayys, while in GO MF, BH3 domain binding had the highest FDR value with 3 genes involved in 6 Pathwayys. KEGG analysis showed that Platinum drug resistance was the mechanism Pathwayy with the highest FDR value, but the Lipid and atherosclerosis mechanism Pathwayy had the highest number of genes (18 genes) involved in the network. Based on the fold enrichment value, Cholesterol metabolism was ranked second (73.9) after Platinum drug resistance (92.8).

In this study, the KEGG Pathwayy findings were integrated with Cytoscape-based topological analysis to provide mechanistic insight into the differential contributions of individual compounds to the lipid and atherosclerosis Pathwayy. Compounds exhibiting the highest degree values in the Cytoscape network namely β-sitosterol and quercetin were found to regulate the greatest number of target genes within this Pathwayy, suggesting that their broad multi-target engagement is the primary driver of the Pathwayy's high gene count. This convergence between high topological centrality in the Cytoscape network and regulatory involvement in the lipid and atherosclerosis Pathwayy strongly suggests that β-sitosterol and quercetin constitute the principal bioactive drivers of the anti-hypercholesterolemic activity attributed to the combination of *H. sabdariffa* and *G. mangostana*.

Hypercholesterolemia is the primary contributor to atherosclerosis and related conditions, including coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. Furthermore, atherosclerosis is a chronic inflammatory condition marked by the narrowing of blood vessels due to lipid-rich plaques that develop within the arterial walls. High levels of low-density lipoprotein (LDL) cholesterol are a significant risk factor for this disease. Oxidized LDL (oxLDL) leads to endothelial dysfunction, which triggers the expression of adhesion molecules and the recruitment of monocytes to the subendothelial space. These monocytes then proliferate, differentiate into macrophages, and absorb lipoproteins, resulting in the formation of enlarged cholesterol "foam cells." Over time, these foam cells undergo cell death, creating a "necrotic core" composed of crystalline cholesterol and cellular debris. Smooth muscle cells then proliferate and migrate to the site, forming a protective layer over the lesion (Jebari-Benslaiman et al., 2022).

β-sitosterol has the highest total degree based on the results of cytoscape analysis (12.6658). While β-sitosterol exhibited log P values exceeding 5 (Table 1) due to low aqueous solubility attributed to its nonpolar nature and structural resemblance to steroid compounds, literature evidence supports its notable activity against hyperlipidemia, including its ability to regulate lipid metabolism in hyperlipidemic mice, reduce fat accumulation in hepatic cells, and decrease the weight of liver and adipose tissue (Saeidnia et al., 2014). To improve the lipid solubility of β-sitosterol and broaden its pharmaceutical applications, efforts have been made to incorporate it into a self-microemulsifying drug delivery system (SMEDDS). Yuan et al. (2019) demonstrated that a β-sitosterol self-microemulsion formulation produced a significantly greater blood lipid-lowering effect in mice compared to the control group. The European Food Safety Authority (EFSA) recommends that consumption of phytosterols and/or stanols of around 1.5–2.4 g/day reduces blood cholesterol levels which reduces the risk of heart disease and is also approved by the FDA (Babu and Jayaraman, 2020).

Table 1. Physicochemical properties of Rosella and Mangosteen plant compounds

Nama Tanaman	Metabolit	Pubchem ID	Molecular Weight	LogP	Rotatable Bonds	Acceptors	Donors
Hibiscus sabdariffa	Trigonelline	134606	173.599	–27867.00	1	1	1
Hibiscus sabdariffa	3,4-Dihydroxybenzoic acid	72	154.121	0.55	1	3	3
Hibiscus sabdariffa	Quercetin	5280343	302.238	1.99	1	7	5
Hibiscus sabdariffa	Citric acid	311	192.123	–1.25	5	4	4
Hibiscus sabdariffa	D-Galactose	6036	180.156	–3.22	1	6	5
Hibiscus sabdariffa	alpha-Terpineol	17100	154.253	2.50	1	1	1
Hibiscus sabdariffa	Malic acid	525	134.087	–1.09	3	3	3
Garcinia mangostana L.	Beta Sitosterol	222284	414.718	8.02	6	1	1

Table 2. Pharmacokinetic properties and toxicity of Rosella and Mangosteen plant compounds

Metabolites	Water solubility	Intestinal absorption (human)	Skin Permeability	VDss (human)	BBB permeability	CYP2D6 substrate	CYP2D6 inhibitior	Total Clearance	Renal OCT2 substrate	Predicted LD50 in mg/kg	Predicted Toxicity Class
Trigonelline	–1.975	100.00	–2.734	–0.679	–0.216	No	No	0.907	No	5,000	5
3,4-Dihydroxybenzoic acid	–2.069	71.174	–2.727	–1.298	–0.683	No	No	0.551	No	2,000	4
Quercetin	–2.925	77.207	–2.735	1.559	–1.098	No	No	0.407	No	159	3
Citric acid	–1.423	0.000	–2.735	–0.418	–1.017	No	No	0.895	No	80	3
D-Galactose	–1.377	21.510	–3.041	0.148	–0.943	No	No	0.626	No	23,000	6
alpha-Terpineol	–2.039	94.183	–2.418	0.207	0.305	No	No	1.219	No	2,830	5
Malic acid	–1.381	13.831	–2.735	–0.998	–0.788	No	No	0.810	No	2,497	5
Beta-Sitosterol	–6.773	94.464	–2.783	0.193	0.781	No	No	0.628	No	89	4

Table 4. Total degree value of 20 anti-hypercholesterolemia target proteins

Name	Betweenness Centrality	Closeness Centrality	Degree	Total
AKT1	0.0583	0.9231	66	66.9814
ALB	0.0338	0.8571	60	60.8909
TNF	0.0314	0.8571	60	60.8886
INS	0.0323	0.8372	58	58.8695
STAT3	0.0283	0.8372	58	58.8655
TP53	0.0270	0.8372	58	58.8642
CASP3	0.0193	0.8182	56	56.8375
JUN	0.0173	0.8182	56	56.8355
PPARG	0.0352	0.8000	54	54.8352
HIF1A	0.0145	0.7826	52	52.7971
CCL2	0.0097	0.7660	50	50.7757
PTGS2	0.0080	0.7660	50	50.7739
CASP8	0.0116	0.7500	50	50.7616
PPARA	0.0476	0.7500	48	48.7976
IL10	0.0070	0.7500	48	48.7570
CAT	0.0053	0.7500	48	48.7553
BCL2L1	0.0119	0.7347	48	48.7466
MAPK14	0.0131	0.7347	46	46.7478
APP	0.0056	0.7200	44	44.7256
HMOX1	0.0028	0.7200	44	44.7228

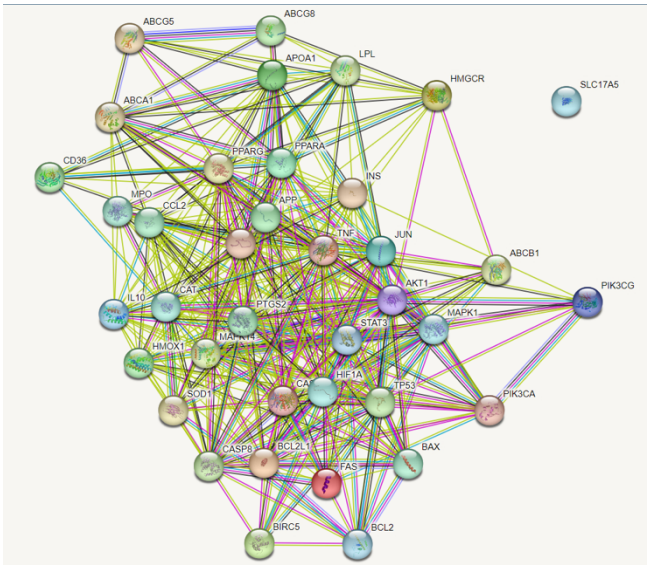


Figure 3. Visualization of PPI network

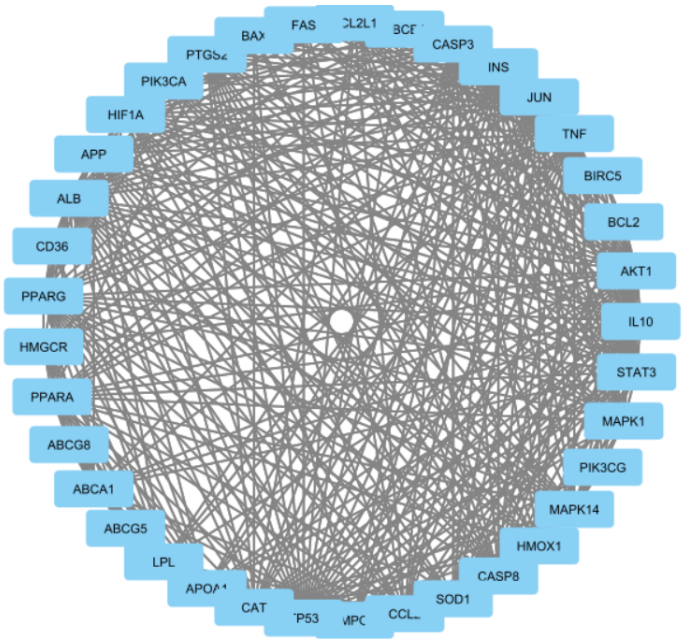
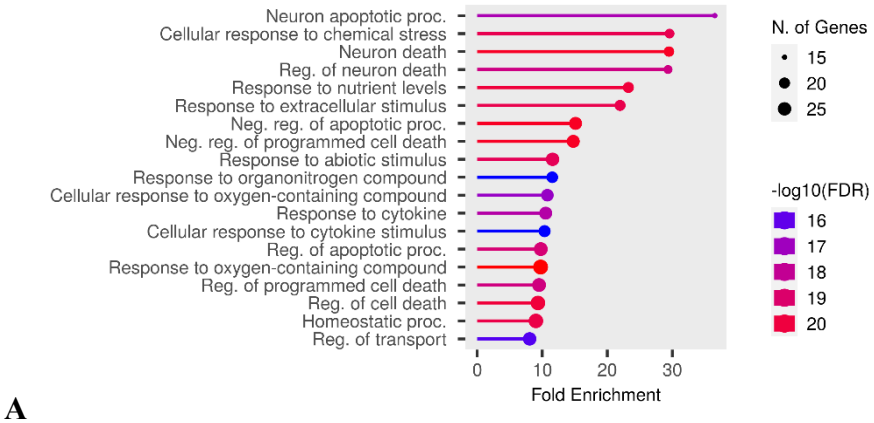


Figure 4. Cytoscape visualization describing PPI



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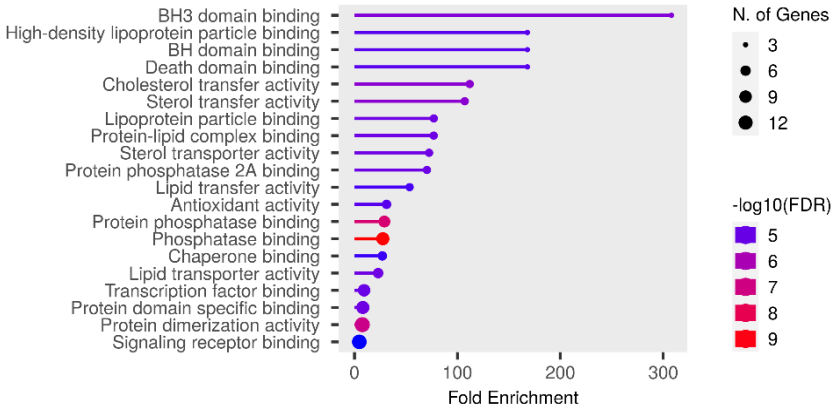
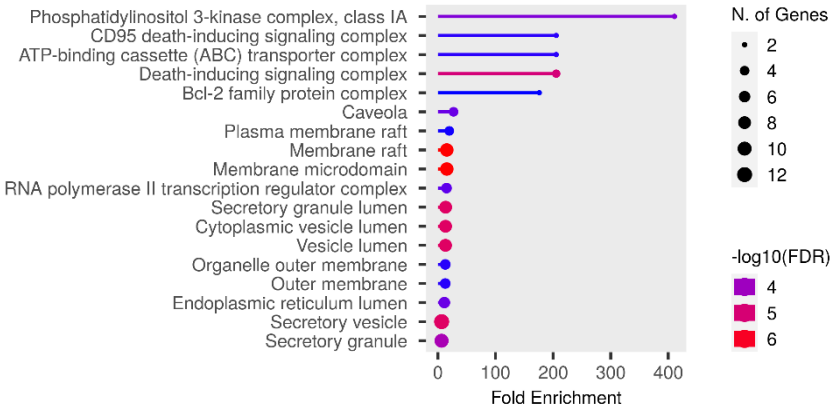
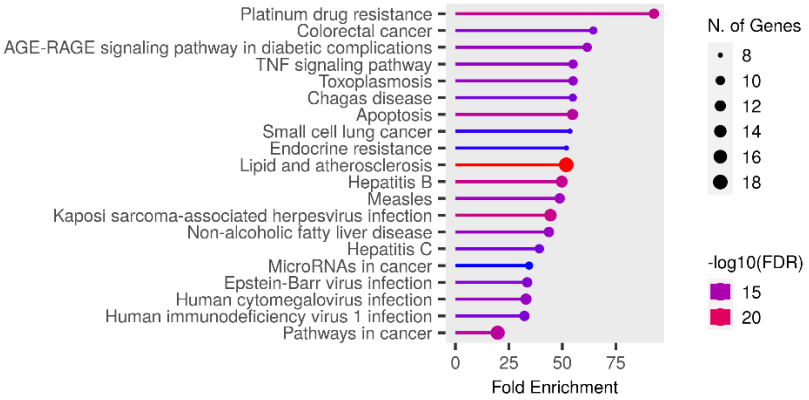


B



C



D

Figure 5. Results of enrichment analysis, A: GO biological process; B: GO molecular function; C: GO cellular components, D: KEGG enrichment results

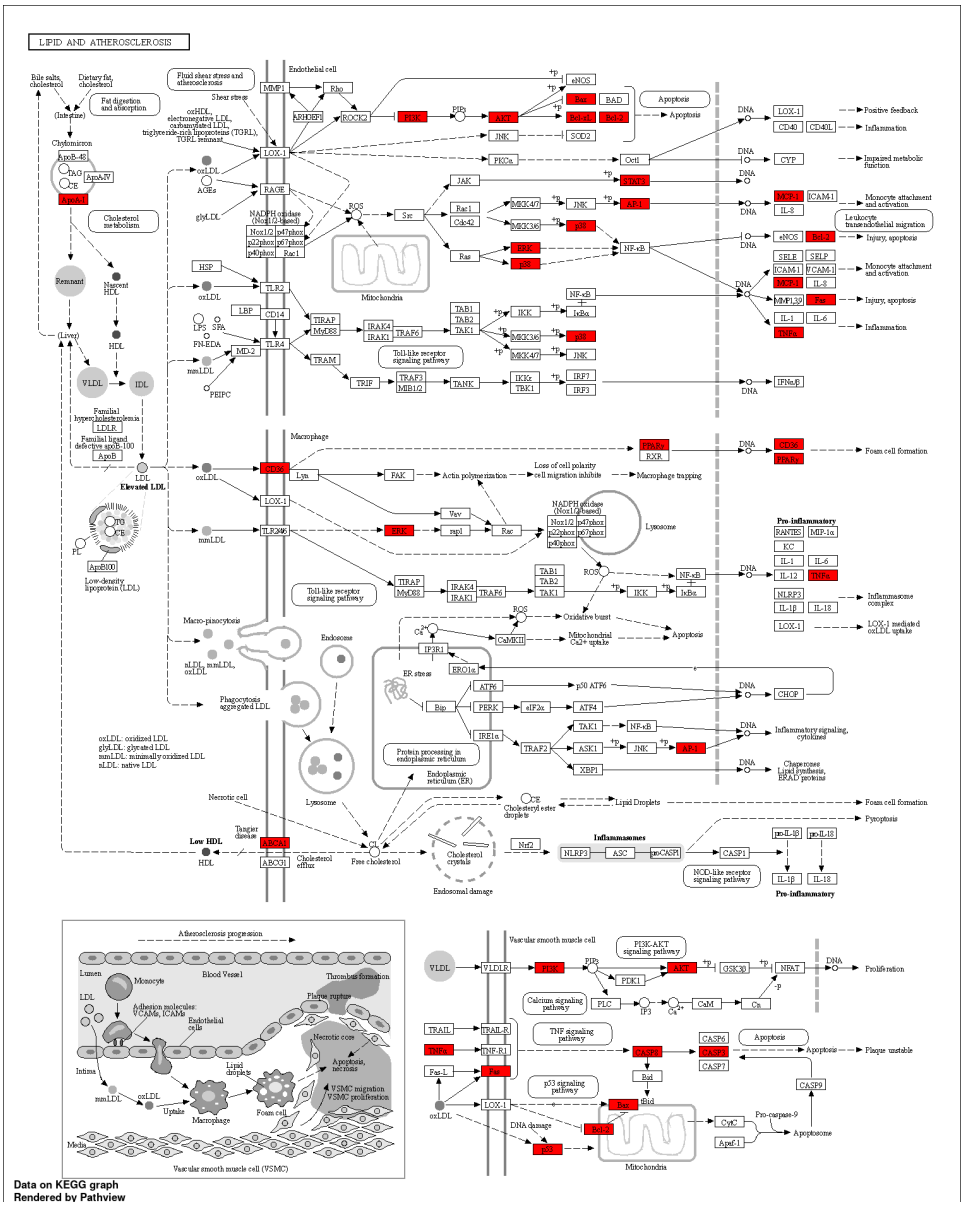


Figure 6. Visualization of lipid and atherosclerosis Pathwayy through KEGG Pathway

Meta-analysis studies related to the effects of HS on lowering cholesterol have been conducted by several researchers. Aziz et al. (2013) showed no significant difference between HS intervention and control groups in regulating TC, LDL-C, HDL-C, and TG, while Zhang et al. (2020) showed that HS could effectively lower TC and LDL-C levels, which is different from the conclusions of Aziz et al. (2013). Several subsequent studies have identified the bioactive components of HS. For instance, research by Chen et al. (2013) demonstrated that polyphenols from HS leaves can promote the removal of cholesterol from macrophages and decrease the accumulation of oxidized LDL by modulating the LXRa/ABCA1 and autophagic Pathwayys, respectively. Using metabolomics and gene expression analysis techniques, Debón et al. discovered that polyphenols from *Hibiscus sabdariffa* (HS) can downregulate genes associated with cholesterol and triglyceride (TG) synthesis, demonstrating multitarget lipid-reducing effects (Beltrán-Debón et al., 2015).

Feng et al. (2018) explained that phytosterol compounds, such as beta-sitosterol and stigmasterol, affect a set of key genes in lipid metabolism and bile acid excretion in the liver and intestines of mice. Increased mRNA levels of enzymes in bile acid synthesis CYP7A1, CYP81 or CYP27A1 and key enzymes in the bile acid biosynthesis pathwayy, were found in both phytosterols. Lower intestinal bile acid levels will decrease dietary lipid absorption, leading to weight loss and lipid accumulation in the liver. In addition, decreased CD36 expression caused by phytosterols can decrease liver fatty acid absorption from the blood (Feng et al., 2018).

CONCLUSIONS

Roselle and mangosteen plant extracts contain several active compounds that can regulate a set of key genes in the pathophysiological Pathwayy of atherosclerosis caused by hypercholesterolemia. Compounds such as beta-sitosterol, 3,4-Dihydroxybenzoic acid, and quercetin can regulate several target genes such as ABCA1, APOA1, BAX, BCL2, CASP3, and CD36. Further research is needed to prove the effectiveness of the combination of extracts or active compounds in these plants, both in vitro and in vivo.

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CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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