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Research Article

Uncovering The Pharmacological Mechanism of *Ficus elastica* as Anti-hyperlipidemia Candidate: LC-HRMS, Network Pharmacology, *In vitro* and *In vivo* Studies

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Abstract
Hyperlipidemia is a major risk factor for cardiovascular diseases. While conventional treatments exist, there is a growing interest in natural remedies with fewer side effects. *Ficus elastica* has promising medicinal properties, yet its potential as an anti-hyperlipidemic agent remains unexplored. This study aimed to investigate the anti-hyperlipidemic effects of *F. elastica* using an integrated approach of LC-HRMS-based chemical bioinformatics and *in vitro*/*in vivo* experimental validation. The anti-hyperlipidemic potential of *F. elastica* and its mechanism of action were screened using integrative computational network pharmacology followed by *in vitro* HMG-CoA reductase inhibition and *in vivo* lipid-lowering activity in a hyperlipidemia rat model. Network pharmacology analysis identified STAT3, HSP90AA1, and TLR4 as potential core targets involved in lipid and atherosclerosis-related KEGG pathways. Molecular docking simulations revealed high-affinity interactions between *F. elastica* compounds and the identified targets, notably compound 41 and compound 61. *In vitro* assay demonstrated that ethanolic extract of *F. elastica* inhibited HMG-CoA reductase with an IC_{50} of 297.73 µg/mL. *In vivo* experiment using a hyperlipidemic rat model showed significant reductions in total cholesterol, triglycerides, and increased HDL levels. The reduction of triglycerides and elevation of HDL level after *F. elastica* ethanolic extract supplementation is similar to the effect from supplementation of simvastatin. These findings suggest that *F. elastica* ethanolic extract possesses notable anti-hyperlipidemic properties, likely mediated through multiple molecular targets and pathways. The study highlights the potential of *F. elastica* ethanolic extract as a promising candidate for anti-hyperlipidemic therapy and underscores the efficacy of integrating computational and experimental approaches in natural product research.

Keywords: *Ficus elastica*, anti-hyperlipidemic, network pharmacology, HMG-CoA reductase, *in vivo* lipid-lowering, LC-HRMS

1. INTRODUCTION

Hyperlipidemia, characterized by abnormally elevated levels of lipids in the blood, is a major risk factor for cardiovascular diseases, which remain the leading cause of mortality worldwide [1]. Approximately 2.6 million deaths and 29.7 million disabilities are caused by cardiovascular diseases due to excess cholesterol and the mortality rate is expected to increase to 23.3 million by 2030 [2]. Excessive cholesterol levels in the blood play a major role in inducing dyslipidemia, leading to the development of cardiovascular diseases through cholesterol accumulation in the arteries, forming plaques that can obstruct blood flow [3].

Synthetic drugs such as statins are the first-line medications used for dyslipidemia therapy [4]. Statins competitively inhibit the HMG Co-A reductase enzyme by binding to the active site of the enzyme and inducing conformational changes in its structure, thus reducing enzyme activity [5] [6]. However, long-term use of statins can cause various side effects, including myalgia, myopathy, and muscle pain [7]. Moreover, statins have also been reported to cause hepatotoxicity, type 2 diabetes mellitus, and peripheral neuropathy [8]. The use of statins is also contraindicated in pregnant and breastfeeding women, as well as patients with chronic liver disease [9][10]. This has led to a growing interest in natural alternatives, particularly plant-based remedies, for managing hyperlipidemia.

Ficus elastica, commonly known as karet merah (Indonesia), has long been used in traditional medicine across various cultures for its potential health benefits [11]. The ethnomedicinal survey revealed that *F. elastica* leaves were used to treat stroke, improve blood circulation, fight free radicals, and manage cholesterol. Recent studies have suggested that certain compounds found in *F. elastica* (i.e. quercitrin, myricitrin, morin, and rutin) may possess lipid-lowering properties, making it a promising candidate for antihyperlipidemic agents

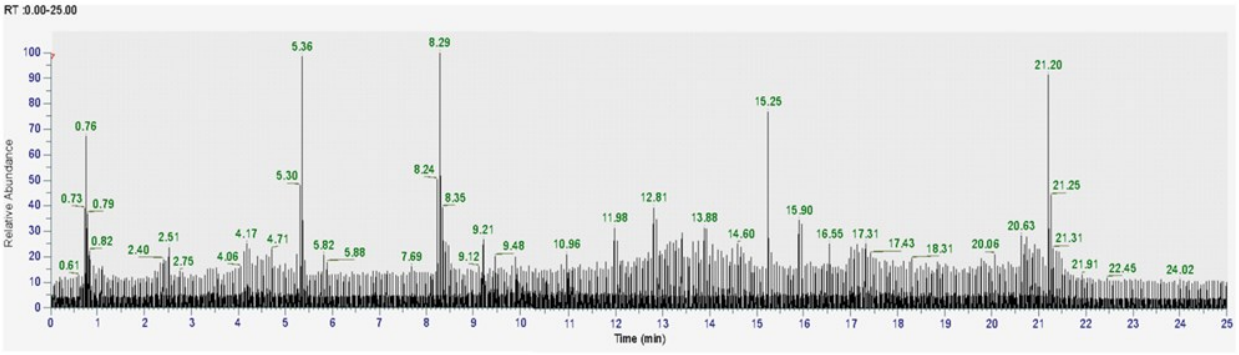

Figure 1. LC Chromatogram of *F. elastica* leaf ethanolic extract.

[12]-[15]. However, the exact mechanisms of action and the full extent of its therapeutic potential in the management of hyperlipidemia remain largely unexplored.

Therefore, this preliminary study aims to evaluate the antihyperlipidemic potential of *F. elastica* and elucidate its potential mechanism of action. By integrating several *in silico* techniques (network pharmacology and molecular docking) with *in vitro* and *in vivo* approaches, we seek to provide a comprehensive mechanistic understanding of which its bioactive components contribute to lipid-lowering activity at the molecular level by observing their molecular targets and interactions. By elucidating the mechanisms of action and demonstrating the efficacy of *F. elastica*, we provide a foundation for future research and potential clinical applications in the field of antihyperlipidemic therapy.

2. MATERIALS AND METHODS

2.1. Materials

HMG-CoA reductase kit assay and cholesterol were purchased from Sigma-Aldrich (Singapore), LC-MS grade of formic acid, acetonitrile and water were provided by Merck (Germany), and technical grade of 96% ethanol was purchased from BrataChem (Indonesia).

2.2. Methods

2.2.1. Sample Preparation and Extraction

Fresh leaves of *F. elastica* were collected from Balai Penelitian Tanaman Rempah dan Obat (BALITRO), Bogor, Indonesia in January 2024. The fresh leaves were sorted, cleaned, and sun-dried for 7 d followed by grounded and sieved using 100 mesh sieves. A 1 kg dry powder of *F. elastica* was extracted using 10 L of 96% ethanol (ratio sample-solvent of 1:10) in an isolated glass chamber for 72 h at room temperature. The liquid extract was filtered using Wathman filter paper in a vacuum Buchner flask. The residues were re-extracted in a similar process as much as two times. The menstruum was then collected and evaporated using a vacuum rotary evaporator (Buchi, Germany) at 70 rpm, 45 °C. Approximately 128.2 g of crude extract (12.82% of dry weight) was obtained and used for further analysis and experiments.

2.2.2. Phytochemical Profiling using LC-ESI-HRMS

The crude extract was characterized using UHPLC (Thermo Scientific) with Binary Pump (Germering, Germany) equipped with Orbitrap™ Exploris 240 HRMS (Bremen, Germany) detector, Optamax™ NG Heated Electrospray Ionization (H-ESI) ion source, and Thermo Scientific™ Accucore™ Phenyl Hexyl column (100 mm length, 2.1 mm ID, 2.6 µm particle size). The separation parameters were as follows: mobile phase water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B); gradient elution with 5% B and increased gradually to 90% in 16 min, hold at 90% for 4 min and back to 5% B until 25 min; flow rate 0.3 mL/min; column temperature 40 °C; injection volume 5 µL. Chemical profiling was implemented using Thermo Scientific Compound Discoverer 3.3 (San Jose, USA).

Table 1. Major compounds found in *F. elastica* leaf extract by LC-ESI-HRMS.

No	RT (min)	Compounds Names	Formula	m/z	Annot. Delta Mass (ppm)	Observed Relative Abundance (%)
1	0.741	Diketo-gulonic acid	$C_6H_8O_7$	191.0199	1.05	2.117813935
2	2.336	Cyclo-(Leu-Ile)	$C_{12}H_{22}N_2O_2$	227.1752	-0.61	4.200081132
3	4.4	1,2,3-Butanetriol	$C_4H_{10}O_3$	107.0702	-0.73	4.676884542
4	4.593	Diethylene glycol *n*-butyl ether	$C_8H_{18}O_3$	163.1327	-0.67	6.519095479
5	5.01	Butoxytriglycol	$C_{10}H_{22}O_4$	207.1590	-0.21	1.970032452
6	5.362	Cyclo(D-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl)	$C_{36}H_{66}N_6O_6$	340.2594	-0.18	26.33182636
7	8.361	Xestoaminol C	$C_{14}H_{31}NO$	230.2476	-0.90	4.000534652
8	8.408	Lauroyl diethanolamide	$C_{16}H_{33}NO_3$	288.2531	-0.62	1.054304466
9	8.467	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	318.3001	-0.51	5.670120273
10	8.96	17-Hydroxypanaxacol	$C_{17}H_{26}O_4$	293.1759	0.45	1.121778032
11	13.408	4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	179.0702	-0.33	1.434980387
12	16.553	Nebrosteroid M	$C_{29}H_{50}O_3$	429.3725	-0.49	1.54461974
13	19.8	(8beta)-8-(1-Phenylvinyl)-1,6:7,14-dicycloprost-7(14)-ene	$C_{28}H_{40}$	377.3200	-0.64	1.78313317
14	21.209	Methylsulfonylisourea	$C_2H_6N_2O_3S$	70.01257	4.78	1.714168667

Note: Annot delta mass ± 5 ppm indicating high accuracy of analysis

2.2.3. In Vitro HMG-CoA Reductase Assay

An enzymatic in vitro assay was conducted to evaluate the inhibitory activity of the extract against HMG Co-A reductase. Spectrophotometric measurements were performed using a 96-well microplate, with the absorbance set at 340 nm employing a kinetic method. Absorbance readings were taken at one-minute intervals over ten minutes. The HMG Co-A reductase assay kit from Sigma-Aldrich was utilized, and it included assay buffer, NADPH, HMG Co-A substrate solution, and HMG Co-A reductase enzyme. Pravastatin was used as a comparative standard in the assay. For the blank sample, 184 µL of buffer solution was combined with 4 µL of NADPH and 12 µL of HMG -CoA, with no pravastatin, samples, or HMGR added. The control preparation consists of 182 µL buffer solution, 4 µL NADPH, 12 µL HMG-CoA, and 2 µL HMGR. For the standard preparation, 181 µL buffer solution was mixed with 1 µL pravastatin, 4 µL NADPH, 12 µL HMG-CoA, and 2 µL HMGR. The extracts preparation contained 181 µL buffer solution, 1 µL of samples, 4 µL NADPH, 12 µL HMG-CoA, and 2 µL HMGR. Following the measurements, the specific activity was calculated using the following equation 1:

$$\text{Units/mgP} = \frac{\left(\frac{\Delta A340}{\text{min sample}} - \frac{\Delta A340}{\text{min blank}} \; x \; TV\right)}{12.44 \; x \; V \; x \; 0.6 \; x \; LP} \quad (1)$$

where TV is total volume of the reaction in 0.2 mL, V is volume of enzyme used in the assay (0.002 mL), and LP is light path in 0.55 cm. The HMG-CoA reductase inhibition (%) was calculated using the following equation 2.

$$\text{Inhibition (\%)} = \frac{(\Delta \text{ Abs control} - \Delta \text{ Abs sample})}{(\Delta \text{ Abs control})} \quad (2)$$

The IC_{50} value was determined from the linear regression equation generated from the calibration curve between concentration vs inhibition (%).

2.2.4. In Vivo Anti-hyperlipidemia

2.2.4.1. Animal and Study Design

The research protocol was officially sanctioned by the National Peripheral Veterinary Authority Animal Ethics Committee, following a positive assessment from the Animal Protocols Evaluation Committee. The approved study was assigned Protocol Number 002422/SITI KHADIJAH PALEMBANG/2024. This rigorous approval process ensured that the research adhered to ethical guidelines and animal welfare standards throughout its execution. The experimental animals used in this research were controlled to be in excellent health with no diseases or medical conditions. Proper nutrition and hydration were maintained at all times. The animals' psychological well being was maintained by keeping animals in an environment free from stressors that could cause fear or chronic stress. To ensure their natural behaviors can be expressed, the animals were housed in appropriately sized enclosures with adequate enrichment facilities that allow for regular physical activity and movement.

This study employed male Wistar rats (*Rattus norvegicus*) (2–3 months; 200 g of weight), which were sourced from Abduh Tikus Palembang, Animal Laboratory breeding stock. Before the commencement of the experiment, all subjects underwent a 7-day acclimatization period to adapt to the laboratory environment. Throughout the study, including the acclimatization phase, the rats

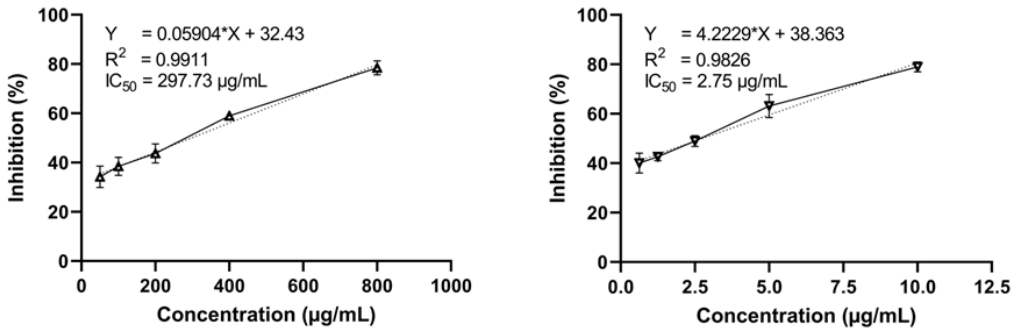

Figure 2. HMG-CoA reductase inhibitory activity of (a) *F. elastica* leaf ethanolic extract in comparison with (b) pravastatin.

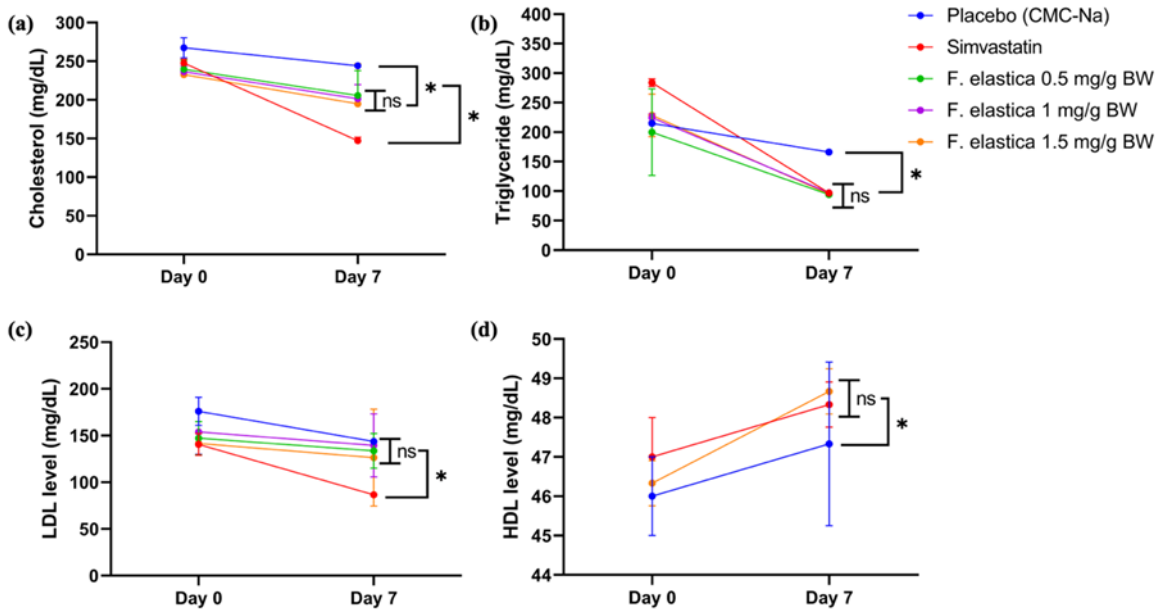

Figure 3. The results of the anti-hyperlipidemic assay of *F. elastica* leaf ethanolic extract on hyperlipidemia rat model. (a) TC, (b) TG, (c) LDL, and (d) HDL levels.

were provided with unrestricted access to standard rodent pellets and fresh water, ensuring ad libitum feeding and hydration.

The experimental protocol involved dividing 30 rats into five distinct groups of six animals each, subjected to different treatments. Three groups received oral suspensions containing *F. elastica* ethanolic extract at 0.5, 1.0, and 1.5 mg/g BW, respectively. The fourth group was administered an oral suspension containing simvastatin at 0.18 mg/200 g BW, serving as a positive control. The fifth group acted as a negative control, receiving a 0.5% CMC-Na suspension orally.

2.2.4.2. Hyperlipidemia Rat Model Induction

A specially formulated high-fat, high-cholesterol diet was administered to induce hyperlipidemia in the test animals. This diet consisted of a precise mixture: 1% pure cholesterol, 5% duck egg yolk, 10% goat fat, and 1% coconut oil with standard animal feed making up the remainder to reach 100%. To ensure freshness, this specialized diet was prepared anew each day. Animals were given 1% cholic acid once a week. The induction phase lasted for three months until the animals reached a state of hyperlipidemia, following an initial acclimatization period. During this time, the animals were consistently fed the prepared high-fat, high-cholesterol diet. The rats were considered suitable for the study only after their blood lipid levels showed a significant increase from their baseline measurements, confirming the successful induction of hyperlipidemia.

2.2.4.3. Blood Serum Collection

Blood sampling from the rats was conducted via the orbital sinus technique. This method involves carefully inserting a capillary pipette at the medial canthus of the eye, at the junction of the upper and lower eyelids. The pipette is then gently rotated and advanced towards the posterior of the eyeball until blood begins to flow through the capillary action. The collected blood was immediately and carefully transferred into microtubes to prevent hemolysis. These samples were then centrifuged at 7000 rpm for 10 min to separate the serum from cellular components. Using a micropipette, the supernatant serum was carefully extracted and transferred to clean storage vials. These serum samples were then refrigerated to maintain their integrity until further analysis. The stored samples were later used for quantitative measurements of total cholesterol, triglycerides, and high-density lipoprotein (HDL) levels.

2.2.4.4. Triglycerides (TG) Level Assay

TG concentrations were measured using an enzyme-based color reaction method. The test

utilized glycerol-3-phosphate oxidase (GPO) as a reaction catalyst. The process involved combining blood plasma samples with a triglyceride kit reagent solution, and then incubating the mixture at 37 °C for 10 min. A reference solution was prepared and subjected to the same treatment and incubation conditions as the samples. Using a spectrophotometer, light absorption was measured for both the samples and the reference against a reagent blank at 500 nm wavelength. Triglyceride level was calculated using the following formula 3.

$$\text{Triglycerides (mg/dL)} = \frac{\text{Abs sample}}{\text{Abs standard}} \text{x concentration of standard} \quad (3)$$

2.2.4.5. High Density Lipoprotein (HDL) Level Assay

HDL cholesterol levels were measured using an enzymatic colorimetric method with an HDL cholesterol kit. A mixture of plasma samples and HDL cholesterol kit reagent solution was incubated for 10 min at 37 °C. The standard mixture and HDL cholesterol kit reagent solution were treated the same way as the samples, being incubated for 10 min at 37 °C. The absorbance of the samples and standard were measured against a blank at 500 nm.

2.2.4.6. Low Density Lipoprotein (LDL) Level Assay

LDL cholesterol levels were indirectly determined using the Friedewald equation 4.

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglicerides}}{5} \text{x HDL cholesterol} \quad (4)$$

2.2.5. Network Pharmacology Analysis

A comprehensive network pharmacology approach, adapted from Putri et al. [16], was implemented to elucidate the potential mechanism of action of *F. elastica* in treating hyperlipidemia. The process began with target identification using the SuperPRED (https://prediction.charite.de) database, inputting the compound's canonical SMILES. Identified gene targets were then standardized using the UniProt (https://www.uniprot.org) database and cross-matched with hyperlipidemia-related genes from the GeneCards database (https://www.genecards.org) using the Venny 2.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/). Subsequently, protein-protein interaction analysis was conducted using the STRING database (https://string-db.org), followed by visualization and topological analysis with Cytoscape 3.10.1. The core targets obtained from this analysis were then used for KEGG enrichment and gene ontology analysis using ShinyGO 0.80 (http://bioinformatics.sdstate.edu/go/), with a false discovery rate (FDR) threshold set to 0.05. The binding affinity of *F. elastica* compound to the target was implemented via molecular docking using Autodock Vina in PyRx 0.8 software as previously described by Susianti et al. [17]. In this case, HSP90AA1 (PDB ID: 5H22) and STAT3 (PDB ID: 6NUQ) were used as they were found as the core targets in this study.

2.2.6. Data Analysis

The experimental data were expressed as mean ± SD values, with each experimental group consisting of six replicates (n=6). Statistical analyses were conducted using two different approaches: an independent t-test for *in vitro* studies to compare differences between two independent groups, and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for in vivo studies involving multiple group comparisons. All statistical analyses were performed using GraphPad Prism software version 10.0.1, with statistical significance set at $p < 0.05$ to indicate a 95% confidence level in the observed differences between groups.

Table 2. Cytotoxicity potential of *F. elastica* on several cell lines at 100 µg/mL.

No.	Cell lines	Cell viability count (%)	References
1.	HepG2	60.4%	[43]
2.	MCF-7	64.1%	[43]
3.	RAW 264.7 macrophage	91.8%	[44]
4.	Brine Shrimp	65.0%	[45]

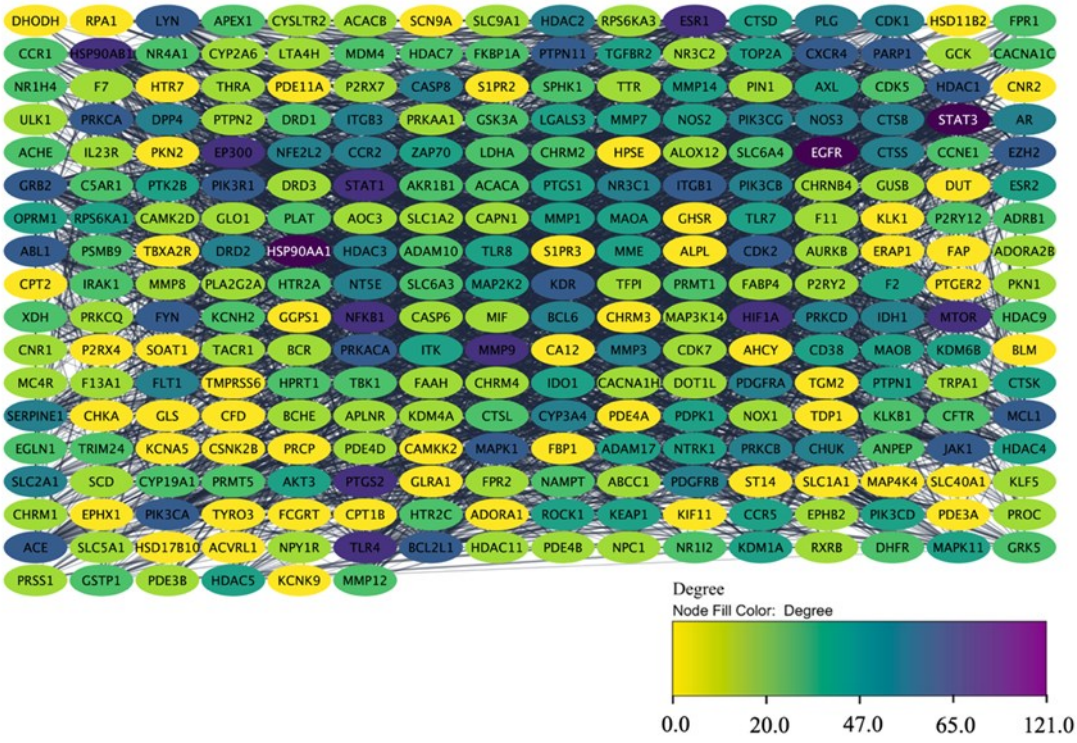

Figure 4. PPI network illustration of all target genes.

3. RESULTS AND DISCUSSIONS

3.1. LC-HRMS Analysis

LC-ESI-HRMS analysis obtained several important data, including molecular structure, molecular weight, retention time, number of components, and the mass spectra of the compound present in the sample. Phytochemical profiling of *F. elastica* ethanolic extract revealed 187 compounds with 14 major constituents, as tabulated in Table 1. Among all major compounds, Cyclo(D-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl) was found to be the most abundant in *F. elastica* ethanolic extract, demonstrating the highest relative abundance (approximately 26.3%), followed by diethylene glycol *n*-butyl ether (6.5%), and 2-amino -1,3,4-octadecanetriol (5.7%). The chromatogram of *F. elastica* ethanolic extract is depicted in Figure 1.

The relationship between compound abundance and biological activity, particularly in lipid-lowering compounds, presents a complex and intriguing area of research that has generated significant scientific discourse. In some cases, there is a positive correlation between the abundance of a natural product compound and its biological activity. This is often observed when the compound in question is the primary bioactive agent within a mixture. For instance, in the study of marine-derived *Penicillium chrysogenum*, a workflow was developed to identify bioactive compounds by linking chemical profiles with biological data. This approach successfully highlighted ergosterol as the main compound responsible for antiproliferative activity, demonstrating a positive correlation between its abundance and biological activity [18]. Similarly, the integration of high-content screening and untargeted metabolomics has been shown to improve the identification of bioactive compounds, suggesting that higher abundance can facilitate the discovery of biologically active constituents [19]. Conversely, there are instances where a negative correlation is observed, particularly when impurities or less abundant compounds contribute to the overall biological activity. The concept of purity-activity relationships (PARs) illustrates this phenomenon, as seen in the study of ursolic acid. Here, it was found that increasing the purity of ursolic acid samples actually reduced their anti-TB bioactivity, suggesting that impurities or less abundant compounds might have synergistic effects that enhance biological activity [20]. This highlights the importance of considering the entire chemical profile of a natural product extract, rather

than focusing solely on the most abundant compounds.

The correlation between the abundance of natural product compounds and their biological activity is not straightforward and can vary depending on the context. While higher abundance can sometimes indicate greater biological activity, as seen with ergosterol, the presence of impurities or synergistic effects from less abundant compounds can also play a significant role, as demonstrated by the study of ursolic acid. Understanding these dynamics is crucial for advancing natural product drug discovery and optimizing the identification of bioactive compounds.

3.2. In vitro HMG-CoA Reductase Assay

The *in vitro* enzyme inhibition assessment was employed to confirm the potential HMG-CoA reductase inhibitory activity of *F. elastica* previously predicted through HTVS. The *in vitro* assay utilized HMG-CoA reductase enzyme as the target with pravastatin as control. Figure 2 demonstrated a concentration-dependent inhibition for both substances, where higher concentrations led to increased enzyme inhibition. The study quantified this effect using IC_{50} values, which represent the concentration required to achieve 50% inhibition. *F. elastica* ethanolic extract showed an IC_{50} of 297.73 mg/mL, while pravastatin had a much lower IC_{50} of 2.75 mg/mL. This significant difference in IC_{50} values indicates that Pravastatin is substantially more potent than the *F. elastica* ethanolic extract, requiring approximately 108 times less concentration to achieve the same level of inhibition.

This substantial difference in potency could be attributed to several key factors related to their chemical nature and mechanism of action. *F. elastica* extract contains a complex mixture of compounds, only some of which may possess HMG -CoA reductase inhibitory activity. In addition, numerous compounds in *F. elastica* might interact with each other including antagonistic activity, potentially leading to interference effects or dilution of active components [21]. This matrix effect necessitates higher concentrations to achieve the same level of inhibition. Moreover, natural compounds demonstrating their lipid-lowering effects often result from multiple mechanisms, not solely through HMG-CoA reductase inhibition [22] [23].

3.3. In vivo Anti-hyperlipidemic Assay

The anti-hyperlipidemia activity of *F. elastica* leaf extract was assessed in a hyperlipidemic rat model subjecting simvastatin as a control. A week's supplementation of *F. elastica* leaf extracts

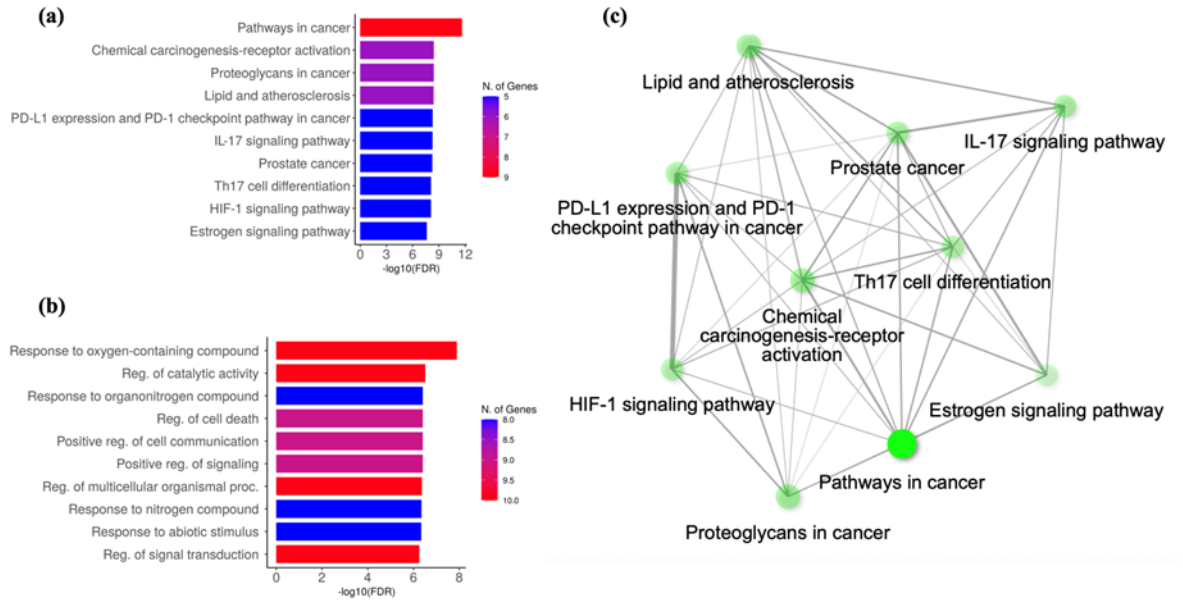

Figure 5. Enrichment results. (a) KEGG pathways, (b) GO biological process, and (c) the connection of each pathway related to 10 core target genes.

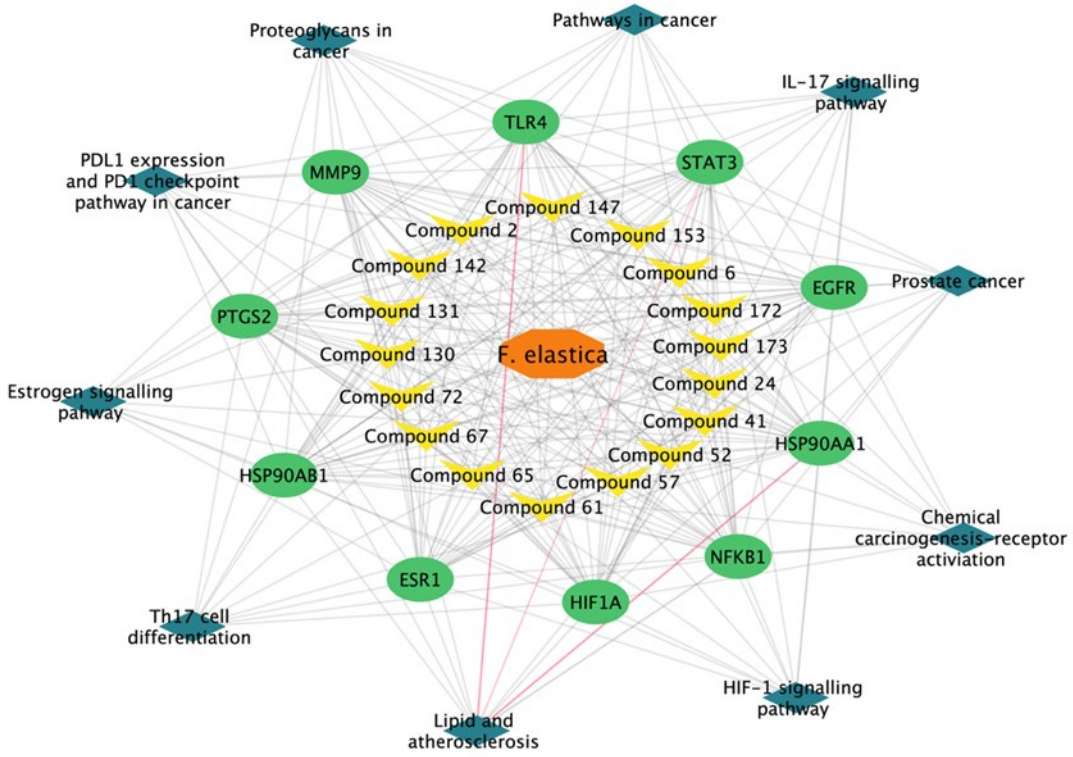

Figure 6. Illustration of a plant-compound-gene-pathways network.

provided a comprehensive overview of its anti-hyperlipidemia activity by regulating several lipid profiles in hyperlipidemia rat model, as depicted in Figure 3. While the extract's effect on total cholesterol (TC) (reducing 14.2% - 16.2% TC) was not as potent as simvastatin (reducing 40.6% TC), its ability to lower TG was comparable with TG reduction of 52.9% - 58.3% (*F. elastica* extract) and 65.9% (simvastatin) with p-value >0.05, demonstrating similar efficacy to this standard medication for this specific lipid parameter. The extract's potent effect on TG is particularly noteworthy, given that hypertriglyceridemia is an independent risk factor for cardiovascular disease and is often challenging to manage with conventional treatments [24]. This highlights *F. elastica's* potential as a targeted intervention for TG management. However, a notable limitation of *F. elastica* was its lack of influence on the LDL profile. The extract showed no significant reduction in LDL levels (9.28–10.7%), with results similar to the placebo group (8.15%) (p-value > 0.05). Interestingly, the supplementation of *F. elastica* showed a tendency to increase HDL levels significantly (3.1–5.8%), even comparable to that of simvastatin (6.1%) (p-value > 0.05). This selective impact on lipid parameters suggests that *F. elastica* may act through specific metabolic pathways, a characteristic not uncommon in natural compounds. For instance, berberine, found in several plants, shows a similar profile with strong effects on TG but less impact on LDL cholesterol [25]. Potential mechanisms could include the inhibition of TG biosynthesis through inhibition of several genes involved in lipid regulation and metabolism including STAT3 [26], NF-KB [27], and HMG-CoA reductase [28]. These hypotheses align with mechanisms observed in other natural products used for lipid management, whether in the realm of plants, i.e., *Cinnamomum burmani* [27][29], *Citrus bergamia* [30]; or bioactive compounds, i.e., apigenin [31][32].

The selective activity of *F. elastica* on TG and HDL rather than LDL and TC presents a fascinating therapeutic profile that deserves careful consideration. The clinical implications of this selective activity are particularly significant in several therapeutic contexts. In mixed dyslipidemia management, *F. elastica* shows promise for patients with high TG and low HDL, a common pattern in metabolic syndrome and type 2 diabetes. This profile could effectively complement statin therapy,

which primarily targets LDL cholesterol [33]. While LDL reduction remains a primary goal in cardiovascular disease prevention, mounting evidence suggests that TG levels and HDL functionality are independent risk factors. *F elastica*'s effects on these parameters could provide additional cardiovascular benefits, especially in populations where high TG and low HDL are prevalent risk factors [34]-[36]. From a therapeutic perspective, several considerations emerge. The selective activity profile suggests potential synergy with statins or other LDL-lowering agents. This combination could provide comprehensive lipid management while potentially allowing for lower statin doses, thereby reducing the risk of side effects [37][38]. *F. elastica* might be particularly suitable for specific patient groups, including those with isolated hypertriglyceridemia, low HDL, statin intolerance, metabolic syndrome requiring multiple therapeutic targets, or residual cardiovascular risk despite adequate LDL control.

F. elastica is potentially developed not only for

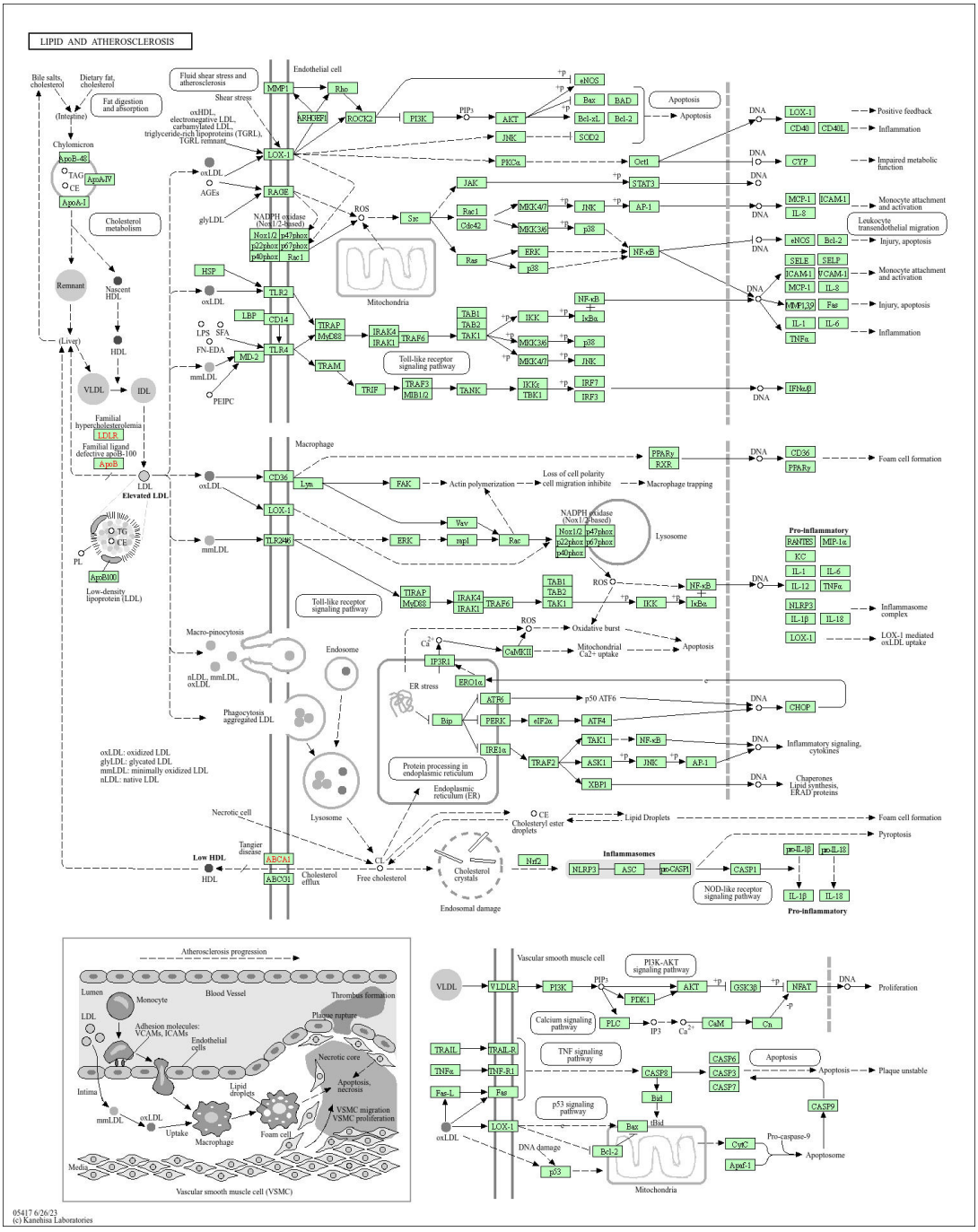

Figure 7. KEGG pathways of *F. elastica* regulate the genes involved in lipid and atherosclerosis.

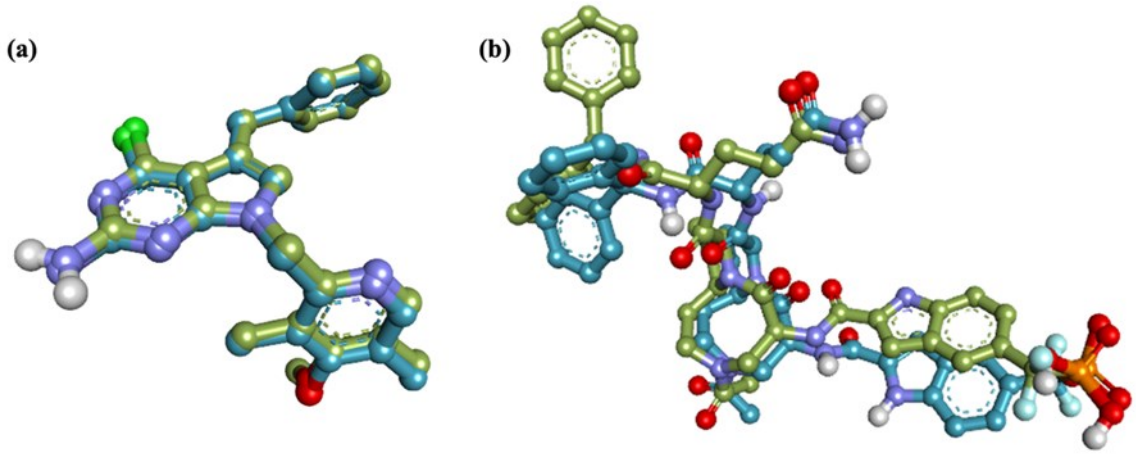

Figure 8. Validation results of molecular docking protocols. (a) Superimposed of HSP90AA1 native ligand (RMSD: 0.97 Å), and (b) Superimposed of STAT3 native ligand (RMSD 1.24 Å). *Note: green for pre-docking state and blue for post-docking state of native ligand.*

its lipid-lowering potential, but also for its safety profile. The acute toxicity assessment results demonstrate the safety profile of *F. elastica* extracts in the experimental mouse model. Throughout the 14-day observation period, test subjects exhibited normal behavioral patterns following extract administration, with no mortalities recorded in any treatment group. The animals maintained consistent behavior during the critical first 24 h post-exposure. The study evaluated three dosage levels (100, 500, and 1000 mg/kg) with 5 rats of each and found that none of these concentrations contained compounds capable of inducing acute lethal effects in the test subjects along with no mortality observed [39].

In addition, several studies have also shown the toxicological profile of *F. elastica* in vivo in rats. *F. elastica* showed no apparent signs of acute toxicity when administered orally at doses of 2000 and 5000 mg/kg body weight without eliciting any concerning changes in physiological or behavioral parameters over a period of 14 d [40]. Furthermore, the subchronic toxicity evaluation, conducted over 28 d with daily doses of 200, 400, and 800 mg/kg body weight, confirmed the extract's safety. Throughout the study, all animals survived, and comprehensive analyses of biochemical, hematological, and physiological parameters showed no statistically significant deviations from the control group [41]. This suggests that *F. elastica* is safe for use at these concentrations in acute settings. The starting dose level was selected on the basis of scientific literature on experimental toxicity studies as the dose expected to produce antihyperlipidemic activity without causing toxic effects.

The toxicity of *F. elastica* extract is not only evidenced by *in vivo* assay but also observed via *in vitro* assessment. As detailed in Table 2, the extract exhibited minimal cytotoxic effects across various cell lines, providing strong evidence for its potential safety in clinical applications. Notably, when tested against different cancer cell lines and in brine shrimp assays, *F. elastica* demonstrated consistently low toxicity levels, with cytotoxicity percentages ranging between 9.0% and 39.6%. This moderate cytotoxic activity against cancer cells suggests that at the tested concentrations (up to 100 µg/mL), *F. elastica* extract would likely have minimal adverse effects on healthy cells, indicating a favorable safety margin for therapeutic use [42].

3.4. Pharmacological Mechanism Elucidation using Network Pharmacology Approach

A network pharmacology approach was used to gain comprehensive insight into how *F. elastica* exerts anti-hyperlipidemic effects and influences lipid metabolism. The study identified 396 target genes associated with 14 compounds found in *F. elastica*. Of these, 279 genes were found to overlap with those related to dyslipidemia, hyperuricemia, or hypercholesterolemia. Delving deeper, the study employed PPI and topological analyses (see Figure 4) to pinpoint the most influential genes in this molecular orchestra. This approach revealed a core

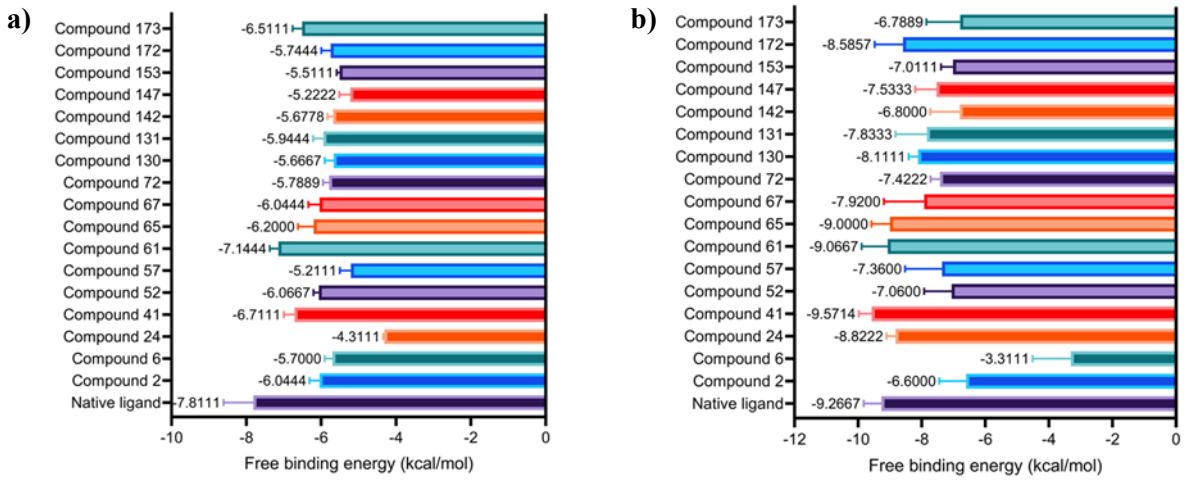

Figure 9. Docking score of 17 best compound of *F. elastica* on (**a**) STAT3 and (**b**) HSP90AA1. Data is presented as mean ± SD (n=10).

group of 10 targets (STAT3, EGFR, HSP90AA1, NFKB1, HIF1A, ESR1, HSP90AB1, PTGS2, MMP9, and TLR4) presumed to be central to both the onset and regulation of hyperlipidemia, acting as crucial nodes in the complex network of metabolic processes.

The study's KEGG pathway analysis pointed to the lipid and atherosclerosis signaling pathway as a potential mechanism for *F. elastica's* effects on hyperlipidemia. Gene Ontology (GO) analysis provided further insights into the compound's diverse impacts. It showed that *F. elastica* plays a role in managing responses to various stimuli, both external and internal, including those related to oxygen, organonitrogen compounds, and general environmental factors. The GO analysis also highlighted *F. elastica's* influence on catalytic processes, cellular communication, and signal transduction pathways. A comprehensive view of the GO enrichment results is presented in Figure 5.

To better understand the complex relationships between *F. elastica*, its key molecular targets, and associated health conditions, a network visualization was created. This visual representation, shown in Figure 6, is based on the core targets identified and their linked diseases, assisting in clarifying the intricate connections uncovered in this work. The mechanism involving the lipid and atherosclerosis signaling pathway, which was implicated in the study, was further developed and refined using the standard pathway map available in the KEGG database (https://www.kegg.jp/pathway/hsa05417), as illustrated in Figure 7. Among the ten core targets, STAT3, HSP90AA1, and TLR4 were found to be the most potential targets, highlighted by their presence in the lipid and atherosclerosis signaling pathways in KEGG analysis results.

Among the ten core targets, STAT3, HSP90AA1, and TLR4 were found to be the most potential targets, highlighted by their presence in the lipid and atherosclerosis signaling pathways in KEGG analysis results. These genes are reported to play important roles in lipid metabolism and regulation [26][46][47]. For instance, in the liver, STAT3 activation has been shown to promote lipogenesis by upregulating the expression of sterol regulatory element-binding protein 1c (SREBP-1c), a key transcriptional regulator of lipogenic genes [48]. This increase in lipogenesis can contribute to the development of fatty liver disease and systemic hyperlipidemia.

In the context of TLR4, its activation has been shown to directly influence lipid metabolism in various tissues, particularly the liver. In hepatocytes, TLR4 signaling can upregulate the expression of key lipogenic enzymes, including fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) [49]. This increased lipogenesis contributes to hepatic steatosis and systemic hyperlipidemia. Moreover, TLR4 activation affects lipoprotein metabolism by decreasing the expression of apolipoprotein E (ApoE) and ATP-binding cassette transporter A1 (ABCA1), both

crucial for cholesterol efflux [50]-[52]. This reduction in cholesterol efflux capacity can lead to cholesterol accumulation in cells and tissues, exacerbating hyperlipidemia. Interestingly, TLR4 acts not only as a mediator of lipid metabolism but also as a sensor of lipid overload. Saturated fatty acids, which are elevated in hyperlipidemic states, can activate TLR4 signaling [53]. This creates a potential feedback loop where hyperlipidemia activates TLR4, which in turn exacerbates lipid dysregulation. Another key contributor found in this work is HSP90AA1, a member of the heat shock protein family recognized for its role in lipid metabolism and the development of hyperlipidemia [46][54][55].

One of the keyways HSP90AA1 influences lipid metabolism is through its interaction with sterol regulatory element-binding proteins (SREBPs). HSP90AA1 has been shown to stabilize SREBP cleavage-activating protein (SCAP), which is essential for SREBP activation and subsequent lipogenic gene expression [56]. Inhibition of HSP90AA1 has been demonstrated to reduce SREBP activity and lipid synthesis, suggesting a potential therapeutic avenue for hyperlipidemia [57]. Furthermore, HSP90AA1 has been implicated in the regulation of peroxisome proliferator-activated receptors (PPARs), key transcriptional regulators of lipid metabolism. HSP90AA1 inhibition has been shown to modulate PPAR activity, potentially affecting lipid storage and oxidation [58].

3.5. Confirmation of Pharmacological Mechanism using Molecular Docking

The potential inhibition of 17 compounds to the potential molecular targets of *F. elastica* was confirmed through a validated molecular docking approach (Figure 8), providing a comprehensive understanding of their affinity and interactions with amino acids of each protein. Figure 9(a) illustrates that compounds 41, 61 and 173 stand out as the most promising bioactive among the tested ligands to STAT3 proteins, demonstrating free binding energy close to the native ligand. Among these, compound 61 was considered the best compound, showing the most negative binding energy (-7.11 kcal/mol) than others. This high affinity of compound 61 to STAT3 is due to its molecular interaction with several essential amino acid residues in STAT3 active site.

As depicted in Figure 10, compound 61 interacted with enormous essential amino acid via hydrogen and pi bond. A distinct interaction is observed in compound 61 with the native ligand, in which native ligand provides more hydrogen bonding (12 hydrogen bonds) than compound 61 (6 hydrogen bonds). The number of hydrogen bonds

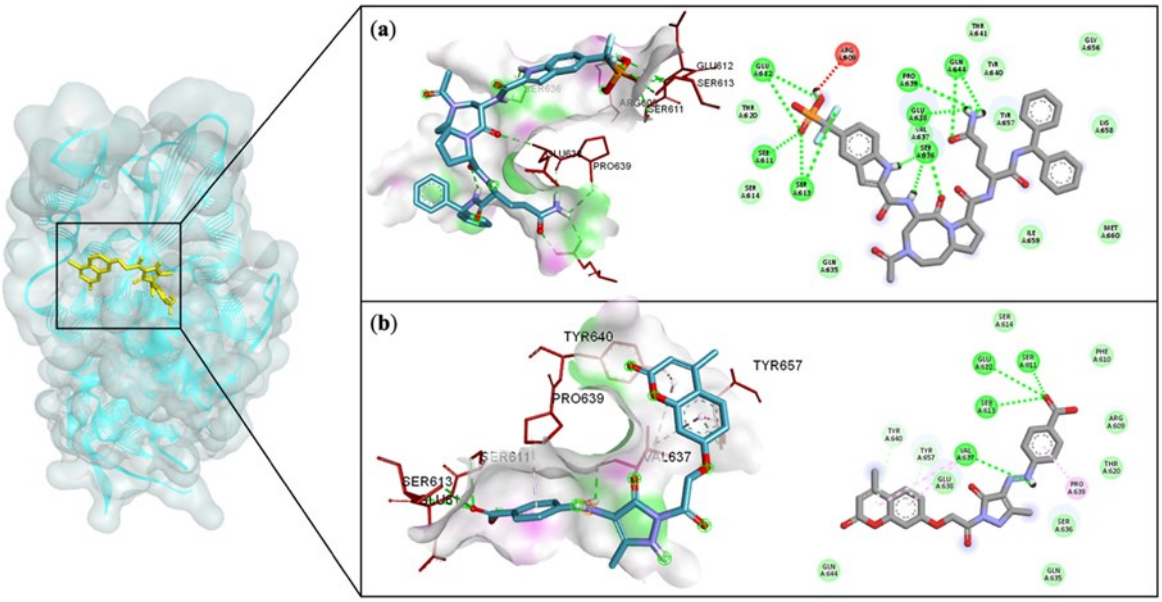

Figure 10. Molecular interactions of (a) native ligand and (b) compound 61 with amino acid residues at STAT3 active site.

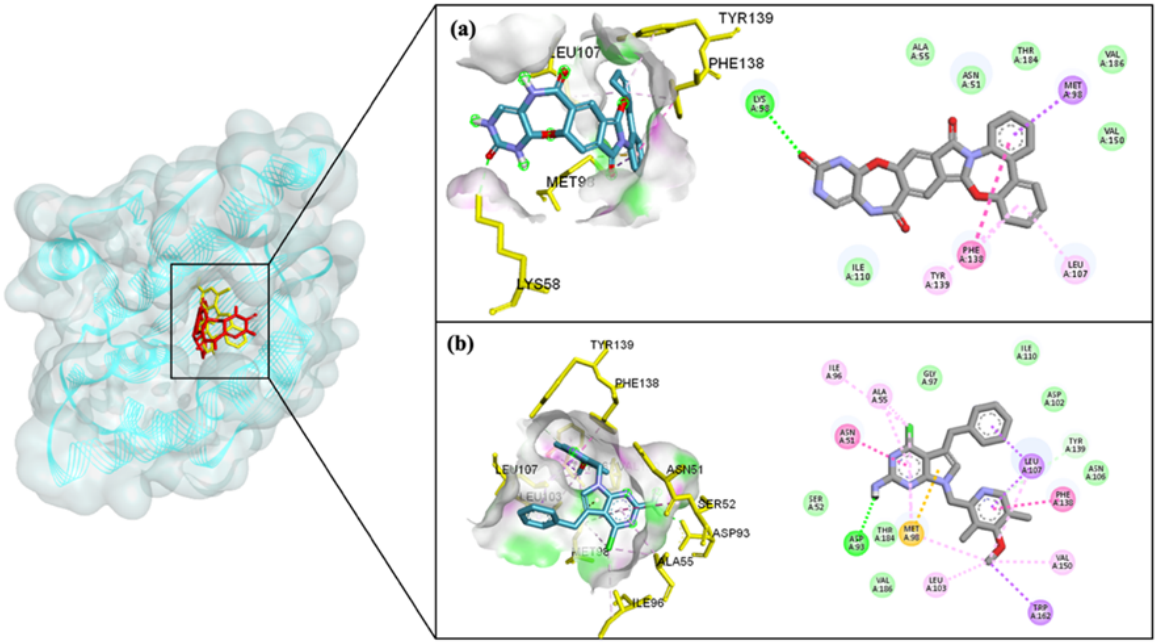

Figure 11. Molecular interactions of (a) native ligand, (b) compound 24, (c) compound 41, (d) compound 61, (e) compound 65, and (f) compound 172 with amino acid residues at HSP90AA1 binding pocket.

continues to be recognized as crucial in determining the affinity of ligand-protein complexes, contributing significantly to both specificity and binding strength [59]. While individual hydrogen bonds are relatively weak, multiple hydrogen bonds can collectively provide substantial binding energy. The formation of hydrogen bonds between a ligand and protein often involves displacing water molecules from the binding site, a process that can be entropically favorable and contribute to the overall binding affinity [60]. Furthermore, hydrogen bonds can induce or stabilize specific conformations in both the ligand and protein, potentially leading to allosteric effects [61].

On the realm of HSP90AA1, several compounds (compounds 24, 41, 61, 65, and 172) demonstrated favorable free binding energy, comparable to native ligand (Figure 9(b)). However, only compound 41 exhibited free binding energy (-9.57 kcal/mol) more negative than native ligand (-9.27 kcal/mol), highlighting its potential as HSP90AA1 inhibitor. This superior binding affinity can be attributed to a combination of key molecular interactions that optimize its fit within the HSP90AA1 binding pocket. As illustrated in Figure 11(a), compound 41 appears to form strong hydrogen bonds with Lys 59, providing a crucial anchor point for its binding. Additionally, its structure incorporates multiple aromatic rings that engage in hydrophobic interactions with residues such as Asn51, Ala55, Ile110, Val150, Thr184, and Val186. These hydrophobic contacts are further enhanced by potential pi-stacking interactions, particularly with Phe138, which contribute to the overall stability of the compound-protein complex [62]. The shape complementarity between compound 41 and the binding site also appears to be excellent, allowing for maximized van der Waals interactions that further strengthen the binding [63]. In comparison to the native ligand (Figure 11(b)), compound 41 seems to establish a more extensive and balanced network of interactions. While the native ligand shares some similar interaction points, such as with Phe138 and Met198, it appears to form different types of interactions.

The comprehensive findings of this study suggest that the compounds derived from *F. elastica* tend to exhibit superior binding interactions with the protein target HSP90AA1 compared to STAT3. This is evident from the superior binding affinities exhibited by the *F. elastica* compounds towards HSP90AA1, surpassing even the affinity of the native ligand for this target. Based on these observations, it can be concluded that HSP90AA1 represents the primary target underlying the anti-hyperlipidemic mechanisms of action of the *F.*

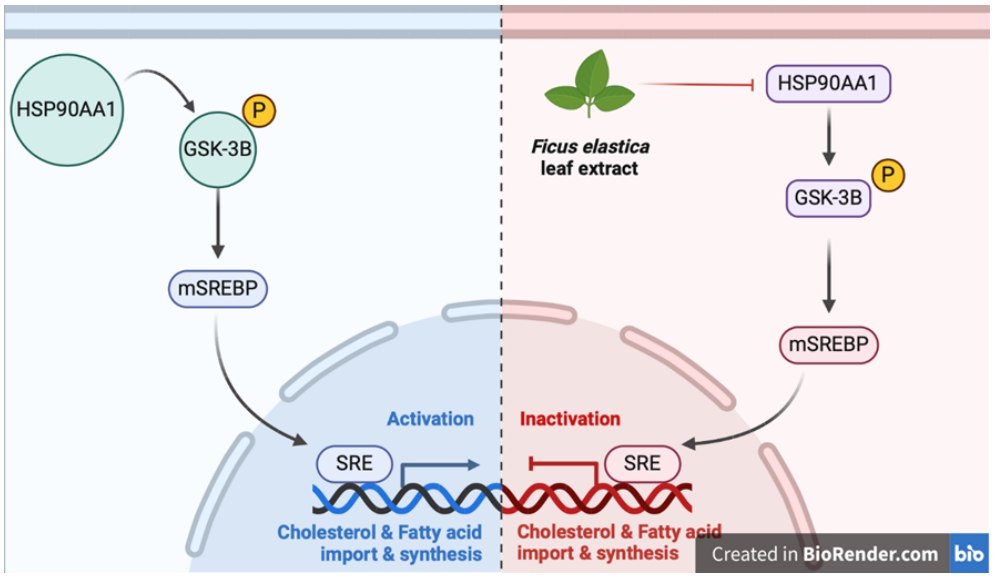

Figure 12. The proposed mechanism of action of *F. elastica* in lowering the lipid profiles.

elastica extracts. The specific molecular mechanisms by which the *F. elastica* compounds exert their lipid-lowering effects through modulation of HSP90AA1 are further illustrated and detailed in Figure 12. This finding is significant, as it suggests that the bioactive constituents present in *F. elastica* leaf ethanolic extract are capable of selectively and potently engaging HSP90AA1. The preferential binding of the *F. elastica* compounds to HSP90AA1 over STAT3 implies a targeted mechanism of action, where modulating HSP90AA1 activity is a critical step in mitigating hyperlipidemic conditions.

4. CONCLUSIONS

This comprehensive study provides compelling evidence for the anti-hyperlipidaemic potential of *Ficus elastica*. By integrating LC-HRMS-based chemical bioinformatics (network pharmacology, molecular docking) and experimental validation, we have elucidated the multi-faceted mechanisms underlying lipid-lowering effects of *F. elastica*. The identification of STAT3, HSP90AA1, and TLR4 as core targets, along with their involvement in lipid and atherosclerosis-related pathways, offers valuable insights into the molecular basis of *F. elastica's* action. The high-affinity interactions observed between *F. elastica* compounds and these targets, particularly compounds 41 and 61, further support the plant's therapeutic potential. Our *in vitro* and *in vivo* experiments corroborate the computational findings, demonstrating *F. elastica's* ability to inhibit HMG-CoA reductase and significantly reduce total cholesterol and triglyceride levels while increasing HDL levels in hyperlipidaemic rats. Notably, the extract's effects on triglycerides and HDL were comparable to those of simvastatin. These results collectively position *F. elastica* as a promising candidate for anti-hyperlipidaemic therapy, potentially offering a natural alternative with fewer side effects compared to conventional treatments. Furthermore, this study underscores the power of integrating computational and experimental approaches in natural product research, paving the way for more efficient drug discovery processes in the future. While these findings are encouraging, further research is warranted to fully elucidate the long-term effects, toxicity, optimal dosing, and potential interactions of *F. elastica* extracts. Nonetheless, this study marks a significant step forward in understanding the anti-hyperlipidaemic properties of *F. elastica* and highlights its potential as a valuable addition to the arsenal of treatments for hyperlipidaemia and related cardiovascular disorders.

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Conflicts of Interest

The authors declare no conflict of interest.

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