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Research Article

Hypolipidemic Effect of Chloroform Extract of *Lagenaria siceraria*: Potential Inhibitory Activity of Phytochemicals Targeting the HMG-CoA Reductase Revealed by Molecular Docking and Simulation Studies

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Conventional systems of medicine play a crucial role in maintaining healthcare. Herbal medicines are intact and less harmful to human beings than synthetic medicines. This study aimed to investigate the phytochemicals and *in vivo* hypolipidemic effect of chloroform extract of *Lagenaria siceraria* in Triton X-100 (100 mg/kg body weight) induced hyperlipidemic Wistar rats. The phytochemical characterization and estimation were performed on the base of the GC-MS approach. The *Lagenaria siceraria* extract (250 and 500 mg/kg bw) was administered orally to hyperlipidemic-induced rats for 7 days to examine its hypolipidemic activity. The experimental animals did not display any acute toxicity. Atorvastatin (10 mg/kg bw) was used as a standard drug. Administration of *Lagenaria siceraria* extract lowers the total cholesterol (TC), triglyceride (TG), and low-density lipoproteins-cholesterol (LDL-C) levels whereas elevating the high-density lipoproteins-cholesterol (HDL-C) level. Histological studies of the liver and heart also showed the hypolipidemic effect of the extract. On the 8th day, no inflammation of the liver, myocardial necrosis, fibrosis, or atypia was seen. Furthermore, binding affinity and plausible binding mode of stigmastan-3-ol with HMG-CoA reductase were predicted by molecular docking studies which showed the same interaction patterns as atorvastatin. Moreover, the docking results were refined by 100 ns MD simulations which revealed that stigmastan-3-ol extract formed a stable complex with protein and did not induce any conformational changes in protein structure.

1. Introduction

Hyperlipidemia is a condition that occurs due to higher levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), and very low-density lipoprotein (VLDL). This disease affects human health badly leading to a variety of cardiovascular disorders including angina pectoris, myocardial infarction, hypertension, atherosclerosis, and congestive heart failure. Hyperlipidemia is divided into two types, first is primary hyperlipidemia (familial hyperlipidemia) which is caused by specific genetic problems. The other type of hyperlipidemia is secondary hyperlipidemia (acquired hyperlipidemia) which is caused due to some disorders leading to abnormalities in plasma lipids and

lipoprotein metabolism [1, 2]. Hyperlipidemia is also a highly anticipated risk factor for atherosclerosis, cerebral vascular disease, and coronary artery disease [3]. Atherosclerosis of coronary arteries is generally a disease of an arterial network called dynamic and silent killer described by the production of abrasions known as atherosclerosis plaques inside the walls of coronary arteries, which cuts down blood flow to the heart and is called CAD (coronary artery disease) [4]. Various shreds of evidence showed that hyperlipidemia is linked to atherosclerosis and clinical tests demonstrated that decreasing serum cholesterol lowers mortality and distress of patients having established CAD [5, 6]. Condiments, herbal plants, and fruits used for daily food have been identified as hypolipidemic in Ayurveda [7].

Conventional systems of medicine play a crucial role in maintaining healthcare. Herbal medicines are intact and less harmful to human beings than synthetic medicines [8]. Several herbal remedies are being used in many medical organizations for the medication and management of different diseases. Usually, herbal compositions include the use of dried or fresh plant parts. Also, herbs provide many important lifesaving medicines that are being used in the modern scheme of medicine. So, laboratories are busy all over the world in the selection and separation of different components of plants for their potential therapeutic applications [8].

Lagenaria siceraria (*L. siceraria*) is an important vegetable that is grown throughout Pakistan and is readily found in the market all over the year. It is an exceptional vegetable for human beings granted by nature which contains all the principal components required for good and normal human health. It is an excellent source of carbohydrates, proteins, minerals, and pectin and also contains the highest level of lipotropic agent choline which is crucial for better functioning of the brain [9]. The ethanolic and methanolic extracts have been reported for various disease targets such as analgesic, anti-inflammatory, antioxidant, hepatoprotective, diuretic, antihyperlipidemic, antidepressant, antiulcer, and antibacterial activities [8, 10]. Its pulp is applied to the soles of feet for the cure of "burning feet." Its seeds have cooling, fattening, and antihelmintic effect [11] whereas lagenin (a ribosome-inactivating protein) has been separated from the lyophilized water extract of its seeds; this extract has antiviral, immunosuppressive, antiproliferative, immunoprotective, antitumor and anti-HIV activities [8]. Although studies have been performed on *L. siceraria*, all the studies used ethanolic or methanolic extracts of this vegetable. In the present study, extraction was performed with chloroform; consequently, gas chromatography coupled with mass spectrometry (GC-MS) was conducted to find the phytochemicals to explore their hypolipidemic activity. Also, chloroform extracts of *L. siceraria* were explored for antibacterial, anti-inflammatory, and antioxidant activities. These findings suggest that *L. siceraria* may have potential therapeutic applications in the treatment of various diseases which involve inflammation and bacterial infections [12, 13]. Furthermore, the use of chloroform as a solvent for the extraction of bioactive compounds from *L. siceraria* is also noteworthy. While other solvents such as ethanol and methanol are commonly used in plant extractions, chloroform has unique properties that make it suitable for the extraction of specific compounds which may not be extracted using other solvents. Therefore, the use of chloroform as a solvent for the extraction of *L. siceraria*'s bioactive compounds adds to the novelty of the research on this plant.

Moreover, in silico studies were performed to check the role of stigmastan-3-ol as ligands against hypolipidemic receptor proteins. Molecular docking studies were performed, and the binding mode of stigmastan-3-ol in the HMG-CoA reductase binding site was analyzed to check its similarity with the atorvastatin binding mode. The bulky hydrophobic group of atorvastatin occupies the binding pocket of the HMG, thus the substrate access to HMGR is blocked [14], which helps in lowering the cholesterol level. So, the same binding pattern of stigmastan-3-ol can mimic the same biological activity. Finally, the stability of stigmastan-3-ol with protein was determined by 100 ns MD simulation.

2. Materials and Methods

2.1. Plant Material. The fresh vegetable samples were collected from a local market in Lahore, Pakistan. Identification and authentication of the plant were performed by plant taxonomists (Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan). A voucher specimen (voucher no. UOLDC-011) was deposited in the herbarium of the university. The samples were washed thoroughly and dried in the shade for a week. The dried fruits were ground to powder.

2.2. Preparation of Crude Extract. To determine the hypolipidemic activities, the powdered sample was soaked in 500 mL chloroform overnight and then extracted using the Soxhlet extraction process with minor modifications in the already reported extraction methodology [15]. The extract obtained was subjected to rotary evaporation for removal of solvent and then placed in a hot air oven to dry the extract completely and placed at 4°C for future use.

2.3. Gas Chromatography-Mass Spectrometry Study (GC-MS). To determine the natural component of *L. siceraria* extract, the considered extract was analyzed by using a GC-MS chromatograph from Agilent Technologies (7890 A, Santa Clara, CA, USA) equipped with an HP-5 (length 30 m, i.d. 250 μm, film thickness 25 μm). 1.0 μL of the sample (1/100, v/v prepared in methanol) was injected in split mode. The mass spectrometer was an Agilent Technologies (5975C) system working on 70 eV with a scan time of 1.0 sec, and the oven temperature ranged from 35°C to 300°C, while the temperature of the injector was 180°C and the mass ranged from 50 to 500 amu.

2.4. Animals and Toxicological Studies. All the animal experimental methods were conducted as per guidelines of the UK Animals (Scientific Procedures) Act 1986, approved by

the Animal Ethical Committee (Ethical certificate: ECDC-002-UL) of The University of Lahore, Pakistan. A total of thirty Wistar albino rats of either sex having a weight between 200 and 250 g were kept under standard conditions in the animal house, Faculty of Pharmacy, The University of Lahore, under the guidelines of the EEC Directive of 1986 (86/609/EEC) use of laboratory animals (NIH Publication no. 80-23; revised 1978). The rats were subjected to toxicological studies tests by giving them 1000 mg/kg dose of *L. siceraria* extract according to OECD oral toxicity guidelines 423 category IV 2001 [16]. Animals were observed individually for any change in the skin, behavioral pattern, mortality, convulsions, lethargy, and coma for a period of 48–72 hrs.

2.5. Treatment and Induction of Hyperlipidemia. Thirty Wistar albino rats were divided randomly into five groups having 6 rats in each group. Hyperlipidemia was induced in Wistar albino rats (group I to V) by injecting intraperitoneal injection of Triton X-100 (100 mg/kg body weight) in saline solution after fasting for 18 h [17, 18]. Group I was administered orally with a standard pellet diet and water (negative control group). After 72 h, group II was given a high-fat diet (which was a mixture of a normal pellet diet and butter) daily for 7 days consecutively (no treatment group). Group III was given standard atorvastatin (10 mg/kg) administered orally for 7 days regularly (positive control). Group IV and V were administered orally with a daily dose of *L. siceraria* extract (250 mg/kg and 500 mg/kg), respectively, for 7 consecutive days after induction of hyperlipidemia. The dose stopped 12 h before taking a blood sample. Plant extracts are generally regarded as natural products and are often assumed to be safer than synthetic drugs. As a result, higher doses may be used in initial biological assays to evaluate the potential toxicological effects or safety margins of the extract. This allows researchers to establish a dose-response relationship and determine the maximum tolerable dose before proceeding to further studies. It is crucial to remember that the dosage utilized in biological assays is not always representative of the dosage used in clinical applications involving humans. The outcomes of these assays are frequently utilized as a starting point to direct additional research and dosage modifications for future medicinal usage.

2.6. Biochemical Analysis and Histopathological Studies. The rats were anesthetized using chloroform on the 8^{th} day of the experiment, and blood samples were collected. The blood samples were centrifuged at 2500 rpm for 10 min. The serum samples were collected and analyzed for the estimation of TL (total lipids), TC (total cholesterol), TG (total glycerides), HDL-C (high-density lipoprotein-cholesterol), LDL-C (low-density lipoprotein-cholesterol), and VLDL-C (very low-density lipoprotein-cholesterol) [19]. After the completion of the experiment, the animals were sacrificed. The liver and heart of all the experimental animals were dissected out and placed in formalin to preserve them. The body parts were washed, and slides were prepared. The slides were stained with hematoxylin-eosin (H&E) and examined under a light microscope.

2.7. Molecular Docking and Simulation Studies. The human HMG-CoA reductase crystal structure (PDB ID: 1HWK) was prepared by using the protein preparation wizard in Maestro. Hydrogens were added, water was removed, residues from side chain atoms were fixed, and charges were added to the receptor. Unnecessary protein chains and ligands were eliminated, creating tautomeric states at a pH of 7.0. In protein was then optimized and minimized by applying the OPLS_2005 force field [20]. To conduct site-specific docking, a cocrystal ligand was selected to generate a receptor grid with internal coordinate values of X, Y, and Z as 6.04, −10.31, and −11.52, respectively. Phytochemicals from the *L. siceraria* extract were prepared by the LigPrep tool in Maestro and docked to the receptor using Glide [21]. The docking poses were analyzed based on the glide score after different ionization states were generated at pH 7 by Epik, and stereoisomers were generated with the OPLS_2005 force field.

The stability of the complexes was confirmed by running a 100 ns long MD simulation using VMD and NAMD [22]. The antechamber module was used to generate the ligand parameters, while the protein structure was processed through the Leap program of the AmberTools21 [23] to generate the protein coordinates and topology files. The pdb4amber module was used to convert the protein residues into amber format. Moreover, GAFF and ff14SB force fields were used to optimize the structures of ligands and proteins, respectively [24]. The optimized structures were solvated using TIP3P water molecules [25] in a periodic box of 10 Å. Both the systems were then neutralized by adding Na^+ and Cl^- counter ions. The systems were minimized to remove steric clashes for 10000 steps and then subjected to water equilibration for another 10000 steps. Water equilibration was further followed by temperature equilibrations at 200, 250, and 300 K. Finally, the systems were subjected to a production run for 100 ns at 310 K temperature and 1 atm pressure using the NPT ensemble. The analysis of the MD trajectory was carried out using the BIO3D package of R [26].

2.8. Statistical Analysis. All the data from the experiment were replicated and their values were reported as mean ± standard error, whereas ANOVA was performed to assess the significant difference between the groups ($p < 0.001$) using SPSS version 15 (SPSS Inc., Chicago, IL, USA) for Windows.

3. Results and Discussion

3.1. GC-MS Analysis. Phytochemicals from *L. siceraria* extract were identified through the GC-MS technique (Figure 1). The different compounds were recognized by comparing the spectral data with the information from the National Institute of Standards and Technology (NIST) library. The compounds' name and their percentage, retention time (RT), formula, molecular mass, and the structure of the fruit extract are shown in Table 1. The most important compound identified in *L. siceraria* extract was stigmastan-3-ol, a phytosterol found in a maximum amount (73.94%). Phytosterol plays a very

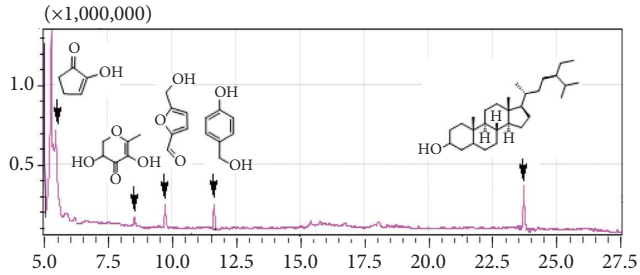
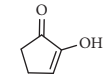
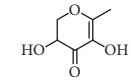
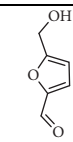
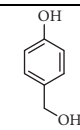
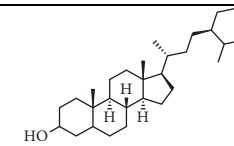


Figure 1: GC-MS of *L. siceraria* extract.

Table 1: Phytochemicals identified in *L. siceraria* extract.

Sr. no.	Compound name (%)	RT (min.)	Formula	Mass (g/mol)	Structure
1	2-Hydroxycyclopent-2-en-1-one (11.09)	5.48	$C_5H_6O_2$	98.04	
2	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (7.18)	8.74	$C_6H_8O_4$	144.13	
3	5-Hydroxymethylfurfural (3.47)	9.94	$C_6H_6O_3$	126.11	
4	4-(Hydroxymethyl)phenol (4.32)	11.75	$C_7H_8O_2$	124.14	
5	Stigmastan-3-ol (73.94)	23.64	$C_{29}H_{52}O$	416.73	

important role in controlling hyperlipidemia and acts as a precursor for vitamin D synthesis. It has been observed from the literature that phytosterols are important metabolites, have great potential, and are a controlling factor against hyperlipidemia, fatty liver, and several heart diseases, and have several health benefits, including antioxidant and anticancer activities [27].

Before evaluating the hypolipidemic effect of *L. siceraria* extract, an acute toxicity study was performed on experimental animals. The experimental animals displayed no behavioral or neurological alterations in their behavior, such as salivation, tremors, diarrhea, lacrimation, sleep, or feeding patterns.

3.2. Effect of Extract on Serum Total Lipids Level. The results (Figure 2(a)) revealed that the TL level in the serum of group II (high-fat diet group) rats was quantified as 415.0 ± 3.85 mg/dl which is quite higher ($p < 0.001$) than the TL level in group I (negative control group, 276.0 ± 2.58 mg/dl) rats which were given a normal diet. Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally for each rat daily) had a serum TL level of 182.3 ± 1.92 mg/dl when estimated on the 8th day. This was convincingly lower ($p < 0.001$) than the TL level in the serum of group II rats (415.0 ± 3.85 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV) and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(a)) showed that the TL level was found to be 198.6 ± 2.09 mg/dl and 186.0 ± 1.68 mg/dl, respectively, when estimated on the 8th day. The TL level in the serum of group V rats (186.0 ± 1.68 mg/dl) was comparable with the TL level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 182.3 ± 1.92 mg/dl) and significantly lower ($p < 0.001$) than the serum TL level found in group I rats (negative control group, 276.0 ± 2.58 mg/dl).

3.3. Effect of Extract on Serum Total Cholesterol Level. The data from Figure 2(b) showed that the TC level in the serum of group II (high-fat diet group) rats was quantified as 72.0 ± 2.08 mg/dl which is quite higher ($p < 0.001$) than the TC level in group I (negative control group, 65.6 ± 2.40 mg/dl) rats which were given a normal diet. Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally for each rat daily) had the serum

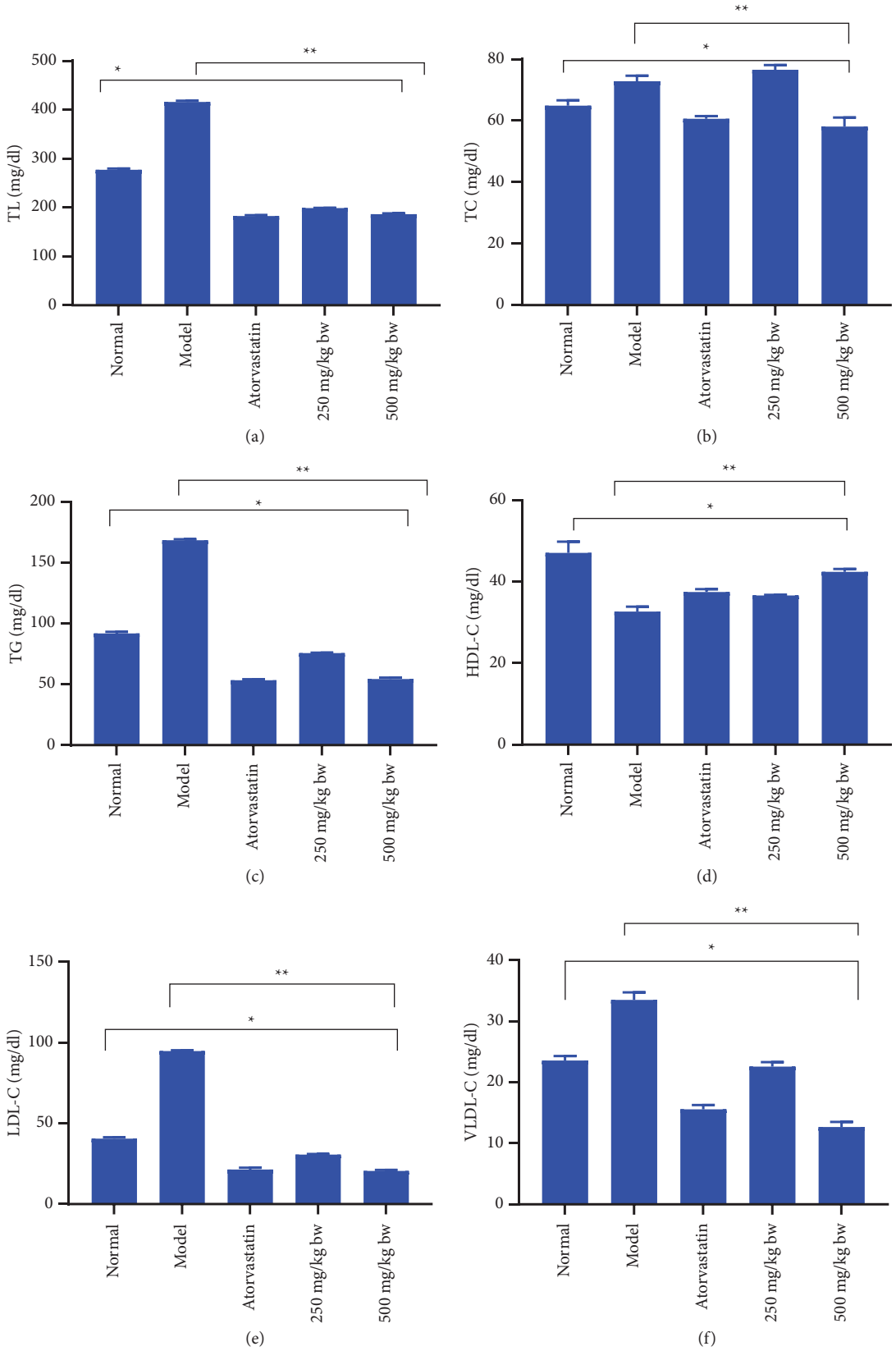


FIGURE 2: Effect of *L. siceraria* extract on different biological parameters of Triton X-100-induced hyperlipidemia rats. Data are expressed as mean ± standard deviation, $n = 6$. $^{*}p < 0.05$, compared with the normal group and $^{**}p < 0.05$, compared with the model group.

TC level of 60.3 ± 0.92 mg/dl when estimated on the 8th day. This was convincingly lower ($p < 0.001$) than the TC level in the serum of group II rats (72.0 ± 2.08 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV), and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(b)) showed that the TC level was found to be 76.0 ± 1.19 mg/dl and 56.8 ± 1.68 mg/dl, respectively, when estimated on the 8th day. The TC level in the serum of group V rats (56.8 ± 1.68 mg/dl) was lower than the TC level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 60.3 ± 0.92 mg/dl) and significantly lower ($p < 0.001$) than the serum TC level found in group I rats (negative control group, 65.6 ± 2.40 mg/dl mg/dl).

3.4. Effect of Extract on Serum Triglyceride Level. The TG level (Figure 2(c)) in the serum of group I rats (negative group) was quantified as 92.3 ± 1.76 mg/dl which was significantly lower ($p < 0.001$) than the serum TG level (168.3 ± 4.41 mg/dl) in rats' group which were given a high-fat diet (group II). Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally for each rat daily) had a serum TG level of 53.3 ± 1.12 mg/dl when estimated on the 8th day. This was convincingly lower ($p < 0.001$) than the TG level in the serum of group II rats (168.3 ± 4.41 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV) and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(c)) showed that the TG level was found to be 75.6 ± 1.21 mg/dl and 54.4 ± 1.21 mg/dl, respectively, when estimated on the 8th day. The TG level in the serum of group V rats (54.4 ± 1.21 mg/dl) was comparable with the TG level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 53.3 ± 1.12 mg/dl) and significantly lower ($p < 0.001$) than the serum TG level found in group I rats (negative control group, 92.3 ± 1.76 mg/dl mg/dl).

3.5. Effect of Extract on Serum High-Density Lipoprotein-Cholesterol. The results (Figure 2(d)) revealed that the HDL-C level in the serum of group II (high-fat diet group) rats was quantified as 33.0 ± 1.52 mg/dl which is significantly lower ($p < 0.001$) than the HDL-C level in group I (negative control group, 47.0 ± 1.08 mg/dl) rats which were given a normal diet. Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally for each rat daily) had a serum HDL-C level of 37.3 ± 0.82 mg/dl when estimated on the 8th day. This was higher than the HDL-C level in the serum of group II rats (33.0 ± 1.52 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV) and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(d)) showed that the HDL-C level was found to be 36.6 ± 1.09 mg/dl and 42.6 ± 1.45 mg/dl, respectively, when estimated on the 8th day. The HDL-C level in the serum of group IV rats (36.6 ± 1.09 mg/dl) was comparable with the HDL-C level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 37.3 ± 0.82 mg/dl), whereas the HDL-C level in the serum of group V rats (42.6 ± 1.45 mg/dl) was higher than the results found from the standard drug.

3.6. Effect of Extract on Serum Low-Density Lipoprotein-Cholesterol. The LDL-C level (Figure 2(e)) in the serum of group I rats (negative group) was quantified as 40.1 ± 1.12 mg/dl which is significantly lower ($p < 0.001$) than the serum LDL-C level (94.6 ± 2.40 mg/dl) in rats' group which were given a high-fat diet (group II). Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally each rat daily) had a serum LDL-C level of 21.0 ± 0.52 mg/dl when estimated on the 8th day. This was significantly lower ($p < 0.001$) than the LDL-C level in the serum of group II rats (94.6 ± 2.40 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV), and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(e)) showed that the LDL-C level was found to be 30.6 ± 1.01 mg/dl and 20.4 ± 0.21 mg/dl, respectively, when estimated on the 8th day. The LDL-C level in the serum of group V rats (20.4 ± 0.21 mg/dl) was almost equivalent to the LDL-C level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 21.0 ± 0.52 mg/dl) and significantly lower ($p < 0.001$) than the serum LDL-C level found in group I rats (negative control group, 40.1 ± 1.12 mg/dl mg/dl).

3.7. Effect of Extract on Serum Very Low-Density Lipoprotein-Cholesterol. The VLDL-C level (Figure 2(f)) in the serum of group I rats (negative group) was quantified as 22.3 ± 0.82 mg/dl which is significantly lower ($p < 0.001$) than the serum VLDL-C level (33.1 ± 1.40 mg/dl) in rats' group which were given a high-fat diet (group II). Triton X-100-treated rats (group III, positive control) administered with atorvastatin (10 mg/kg, orally for each rat daily) had a serum VLDL-C level of 15.3 ± 0.76 mg/dl when estimated on the 8th day. This was significantly lower ($p < 0.001$) than the VLDL-C level in the serum of group II rats (33.1 ± 1.40 mg/dl). The Triton X-100-treated rats were administered with extract 250 mg/kg (group IV), and 500 mg/kg (group V) orally for each rat daily. The results (Figure 2(f)) showed that the VLDL-C level was found to be 22.3 ± 0.81 mg/dl and 12.4 ± 0.45 mg/dl, respectively, when estimated on the 8th day. The VLDL-C level in the serum of group V rats (12.4 ± 0.45 mg/dl) was convincingly lower than the VLDL-C level in the serum of rats administered with standard hypolipidemic drug (atorvastatin, 15.3 ± 0.76 mg/dl) and significantly lower ($p < 0.001$) than the serum VLDL-C level found in group I rats (negative control group, 22.3 ± 0.82 mg/dl).

3.8. Histopathological Studies

3.8.1. Lipid Accumulation in the Liver. There was no lipid accumulation seen in the liver of the rats' group without treatment and labeled as a normal control group. Also, no inflammation, hydropic degeneration, necrosis, fibrosis, malignancy, or atypia was seen (Figure 3(a)). Histological changes in high-fat diet rats showed degeneration of hepatocytes. A minimum accumulation of lymphocytes was seen in portal tracts. Severe lipid accumulation was seen in hepatocytes (Figure 3(b)). Histological changes in the rat's standard (atorvastatin)-treated group rats showed normal hepatic architecture and low-grade lipid accumulation. Focal

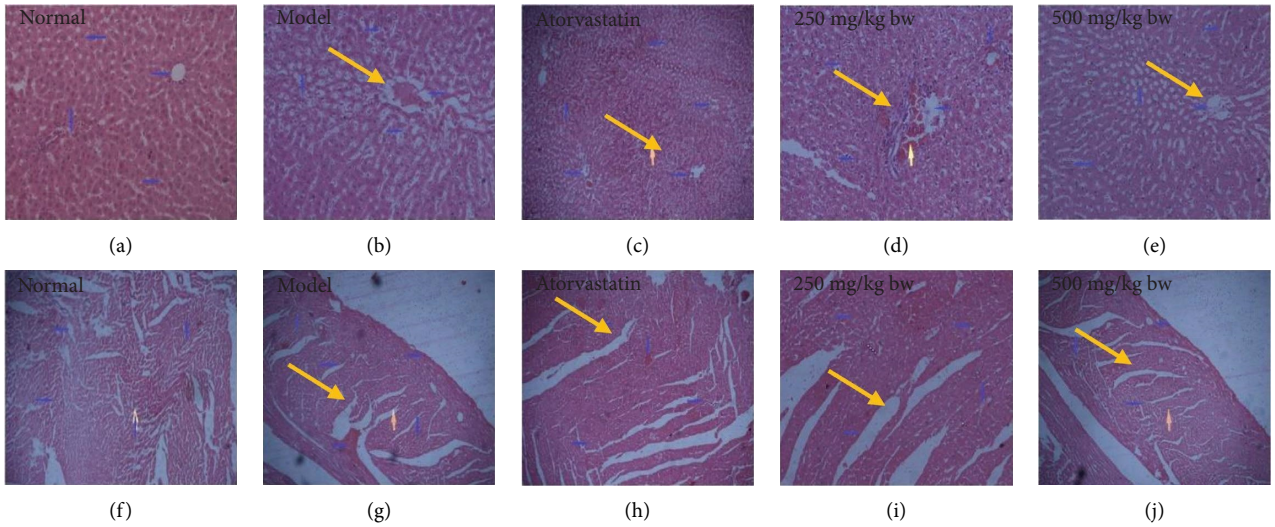


Figure 3: Histopathological changes on liver and heart of animals induced by Triton X-100. Hematoxylin and eosin (H&E) ×100.

central vein dilation was also seen. No inflammation, hydropic degeneration, necrosis, fibrosis, malignancy, or atypia was seen (Figure 3(c)). Histological changes in the rats of the 250 mg/kg fruit extract group showed sparsely present hydropic degeneration of hepatocytes. Moderate-grade lipid accumulation is seen. No inflammation, necrosis, fibrosis, malignancy, or atypia was seen (Figure 3(d)). Histological changes in the rats of the 500 mg/kg extract group showed normal hepatic architecture and central veins pattern. Low-grade lipid accumulation was evident. No hydropic degeneration, inflammation, necrosis, fibrosis, malignancy, or atypia was seen (Figure 3(e)).

3.8.2. Lipid Accumulation in the Heart. Histological examination of the normal group rats showed normal myocardium. There was no evidence of fatty changes. No myocardial necrosis, fibrosis, atypia, or malignancy was seen in the heart (Figure 3(f)). Histological examination of the high-fat diet group rats revealed normal myocardium pattern and arrangement. Severe lipid accumulation was evident. A minimal deposition between myofibroblasts was seen. Myocardial necrosis is seen. No edema, fibrosis, atypia, or malignancy was seen (Figure 3(g)). Histological examination of the rats of standard (atorvastatin) group rats revealed normal myocardium. There was no myocardial necrosis, edema, fibrosis, atypia, or malignancy seen (Figure 3(h)). Histological examination of the rats of the 250 mg/kg fruit extract group revealed normal-looking myocardium. There was minimum deposition between myofibroblasts. No myocardial necrosis, edema, fibrosis, atypia, or malignancy was seen (Figure 3(i)). Histological examination of the rats of the 500 mg/kg extract group revealed normal myocardium. Focal edema between myofibroblasts was seen. No myocardial necrosis, fibrosis, atypia, or malignancy was seen (Figure 3(j)).

The whole dried and finely powdered fruit of *L. siceraria* was extracted using the Soxhlet extraction method with chloroform. Phytochemicals such as 2-hydroxycyclopent-2-en-1-one, 3, 5-dihydroxy-6-methyl-2, 3-dihydro-4*H*-pyran-4-one, 5-hydroxymethylfurfural, 4-(hydroxymethyl) phenol, and stigmastan-3-ol were identified and quantified by the GC-MS technique. It has been known that phytochemicals play a vital role in the major causes of atherosclerosis and hyperlipidemia [28, 29].

Usually feeding cholesterol has been utilized to increase serum TC levels to find out metabolic disturbances such as hypercholesterolemia in animals. However, the serum triglyceride level has not only been changed by feeding cholesterol. It is thought that elevated levels of fats along with cholesterol are necessary to increase serum TG levels significantly in rats. In phase I, medicines that interfere with the biosynthesis of cholesterol are active, while in phase II medicines that interfere with metabolism and excretion of cholesterol are active. In phase I as well as in phase II, the rats' group (IV and V) administered with extract showed lowered TL. The hypolipidemic activity of the extract was obvious in both production and excretion of Triton X-100-induced hyperlipidemia rats.

Food that consists of saturated fats elevates HMG-CoA reductase levels, which is the enzyme that determines the rate of cholesterol biosynthesis, and this is because of the availability of acetyl CoA that triggers the rate of cholesterogenesis. Moreover, it could also be correlated with a decreased control in LDL receptors by saturated fatty acids and cholesterol being a part of our diet, which also could explain the increase of the serum LDL-C levels either by altering hepatic LDL receptor (LDLR) activity or by the rate of LDL-C synthesis. LCAT (lecithin-cholesterol acyltransferase) enzyme is associated with the completion of HDL-C, transesterification of cholesterol, and the change of cholesterol from cell membranes to HDL [30].

In this present study, the Triton X-100-induced system was used for the introduction of hyperlipidemia. The Triton X-100 system is utilized as a subtle model for the introduction of hyperlipidemia. Rats which were given Triton X-100 (intraperitoneal injection) showed convincingly elevated levels of TC, TG, and LDL, whereas lowered levels of HDL-C and VLDL-C were noted as compared to total serum lipid levels in high-fat diet control rats. The abovementioned guidelines were convincingly inverted with the medication of atorvastatin, which is used as a standard drug, with lowered levels of TG, TC, VLDL, and LDL, and elevated levels of HDL-C, respectively. Rats fed with a high-fat diet intraperitoneally demonstrated significantly elevated levels of TC, TG, and LDL, whereas lowered levels of HDL and VLDL were noted as compared to total serum lipid levels in high-fat diet control rats. The abovementioned guidelines were convincingly inverted with the medication of atorvastatin (standard drug) and lowered levels of TG, TC, VLDL, LDL, and HDL-C, respectively. The medication with extract 250 mg/kg and 500 mg/kg also convincingly decreased the increased levels of TC, TG, and VLDL, respectively. Triton X-100 is used as a medium (surfactant) and lowers the activity of lipase to cut off the lipoproteins uptake from circulation by extrahepatic tissues, thus elevating lipid levels in blood [17, 31]. There was also an elevation in the HDL-C levels after the treatment with extracts (250 mg/kg and 500 mg/kg). Hyperlipidemia is a condition that shows an abnormal elevation of lipid levels in the blood causing an anticipated hazard for CAD (coronary artery disease), atherosclerosis, and cerebral vascular diseases. Atherosclerosis is usually a familiar mode of arteriosclerosis which is marked by the deposition of cholesterol-lipid calcium inside the arteries. The atherosclerosis plaque builds up on the walls of coronary arteries, decreasing the flow of blood to the heart, and causing a medical problem known as CAD (coronary artery disease). Stigmastan-3-ol present in the juice of *L. siceraria* lowers the serum cholesterol and reduces depression and mortality rate from CAD (coronary artery disease) [32–37]. Elevated plasma cholesterol, particularly LDL and TG levels, are the primary cause of hypercholesterolemia, which can also lead to other illnesses such as obesity, diabetes, and cancer [38–41]. HMG-CoA reductase, the rate-limiting enzyme in the synthesis of cholesterol, has a catalytic role in the conversion of it to mevalonate. The inhibition of HMG-CoA reductase successfully lowers the level of cholesterol in humans and the majority of other animals by activating sterol regulatory element-binding protein-2, which in turn upregulates the HMG-CoA reductase and LDL receptor [38, 42]. Although statins are well-known HMG-CoA reductase inhibitors, prolonged use of these drugs results in serious side effects including rhabdomyolysis, abrupt renal failure, and damage to the liver and muscles. However, it is important to note that HMG-CoA reductase activity and HMG-CoA reductase levels are not the same thing. While reducing the activity of the enzyme can lead to a decrease in its levels, there may be other factors that can influence the levels of HMG-CoA reductase in the body as well. Although the precise mechanism by which *L. siceraria* reduces serum cholesterol is unknown, the observation of increased excretion of bile acids (cholic acid and deoxycholic acid, which are the end products of cholesterol metabolism) suggests that it may act by influencing the endogenous synthesis of cholesterol in the liver and by increasing the excretion of cholesterol end products [43]. Several sterols found in *L. siceraria* fruit extract may help inhibit cholesterol production by inhibiting HMG-CoA reductase. *L. siceraria* fruit extract had significant HMG-CoA reductase inhibitory activity *in vitro*. The study also found that the extract had relatively low cytotoxicity, meaning that it did not have harmful effects on cells. Data collected from experiments show that dried powder of *L. siceraria* has been used as a cardioprotective agent against cardiotoxicity caused by doxorubicin in rats [44–46]. The cardiotoxicity produced by doxorubicin is prevented by oral intake of *L. siceraria*, and myocardial diseases are lowered by the conservation of remote antioxidants (such as reduced glutathione and superoxide dismutase) and peroxidation of lipids in rats [47, 48]. Heart patients can have compelling comfort via daily intake of fresh juice of *L. siceraria*. Moreover, fresh juice lowered blood cholesterol levels, LDL, and TG while increasing HDL. Parikh and Kumar et al. [49, 50] worked on various solvent extracts of *L. siceraria* in Triton X-100-induced rats and checked hypolipidemic activity. Oral intake of 250 mg/kg and 500 mg/kg of *L. siceraria* extract lowered TC, TG, and LDL levels, whereas it elevated HDL levels. *L. siceraria* is found to have more soluble dietary fibers (SDFs) than insoluble dietary fibers (IDFs) also known as insoluble cellulose fibers which lower blood cholesterol levels [51]. As *L. siceraria* is part of our diet and used as a vegetable in different forms, this treatment does not need extract. This is a bioassay-guided study, and it has been further extended towards the isolation, purification, and characterization of potent compounds identified in the extract for screening against hyperlipidemia and synthesizing in the lab. The results of the extract of *L. siceraria* are very convincing and found effective in lowering the TL, TG, LDL-C, and VLDL-C levels as shown in this study. Freshly produced fruit juice of *L. siceraria* taken daily on an empty stomach for 90 days can dramatically lower blood levels of TC, TG, HDL, LDL, and VLDL, according to a prior study conducted in human subjects with dyslipidemia and those with normal health, validating our findings [52].

3.9. Molecular Docking and Simulation Studies. The molecular interactions and plausible binding modes of the phytochemicals in the active site of protein were predicted by molecular docking studies. The active site of the receptor consists of the following residues: Glu559, Gly560, Lys735, Ala751, His752, Asn755, Leu853, Ala856, Leu857, Gly860, Arg590, Met655, Met657, Asn658, Ser661, Glu665, Val683, Ser684, Asp690, Lys690, Lys692, Asp767, and Gly807. 2-Hydroxycyclopent-2-en-1-one was involved in three hydrogen bonds with Ser684, Lys692, and Lys735, and one hydrophobic interaction with Leu853. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one made five hydrogen bonds with Glu559, Arg590, Asp690, Lys691, Asn755, and one pi-alkyl bond with Met657. 5-Hydroxymethylfurfural

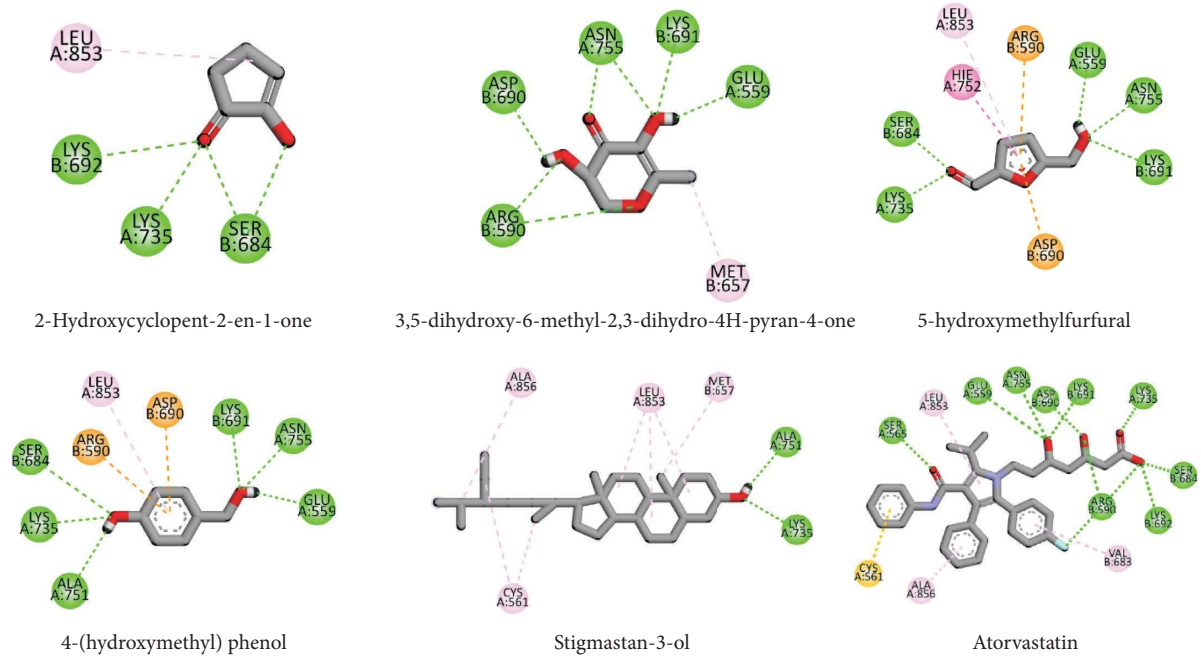
was also involved in five hydrogen bonds and two alkyl bonds. It was additionally involved in pi-anion interactions. 4-(Hydroxymethyl)phenol made six hydrogen bonds and two pi-anion bonds. Stigmastan-3-ol made two hydrogen bonds and four pi-alkyl interactions with the cis loop and large La6 domain. Lastly, the molecular interactions of atorvastatin were also observed which made nine hydrogen bonds, one pi-sulfur bond, and three hydrophobic interactions with similar residues (Figure 4). The binding modes of the docked phytochemicals were analyzed by aligning on the cocrystal structure, and it was observed that all the ligands occupied a similar space in the protein binding pocket. It was observed that stigmastan-3-ol was well fitted onto the cocrystal ligand (atorvastatin) (Figure 5). Hence, the binding pose of the stigmastan-3-ol was selected to perform the MD simulation to find the stability of the protein-ligand complex.

To assess the stability of the protein-ligand complex, the root mean square deviation (RMSD) of the protein backbone atoms and ligand atoms was calculated and compared to the atorvastatin complex throughout a 100 ns simulation, as shown in Figure 6. The complexes were equilibrated for the first 10 ns of the simulation. After the equilibration period, the RMSD of the stigmastan-3-ol complex stabilized within the range of approximately 1.5–2 Å and maintained this stability until around 80 ns. However, after 80 ns, the RMSD gradually increased, reaching approximately 2.25 Å towards the end of the simulation. This indicates a slight increase in structural deviation or fluctuations of the stigmastan-3-ol complex in the later stages of the simulation. While the RMSD of ligand atoms increased to ~2 Å at 20 ns and then attained stability in the range of ~1–1.25 Å till the end of the simulation. In contrast, the RMSD of the atorvastatin complex achieved stability within the range of approximately 2–2.5 Å during the simulation and maintained this stability until the end of the simulation. The atorvastatin complex displayed a relatively higher level of structural deviation than the stigmastan-3-ol complex. A similar trend was observed in the RMSD of atorvastatin atoms as the RMSD values showed deviations up to ~2.5 Å till 70 ns but then attained stability towards the end of the simulation. By comparing the RMSD profiles of the two complexes, it can be observed that both complexes reached a stable state during the simulation, but with different levels of structural deviation. The stigmastan-3-ol complex exhibited a relatively lower RMSD and maintained a more stable conformation within the range of approximately 1.5–2.25 Å, while the atorvastatin complex displayed a slightly higher RMSD within the range of approximately 2–2.5 Å throughout the simulation.

To evaluate the protein compactness during the simulation, the radius of gyration (Rg) was calculated. The Rg provides information about how compact or unfolded the protein structure is throughout the simulation period. Higher Rg values indicate a more extended or unfolded protein conformation, while lower or stable Rg values suggest a compact protein structure. In Figure 7, the Rg plots of the complexes can be observed. The Rg value of the stigmastan-3-ol complex was approximately 27.2 Å at the beginning of the simulation. Throughout the simulation, the Rg value did not increase significantly and remained within the range of approximately 27.2–27.4 Å. Towards the end of the simulation, the Rg value slightly increased to approximately 27.5 Å. The deviation in the Rg value was only 0.2 Å, indicating that the protein maintained its compactness during the simulation and did not undergo any significant unfolding events. Similarly, the Rg value of the atorvastatin complex remained within the range of approximately 27.2–27.4 Å throughout the entire simulation, indicating a stable and compact protein structure. Based on the Rg analysis, both complexes exhibited stable and compact protein conformations throughout the simulation period. The small deviations in the Rg values suggest that there were no major unfolding events or significant changes in the protein compactness during the simulation.

The flexibility of amino acid residues during the simulation was analyzed by calculating the root mean square fluctuation (RMSF). The RMSF values provide insights into the dynamic behavior of individual residues, with higher values indicating greater flexibility compared to more rigid regions such as alpha helices and beta sheets. Figure 8 depicts the RMSF plot of the complex. Most of the residues displayed RMSF values below 1.5 Å, indicating relatively low flexibility. However, residues 400 to 410 in the stigmastan-3-ol complex exhibited an RMSF value of approximately 4 Å, while the corresponding residues in the atorvastatin complex showed slightly lower RMSF values of around 3.5 Å. These higher RMSF values suggest the presence of loop regions in the protein structure, which tend to be more flexible and exhibit greater conformational variability. Overall, the protein structure remained relatively rigid during the simulation, as indicated by the low RMSF values for most residues. This suggests the stability of the protein-ligand complex throughout the simulation period.

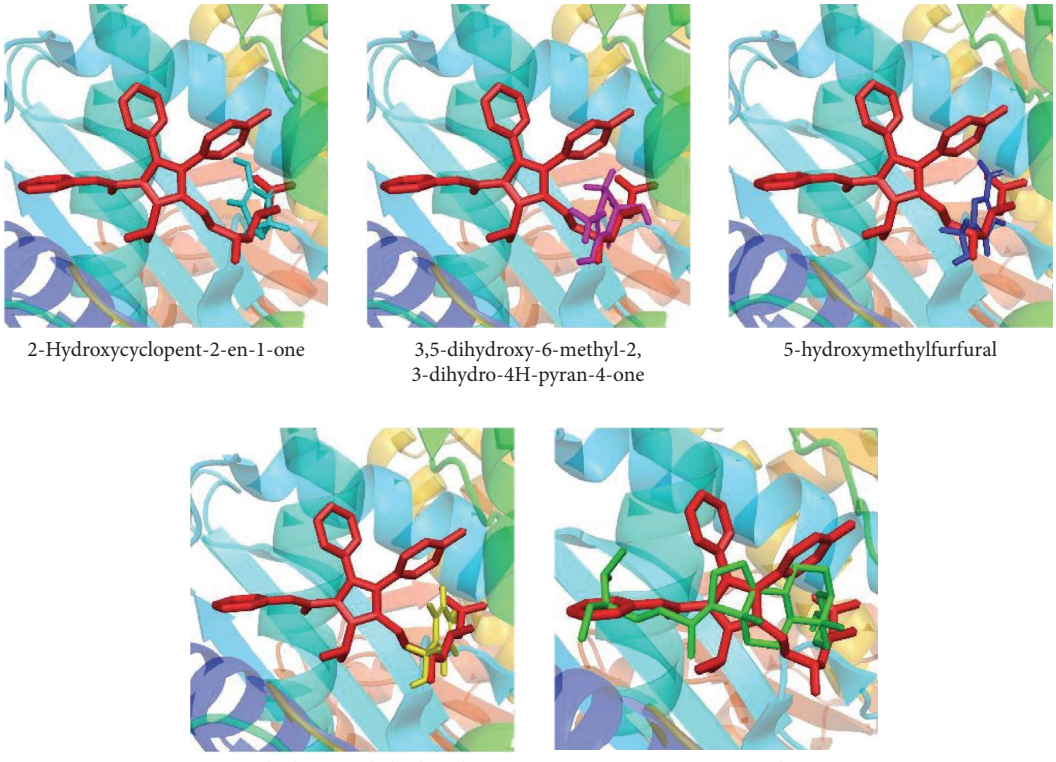
To analyze the dynamic behavior of the protein, principal component analysis (PCA) was conducted, which helps identify collective motions in MD trajectories. The PCA plots of the complexes are presented in Figure 9. The proportion of variance, represented by eigenvalues, was plotted against the number of principal components (PCs) to capture the major fluctuations. In this case, only three PCs were plotted as they accounted for significant variance. In the stigmastan-3-ol complex, PC1 exhibited the highest variation, accounting for 19.13% of the total variance. On the other hand, PC1 in the atorvastatin complex accounted for 13.04% of the variance. The PCA analysis indicated conformational changes across all clusters within the PC subspace. The color-coded representation in the plots showed the degree of movement, with blue regions indicating the greatest degree of movement, white regions representing intermediate levels of flexibility, and red regions indicating the least significant movement. In addition, the correlation among protein residues was investigated through a cross-correlation matrix, as shown in Figure 10. The matrix depicted the correlation between residues during the simulation. Cyan color represented positively correlated residues, while magenta color represented anticorrelated residues. It was observed that most residues exhibited



2-Hydroxycyclopent-2-en-1-one | 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one | 5-hydroxymethylfurfural

4-(hydroxymethyl) phenol | Stigmastan-3-ol | Atorvastatin

Figure 4: The types of molecular interactions formed by ligands with HMG-CoA reductase binding site. Green lines show hydrogen bonding, orange lines show pi-anion bonds, while magenta shows the pi-alkyl bonds.



2-Hydroxycyclopent-2-en-1-one | 3,5-dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one | 5-hydroxymethylfurfural

4-(hydroxymethyl) phenol | Stigmastan-3-ol

Figure 5: The plausible binding modes of the phytochemicals superimposed on the cocrystal ligand. Green sticks (stigmastan-3-ol) aligned well on the cocrystal ligand (atorvastatin), indicating the importance of binding mode.

a positive correlation, indicating that they moved in a coordinated manner. The anticorrelated residues were observed to be relatively short and less pronounced. The diagonal lines in the matrix indicated the positive correlation among topologically proximate residues. This analysis suggested that the protein residues displayed a high

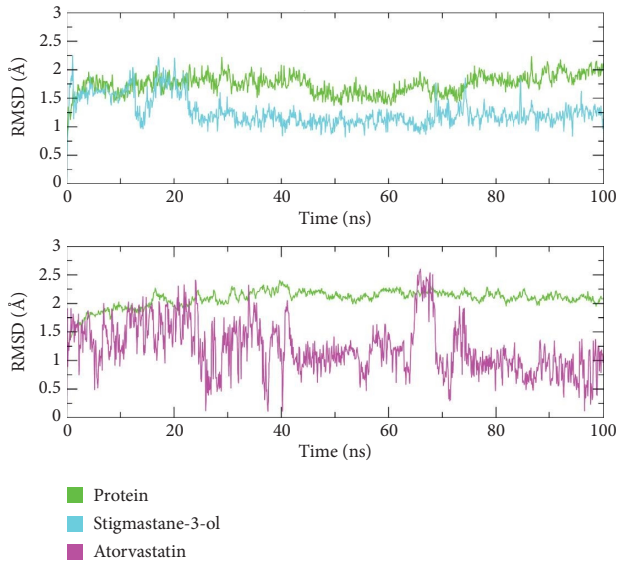


Figure 6: The protein-ligand stability analysis by calculation of protein backbone and ligand atoms' RMSD during simulation. Green plots show the RMSD of protein backbone atoms with the respective ligands.

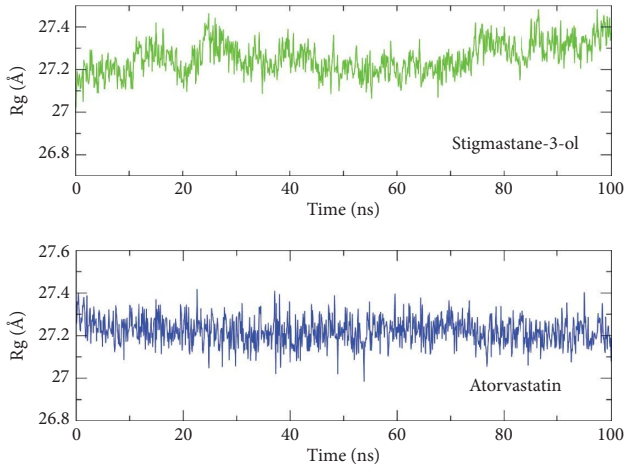


Figure 7: The exploration of protein structure compactness bound to stigmastan-3-ol and atorvastatin, by measuring the radius of gyration.

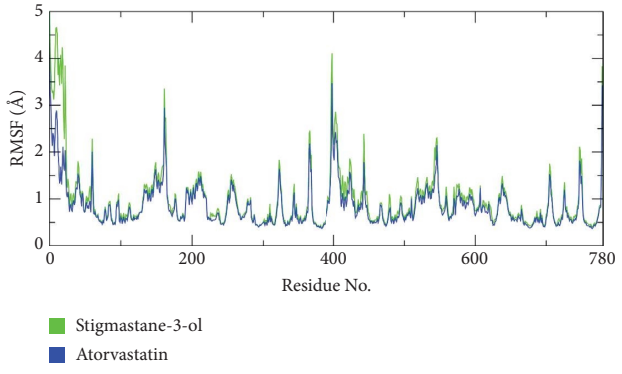


Figure 8: The RMSF plots of the complexes to analyze the residues' flexibility during simulation.

correlation with each other during the simulation, indicating coordinated motions and a coupled dynamic behavior within the protein structure.

3.10. Molecular Mechanics/Poisson–Boltzmann Surface Area. The molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) method was used to calculate the total binding free energy (ΔG_{total}) for both complexes. ΔG_{total} value is usually used to estimate the stability of the protein-ligand complex. The lower values of ΔG_{total} indicate that the complex is more stable and vice versa. It was computed as a sum of the protein-ligand complex and the difference of protein and its ligands' free energies. The total binding free energy estimated using the MM/PBSA model is the outcome

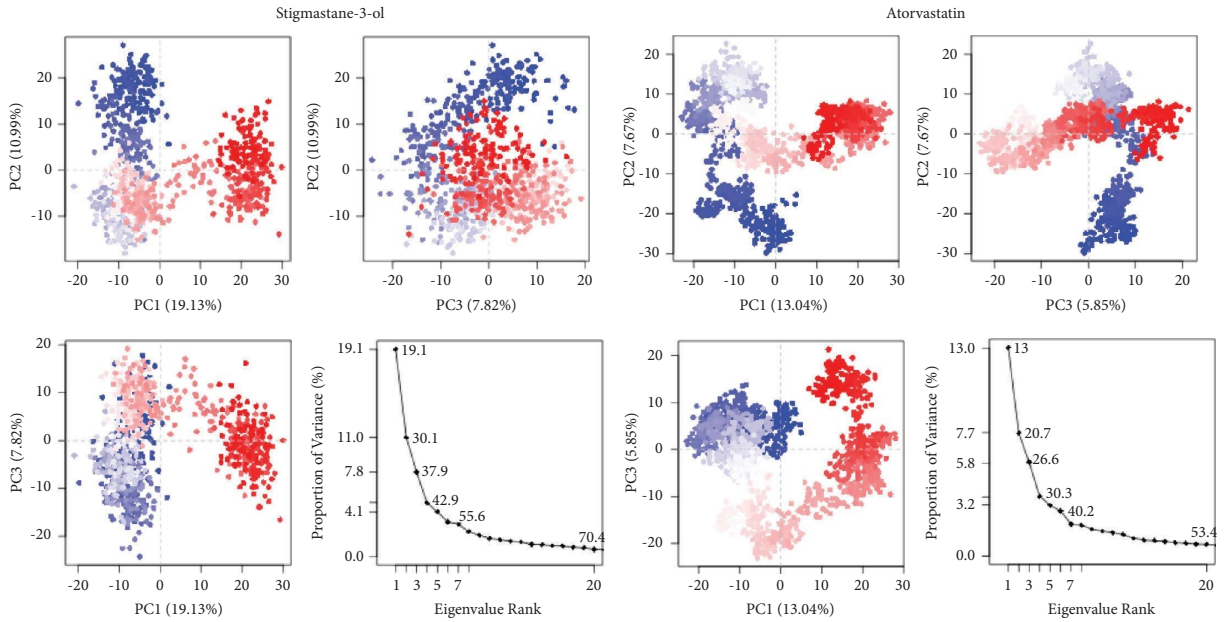


Figure 9: The investigation of the dynamic behavior of protein at different hyperspaces through PCA analysis. The PCA plot of the stigmastan-3-ol complex shows 37.94% variations in three PCs. The PCA plot of the atorvastatin complex shows 26.56% variations in three PCs.

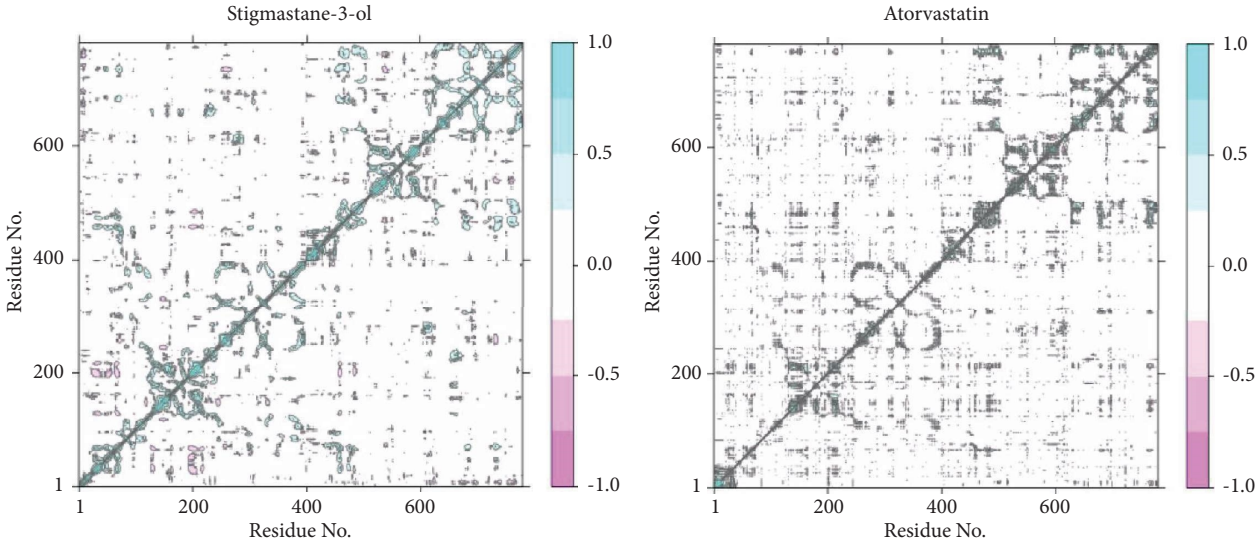


Figure 10: The estimation of positive and negative correlation among amino acid residues in stigmastan-3-ol and atorvastatin complexes.

Table 2: The binding free energies of the complexes calculated by implying the MM/PBSA module.

Energy components (kcal/mol)	Stigmastan-3-ol	Atorvastatin
ΔE_{vdW}	−41.35 ± 0.14	−43.62 ± 0.15
ΔE_{ele}	−3.47 ± 0.08	−4.51 ± 0.09
ΔG_{nonpol}	−30.28 ± 0.15	−27.69 ± 0.12
ΔG_{DIS}	44.31 ± 0.06	48.77 ± 0.09
ΔE_{PB}	21.24 ± 0.08	19.45 ± 0.10
ΔG_{gas}	−44.82 ± 0.17	−43.57 ± 0.16
ΔG_{solv}	45.28 ± 0.09	44.67 ± 0.10
ΔG_{total}	−9.09 ± 0.15	−6.50 ± 0.17

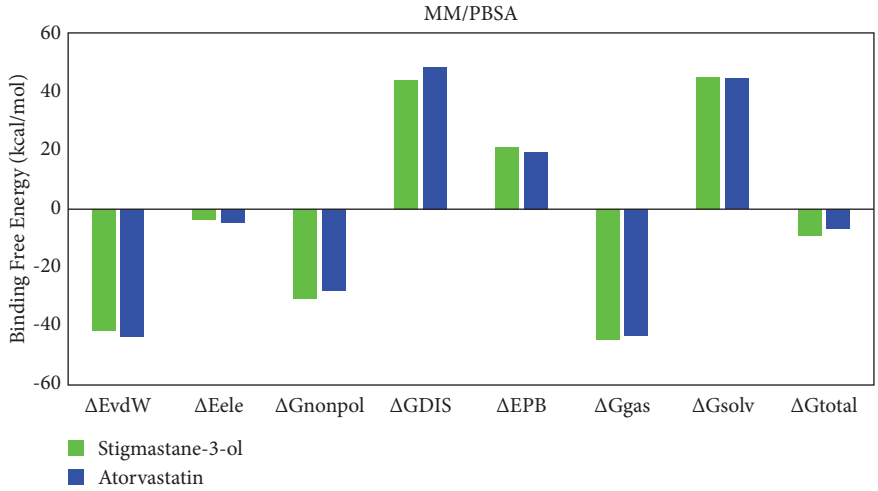


Figure 11: Binding free energy contributions of the energy components.

of the contribution of various protein-ligand interactions such as van der Waals energy (ΔE_{vdW}), electrostatic energy (ΔE_{ele}), and ΔE_{PB} (electrostatic contribution to the solvation free energy by Poisson–Boltzmann surface area). The total binding free energies are given in Table 2. The ΔE_{vdW} contribution of the atorvastatin complex was more than the stigmastan-3-ol complex, and a similar trend was observed in terms of electrostatic contributions. However, it is important to note that the total binding free energy of the stigmastan-3-ol complex was −9.09, indicating a more favorable and stable complex, while the total binding free energy of the atorvastatin complex was −6.5, as depicted in Figure 11. This suggests that, based on the MM/PBSA calculations, the stigmastan-3-ol complex exhibits higher stability than the atorvastatin complex.

4. Conclusion

The Soxhlet extraction process using chloroform followed by GC-MS analysis was performed to identify and quantify the compounds found in *L. siceraria*. *In vivo*, the hypolipidemic activity was performed on Triton X-100-induced hyperlipidemia Wistar rats. The data collected from the study showed that *L. siceraria* extract can be a potential source of antihyperlipidemic drugs. The TL, TG, and LDL-C levels in the serum of group V rats (500 mg/kg, extract) were comparable to the serum of rats administered with a standard hypolipidemic drug (atorvastatin). The TC and VLDL-C levels in the serum of group V rats (500 mg/kg, extract) were convincingly lower than in the serum of rats administered with a standard hypolipidemic drug (atorvastatin). The HDL-C level in the serum of group IV rats (250 mg/kg, extract) was comparable with the HDL-C level in the serum of rats administered with standard hypolipidemic drug (atorvastatin), whereas the HDL-C level in the serum of group V rats (500 mg/kg, extract) was higher than the results found from the standard drug. Histological examination of the rats treated with extracts revealed normal-looking myocardium. This mechanism has a clear indication of lowering triglyceride and cholesterol synthesis. Also, it is observed from histopathological studies of rats' liver and heart that there is a significant decrease in cholesterol, LDL, and VLDL levels and a significant increase in HDL levels. Molecular docking studies confirmed that stigmastan-3-ol binds in a similar mode as atorvastatin to HMG-CoA reductase, thus blocking the protein sites for natural substrates. The MD simulation revealed that it formed a stable complex with protein and did not induce any structural changes that can lead to unfolding events in protein structure. Overall, while there is some evidence to suggest that *L. siceraria* may have a positive effect on serum cholesterol levels, more research is needed to confirm these findings and determine the optimal dosage and duration of treatment. Furthermore, this study proposed that *L. siceraria* extract in powder form might be introduced as a suitable additive in syrups or foods with hypolipidemic capacity after a medically proven prescribed dose of the extract.

Data Availability

No data were used to support the study.

Ethical Approval

All the animal experiments were performed with approval of the Ethical Committee of The University of Lahore, Pakistan.

Consent

This study was not performed on humans.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qudsia Kanwal supervised the study, developed the methodology, and performed the formal analysis. Mahmood Ahmed reviewed and edited the manuscript and wrote the original draft. Atiq-ur-Rehman reviewed the manuscript.

Aneela Anwar reviewed and edited the manuscript. Samiah Shahid investigated the study and performed the formal analysis. Atif Shahzad and Muhammad Ahmad validated the study. Attaullah Shah Bukhari performed the formal analysis and reviewed the manuscript. Numan Yousaf performed the formal analysis. Muhammad Muddassar reviewed the manuscript and performed the docking and simulation.

References

[1] P. M. Kris-Etherton, L. Sanders, O. Lawler, T. Riley, and K. Maki, "Hyperlipidemia," in *Encyclopedia of Human Nutrition*, pp. 361–379, Elsevier, Amsterdam, Netherlands, 2023.

[2] L. N. Maslov, R. Nimer, and R. Samara, "Relationship between hyperlipidemia, cardiovascular disease and stroke: a systematic review," *Current Cardiology Reviews*, vol. 17, no. 6, pp. 52–66, 2021.

[3] T. Sun, M. Chen, H. Shen et al., "Predictive value of ldl/hdl ratio in coronary atherosclerotic heart disease," *BioMed Central Cardiovascular Disorders*, vol. 22, no. 1, p. 273, 2022.

[4] M. Gaggini, F. Gorini, and C. Vassalle, "Lipids in atherosclerosis: pathophysiology and the role of calculated lipid indices in assessing cardiovascular risk in patients with hyperlipidemia," *International Journal of Molecular Sciences*, vol. 24, no. 1, p. 75, 2022.

[5] Å. L. Amundsen, L. Ose, M. S. Nenseter, and F. Y. Ntanios, "Plant sterol ester–enriched spread lowers plasma total and ldl cholesterol in children with familial hypercholesterolemia," *The American Journal of Clinical Nutrition*, vol. 76, no. 2, pp. 338–344, 2002.

[6] J. Stewart, T. McCallin, J. Martinez, S. Chacko, and S. Yusuf, "Hyperlipidemia," *Pediatrics in Review*, vol. 41, no. 8, pp. 393–402, 2020.

[7] A. Lal, T. Kumar, P. B. Murthy, and K. S. Pillai, *Hypolipidemic Effect of Coriandrum Sativum L. In Triton-Induced Hyperlipidemic Rats*, NISCAIR-CSIR, India, 2004.

[8] A. Kumar, S. Partap, N. K. Sharma, and K. Jha, "Phytochemical, ethnobotanical and pharmacological profile of lagenaria siceraria:-a review," *Journal of Pharmacognosy and Phytochemistry*, vol. 1, pp. 24–31, 2012.

[9] D. Kumar, C. Sharma, B. Singh, and D. Singh, "Pharmacognostical, phytochemical and pharmacological profile of natural remedy lagenaria siceraria (mol.) standly: a review," *British Journal of Pharmaceutical Research*, vol. 7, no. 5, pp. 340–352, 2015.

[10] R. Prajapati, R. Umbarkar, S. Parmar, and N. Sheth, "Antidepressant like activity of lagenaria siceraria (molina) standley fruits by evaluation of the forced swim behavior in rats," *International Journal of Nutrition, Pharmacology, Neurological Diseases*, vol. 1, no. 2, p. 152, 2011.

[11] R. P. Prajapati, M. Kalariya, S. K. Parmar, and N. R. Sheth, "Phytochemical and pharmacological review of lagenaria sicereria," *Journal of Ayurveda and Integrative Medicine*, vol. 1, no. 4, p. 266, 2010.

[12] B. S. Yadav, R. Yadav, R. B. Yadav, and M. Garg, "Antioxidant activity of various extracts of selected gourd vegetables," *Journal of Food Science and Technology*, vol. 53, no. 4, pp. 1823–1833, 2016.

[13] P. Dash and G. Ghosh, "Amino acid profiling and antimicrobial activity of cucurbita moschata and lagenaria siceraria seed protein hydrolysates," *Natural Product Research*, vol. 32, no. 17, pp. 2050–2053, 2018.

[14] E. S. Istvan and J. Deisenhofer, "Structural mechanism for statin inhibition of hmg-coa reductase," *Science*, vol. 292, no. 5519, pp. 1160–1164, 2001.

[15] B. Ghule, M. Ghante, A. Saoji, and P. Yeole, "Hypolipidemic and antihyperlipidemic effects of lagenaria siceraria (mol.) fruit extracts," *Indian Journal of Experimental Biology*, vol. 44, no. 11, pp. 905–909, 2006.

[16] Oecd, *Oecd Guidelines for the Testing of Chemicals*, Organization for Economic, Paris, France, 1994.

[17] M. A. Hashem, N. A. Abd-Allah, E. A. Mahmoud, S. A. Amer, and M. Alkafafy, "A preliminary study on the effect of psyllium husk ethanolic extract on hyperlipidemia, hyperglycemia, and oxidative stress induced by triton x-100 injection in rats," *Biology*, vol. 10, no. 4, p. 335, 2021.

[18] S. Syed Zameer Ahmed, S. Z. A. Khader, K. Radhakrishnan et al., "Antiobesity and antihyperlipidemic effect of ixora coccinea on triton x-100 induced hyperlipidemia in rats: an approach to evaluate asymmetrical temperature distribution analysis using thermography," *Chinese Herbal Medicines*, vol. 11, no. 3, pp. 326–331, 2019.

[19] S. S. Ghori, M. R. Khan, and A. H. A. Kaab e Alam, "Evaluation of antihyperlipidemic activity of ethanolic extract of glycosmis pentaphylla in hyperlipidemic wistar rats," *International Journal of Pharma Sciences and Research*, vol. 6, pp. 288–292, 2015.

[20] S. Jaganathan, G. J. Christobel Robinson, A. Padmanaban, S. Samuel, and V. J. Radhakrishnan, "In silico and in vitro perspective to elucidate the anticancer effect of natural compound hesperetin in inhibiting mammalian target of rapamycin pathway," *Indian Journal of Pharmaceutical Sciences*, vol. 83, no. 5, pp. 989–999, 2021.

[21] M. S. Pingali, A. Singh, V. Singh, A. K. Sahoo, P. K. Varadwaj, and S. K. Samanta, "Docking and molecular dynamics simulation for therapeutic repurposing in small cell lung cancer (sclc) patients infected with covid-19," *Journal of Biomolecular Structure and Dynamics*, vol. 41, pp. 16–25, 2021.

[22] J. C. Phillips, D. J. Hardy, J. D. Maia et al., "Scalable molecular dynamics on cpu and gpu architectures with namd," *The Journal of Chemical Physics*, vol. 153, no. 4, Article ID 044130, 2020.

[23] D. A. Case, H. M. Aktulga, K. Belfon et al., *Amber 2021*, University of California, San Francisco, CA, USA, 2021.

[24] Y. Duan, C. Wu, S. Chowdhury et al., "A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations," *Journal of Computational Chemistry*, vol. 24, no. 16, pp. 1999–2012, 2003.

[25] W. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, "Comparison of simple potential functions for simulating liquid water," *The Journal of Chemical Physics*, vol. 79, no. 2, pp. 926–935, 1983.

[26] B. J. Grant, L. Skjærven, and X. Q. Yao, "The bio3d packages for structural bioinformatics," *Protein Science*, vol. 30, no. 1, pp. 20–30, 2021.

[27] C.-Y. Huang, Y.-L. Chu, K. Sridhar, and P. Tsai, "Analysis and determination of phytosterols and triterpenes in different inbred lines of djulis (chenopodium formosanum koidz.) hull: a potential source of novel bioactive ingredients," *Food Chemistry*, vol. 297, Article ID 124948, 2019.

[28] M. Rafieian-Kopaei, M. Sedighi, M. Bahmani, S. Asgary, and F. Beyranvand, "A review of plant-based compounds and medicinal plants effective on atherosclerosis," *Journal of Research in Medical Sciences*, vol. 22, no. 1, p. 30, 2017.

[29] K. Laka, L. Makgoo, and Z. J. Mbita, "Cholesterol-lowering phytochemicals: targeting the mevalonate pathway for anticancer interventions," *Frontiers in Genetics*, vol. 13, Article ID 841639, 2022.

[30] K. Yokoyama, S. Tani, R. Matsuo, and N. Matsumoto, "Association of lecithin-cholesterol acyltransferase activity and low-density lipoprotein heterogeneity with atherosclerotic cardiovascular disease risk: a longitudinal pilot study," *BioMed Central Cardiovascular Disorders*, vol. 18, pp. 224–310, 2018.

[31] A. Parwin, A. K. Najmi, M. V. Ismail, M. Kaundal, and M. Akhtar, "Protective effects of alendronate in triton x-100-induced hyperlipidemia in rats," *Turkish Journal of Gastroenterology*, vol. 30, no. 6, pp. 557–564, 2019.

[32] L. Calpe-Berdiel, J. C. Escolà-Gil, and F. Blanco-Vaca, "New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism," *Atherosclerosis*, vol. 203, no. 1, pp. 18–31, 2009.

[33] E. A. Trautwein, G. S. Duchateau, Y. Lin, S. M. Mel'nikov, H. O. Molhuizen, and F. Y. Ntanios, "Proposed mechanisms of cholesterol-lowering action of plant sterols," *European Journal of Lipid Science and Technology*, vol. 105, no. 3-4, pp. 171–185, 2003.

[34] M. Vijayakumar, M. Selvi, and S. Krishnakumari, "Cardioprotective effect of lagenaria siceraria (mol) on antioxidant tissue defense system against isoproterenol-induced myocardial infarction in rats," *Inventi Impact: Ethnopharmacology*, vol. 1, pp. 207–210, 2010.

[35] V. R. Mali, V. Mohan, and S. L. Bodhankar, "Antihypertensive and cardioprotective effects of the lagenaria siceraria fruit in n g-nitro-l-arginine methyl ester (l-name) induced hypertensive rats," *Pharmaceutical Biology*, vol. 50, no. 11, pp. 1428–1435, 2012.

[36] M. Hafez Hetta, A. S. Moawad, M. Abdel-Aziz Hamed, and A. I. Sabri, "In-vitro and in-vivo hypolipidemic activity of spinach roots and flowers," *Iranian Journal of Pharmaceutical Research: International Journal of Psychological Research*, vol. 16, no. 4, pp. 1509–1519, 2017.

[37] T. F. Owoeye, K. D. Akinlabu, and O. O. Ajani, "Proximate composition, phytochemical screening and mineral content studies of leaves extract of adenanthera pavonina," *Arab Journal of Basic and Applied Sciences*, vol. 30, no. 1, pp. 317–328, 2023.

[38] G. Baskaran, S. Salvamani, S. A. Ahmad, N. A. Shaharuddin, P. D. Pattiram, and M. Y. Shukor, "Hmg-coa reductase inhibitory activity and phytocomponent investigation of basella alba leaf extract as a treatment for hypercholesterolemia," *Drug Design, Development and Therapy*, vol. 9, pp. 509–517, 2015.

[39] A. Gholamhose, B. Shahouzehi, and F. Sharifi-Fa, "Inhibitory activity of some plant methanol extracts on 3-hydroxy-3-methylglutaryl coenzyme a reductase," *International Journal of Pharmacology*, vol. 6, no. 5, pp. 705–711, 2010.

[40] D. S. Randive, M. A. Bhutkar, S. D. Bhinge et al., "Hypoglycemic effects of lagenaria siceraria, cynodon dactylon and stevia rebaudiana extracts," *Journal of Herbmed Pharmacology*, vol. 8, no. 1, pp. 51–55, 2019.

[41] S. Bhattacharya and B. Das, "Anti-diabetic activity of lagenaria siceraria pulp and seed extract in normal and alloxan-induced diabetic rats," *International Journal of Pharmaceutical Sciences and Research*, vol. 3, p. 3362, 2012.

[42] J. A. Friesen and V. W. Rodwell, "The 3-hydroxy-3-methylglutaryl coenzyme-a (hmg-coa) reductases," *Genome Biology*, vol. 5, pp. 1–7, 2004.

[43] B. Ghule, M. Ghante, A. Saoji, and P. Yeole, "Anti-hyperlipidemic effect of the methanolic extract from lagenaria siceraria stand. Fruit in hyperlipidemic rats," *Journal of Ethnopharmacology*, vol. 124, no. 2, pp. 333–337, 2009.

[44] E. Fox, B. C. Widemann, D. Pastakia et al., "Pharmacokinetic and pharmacodynamic study of tariquidar (xr9576), a p-glycoprotein inhibitor, in combination with doxorubicin, vinorelbine, or docetaxel in children and adolescents with refractory solid tumors," *Cancer Chemotherapy and Pharmacology*, vol. 76, no. 6, pp. 1273–1283, 2015.

[45] V. R. Mali and S. L. Bodhankar, "Cardioprotective effect of lagenaria siceraria (ls) fruit powder in isoprenalin-induced cardiotoxicity in rats," *European Journal of Integrative Medicine*, vol. 2, no. 3, pp. 143–149, 2010.

[46] M. Zahoor, M. Ikram, N. Nazir et al., "A comprehensive review on the medicinal importance; biological and therapeutic efficacy of lagenaria siceraria (mol)(bottle gourd) standley fruit," *Current Topics in Medicinal Chemistry*, vol. 21, no. 20, pp. 1788–1803, 2021.

[47] M. K. Singh, A. Ayaz, S. Ankur, Y. Jyoti, and F. Mohd, "Protective effect of lagenaria siceraria against doxorubicin induced cardiotoxicity in wistar rats," *International Journal of Drug Development & Research*, vol. 4, pp. 298–305, 2012.

[48] A. I. Abushouk, A. Ismail, A. M. A. Salem, A. M. Afifi, and M. M. Abdel-Daim, "Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity," *Biomedicine and Pharmacotherapy*, vol. 90, pp. 935–946, 2017.

[49] A. Senior, S. Kumar, and M. Kumar, "Comparison between hypolipidemic effect of lagenaria siceraria and lovastatin in hypercholesterolemic rats," *Paripex Indian Journal of Research*, vol. 8, pp. 13-14, 2019.

[50] N. H. Parikh, P. K. Parikh, and C. Kothari, "Indigenous plant medicines for health care: treatment of diabetes mellitus and hyperlipidemia," *Chinese Journal of Natural Medicines*, vol. 12, no. 5, pp. 335–344, 2014.

[51] I. Ahmad, M. Irshad, and M. M. A. Rizvi, "Nutritional and medicinal potential of lagenaria siceraria," *International Journal of Vegetable Science*, vol. 17, no. 2, pp. 157–170, 2011.

[52] S. Banerjee, A. Tiwari, A. Kar et al., "Combining lc-ms/ms profiles with network pharmacology to predict molecular mechanisms of the hyperlipidemic activity of lagenaria siceraria stand," *Journal of Ethnopharmacology*, vol. 300, Article ID 115633, 2023.