Title:

Phytochemical Analysis and Anti-dyslipidemia and Antioxidant Activities of *Pluchea dioscoridis*: *In Vitro*, *In Silico* and *In Vivo* Studies

Running title:

Pluchea dioscoridis attenuates dyslipidemia and oxidative stress

Authors and affiliations:

Wageha S. Sultan[1], Ayman M. Mahmoud[2,3]*, Shimaa A. Ahmed[4], Reem S. Alruhaimi[5], Mohammed A. Alzoghaibi[6], Ashraf A. El-Bassuony[3], Nabil A. Hasona[7], Emadeldin M. Kamel[4]

[1]Department of Chemistry, Research Institute of Medicinal and Aromatic Plants, Beni-Suef University, Beni-Suef 62514, Egypt
[2]Department of Life Sciences, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK
[3]Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt.
[4]Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt.
[5]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia.
[6]Physiology Department, College of Medicine, King Saud University, Riyadh, 11461, Saudi Arabia
[7]Biochemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt.

Corresponding author:
Ayman M. Mahmoud, PhD
Department of Life Sciences, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK.
E-mail: a.mahmoud@mmu.ac.uk
ORCID: 0000-0003-0279-6500

Abstract

Pluchea dioscoridis (L.) DC. is a flowering wild plant used traditionally in the treatment of rhematic disorders. This study investigated the phytochemical and *in vitro* radical scavenging activity (RSA), and *in vivo* anti-hyperlipidemic, antioxidant and anti-inflammatory properties of *P. dioscoridis*. The antihyperlipidemic efficacy was determined in a rat model of dyslipidemia. The extract and fractions of *P. dioscoridis* showed RSA with the ethyl acetate (EA) fraction exhibiting the most potent activity. The phytochemical analysis of *P. dioscoridis* EA fraction (PDEAF) led to the isolation of five compounds (lupeol, quercetin, lupeol acetate, stigmasterol, and syringic acid). To evaluate its anti-hyperlipidemic effect, three doses of PDEAF were supplemented to rats for 14 days and poloxamer-407 was administered on day 15 to induce dyslipidemia. All doses of PDEAF decreased plasma triglycerides, cholesterol, low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (vLDL-C), and increased plasma lipoprotein lipase (LPL). PDEAF upregulated hepatic LDL receptor and suppressed 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, decreased lipid peroxidation and tumor necrosis factor (TNF)-α and enhanced reduced glutathione (GSH) and enzymatic antioxidants in dyslipidmeic rats. *In silico* findings revealed the binding affinity of the isolated compounds towards LPL, HMG-CoA reductase, and LDL receptor. In conclusion, *P. dioscoridis* is rich in phytoconstituents, exhibited RSA and its EA fraction effectively prevented acute dyslipidemia and its associated oxidative stress and inflammatory response.

Keywords: Dyslipidemia; Inflammation; Oxidative stress; *Pluchea*.

1. Introduction

Dyslipidemias refer to the presence of irregular levels of triglycerides (TG) and/or cholesterol (CHOL) in the bloodstream, along with their associated lipoprotein variations [1]. Dyslipidemia

is known to elevate the chances of developing cardiovascular disease (CVD) and is commonly associated with an increased risk of atherosclerotic CVD. Atherosclerosis is often characterized by elevated levels of CHOL in the bloodstream, which can contribute to severe health conditions like ischemic heart disease (IHD), myocardial infarction (MI), and stroke. It is crucial to manage and treat dyslipidemia, particularly when multiple risk factors are present, to mitigate the potential harm it can cause [1-2]. The liver produces most of the CHOL and TG found in the bloodstream, while diet also plays a significant role. The regulation of lipid metabolism in mammals is closely controlled by sterol regulatory-element binding proteins (SREBPs) which have a controlling influence on the expression of genes responsible for CHOL synthesis, fatty acid synthesis, and TG production [3]. SREBP-1 regulates genes associated with TG biosynthesis. On the other hand, SREBP-2 primarily controls crucial genes involved in CHOL synthesis and uptake, including HMG-CoA reductase (HMGCR) and LDL receptor (LDL-R) [3]. The enzyme HMGCR, which is primarily found in the liver, regulates the rate of CHOL synthesis in the body [4]. The increasing occurrence of non-communicable diseases and their association with dyslipidemias has reached alarming levels, requiring increased attention to their impact on premature mortality, especially among young individuals. The lost lives due to CVD with a metabolic foundation has risen from the fourth to the top position between 1990 and 2017 [5]. Dyslipidemia is connected to redox imbalance and oxidative stress (OS) [6]. Lipids building up in cells can cause the release of reactive oxygen species (ROS), resulting in OS that can have adverse effects on various organs [7]. Hyperlipidemia-associated OS is commonly caused by LDL-C, glucose, and protein oxidative modifications. OS is further exacerbated by activated NADPH oxidase and changes in the properties of the mitochondrial membrane, which leads to the release of ROS. These factors contribute to the development of hyperlipidemia and its associated OS [6].

Numerous plant species and their bioactive compounds have demonstrated advantageous effects in reducing high lipid levels and OS in preclinical models [8]. Many plants produce secondary metabolites naturally, such as flavonoids, coumarins, and polyphenols, which function as antioxidants and are also essential for a variety of biological processes [8d, 9]. As a result, plants and their ingredients represent a significant source of antioxidants that can scavenge free radicals and defend against OS associated with metabolic conditions such as dyslipidemia. Egyptian folk medicine has a long history of development and is a product of numerous cultural influences [10]. *Pluchea dioscoridis* (L.) is a flowering wild plant belongs to the family *Asteraceae* and widely distributed in different parts of Egypt. The Nile area, the Western and Eastern Deserts, the Sinai Peninsula, and the oasis along the Mediterranean coast are the areas where it is most prevalent [11]. This species is traditionally used in folk medicine for the treatment of different ailments, including rhematic disorders, epilepsy, ulcers and as a carminative agent [11]. Due to its ability to repel insects, farmers in Egypt refer to *P. dioscoridis* as the "mosquito tree". Scientifically based bioactivity studies demonstrated that *P. dioscoridis* extracts exhibit antioxidant, antidiabetic [12], anticancer, anti-aging [13], antinociceptive, anti-inflammatory and anti-pyretic efficacies [14]. Despite the reported activities, the effect of *P. dioscoridis* on dyslipidemia and its associated OS has not been explored yet. This study investigated the chemical constituents and *in vitro* radical scavenging activity (RSA) of *P. dioscoridis*, and its effect on dyslipidemia, OS, and inflammation in poloxamer-407 (P-407)-provoked acute dyslipidemia in rats.

2. Materials and methods

2.1. Plant collection extraction, and isolation

P. dioscoridis was collected from the western desert (Egypt) in July 2021 (latitude - longitude: 29.421088 - 31.14723, Beni-Suef, Egypt) and the species was identified by expert taxonomists at the Botany and Microbiology Department, Beni-Suef University (Egypt). A voucher

specimen (No.: BSU-CH2021-517) was meticulously archived in the Herbarium of the Faculty of Science (Registration code: BSU- HERB21517A).

Two kg of the aerial parts of *P. dioscoridis* was cleaned, air-dried, grounded, and soaked in 70 % ethanol (4 x 3 L). The extraction solvent was removed under reduced pressure to afford 349.2 g dark amorphous crude extract. For fractionation, 200 g was dissolved in 0.5 L distilled water and 100 ml ethanol, then hexane was employed for crude extract defatting. The defatted extract underwent successive solvent-solvent partition using *n*-hexane, dichloromethane (DCM), ethyl acetate (EA), and *n*-butanol to afford 13.2, 12.3, 13.9, and 33.5 g fractions, respectively. The EA fraction was fractioned over a silica gel column (60, 70–230 mesh) eluted with ether, *n*-hexane, and ethyl acetate (EtOAc) followed by EtOAc:methanol (MeOH) (9:1) and EtOAc:MeOH (5:5). Fractions were collected from the column by band migration observation using UV lamp. Seventeen subfractions were collected and combined into four (W1-W4) subfractions based on their TLC profile. Pure crystals of compound **1** (16.8 mg) were precipitated by adding MeOH to subfraction W1. Subfraction W2 was purified over silica gel column employing isocratic elution using the solvent system of hexane:EtOAc (90%: 10%) to afford the purified compound **2** (17 mg). Next, compound **3** (19 mg) was obtained by chromatographing subfraction W3 on a silica gel column using hexane:EtOAc (80%: 20%) as an isocratic eluent. Subfraction W4 was applied to the top of a silica gel column eluted with EtOAc:MeOH to afford a total of 12 subfractions. The collected fractions were examined and combined into five subfractions (S1-S5). S1 was chromatographed over a silica gel column eluted with EtOAc:MeOH to afford four main subfractions (A1-A4). A4 was further purified over Sephadex LH-20 column eluted with MeOH to give compound **4** (19 mg). Subfraction A3 was purified over silica eluted with MeOH to produce compound **5** (16 mg).

2.2. *In vitro* RSA

The RSA activity of the extract and fractions of *P. dioscoridis* was determined according to the method of Cheel et al [15]. Briefly, different concentrations of the samples were incubated with 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in MeOH for 30 min and the absorbance was measured at 517 nm. Ascorbic acid was employed as a positive control.

2.3. *In vivo* anti-hyperlipidemic study

2.3.1. Experimental animals and treatments

The experiment was approved by the Animal Care and Use Committee of Beni-Suef University (Approval number: 022-465). Forty-two adult male Wistar rats (160-180 g) were kept in standard conditions (temperature 23±1°C and humidity 50-60%) and provided food and water *ad libitum*. To investigate the anti-hyperlipidemic effect of *P. dioscoridis* EA fraction (PDEAF), a single dose of P-407 (500 mg/kg) was injected intraperitoneally (i.p.) to induce hyperlipidemia [16] and control rats received physiological saline. The rats were divided into two normal groups (I and II) and five dyslipidemic groups (III-VII), each is six (n = 6), as outlined below:

Group I: received the vehicle.

Group II: received 400 mg/kg PDEAF.

Group III: received the vehicle.

Group IV: received 100 mg/kg PDEAF.

Group V: received 200 mg/kg PDEAF.

Group VI: received 400 mg/kg PDEAF.

Group VII: Rats received 10 mg/kg of atorvastatin (ATOR).

PDEAF and ATOR were suspended in 0.5% carboxymethyl cellulose and supplemented via oral gavage for 14 days and P-407 was administered on day 15. Blood samples were collected from tail vein before P-407 and at 12, 24 and 48 h for the assay of TG and CHOL. After 48 h, the animals were anesthetized with ketamine, sacrificed and liver samples were collected.

2.3.2. Biochemical assays

Plasma TG, CHOL and high-density lipoprotein (HDL)-C were determined using Biosystems (Spain) kits. Plasma LPL and TNF-α were determined Solarbio (China) and (Cusabio, China) kits, respectively. Thiobarbituric acid reactive substances (TBARS), GSH, superoxide dismutase (SOD), and catalase were assayed in plasma using kits supplied by Bio-Diagnostic (Egypt). Liver samples were homogenized in (10% w/v) in Tris-HCl buffer (pH 7.4) and the homogenate was centrifuged, and the clear supernatant was used for HMGCR activity assay using a reagent kit supplied by Sigma (USA).

vLDL and LDL were calculated as following:

$$vLDL = Trilycerides/5$$

$$LDL = Total\ Cholesterol - (HDL + vLDL)$$

2.3.3. Determination of LDL-R expression

Changes in the mRNA levels of LDL-R between groups were determined using qRT-PCR as we previously reported [16b]. Briefly, RNA was isolated from liver samples using Trizol reagent and samples showed A260/A280 value ≥ 1.8 after purification were reverse transcribed. The obtained cDNA was amplified using SYBR Green Master Mix and the primers F: 5'CATTTTCAGTGCCAACCGCC3' and R: 5'TGCCTCACACCAGTTTACCC3' for Ldlr, and F: 5'AGGAGTACGATGAGTCCGGC3' and R: 5'CGCAGCTCAGTAACAGTCCG3' for β-actin (Actb). The data were analyzed using the $2^{-\Delta\Delta Ct}$ method [17] and normalized to β-actin.

2.3.4. Statistical analysis

The data were analyzed using the one-way ANOVA followed by Tukey's test on GraphPad 8. A p value <0.05 was considered significant and the data are represented as mean ± SEM.

2.4. Molecular docking

The possible binding of compounds **1-5** with LPL (PDB ID: 6OB0), HMGCR (PDB ID: 1HWI), and LDL-R binding domain of PCSK9 (PDB ID: 3GCX) was investigated using AutoDock Vina software package [18]. Autodock Tools (ADT) v1.5.6 was used for the

optimization of targets and UCSF Chimera was utilized to visualize and extract macromolecules from ligands, solvents, and unnecessary residues [19]. The analysis and visualization of the results were performed using PyMOL v2.3.2.

3. Results

3.1. Phytochemical analysis

Phytochemical analysis of *P. dioscoridis* resulted in isolation of five compounds from the EA fraction (Fig. 1). The structure elucidation was performed by spectroscopic data (Suppl. Fig. 1-10), comparison with the published literature, and by PC and TLC comparisons with authentic samples. The analysis revealed that the compounds are stigmasterol (**1**), lupeol acetate (**2**), lupeol (**3**), quercetin (**4**), and syringic acid (**5**) (Suppl. material).

3.2. *In vitro* RSA

The RSA of *P. dioscoridis* extract and its fractions was determined using the DPPH assay. The results represented in Figure 2 show that the EA fraction exhibited the highest RSA with IC_{50} value of 11.37 ± 0.88 µg/ml (Fig. 2B) followed by the n-butanol (14.52 ± 0.57 µg/ml; Fig. 2C), crude extract (26.82 ± 1.38 µg/ml; Fig. 2A), DCM (48.58 ± 4.18 µg/ml; Fig. 2D), and the n-hexane fraction (15.60 ± 7.81 µg/ml; Fig. 2E). Ascorbic acid showed IC_{50} value of 9.19 ± 0.38 µg/ml (Fig. 2F).

3.3. Anti-dyslipidemia activity of PDEAF

The data depicted in Fig. 3A-B reveal significant increase in plasma CHOL levels at 12, 24 and 48 h in P-407-treated rats (p<0.001). Similarly, TG (Fig. 3C-D) was elevated in the plasma of rats that received P-407 (p<0.001). PDEAF at all doses ameliorated plasma CHOL and TG significantly in dyslipidemic rats and its effect was dose-dependent. The assessment of plasma LDL-C (Fig. 4A) and vLDL-C (Fig. 4B) revealed remarkable elevation in dyslipidemic rats (p<0.001). While the 400 mg/kg PDEAF and ATOR decreased plasma LDL-C significantly, all doses of PDEAF and ATOR ameliorated plasma vLDL-C levels. HDL-C levels showed

non-significant changes as shown in Fig. 4C. Of note, the high dose of PDEAF had no effect on plasma lipids in normal rats.

3.4. Effect of PDEAF on LPL, LDL-R, and HMGCR in dyslipidemic rats

The effects of the PDEAF on the activities of LPL and HMGCR, and the expression of LDL-R were assessed in dyslipidemia rats. As illustrated in Figure 5A, P-407 decreases plasma LPL (p<0.001) and all doses of PDEAF as well as ATOR significantly ameliorated plasma LPL. The effect of PDEAF on plasma LPL was dose-dependent. The affinity of the isolated compounds towards LPL was studied *in silico* (Fig. 5B-C). All phytochemicals showed binding energies ranged between -6.0 and -11.3 kcal/mol with compound **1** (stigmasterol) exhibiting the lowest energy (Fig. 5B and Table 1). The binding of isolated compounds with LPL is mainly hydrophobic and polar interactions were detected with compounds **4** and **5** (Table 1).

LDL-R mRNA was decreased in the liver of dyslipidemic rats (p<0.001) and treatment with all doses of PDEAF and ATOR upregulated it (Fig. 6A). Molecular docking revealed the afiininty of the isolated compounds towards LDL-R binding domain of PCSK9 with energies ranged from -6.3 to -9.2 kcal/mol (Fig. 6B-C and Table 1). All compounds exhibited hydrophobic interactions and **2**, **4** and **5** showed polar bonding.

P-407-induced dyslipidemia was associated with increased HMGCR activity (p<0.001) in the liver of rats, and PDEAF ameliorated HMGCR activity at all doses (Fig. 7A). *In silico*, all compounds exhibited binding affinity with HMGCR with binding energies range from -5.1 to -7.9 kcal/mol as depicted in Figure 7B-C and Table 1.

3.5. PDEAF mitigated OS and inflammation in dyslipidemic rats

Dyslipidemic rats exhibited remarkable elevation of TBARS (Fig. 8A) and TNF-α (Fig. 8B) (p<0.001). In contrast, GSH (Fig. 8C), SOD (Fig. 8D) and CAT (Fig. 8E) were declined in P-407-administered rats. All doses of PDEAF decreased TBARS and TNF-α, and enhanced

antioxidants in dyslipidemic rats. The effect of PDEAF on TBARS and antioxidants, but not on TNF-α, was dose-dependent. The 400 mg/kg dose of PDEAF had no effect on the assayed redox and inflammation parameters in normal rats.

4. Discussion

P. dioscoridis has been traditionally employed in numerous countries to treat various health ailments. This study showed its phytoconstituents and *in vitro* RSA, and the beneficial effect of its EA fraction against dyslipidemia and its associated OS and inflammatory response. Using molecular docking, this study revealed the affinity of *P. dioscoridis* phytoconstituents towards LPL, LDL-R and HMGCR.

The *in vitro* study revealed the RSA of the crude extract and the fractions of *P. dioscoridis* with the EA fraction exhibiting the most potent efficacy. The antioxidant activity of the extract of parts of *P. dioscoridis* has been demonstrated previously. The essential oils of this species showed RSA against $DPPH^{\bullet}$ and safety at doses higher than 5000 mg/kg [14]. The RSA of *P. dioscoridis* and its fractions is attributed to the phytochemical constituents, mainly phenolics and flavonoids. Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, rutin, vitexin, isovitexin, quercetin-3-*O*-*α*-L- rhamnopyranoside, quercetin 3-*O*-*β*-D-glucopyranosyl, kaempferol 3-*O*-*β*-D-glucopyranosyl, kaempferol, and other compounds with RSA have been reported in different parts of *P. dioscoridis* [13, 20]. The main secondary metabolites in the EA fraction of *P. dioscoridis* are flavonoids and phenolics [13, 20-21]. Flavonoids and phenolics have been the subject of numerous studies documenting their antioxidant activity and other favorable influences [8c, 8d, 9a, 9b, 22]. Given the superior RSA of PDEAF, we demonstrated its phytochemical constituents and evaluated its effectiveness on acute dyslipidemia in P-407-administered rats.

P-407 is a non-toxic and non-ionic copolymer surfactant that notably elevates blood CHOL and TG in various rodents [23]. Here, P-407-treated rats showed significant elevation in circulating

CHOL and TG at 12, 24 and 48 h as previously demonstrated [16b, 24]. Along with TG and CHOL, LDL-C and vLDL-C were remarkably elevated in the blood of P-407-treated rats, whereas HDL-C showed non-significant change. The obtained dyslipidemia following P-407 administration is related to its ability to suppress LPL and consequently TG hydrolysis and promoting cholesterolgenesis [23-24]. Accordingly, plasma LPL activity and liver LDL-R mRNA were decreased, and the activity of hepatic HMGCR was elevated in rats with dyslipidemia in this study. All doses of PDEAF decreased plasma CHOL and TG at 12, 24 and 48 h post P-407 injection and decreased LDL-C and vLDL-C at 48 h. The effect of PDEAF on plasma CHOL and TG was dose dependent. These findings were accompanied by upregulated plasma LPL activity and liver LDL-R, and suppressed hepatic HMGCR activity, demonstrating that enhanced TG hydrolysis and LDL-C uptake and suppressed CHOL synthesis represent the main mechanism underlying the anti-dyslipidemia effect of *P. dioscoridis*. This beneficial effect of PDEAF on dyslipidemia could be attributed to the contained phytoconstituents. For instance, quercetin decreased the synthesis of fatty acids and TG in rat liver cells [25], promoted CHOL-to-bile acid conversion and CHOL efflux in rats [26], however, both studies showed non-significant effect on HMGCR. In contrast, quercetin decreased HMGCR and alleviated dyslipidemia in early stage diabetic nephropathy [27]. In rats fed a high CHOL diet, lupeol and its ester ameliorated dyslipidemia and cardiac OS [28]. Similar findings were reported in diabetic rats where syringic acid decreased circulating TG and CHOL and suppressed OS in the liver and kidney [29]. Moreover, stigmasterol reduced plasma CHOL and inhibited intestinal CHOL absorption and cholsterologenesis in rats [30].

Next, we examined the affinities of the isolated phytochemicals towards LPL, LDL-R and HMGCR using molecular docking. LPL plays a role in the breakdown of lipoproteins, particularly TG-rich lipoproteins like chylomicrons (CM) and vLDL, releasing TG into circulation and tissues for energy or as precursors for physiological substances in the plasma

membrane [31]. Activated LPL facilitates the clearance of TG-rich lipoproteins and is generally considered to have a favorable impact on lipoprotein metabolism [31]. Limited research has investigated the effect of phytochemicals on LPL activity [32]. Some studies have shown that certain natural compounds decrease LPL expression levels, but the activity and protein levels of LPL were not measured, leaving room for the possibility that natural phytochemicals can modulate LPL activity and consequently modulate dyslipidemia and atherosclerosis development [33]. Here, the isolated phytochemicals showed binding energies ranged between -6.0 and -11.3 kcal/mol towards LPL. The tested compounds were shown to dock into the main binding site of the enzyme with many common amino acid residues. The binding of isolated compounds with the target enzyme is mainly hydrophobic in nature as estimated by the dense extent of hydrophobic interactions. However, polar interactions were detected in compounds **4** and **5** with significant key residues, enabling the generation of energy minimized drug-enzyme interaction. Thus, isolated compounds were found to occupy the distal binding pocket on the enzyme surface, indicating that these compounds are likely to have a significant impact on LPL activity.

Elevated levels of LDL-C in the bloodstream contribute to atherosclerosis and P-407 promotes early stages of atherosclerosis [34]. Hepatocytes, which contain LDL-R on their surface, play a crucial role in capturing LDL particles and transporting them to the liver for degradation [35]. Consequently, LDL-R·LDL complexes present on hepatocytes surface undergo clathrin-mediated endocytosis, entering endosomes [35]. The acidic environment within these endosomes triggers rearrangement of the LDLR extracellular domain, leading to the release of bound lipoproteins [36]. With the lipoproteins detached, the LDL-R is recycled back to the plasma membrane [36]. In this study, PDEAF decreased plasma LDL-C and upregulated hepatic LDL-R. To explore whether the isolated compounds from PDEAF exhibit binding affinity with LDL-R, we carried out molecular docking that showed the interaction of all compounds with

LDL-R binding domain of PCSK9 with energies ranged from -6.3 to -9.2 kcal/mol, indicating a high probability for these phytochemicals to invade the binding site of the target protein. PCSK9 functions as a protease that can degrade LDL-R by interacting with its extracellular domain [37]. The binding of LDL to LDL-R is crucial for reducing circulating LDL, however, when PCSK9 binds to LDL-R, it prevents the binding of LDL and promotes CHOL accumulation [38]. Therefore, inhibiting PCSK9 and its association with LDL-R is essential in preventing dyslipidemia and its consequent atherosclerosis and coronary artery disease.

The isolated compounds showed binding affinities towards HMGCR, the rate-limiting step of CHOL synthesis [4]. The obtained low binding energies (-5.1 to -7.9 kcal/mol) suggest the potential of the isolated compounds as inhibitors of HMGCR. All compounds occupied the same binding pocket, with many common amino acid residues. While compounds **2** and **3** exhibited the lowest binding energies, they did not engage in polar interactions with HMGCR. This finding is noteworthy as it highlights the hydrophobic nature of the HMGCR binding pocket. The dense networks of hydrophobic interacting residues further confirm the compatibility of *P. dioscoridis* phytochemicals with the active site of HMGCR.

The involvement of OS in dyslipidemia has been demonstrated in many studies [6], and P-407-induced dyslipidemia was associated with elevated TBARS and decreased antioxidants as demonstrated in our previous work [16b]. Here, TBARS and TNF-α were elevated whereas GSH and enzymatic antioxidant were declined, demonstrating OS and inflammation. Under dyslipidemia, OS promotes LDL oxidation that activates the vascular endothelium to recruit inflammatory cells resulting in an inflammatory response [39]. Accordingly, chronic inflammation characterized by disrupted cytokine regulation is driven by dyslipidemia [40]. The increase in TNF-α in P-407-induced dyslipidemia has been previously reported in blood and hippocampus [41]. PDEAF decreased TBARS and TNF-α and enhanced antioxidant defenses in P-407-admisniteerd animals, findings highlighting its antioxidant and anti-inflammatory

activities. These beneficial effects supported the previously demonstrated antioxidant activity of *P. dioscoridis* and its phytochemicals [13-14, 28-30].

5. Conclusion

This study demonstrated the phytochemical constituents, antioxidant, and anti-dyslipidemia effects of the traditionally used plant species *P. dioscoridis*. The extract and fractions of the aerial parts of *P. dioscoridis* exhibited RSA and the EA fraction ameliorated dyslipidemia by promoting TG hydrolysis and suppressing CHOL biosynthesis. PDEAF increased plasma LPL and liver LDL-R and suppressed liver HMGCR activity. In addition, PDEAF decreased TBARS and TNF-α and enhanced antioxidants in dyslipidemic rats. The phytoconstituents of *P. dioscoridis* showed *in silico* binding affinity towards LPL, LDL-R and HMGCR. Therefore, *P. dioscoridis* is effective in ameliorating dyslipidemia and its associated OS and inflammatory response. It can also be inferred that the isolated compounds from *P. dioscoridis* showed promising potential as candidates for further investigation and potential development of new medicines. Their pharmacophore structure suggests that they have the potential to act as inhibitors for PCSK9 binding to LDL-R, making them valuable for future research and drug design endeavors. Further studies to elucidate other mechanisms underlying the anti-hyperlipidemia efficacy of *P. dioscoridis* and its phytoconstituents are recommended.

Conflict of Interests

The authors declare no conflict of interest.

Availability of data and materials

The manuscript and supplementary material contain all data supporting the reported results.

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Authors' Contributions:

Conceptualization: A.M.M. and E.M.K.; Methodology: W.S.S., S.A.A., N.A.M., M.A.A., R.S.A., A.A.E., E.M.K. and A.M.M.; Investigation: W.S.S., S.A.A., R.S.A., E.M.K. and A.M.M.; Data curation: A.M.M., E.M.K., W.S.S., and S.A.A.; Formal analysis: A.M.M., and E.M.K.; Resources: M.A.A., R.S.A., A.A.E., E.M.K. and A.M.M.; Validation: A.M.M.; Supervision: A.M.M.; Writing-Original draft: A.M.M., E.M.K., and W.S.S.; Writing-review and editing: A.M.M.

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Tables:

Table 1. Binding affinities and interactions of phytochemicals (**1**-**5**) isolated from *P. dioscoridis* with LPL, LDL-R binding domain of PCSK9 and HMGCR.

	LPL			LDLR binding domain of PCSK9			HMGCR		
	Affinity (kcal/mol)	Polar bonds	Hydrophobic interactions	Affinity (kcal/mol)	Polar bonds	Hydrophobic interactions	Affinity (kcal/mol)	Polar bonds	Hydrophobic interactions
1	-11.2		His120, Phe415, Pro187, Tyr121, Ala185, Ile221, Ser159, Lys265, His268, Trp421, Trp82, and Trp420	-8.1		Arg434, Arg657, Glu426, Gln621, Thr437, Asp422, Asn439, Leu440, Asp651, Ile416, Pro438, Thr459, Val650, Val435, and Tyr648	-6.9	Gln766 and Tyr761	Ile762, Gln814, Ile531, Val530, Asn529, Cys527, Gly532, Val522, Leu512, Tyr533, Met534, and Gly765
2	-11.3		Trp420, His120, Val84, Tyr121, Ile221, Trp421, Phe415, Trp82, and Ser418	-8.0	Asn317	Lys258, Phe150, Gln152, Leu348, Ala151, Phe318, and Trp72	-7.8		Ala695, Ile696, Ile699, Glu700, His635, Tyr687, Tyr644, Gly685, and Asn686
3	-10.7		Ile221, Trp82, Phe415, Trp421, Ser418, Trp420, His120, Val84, and Tyr121	-7.5		Ser383, Gln382, Gln152, Asn317, Ala151, Phe318, Phe150, Gln256, and Gly257	-7.9		Ala695, Ile696, Ile699, His635, Tyr687, Tyr644, and Gly685
4	-8.9	Ser159, Arg219, Ser422, and His268,	Pro187, Tyr121, Ala185, Trp421, and Ile221	-9.2	Val435, Leu436, Thr437, and Glu426	Asn439, Arg434, Val650, Tyr648, Asp651, Ile416, Arg458, Thr459, and Pro438	-7.3	Met655 and Met659	Asn658, Gly807, Gly656, Gly806, Gln766, Asp767, and Val805
5	-6.0	Ser159, His268, and Gln262	Phe212, Lys265, Trp82, and Ile221	-6.3	Val435, Thr459, Arg525, and Ala649	Val650, Leu436, Trp461, Pro438, and Asp651	-5.1	Met657 and Gly808	Gly656, Ala654, Asp767, Gly803, Gly807, Gly806, Thr809, Gln766, and Met655

Figure legends:

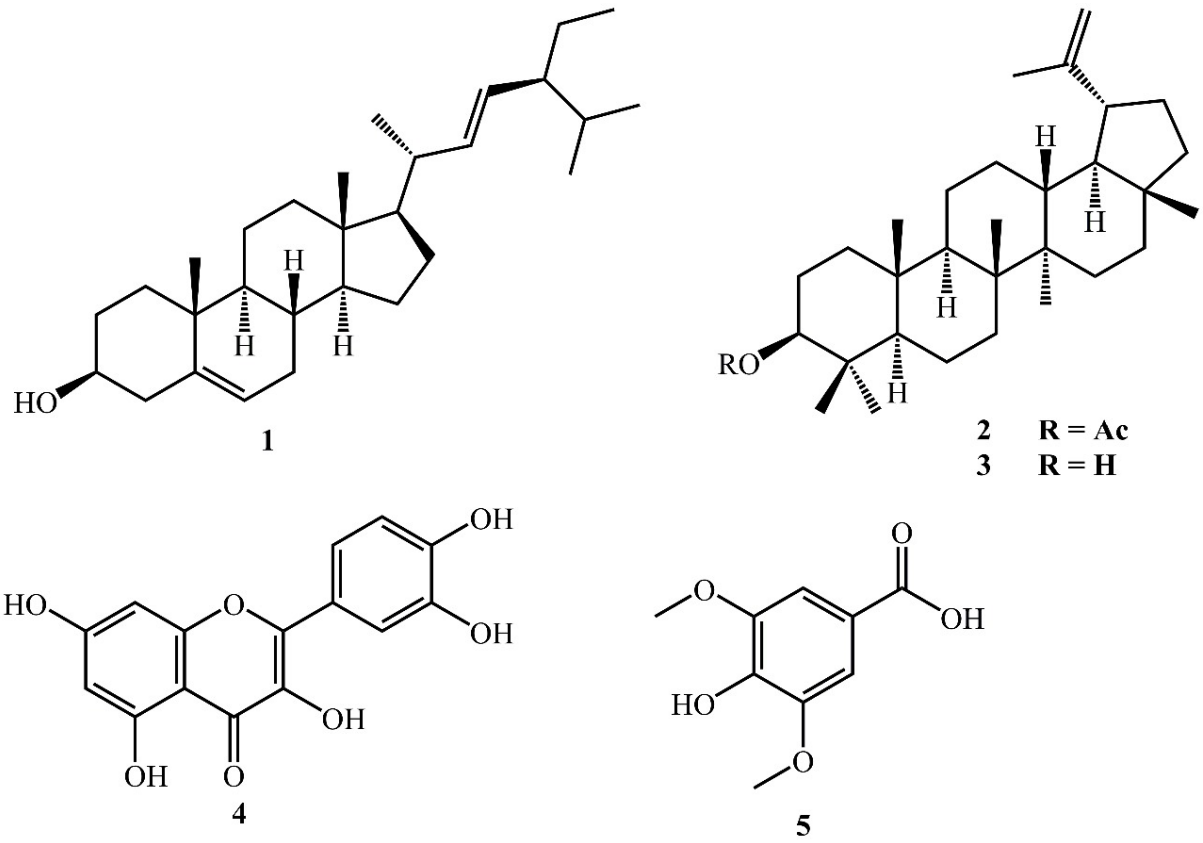
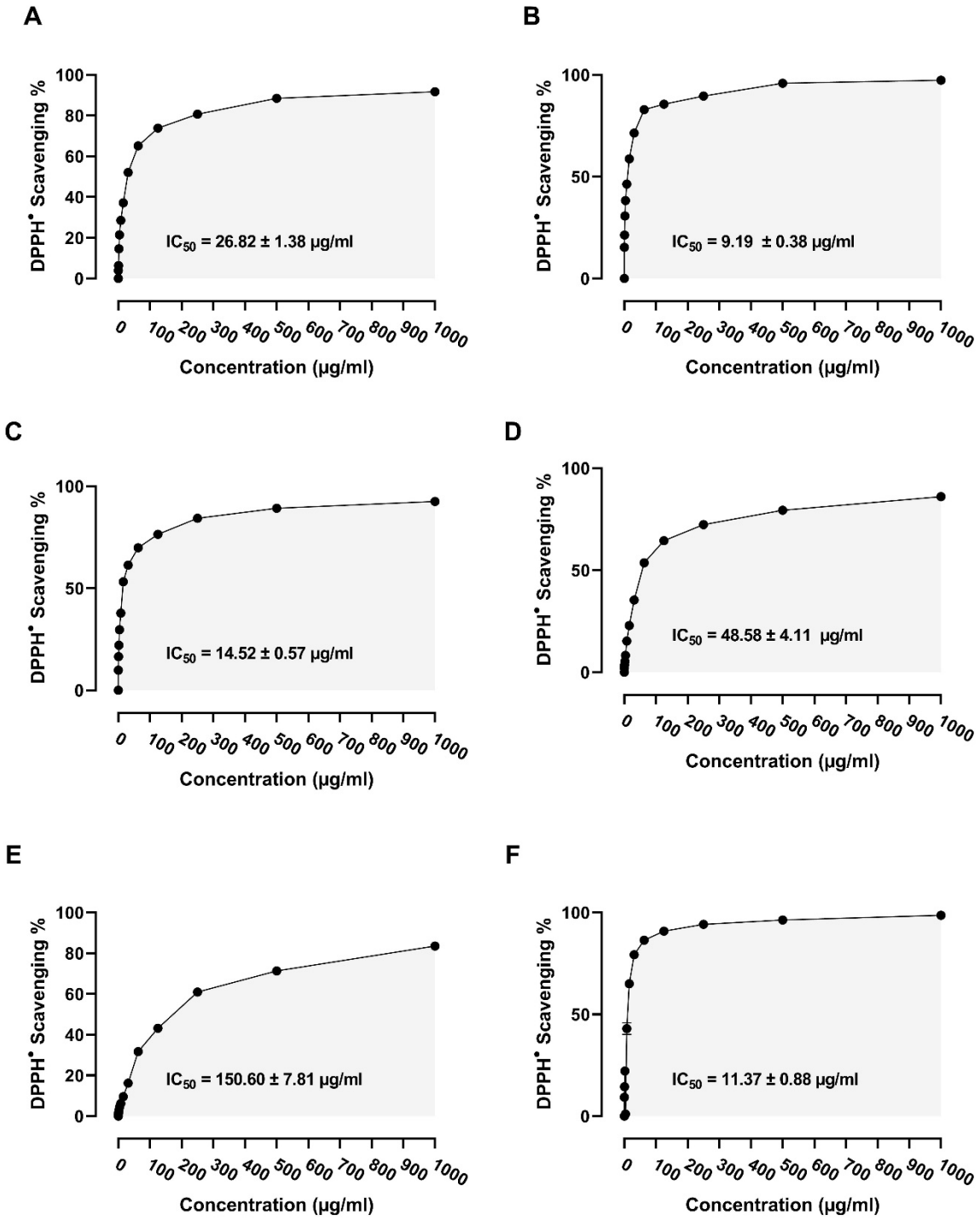


Fig. 1. Chemical structures of the compounds isolated from *P. dioscoridis*.



464

465 Fig. 2. DPPH radical scavenging % and IC_{50} values of *P. dioscoridis* (A) extract, (B) EA, (C)
466 n-butanol, (D) DCM and (E) n-hexane fractions, and (F) Ascorbic acid. Data are mean ± SD,
467 (N = 3).

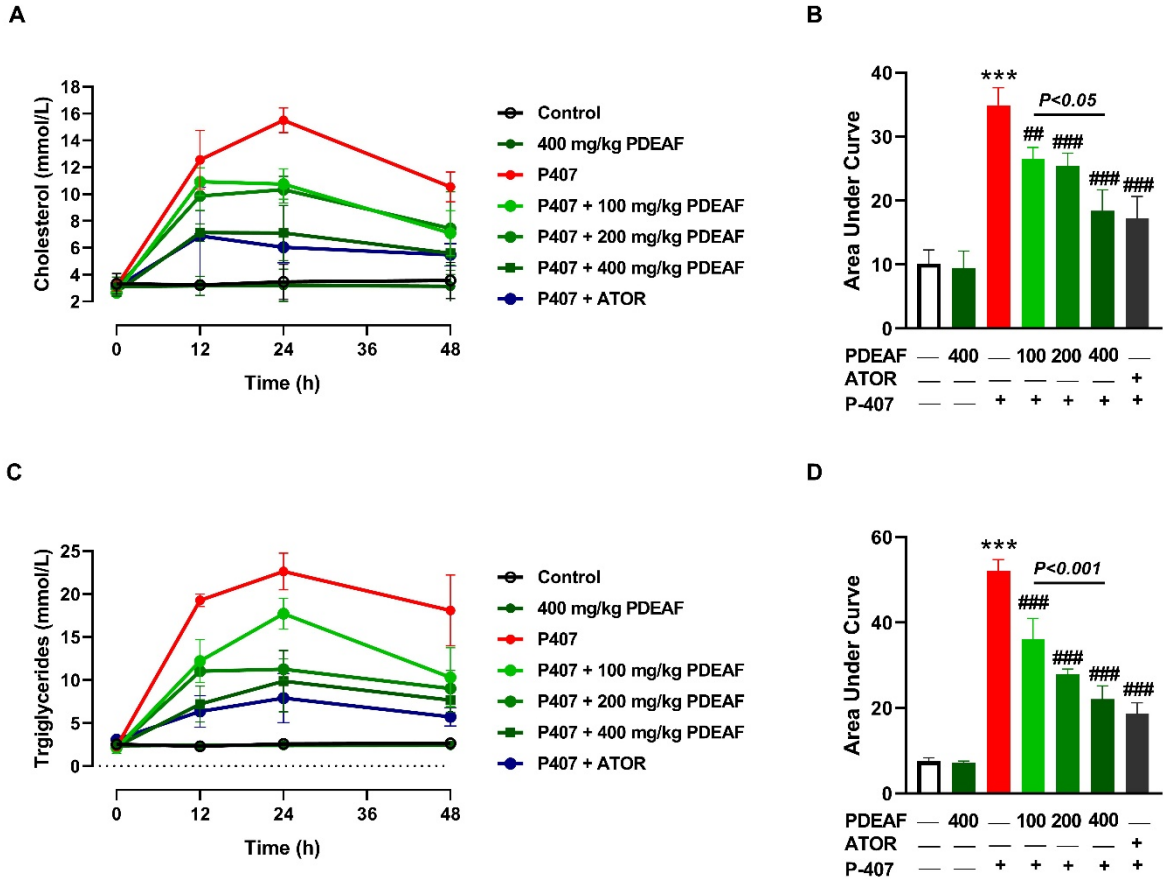
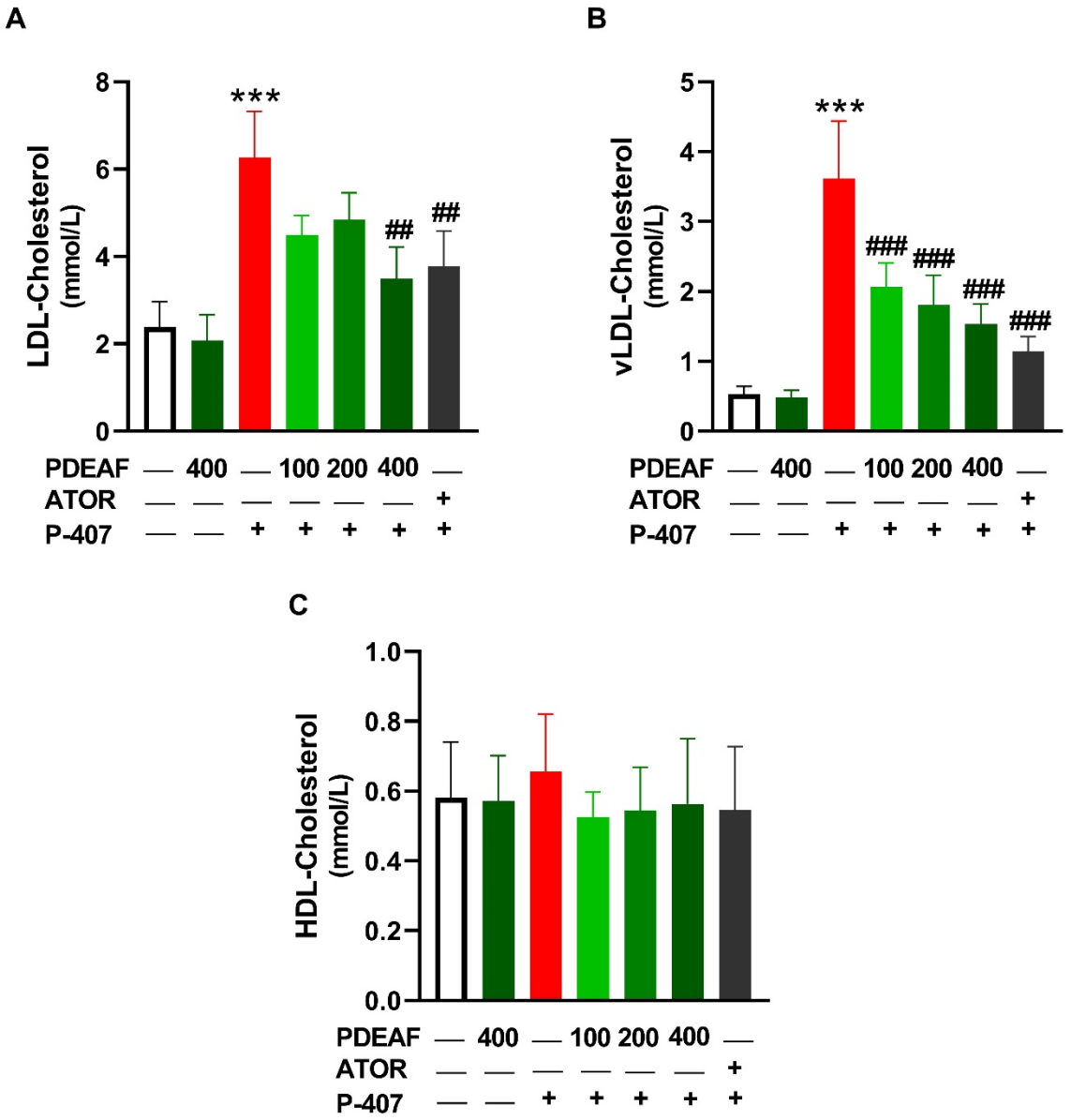


Fig. 3. PDEAF decreased plasma (A-B) CHOL and (C-D) TG significantly at 12, 24 and 48 h in P-407-adminsitered rats. Data are mean ± SEM, (n = 6). ***p<0.001 versus Control. ##p<0.01 and ###p<0.001 versus P-407.



473

474 Fig. 4. PDEAF decreased plasms (A) LDL-C and (B) vLDL-C at 48 h in P-407-adminsitered
475 rats. (C) HDL-C showed non-significant changes between the groups. Data are mean ± SEM,
476 (n = 6). ***p<0.001 versus Control. ##p<0.01 and ###p<0.001 versus P-407.

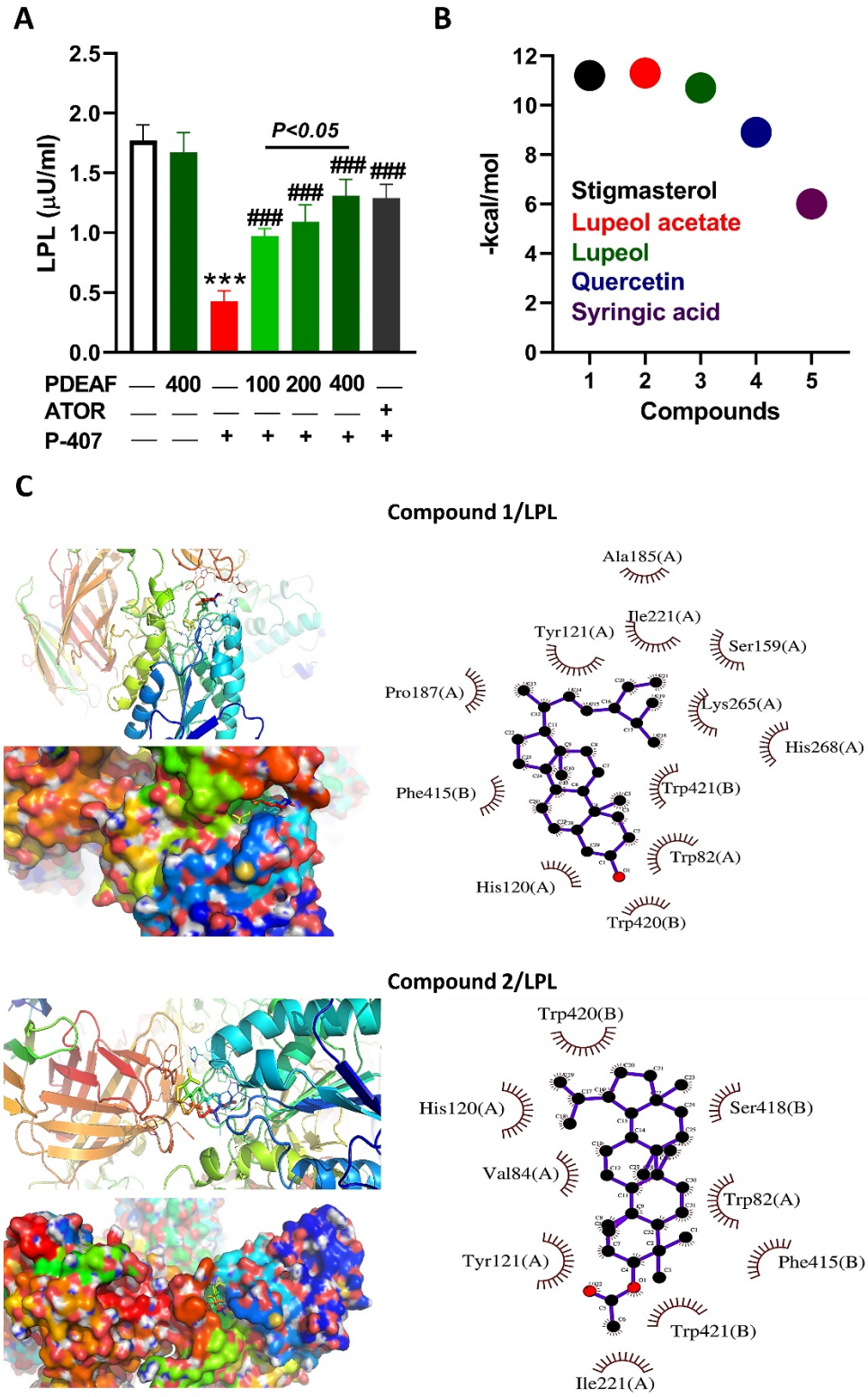
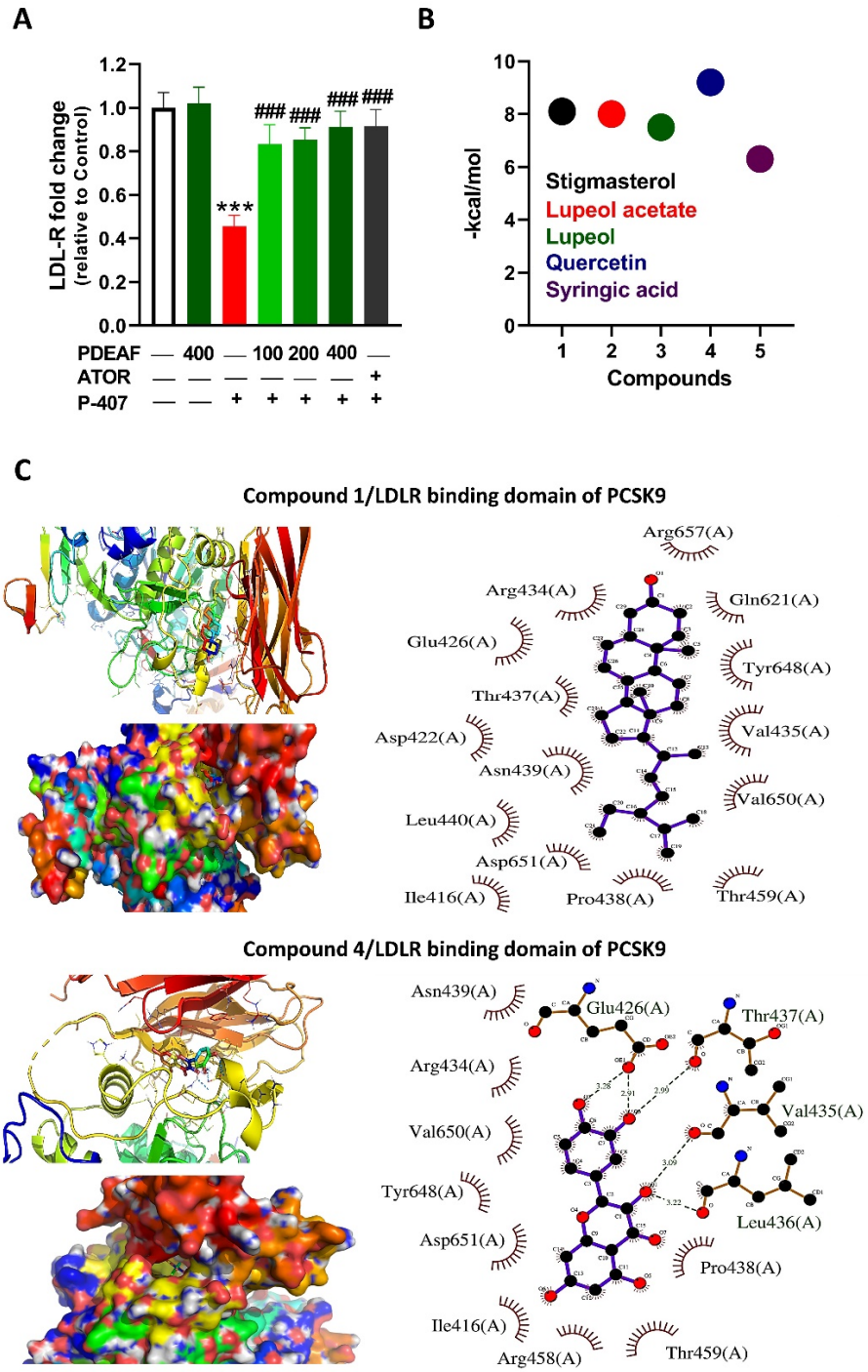


Fig. 5. PDEAF ameliorated plasma LPL activity in P-407-adminsitered rats (A). Data are mean ± SEM, (n = 6). ***p<0.001 versus Control and ###p<0.001 versus P-407. (B) lowest binding energies of the interaction between the compounds isolated from *P. dioscoridis* and LPL. (C) Binding interactions of compounds **1** and **2** with LPL.



482

483 Fig. 6. PDEAF upregulated LDL-R mRNA in the liver of P-407-adminsitered rats (A). Data
484 are mean ± SEM, (n = 6). ***p<0.001 versus Control and ###p<0.001 versus P-407. (B) lowest
485 binding energies of the interaction between the compounds isolated from *P. dioscoridis* and
486 LDL-R binding domain of PCSK9. (C) Binding interactions of compounds **1** and **4** with LDL-
487 R binding domain of PCSK9.

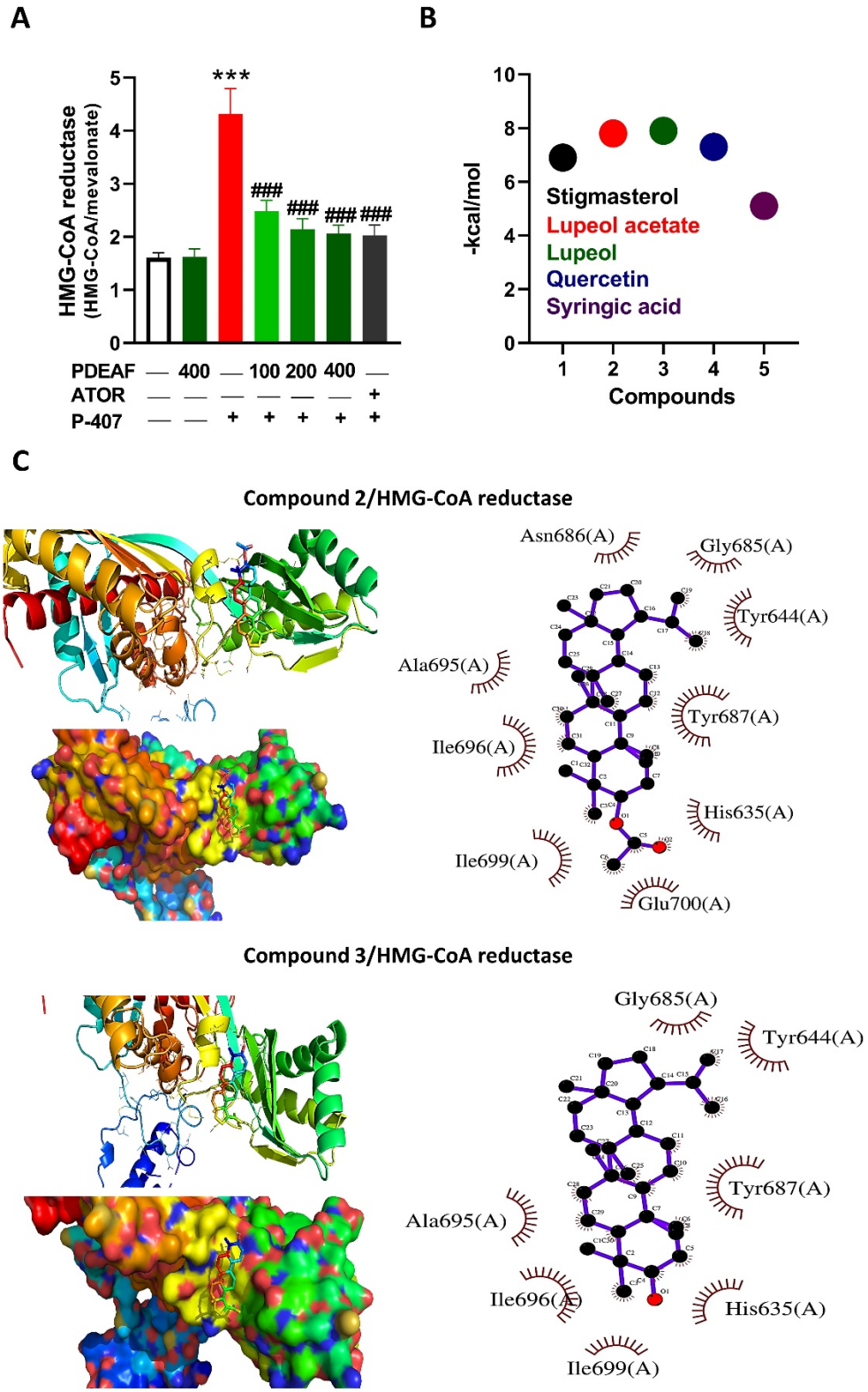
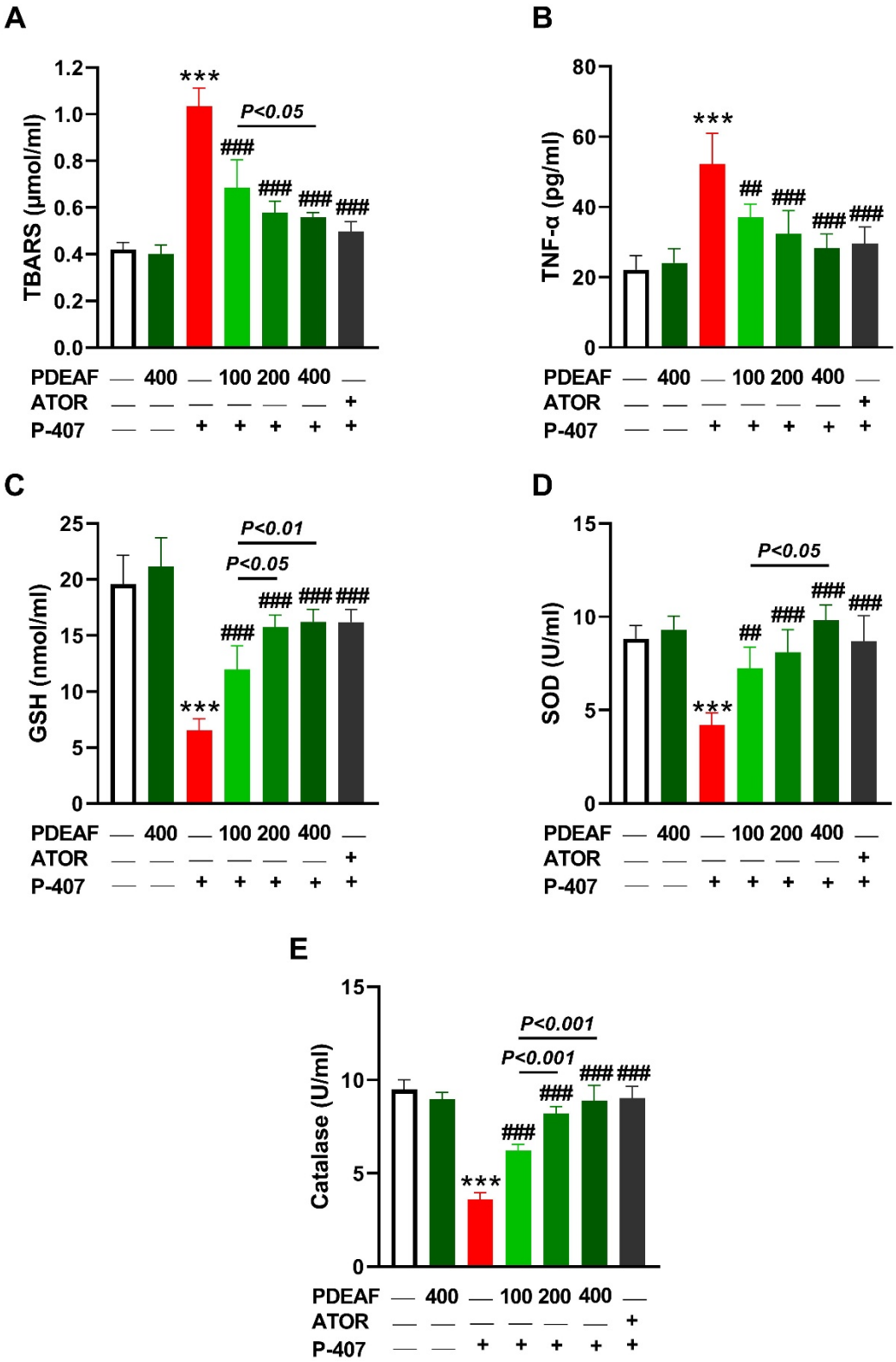


Fig. 7. PDEAF suppressed HMGCR activity in the liver of P-407-adminsitered rats (A). Data are mean ± SEM, (n = 6). ***p<0.001 versus Control and ###p<0.001 versus P-407. (B) lowest binding energies of the interaction between the compounds isolated from *P. dioscoridis* and HMGCR. (C) Binding interactions of compounds **2** and **3** with HMGCR.



493

494 Fig. 8. PDEAF decreased TBARS (A) and TNF-α (B),a dn increased GSH (C), SOD (D) and
495 catalase (E) in P-407-adminsitered rats (A). Data are mean ± SEM, (n = 6). ***p<0.001 versus
496 Control. ##p<0.01 and ###p<0.001 versus P-407.