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# Phytochemical Composition and Antioxidant Properties of Avocado (*Persea americana*) Seed Extract from Aceh, Indonesia: Implications for Antihyperlipidemic Use in Postmenopausal Women

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**Abstract**

Avocado (*Persea americana*) is widely recognized for its high antioxidant capacity. Its rich phytochemical composition is crucial in mitigating oxidative stress and managing chronic conditions such as cardiovascular disease and hyperlipidemia. This study aimed to investigate the phytochemical profile, antioxidant activity, and antihyperlipidemic potential of ethanol extracts derived from avocado seeds. Phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were conducted to identify key chemical constituents, while antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In addition, in-silico techniques were employed to evaluate the antihyperlipidemic potential of the bioactive compounds. Phytochemical analysis revealed a variety of bioactive compounds, including volatile compounds, steroids, and fatty acids, contributing to the extract's biological activity. The extract demonstrated strong antioxidant capacity, with an $IC_{50}$ value of 20.83 ppm, indicating potent free radical scavenging ability. GC-MS analysis identified significant compounds such as Undec-10-ynoic acid, tetradecyl ester, and 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester, which were further analyzed through molecular docking studies. These studies indicated their potential as inhibitors of hyperlipidemia-associated proteins, with binding energy values exceeding -6 kcal/mol. Moreover, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis demonstrated favorable pharmacokinetic profiles, including good absorption and low toxicity, positioning these compounds as promising candidates for therapeutic development. The findings of this study underscore the potential of avocado seed extract as a natural source of antioxidants and antihyperlipidemic agents. The identified bioactive compounds offer a promising therapeutic strategy for managing oxidative stress and lipid disorders, particularly in populations at heightened risk, such as postmenopausal women.



## 1. Introduction

Antioxidants have been widely recognized for their biological activities, such as anti-aging and anti-inflammatory effects [1–3]. Natural antioxidants are often associated with the development of modern pharmaceuticals. These compounds protect against damage caused by free radicals and play a crucial role in treating chronic diseases, including cardiovascular disease, aging, heart disease, anemia, cancer, and inflammation [4–6]. The body naturally defends against the formation of free radicals through enzymes such as

superoxide dismutase (SOD) and catalase [7]. However, in menopausal women, the expression of SOD and catalase decreases, which is associated with the gradual loss of estrogen during menopause [8]. This reduction in antioxidant activity triggers increased cellular oxidative stress, adversely impacting cardiovascular protection [4].

Hyperlipidemia is a condition characterized by abnormal plasma lipid levels. Menopausal women often experience a decrease in high-density lipoprotein (HDL) levels and an increase in low-density lipoprotein (LDL) levels, which can lead to hyperlipidemia and related conditions such as coronary heart disease and atherosclerosis [9, 10].

Avocado (*Persea americana*) seeds have garnered significant attention due to their rich phytochemical profile and potential health benefits. These seeds are abundant in bioactive compounds such as phenolics, flavonoids, alkaloids, saponins, and sterols, contributing to their antioxidant and antihyperlipidemic properties. Studies suggest that avocado seeds exhibit potent antioxidant activity due to their high concentration of polyphenols capable of scavenging free radicals and inhibiting lipid peroxidation [11]. This antioxidant potential is crucial for their application in treating oxidative stress-related conditions, such as hyperlipidemia and cardiovascular diseases.

Furthermore, research confirms that avocado seeds contain compounds like catechins, procyanidins, and hydroxycinnamic acids, which enhance their biological activities, including antioxidant and anti-inflammatory effects [12]. These seeds' ability to reduce lipid peroxidation and protect against oxidative damage aligns with their potential as therapeutic agents for conditions like hyperlipidemia, which are exacerbated by oxidative stress.

Methanol extracts from avocado leaves have demonstrated antioxidant activity and the potential to protect against diseases caused by oxidative stress [13]. Avocado fruit peel has also shown a high antioxidant content with an $IC_{50}$ value of 185.891 ± 1.598 ppm [14]. Extracts from avocado seeds prepared using a mixture of ethanol and water (50:50) exhibited higher antioxidant activity than those prepared with methanol, although both were categorized as active extracts [15]. Additionally, avocado has been shown to lower total cholesterol and LDL levels, highlighting its potential as a treatment for hypercholesterolemia. This activity is closely associated with its high antioxidant properties [16].

Postmenopausal women are at increased risk of hyperlipidemia due to hormonal changes that affect lipid metabolism, making them more susceptible to cardiovascular issues. Avocado seed extracts have demonstrated the ability to reduce total cholesterol and LDL levels while increasing HDL levels in hypercholesterolemic models, showcasing their potential as antihyperlipidemic agents [11]. These lipid-lowering effects and their antioxidant properties suggest that avocado seed extract could be a valuable natural remedy for managing postmenopausal hyperlipidemia.

This study's primary aim is to investigate the phytochemical composition, antioxidant activity, and antihyperlipidemic potential of ethanol-extracted avocado seeds. Specifically, we aim to elucidate the molecular mechanisms by which compounds from avocado seeds may exert antihyperlipidemic effects using in-silico techniques, including molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis.

## 2. Materials and Methods

### *2.1. Materials*

Materials in this study were ethanol, methanol, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, anhydrous acetic acid, concentrated $H_2SO_4$, $FeCl_3$ solution, gelatin, Mg powder, concentrated HCl, anhydrous acetic acid, and 2.2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) powder. Apparatus: Buchi Rotavapor R-114, maceration flask, spatula, Erlenmeyer, volumetric flask, measuring cylinder, funnel, test tubes, test tube rack, Sartorius CPA 6235 scale, volumetric pipette, micropipette, Bransted International vortex mixer 50 Hz, UV-Vis spectrophotometer (Shimadzu 1200), GC-MS spectrometer 70 eV (Shimadzu QP2000A), and other necessary glassware.

### *2.2. Extraction Process*

Avocado seeds used in this study were sourced from the Bener Meriah Regency in Aceh Province, Indonesia. It is located at 4.832371° North Latitude and 96.748394° East Longitude, at an elevation of 1,134 meters above sea level. The seeds were carefully selected from mature fruits to ensure consistency in their phytochemical composition.

After collection, the seeds were thoroughly cleaned to remove residual pulp and contaminants. Their water content was removed through controlled drying to prepare the seeds for extraction. This involved air-drying at room temperature for several days, ensuring moisture loss while preserving the integrity of the bioactive compounds. Once dried, the seeds were ground into a fine powder using a mechanical grinder, ensuring an

**Table 1.** Phytochemical analysis 96% ethanol extract of avocado seeds.

| Secondary metabolites | Reagent | Result of Phytochemical |
|---|---|---|
| Alkaloids | Mayer | - |
| | Wagner | - |
| | Dragendrorff | - |
| Steroids | Liebermann-Burchard | + |
| Terpenoids | Liebermann-Burchard | - |
| Saponins | Foam Test | - |

Descriptions: (-) = absence in ethanolic extract of avocado seeds and (+) = presence in ethanolic extract of avocado seeds

appropriate particle size for efficient extraction of bioactive components.

The powdered avocado seeds were then macerated with 96% ethanol, a widely used solvent capable of extracting both polar and non-polar compounds. The maceration process was conducted at room temperature, allowing the ethanol to dissolve the bioactive compounds in the seed powder. The mixture was stirred occasionally to facilitate thorough extraction. Following maceration, the ethanol extract was filtered to remove solid residues.

The ethanol solution was evaporated using a rotary evaporator to concentrate the extract. This step was essential to remove the ethanol while preserving the bioactive compounds. The evaporation process was closely monitored to prevent overheating and degradation of the extract's constituents. After complete solvent removal, a concentrated extract was obtained and stored under appropriate conditions for subsequent analysis.

*2.3. Compounds Analysis*

The compound content in the extract was analyzed using the phytochemical method following Harborne method guidelines [17] and GC-MS using a Shimadzu QP2000A 70 eV. The extract was characterized with a minimum volume of 1 µL. The GC-MS was operated using a 25-m-long glass column, 0.25 mm in diameter, 0.25 µm thickness, and a 5CB CP-Sil stationary phase. The mobile phase was helium gas with a pressure of 12 kPa, a total rate of 30 mL/min, and a split ratio 1:50.

*2.4. DPPH radical-scavenging activity*

The antioxidant activity of the extract was evaluated using the stable radical 2.2-diphenyl-1-picrylhydrazyl (DPPH), according to the method described by Brand-Williams [18, 19]. The concentration of the extract in methanol solvent was made with various variations, namely 20; 40; 60; 80; and 100 ppm. The DPPH stock solution was made with a concentration of 0.4 mM in methanol. The absorbance was calculated after 30 minutes at $\lambda_{max}$ = 517 nm. The percentage inhibition results of the extract were calculated using Equation 1:

$$Inhibition\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \qquad (1)$$

where $A_0$ is the absorbance of the DPPH control solution, and $A_1$ is the absorbance of the extract. The $IC_{50}$ value is obtained from the linear equation from the graph plot x = concentration and y = percent inhibition [20].

*2.5. Molecular Docking Analysis*

The compound structures were obtained from the PubChem database (www.pubchem.ncbi.nlm.nih.gov), and the protein structures HMG-CoA Reductase (PDB ID: 1HW9), PCSK9 (PDB ID: 6U3X), and ATP Citrate Lyase (PDB ID: 6O0H) were obtained from the Protein Data Bank (PDB) (www.rscb/pdb.org). The protein structures were prepared by removing water molecules, repairing missing residues, and identifying the protein's active sites using Discovery Studio Visualizer [21]. The prepared structure was saved in MOE (.moe) file format. Molecular docking analysis was carried out using MOE software. The default MOE parameters were used in this method. The top-ranked conformations based on docking scores were selected and visualized using Discovery Studio Visualizer to show the formed bond interactions [22].

*2.6. ADMET Prediction Analysis*

The prediction of Absorption, Distribution, Metabolism, and Toxicity (ADMET) values was analyzed using the pkCSM website (https://biosig.lab.uq.edu.au/pkcsm/). The compounds with the best docking results were uploaded in .SDF or SMILES format to the online server to calculate ADMET properties using default parameters [23, 24].

## 3. Results and Discussion

*3.1. Chemical Compounds of Ethanolic Extract of Avocado Seeds*

Phytochemical analysis showed positive results for steroid secondary metabolite in ethanolic extract of avocado seeds (Table 1). Determination of composition through GC-MS showed that ethanolic extract of avocado seeds contained secondary metabolite compounds of

**Table 2**. GC-MS analysis of ethanolic extract of avocado seeds.

| No. | Compound name | Area (%) |
|---|---|---|
| 1 | Avocadenofuran | 6.70 |
| 2 | Hexadecanoic acid, Methyl ester | 1.01 |
| 3 | *n*-Hexadecanoic acid | 6.15 |
| 4 | €-2-(Pentadec-2-en-1-yl)furan | 1.03 |
| 5 | Methyl 12.13-tetradecadienoate | 0.70 |
| 6 | 2-Hexadecenoic acid, methyl ester | 0.74 |
| 7 | *cis*-Vaccenic acid | 9.60 |
| 8 | Octadecanoic acid | 0.60 |
| 9 | 2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)furan | 5.35 |
| 10 | Geranyl vinyl ether | 1.93 |
| 11 | 9-Tetradecen-1-ol, acetate | 0.91 |
| 12 | E-11-Methyl-12-tetradecen-1-ol acetate | 11.37 |
| 13 | Cyclopropaneoctanoic acid, 2-[[2-[(2ethylcyclopropyl)methyl]cyclopropyl]methyl] -, methyl ester | 6.64 |
| 14 | E,E,Z-1,3,12-Nonadecatriene-5,14-diol | 6.17 |
| 15 | 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)1-[(acetyloxy)methyl]ethyl ester, (Z.Z.Z) | 0.83 |
| 16 | Cyclopropaneoctanoic acid, 2-[[2-[(2ethylcyclopropyl)methyl]cyclopropyl]methyl] -, methyl ester | 2.72 |
| 17 | 12-Tridecynoic acid, methyl ester | 9.39 |
| 18 | Cyclopropaneoctanoic acid. 2-[[2-[(2ethylcyclopropyl)methyl]cyclopropyl]methyl] -, methyl ester | 1.10 |
| 19 | 12-Tridecynoic acid, methyl ester | 9.80 |
| 20 | Hexadecanoic acid. 2-hydroxy-1(hydroxymethyl)ethyl ester | 4.09 |
| 21 | Methyl 2-hydroxy-octadeca-9,12,15trienoate | 1.46 |
| 22 | Undec-10-ynoic acid, tetradecyl ester | 1.14 |
| 23 | 7-Methyl-Z-tetradecen-1-ol acetate | 1.07 |
| 24 | 9,12-Octadecadienoic acid (Z.Z)-,2-hydroxy1-(hydroxymethyl)ethyl ester | 4.98 |
| 25 | β-sitosterol | 3.39 |
| 26 | Stigmast-4-en-3-one | 1.11 |

**Table 3.** DPPH inhibition of 96% ethanol extract of avocado seeds and ascorbic acid.

| Concentration (ppm) | Inhibition (%) | |
|---|---|---|
| | Ethanolic Extract Avocado seed | Ascorbic acid |
| 20 | 46.95 | 50.74 |
| 40 | 60.72 | 66.36 |
| 60 | 69.4 | 78.6 |
| 80 | 74.53 | 86.18 |
| 100 | 83.50 | 88.9 |

steroids, namely Stigmast-4-en-3-one and β-sitosterol. Besides steroids, avocado seeds contain various volatile organic compounds listed in Table 2.

The most dominant component in this extract was E-11-methyl-12-tetradecen-1-ol acetate (11.37%). A previous study has reported that bio-oil from ethanol extraction of avocado seeds contained E-11-methyl-12-tetradecen-1-ol acetate [25]. Interpretation of other compounds is also supported by previous studies, where avocado seed extract using absolute ethanol and acetone showed the presence of β-sitosterol and several similar fatty acids, such as *n*-Hexadecanoic acid and 9.12-Octadecanoic acid [26, 27].

### *3.2. Antioxidant capacity*

The antioxidant activity of the ethanol extract of avocado seeds is summarized in Table 3 and illustrated in Figure 1. The study demonstrated that the extract effectively reduced DPPH (2,2-Diphenyl-1-picrylhydrazyl), increasing its scavenging ability proportionally with higher extract concentrations. The reduction of DPPH occurs through the direct donation of hydrogen atoms to free radicals, stabilizing them into non-reactive compounds [28].

The antioxidant activity of the avocado seed extract was compared to the positive control, ascorbic acid, a well-established potent antioxidant often used as a reference in antioxidant testing. The results showed that the antioxidant capacity of the avocado seed extract was comparable to that of ascorbic acid. Specifically, the extract exhibited an $IC_{50}$ value of 20.83 ppm, while ascorbic acid demonstrated an $IC_{50}$ of 9.75 ppm. These values indicate that the avocado seed extract possesses strong antioxidant activity ($IC_{50}$ < 100 ppm), reinforcing its potential as a source of natural antioxidants.

Phytochemical analysis of the ethanol extract revealed the presence of steroid compounds and volatile organic compounds, which are likely contributors to its DPPH-reducing capability. Steroids and essential oils are known for their effectiveness as natural antioxidants, primarily due to their ability to neutralize free radicals and protect cells from oxidative damage. One such compound, β-sitosterol, has been shown to significantly reduce free radicals, with a reduction of up to 78.12% at a concentration of 250 µg/mL. These findings highlight the strong antioxidant potential of β-sitosterol [29].

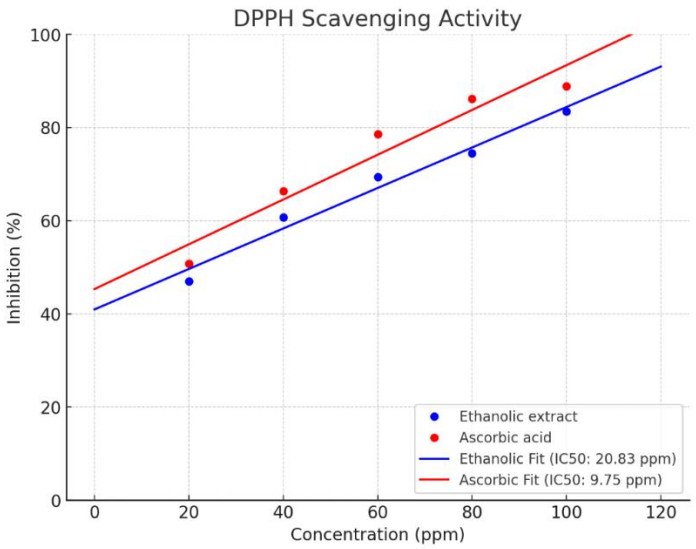


**Figure 1.** DPPH scavenging activity of ethanol extract of avocado seeds (blue line) and ascorbic acid (red line) with different concentrations i.e., 20. 40. 60. 80 and 100 ppm.

**Table 4.** Protein target with hyperlipidemia.

| Protein | Effects on hyperlipidemic | Reference |
|---|---|---|
| HMG-CoA Reductase | Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors reducing total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels. | [30] |
| Proprotein convertase subtilisin/kexin type 9 (PCSK9) | PCSK9 enzyme inhibitors play an important role in the breakdown of LDL receptors in the liver. | [31] |
| ATP Citrate Lyase | ATP-citrate lyase (ACL) is an enzyme involved in lipid biosynthesis through extramitochondrial acetyl-CoA production. | [32] |

In contrast, the compound stigmast-4-en-3-one exhibited relatively lower antioxidant activity but demonstrated notable potential as an anti-inflammatory agent [33, 34]. Previous research has also identified volatile sesquiterpenoids, unsaturated fatty acid esters, and polyunsaturated fatty acids in avocado seed extracts obtained using acetone and ethanol solvents. These extracts were reported to be active antioxidants, with DPPH scavenging values of 212.75 and 183.75 mg Trolox/100 g, respectively [35].

*3.3. Molecular Docking Analysis*

Molecular docking studies were conducted to evaluate the interactions of compounds in the ethanol extract of avocado seeds with three target proteins: HMG-CoA reductase, proprotein convertase subtilisin/kexin type 9 (PCSK9), and ATP citrate lyase. These proteins are key players in developing hyperlipidemia (Table 4). The results revealed that active compounds in the extract exhibited strong binding affinities to the target proteins (Table 5, Figure 2). The analysis focused on 12 compounds with the highest concentrations in the extract.

Among the compounds studied, Undec-10-ynoic acid, tetradecyl ester, and 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester demonstrated the most promising interactions with all three target proteins. These compounds showed the lowest binding energy compared to the positive controls and other tested molecules. The low binding energy suggests that these compounds have significant potential as inhibitors of the target proteins, thereby reducing hyperlipidemic activity.

Although the antihyperlipidemic activity of Undec-10-ynoic acid, tetradecyl ester, and 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester has not been previously reported, extracts containing similar compounds have demonstrated antioxidant activity and the ability to prevent or reduce LDL oxidation [36, 37]. These properties further support the potential of these compounds in managing hyperlipidemia.

While molecular docking provides valuable insights into the interactions between compounds and target proteins, it is important to note that docking energy values alone cannot predict in vivo efficacy. Further studies, such as in vitro enzyme inhibition assays or receptor binding experiments, are necessary to validate the therapeutic potential of these compounds and

**Table 5.** Docking Score of active compounds from avocado extract.

| Ligand | HMG-CoA Reductase | | PCSK9 | | ATP Citrate Lyase | |
|---|---|---|---|---|---|---|
| | Docking score | RMSD | Docking score | RMSD | Docking score | RMSD |
| Native ligand | -5.922 | 1.164 | -9.217 | 0.860 | -8.972 | 0.170 |
| Hexadecanoic acid. methyl ester | -6.027 | 1.894 | -8.235 | 1.897 | -8.484 | 1.705 |
| *n*-Hexadecanoic acid | -5.737 | 2.593 | -8.026 | 1.578 | -8.218 | 2.028 |
| *cis*-Vaccenic acid | -6.272 | 0.949 | -8.288 | 1.575 | -8.441 | 3.019 |
| 9,12,15-Octadecatrienoic acid. 2-(acetyloxy)1-[(acetyloxy)methyl]ethyl ester | -7.219 | 2.450 | **-10.579** | 2.582 | **-10.836** | 1.766 |
| Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester | -6.561 | 1.717 | -9.276 | 2.353 | -9.264 | 1.969 |
| 9,12-Octadecadienoic acid (Z.Z)-,2-hydroxy1-(hydroxymethyl)ethyl ester | -6.232 | 2.182 | -9.425 | 1.948 | -9.029 | 3.001 |
| β-sitosterol | -6.238 | 2.546 | -8.535 | 1.611 | -7.916 | 1.191 |
| Stigmast-4-en-3-one | -5.967 | 1.922 | -8.842 | 1.965 | -9.064 | 2.925 |
| Methyl 12,13 tetradecadienoate | -5.663 | 1.276 | -7.529 | 2.265 | -7.648 | 1.854 |
| Undec-10-ynoic acid, tetradecyl ester | **-7.324** | 1.702 | -9.962 | 1.707 | -9.812 | 1.964 |
| Geranyl vinyl ether | -5.116 | 1.826 | -6.299 | 1.599 | -6.462 | 1.163 |
| 2-((8Z.11Z)-Heptadeca-8.11- dien-1-yl)furan | -6.233 | 1.978 | -8.965 | 1.284 | -8.473 | 2.251 |

Description: RMSD = Root mean square deviation

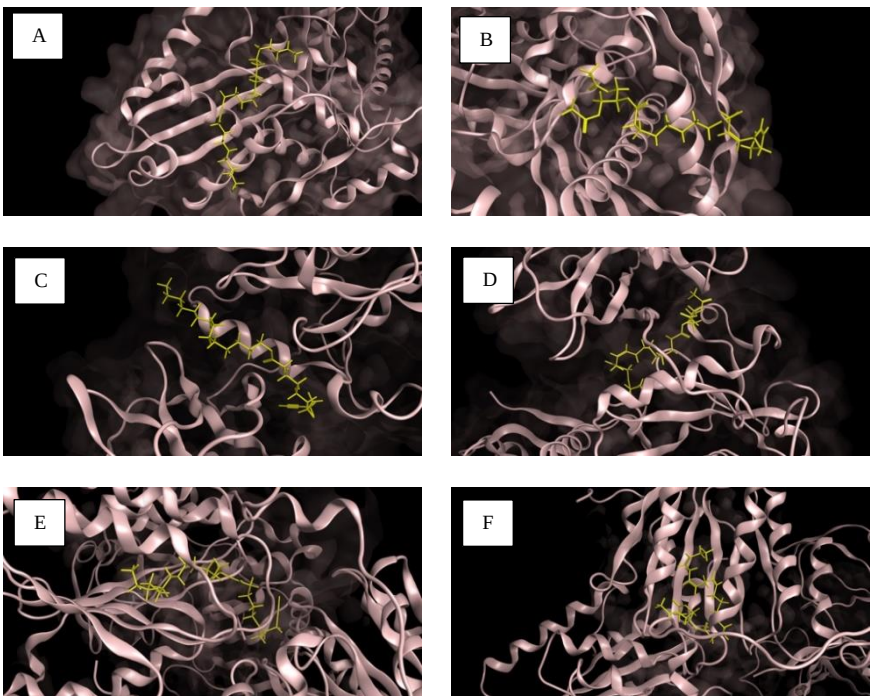


**Figure 2.** 3D model of Undec-10-ynoic acid, tetradecyl ester with HMG-CoA Reductase (A), PCSK9 (C), ATP Citrate Lyase (E) and ,12,15-Octadecatrienoic acid, 2-(acetyloxy)1-[(acetyloxy)methyl] ethyl ester with HMG-CoA Reductase (B), PCSK9 (D), ATP Citrate Lyase (F).

provide robust evidence for their antihyperlipidemic activity.

### *3.4. ADMET Predictions*

In accordance with pKCSM guidelines (Table 6), the compounds Undec-10-ynoic acid, tetradecyl ester, and 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester demonstrated notable pharmacokinetic characteristics. CaCO2 permeability values greater than 0.90 × $10^{-6}$ cm/s indicate effective intestinal absorption of a compound [23]. Undec-10-ynoic acid, tetradecyl ester exhibited a CaCO2 permeability value of 1.341 × $10^{-6}$ cm/s, indicating excellent absorption through the intestine and suitability for oral administration. Similarly, 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester showed good oral administration potential, though its intestinal absorption was moderate, with a CaCO2 permeability value of 0.537 × $10^{-6}$ cm/s.

**Table 6.** Pharmacokinetics predictions of active compounds based on pKCSM.

| Property | Model | Unit | Undec-10-ynoic acid, tetradecyl ester | 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)1-[(acetyloxy)methyl] ethyl ester |
|---|---|---|---|---|
| Absorption | Water Solubility | Numeric (log mol/L) | -6.858 | -5.969 |
| | Caco2 permeability | Numeric (log Papp in $10^{-6}$ cm/s) | 1.341 | 0.537 |
| | Intestinal Absorption (human) | Numeric (% absorbed) | 90.69 | 94.033 |
| | Skin permeability | Numeric (log Kp) | -2.759 | -2.723 |
| | P-glycoprotein substrate | Categorical (Yes/No) | No | No |
| | P-glycoprotein I inhibitor | Categorical (Yes/No) | No | Yes |
| | P-glycoprotein II inhibitor | Categorical (Yes/No) | Yes | Yes |
| Distribution | VDss (human) | Numeric (Fu) | 0.064 | -0.304 |
| | Fraction unbound (human) | Numeric (log BB) | 0 | 0.072 |
| | BBB permeability | Numeric (log PS) | 0.909 | -0.97 |
| | CNS permeability | Categorical (Yes/No) | -1.33 | -0.094 |
| Metabolism | CYP2D6 substrate | Categorical (Yes/No) | No | No |
| | CYP3A4 substrate | Categorical (Yes/No) | Yes | Yes |
| | CYP1A2 inhibitor | Categorical (Yes/No) | No | No |
| | CYP2C19 inhibitor | Categorical (Yes/No) | No | No |
| | CYP2C9 inhibitor | Categorical (Yes/No) | No | No |
| | CYP2D6 inhibitor | Categorical (Yes/No) | No | No |
| | CYP3A4 inhibitor | Categorical (Yes/No) | No | No |
| Toxicity | AMES toxicity | Categorical (Yes/No) | No | No |
| | Max. Tolerated dose (human) | Numeric (mol/kg/day) | 0.131 | 0.321 |
| | hERG I inhibitor | Categorical (Yes/No) | No | No |
| | hERG II inhibitor | Categorical (Yes/No) | Yes | No |
| | Oral Rat Acute Toxicity (LD50) | Numeric (mol/kg) | 1.466 | 1.793 |
| | Oral Rat Chronic Toxicity (LOAEL) | Numeric (log mg/kg bw/day) | 3.091 | 0.44 |
| | Hepatotoxicity | Categorical (Yes/No) | No | No |
| | Skin sensitisation | Categorical (Yes/No) | Yes | No |
| | Skin *T. pyriformis* toxicity | Numeric (log ug/L) | 0.581 | 0.479 |
| | Minow toxicity | Numeric (log mM) | -3.099 | -0.552 |

Skin permeability analysis revealed that both compounds had very low permeability through the skin. Regarding distribution properties, both compounds exhibited low distribution volumes in tissue, with a tendency to remain primarily in blood plasma. The compounds were also evaluated for blood-brain barrier (BBB) permeability based on logBB values. A logBB greater than 0.3 indicates the compound can effectively cross the BBB, while a value below -1 suggests poor distribution to the brain [23]. Undec-10-ynoic acid, tetradecyl ester, had a logBB value > 0.3, suggesting its ability to penetrate the BBB and potentially reach the central nervous system (CNS). Conversely, 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester had a logBB value of -0.309, indicating limited BBB penetration and reduced effectiveness for CNS-targeted applications.

Both compounds were metabolized by the CYP3A4 enzyme, a major subfamily within the cytochrome P450 superfamily that processes endogenous substrates such as hormones, bile acids, and non-pharmaceutical xenobiotics [38]. Additionally, neither compound acted as an inhibitor of the metabolic enzymes CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4, indicating a relatively low risk of drug-drug interactions due to enzyme inhibition.

Toxicity analysis revealed that Undec-10-ynoic acid, tetradecyl ester was not mutagenic (AMES test) and did not cause liver damage (hepatotoxicity). However, it did exhibit skin sensitization potential and inhibited the hERG II ion channel, which is critical for maintaining proper heart rhythm [39]. The maximum tolerated dose for this compound was low, at 0.131 log mg/kg/day, and it demonstrated acute toxicity with an $LD_{50}$ value of 1.466 mol/kg and a LOAEL of 3.091 log mg/kg body weight per day.

In contrast, 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester, was neither mutagenic nor hepatotoxic and did not inhibit hERG I or II ion channels. This compound had a slightly higher maximum tolerated dose of 0.321 log mg/kg/day compared to Undec-10-ynoic acid, tetradecyl ester, though it remained within the low tolerability category.

Overall, these findings reinforce the medicinal potential of avocado seeds, particularly in managing oxidative stress and lipid disorders. These compounds' pharmacokinetic properties and low toxicity profiles highlight their promise as natural therapeutic agents. These results are especially significant for populations at high risk of chronic conditions, such as postmenopausal women. The study lays a foundation for future research to validate the therapeutic efficacy of avocado seed extracts. It supports their potential as natural alternatives or complementary treatments for hyperlipidemia and cardiovascular diseases.

## 4. Conclusions

This study highlights avocado (*Persea americana*) seed extract as a promising natural source of antioxidants and antihyperlipidemic agents. The study's key findings demonstrate that avocado seed extract contains a rich array of bioactive compounds, including undec-10-ynoic acid, tetradecyl ester, and 9,12,15-octadecatrienoic acid, which exhibited strong antioxidant activity with an $IC_{50}$ value of 20.83 ppm. Molecular docking results indicated that these compounds possess high binding affinities for proteins involved in lipid metabolism, suggesting potential mechanisms by which they may reduce lipid levels and manage hyperlipidemia. Additionally, ADMET analysis revealed favorable pharmacokinetic profiles for these compounds, including good absorption and low toxicity, supporting their potential as safe and effective drug candidates. This study establishes that avocado seed extracts could be a viable natural source of antioxidants and antihyperlipidemic agents.

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