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Research Article

Bioinformatics Analysis of the Anti-inflammatory Potential of Persea americana Mill. Leaves through IL-18 Inhibition Mediated by the NLRP3 Inflammasome

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Abstract

Acute Kidney Injury (AKI) is a severe condition marked by a rapid decline in kidney function, often linked to inflammation and oxidative stress. Activation of the NLRP3 inflammasome triggers inflammation by releasing interleukin-18 (IL-18), exacerbating renal damage. *Persea americana* Mill. (avocado) contains bioactive compounds with known anti-inflammatory and antioxidant properties. This study aimed to evaluate the anti-inflammatory potential of P. americana leaves in inhibiting IL-18 mediated by the NLRP3 inflammasome using a bioinformatic approach. Active compounds were identified through The Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) and KNApSAcK databases. Molecular docking simulations assessed their binding affinity to the NLRP3 inflammasome, while network pharmacology analysis explored interactions with inflammation-related pathways. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis highlighted the biological processes influenced by these compounds. The results showed that flavonoids such as luteolin and apigenin exhibited strong binding affinity to the NLRP3 inflammasome, with docking scores of -8.6850 kcal/mol and -8.2520 kcal/mol, respectively, indicating their potential to modulate inflammatory pathways. Network pharmacology analysis indicated that these compounds are linked to apoptosis and oxidative stress regulation, both critical in AKI progression. These findings suggest that *P. americana* leaves have promising anti-inflammatory potential by targeting IL-18 inhibition through modulation of the NLRP3 inflammasome, highlighting their value as a natural therapeutic agent for managing AKI.

Keywords: *Persea americana*; NLRP3 inflammasome; Interleukin-18; Acute Kidney Injury; Molecular docking

1. Introduction

Acute Kidney Injury (AKI) is a critical medical condition characterized by a rapid decline in kidney function and is often associated with inflammation and oxidative stress (Ronco et al., 2019). The prevalence of AKI continues to increase globally, with the incidence varying depending on the population and the underlying risk factors. Studies have shown that AKI occurs in approximately 10–15% of hospitalized patients and in more than 50% of patients in intensive care units (Hoste et al., 2018). Inflammation is the protective response of the body to infection, injury, or tissue damage. However, chronic inflammation can also contribute to the development of various diseases (Swanson et al., 2019).

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One of the key mechanisms of inflammation is the activation of a protein complex known as the inflammasome, specifically NLRP3 (NOD-like receptor family pyrin domain containing 3). The NLRP3 inflammasome plays a role in triggers the inflammatory cascade by activating interleukin-18 (IL-18), a proinflammatory cytokine involved in the innate immune response (He et al., 2016). This inflammatory mediator contributes to kidney tissue damage, tubular necrosis, and fibrosis, further exacerbating kidney injury (Wang et al., 2023). Interleukin-18 (IL-18) has been identified as an important biomarker for the pathogenesis of AKI. Elevated IL-18 levels are found in patients with AKI, and high IL-18 levels correlate with the severity of kidney damage (Lin et al., 2015). Activation of the NLRP3 inflammasome, which triggers the release of IL-18, can be triggered by various factors such as ischemia-reperfusion, toxins, or infections (Komada & Muruve, 2019). Therefore, inhibition of this pathway may have a protective effect on the kidneys.

Persea americana Mill, better known as avocado, has long been known as a plant with various health benefits, including antioxidant and anti-inflammatory activities. Avocado leaves contain bioactive compounds such as flavonoids, phenols, and saponins, which are thought to inhibit inflammatory pathways (Dabas et al., 2013). Previous studies have shown that avocado leaf extracts have pharmacological effects, including antimicrobial, antidiabetic, and anti-inflammatory activities (Ngbolua, 2019). Additionally, Purwono et al. (2024) using an in silico approach showed that avocado leaf extract has the potential to inhibit calcium oxalate stone formation. This study indicated that compounds in avocado leaves can interact with molecular targets involved in the nephrolithiasis process, thereby reducing the risk of kidney injury due to urolithiasis. However, bioinformatics studies on the effects of avocado leaves on IL-18 inhibition mediated by NLRP3 inflammasome are limited. Bioinformatic techniques, such as molecular docking and network pharmacology, provide valuable insights into the interactions between bioactive compounds and target proteins involved in inflammation (Liu et al., 2018). This study aimed to analyze the anti-inflammatory potential of Persea americana leaves using an in silico approach, with a particular focus on the inhibition of IL-18 mediated by the NLRP3 inflammasome.

2. Material and Method

Prediction active ingredients of *Persea americana* Mill. and Acute Kidney Injury drug targets

The Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) and KNApSAcK databases were searched using the keywords "*Persea americana*" and "*Avocado".* The results obtained from IMPPAT and KNApSAcK were then filtered with parameters of *oral bioavailability* (OB) ≥ 30% and *drug-likeness* (DL) ≥ 0.18 (Shen et al., 2017). The filtered results were further analyzed to identify the active compounds in the rhizome of *Persea americana Mill.* which has the potential to exert therapeutic effects on *acute kidney injury* (AKI). The two-dimensional (2D) molecular structures of these active compounds were obtained from the PubChem database. The SwissTargetPrediction

platform was used to predict potential targets of these compounds. Active compounds from the rhizome of *Persea americana Mill.* along with their targets were then imported into Cytoscape 3.7.2, to visualize the interaction network of the active compounds and their targets.

The keyword *acute kidney injury* was used to identify disease targets in the DrugBank and GeneCards databases (Suresh et al., 2023). Relevant disease targets were collected, organized, and imported into the UniProt platform for gene standardization. Targets of active compounds in *Persea americana* Mill*.* Leaves and AKI drug targets were analyzed using Venny 2.1.0 to identify common targets (Dong et al., 2023). The data were then processed in STRING to create a protein interaction (PPI) network, which was visualized in Cytoscape 3.7.2, and filtered based on degree value *and* closeness *centrality* .

Analysis of GO Function and KEGG Pathway Enrichment

Gene Ontology (GO) and *Kyoto Encyclopedia of Genes and Genomes (KEGG)* enrichment analyses were performed and graphically visualized using ShinyGO (version 0.76.3) (http://bioinformatics.sdstate.edu/go/) (Ge et al., 2020). The significance threshold for enrichment was set at *false discovery rate* (FDR) ≤ 0.05.

Molecular Docking Simulation of Active Compounds

The protein structure of NLRP3 (PDB ID: 7ALV) was downloaded from the *.PDB format from the RCSB PDB (www.rcbs.org/pdb). The three-dimensional (3D) structure of the target protein was then opened *.pdb format using YASARA. The co-crystallin ligand was separated from the target protein and saved in PDB file format (*.pdb). The preparation of the reference ligands and tests were performed using the 3D structures in *.sdf format of Benzyl Benzoate, Apigenin, Luteolin, D-Limonene, and Eugenol compounds were obtained from PubChem (https://pubmed.ncbi.nlm.nih.gov/).

Molecular docking simulations were performed using the YASARA Structure software. The prepared protein files were loaded back into the YASARA Structure, where the docking sites were determined and saved in *.sce format. The docking process was performed with the extend setting based on the validation results with the best binding energy used as the grid box. All test ligands in *.PDB format was imported, and the protein structure was locked. The AMBER14 force field was selected to perform docking. The ligand-protein complex was then stored in *.complex.sce format. Molecular docking was performed 50 times to generate the files in *.yob and *.txt format containing binding energy data, dissociation constants (Kd), and contact residues (Dash et al., 2019; Land & Humble, 2018).

3. Results and Discussion

3.1. Results

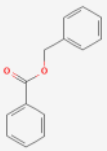
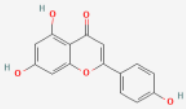
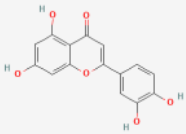
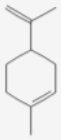
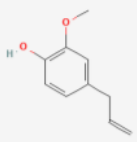
Heavy Metals Concentration in Wastewater

The analysis of heavy metal concentrations in wastewater reveals concerning pollution levels, particularly from the batik industry in Yogyakarta (Table 1). Arsenic,

cadmium, and lead in this wastewater significantly exceed regulatory limits according to PP RI no 22 (2021). These contaminations highlighted the inadequate treatment of industrial effluents especially from the dyeing process. These metals are highly toxic and can accumulate in aquatic ecosystems, posing risks to both the environment and human health. Sand mine wastewater also shows elevated cadmium levels, although arsenic, chromium, and lead remain within acceptable limits. Mining activities often disrupt natural systems, and even metals within permissible levels can accumulate over time. Household wastewater, on the other hand, shows much lower concentrations of heavy metals, suggesting minimal contribution to pollution. However, growing chemical use in homes could present future challenges if not managed responsibly. These findings suggest the potential of as a phytoremediation agent to address heavy metal contamination in wastewater.

Bioactive Compounds of *Persea americana Mill.* Rhizomes and Their Potential Targets

Table 1. Information on the 5 active components that were filtered

Mol ID	Molecule Name	OB (%)	DL	MW	Structure
MOL2345	Benzyl Benzoate	45.0	0.73	212.26	
MOL5280443	Apigenin	42.0	0.63	270.24	
MOL 5280445	Luteolin	40.0	0.51	286.24	
MOL22311	D-Limonene	60.0	0.49	136.23	
MOL3314	Eugenol	56.24	0.49	164.2	

Collected from the IMPPAT and KNApSAcK database. OB, oral bioavailability; DL, drug likeness; MW, molecular weight

Avocado leaves, known as *Persea americana* Mill. and belonging to the Lauraceae family have been widely used in traditional medicine, especially in tropical regions. Although avocado leaves are known for their culinary uses, they have a variety of pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial effects (Ochoa-Zarzosa et al., 2021). In Indonesian herbal medicine, these leaves are often used as natural remedies for hypertension, diabetes, and inflammatory disorders (Pratiwi et al., 2024).

Screening identified several bioactive compounds that contribute to the therapeutic potential of *P. americana*. Among these compounds, five major active components, Benzyl Benzoate, Apigenin, Luteolin, D-Limonene, and Eugenol, are known to have anti-inflammatory effects (Marwat et al., 2011). Flavonoids such as Apigenin and Luteolin play an important role in modulating inflammatory pathways by inhibiting NF-κB and COX-2 expression, while Eugenol and D-Limonene exhibit antioxidant and analgesic properties that contribute to cellular protection (C.-J. Chen et al., 2023).

Analysis and Construction of the PPI Network

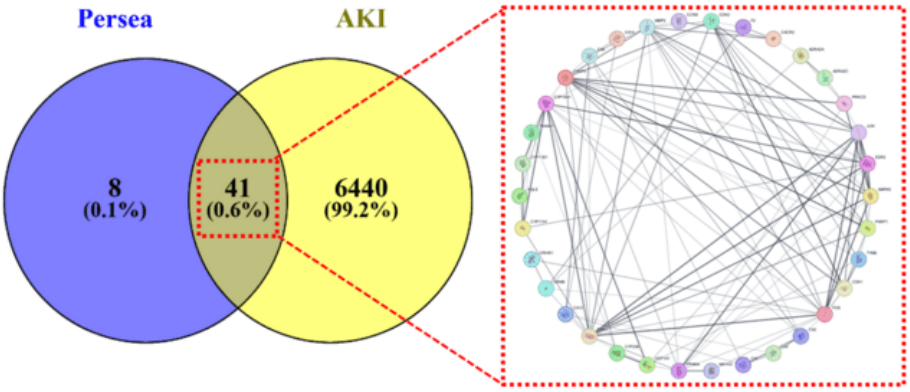


Figure 1. Target Identification and PPI Network Diagram of Persea americana Mill. for AKI Treatment

P. americana rhizome targets and those associated with AKI were identified using Venny 2.1.0, resulting in a Venn diagram (Figure 1) that identified 49 overlapping targets. The STRING platform (Version 11.0) was used to import these targets, selecting *Homo sapiens* as the reference organism to construct a protein-protein interaction network (PPI *network)*. Further analysis using the SwissTargetPrediction and STITCH 5.0 databases revealed several proteins that were affected by *P. americana* active compounds . SwissTargetPrediction showed that Benzyl Benzoate interacted with two proteins and D-limonene interacted with three proteins with the highest probability scores, while STITCH 5.0 identified interactions of A pigenin with two proteins, eugenol with one protein, and luteolin with three proteins. The PPI network was visualized using Cytoscape 3.7.2 (Figure 1) after all interaction data were imported and further evaluated using the combination score parameter.

Table 2. Active compound-target proteins of P. Americana

Node 1	Node 2	Description	Combination Score
Apigenin	CASP3	Caspase-3	0.947
	MMP9	Matrix Metalloproteinase-9	0.842
Eugenol	CASP3	Caspase-3	0.722
luteolin	MAPK8	Mitogen-Activated Protein Kinase 8	0.951
	JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit	0.946
	FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit	0.944
Benzyl Benzoate	PRKCD	Protein Kinase C Delta	0.060
	CXCR2	CXC Motif Chemokine Receptor 2	0.060
D-Limonene	PPARA	Peroxisome Proliferator-Activated Receptor Alpha	0.156
	CNR2	Cannabinoid Receptor 2	0.156
	NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3	0.044

The interaction between apigenin and CASP3 and MMP9 has the potential to modulate apoptosis and inflammatory responses. Activation of the NLRP3 inflammasome can trigger CASP3 as a mediator of the inflammatory apoptosis process (pyroptosis). Apigenin is known to have anti-inflammatory properties through inhibition of CASP3 activation, which has the potential to reduce the release of IL-18 from the NLRP3 inflammasome. By targeting CASP3, apigenin can modulate this pathway and reduce renal tissue damage caused by inflammation (H. Du et al., 2015). MMP9 is an enzyme that plays a role in the degradation of the extracellular matrix and contributes to the inflammatory process by disrupting the epithelial barrier (Derkacz et al., 2021). MMP9 overexpression is associated with increased inflammation and activation of the NLRP3 inflammasome. The interaction between apigenin and MMP9 can suppress the expression of this enzyme, thereby reducing immune cell infiltration and IL-18 release (X. J. Chen et al., 2016).

Luteolin along with MAPK8, JUN, and FOS indicates its role in modulating the MAPK and AP-1 signaling pathways, which regulate cellular responses to stress and inflammation. MAPK8 activation has been associated with AKI because of its role in oxidative stress and inflammatory damage (Pavlakou et al., 2017). This compound can also inhibit the phosphorylation of c-Jun and c-Fos, thereby reducing Interleukin-6 (IL-6) production in microglia (Jang et al., 2008; Lim et al., 2013). JUN is a key transcription factor in the AP-1 activation pathway, which regulates *proinflammatory* gene expression (X. Yu et al., 2023). The interaction between eugenol and JUN suppressed the expression of the NLRP3 inflammasome and reduced the release of IL-18. MAPK8, also known as JNK1, is a key component of the MAPK signaling pathway that regulates cellular inflammatory and stress responses (Grynberg et al., 2017). MAPK8 activation triggers the expression of NLRP3 inflammasome and increases IL-18 production. This suggests that

Luteolin and Eugenol may have protective effects against AKI by modulating oxidative stress and inflammation (W. Yu et al., 2025). CASP3 in combination with eugenol has shown its potential to modulate apoptosis (J. Du et al., 2014). Although its interaction score is lower than that of apigenin, apoptotic cell death is a characteristic of AKI, and eugenol compounds targeting CASP3 can suppress caspase-3 activation, which indirectly decreases IL-18 production by inhibiting the NLRP3 inflammasome.

Benzyl Benzoate could bind to PRKCD and CXCR2, both with a combined score of 0.060. PRKCD is involved in various cellular processes, including apoptosis and immune signaling, whereas CXCR2 plays a role in neutrophil recruitment, which contributes to inflammation in AKI (Li et al., 2022; Zhu et al., 2020). PPARA, CNR2, and NR1H3 with D-Limonene have been shown to play a role in lipid metabolism and modulation of inflammatory responses. PPARA activation is known to have a renoprotective effect in AKI by modulating lipid metabolism and reducing inflammation (Comella et al., 2024). CNR2 has been associated with anti-inflammatory and anti-fibrotic responses in kidney injury models by reducing proinflammatory immune cell infiltration, decreasing oxidative stress, and inhibiting fibrosis. These interactions suggest that d-limonene may contribute to kidney protection through lipid homeostasis and anti-inflammatory pathways. NR1H3 activation has been associated with anti-inflammatory effects through the suppression of proinflammatory cytokine expression and increased cholesterol transporter expression, which may help reduce oxidative stress in injured kidneys (Deng et al., 2023; Ratnadewi, 2023; Shi et al., 2018).

3.2.Discussion

GO Function and KEGG Pathway Enrichment Analysis of Common Targets

Gene Ontology (GO) is a classification system used to group genes and proteins based on their biological functions. GO analysis consists of three main aspects: *Biological Process* (BP), *Molecular Function* (MF), and *Cellular Component* (CC) (L. Chen et al., 2017). *Kyoto Encyclopedia of Genes and Genomes)* pathway analysis was used to identify metabolic and signaling pathways associated with expressed genes (J. Du et al., 2014).

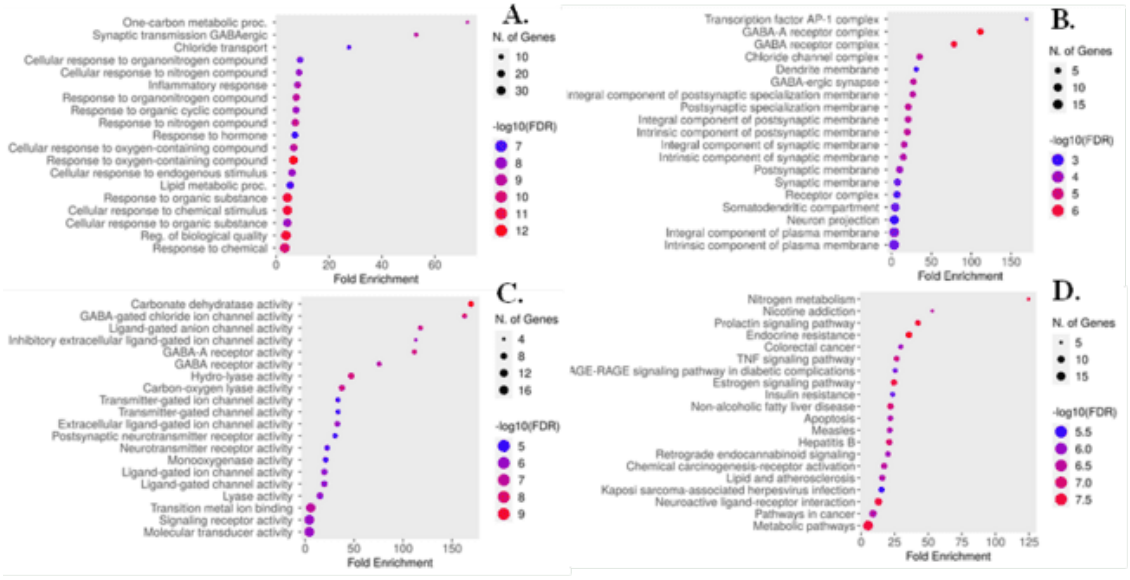


Figure 2. GO and KEGG Enrichment Analysis Bubble Diagram: Biological Processes (A), Molecular Functions (B), Cellular Components (C), and KEGG Pathways (D).

The results of GO analysis in *the Biological Process category* showed that the genes studied were involved in various biological processes related to nephroprotection and inflammation. Enriched processes include the regulation of inflammatory responses, oxidative stress, and metabolic mechanisms of active compounds that contribute to nephroprotective effects. One prominent biological process is the regulation of inflammatory signaling pathways, which reflects the role of active compounds in modulating the immune response to kidney injury (Rapa et al., 2019). In addition, exposure to certain chemical compounds such as nephrotoxic substances (ethylene glycol) can cause AKI. Genes involved in the response to chemical compounds may affect the susceptibility of the kidneys to toxins.

Molecular Function category in GO analysis revealed that the studied genes were involved in various molecular functions relevant to nephroprotective effects. The main enriched functions included antioxidant enzyme activity, specific ligand binding, and regulation of inflammatory receptor activity. The results of this analysis showed that several proteins have enzymatic activities that can help in the degradation of peroxides and free radicals. Monooxygenase enzyme activity is involved in drug metabolism and detoxification (Rendić et al., 2022). Kidney damage can affect the body's ability to metabolize and eliminate nephrotoxic drugs, which can worsen AKI. In addition, oxidative stress, which can be influenced by the binding activity of transition metal ions, is also an important risk factor in the pathogenesis of AKI because metal ions such as iron and copper can produce free radicals that damage kidney cells (Valko et al., 2016).

Cellular Component analysis revealed that the proteins involved in this study were distributed in various cellular compartments, including the cell membrane, cytoplasm,

and mitochondria. The presence of proteins in mitochondria suggests their involvement in the regulation of oxidative stress and cellular energy production, which play a role in nephroprotective mechanisms (Candas & Li, 2014). In addition, several proteins were found to be located in the cell membrane and associated with transmembrane receptors, indicating their involvement in inflammatory signaling mechanisms and the transport of active substances. The renal plasma membrane is important for the filtration and transport of molecules (Breshears & Confer, 2017). Damage to the plasma membranes of renal cells can disrupt filtration and reabsorption functions, which are key mechanisms in the pathogenesis of AKI.

KEGG analysis showed that several major pathways involved in nephroprotective effects were inflammation, oxidative stress, and apoptosis. Disturbances in nitrogen metabolism contribute to the accumulation of waste products such as urea and creatinine, which are major indicators of kidney dysfunction (Weiner et al., 2015). According to Al-Lamki & Mayadas (2015), excessive activation of *the Tumour Necrosis Factor* TNF signaling pathway triggers inflammation and apoptosis of renal tubular cells. The involvement of the *receptor for advanced glycation endproducts* (AGE-RAGE) pathway further exacerbates kidney damage, especially in patients with diabetes (Wu et al., 2021). In addition, insulin and lipid resistance, and atherosclerosis play a role in worsening kidney conditions through oxidative stress and impaired renal blood flow, causing ischemia (Podkowińska & Formanowicz, 2020) . Increased apoptosis due to ischemia, toxins, or inflammation are also major factors in AKI (Petejova et al., 2019). Exposure to nephrotoxic compounds in the chemical carcinogenesis pathway exacerbates AKI by causing direct injury to kidney cells, and metabolic pathway disturbances, such as energy imbalance and metabolic acidosis, further worsen kidney function (Barnett & Cummings, 2018).

Molecular Docking Analysis of Active Compounds and Core Targets

Molecular docking analysis was performed to evaluate the binding affinity of the active compounds contained in *Persea americana* Mill. leaves to core protein targets involved in the inflammatory pathway, especially IL-18, which is mediated by the NLRP3 inflammasome. The results of the *virtual screening process* are summarized in Table 3, which lists the dissociation constant (Kd) and binding free energy (ΔG) for each ligand-protein interaction.

Table 3. Selected ligands from *virtual screening results*

Compound	Dissociation Constant (Kd) (µM)	Binding Free Energy (ΔG) (kcal/mol)
RM5	0.3656	-10.6030
Luteolin	0.4136	- 8.6850
Apigenin	0.4126	- 8.2520
Benzyl Benzoate	0.4516	- 7.2260
D-Limonene	0.5634	- 5.6340
Eugenol	0.4465	- 5.3580

Yellow box: Natural ligand

The dissociation constant (Kd) and binding free energy (ΔG) values are important parameters for assessing the strength and stability of ligand-protein interactions. Lower Kd values indicate stronger binding affinities, whereas more negative ΔG values indicate more favorable binding interactions. Among the selected ligands, luteolin showed the lowest Kd value (0.4136 µM) and a moderately negative ΔG value (-8.6850 kcal/mol), indicating good binding affinity and stability with the NLRP3 inflammasome compared to other compounds. Luteolin and Apigenin, both natural flavonoids, showed significant binding affinities, with Kd values of 0.4136 and 0.4126 µM, respectively. The ΔG values of -8.6850 kcal/mol and -8.2520 kcal/mol further support their potential as effective inhibitors. These findings are in line with those of previous studies, highlighting the anti-inflammatory properties of flavonoids, which are known to effectively modulate inflammatory pathways.

Benzyl Benzoate, D-Limonene, and Eugenol, despite showing relatively higher Kd values, still showed moderate binding affinities. Their ΔG values suggest that they can form stable interactions with the NLRP3 inflammasome, although they are less favorable than Luteolin and Apigenin. These compounds, especially eugenol, have been reported to possess anti-inflammatory and antioxidant activities, which may contribute to their therapeutic potential. In the context of conventional drug use, it is important to consider AKI, a serious condition in which the kidneys suddenly lose filtration function. Uncontrolled inflammation, especially that involving the NLRP3 and IL-18 pathways, may contribute to the development of AKI. Therefore, the development of natural anti-inflammatory compounds such as luteolin and apigenin from *Persea americana* Mill. may be a promising alternative for reducing the risk of excessive inflammation that can lead to AKI.

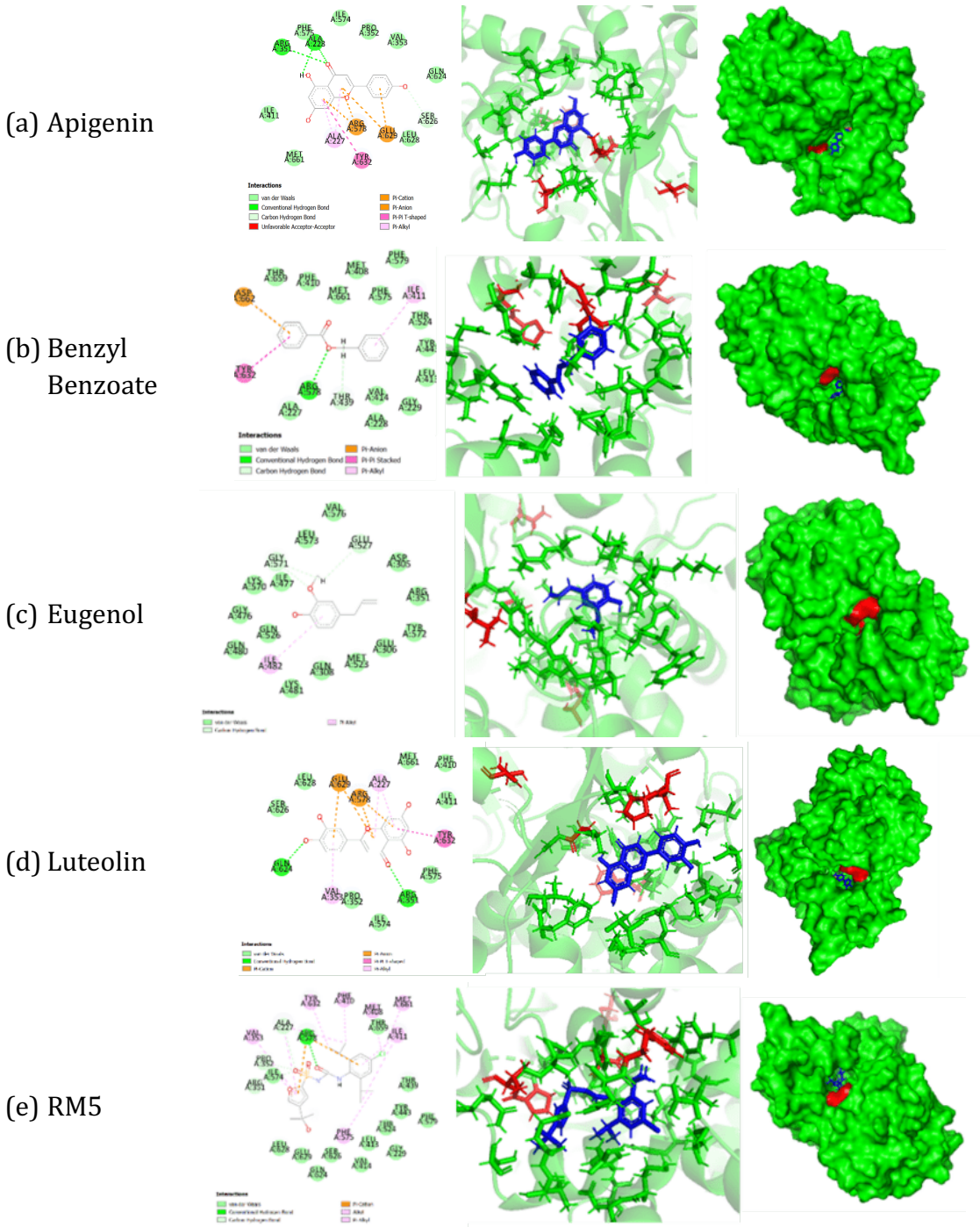


Figure 3. Interaction of Active Compounds with Core Targets of *Persea americana* Mill. Leaves via Molecular Docking

Conclusion

This study demonstrated the potential anti-inflammatory effects of *Persea americana leaves* in inhibiting IL-18 via modulation of the NLRP3 inflammasome. Bioinformatics approaches, including *molecular docking simulations* and *network pharmacology analysis*, revealed that active compounds, such as luteolin and apigenin, have strong binding

affinities to key inflammatory proteins. These findings suggest that *P. americana leaves* have potential as natural therapeutic agents for the management of *Acute Kidney Injury* (AKI) by reducing inflammation and oxidative stress. Further validation through in vitro and in vivo studies is required to confirm these computational predictions and explore their clinical applications.

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