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Integrating Chemical Profiling, In Vivo Study, and Network Pharmacology to Explore the Anti-inflammatory Effect of *Pterocarpus dalbergioides* Fruits and Its Correlation with the Major Phytoconstituents

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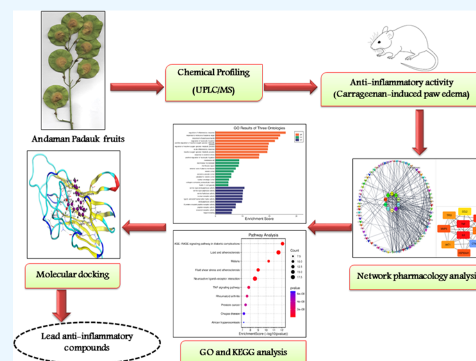
ABSTRACT: The purpose of this study is to explore the anti-inflammatory activity of *Pterocarpus dalbergioides* fruit extract (PFE) and the underlying mechanism. Chemical profiling using ultraperformance liquid chromatography/mass spectrometry identified 28 compounds in PFE (12 flavonoids, 5 fatty acids, 4 phenolic compounds, 3 alkaloids, 2 sesquiterpenes, and 2 xanthophylls). PFE (2 g/kg) significantly inhibited carrageenan-induced rat paw edema after 4 h of administration (42% inhibition). A network-based strategy and molecular docking studies were utilized to uncover the anti-inflammatory mechanism. Out of the identified compounds, 16 compounds with DL ≥ 0.18 and *F* ≥ 30% were selected using bioavailability (*F*) and drug-likeness (DL) metrics. The network analysis revealed that 90 genes are considered key targets for the selected compounds and linked to the anti-inflammatory effect. Among all compounds, linoleic acid was found to be the top-most active constituent as it targets maximum genes. Four targets (TNF, IL6, AKT1, and CCL2) among the top 10 genes were found to be the main target genes that may contribute to the anti-inflammatory potential of PFE. Furthermore, KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis revealed that PFE might regulate inflammation through five pathways: neuroactive ligand−receptor interaction, lipid and atherosclerosis, fluid shear stress and atherosclerosis, TNF signaling pathway, and rheumatoid arthritis. The docking study predicted the significant binding affinity between the top four active constituents (linoleic acid, 9-octadecenoic acid, 11,12,13-trihydroxy-9-octadecenoic acid, and rhamnetin-3-*O*-rhamnoside) and the selected target proteins (TNF and AKT1). The findings highlight PFE as a promising drug lead for controlling inflammation.



1. INTRODUCTION

Inflammation is the immune system's response to a stimulant. The immune system is activated when the body is subjected to pathogens (bacteria, viruses, or poisonous substances) or suffers injury. Inflammatory cells and cytokines are the initial responses released by your immune system. These kinds of cells produce an inflammatory response to engulf pathogens or to begin the healing process.[1] Chronic state of inflammation seems to be linked to many health problems, especially cardiovascular diseases, bronchial asthma, arthritis, diabetes mellitus, Alzheimer's disease, and cancer.[2] The most popular synthetic medications for treating inflammation are nonsteroidal anti-inflammatory drugs and corticosteroids. Unfortunately, these drugs have well-known side effects that affect the hepatic, renal, cardiovascular, and hematologic systems alongside the gastric mucosa.[3,4] As a result, the discovery of new anti-inflammatory drugs is of interest. Plant secondary metabolites are a valuable basis for the discovery of new anti-inflammatory medicines.[5] Many phytoconstituents, including quercetin, epigallocatechin-3-gallate, curcumin, capsaicin, colchicine, and resveratrol are now in clinical usage as anti-inflammatory treatments.[6] The number of new anti-inflammatory herbal ingredients rises continuously. The main challenge in this area is converting preclinical information into evidence-based clinical advancement.

Pterocarpus (Fabaceae) comprises around 46 species found in Asia, Africa, and many American countries. Plants in this genus

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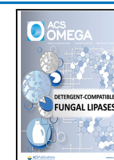

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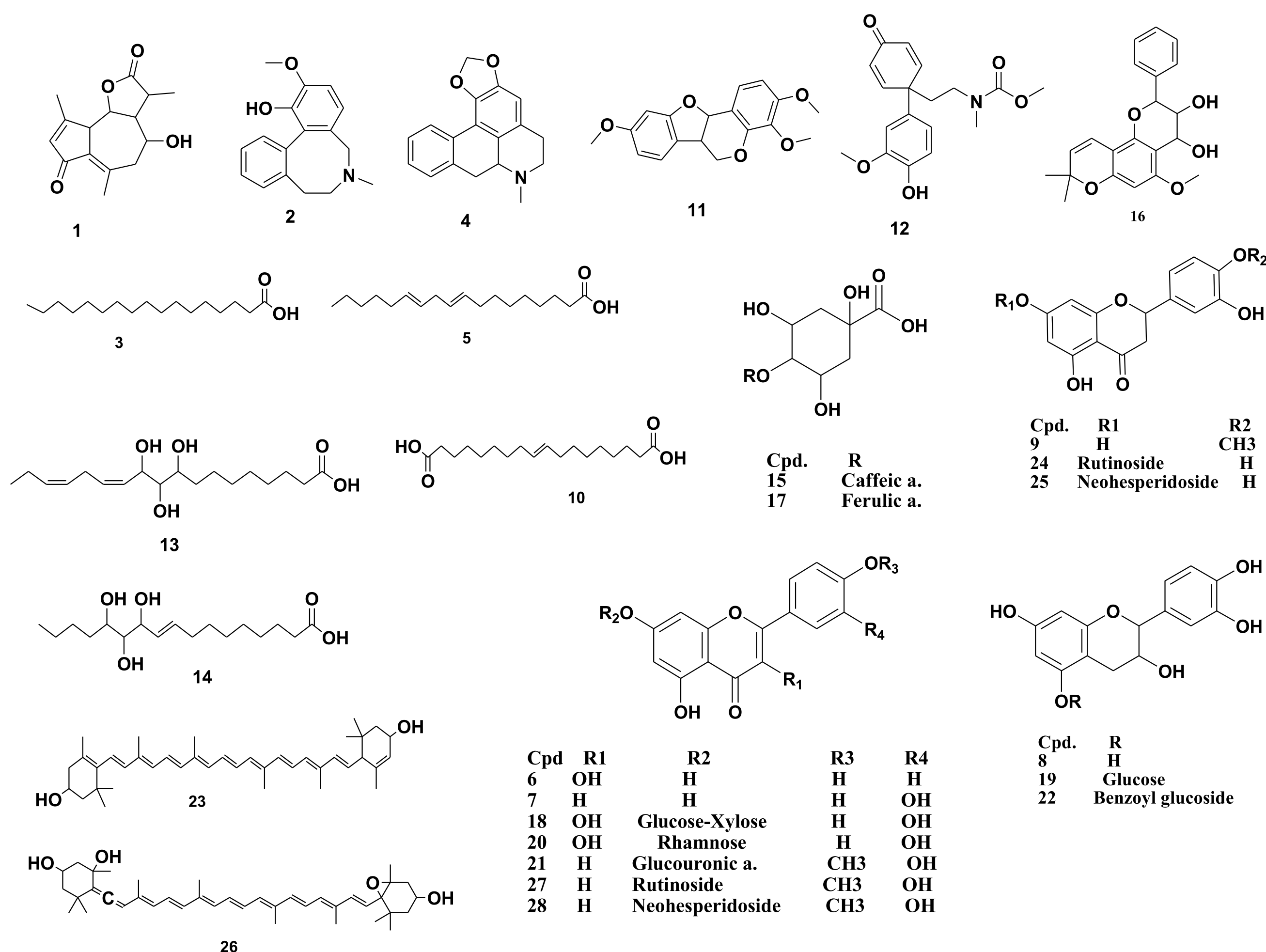

Figure 1. Chemical structures of phytochemicals in PFE identified by UPLC/MS.

have been used in traditional medicine to treat inflammatory conditions, mouth ulcers, boils, gonorrhea, infection, cough, diarrhea, and as an analgesic.[7] Andaman padauk (*Pterocarpus dalbergioides*) is a medium to large evergreen tree belonging to Fabaceae family. It is indigenous to the Andaman Islands in the Indian Ocean. Sometimes it is grown in Nicobar and East Madagascar.[8,9] The fruit is a lomentum, disc-shaped, flattened, and has a broadly winged margin, giving it a "flying saucer" appearance. The unripe fruits are light green, pubescent, and turn coffee brown when they dry. They have a central woody part containing one seed.[10] About the plant phytoconstituents, Seshadri[11] identified 3′-hydroxy formononetin and maackiain in the heartwood. Genistin, gentisic acid, and gallic acid were isolated from the plant's bark.[12] Alcoholic extracts of flowers,[9] bark,[12] and wood[13] demonstrated anti-diabetic effect. Numerous studies have been conducted on the anti-inflammatory activities of phytochemicals found in the genus *Pterocarpus*.[14−19] These findings are consistent with the plant's traditional use in the treatment of inflammation and boils.[8] Only one report was found about the anti-inflammatory activity of the bark;[12] however, nothing has been reported about the biological activity and compounds of Andaman padauk fruits.

Ultra-performance liquid chromatography (UPLC) is a relatively recent method that offers new opportunities for reducing the analysis time and solvent use. The UPLC combined mass spectrometry (MS) approach has been proven to provide satisfactory resolution of complex samples, along with improved separation capacity and mass information. UPLC/MS can provide MS information for each constituent in a chromatogram and has been demonstrated to be a valuable tool for qualitative and quantitative assessment of components in botanical extracts and medicinal herbs.[20,21]

Network pharmacology is an emerging technique for understanding multicomponent drugs' mechanism of action. Its basic idea is to maximize treatment options based on a biological network comprising disease characteristics, bioactive agents, and therapeutic targets that are all linked.[22] Bioinformatics and systems biology advances are making network pharmacology a viable approach for identifying active compounds and elucidating the underlying pharmacological mechanisms. This approach can reliably identify the molecular pathways influenced by the active chemicals in the drug. This is accomplished by integrating chemical component identification, target prediction, and network construction. Therefore, network pharmacology is an effective method for examining the mechanism of action of drugs containing multiple active ingredients.[23] Molecular docking studies employ computer simulations to investigate the interactions between receptors and compounds that bind to them. Being able to predict specific properties, for instance, the location and affinity of chemical interactions, makes it a powerful tool in drug design and chemical screening.[24]

The present study aimed to explore the anti-inflammatory activity of *P. dalbergioides* fruit extract (PFE) and the underlying

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mechanism. First, we profiled the metabolite of Andaman padauk fruits using UPLC/MS. Then, the extract was in vivo investigated for the anti-inflammatory efficacy via a carrageenan-induced rat paw edema model. Both the network-based strategy and molecular docking study were utilized to elucidate its anti-inflammatory mechanism.

2. RESULTS AND DISCUSSION

2.1. Chemical Profiling of PFE. Natural extracts generally contain a large number of metabolites. The bioactivity of the extracts is represented by the synergism between multiple components of these metabolites. Since the isolation of each compound from extracts is not usually possible because of the complex chemical makeup of crude natural extracts,[25] secondary metabolites that may act as intermediates in the anti-inflammatory effect of PFE were identified using UPLC coupled with MS. The majority of the identified compounds were detected in the negative mode. As depicted in Table S1, 28 compounds were identified[26−51] in PFE, including 12 flavonoids, 5 fatty acids, 4 phenolic compounds, 3 alkaloids, 2 sesquiterpenes, and 2 xanthophylls (Figure 1). The chemical profile of *P. dalbergioides* fruits has not been thoroughly described in the literature; however, flavonoids are very distinct compounds in the genus *Pterocarpus*. It is reported that flavonoids are the dominant plant constituents that significantly influence *Pterocarpus*'s biological activity.[8] It is worth mentioning that this is the first in-depth investigation into the chemical profile of *P. dalbergioides* fruits.

2.2. Effect of PFE on Carrageenan-Induced Rat Paw Edema. Several anti-inflammatory drugs have been tested using carrageenan-induced rat paw edema as a model of inflammation. The therapeutic impact of *P. dalbergioides* on inflammation was assessed in the carrageenan-induced rat paw edema model. Rats that received PFE (2 g/kg) significantly inhibited the paw edema (Table 1) after 4 h of administration (42% inhibition) compared to the standard drug indomethacin at a dose of 0.2 g/kg (64.8% inhibition). Carrageenan injection causes acute and local edema. Histamine, serotonin, and bradykinin are the initial mediators involved in the early phase (0−1 h). However, prostaglandins and different cytokines such as interleukins and tumor necrosis factor (TNF) are active in the second phase. PFE's anti-inflammatory activity may be attributed to inhibition of such inflammatory mediators.[52]

Table 1. Effect of PFE on Paw Edema Induced by Carrageenan in Rats[a]

groups	% edema	% inhibition
control (saline)	61.9 ± 2.1	
PFE	35.9 ± 1.1	42
indomethacin	21.8 ± 0.4	64.8

[a]All data are presented as (mean ± S.E.).

2.3. Screening of Targets and Active Compounds. To unravel the anti-inflammatory mechanism of PFE, we utilized a network pharmacology strategy to predict the active compounds in PFE and their possible targets.[53] First, *F*30, which represents 30% bioavailability (the quantity of drug that enters the systemic circulation after being absorbed) of compounds, and drug-likeness (DL), which is the measure of tendency of any compound to behave like a drug, were evaluated. Out of the identified compounds, 16 compounds with DL ≥ 0.18 and *F* ≥ 30% were selected for further analysis. A total of 751 target genes were identified for selected 16 compounds by using the SwissTargetPrediction database. DisGeNET database was retrieved for inflammation-related target genes, and a total of 467 target genes were obtained. After that, intersecting genes between inflammation and selected compounds were predicted. Ninety overlapping genes that may contribute to the anti-inflammatory effect of *Pterocarpus dalbergoides* were selected and were deemed to be the key targets for further analysis. The selected compounds along with their properties are presented in Table 2.

Table 2. Selected Active Compounds and Their DL and *F* Properties

compound no	name of compound	DL	bioavailability
3	heptadecanoic acid	0.37	0.99
5	linoleic acid	0.31	0.84
6	kaempferol	0.54	0.99
7	luteolin	0.51	1
9	hesperetin	0.78	0.98
10	9-octadecenedioic acid	0.30	0.97
12	4-{2-(acetylmethylamino)ethyl}-4-(4-hydroxy-3-methoxyphenyl)-2,4-cyclohexadien-1-one	0.89	0.90
13	9,10,11-trihydroxy-12,15-octadecadienoic acid	0.29	1
14	11,12,13-trihydroxy-9-octadecenoic acid	0.29	0.99
15	5-caffeoylquinic acid (chlorogenic acid)	0.23	0.99
17	5-feruloylquinic acid	0.34	0.99
18	quercetin-7-glycosides; 7-*O*-*β*-D-xylopyranoside	0.27	0.99
19	catechin-5-*O*-glucoside	0.26	1
20	rhamnetin-3-*O*-rhamnoside	0.30	0.96
21	diosmetin-7-*O*-glucuronide	0.29	0.99
28	diosmetin-7-*O*-rutinoside	0.18	0.99

2.4. Construction of a Compound-Target Network. Network analysis is an effective approach for improving our understanding of multicomponent drugs' action mechanism. Sixteen selected compounds along with their targets were imported to Cytoscape for generating a compound-target network. Each of the selected compounds corresponds to multiple targets. The efficacy of these 16 compounds was then assessed in the compound-targeted gene pathway network (Table 3). As shown in Table 3, fatty acids, phenolic acids, flavonoids, and alkaloids had high connectivity levels. Among all compounds, linoleic acid was found to be the top-most active constituent as it targets maximum genes among all other compounds.

Previous research has shown that some PFE's identified constituents have anti-inflammatory properties. Linoleic acid inhibited NO production in RAW264.7 cells and suppressed the release of pro-inflammatory cytokines (TNF-*α*, IL-6, IL-1*β*, and NOS2). Additionally, it restored PPAR*α* and reduced NF-B-p50 expression.[51] Octadecenedioic acid is a mono-unsaturated dioic acid (C18:1). It demonstrated anti-inflammatory properties via binding to the peroxisome proliferator-activated receptor (PPAR).[54,55] Rhamnetin (*O*-methylated flavonoid) significantly inhibited edema formation in rat paws.[56] In another study, it suppressed mTNF-*α*, mMIP-1, mMIP-2 cytokine, and NO production in LPS-stimulated RAW264.7 by acting on the p38-MAPK, ERK, c-JNK, and COX-2 pathways.[58]

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ACS Omega 2023, 8, 32544−32554

Table 3. Degree Value of Selected Phytoconstituents

phytoconstituents	degree value	class
heptadecanoic acid	11	fatty acid
linoleic acid	22	fatty acid
kaempferol	17	flavonoid
luteolin	17	flavonoid
hesperetin	12	flavonoid
9-octadecenedioic acid	19	fatty acid
4-{2-(acetylmethylamino)ethyl}-4-(4-hydroxy-3-methoxyphenyl)-2,4-cyclohexadien-1-one	14	alkaloid
9,10,11-trihydroxy-12,15-octadecadienoic acid	13	fatty acid
11,12,13-trihydroxy-9-octadecenoic acid	19	fatty acid
5-caffeoylquinic acid (chlorogenic acid)	15	phenolic acid
5-feruloylquinic acid	17	phenolic acid
quercetin-7-glycosides; 7-*O*-*β*-D-xylopyranoside	14	flavonoid
catechin-5-*O*-glucoside	15	flavonoid
rhamnetin-3-*O*-rhamnoside	18	flavonoid
diosmetin-7-*O*-glucuronide	16	flavonoid
diosmetin-7-*O*-rutinoside	15	flavonoid

2.5. Construction of a Protein–Protein Interaction Network. A protein–protein interaction (PPI) network (Figure 2) was generated by submitting 90 intersecting genes to the STRING database. The PPI network represents the interrelationship between different disease targets during disease development and progression. The more the degree value of any gene, the more evident it will be in disease pathogenesis. The cytoHubba plugin tool was then used to analyze the network, and top ten genes were selected based on their degree value. According to results of analysis TNF (28), IL6 (28), EGFR (24), MMP9 (23), HSP90AA1 (23), VEGFA (22), TP53 (20), AKT1 (20), CTNNB1 (18), and CCL2 (17) were found to be the top ten target genes. Comparison of PPI analysis with enrichment analysis reveals that among the top 10 genes TNF, IL6, AKT1, and CCL2 were found to be the main target genes that may contribute to the anti-inflammatory potential of PFE. Moreover, TNF and AKT1 were further analyzed for molecular docking between selected target genes and PFE compounds.

2.6. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Pathway Analyses. PFE's probable biological functions were uncovered by functional annotation and enrichment analyses. Gene ontology (GO) functional analysis indicated that the selected compounds were correlated to regulation of inflammatory response, response to bacterial molecule, response to lipopolysaccharide and so forth (Figure 3). Afterward, the Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis was carried out to identify the major signaling pathways associated with PFE anti-inflammatory activity. Notably, PFE might regulate inflammation through the following pathways: neuroactive ligand–receptor interaction (19), lipid and atherosclerosis (18), fluid shear stress and atherosclerosis (13), TNF signaling pathway (11), and

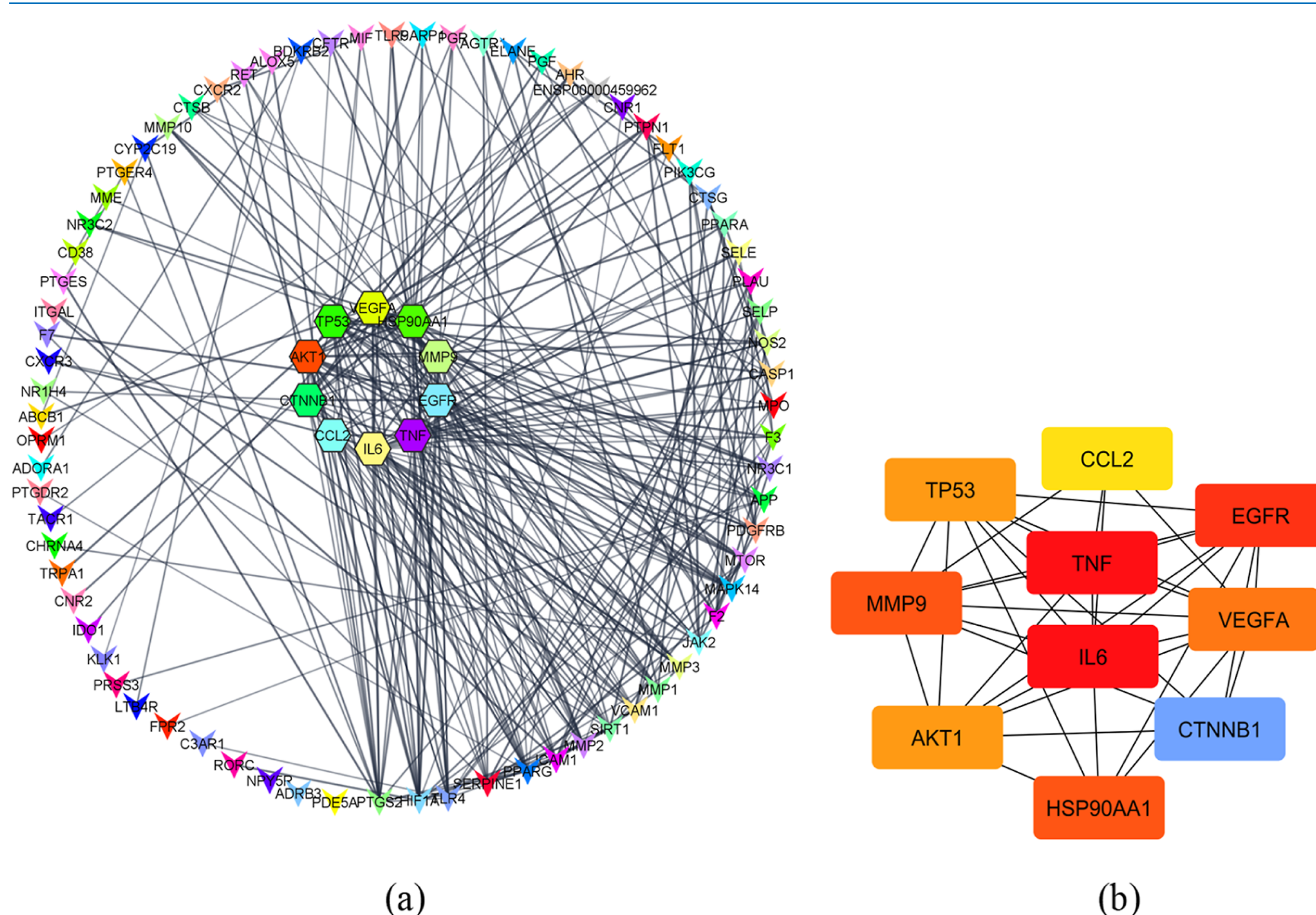

Figure 2. (a) PPI network of 90 overlapping genes and (b) top ten genes with respect to the degree value.

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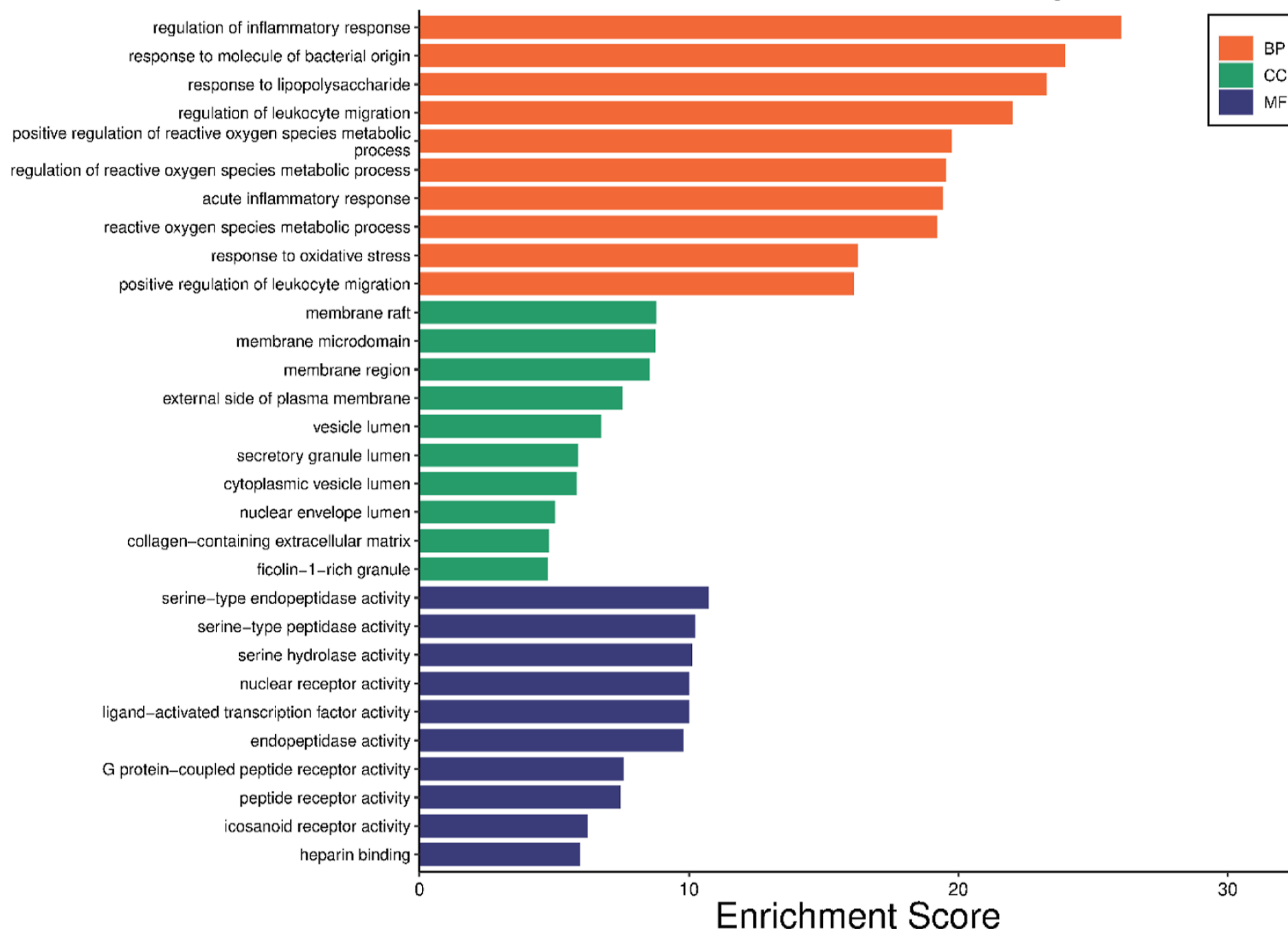

Figure 3. GO enrichment analysis of 90 intersecting genes where orange color represents biological processes (BP), green color represents cellular components (CC), and blue color represents molecular functions (MF).

rheumatoid arthritis (10) as the majority of the genes were connected to these pathways (Figure 4).

2.7. Molecular Docking. In order to further verify the validity of the target prediction results, key molecules were selected based on their importance within the component-target-pathway network. The top four active constituents (linoleic acid, 9-octadecenedioic acid, 11,12,13-trihydroxy-9-octadecenoic acid, and rhamnetin-3-*O*-rhamnoside) were docked with selected target genes TNF and AKT1. TNF plays an important role in regulating inflammatory responses. When TNF enters the vascular system, endothelial cells undergo a number of pro-inflammatory changes, leading to increased leukocyte adhesion, transendothelial migration, and vascular leakage causing edema.[56] AKT1 plays an important role in acute inflammation and histamine-mediated vascular leakage. In mice lacking AKT1, edema was markedly reduced, and neutrophil infiltration and monocyte proliferation were dramatically decreased.[57] In addition, mice lacking AKT1 showed dramatic reductions in carrageenan-induced edema and bradykinin and histamine directed permeability actions.[59]

Target protein's TNF (PDB ID: 2AZ5) and AKT1 (PDB ID: 3QKK) crystal structures were extracted from PDB. The docking study predicted the significant binding affinity between the compounds and the binding pockets of two target proteins. Based on these findings, we can further verify predicted interactions between PFE components and their targets. Binding energy and docking scores were used as important criteria for compounds' screening (Table S2). 2D and 3D binding mode analyses for selected compounds are provided in Figure 5. These results provide additional support for the anti-inflammatory properties of PFE.

3. CONCLUSIONS

Inflammation is linked to many health problems, such as heart diseases, asthma, arthritis, diabetes, Alzheimer's disease, and cancer. To summarize, an integrated approach, including chemical profiling, network pharmacology, molecular docking, and in vivo experimental validation, was used to explore the anti-inflammatory mechanism of PFE. UPLC/MS identified 28 compounds, 16 of which were selected based on *F* and DL parameters. According to the network analysis, 90 genes are considered key targets for the selected compounds and are linked to their anti-inflammatory effect. Among all compounds, linoleic acid was found to be its most active constituent since it targets most genes. Among the top ten genes, four targets (TNF, IL6, AKT1, and CCL2) were identified as the main target genes that may contribute to PFE's anti-inflammatory potential. Furthermore, KEGG pathway analysis suggested that PFE may regulate inflammation via five pathways: neuroactive ligand−receptor interaction, lipid and atherosclerosis, fluid

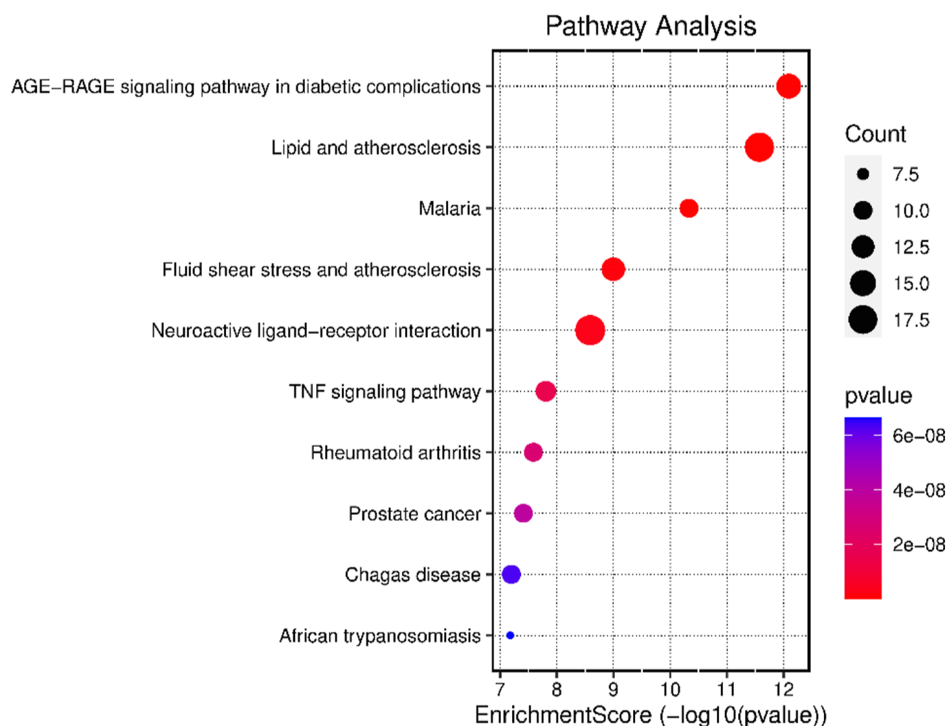

Figure 4. KEGG pathway analysis of PFE-targeting genes.

shear stress and atherosclerosis, TNF signaling pathway, and rheumatoid arthritis. The docking study predicted the significant binding affinity between the top four active constituents (linoleic acid, 9-octadecenoic acid, 11,12,13-trihydroxy-9-octadecenoic acid, and rhamnetin-3-*O*-rhamnoside) and the selected target proteins (TNF and AKT1). The findings highlight PFE as a promising drug lead for controlling inflammation. Interestingly, rat paw edema induced by carrageenan was significantly inhibited by PFE in an in vivo experiment. This study's findings could pave the way for more research into the molecular mechanism of PFE as an anti-inflammatory medication. It could potentially open the way for future clinical trials.

4. MATERIALS AND METHODS

4.1. Plant Material. Fruits of *P. dalbergioides* were collected in April 2022 from El-Orman Botanical Garden, Giza, Egypt. Mrs. Therese Labib, Head of Taxonomists at El-Orman Botanic Garden, confirmed the plant material's identification. The voucher specimen (no. 5.4.2022) was stored in the herbarium of the Department of Pharmacognosy, College of Pharmacy, Jouf University.

4.2. Preparation of the Fruit Extract. Fresh fruits (500 g) were extracted with methanol (5 × 500 mL) until exhaustion using an Ultra-Turrax homogenizer. This was followed by evaporation under vacuum (Buchi-210, Switzerland) to obtain the dried residue (50 g), which was then stored at 4 °C for future analysis.

4.3. Chemicals and Reagents. UPLC/MS analysis: HPLC grade methanol, water, and acetonitrile were purchased from Thermo Fisher Scientific Inc., Dublin, Ireland. Other chemicals of analytical grade used in the current study were purchased from Sigma-Aldrich Chemical Co. (Ireland). Anti-inflammatory investigation: indomethacin was obtained from EIPICO, Egyptian Pharmaceutical Company under license from Merck and carrageenan was purchased from Sigma Company, USA.

4.4. UPLC/MS Analysis. Metabolite profiling of PFE was carried out using a Waters Acquity UPLC system (Waters, Manchester, U.K.) coupled with an Orbitrap-type HRMS system (Exactive, Thermo Fisher, Bremen, Germany) in accordance with a previously reported method.[25] Metabolites were identified by comparing the retention time and MS data with those in the current literature and using the "Dictionary of Natural Products", CRC database.

4.5. Animals. Male albino Sprague−Dawley rats (130−150 g) were obtained from the National Research Centre's animal house colony in Giza, Cairo. The same hygienic conditions and normal laboratory diet were used for all of the animals' care. Animal experiments were conducted following the guidelines of the Animal Ethics Committee of the National Research Centre.

4.6. Carrageenan-Induced Rat Paw Edema Test. Acute anti-inflammatory activity was assessed using a rat paw edema model, with edema triggered by carrageenan.[60] Rats were divided into three groups (n = 6). The first group (control) received 1 mL saline and the second group (standard) received indomethacin (0.2 g/kg) orally. The third group received PFE (2 g/kg) orally. One hour later, all animals received 0.1 mL of 1% carrageenan/saline by subcutaneous injection into the right paw and 0.1 mL of saline in the left paw. Animals were scarified 4 h after oral administration, and the weight of edema was determined by weighing each of the two paws individually.

$$\%\ \text{edema} = \frac{\text{right paw weight} - \text{left paw weight}}{\text{left paw weight}} \times 100$$

$$\%\ \text{inhibition} = \frac{\text{edema in control rats} - \text{edema in drug} - \text{treated rats}}{\text{edema in control rats}} \times 100$$

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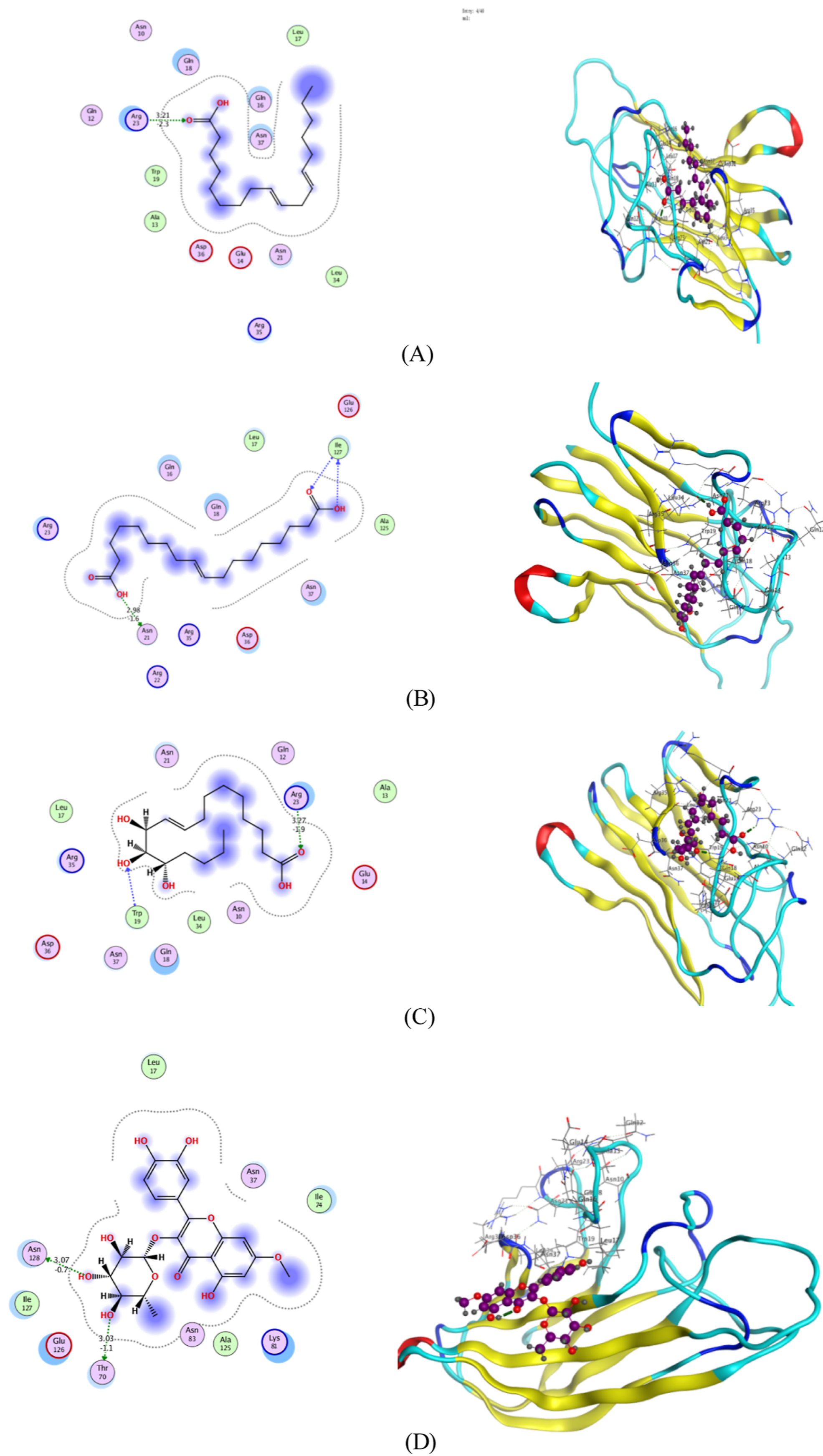

Figure 5. continued

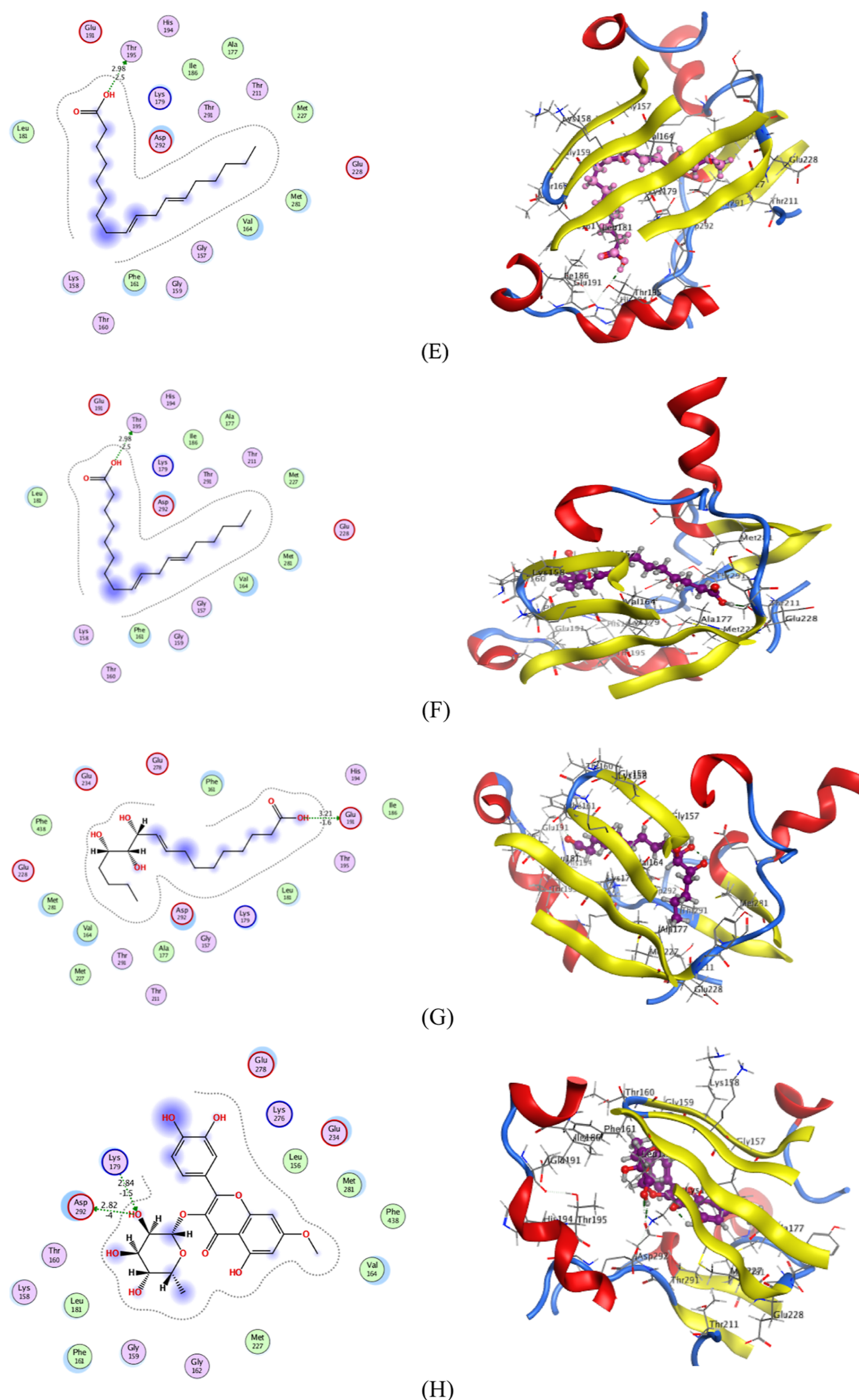

Figure 5. 2D and 3D binding mode analyses of top four active constituents with TNF and AKT1. (A) Linoleic acid−TNF complex, (B) 9-octadecenedioic acid−TNF complex, (C) 11,12,13-trihydroxy-9-octadecenoic acid−TNF complex, (D) rhamnetin-3-*O*-rhamnoside−TNF complex, (E) linoleic acid−AKT1 complex, (F) 9-octadecenedioic acid−AKT1 complex, (G) 11,12,13-trihydroxy-9-octadecenoic acid−AKT1 complex, and (H) rhamnetin-3-*O*-rhamnoside−AKT1 complex.

4.7. Selection of Compounds. The bioactive components of PFE were computationally evaluated using *F* and DL metrics, which are important in drug absorption, distribution, metabolism, and excretion (ADME) features. Compounds were only retained if DL $\geq$ 0.18 and $F \geq 30\%$ met ADME requirements. In this context, the *F*30% and DL of all chemical compounds were

determined using SwissADME (http://www.swissadme.ch/)and ADMETlab (https://admetmesh.scbdd.com/).

4.8. Inflammation-Related Target Genes and Selected Compounds. Binding DB retrieved on December 15 2022 (https://www.bindingdb.org/bind/index.jsp) was used to estimate target genes of selected compounds based on SMILES via the "homo sapiens" setting. During Binding DB prediction, the "minimum needed interaction score" was selected to "high confidence (0.700)". The public DisGeNET database (http://www.disgenet.org/) was utilized to identify inflammation-related target genes on December 20, 2022.

4.9. Network Construction: Compounds–Overlapping Genes Interactions. Cytoscape 3.9.1 (accessed 19 December 2022, https://cytoscape.org/) was utilized to build, show, and explore the network interactions of Binding DB prediction outcomes for compounds and intersecting genes.[61] Nodes in the network represent biologically active compounds and genes, whereas edges represent relations between compounds and genes. PEF anti-inflammatory components and hub genes were determined by assessing the network's topological construction and adjusting the "degree value" of compounds or genes, respectively.[62] The degree value of a compound or gene reflects how many phytoconstituents or genes the network contain. The anti-inflammatory impact of PFE improved when the compound targeted more disease-inducing genes.

4.10. Constructing a PPI Network. The information on PPIs between the targets of selected PEF components was collected by STRING, version 11.5. The website assigned a score to mutual information of each protein. The more the interaction between the two target proteins, the higher the score. The study was rated reliable because high-confidence data >0.7 were used. The Cytoscape 3.9.1 program was used to create a protein interaction network using the provided protein interaction data. The cytoHubba plug-in was used to identify hub genes.[63]

4.11. GO and KEGG Enrichment Analyses of Target Proteins. Using DAVID v 6.8 software, the identified target genes were evaluated for GO and KEGG analyses. GO analysis was performed on cellular components (CC), molecular functions (MF), and biological processes (BP). To predict the possible anti-inflammatory molecular mechanism of PEF, a KEGG pathway enrichment analysis was performed. SRPLOT (accessed on 20 November 2022, http://bioinformatics.com.cn/) was used to create GO and KEGG pathway bar charts.[64]

4.12. Molecular Docking. The molecular docking tool used was MOE 2015 (Molecular Operating Environment). TNF and AKT1 crystal structures were available in the protein data bank (PDB ID: 2AZ5, 3QKK). The structures of linoleic acid, 9-octadecenedioic acid, 11,12,13-trihydroxy-9-octadecenoic acid, and rhamnetin-3-*O*-rhamnoside were all developed with MOE's builder tool. The energy minimization method of the MOE program was used to minimize the energy of the protein molecule via the following variables: current geometry chiral constraint, 0.05 gradient, and MMFF94X + solvation force field. Energy minimization was halted once the root-mean-square gradient >0.05. When the active site was determined, ten different docked conformations for each compound were generated. To examine binding patterns, the compounds' lowest energy conformation was chosen. The docking template was based on the minimized protein structure.[64]

4.13. Statistical Analysis. Data were reported as mean ± standard error. The significance of difference was established by Student's *t*-test. Values of *P* less than 0.01 were considered significant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02940.

UPLC/MS dereplicated compounds in *PFE*, molecular docking study results of TNF (PDB ID: 2AZ5) and AKT1 (PDB ID: 3QKK) by various compounds, and total ion chromatogram of *PFE* in the negative ionization mode and positive ionization mode (PDF)

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Notes

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