Journal of Applied Pharmaceutical Science Vol. 15(04), pp 162-178, April, 2025
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2025.217871
ISSN 2231-3354

LC-MS/MS profiling and *in silico* molecular docking analysis of water-soluble bioactive compounds from *Pleurotus pulmonarius* as potential immunomodulators on monocyte immune response

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ARTICLE HISTORY

Received on: 30/08/2024
Accepted on: 04/12/2024
Available Online: 05/03/2025

Key words:
Edible mushroom, monocyte, dihydroresveratrol, anti-inflammatory activity, molecular docking, LC-MS/MS.

ABSTRACT

Pleurotus pulmonarius is an affordable edible fungus extensively utilized in food and traditional medicine. This study aims to comprehensively profile the water-soluble bioactive compounds in *P. pulmonarius* and identify those responsible for modulating monocyte immune response. The crude extract, prepared through hot water extraction, was analyzed for its biological activities using untargeted LC/MS-MS analysis and molecular docking. The potential compound-targeted proteins in monocytes were investigated and further identified using the MCODE algorithm. The pharmacokinetic properties and the biological activity of tentative compounds were studied using absorption, distribution, metabolism, excretion, and toxicity (ADMET) and PASS analysis. The findings revealed that 164 chromatographic peaks were detected in ESI-positive mode, and 36 bioactive compounds were proposed. The top seven candidate compounds found abundantly in the mushroom were selected, including diacylglycerol, phosphatidylethanolamine, phosphatidylinositol, glutathione, quercetin 3-(6-O-acetyl-beta-glucoside), dihydroresveratrol, and aspartic acid. Notably, glutathione, quercetin, and dihydroresveratrol demonstrated promising potential proinflammatory inhibitors, with their binding affinities surpassing those of known inhibitors (−2.32 kcal/mol for glutathione; −6.76 kcal/mol for quercetin and −5.02 to −7.02 kcal/mol for dihydroresveratrol). Additionally, dihydroresveratrol showed promise as an immunomodulatory agent due to its favorable absorption, distribution, and safety profile. These findings highlight the potential of *P. Pulmonarius,* particularly dihydroresveratrol as a natural alternative immunomodulator for addressing monocyte-related inflammation.

INTRODUCTION

Monocytes are pivotal components of the innate immune system. They are crucial in combating infections, controlling inflammation, and tissue repair and remodeling via inflammatory and anti-inflammatory responses. The dysregulation of monocytes is characterized by increased proinflammatory phenotypes. This could potentially initiate or exacerbate immunopathological conditions such as allergies, autoimmune diseases, chronic inflammation, and immunosenescence in elderly individuals [1,2]. Recently, the concept of trained immunity has been introduced which involves the epigenetic changes and metabolic reprogramming of the innate immune cells including monocytes. This adaptive-like response of the innate immune system enhances its reaction to subsequent infections or stressors after an initial encounter [3]. Studies on trained immunity have implications for developing vaccines and therapies, as enhancing innate immune memory might offer improved defenses against infectious diseases and immune modulation in chronic inflammatory conditions [4].

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Therefore, the strategies to modulate the balance of monocyte immune response are challenging.

A healthy diet or functional food has gained attention for its impact on modulating the human immune system. Functional foods contain antioxidants, fiber, prebiotics, or phytochemicals that can prevent diseases or improve overall health. Growing evidence reveals that bioactive components in such foods influence the molecular regulatory mechanisms such as epigenetics involved in gene expression [5,6]. Mushrooms, one of the functional foods, are now recognized as a source of nutraceuticals and have been revered in traditional medicine for centuries [7]. Extensive research has unequivocally shown the remarkable health benefits of edible mushrooms for their anti-cancer, antioxidant, anti-microbial, and immunomodulatory effects. However, these advantages have been demonstrated in some mushroom species, whereas most mushrooms are still unknown [8,9]. Furthermore, the insight mechanisms that lead to the activity have not been thoroughly explored. Several pieces of evidence demonstrated that different mushroom strains, species, and extraction methods affect bioavailability and bioactivity [10–12]. Thus, an investigation into commonly available and affordable mushroom species, alongside an analysis of their nutritional components through extraction methods mirroring traditional culinary techniques, could yield accurate data that more authentically reflects the transformations occurring in real cooking processes.

Pleurotus pulmonarius (*P. pulmonarius*), the Indian oyster, is an easily cultivated and affordable mushroom that is widely consumed worldwide and has significant economic value. *P. pulmonarius* is high in dietary fiber and rich in bioactive compounds such as polysaccharide components, proteins, vitamins, and minerals. Literature studies have reported the anticancer, antioxidant, and immunomodulatory effects of *P. pulmonarius* extracts [12]. The studies on the immunomodulatory activities of *P. pulmonarius* are limited and mainly focused on the effect of bioactive compounds extracted from methanol or organic solvent [12–15]. The specific bioactive compounds extracted from hot water that are responsible for immunomodulatory activities on monocytes and the pharmacological network have not been thoroughly explored.

This study is the first to demonstrate the profile of water-soluble compounds in *P. pulmonarius*. *Pleurotus pulmonarius* was extracted using hot water to simulate traditional food and alternative medicine use. A comprehensive analysis using untargeted liquid chromatography with tandem mass spectrometry (LC-MS/MS) was performed to identify bioactive compounds in *P. pulmonarius.* Genes associated with monocyte immune responses, metabolic control, and epigenetic regulation were gathered and the tentative compound-target proteins were identified. Furthermore, the interactions between these bioactive compounds and monocyte-targeted proteins were investigated using an *in silico* molecular docking approach. Additionally, the pharmacokinetic properties and the biological activity of potential compounds were evaluated using ADMET and PASS analysis, respectively. Overall, this study provides valuable insights into the immunomodulatory benefits of *P. pulmonarius*, especially in monocyte function, and highlights specific compounds for further investigation into potential preventive or therapeutic applications in inflammation-related diseases.

MATERIALS AND METHODS

Pleurotus pulmonarius crude extract preparation

The mushroom was identified as a Voucher specimen: MZ395974. The extraction of *P. pulmonarius* was performed using hot water followed by ethanol precipitation [16,17]. Initially, the dried mushrooms were ground into a fine powder. Subsequently, 100 mg of this powder was extracted with 1,000 ml of distilled water at a ratio of 1:10 (w/v) at a temperature of 95°C ± 5°C for 1 hour. The mixture was then meticulously filtered through 11 µm of filter paper (Whatman, England), allowing the mushroom residue to undergo two additional extraction rounds, with the supernatant being collected each time. After this, the supernatant was further filtered through 0.2 µm of SFCA syringe filter (Corning, USA) and precipitated for 18 hours in 80% ethanol (v/v) at 4°C. The mixture was then centrifuged for 10 minutes at 4,500 rpm and 4°C to facilitate component separation. After removing the supernatant, the residue was washed with absolute ethanol and incubated at 70°C to ensure complete evaporation of the ethanol.

Component analysis by liquid chromatography with tandem mass spectrometry

To find bioactive molecules comprised in *P. pulmonarius,* a crude extract was submitted to the Institute of Systems Biology (*Universiti Kebangsaan*, Malaysia). The untargeted compound was analyzed using LC-MS/MS. In brief, 20 ul of *P. pulmonarius* solution was injected. Then the gradient elution was performed through a Thermo Scientific C18 column operating on an UltiMate 3000 UHPLC system (Dionex), with a 22-minute total run time. Using ESI positive ionization, high-resolution mass spectrometry was performed through MicroTOF QIII (Bruker Daltonic, USA). M/Z was analyzed using Compass Data Analysis software (Bruker Daltonic, USA). Subsequently, *in silico* MS/MS fragmentation was performed, and the compounds were identified by comparing M/Z with the theoretical masses accessible through the public database.

In silico studies of potential compound-targeted proteins on monocyte immune response

Compound-protein target interaction and GO and Reactome analyses

To identify potential human protein targets for the proposed bioactive molecules from *P. pulmonarius*. The proposed bioactive compounds were ranked in ascending order based on their Area (%) values. The top seven compounds have been selected as potential immunomodulators based on their area percentage and being identified in mushrooms. Then, the compounds' simplified molecular input line entry system (SMILES) was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and imported into the SwissTargetPrediction web tool (http://www.swisstargetprediction.ch/). The targets of proposed bioactive compounds acquired from

SwissTargetPrediction with a probability score greater than 0.04 were chosen as potential targets in this study. Those compounds without target information were excluded. [18].

Next, the GeneCards database (https://www.genecards.org/) was used to select the targets that have been reported to be expressed in monocytes together with those involved in immune response, metabolic pathways related to immune response, and epigenetic regulation of immune response. In addition, CD36, a well-known receptor for long chain-fatty acid chains, was selected as the target protein for diacylglycerol [19]. A Venn diagram to identify intersecting genes was plotted. These genes were then used to construct a protein-protein interaction (PPI) network in the STRING database.

Furthermore, the MCODE analysis (default parameter) in Cytoscape software (Version 3.10.2) was applied to identify core target proteins based on the highest clustering scores in the network [20]. Subsequently, enrichment analysis on these core target proteins was studied using GO ontology and Reactome pathway analysis to determine their functions. Then, a bar plot was generated using R studio (Version 2024.04.2).

Molecular docking and PASS analysis

Bulk molecular docking was conducted on the core target proteins and compounds to determine which compounds primarily bind to each target. The structure of core target proteins was obtained from the Protein databank (PDB) (https://www.rcsb.org/). The structure of predicted compounds was generated by the IUPAC name using Biovia Draw (Version 24.1.NET). Using the Discovery Studio visualizer, water molecules were removed, and energy was minimized. Molecular docking was performed by Autodock tools (Version 1.5.7). Hydrogen atom and Kallmandata charge were added, and the coordinate files were generated. Torsion root detection was performed for ligand preparation, and the coordinate files were generated. The size and coordinates of the grid boxes were set with a spacing of 0.375°A, the center of the grid box and docking parameters are shown in Table 1. Docking analysis was performed using the Lamarckian genetic algorithm with default parameters. The Discovery Studio visualizer was used to visualize the hydrogen bond between the targeted protein and ligand.

For molecular docking validation, the redocking method was used to verify the molecular docking method and parameters. The co-crystal structure of the targeted proteins and native ligands was downloaded from the PDB (https://www.rcsb.org/). The structure was then separated, and molecular docking was performed, as mentioned above. Moreover, the potential of a substance to exhibit immunomodulatory activity was assessed using the PASS server (https://www.way2drug.com /passonline/predict.php) [21]. The schematic diagram of the methodology is presented in Figure 1.

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis, and pharmacokinetic properties

The pharmacokinetic properties of the candidate compounds were assessed using ADMET analysis (the SwissADME and pkCSM online tools). The analysis included absorption, distribution, metabolism, excretion, toxicity, and drug-likeness evaluation based on Lipinski's rule of five, allowing no more than one violation. Intestinal absorption and hepatotoxicity were also considered. The compound's structure was analyzed using the SMILES notation.

Table 1. Grid box of the x, y, and z centers for each target compound.

Targeted protein	Grid box		
	X	Y	Z
CD36	−41.907	−27.468	24.967
GSTP1	19.296	0.051	20.048
PTGS2	21.646	−4.965	79.46
MET	4.481	9.317	21.846
CKD4	17.006	−32.725	0.355
IGFR1	17.41	3.988	46.497
GSK3B	27.053	48.344	38.241
HSP90AA1	0.475	14.642	20.599

RESULTS AND DISCUSSION

Identification of PP crude extract contains bioactive molecules using untargeted LC-MS/MS analysis

Pleurotus pulmonarius was extracted by hot water extraction to recapitulate its traditional culinary and folk medicine applications. LC-MS/MS analysis was performed to identify water-soluble bioactive compounds in *P. pulmonarius* crude extract. A total of 169 chromatographic peaks were detected in ESI-positive mode. *In silico* MS/MS fragmentation was carried out using molecular ion peak $[M^{+}H]^{+}$ compared with the theoretical mass, 36 bioactive molecules were proposed (Table 2). We found that 12 out of 36 proposed bioactive compounds, namely diacylglycerol, phosphatidylethanolamine, phosphatidylinositol, glutathione, quercetin, dihydroresveratrol, aspartic acid, isorhamnetin, catechin, myricitrin, apigenin, and oleic acid had previously been reported in mushrooms [12,22–28]. The proposed twelve bioactive compounds reported in mushrooms are labeled (Fig. 2), and the immunomodulatory activities of each compound are presented in Table 3. These water-soluble bioactive compounds are mainly involved in antioxidant activity and anti-inflammatory activity. Moreover, diacylglycerol and glutathione have been revealed to enhance monocyte and macrophage responses via trained immunity [19,29,30]. Previous studies have shown that mushrooms are rich sources of β-glucans and natural polysaccharides [31]. However, due to the large molecular size of these compounds, the limitations of LC/MS-MS methods prevent their analysis. In this study, we detected disaccharides, but their specific types could not be identified (data not shown). Therefore, additional analytical methods should be employed to further investigate such compounds.

Compound-protein target interaction and pharmacy network of *P. pulmonarius* crude extract

The proposed bioactive compounds were ranked in ascending order based on their area percentage values to predict the functions and interactions between bioactive compounds and monocytes. The top seven compounds

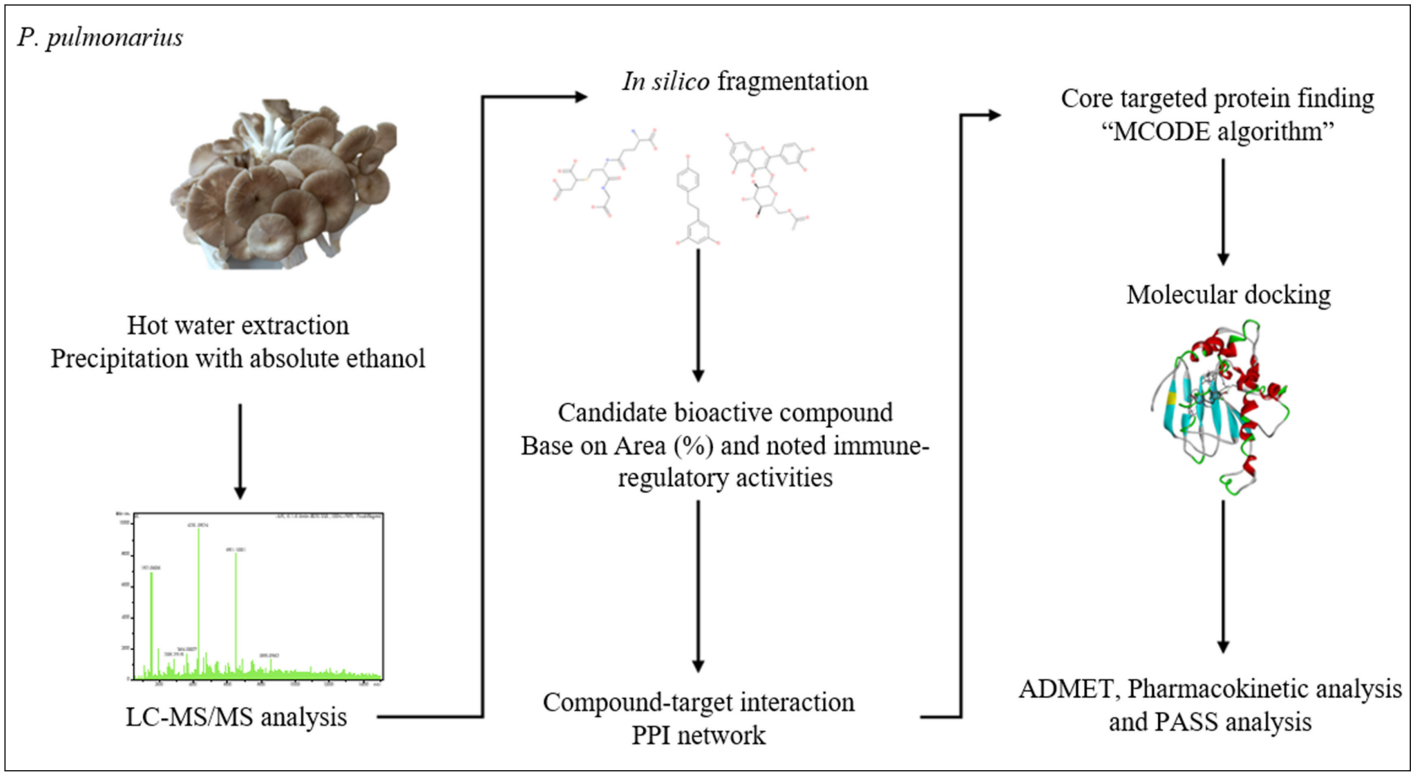


Figure 1. Schematic diagram of methodology.

based on their area percentage were selected and proposed as potential immunomodulators, including diacylglycerol, phosphatidylethanolamine, phosphatidylinositol, glutathione, quercetin 3-(6-O-acetyl-beta-glucoside), dihydroresveratrol, and aspartic acid. Potential target proteins for each compound were identified using the SwissTargetPrediction. Intersecting between these potential target proteins and monocyte-related target proteins was discovered and visualized in the Venn diagram (Fig. 3A). We could not find the intersecting targets between aspartic acid and monocyte. Although, the aspartate ion form of aspartic acid has been reported to induce inflammation in macrophages [32]. Since amino acid metabolism contributes to immune responses, we hypothesized that aspartic acid possibly promotes inflammation through its role as a precursor rather than directly interacting with proteins.

Moreover, the MCODE analysis revealed the core target proteins for glutathione, quercetin, and dihydroresveratrol, as presented in the cluster network (Fig. 3B). However, the core target proteins of diacylglycerol could not be identified. This may be due to the low abundance of the protein or a lack of connectivity of this protein (Fig. 3B). Furthermore, GO ontology and Reactome pathway analyses were performed to identify the function of each core targeted protein, which is involved in immune regulation and homeostasis in inflammatory responses (Fig. 3C).

Molecular docking of *P. pulmonarius*-derived candidate compounds and the potential target proteins

Next, molecular docking analysis of selected core target proteins was performed to investigate the candidate compounds' interaction with the potential target proteins. The crystal of the possible target proteins was obtained from a PDB. We performed the redocking of the native ligand with the targeted protein to validate the molecular docking protocol. Subsequently, the validated protocol was applied to the *in-silico* screening of the bioactive compound and target protein interaction. The binding affinities of native ligands from redocking experiments are presented in Table 4. Molecular docking of well-known activators or inhibitors sourced from the Drug Bank database is shown in Table 5, detailing their binding affinities. Molecular docking results of the candidate compounds, including binding affinity and 2D diagram structure of binding, are shown in Table 6 and Figure 4, respectively.

The finding demonstrated the unfavorable interactions between diacylglycerol and the CD36 fatty acid transporter, which may be related to the high molecular weight of these compounds. Additionally, no interactions between phosphatidylethanolamine, phosphatidylinositol, and the targeted protein were observed (data not shown). Interestingly, glutathione, quercetin, and dihydroresveratrol demonstrated promising potential proinflammatory inhibitors, with their binding affinities surpassing those of known inhibitors (−2.32 kcal/mol for glutathione; −6.76 kcal/mol for quercetin and −5.02 to −7.02 kcal/mol for dihydroresveratrol).

Molecular docking results uncovered the interaction between glutathione and glutathione S-transferase P (GSTP1). This interaction might support cellular redox status and modulation of host immune defense mechanisms to prevent cellular damage [33]. Moreover, the finding revealed that Quercetin 3-(6-O-acetyl-beta-glucoside) and dihydroresveratrol

Table 2. The compounds found in the extract of *Pleurotus pulmonarius* analyzed by LC-MS/MS and *in silico* fragmentation, ordered by retention time (RT).

Peak No.	RT (Min)	$(M^+H)^+$ (m/z)	Area (%)	Proposed compound	Theoretical mass	Database
2	1.1	218.9,918	0.07	5-Sulfosalicylate	217.9,885	MetFrag
3	1.3	209.0172	0.08	(Z)-[3-(Methylsulfinyl)-1-propenyl] 2-propenyl disulfide	209.0,123	METLIN
4	1.3	290.858	0.07	Catechin	290.0,790	MassBank
6	1.5	223.0,825	0.02	6-Acetyl-D-glucose	222.0,740	MetFrag
7	1.6	1080.398	0.05	Docosapentaenoyl-CoA	1079.3,608	METLIN
8	1.6	163.0,684	0.07	R-3-Amino-5-(methylthio) pentanoic acid	162.0,681	MassBank
9	1.7	1122.409	0.08	Disaccharide (HEX-HEX)	342.2,970	MassBank
11	1.8	134.051	0.35	Aspartic acid*	133.0,3751	MassBank
16	2.5	231.0,525	0.36	Dihydroresveratrol*	230.2,630	MetFrag
18	2.6	348.0,785	0.22	Inosinic acid	135.0,532	METLIN
20	2.8	261.0,463	0.26	Austricine	262.1,205	MassBank
21	3	424.1,144	2.93	S-(1,2-dicarboxyethyl) glutathione*	424.1,020	METLIN
24	3.3	586.1,675	0.24	Baccatin III	586.2,414	MassBank
26	3.7	277.0,365	0.03	Capensine	276.0,998	MassBank
27	4.2	152.0,625	0.03	L-Phenylglycine	151.0,634	MetFrag
29	4.9	630.1,033	0.03	Peridinin	630.3,557	MassBank
32	5.7	428.0,456	0.19	8-benzyl-9,10-dimethoxy-5,8-dihydro-6H-[1,3]dioxolo[4,5-g] isoquino[3,2-a]isoquinoline	427.1,784	MassBank
33	5.7	136.0,686	0.20	4-Hydroxy-L-threonine	135.0,532	MetFrag
38	7	615.762	0.27	Amarouciaxanthin A	614.3,971	MassBank
52	8	978.8,897	0.09	4'-O-(Glucuronyl-(1-3)-2'-E-Feruloyl-O-glucuronyl)-(1-2)-glucuronyl Apigenin	974.1,964	MassBank
53	8	624.9,615	0.00	Isorhamnetin-3-O-rutinoside*	624.1,690	MassBank
57	9.1	464.0,934	0.03	Myricitrin*	464.0,955	MassBank
58	9.2	274.2,858	0.02	Margaric acid(d3)	274.2,820	METLIN
59	9.2	230.2,588	0.02	Xestoaminol C	230.2,478	METLIN
62	9.4	288.3,005	0.27	Oleic acid*	287.2,873	METLIN
63	9.4	288.3,009	0.09	Eriodictyol	288.0,634	MassBank
64	9.9	316.3,339	0.04	Isorhamnetin*	316.05,829	MassBank
70	10.6	386.3,154	0.01	(1,2,9,10-tetramethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de, g]quinolin-3-yl) methanol	385.1,889	MassBank
79	11.4	340.3,333	0.13	Coumarin 338	340.07,944	MassBank
84	11.8	415.2,236	0.55	Daidzein	415.2,115	METLIN
95	12.8	506.3,466	1.87	Quercetin 3-(6-O-acetyl-beta-glucoside)*	506.1,060	MassBank
103	12.9	383.2,162	1.17	Cinncassiol C3	382.2,145	METLIN
116	13.7	425.2,273	0.94	Didrovaltratum	424.2,251	MetFrag
132	14.8	619.5,449	6.57	Diacylglycerol*	618.5,223	METLIN
135	15	965.6,391	3.56	Phosphatidylinositol*	965.6,114	METLIN
158	16.8	716.5,402	3.59	Phosphatidylethanolamine*	715.5,120	METLIN

* Referring to compounds that have been reported in mushrooms.

hold potential as anti-inflammatory agents through their ability to inhibit prostaglandin G/H synthase-2 (PTGS2). The PTGS2 enzyme plays a crucial role in converting arachidonic acid to prostaglandin H2, which promotes inflammatory immune responses [34]. Furthermore, dihydroresveratrol demonstrated the potential to bind to hepatocyte growth factor receptor (MET), cyclin-dependent kinase 4 (CKD4), glycogen synthase kinase-3 beta (GSK3B), and heat shock protein HSP 90-alpha (HSP90AA1). Such target proteins are involved in numerous signal transduction pathways linked to immune responses, including monocyte migration and proinflammatory cytokine production [35–38].

Previous studies have highlighted the role of PTGS2 in trained immunity [39], while the epigenetic regulation by

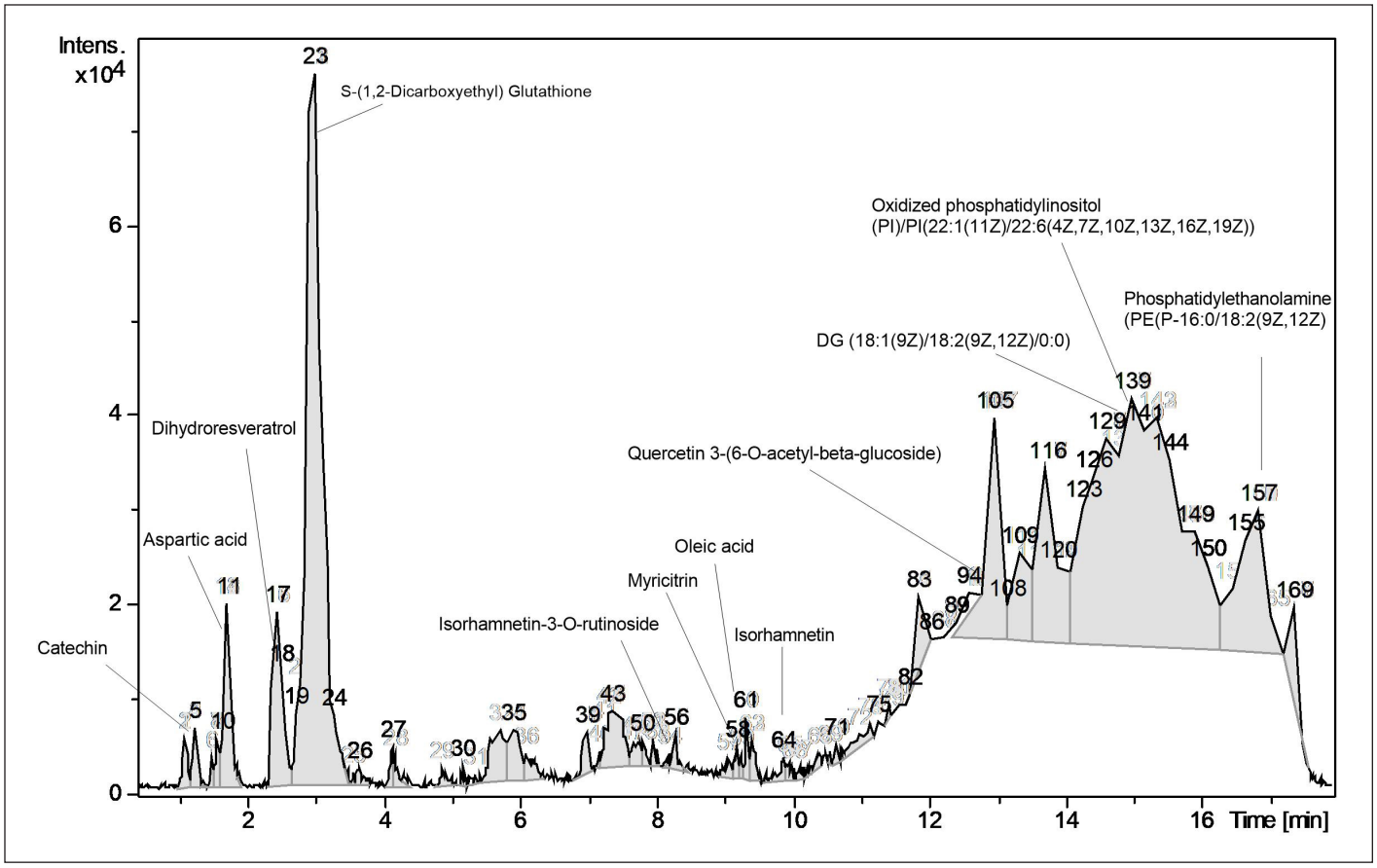


Figure 2. *In silico* MS/MS fragmentation with M/Z was used to predict the compounds. Thirty-six bioactive molecules were proposed. Chromatograms are presented, and the 12 bioactive compounds that have been previously reported in mushrooms are labeled.

GSK3B affects the expression of specific NF-κB-regulated genes [40]. Furthermore, the activity of GSK3B also influences the expression of MCP-1 and IL-6 [41]. Trained immunity or innate immune memory strengthens the responses of monocytes to subsequent stimuli; however, its dysregulation could result in hyperinflammation [42]. Therefore, modulating the activities of PTGS2 and GSK3B using Quercetin 3-(6-O-acetyl-beta-glucoside) and dihydroresveratrol may help control inflammation and slow disease progression in hyperinflammatory conditions. Notably, dihydroresveratrol can interact with multiple signaling pathways involved in immune regulation, including MET and CDK4. This offers new therapeutic opportunities for chronic inflammation-related diseases.

Research has established a significant connection between functional foods and immune responses, demonstrating their remarkable ability to promote inflammation and provide anti-inflammatory benefits [5,43]. Targeting specific proteins with bioactive compounds like quercetin 3-(6-O-acetyl-beta-glucoside) and dihydroresveratrol, found in *P. pulmonarius*, may play a crucial role in mitigating the inflammatory phenotype of monocytes. This strategic approach holds significant promise, particularly for conditions such as inflammatory bowel disease, which is characterized by excessive inflammation driven by monocytes [44–46]. On the other hand, insulin-like growth factor 1 (IGF1R) is pivotal in anti-inflammatory response [47]. Inhibiting IGF1R by dihydroresveratrol may contribute to supporting inflammatory phenotype.

Resveratrol has demonstrated both pro-inflammatory and anti-inflammatory effects, influencing immune responses in monocytes and macrophages in a cell type-specific and dose-dependent manner. These effects are associated with the differential upregulation of the Aryl hydrocarbon receptor and cytochrome P450 1B1 in these cell types [48].

Furthermore, the results from PASS analysis support the anti-inflammatory properties of quercetin 3-(6-O-acetyl-beta-glucoside) and dihydroresveratrol (Table 7). Quercetin 3-(6-O-acetyl-beta-glucoside) exhibited strong anti-inflammatory (Pa 0.773) and immunosuppressant (Pa 0.713) potential. This finding aligns with previous studies that have demonstrated quercetin's ability to inhibit pro-inflammatory cytokines and downregulate immune cell activation [49]. Dihydroresveratrol displayed modest anti-inflammatory (Pa 0.366) and immunosuppressive effects (Pa 0.285). Notably, the parent compound resveratrol has shown both inflammatory and anti-inflammatory activities [48]. Therefore, further experimental validation and *in vivo* studies are crucial to fully verify its therapeutic potential.

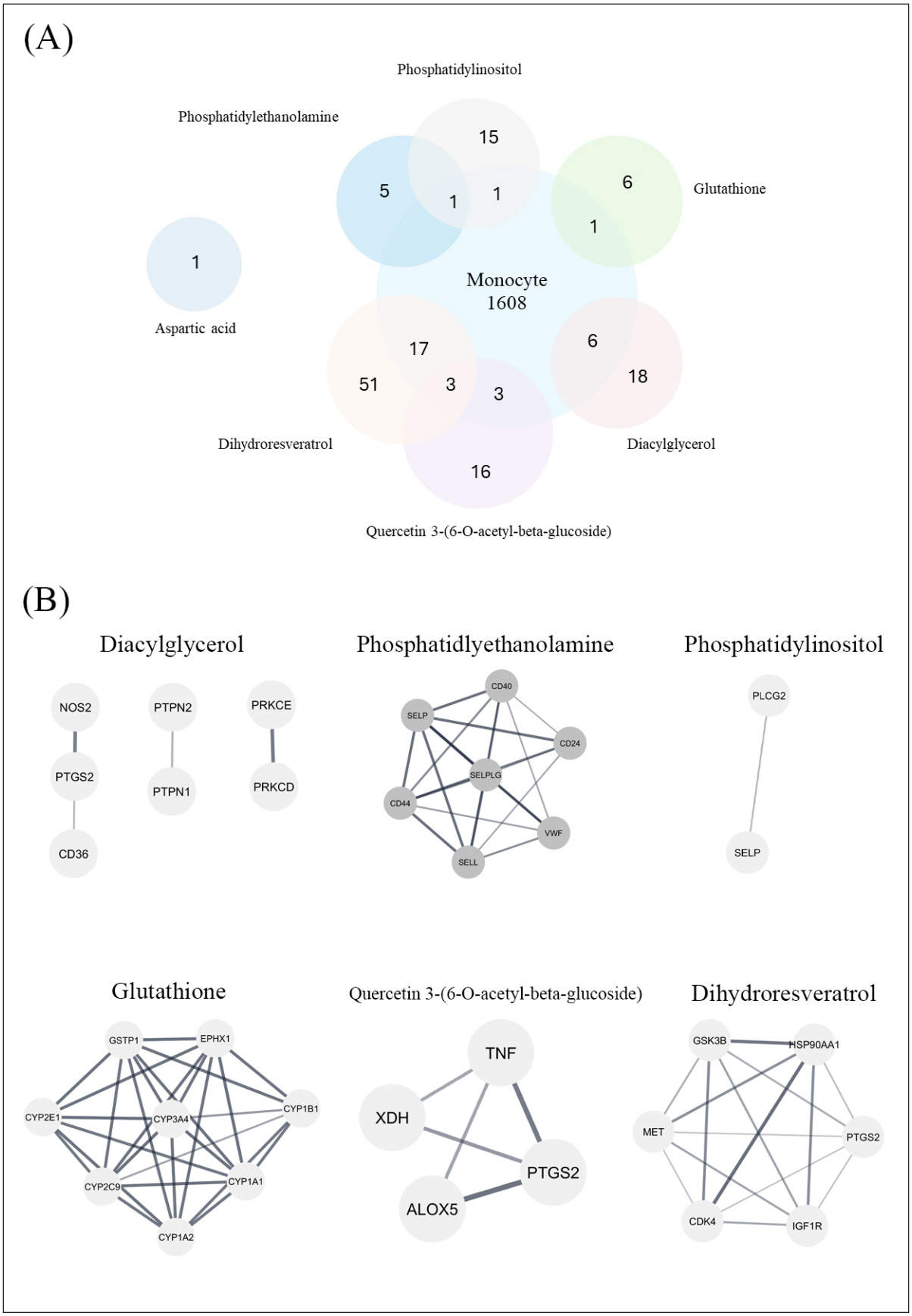
Table 3. Immunomodulatory properties of the proposed bioactive compounds ordered by area (%).

Compound (source)	Model	Immunomodulatory effect	Mechanism	References
Diacylglycerol (N/A)	-	Induce inflammation, trained immunity	Endogenous diacylglycerol activated protein kinase C signaling leading to an inflammatory immune response.	[58] [19,29]
			Diacylglycerol and ceramides metabolized from palmitic acid induced inflammatory immune response and trained immunity effect.	
Phosphatidylethanolamine (Soybean)	CFLP mice	Anti-inflammatory activity	Dietary containing PC, PE, NAPE down-regulated pleurisy in carrageenan-induced mice.	[59]
Phosphatidylinositol (Soybean oil)	C57BL/6 mice	Anti-inflammatory activity	Dietary containing phosphatidylinositol suppressed IFN-γ, TNF-α and MCP-1 production in Concanavalin A-induced liver injury mice.	[60]
Glutathione (N/A)	RAW 264.7 macrophage	Antioxidant, ROS scavenger, and proinflammatory activity	Reduced glutathione suppressed ROS production in LPS-stimulated RAW 264.7 macrophages.	[52]
			Proinflammatory cytokine production was upregulated in reduced glutathione-treated macrophages in a dose-dependent manner.	
	Wistar rat	Anti-inflammatory activity	Glutathione combination with ceftriaxone suppressed TNF-α production in *E.coli*-induced peritonitis mice.	[53]
	Human monocyte, *Gclc*$^{-/-}$ mice	Trained immunity	Intracellular glutathione indirectly supported the induction of trained immunity by acting as a redox buffer, helping to regulate ROS levels.	[30]
Quercetin (*Pleurotus pulmonarius*)	RAW 264.7 macrophage	Anti-inflammatory activity	*P. pulmonarius* extract with quercetin inhibited NO production in LPS-stimulated RAW 264.7 macrophages.	[13]
Resveratrol (*Pleurotus pulmonarius*)	RAW 264.7 macrophage	Anti-inflammatory activity	*P. pulmonarius* extract with resveratrol inhibited NO production in LPS-stimulated RAW 264.7 macrophages.	[13]
Aspartic acid (N/A)	*In vitro*: peritoneal macrophages from mice *In vivo*: mice and piglets	Induce inflammation, and bactericidal activity	Exogenous aspartate and its metabolite, asparagine, supported M1 macrophage polarization, proinflammatory cytokine production and bactericidal activity.	[32]
Oleic acid (*Grifola frondosa*)	-	Antioxidant activity	Fatty acid fraction of *G. frondose* mycelia contained palmitic, oleic and linoleic inhibited cyclooxygenase activity.	[61]
Apigenin (N/A)	THP-1-induced macrophage	Anti-inflammatory activity	Apigenin inhibited proinflammatory cytokines production in LPS-stimulated macrophages.	[62]
Catechin (*Pleurotus pulmonarius*)	RAW 264.7 macrophage	Anti-inflammatory activity	*P. pulmonarius* extract with catechin inhibited NO production in LPS-stimulated RAW 264.7 macrophages.	[13]
Isorhamnetin (*Inonotus sanghuang*)	RAW 264.7 macrophage	Anti-inflammatory activity	*I. sanghuang* ethyl acetate fraction with isorhamnetin suppressed NO, TNF-α, IL-6, and MCP-1 in LPS- or 3T3-L1 adipocytes activated RAW 264.7 macrophages.	[63]
Myricitrin (*Pleurotus eryngii*)	RAW 264.7 macrophage	Anti-inflammatory activity	*P. eryngii* ethanolic extract with myricitrin suppressed NO and PGE2 production in LPS-stimulated RAW 264.7 macrophages.	[64]

N/A = not available.

ADMET analysis and pharmacokinetic properties

To investigate the pharmacokinetic properties and drug-likeness of candidate bioactive compounds, we conducted an ADMET analysis, focusing on gastrointestinal (GI) absorption and hepatotoxicity. The results are summarized in Table 8. The predictions indicated that the GI system rarely absorbs diacylglycerol, glutathione, and quercetin 3-(6-O-acetyl-beta-glucoside). In contrast, resveratrol is well absorbed through passive human gastrointestinal absorption and can cross the blood-brain barrier (Fig. 5A). The physicochemical properties of each compound are illustrated in a bioavailability radar, which includes attributes such as lipophilicity, size, solubility, polarity, saturation, and flexibility (Fig. 5B). The colored area of the radar shows the most desirable zone for each bioavailability characteristic. According to Lipinski's rule of five and the bioavailability properties assessed, dihydroresveratrol emerges as the only compound with a favorable absorption, distribution, and safety profile, indicating its potential as a drug-like compound.



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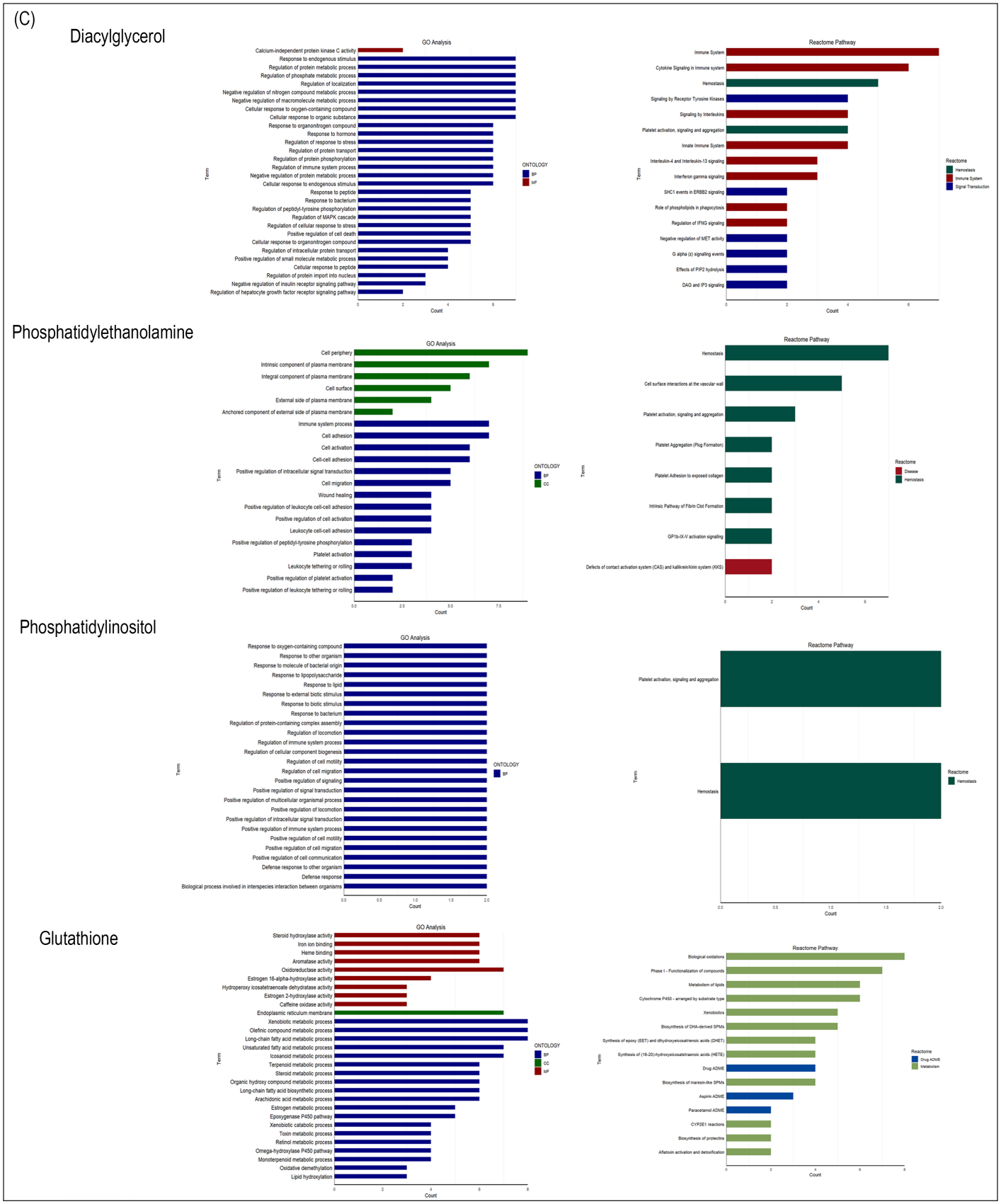


Figure 3. *Continued*..

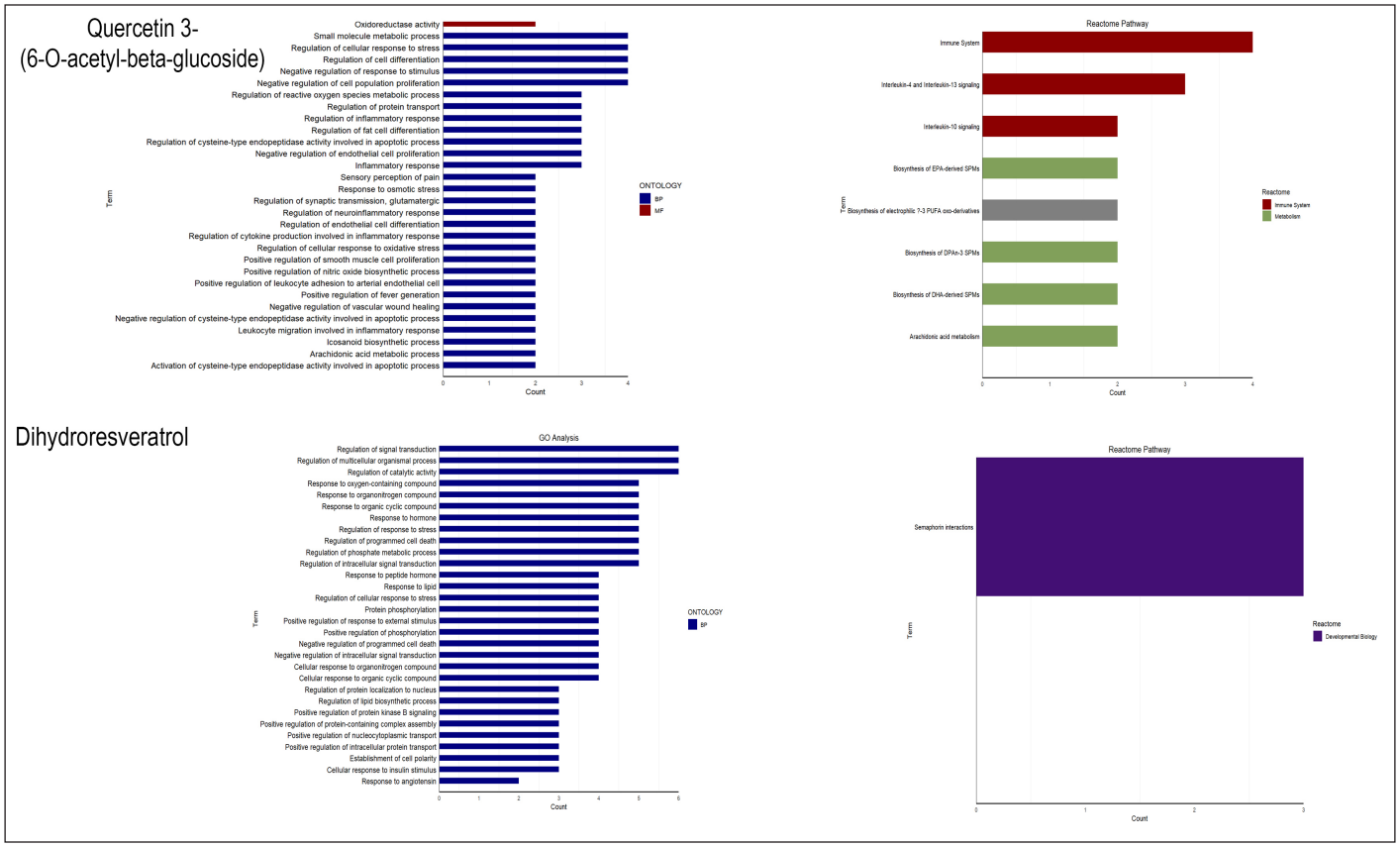


Figure 3. The illustration of the Venn diagram demonstrated an intersecting target between monocyte proteins and potential targets of each bioactive compound (a), the core cluster of the PPI network (b), and functional analysis of core target proteins (c). *GO* Gene Ontology, *BP* biological process, *CC* cellular component, *MF* molecular function.

Limitations and future directions

This study identified water-soluble bioactive compounds from *P. pulmonarius* utilizing LC-MS/MS profiling and *in silico* molecular docking, emphasizing their potential as immunomodulators targeting monocyte immune responses. By employing advanced computational tools such as PASS analysis, ADMET profiling, and Reactome pathway analysis, we predicted interactions between bioactive compounds and immune-related proteins. This approach provides a comprehensive framework for understanding their immunomodulatory effects. However, it is essential to recognize the necessity for further experimental validation alongside a broader environmental context.

Biomedical implications

The bioinformatics analyses revealed compounds such as glutathione, quercetin, and dihydroresveratrol, which demonstrated significant binding affinities to monocyte-related target proteins involved in inflammatory processes. These findings are supported by existing experimental evidence on related compounds, underscoring their relevance [13,50–54]. Although the current study does not encompass wet laboratory experiments, the results provide a roadmap for future validation. It is recommended that cytokine modulation assays be conducted using monocyte-derived cell lines or primary monocytes to evaluate pro- and anti-inflammatory cytokine production through ELISA assays. In addition, *in vitro* binding studies should be performed to confirm the predicted interactions between compounds and proteins. Pathway-specific analyses targeting key immune pathways, such as NF-κB and JAK/STAT, may be investigated using qPCR, Western blotting, or reporter assays. Furthermore, *in vivo* models would serve as a suitable tool for assessing the bioavailability, pharmacokinetics, and therapeutic efficacy of the compounds. These experimental approaches are vital for translating computational findings into actionable biomedical applications.

Environmental implications

While the primary focus of this study is biomedical applications, the ecological behavior of *P. pulmonarius*-derived bioactive compounds warrants further research. Their interactions with environmental pollutants and behavior in aquatic ecosystems have not been thoroughly explored. Glutathione has been shown to reduce oxidative stress caused by heavy metals and pesticides in aquatic environments [55], and dihydroresveratrol possesses antioxidant properties that may help mitigate environmental oxidative damage [56,57]. Therefore, investigating the biodegradability and environmental fate of these compounds, along with their stability and interactions with pollutants, is essential. Moreover, exploring their potential in bioremediation to address pollutant

Table 4. Hydrogen bond interaction between targeted protein and original unique ligand.

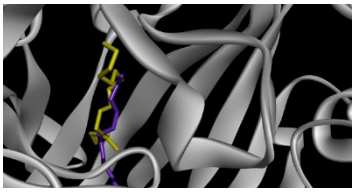
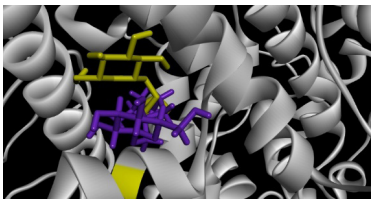
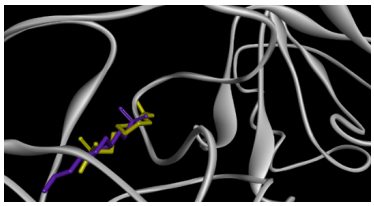
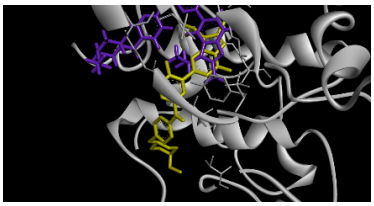
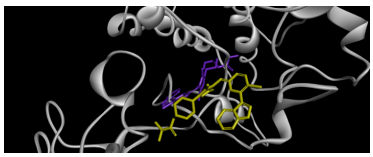
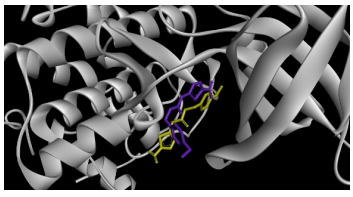
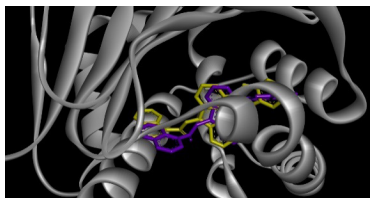
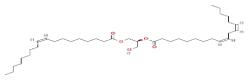
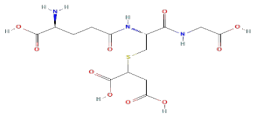
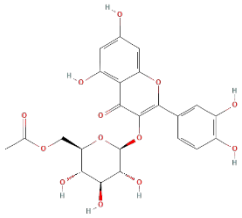
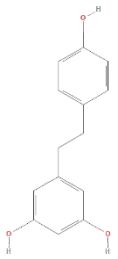
Targeted protein-unique ligand interaction	Binding energy (kcal/mol)	Hydrogen bonding		3D diagrams
		Number	Amino acid interaction	
CD36	−5.48	2	ARG386, PLM513	
PTGS2	−2.84	6	ARG311, SER563, SER566, LEU567, CYS569, ASN570	
MET	−5.13	1	GLN150	
CKD4	−9.58	1	LYS35	
IGFR1	−9.85	1	ASP1153	
GSK3B	−4.75	3	THR138, GLN185, LYS85	
HSP90AA1	−14.04	1	ASN51	

Table 5. Molecular docking results of activators and inhibitors on the targeted protein.

No.	PubChem ID	Name	Target protein		Result		
			PDB ID	Name	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant (Kpi)
1	985	Palmitic acid	5LGD	CD36	−5.48	−0.3	96.45
2	65359	Glutathione	2A2R	GSTP1	−1.02	−0.05	178.82
3	446284	Icosapent	5IKR	PTGS2	−2.9	−0.13	7.53
4	11626560	Crizotinib	1BHT	MET	−9.18	−0.31	186.04
5	5330286	Palbociclib	7SJ3	CKD4	−9.04	−0.27	235.03
6	68165256	Brigatinib	5FXS	IGFR1	−4.96	−0.12	232.87
7	11671467	Fostamatinib	1Q5K	GSK3B	−1.76	−0.04	50.9
8	5288674	Alvespimycin	2YKE	HSP90AA1	−5.85	−0.13	51.79

Table 6. Chemical structure and molecular docking results.

No.	Ligand			Target protein		Result		
	PubChem ID	Name	Chemical structure	PDB ID	Name	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant (Kpi)
1	9543722	DG (18:1(9Z)/18:2(9Z,12Z)/0:0) [iso2]		5LGD	CD36	299.09	6.80	288.65
2	9802383	S-(1,2-dicarboxyethyl) glutathione		2A2R	GSTP1	−2.32	−0.06	19.93
3	10006384	Quercetin 3-(6-O-acetyl-beta-glucoside)		5IKR	PTGS2	−6.76	−0.19	11.16
4	185914	Dihydroresveratrol		5IKR	PTGS2	−5.02	−0.3	210.66
				1BHT	MET	−7.02	−0.41	7.14
				7SJ3	CKD4	−6.34	−0.37	22.35
				5FXS	IGFR1	−6.58	−0.39	15.02
				1Q5K	GSK3B	−5.42	−0.32	107.07
				2YKE	HSP90AA1	−6.77	−0.4	10.82

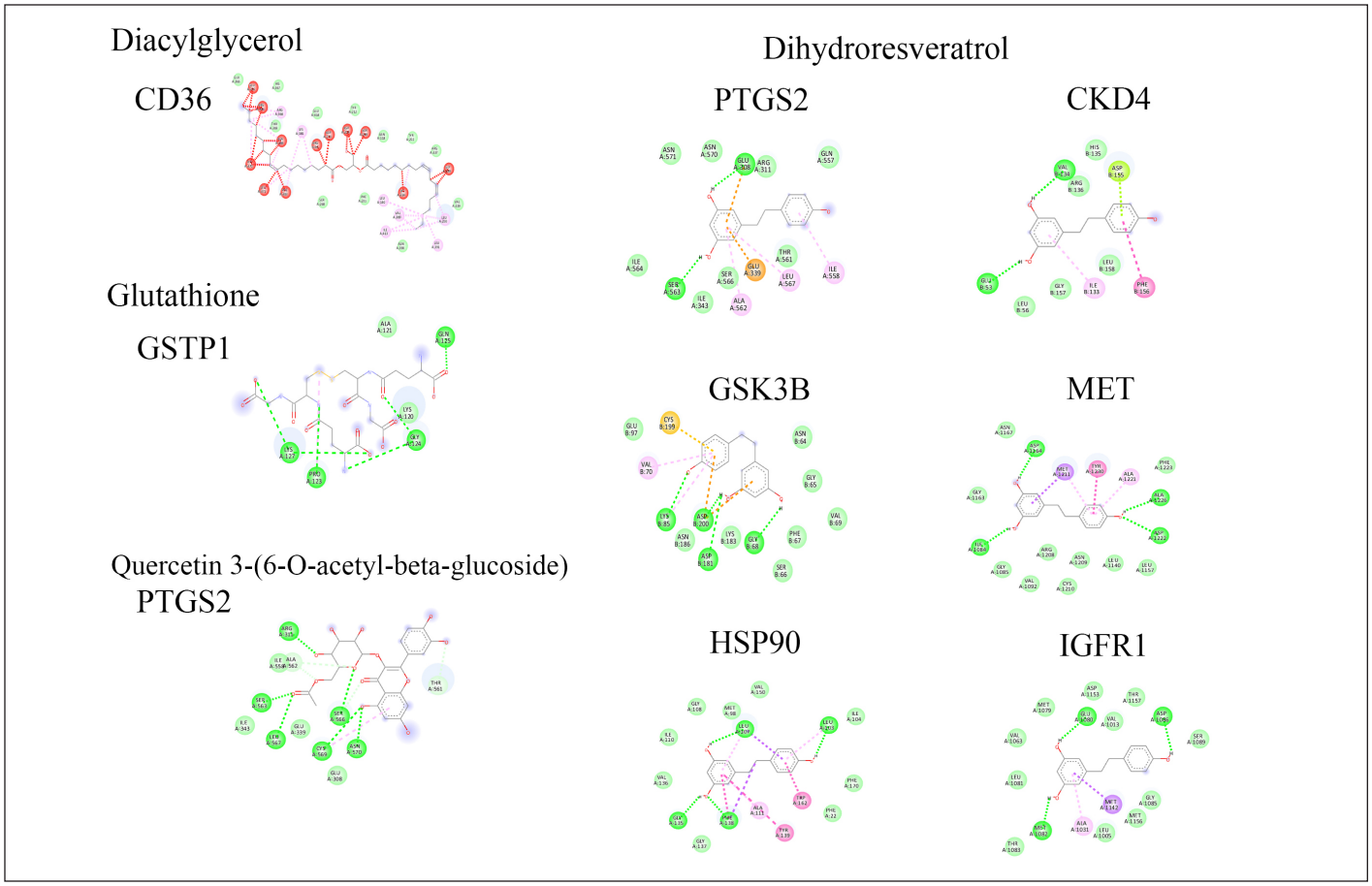


Figure 4. A 2D diagram represents the molecular docking analysis of *P. pulmonarius* candidate compounds and potential targeted proteins.

Table 7. PASS analysis of potential target compounds for inflammatory and anti-inflammatory effects.

Activities	PASS prediction (P_a/P_i)			
	Diacylglycerol	Glutathione	Quercetin 3-(6-O-acetyl-beta-glucoside)	Dihydroresveratrol
Immunostimulant	0.753/0.011	0.604/0.023	0.597/0.024	0.2620.094
Macrophage stimulant	0.861/0.002	0.447/0.024	0.306/0.066	-
Immunosuppressant	0.620/0.026	-	0.713/0.015	0.285/0.124
Anti-inflammatory	0.766/0.009	0.274/0.188	0.773/0.009	0.366/0.114
TNF expression inhibitor	0.702/0.006	0.255/0.138	-	0.573/0.016

P_a = Probability to be active, P_i = Probability to be inactive.

Table 8. ADMET analysis and pharmacokinetic properties.

Properties	Compounds			
	Diacylglycerol	Glutathione	Quercetin 3-(6-O-acetyl-beta-glucoside)	Dihydroresveratrol
MW (g/mol)	618.97	423.4	506.41	230.26
Num. H-bond acceptors (≤ 10)	5	11	13	3
Num. H-bond donors (≤ 5)	1	7	7	3
Log Po/w (≤ 5)	10.75	−2.84	−0.11	2.49
Violation (≤ 1)	2	2	3	0
GI absorption	Low	Low	Low	High
Hepatoxicity	No	No	No	No

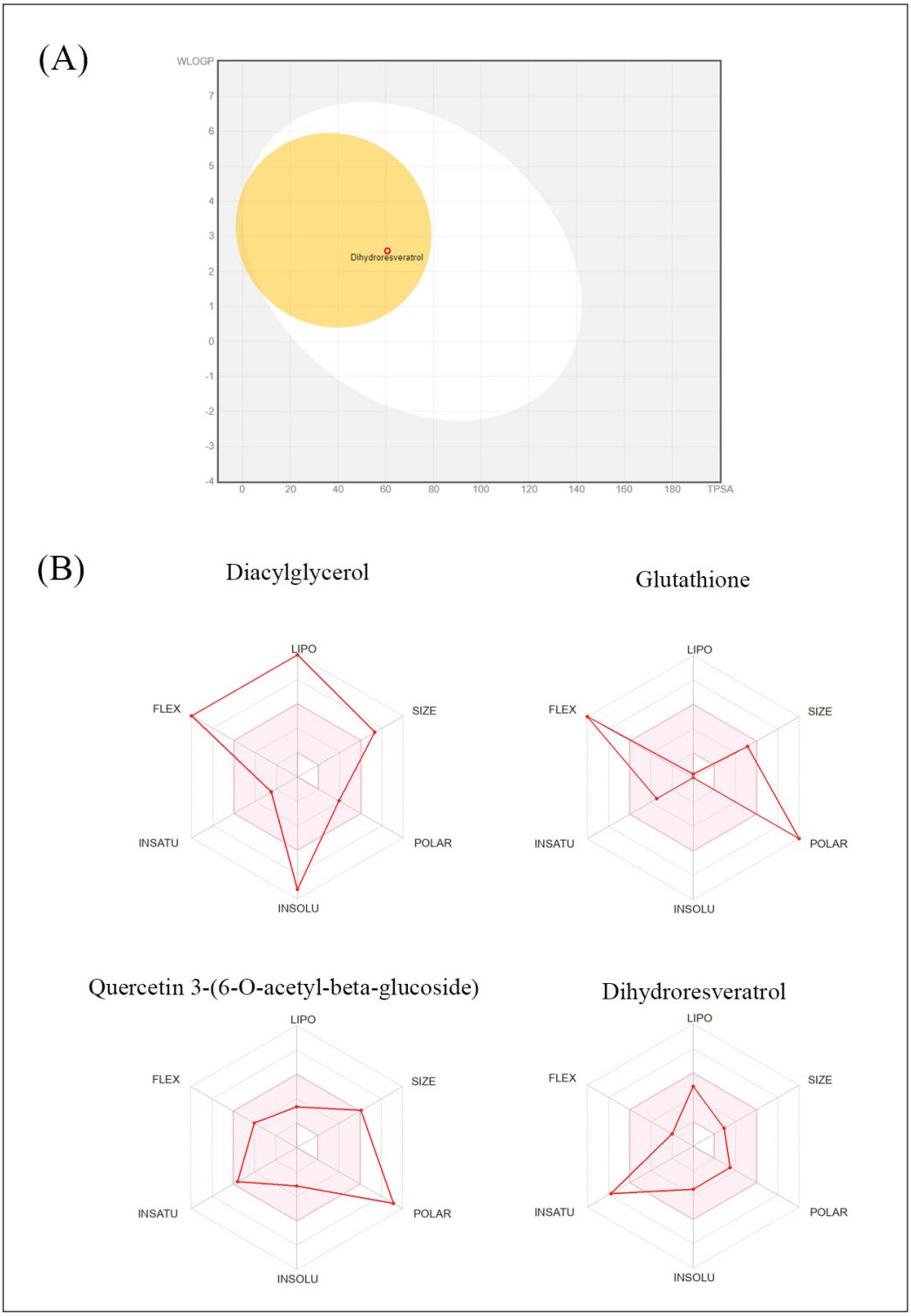


Figure 5. The illustration of BOILED-Egg diagram (a) and bioavailability radar related to physiochemical properties of *P. pulmonarius* candidate compounds (b).

accumulation in water bodies could be beneficial. Such studies could contribute to the broader understanding of natural immunomodulators and their potential applications in human health and ecosystem restoration.

CONCLUSION

Pleurotus pulmonarius is a highly accessible and cost-effective edible fungus worldwide. Our study involved the comprehensive profiling of water-soluble bioactive compounds in

P. pulmonarius using untargeted LC-MS/MS analysis and revealed 36 bioactive compounds from the mushroom with hot-water extraction. The top seven candidate compounds found abundantly in the mushroom were selected, including diacylglycerol, phosphatidylethanolamine, phosphatidylinositol, glutathione, quercetin 3-(6-O-acetyl-beta-glucoside), dihydroresveratrol, and aspartic acid. The bioactive compounds responsible for modulating immune response in monocytes were further identified by *in silico* screening. We unveiled the potential anti-inflammatory activity, mainly attributed to glutathione, quercetin, and dihydroresveratrol in the extract. Furthermore, our ADMET analysis highlighted the promising potential of dihydroresveratrol as an immunomodulatory target due to its favorable absorption and bioavailability. This study provides valuable insights into the immunomodulatory potential of *P. pulmonarius* bioactive compounds, offering a strong foundation for future experimental validation which could contribute to significant implications for their application in functional food and alternative medicine, especially in monocyte-related inflammation in the future.

ACKNOWLEDGMENTS

The authors thank Miss Sunita Nilkhet for her technical support in the molecular docking analysis. The English editing of this manuscript was kindly performed in part by Dr. James Michael Brimson, Research, Innovation and International Affairs, Faculty of Allied Health Sciences, Chulalongkorn University. A.A. would like to thank the Graduate School, Chulalongkorn University for the research assistance scholarship.

LIST OF ABBREVIATIONS

ADMET, Absorption, distribution, metabolism, excretion, and toxicity; CKD4, Cyclin-dependent kinase 4; GSK3B, Glycogen synthase kinase-3 beta; GSTP1, Glutathione S-transferase pi 1; HSP90AA1, Heat Shock Protein 90 alpha family class A member 1; IGF1R, Insulin-like growth factor 1 receptor; LC-MS/MS, Liquid chromatography with tandem mass spectrometry; MET, Mesenchymal-epithelial transition, tyrosine kinase receptor; *P. pulmonarius*, *Pleurotus pulmonarius*; PPI, Protein-protein interaction; PTGS2, Prostaglandin G/H synthase-2; SMILES, Simplified molecular input line entry system.

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

This work was supported by Research Funding from the Faculty of Allied Health Sciences, Chulalongkorn University (AHS CU 62003).

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

CONSENT TO PARTICIPATE

All authors agree to publish the article.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Arromsava A, Chuchawankul S, Amornsupak K. LC-MS/MS profiling and *in silico* molecular docking analysis of water-soluble bioactive compounds from *Pleurotus pulmonarius* as potential immunomodulators on monocyte immune response. J Appl Pharm Sci. 2025;15(04):162–178.