



Mechanistic insights into *Plantago major* L. for Alzheimer's disease treatment: Untargeted metabolomic-network pharmacology and molecular docking approach

[Perspectivas mecánicas sobre *Plantago major* L. para el tratamiento de la enfermedad de Alzheimer: farmacología de redes metabolómicas no dirigida y enfoque de acoplamiento molecular]

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Abstract

Context: Alzheimer's disease (AD) is a progressive neurodegenerative disorder with limited therapeutic options. *Plantago major*, an herbal plant with prominent anti-inflammatory and antioxidant properties, has shown potential antineuroinflammatory effects for AD treatment.

Aims: To elucidate the mechanistic basis of *P. major* in AD treatment using integrated untargeted metabolomics, network pharmacology, and molecular docking.

Methods: Dried *P. major* leaves were extracted using ultrasonic-assisted extraction, and the extract was analysed by UHPLC-HRMS. Identified bioactive metabolites were subjected to ADMET screening and profiling. These metabolites were then evaluated using network pharmacology, validated through molecular docking, and prioritised using the SAW method.

Results: Fifteen metabolites met drug-likeness criteria, and network analysis identified 455 overlapping protein targets associated with AD, including ten key nodes such as SRC, PIK3R1, and MAPK1. Aucubin, scutellarein, luteolin, hispidulin, and ursolic acid exhibited strong binding affinities to these targets and ranked among the top six in SAW prioritization. These metabolites were further implicated in PI3K-AKT, JAK-STAT, and MAPK signaling pathways linked to neuroinflammation.

Conclusions: The findings support the therapeutic potential of *P. major* in AD through the modulation of neuroinflammatory mechanisms, particularly via the JAK-STAT, MAPK, and PI3K-AKT pathways, and highlight its potential for further *in vitro* and *in vivo* investigations.

Keywords: Alzheimer's disease; molecular docking; network pharmacology; neuroinflammation; *Plantago major* L.; simple additive weighting.

Resumen

Contexto: La enfermedad de Alzheimer (EA) es un trastorno neurodegenerativo progresivo con opciones terapéuticas limitadas. *Plantago major*, una planta herbácea con destacadas propiedades antiinflamatorias y antioxidantes, ha mostrado potencial para el tratamiento de la EA mediante metabolómica no dirigida integrada, farmacología de redes y acoplamiento molecular.

Objetivos: Elucidar la base mecánica de *P. major* en el tratamiento de la EA mediante metabolómica no dirigida integrada, farmacología de redes y acoplamiento molecular.

Métodos: Se extrajeron hojas secas de *P. major* mediante extracción asistida por ultrasonidos y el extracto se analizó por UHPLC-HRMS. Los metabolitos bioactivos identificados se sometieron a cribado y perfilamiento ADMET. Posteriormente, estos metabolitos se evaluaron mediante farmacología de redes, se validaron mediante acoplamiento molecular y se priorizaron mediante el método SAW.

Resultados: Quince metabolitos cumplieron los criterios de similitud con fármacos, y el análisis de redes identificó 455 proteínas diana superpuestas asociadas con la EA, incluyendo diez nodos clave, como SRC, PIK3R1 y MAPK1. La aucubina, la escutelareína, la luteolina, la hispidulina y el ácido ursólico mostraron una alta afinidad de unión a estas dianas y se ubicaron entre los seis primeros en la priorización SAW. Estos metabolitos también se relacionaron con las vías de señalización PI3K-AKT, JAK-STAT y MAPK, todas vinculadas a la neuroinflamación.

Conclusiones: Los hallazgos respaldan el potencial terapéutico de *Plantago major* en la enfermedad de Alzheimer mediante la modulación de mecanismos neuroinflamatorios, en particular a través de las vías JAK-STAT, MAPK y PI3K-AKT, y destacan su potencial para futuras investigaciones *in vitro* e *in vivo*.

Palabras Clave: enfermedad de Alzheimer; acoplamiento molecular; farmacología de redes; neuroinflamación; *Plantago major* L.; ponderación aditiva simple.

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Abbreviations: AD: Alzheimer's disease; ADMET: Absorption, distribution, metabolism, excretion, and toxicity; BBB: blood-brain barrier; DILI: drug-induced liver injury; EGFR: epidermal growth factor receptor; FDR: false discovery rate; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; iNOS: inducible nitric oxide synthase; JAK-STAT: Janus kinase/signal transducer and activator of transcription; KEGG: Kyoto Encyclopedia of Genes and Genomes; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; NF- κ B: nuclear factor kappa B; NP: network pharmacology; PI3K-AKT: phosphoinositide 3-kinase-protein kinase B; PLIF: protein-ligand interaction fingerprinting; PMT: plant-metabolite-target; PDB: Protein Data Bank; ROF: rule of five; RMSD: root-mean-square deviation; SAW: Simple Additive Weighting; SRC: proto-oncogene tyrosine-protein kinase Src; STAT3: signal transducer and activator of transcription 3; SRPlot: scientific research plot; TNF- α : tumor necrosis factor alpha; UHPLC-HRMS: ultra high-performance liquid chromatography coupled with high-resolution mass spectrometry.

INTRODUCTION

Alzheimer's disease (AD), the most prevalent form of dementia, currently affects approximately 55 million individuals globally and is projected to rise dramatically by 2050 (Reitz et al., 2023). In Indonesia, the disease accounts for a substantial portion of dementia cases, particularly on the islands of Java and Bali (Farina et al., 2023). Despite increasing research into the pathophysiology of AD, therapeutic progress remains limited to targeting a single pathway (Yiannopoulou et al., 2019). Existing pharmacological agents, including acetylcholinesterase inhibitors and NMDA receptor antagonists, offer symptomatic relief; they fail to halt or reverse disease progression and are frequently associated with adverse effects (Zhang et al., 2024). These limitations underscore the urgent need for alternative therapeutic strategies that address the underlying mechanisms of AD, particularly neuroinflammation.

Neuroinflammation is a key pathophysiological factor that plays a critical role in the progression and exacerbation of AD (Heneka et al., 2015). Numerous studies have demonstrated that neuroinflammation contributes to the neurodegenerative processes commonly observed in AD (Mhatre et al., 2015). This inflammatory response can exacerbate the disease by amplifying amyloid beta peptide and tau pathology, primary biomarkers of AD (Zotova et al., 2010). As a result, there is a pressing need for more effective and efficient therapeutic strategies. A promising approach involves the adoption of multi-target therapies, which may better address the complexity of AD's pathogenesis and potentially lead to significant clinical improvements (Yiannopoulou et al., 2019). One such alternative is the use of medicinal plants.

Ginkgo biloba remains a popular medicinal plant used in the therapy of AD. However, its raw materials are still imported, and it is known to have relatively low bioavailability and a short half-life (Zhang et al., 2024). Furthermore, *G. biloba* contains ginkgotoxin and ginkgolic acid, which have been shown to have toxic and mutagenic properties (Yao et al., 2025). These concerns underscore the urgency of exploring native Indonesian medicinal plants that are not only safer but also capable of providing effective therapeutic solutions, while supporting the country's self-sufficiency in medicinal raw materials.

Plantago major L. (family *Plantaginaceae*) emerges as a promising candidate due to its widespread availability across Indonesia, particularly on the islands of Java, Sumatra, and Sulawesi (Kartini et al., 2019). This plant has been extensively utilised in traditional medicine and contains various active compounds with medicinal properties found throughout the plant, including in the seeds, leaves, flowers, and roots. Its bioactivity is closely linked to these chemical constituents (Adom, 2017; Gonçalves & Romano, 2016; Kartini et al., 2021).

From an ethnopharmacological perspective, *P. major* has been traditionally used in various regions as a wound healing agent. Historical records indicate that Dioscorides, in *De Materia Medica* (1st century), used this plant to treat dog bites. According to Samuelsen (2000), the Vikings also used it for wounds (*Vølsuga saga*). Ibn El Beithar (12th–13th century) also adopted its use from Greek medicine. In *Liber Harbarum*, Henrik Harpestreng (†1244) stated that *P. major* could heal wounds, especially when mixed with honey or boiled with butter. Additionally, Simon Paulli, in *Flora Danica* (1648), described *P. major* as a highly wound-healing remedy. These ethnomedical accounts suggest underlying anti-inflammatory properties, such as wound-healing effects, which may be particularly relevant to conditions associated with neuroinflammation, including AD.

Several studies have highlighted the potential of *P. major* as a candidate for Alzheimer's disease (AD) treatment. Kang (2017) further confirmed that *P. major* extract inhibited neuroinflammation in lipopolysaccharide (LPS)-activated BV-2 microglial cells, reducing key inflammatory mediators, including nitric oxide and TNF- α . Additionally, *P. major* has been shown to selectively inhibit monoamine oxidase A and B (Akincioglu et al., 2019) and reduce amyloid fibril production (Shiri et al., 2023). Recent research by Akincioglu et al. (2025) further validated its neuroprotective potential, with methanol extracts demonstrating inhibition of acetylcholinesterase, butyrylcholinesterase, and MAO. Moreover, *P. major* exhibits antidiabetic effects, which further support its potential for treating neurodegenerative disorders.

To explore the mechanism of action of multicomponent phytochemicals in *P. major*, the network pharmacology (NP) approach offers a comprehensive

projection. This approach has gained interest in the field of medicinal plant research. At the same time, it enables the analysis of complex relationships between plant metabolites and their biological targets, providing a more comprehensive picture of the mechanisms (Noor et al., 2022). Additionally, technologies such as high-resolution mass spectrometry (HRMS) enable the untargeted metabolomics analysis of *P. major* extracts to identify key bioactive compounds (Karomah et al., 2023). The results can facilitate validation through molecular docking to predict metabolite interactions with key proteins associated with Alzheimer's (Noor et al., 2022).

Despite the increasing interest in botanicals for AD therapy, current candidates such as *G. biloba* have demonstrated safety concerns, including adverse drug reactions and mutagenic constituents. These limitations underscore the necessity to explore alternative medicinal plants with multi-target neuroprotective properties. *P. major*, long known for its ethnopharmacological use in wound healing and inflammation, contains metabolites with potential neuroinflammatory modulation. However, its mechanism of action in AD remains poorly defined. This study addresses this gap by integrating metabolomics and systems biology tools to elucidate its mechanistic relevance.

MATERIAL AND METHODS

Preparation of *P. major* extract

This stage aims to provide *P. major* extract and to analyze its metabolites. A total of 2 kg of *P. major* leaves were collected from the Balai Pengkajian Teknologi Pertanian (BPTP), Bogor, Indonesia, at a maximum plant age of 30 days. The collection site was located in the vicinity of the Kebun Raya Bogor at coordinates 6°35'52"S, 106°47'44"E, with an altitude of 263 m above sea level. Taxonomic identification of the plant material was carried out by the Unit Pelayanan Farmasi (UPF) YANKESTRAD, RSUP Dr. Sardjito, Tawangmangu, Indonesia, with the test reference number TL.02.04/D.XI.5/16536.268/2023. The Ultrasonic-Assisted Extraction (UAE) was selected based on prior optimization of extraction conditions for antioxidant and anti-inflammatory activity. Precisely 1.186 g of dried *P. major* leaves was extracted with 70% ethanol at a solid-to-solvent ratio of 1:5 (g/mL) under controlled conditions (20 °C, 20 min) using a bath sonicator operating at 40 Hz (B-ONE, Indonesia). Following extraction, the filtrate was concentrated by rotary evaporation at 60°C to yield the concentrated crude extract.

Untargeted metabolomic analysis of *P. major* extract

An untargeted metabolomics workflow was applied to characterize the chemical profile of the concentrated crude extract by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), using a Thermo Scientific Dionex UltiMate 3000 RSLCnano UHPLC system coupled to a Thermo Scientific Q Exactive instrument. This approach was used to detect putative bioactive metabolites in the concentrated extract (La Basy et al., 2024). The mobile phase used consisted of 0.1% formic acid (A) and methanol (B). The stationary phase used was a Hypersil Gold analytical column (50 mm × 1 mm × 1.9 µm) (Thermo Fisher Scientific, USA) with a temperature of 30°C, a flow rate of 1 mL/min, an injection volume of 5 µL, and an isocratic elution with an analysis time of 25 min. The untargeted screening was performed using full MS/dd-MS² acquisition mode over the range 66.7–1000 m/z. The resolutions used were 70,000 for full MS and 17,500 for dd-MS², with both positive and negative ionization. Raw data were processed with Thermo Scientific Xcalibur software (version 4.4). During data curation, the biological relevance of detected metabolites to the plant matrix was used as a selection criterion; putative contaminants (e.g., phthalate plasticizers) and primary metabolites (e.g., carbohydrates) were excluded from the list of bioactive metabolites.

ADMET study

This stage served as the initial selection process for *P. major* metabolites prior to network analysis. Selected LC-HRMS data were used as the basis for identifying potential bioactive metabolites. The ADMET properties of these compounds were predicted using three independent platforms: SwissADME (Daina et al., 2017), Deep-PK (Myung et al., 2024), and ADMETlab 3.0 (Fu et al., 2024). Using multiple databases allowed cross-validation of pharmacokinetic and toxicity predictions, thereby increasing the robustness of the analysis.

The evaluation included compliance with Lipinski's Rule of Five (ROF), oral bioavailability, blood-brain barrier (BBB) permeability, P-glycoprotein (P-gp) inhibition, cytochrome P450 (CYP2D6 and CYP3A4) inhibition, drug-induced liver injury (DILI), and mutagenic potential. ROF compliance was applied as the primary selection criterion to define "drug-like" metabolites, whereas the other parameters were used for comprehensive ADMET characterization. The integration of toxicity predictions (hepatotoxicity and mutagenicity) complemented the pharmacokinetic profiles and provided an additional layer of evaluation.

The outcome of this stage was the identification of *P. major* metabolites that met drug-likeness criteria

based on ROF compliance. These selected metabolites, together with their ADMET evaluation results from the three databases, were subsequently advanced to NP analysis.

Data mining and target identification

This stage aimed to identify potential molecular targets of *P. major* bioactive metabolites for application in AD through an integrated NP and molecular docking approach. AD-related targets were retrieved from databases including the Comparative Toxicogenomics Database (CTD) (Davis et al., 2023), NCBI, and GeneCards (Safran et al., 2021). Prediction of potential targets for drug-like bioactive metabolites was carried out using SwissTargetPrediction (Daina et al., 2019) and TargetNet (Yao et al., 2016). The overlapping targets between metabolite-associated targets and AD-related targets were identified and visualized using InteractiVenn (Heberle et al., 2015). Subsequently, the UniProt database was employed to filter and ensure that the selected targets were specific to *Homo sapiens*. The final set of targets was then prioritized for further validation as candidate bioactive targets of *P. major* in the context of AD.

Network construction and pathway analysis

This step aims to understand the relationships among key metabolites of *P. major*, targets, and biological pathways involved in Alzheimer's disease. The plant-metabolite-target (PMT) network was constructed in Cytoscape (Otasek et al., 2019), with key targets identified based on degree centrality. Protein-protein interactions (PPI/PPIs) were identified through STRING with high confidence values (0.9) (Szklarczyk et al., 2023). Network topology analysis was performed in Cytoscape to determine elements with the most significant scores. Biological pathway analysis was performed through WebGestalt (Liao et al., 2019), and the results were visualized using the scientific research plot (SRPlot).

Molecular docking studies

This stage aims to assess how *P. major* metabolites interact with molecular targets. A total of three key targets from PPI, which have the highest degree, were selected as target proteins for molecular docking based on their involvement in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, relevance supported by the scientific literature, and the availability of corresponding structural data in the Protein Data Bank (PDB). Protein structure preparation was performed using UCSF Chimera (Pettersen et al., 2004), while flexible docking simulations were carried out using AutoDock Vina software integrated into UCSF Chimera (Eberhardt et al., 2021). The grid box was constructed

based on the location of the active site of the target protein that binds to the native ligand (e.g., saracatinib for proto-oncogene tyrosine-protein kinase Src (SRC) (PDB ID: 2H8H), 4Q2 for PIK3R1 (PDB ID: 4ZOP), and norathyriol for MAPK1 (PDB ID: 3SA0)). Reference drugs (saracatinib and dasatinib) were also included as benchmarks for docking-affinity comparisons. Binding affinity scores were predicted using AutoDock Vina and visualized with SRPlot. To ensure simulation validity, redocking was performed by comparing the native ligand position with the simulation results. Valid results were obtained if the root-mean-square deviation (RMSD) value is $<2.00 \text{ \AA}$ (Lieanto et al., 2022).

Interaction analysis and visualization

This step focuses on visualizing and evaluating the interaction pattern between proteins and ligands to ensure that the identified targets are biologically relevant. Interactions were evaluated using BIOVIA Discovery Studio Visualizer 2024 Client software. Binding patterns were analyzed using the Protein-Ligand Interaction Fingerprinting (PLIF) method, and the results were visualized using SRPlot. This representation provides an in-depth view of the interactions of bioactive metabolites, native ligands, and reference drugs with amino acid residues in the target protein.

Prioritization of bioactive metabolites from *P. major* in Alzheimer's disease therapy

This stage aimed to prioritize the relative influence of metabolites present in *P. major* extract on their potential activity in AD therapy. The method employed was the Simple Additive Weighting (SAW) approach with max-min normalization (Vafaei et al., 2022). The model incorporated metabolite abundance (peak areas) and predicted binding affinities (docking scores) for three key protein targets: SRC, PIK3R1, and MAPK1. The outcome of this stage was the identification of five key metabolites with the highest SAW scores, indicating their potential as major contributors to the anti-neuroinflammatory activity of *P. major*.

Ethical statement

No human or animal subjects were involved in this study; therefore, ethical approval was not required.

RESULTS

Bioactive metabolites and Lipinski's Rule of Five (ROF) screening

The extraction process resulted in a yield of 20.1% yield. A total of 220 metabolites were identified (Supplementary Data 1) from the extract through untargeted metabolomics analysis, of which 17 bioactive

metabolites relevant to *P. major* were selected from the total identified metabolites (Table 1). Further screening based on the ROF criteria, which include molecular weight ≤ 500 Da, $\log P \leq 5$, hydrogen bond donors ≤ 5 , and hydrogen bond acceptors ≤ 10 , resulted in 15 metabolites that met the drug-likeness requirements. These drug-like metabolites were then used in the next stage of the study. In contrast, metabolites such as calceolarioside B and hellicoside did not meet the ROF criteria and were therefore excluded.

A total of 17 metabolites were categorized as bioactive metabolites in this study. The fragmentation patterns of these metabolites were annotated against two databases, namely mzCloud and ChemSpider, with the inclusion criterion requiring at least one annotation from either database to display a full match, thereby ensuring that the identified compounds were truly present (Table 1 and Supplementary Data 2). In addition, contaminant compounds and primary metabolites were excluded from the analysis. Furthermore, each metabolite was cross-checked in PubChem to confirm its occurrence in plants and to highlight its potential biological activities. This combined annotation strategy has been widely applied in untargeted metabolomics studies to reduce false positives, and adopting a minimum full-match criterion enhances the reliability of metabolite identification in the absence of authentic reference standards.

The ADMET analysis (Table 1 and Supplementary Data 4) indicated that, in general, all compounds exhibited favorable predicted bioavailability, except for those that failed the ROF screening, such as hellicoside and calceolarioside B. With regard to predicted BBB permeability, only ursolic acid, α -linolenic acid, 4-coumaric acid, asiatic acid, ferulic acid, benzaldehyde, and the reference drugs saracatinib and dasatinib showed favorable distribution in at least one database. Several metabolites, including aucubin, calceolarioside B, musaenosidic acid, asiatic acid, and benzaldehyde, were predicted to act as potential P-gp inhibitors, potentially increasing the systemic exposure of co-administered drugs by altering their absorption and distribution. This effect was predicted to be more pronounced for the reference drugs saracatinib and dasatinib, as they were positive in two independent databases. In terms of metabolic stability, almost all *P. major* bioactive metabolites showed no inhibitory potential toward CYP2D6 or CYP3A4, except for scutellarein, luteolin, hispidulin, and gallic acid (CYP3A4 only), as well as the two reference drugs. On the other hand, nearly all *P. major* metabolites showed potential hepatotoxicity, as did the reference drugs. Notably, metabolites such as aucubin, caffeic acid, geniposide, geniposidic acid,

p-coumaric acid, asiatic acid, ferulic acid, and benzaldehyde were predicted to be non-mutagenic, in contrast to the reference drugs, which exhibited mutagenic potential.

PPI and analysis of key targets

Key targets for AD treated with *P. major* metabolites have been identified, as shown in Fig. 1.

A total of 455 overlapping targets from 25,760 AD-related targets (Supplementary Data 3) and 522 active metabolite targets of *P. major* (Fig. 1A) were identified. Data normalization using the UniProt database refined the list to 454 Alzheimer-*P. major* targets specific to the human species (Supplementary Data 4). These targets were then input into the STRING database to construct a protein-protein interaction (PPI) network (Fig. 1B).

The topology analysis of this network identified 10 key targets with the highest degree values, namely SRC, signal transducer and activator of transcription 3 (STAT3), PIK3R1, PIK3CA, PIK3CB, HSP90AA1, AKT1, MAPK1, epidermal growth factor receptor (EGFR), and MAPK3. This PPI network is provided in Supplementary Data 5 (Cytoscape file) and visualized in Fig. 1B-C. In addition, functional enrichment analysis identified 17 signaling pathways relevant to AD, selected from 154 pathways with a false discovery rate (FDR) of less than 0.01 (Fig. 1D and detailed table in Supplementary Data 4). Among these, KEGG pathways such as nuclear factor kappa B (NF- κ B) signaling, TNF signaling, Toll-like receptor signaling, PI3K-Akt signaling, and MAPK signaling exhibit strong relevance to neuroinflammation, as supported by previously reported literature (Almasoudi et al., 2025; Chu et al., 2021; Jayaraman et al., 2021; Momtazmanesh et al., 2020; Sun et al., 2022).

P-M-T network, analysis of key targets, and validation via molecular docking

The key metabolites identified from *P. major* extracts and their docking affinity scores relevant to AD therapy are illustrated in Fig. 2, whereas Table 2 summarizes the docking parameters applied in the study.

The plant-metabolite-target (PMT) network was constructed using data from 15 bioactive metabolites of *P. major* and 454 AD-related *P. major* targets (Fig. 1A). Network topology analysis identified 10 metabolites with the highest degree values: benzaldehyde (C14), gallic acid (C15), luteolin (C10), scutellarein (C7), α -linolenic acid (C4), caffeic acid (C2), ferulic acid (C13), hispidulin (C12), coumaric acid (C8), and ursolic acid (C3), respectively as shown in Fig. 2B (see also Supplementary Data 5, Cytoscape file).

Table 1. Bioactive metabolites identified in *P. major* leaf extract by UPLC-HRMS and their ADMET predictions based on three computational databases.

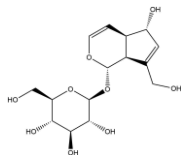
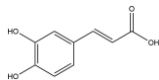
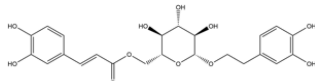
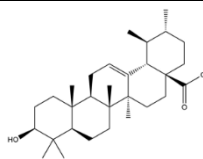
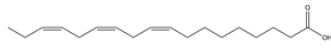
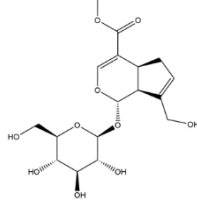
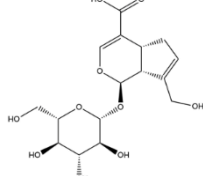
No	Name	Chemical structure	UPLC-HRMS				ADMET predictions							
			RT [min]	Ant. MZC (%BM)	Ant. CS	MP (%)	ROF #V *	BA score **	P-gp I **	BBB P **	CYP2D6 I**	CYP3A4 I**	DILI #	MT #
1	Aucubin (NP-009911)		1.227	FM (89.2)	FM	2.74331	1	1	1	0	0	0	1	0
2	Caffeic acid		6.092	FM (96.7)	FM	2.39397	0	2	0	0	0	0	2	0
3	Calceolarioside B		5.68	FM (98.7%)	PM	1.06282	2	0	1	0	0	0	1	2
4	Ursolic acid		14.561	FM (93.9)	FM	0.84479	1	2	0	2	0	0	2	1
5	α -Linolenic acid		14.58	FM (98.9)	F<	0.66005	1	3	0	1	0	0	0	1
6	Geniposide		4.961	FM (73.5)	FM	0.41367	0	1	0	0	0	0	1	0
7	Geniposidic acid		1.376	FM (86.3)	FM	0.38450	1	1	0	0	0	0	1	0

Table 1. Bioactive metabolites identified in *P. major* leaf extract by UPLC-HRMS and their ADMET predictions based on three computational databases (continued...)

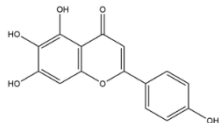
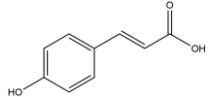
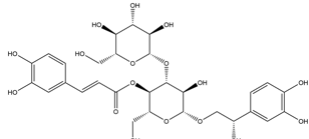
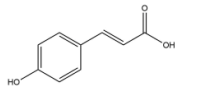
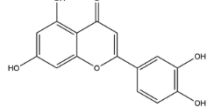
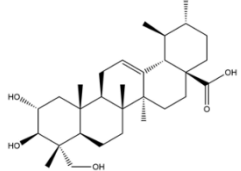
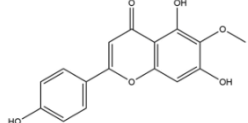
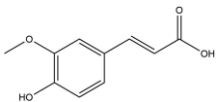
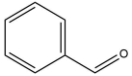
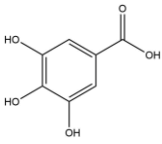
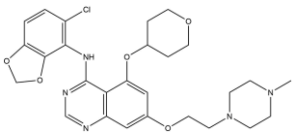
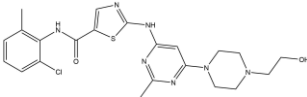
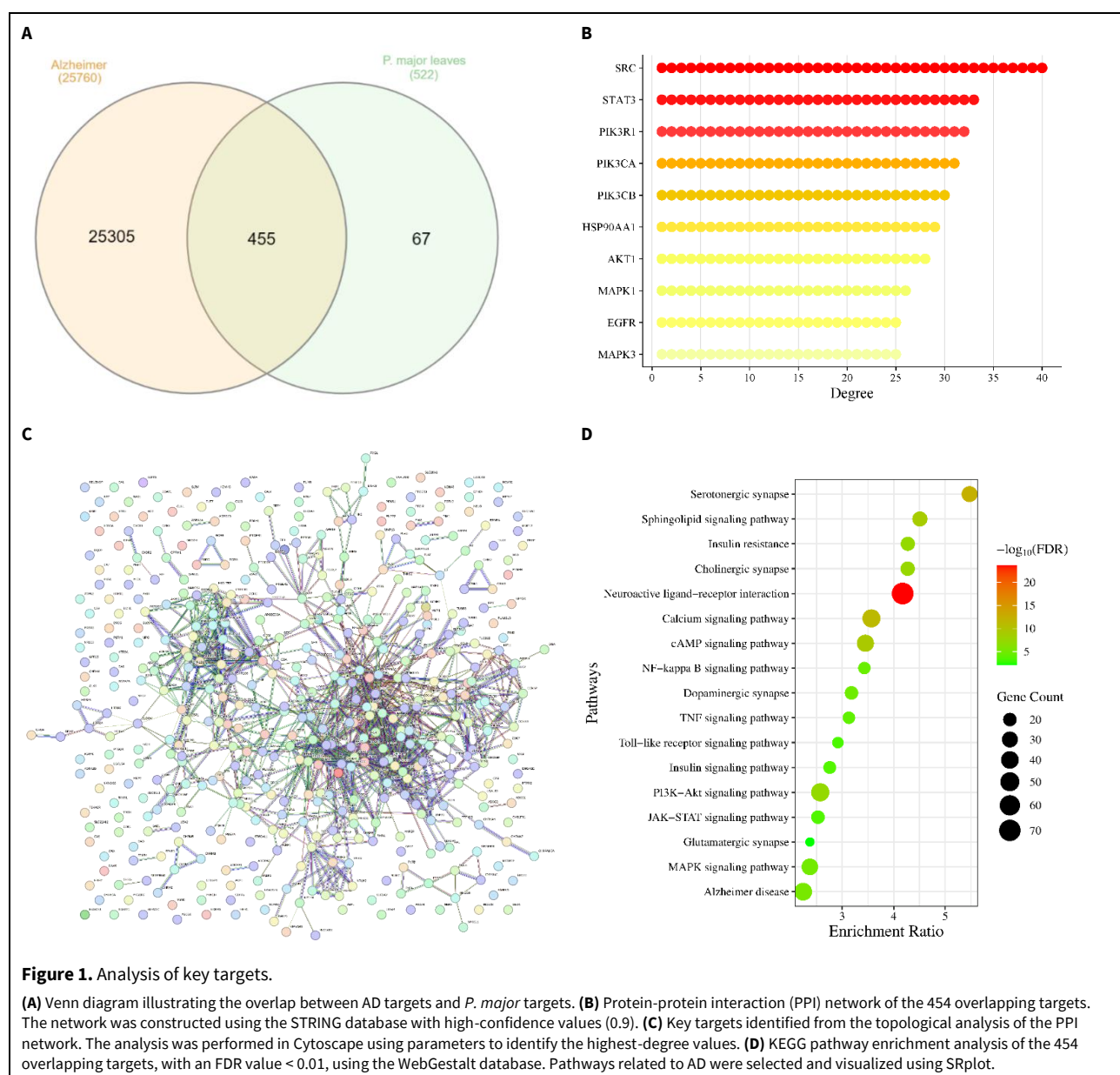
N o	Name	Chemical structure	UPLC-HRMS				ADMET predictions							
			RT [min]	Ant. MZC (%BM)	Ant. CS	MP (%)	ROF #V *	BA score **	P-gp I **	BBB P **	CYP2D6 I**	CYP3A4 I**	DILI #	MT #
8	Scutellarein (NP-013249)		5.953	FM (99.8)	NTH	0.26415	0	1	0	0	1	2	2	1
9	4-Coumaric acid		3.803	FM (92.7)	FM	0.25989	0	2	0	1	0	0	2	0
10	Hellicoside		4.863	NR (0)	FM	0.25315	3	0	0	0	0	0	1	1
11	Mussaenosidic acid		2.274	FM (82.7)	PM	0.13662	1	1	1	0	0	0	1	1
12	Luteolin		6.517	FM (83.8)	OM	0.10851	0	1	0	0	2	3	2	1
13	Asiatic acid		11.158	FM (93.6)	FM	0.07163	0	2	2	2	0	0	2	0
14	Hispidulin		7.955	FM (97.8)	NTH	0.03561	0	3	0	0	2	3	2	1

Table 1. Bioactive metabolites identified in *P. major* leaf extract by UPLC-HRMS and their ADMET predictions based on three computational databases (continued...)

N o	Name	Chemical structure	UPLC-HRMS				ADMET predictions							
			RT [min]	Ant. MZC (%BM)	Ant. CS	MP (%)	ROF #V *	BA score **	P-gp I **	BBB P **	CYP2D6 I**	CYP3A4 I**	DILI #	MT #
1 5	Ferulic acid		6.822	FM (80.4)	FM	0.03339	0	2	0	1	0	0	2	0
1 6	Benzaldehyde		4.968	NR (0)	FM	0.03172	0	3	1	3	0	0	0	0
1 7	Gallic acid		1.328	NR (0)	FM	0.02843	0	3	0	0	0	1	2	1
R 1	Saracatinib						1	3	2	2	2	3	1	2
R 2	Dasatinib (reference drug)						0	0	2	1	1	2	1	1

(*) Data were obtained from SwissADME. (**) Data were integrated from SwissADME, Deep-PK, and ADMETLab 3.0. (#) Data were derived from Deep-PK and ADMETLab 3.0.

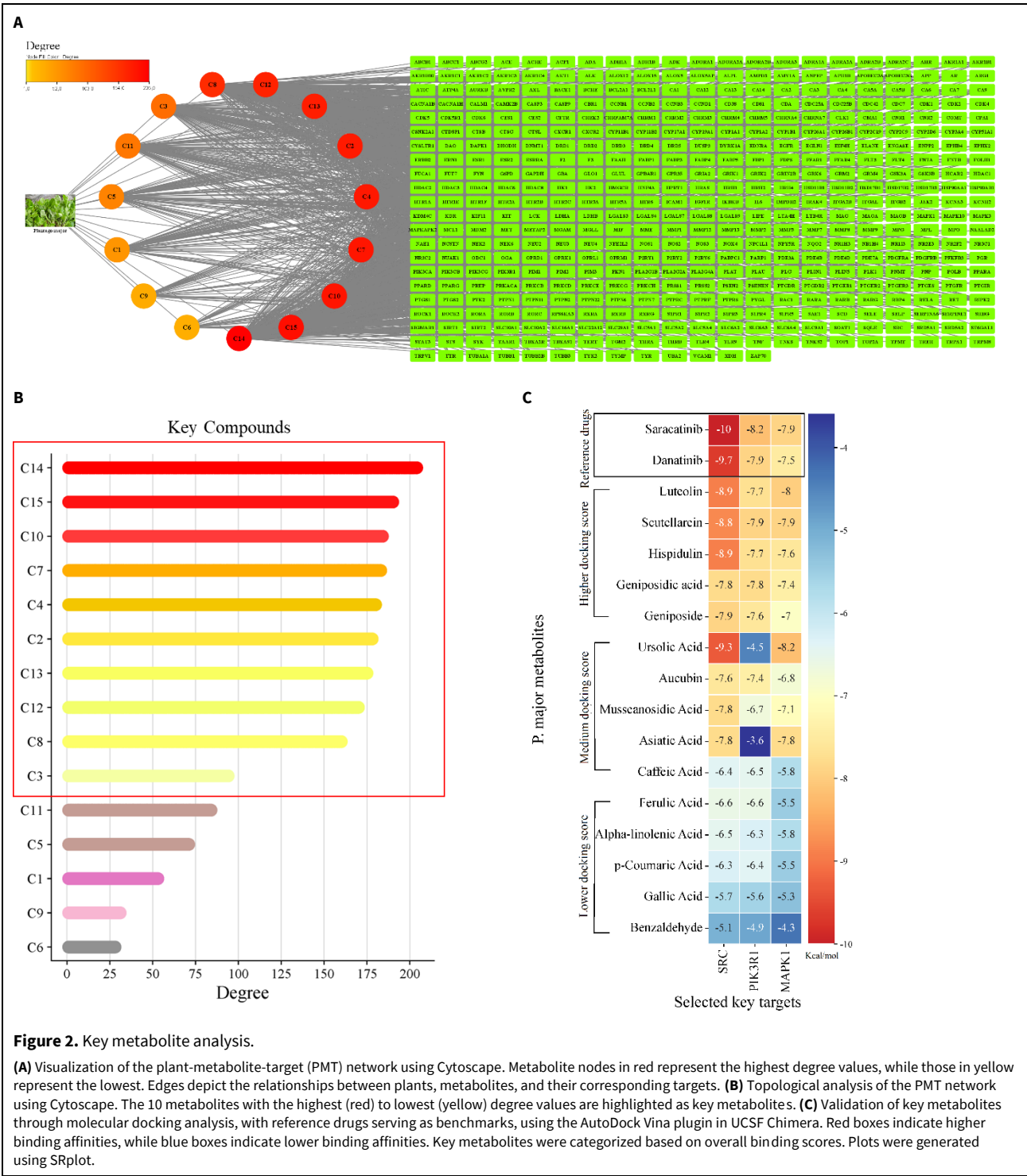
(RT, min) : retention time; Ant.MZC (%BM): Annotation mzCloud (% best match); Ant.CS: Annotation ChemSpider; MP (%): metabolite proportion; ROF V#: number of Lipinski's Rule of Five violations; BA: bioavailability score; P-gp I: P-glycoprotein inhibitors; BBB P: blood-brain barrier permeability; CYP2D6 I: Cytochrome P450 2D6 Inhibitors; CYP3A4 I: Cytochrome P450 2D6 Inhibitors; DILI: drug-induced liver injury; MT: mutagenesis; Scoring system in UPLC-HRMS: FM: full match, PM: partial match, NTH not the top hit, NR: no results. Scoring system in ADMET: 1: positive in one database, 2: positive in two, 3: positive in all three databases.



The results of this network were further validated through molecular docking analysis to ensure methodological reliability. From the 10 identified key targets, three proteins were selected for molecular docking analysis. This selection was based on the relevance of the enriched KEGG pathways supported by existing literature and the availability of structural data in public databases. Specifically, PIK3R1 and MAPK1 were chosen as representatives of their respective KEGG pathways that contribute to neuroinflammatory processes, while SRC, identified as the target with the highest degree value, was selected due to its upstream regulatory potential in multiple AD pathways and its role as a critical regulator of neurodegeneration (Portugal et al., 2022). The docking parameters are provided in Table 2, and the protocol was confirmed to be valid based on RMSD values < 2 Å. Moreover, the

strong predicted binding affinities, together with the reported IC₅₀ values for each native ligand, further support the robustness and reliability of this approach.

Molecular docking analysis revealed that several metabolites exhibited favorable docking scores (kcal/mol) against three key target proteins implicated in neuroinflammation in AD pathology, namely SRC, PIK3R1, and MAPK1 (Fig. 2C). Ursolic acid, hispidulin, and luteolin demonstrated docking scores for SRC that were slightly lower but comparable to the reference drug dasatinib (Figs. 2C and 3A). In contrast, scutellarein and geniposidic acid exhibited the strongest predicted binding affinity toward PIK3R1 (Figs. 2C and 3B). For MAPK1, ursolic acid and luteolin showed the most favorable binding interactions, consistent with high affinity values (Figs. 2C and 3C; Table 3).



Docking pose, PLIF analysis, and SAW prioritization

The docking pose results for the key target protein and PLIF analysis are shown in Fig. 3.

PLIF analysis of the SRC protein revealed that key residues frequently interacting with saracatinib and *P. major* metabolites include LYS295, ALA403, LEU393, and VAL281. Residue MET341 exhibited strong

interaction with saracatinib and also bound to luteolin, hispidulin, and ursolic acid. LYS295 interacted strongly with saracatinib, aucubin, geniposidic acid, and hispidulin. In addition, ASP404 showed strong interactions with gallic acid, benzaldehyde, ferulic acid, p-coumaric acid, scutellarein, caffeic acid, and aucubin; however, this interaction was absent in saracatinib (native ligand) but present in dasatinib (Fig. 3A).

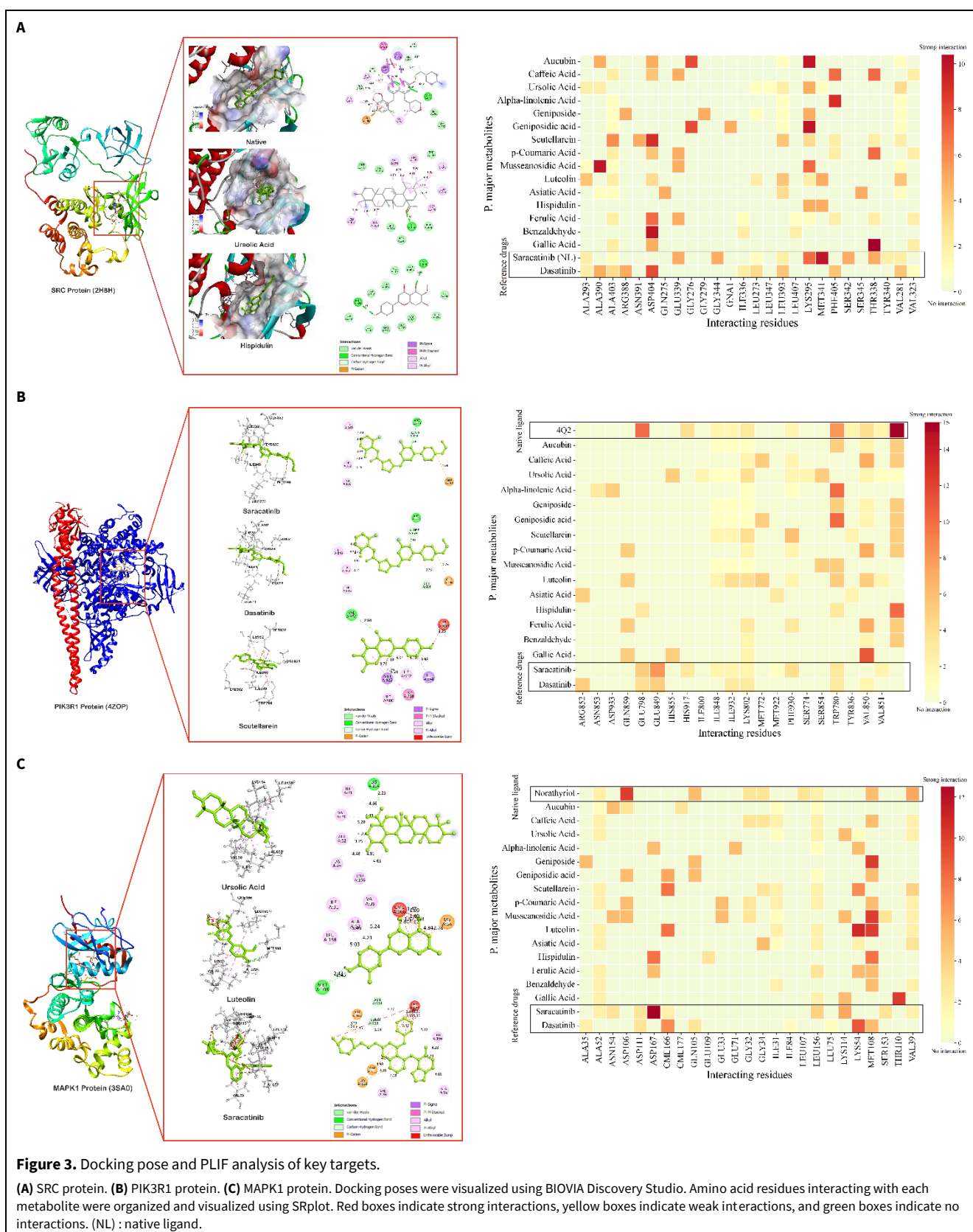
Table 2. Docking variables

Variable	SRC	PIK3R1	MAPK1
PDB code	2H8H	4ZOP	3SA0
Resolution of crystal structure (Å)	2.20	2.62	1.59
Docking software and scoring function	UCSF Chimera 1.17.3 with Autodock Vina v1.2.x plug-in	UCSF Chimera 1.17.3 with Autodock Vina v1.2.x plug-in	UCSF Chimera 1.17.3 with Autodock Vina v1.2.x plug-in
Protein preparation	DockPrep (Chimera)	DockPrep (Chimera)	DockPrep (Chimera)
Native ligand	Saracatinib (H8H)	(2s,3r)-N~1~-(8-Tert-Butyl-4,5-dihydro[1,3]thiazolo[4,5-H]quinazolin-2-yl)-3-methylpyrrolidine-1,2-dicarboxamide (4Q2)	Norathyriol (NRA)
Grid coordinates (Å)	x = 21, y = 21, z = 58	x = -2, y = 10, z = -16	x = -15, y = 14, z = 42
Grid size (Å)	x = 20, y = 20, z = 13	x = 15, y = 15, z = 15	x = 15, y = 15, z = 15
Exhaustiveness / Max energy difference (kcal/mol)	8 / 3	8 / 3	8 / 3
Redocking RMSD (Å)	1.773 (protocol validated)*	0.555 (protocol validated)*	0.778 (protocol validated)*
Docking score of native ligand (kcal/mol)	-10	-10.1	-7.2
Activity of native ligand	IC ₅₀ = 1–3 nM (n = 3 assays)	IC ₅₀ = 4–6 nM (n = 2 assays)	Not determined

Table 3. SAW analysis using peak area and docking score value.

Compound name	PA (a.u.)	Docking score			SAW with max-min normalization					R
		SRC	PIK3R1	MAPK1	PA (a.u.)	SRC	PIK3R1	MAPK1	FS	
Aucubin	1111354054	-7.6	-7.4	-6.8	1.000	0.595	0.884	0.641	0.780	1
Caffeic acid	969829159	-6.4	-6.5	-5.8	0.871	0.310	0.674	0.385	0.560	8
Ursolic acid	342234372	-9.3	-4.5	-8.2	0.301	1.000	0.209	1.000	0.627	6
Alpha-linolenic acid	267396015	-6.5	-6.3	-5.8	0.233	0.333	0.628	0.385	0.395	10
Geniposide	167583728	-7.9	-7.6	-7	0.142	0.667	0.930	0.692	0.608	7
Geniposidic acid	155768214	-7.8	-7.8	-7.4	0.131	0.643	0.977	0.795	0.636	5
Scutellarein	107011463	-8.8	-7.9	-7.9	0.087	0.881	1.000	0.923	0.723	2
p-Coumaric acid	105287012	-6.3	-6.4	-5.5	0.085	0.286	0.651	0.308	0.332	13
Musseanosidic acid	55348126	-7.8	-6.7	-7.1	0.040	0.643	0.721	0.718	0.530	9
Luteolin	43956917	-8.9	-7.7	-8	0.029	0.905	0.953	0.949	0.709	3
Asiatic acid	29016354	-7.8	-3.6	-7.8	0.016	0.643	0.000	0.897	0.389	11
Hispidulin	14425837	-8.9	-7.7	-7.6	0.003	0.905	0.953	0.846	0.677	4
Ferulic acid	13526341	-6.6	-6.6	-5.5	0.002	0.357	0.698	0.308	0.341	12
Benzaldehyde	12848452	-5.1	-4.9	-4.3	0.001	0.000	0.302	0.000	0.076	15
Gallic acid	11518339	-5.7	-5.6	-5.3	0.000	0.143	0.465	0.256	0.216	14

PA: Peak Area based on UPLC-HRMS analysis. FS: Final Score. R: Ranking.



For the PIK3R1 protein, key residues interacting with 4Q2 (native ligand) were identified as VAL851, TYR836, ILE932, and SER854. Among them, VAL851 formed strong interactions with both 4Q2 and *P. major* metabolites, particularly scutellarein and geniposidic

acid, which demonstrated high predicted binding affinity (Fig. 3B).

Analysis of MAPK1 revealed that MET108, LEU156, ALA52, and VAL39 were the main residues

interacting with norathyriol. Residue ASP106 displayed the strongest interaction with norathyriol and also bound to several *P. major* metabolites, including aucubin, geniposidic acid, p-coumaric acid, and musseanosidic acid (Fig. 3C).

The SAW analysis, which integrated peak area (reflecting metabolite abundance) with docking scores against three AD-related proteins (SRC, PIK3R1, and MAPK1), identified six metabolites predicted to play the most significant roles in the anti-neuroinflammatory activity of *P. major*. These were aucubin, scutellarein, luteolin, hispidulin, geniposidic acid, and ursolic acid, ranked in descending order. Aucubin achieved the highest rank primarily due to its remarkably high abundance in the *P. major* leaf extract; however, its binding affinities toward the three protein targets were comparatively weaker than those of scutellarein and luteolin. Overall, five of the top six metabolites (excluding aucubin and geniposidic acid) exhibited strong binding activity against SRC, with docking energies ranging from -8.8 to -9.3 kcal/mol (Table 3). Ursolic acid, which demonstrated the strongest predicted binding affinities toward both SRC (-9.3 kcal/mol) and MAPK1 (-8.2 kcal/mol), was able to compete effectively with the reference drugs and native ligands of these proteins, even showing stronger binding than saracatinib (-7.9 kcal/mol) (Fig. 2C) and norathyriol (-7.2 kcal/mol) (Table 2) for MAPK1. For PIK3R1, scutellarein showed a docking score of -7.9 kcal/mol, identical to that of dasatinib and slightly lower than saracatinib (-8.2 kcal/mol) (Fig. 2C). Collectively, this ranking underscores the relative contribution of individual metabolites to the predicted anti-neuroinflammatory activity of *P. major*. Importantly, the SAW method, which has been widely applied across various scientific disciplines for decision-making (Aliyeva et al., 2023; Arsyah et al., 2021; Chou et al., 2008; Kaliszewski & Podkopaev, 2016), may serve as a valuable framework for multi-target and multi-parameter prioritization in in silico pharmacological studies.

DISCUSSION

P. major, known in Indonesia as "Daun Sendok," is an herbal plant with a wide array of health benefits attributed to its antioxidant and anti-inflammatory properties (Adom et al., 2017). Evidence from pharmacological studies supports its potential as a novel therapeutic candidate for alleviating neuroinflammation in patients with AD. In the present study, an NP approach, informed by untargeted metabolomics data, was employed to systematically investigate this potential. Subsequent validation was performed through molecular docking and PLIF analyses to elucidate the molecular mechanisms underlying its therapeutic effects. Additionally, pharmacokinetic predictions were

conducted to assess the drug-likeness of each metabolite. Finally, metabolite prioritisation was carried out using the SAW method with max-min normalisation to assess their relative contributions to anti-neuroinflammatory activity.

Untargeted metabolomics analysis using UHPLC-Q-Orbitrap-HRMS detected the presence of putative metabolites from *P. major*, including aucubin, caffeic acid, ursolic acid, and α -linolenic acid. Through NP analysis, these bioactive metabolites were found to target several key signalling pathways involved in neuroinflammation. These pathways include phosphoinositide 3-kinase-protein kinase B (PI3K-AKT), MAPK, JAK-STAT, NF- κ B, as well as pathways specific to Alzheimer's based on gene enrichment analysis (Fig. 1D). This finding was further supported by PPI analysis, which revealed 10 hub proteins with high degree values, predominantly associated with neuroinflammatory processes in AD (Fig. 1B-C). These results are consistent with previous studies that have demonstrated the relevance of inflammatory proteins in Alzheimer's pathology, including SRC (Portugal et al., 2022), PIK3R1 (Chu et al., 2021), and MAPK1 (Almasoudi et al., 2025). Similarly, an NP investigation of *Scutellaria baicalensis* also identified SRC, PIK3R1, and STAT3 as key targets for inflammation in AD (Peng & Zhou, 2024). Neuroinflammation can activate beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) and amyloid precursor protein (APP), genes that promote the formation of amyloid beta, worsening AD (Heneka et al., 2024). These results align with previous literature, confirming the pharmacological relevance of *P. major* and strengthening its potential for AD therapy.

In this study's research methodology, the results of the NP analysis were validated by molecular docking and further complemented by ADMET predictions to assess the therapeutic potential of *P. major* metabolites. Molecular docking against three core targets highlighted five metabolites with higher binding affinities compared to others: luteolin, scutellarein, hispidulin, geniposidic acid, and geniposide (Fig. 2C). These findings were largely consistent with the NP analysis, where luteolin, scutellarein, and hispidulin ranked among the top 10 key metabolites with high degree values (Fig. 1D). An exception was observed with benzaldehyde (C14), which exhibited the highest degree value but the lowest docking score. Recent developments on benzaldehyde have demonstrated that several structural modifications (benzaldehyde derivatives) exhibit strong potential as therapeutic agents in the treatment of AD (Chen et al., 2023; Costa et al., 2020; Eno et al., 2022). This suggests that NP can broadly capture the activity spectrum of compounds but is less predictive of compound-specific efficacy,

highlighting the limitation of relying on this method alone.

To address this, the SAW method, a multi-criteria decision-making approach, was utilised to systematically evaluate which metabolites of *P. major* are most likely to contribute to neuroinflammatory activity. Although SAW has been widely used in decision-making studies (Aliyeva et al., 2023; Arsyah et al., 2021; Chou et al., 2008; Kaliszewski & Podkopaev, 2016), and even in virtual screening studies of *Azadirachta indica* bioactive metabolites (Anuradha & Jaganathan, 2025). To the best of our knowledge, this study is among the first to apply the SAW method in an *in silico* framework integrating multi-target molecular docking parameters. The analysis highlighted six metabolites with the highest final scores: aucubin, scutellarein, luteolin, hispidulin, geniposidic acid, and ursolic acid, respectively (Table 3).

Aucubin, identified in the present study as the most abundant metabolite in *P. major* (2.74331%, Table 1), has been previously reported by Wang et al. (2024) using a pharmacological network approach. Aucubin has demonstrated potential therapeutic effects in AD by inhibiting ERK1/ERK2 (MAPK1) phosphorylation within the MAPK signalling pathway. This inhibition has been demonstrated *in vitro* to reduce glial cell activation and decrease levels of pro-inflammatory mediators, including IL-1 β , IL-6, TNF- α , COX-2, and iNOS, while also inhibiting microglial activation. Furthermore, *in vivo* experiments show that aucubin suppresses neuroinflammation by inhibiting glial cell activation in the brains of memory-deficient mice. These findings are corroborated by Park et al. (2022), who demonstrated that aucubin induces an anti-neuroinflammatory response by reducing the expression of inflammatory mediators, including NO and PGE2, as well as pro-inflammatory mediators such as IL-1 β , IL-6, and TNF- α , in LPS-induced BV2 microglia and astrocytes. This effect is achieved by blocking the NF- κ B, PI3K/Akt, and MAPK signalling pathways.

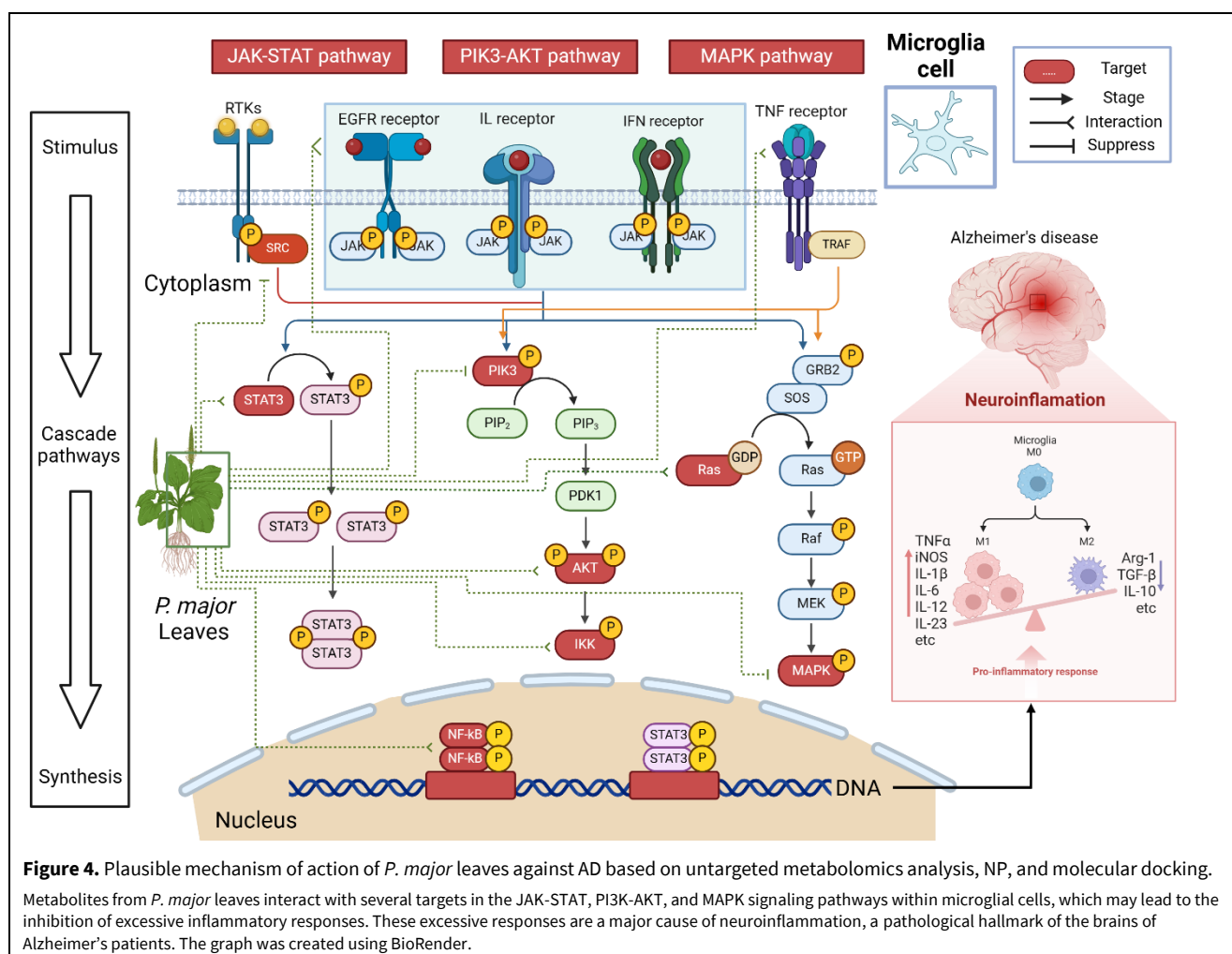
In contrast, ursolic acid, which achieved the highest docking score against SRC and MAPK1 in the present study, aligns with the findings of Shanmugam et al. (2011), who reported that ursolic acid effectively inhibits SRC, JAK2, STAT3, and NF- κ B signalling. Its ability to cross the BBB further enhances its therapeutic potential in treating neuroinflammatory conditions (Table 1). Additionally, ursolic acid has been shown to inhibit the phosphorylation of ERK, p38, and JNK kinases, which are integral components of the MAPK signalling pathway (Shan et al., 2009). Two other metabolites exhibit similar target actions. Ha et al. (2021) reported that scutellarein exhibits promising effects by modulating the PI3K-AKT, NF- κ B, and MAPK pathways,

thereby reducing neuroinflammatory responses in a preclinical AD model. This is consistent with Kou et al. (2022), who found that luteolin inhibits activation of the NF- κ B and p38 inflammatory pathways induced by endoplasmic reticulum stress, reducing levels of inflammatory cytokines, COX-2, and iNOS expression, and ultimately improving learning and memory abilities in 3 $\times$ Tg-AD mice.

The NP approach used in this study identified ten key protein targets, including SRC, PI3K1, MAPK1, and AKT, which play critical roles in the cellular mechanisms underlying the pharmacological effects of *P. major* metabolites in AD. These targets are involved in signalling pathways associated with neuroinflammation. Inhibition of SRC can reduce the release of pro-inflammatory factors from microglia. At the same time, EGFR activation affects key signalling pathways, such as PI3K-AKT, MAPK, and JAK-STAT, all of which are implicated in the development of neuroinflammation (Abdelhamed et al., 2016; Yang et al., 2020). Ursolic acid, a key metabolite of *P. major*, has been shown to inhibit EGFR phosphorylation and its downstream MAPK pathway, thereby suppressing PI3K and AKT phosphorylation, further strengthening its potential to address neuroinflammation (Zafar et al., 2022).

Luteolin has been shown to inhibit phosphorylation of key elements in the PI3K-AKT and MAPK pathways, including PI3K, AKT, and ERK1/2. Additionally, luteolin is also capable of blocking the activity of SRC and STAT3 (Lu et al., 2017). This activity supports the effectiveness of luteolin in reducing neuroinflammatory symptoms in a triple-transgenic mouse model of AD. Treatment with luteolin showed improved spatial learning and ameliorated memory deficits in a dose-dependent manner (Kou et al., 2022). Meanwhile, scutellarein (SCU) can suppress NF- κ B activation and reduce pro-inflammatory responses by inhibiting p65 phosphorylation and I κ B degradation in microglial cells (You et al., 2018; Zeng et al., 2018). These findings demonstrate the multitarget approach of scutellarein, luteolin, ursolic acid, and aucubin, making these metabolites very interesting candidates for further research.

The results confirmed that several bioactive metabolites in *P. major*, including aucubin, ursolic acid, scutellarein, and luteolin, exhibit significant anti-neuroinflammatory activity. These metabolites function by inhibiting microglial activation via three major interconnected signalling pathways: PI3K-AKT, MAPK, and JAK-STAT. Suppression of these pathways can reduce the production of pro-inflammatory factors by glial cells, which is known to be a primary cause of excessive neuroinflammation (Fig. 4).



This study has certain limitations. The NP validation was primarily conducted through molecular docking, which, as an *in silico* method, may inherently limit the direct translational applicability of the findings to the development of herbal preparations or metabolites. Moreover, the use of AutoDock Vina is not without caveats, including the rigid receptor approximation that neglects protein conformational flexibility, the reliance on a non-polarizable force field that oversimplifies complex molecular interactions, and the limited representation of π - π aromatic interactions, all of which may compromise the accuracy of ligand-target binding predictions (Sarkar et al., 2024). A similar limitation applies to the pharmacokinetic assessment of each compound, particularly in predicting BBB penetration. To strengthen the reliability of the findings, the docking protocol was validated through the evaluation of RMSD values, and the analysis was further extended to include ADME-toxicity predictions from two independent databases. However, it is acknowledged that the gold standard for metabolite identification remains confirmation through the use of authentic standards and MS/MS fragmentation spectra. (Sumner et al., 2007; Wishart, 2011). Nevertheless, in the present study, systematic annotation was performed using two

fragmentation databases (mzCloud and ChemSpider), with an inclusion criterion of at least one full match, which was achieved by almost all metabolites. Additionally, each compound was cross-checked in PubChem to confirm its presence in plants and to emphasize its reported biological activities. Likewise, experimental pharmacodynamic and pharmacokinetic validation remains to be addressed in future work; these complementary approaches provide a more robust basis for interpreting the current findings and supporting their potential relevance in subsequent experimental studies.

In addition, despite the promising pharmacological effects of aucubin, ursolic acid, luteolin, and scutellarin, their therapeutic potential is limited by stability and bioavailability challenges. Aucubin is unstable in acidic gastric fluids, exhibits poor absorption in the gastrointestinal tract due to its low lipophilicity, and undergoes significant first-pass metabolism. As a result, its bioavailability is higher after intraperitoneal administration (76.8%) than after oral administration (19.8%) (Kartini et al., 2023). Ursolic acid, due to its low water solubility, faces absorption limitations, and its rapid metabolism further reduces its systemic

availability, thereby restricting its clinical applicability (Mlala et al., 2019). Similarly, luteolin suffers from poor oral bioavailability, low water solubility, and extensive hepatic metabolism, all of which hinder its pharmacological efficacy (Mod Razif et al., 2024; Wu et al., 2024). Scutellarin is extensively metabolised to scutellarein in the gastrointestinal tract before absorption, and it is predominantly excreted in the faeces, resulting in very low oral bioavailability (Xing et al., 2011). These limitations underscore the need for optimised formulation strategies or alternative administration routes to enhance the therapeutic effectiveness of these metabolites in treating neuroinflammatory and neurodegenerative diseases.

Further studies are needed to validate these findings through experimental approaches, particularly in Alzheimer's animal models and cell-based assays, to confirm the effectiveness of *P. major*. Pharmacokinetic and pharmacodynamic experimental studies of key metabolites, such as using PAMPA-BBB or co-culture models, are also required to evaluate their ability to penetrate the BBB and their effects on the central nervous system. On the other hand, the development of pharmaceutical formulations that enhance the bioavailability and stability of these metabolites represents a crucial next step. Nanocarrier encapsulation technology can be explored to enhance the effectiveness, bioavailability, and central nervous system penetration of these metabolites in clinical applications (Iqbal et al., 2024).

CONCLUSION

The results of this study demonstrate that several bioactive metabolites of *P. major*, including aucubin, ursolic acid, scutellarein, and luteolin, may exert neuroprotective effects by modulating inflammation-associated signalling pathways implicated in AD. Specifically, these metabolites inhibit microglial activation through three major interconnected signalling pathways: the PI3K-AKT, MAPK, and JAK-STAT pathways. Suppression of these pathways reduces the production of pro-inflammatory mediators by glial cells, which is a key driver of excessive neuroinflammation. Collectively, these findings support the therapeutic potential of *P. major* as a candidate for the development of anti-neuroinflammatory drugs. However, further experimental and formulation studies are required to confirm its efficacy and bioavailability.

The reliance on molecular docking methods requires further experimental validation. This study provides an important foundation for developing a suitable model for *in vitro* and *in vivo* studies. Given the urgency of bioactive metabolites crossing the BBB, nanomaterial-based technologies are a promising approach

to improve the bioavailability and therapeutic efficacy of these metabolites from the *P. major* extract.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used Grammarly Inc. to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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AUTHOR CONTRIBUTION:

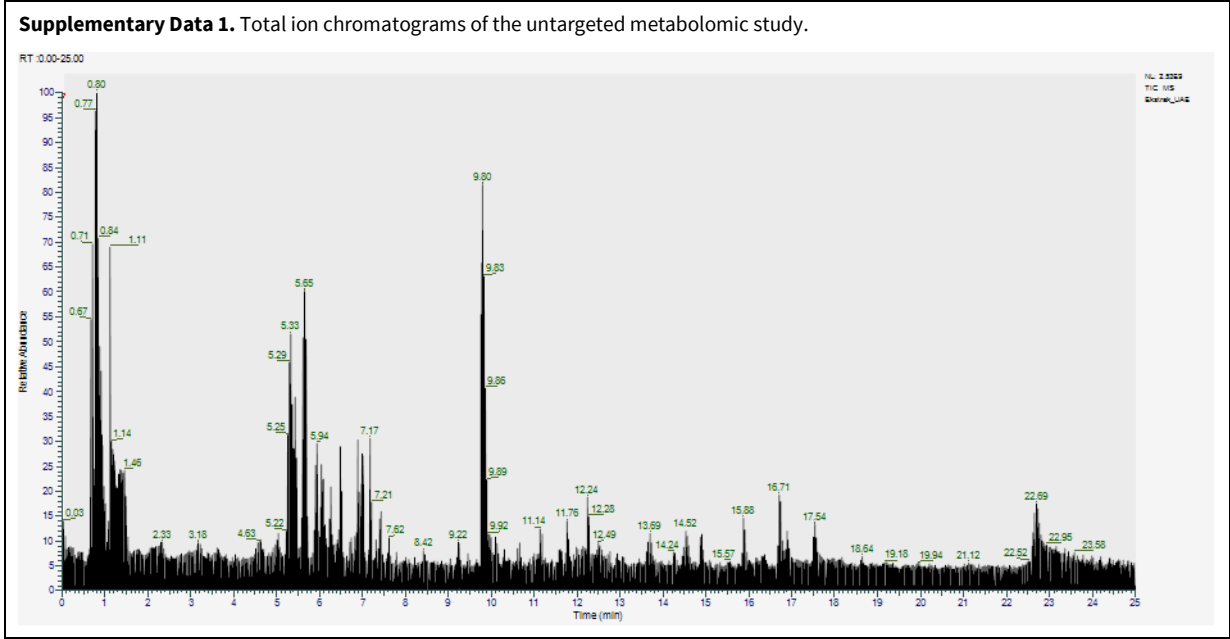
Contribution	Hertiani T	Khafi M	Ikawati Z	Ujiantari NSO	Marzuki A
Concepts or ideas	x	x			
Design	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x
Literature search		x		x	x
Experimental studies	x	x		x	
Data acquisition	x	x		x	
Data analysis	x	x		x	
Statistical analysis	x	x		x	
Manuscript preparation	x	x			
Manuscript editing	x	x			x
Manuscript review	x	x	x	x	x

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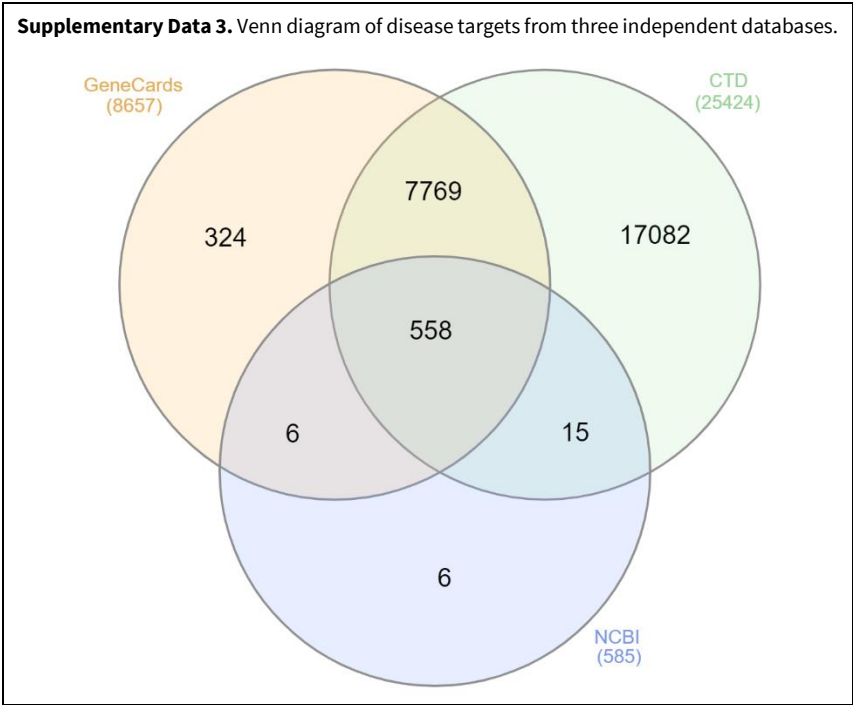
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Supplementary data



Supplementary Data 2. Raw data of UPLC-HRMS analysis provided by BRIN, Indonesia.

<https://data.brin.go.id/privateurl.xhtml?token=ca783d48-5397-4c32-bbbe-2ff65297cbb5>



Supplementary Data 4. Excel file containing ADMET profiling, KEGG enrichment analysis, and UniProt ID mapping.

<https://docs.google.com/spreadsheets/d/15eNaEf9LLxlyIngLaTlj4RBTPGysHlV/edit?usp=sharing&ouid=110627999812463258956&trtpof=true&sd=true>

Supplementary Data 5. Cytoscape file of network pharmacology and PPI analysis.

<https://drive.google.com/drive/folders/13mNc9BWWQcysL1l0OqgBdzFlibVC-f0?usp=sharing>