

Integrated LC-HRMS Analysis, Network Pharmacology, and Molecular Docking to Predict the Potential of *Syzygium polyanthum* in Alleviating Rheumatoid Arthritis

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ABSTRAK

Rheumatoid arthritis (RA) adalah penyakit autoimun kronis dengan hasil terapi yang terbatas dan efek samping yang signifikan terkait dengan pengobatan saat ini. Studi ini menggunakan LC-HRMS, farmakologi jaringan, dan docking molekuler untuk menganalisa senyawa bioaktif dan mekanisme dasar Syzygium polyanthum sebagai terapi komplementer potensial untuk RA. Ekstrak diperoleh dengan metode maserasi menggunakan etanol 90% selama 2x24 jam. Profiling metabolit menggunakan LC-HRMS mengidentifikasi tujuh senyawa kandidat dengan kelimpahan relatif lebih dari 1%, di mana 2,6-dimethylquinoline memiliki kelimpahan paling tinggi (5,77%). Farmakologi jaringan mengungkapkan 121 target tumpang tindih antara target RA dan senyawa-senyawa ini, dengan PTGS2 dan MAPK14 dipilih untuk docking molekuler terhadap tiga ligan representatif: 2,6-dimethylquinoline, 6-methylquinoline, dan myricetin. Myricetin menunjukkan afinitas ikatan terkuat terhadap PTGS2 dan MAPK14, dengan energi ikatan masing-masing -8,915 dan -7,849 kcal/mol. Hasil studi menunjukkan bahwa ekstrak etanol S. polyanthum (EESP) memiliki potensi terapeutik untuk mengurangi inflamasi pada RA.

Kata Kunci: Daun Salam, Inflamasi, Penyakit Autoimun, Siklooksigenase 2, Hasil Terapi

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease with limited therapeutic outcomes and considerable adverse effects associated with current treatments. This study employed LC–HRMS, network pharmacology, and molecular docking to investigate the bioactive compounds and underlying mechanisms of *Syzygium polyanthum* as a potential complementary therapy for RA. The extract was obtained by maceration using 90% ethanol for 2x24 hours. Metabolite profiling by LC–HRMS identified seven candidate compounds with relative abundance greater than 1%, of which 2,6-dimethylquinoline was the most abundant (5.77%). Network pharmacology revealed 121 overlapping targets between RA and these compounds, with PTGS2 and MAPK14 selected for molecular docking against three representative ligands: 2,6-dimethylquinoline, 6-methylquinoline, and myricetin. Myricetin exhibited the strongest binding affinity to PTGS2 and MAPK14, with binding energies of –8.915 and –7.849 kcal/mol, respectively. These findings suggest that *S. polyanthum* ethanol extract (EESP) possesses therapeutic potential in attenuating RA-related inflammation.

Keywords: Bay Leaf, Inflammation, Autoimmune Disease, Cyclooxygenase 2, Treatment Outcome

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by persistent synovial inflammation, bone erosion, and progressive cartilage destruction, ultimately leading to joint deformity and disability. Its multifactorial etiology involves genetic susceptibility, infectious triggers, and environmental influences. Clinically, RA manifests as pain in the shoulders, neck, and pelvic girdle, accompanied by systemic symptoms such as fatigue, malaise, and weight loss (Uke et al., 2025). The global prevalence of RA is estimated at 0.5–1%, remaining relatively stable across populations. In Indonesia, data from the National RA Registry (2019–2020, across 16 institutions) reported a

remission rate of only 24.5%, underscoring the need for more effective and safer therapeutic strategies (Hidayat et al., 2021). Current pharmacological management primarily relies on disease-modifying anti-rheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs). Although these agents improve symptoms and slow disease progression, their long-term use is frequently associated with adverse effects, highlighting the urgent demand for alternative or complementary therapeutic options (Kciuk et al., 2024).

Medicinal plants have long been recognized as valuable sources of bioactive compounds with many therapeutic potentials. (Samy et al., 2025). Among them, *Syzygium polyanthum* (Indonesian

bay leaf), a member of the Myrtaceae family widely used in Indonesian and Malaysian cuisine, has gained attention for its medicinal properties. Traditionally, it has been employed to manage diabetes, infections, inflammation, hyperuricemia, osteoarthritis, and gastrointestinal disorders (Darussalam & Rukmi, 2019; Malik & Ahmad, 2013; Rochmat et al., 2022; Syabana et al., 2021). Phytochemical studies reveal that the 96% ethanol extract of *S. polyanthum* using ultrasound-assisted extraction contains abundant flavonoids, phenolic compounds, and antioxidants, which may contribute to its anti-inflammatory activity (Renny et al., 2023).

In recent years, network pharmacology has emerged as a powerful interdisciplinary approach for elucidating the complex interactions between bioactive compounds, molecular targets, and signaling pathways. Integrating bioinformatics with pharmacological evidence enables a systems-level understanding of drug-disease relationships. When combined with molecular docking, it allows for the prediction of ligand–target interactions at the structural level, strengthening the mechanistic basis for drug discovery (Yang et al., 2025).

The 90% ethanol extract of *S. polyanthum*, obtained through maceration, is suspected to contain secondary

metabolites with potential activity against rheumatoid arthritis (RA). However, analysis of its constituents using liquid chromatography–high resolution mass spectrometry (LC-HRMS) and evaluation of its anti-inflammatory potential for RA through pharmacological network prediction and molecular docking have not yet been conducted.

Building upon these advances, the present study employs liquid chromatography–high resolution mass spectrometry (LC-HRMS) to identify the phytochemical constituents of *S. polyanthum* extract, followed by integrated network pharmacology and molecular docking analyses to investigate their potential mechanisms against RA. This integrated approach provides novel insights into the molecular basis of the anti-inflammatory effects of *S. polyanthum*, thereby offering a scientific foundation for its development as a complementary therapeutic agent in RA management.

II. METHODS

A. Extraction

Powdered leaves of *S. polyanthum* (Mesh 60) were obtained from the Herbal Center of Merapi Farma, Yogyakarta, Indonesia, and authenticated at the Department of Pharmaceutical Biology, Universitas Gadjah Mada (Voucher No.

36.88/UN1/FFA.2/BF/PT/2023) by Dr. Joko Santoso, Apt.

Powdered leaves of *S. polyanthum* were extracted twice by maceration with 90% ethanol (5:1, v/w). The filtrates were evaporated using a rotary evaporator at 50°C, and concentrated to obtain the crude ethanol extract using a waterbath, and stored at 2–8 °C for further analysis.

B. Phytochemical Screenings

Each extract underwent a qualitative phytochemical screening, including assays for alkaloids, carbohydrates, tannins, saponins, phenolics, flavonoids, triterpenes, and diterpenes according to standard methods.

C. LC-HRMS Analysis

The phytochemical profiling of ethanol extract of *S. polyanthum* (EESP) was performed with a Thermo Scientific™ Vanquish™ Horizon UHPLC along with a Thermo Scientific™ Orbitrap™ Exploris 240 HRMS. Separation was accomplished using an Accucore™ Phenyl Hexyl column (100 × 2.1 mm, 2.6 µm) with a gradient elution of water (0.1% formic acid) and acetonitrile (0.1% formic acid) at a flow rate of 0.3 mL/min over a duration of 25 minutes. Mass spectrometry was conducted in both positive and negative ion modes with polarity swapping, obtaining complete MS (m/z 70–800) and dd-MS² data with H-

ESI. Compound annotation was conducted using Compound Discoverer™ 3.3, utilizing the mzCloud and ChemSpider databases.

D. Network Pharmacology

The selected bioactive compounds identified from the ethanol extract of *S. polyanthum* (EESP) by LC-HRMS were first converted into canonical SMILES format using PubChem (<https://pubchem.ncbi.nlm.nih.gov>).

Potential protein targets of these compounds were predicted using TargetNet (<http://targetnet.scbdd.com/>). However, RA-related genes were retrieved from GeneCards (<https://www.genecards.org/>) and NCBI (www.ncbi.nlm.nih.gov). Overlapping targets between *S. polyanthum* compounds and RA-related genes were identified using a Venn diagram (www.interactivenn.net) to obtain potential targets of RA (Heberle et al., 2015).

The compound–target interaction network was constructed and visualized in Cytoscape 3.9.1 (<https://cytoscape.org/>). Protein–protein interaction (PPI) analysis was performed using STRING database version 11.5 (<https://string-db.org/>), with the species set to *Homo sapiens* and a confidence score >0.9. The PPI network was further analyzed in Cytoscape using the CytoHubba plugin to identify hub genes

based on degree centrality (Shannon et al., 2003).

Moreover, pathway enrichment analysis, including Gene Ontology (GO: biological process, cellular component, molecular function) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, was conducted using Web-based Gene Set Analysis Toolkit (www.webgestalt.org), and enrichment results were considered significant at $p < 0.05$ (Elizarraras et al., 2024).

E. Molecular Docking Study

The 3D structure of the target proteins was retrieved from the Protein Data Bank (PDB) and prepared using PyMOL by removing water molecules, adding polar hydrogens, and optimizing receptor and ligand structures. The selected ligands identified from LC-HRMS and receptors were prepared and converted into pdbqt format using AutoDock Tools 1.5.7. Docking simulations were performed with the grid box centered at coordinates (x,y,z) and dimensions adjusted to cover the active site region. Cross-docking validation was carried out by re-docking the native ligand, and the resulting root mean square deviation (RMSD) values $< 2.0 \text{ \AA}$ were considered reliable. Binding affinities (ΔG , kcal/mol) were calculated from Vina output, and the docking interactions were further analyzed and visualized in both 2D

and 3D using BIOVIA Discovery Studio Visualizer 2024.

III. RESULTS AND DISCUSSION

A. Qualitative Phytochemical Screenings

Ethanol extract of *S. polyanthum* contained flavonoids, tannins, alkaloids, saponins, phenols, diterpenes, triterpenes, and carbohydrates. Those phytochemicals play a role in antioxidant activity and improving the amelioration of RA, such as inhibiting the release of tumour necrosis factor α (TNF α), interleukin 6 (IL-6), and interleukin 17 (IL-17) release (Kang et al., 2022; Shan et al., 2019). Alkaloids may diminish synovial hyperplasia, while saponins can inhibit angiogenesis and osteoclastogenesis (Liang et al., 2016; Song et al., 2022). Moreover, triterpene also increases superoxide dismutase activity to reduce oxidative stress, and diterpenes can modulate the expression of matrix metalloproteinases (MMPs) that play a role in cartilage damage (Islam et al., 2022). The active compounds in EESP may enhance the effectiveness in treating RA.

B. Phytochemical Analysis using LC-HRMS

LC-HRMS analysis of EESP yielded a clear base peak chromatogram with well-resolved signals spanning a retention time of 0–25 minutes (Figure 1). Data acquired in both positive and negative

ionization modes provided complementary metabolite coverage, with a mass accuracy of <5 ppm, ensuring precise and reliable molecular feature identification. Compounds were selected based on percentage of relative abundance (%RA $\geq$ 1.0) and cross-referenced with the database of natural compounds, coconut (www.coconut.naturalproducts.net), as well as previous reports being potential natural product metabolites, as listed in Table I. 2,6-Dimethylquinoline is the most abundant natural metabolite in EESP. All compounds analyzed by LC-HRMS are presented in Supplementary 1. The quinoline group has been shown to attenuate the inflammatory response of fibroblast-like synoviocytes (FLS) stimulated by TNF- α , thereby exerting anti-inflammatory effects in RA. Similar to other quinoline derivatives, 6-Methylquinoline has been reported to

inhibit COX-2 activity, thereby modulating the inflammatory response (Yang et al., 2024). Moreover, myricetin, a compound from the Myrtaceae family, demonstrates synergistic activity with the COX-2 inhibitor celecoxib, reducing neutrophil extracellular trap (NET) release and consequently alleviating inflammation in RA (Shu et al., 2024). The relationship between the abundance of natural product compounds and their biological activity is complex and context-dependent (Susanti et al., 2025). Therefore, we selected three compounds—2,6-dimethylquinoline, 6-methylquinoline, and myricetin—as chemical markers, and subsequently subjected them to network pharmacology and in silico molecular docking analyses, supporting their potential as candidate molecules for further pharmacological investigation.

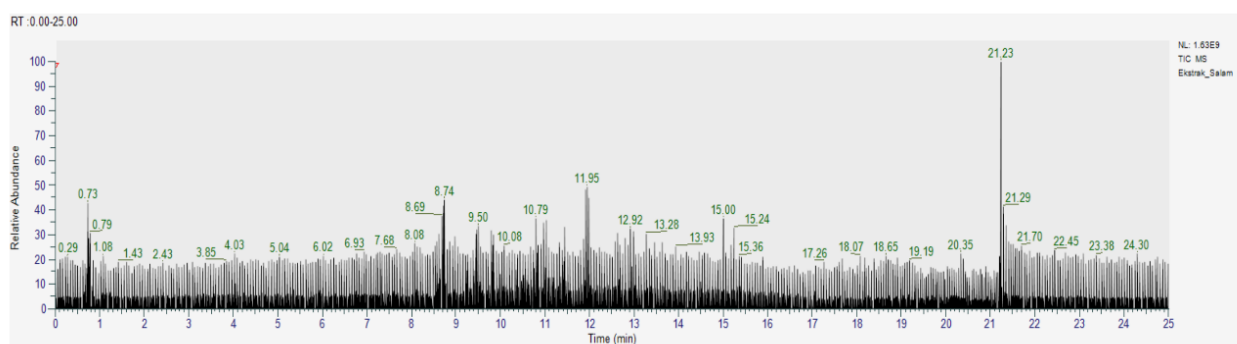


Figure 1. LC-HRMS total ion chromatogram of EESP (0-25 min), with only peaks exceeding 1% relative abundance considered for further analysis

Table I. The selected bioactive compound of EESP was detected by LC-HRMS

No	Compound Name	Formula	Molecular Weight	Retention Time (min)	Area (Max.)	Relative Abundance (%)
1	2,6-Dimethylquinoline	C ₁₁ H ₁₁ N	157.089	0.788	208343758.1	5.77
2	Lauramidopropyl dimethylamine	C ₁₇ H ₃₆ N ₂ O	284.283	8.252	163087455.1	4.52
3	Medelamine A	C ₁₄ H ₃₁ N	213.245	8.878	107830505.1	2.99
4	6-methylheptane-1,2,3,4,5-pentaol	C ₈ H ₁₈ O ₅	194.115	1.65	61629130.7	1.71
5	Myricetin	C ₁₅ H ₁₀ O ₈	318.037	4.044	61456524.2	1.70
6	6-Methylquinoline	C ₁₀ H ₉ N	143.073	0.766	45886560.8	1.27
7	Stigmasta-3,5-diene	C ₂₉ H ₄₈	396.375	17.118	40543715.1	1.12

C. Network Pharmacology

This network pharmacology study was conducted to elucidate the potential mechanisms by which EESP exerts therapeutic effects against RA, enabling the identification of key targets associated with its anti-inflammatory activity. Gene targets related to RA in *Homo sapiens* were retrieved from the NCBI and GeneCards databases, yielding 1536 and 4534 targets, respectively. After deduplication using a Venn diagram, 4712 targets were obtained. These were subsequently standardized through UniProt, resulting in a final set of 4,646 RA-related gene targets as seen in Supplementary 2.

From the seven compounds identified by LC-HRMS analysis, 444 predicted targets were obtained. After deduplication, 183 RA-related targets of EESP were identified after deduplication. A

Venn diagram was then used to determine the overlapping targets, resulting in 121 targets potentially involved in the treatment of RA (Figure 2A). These were imported into the STRING database for protein–protein interaction (PPI) analysis, which revealed 121 nodes, 107 edges, an average node degree of 1.77, and a PPI enrichment *p*-value of 1.0×10^{-16} , indicating significantly higher interconnectivity than expected by chance (Figure 2B).

Network analysis results were imported into Cytoscape to identify top targets using the criteria of degree, closeness centrality (CC), and betweenness centrality (BC) > median (Figure 2C). The detail results of these parameters are presented in Supplementary 2. This analysis yielded 20 chosen gene targets (Table II), representing key nodes within the RA metabolic network.

Table II. The Values of Degree, CC, BC of 20 targets

Gene code	Gene name	Degree	Closeness centrality	Betweenness centrality
HDAC1	Histone Deacetylase 1	9	21.3496	378.83333
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19	9	21.95595	1164.1381
ESR1	Estrogen Receptor 1	9	22.96746	1019.96667
PTGS2	Prostaglandin-Endoperoxide Synthase 2	8	19.84802	283.34286
CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1	7	21.18571	1023.53333
CYP2C9	Cytochrome P450 Family 2 Subfamily C Member 9	7	20.75595	339.6381
PTK2	Protein Tyrosine Kinase 2	6	17.23182	612.33333
CYP17A1	Cytochrome P450 Family 17 Subfamily A Member 1	6	20.41905	1053.26667
HDAC2	Histone Deacetylase 2	6	19.20198	89.06667
PTGS1	Prostaglandin-Endoperoxide Synthase 1	6	18.51468	49.85714
ALOX15	Arachidonate 15-Lipoxygenase	6	19.70595	86.1381
SIRT1	Sirtuin 1	5	18.81746	487.26667
HSD17B3	Hydroxysteroid 17-Beta Dehydrogenase 3	5	18.01905	0
SRD5A2	Steroid 5 Alpha-Reductase 2	5	18.01905	0
SRD5A1	Steroid 5 Alpha-Reductase 1	5	18.01905	0
CYP11B2	Cytochrome P450 Family 11 Subfamily B Member 2	5	18.01905	0
MAPK14	Mitogen-Activated Protein Kinase 14	5	19.2377	876
CYP1A2	Cytochrome P450 Family 1 Subfamily A Member 2	5	20.90952	1213.04762
ALOX5	Arachidonate 5-Lipoxygenase	5	18.01468	0
RELA	RELA Proto-Oncogene, NF- κ B Subunit	4	18.8496	88.1

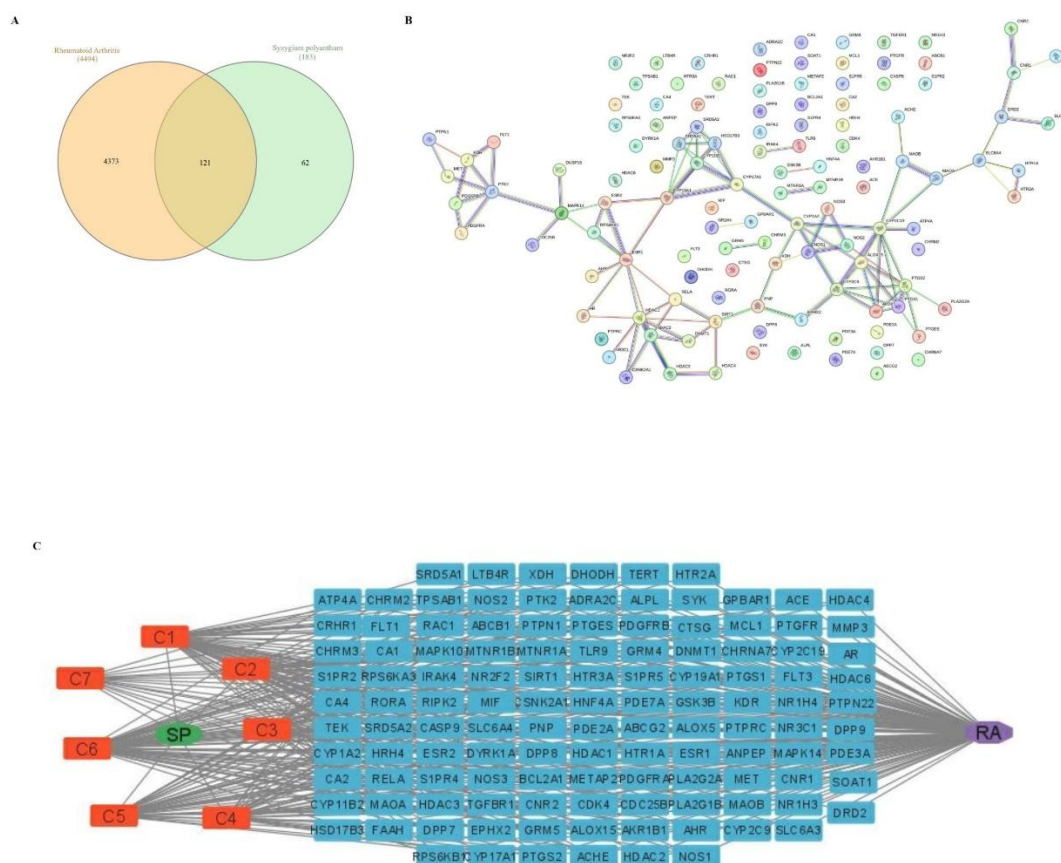


Figure 2. Network pharmacology analysis of EESP (A). Overlapping target between *S. polyanthum* and RA using interactive Venn (B), construction of a PPI network using STRING database (C), Herb-Compound-Gene-Disease relationship of 7 active compounds of EESP

Among the 5 top-ranked targets based on the degree value, HDAC1, ESR1, and PTGS2 appear to play central roles in the inflammatory mechanisms underlying RA. We excluded CYP targets because they tend to influence the endocrine context rather than driving RA pathology. HDAC1 is an epigenetic regulator that modulates chromatin structure and promotes the transcription of proinflammatory cytokines such as TNF- α and IL-6, crucial inflammatory drivers of RA pathogenesis. Pharmacological inhibition of HDAC1 has

been shown to attenuate inflammatory responses in immune cells and synovial fibroblasts, supporting its potential as a therapeutic RA target (Guo et al., 2021). Moreover, ESR1 provides an important link between hormonal regulation and immune function (Polanczyk et al., 2004). Estrogen signaling through ESR1 has been implicated in modulating T and B cell responses, enhancing the production of inflammatory mediators. ESR1 may perpetuate inflammation within the synovial microenvironment (Recalde et al.,

2018). In parallel, PTGS2 (COX-2) is a key inflammatory mediator that trigger prostaglandin synthesis, leading to pain, swelling, and cartilage damage in RA (Yoon et al., 2013). Its identification validates our network analysis, as PTGS2 is the primary target of COX-2 inhibitors commonly used in RA therapy.

The results of Gene Ontology (GO) and KEGG pathway enrichment analyses

are presented in Figure 3A–D. Table III provides a detailed summary of the enriched GO terms and KEGG pathways, highlighting those closely associated with the pathogenesis of RA. This integrative GO and KEGG analysis supports the notion that the tested compounds may exert multi-target effects, modulating both pro-inflammatory and protective pathways to restore immune balance in RA.

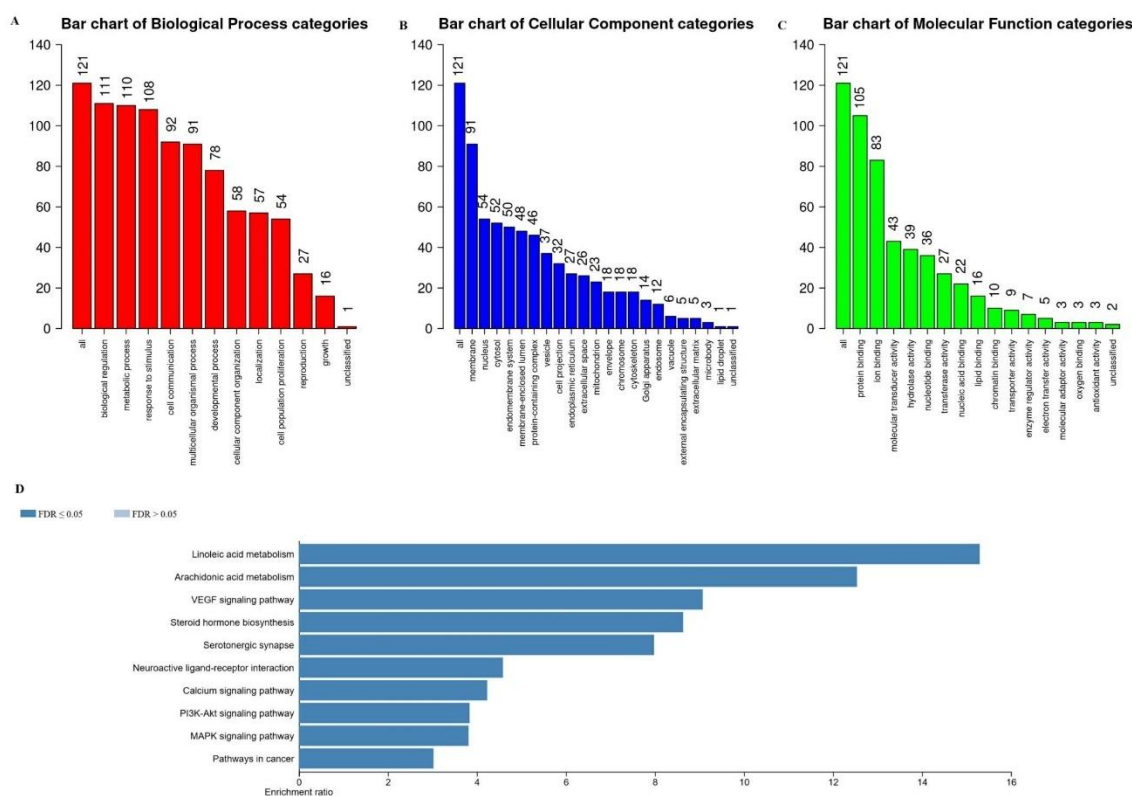


Figure 3. GO analysis and KEGG pathway (A) GO analysis based on biological process (B) GO analysis based on cellular component (C) GO analysis based on molecular function (D) KEGG enrichment analysis

Table III. Integrated Enrichment Results of GO and KEGG Related to RA

Category	Enriched Term		RA relevance
Biological Process (BP)	Eicosanoid metabolic process		Generates inflammatory lipid mediators central to RA (Hoxha, 2018)
	Response to a molecule of bacterial origin		Toll-like receptor (TLR) activation by bacterial components contributes to

Category	Enriched Term	RA relevance
		synovial inflammation (Duan et al., 2022)
	Cellular response to biotic stimulus	Reflects immune activation triggering chronic inflammation (Chen et al., 2017)
	Regulation of inflammatory response	Core mechanism of RA pathogenesis (Gao et al., 2020)
Cellular component (CC)	Histone deacetylase complex	Epigenetic control of cytokine expression; HDAC inhibitors as potential RA therapy (Pai et al., 2024)
	Membrane microdomain	Lipid rafts facilitate T/B cell receptor and cytokine signaling (Warda et al., 2025)
	Receptor complex	Include TNF, IL-6, and TLR signaling complexes in central RA inflammation. (Yu et al., 2018)
	Transcription regulator complex	Controls NF- κ B/STAT-driven pro-inflammatory gene expression (Nejabatbakhsh Samimi et al., 2020)
Molecular Function (MF)	Ligand-activated transcription factor activity	Nuclear receptors (e.g., glucocorticoid receptor, PPARs) regulate inflammation (Eiers et al., 2024)
	Deacetylase activity	HDACs regulate synovial fibroblast proliferation and cytokine expression (Liu et al., 2024)
	Protein tyrosine kinase activity	JAKs and SYK are critical in cytokine signaling; targeted by JAK inhibitors in RA therapy (Ciobanu et al., 2020)
	Steroid binding	Basis for glucocorticoid therapy in RA (Paolino et al., 2017)
KEGG Pathway	Arachidonic acid metabolism	Produces prostaglandins and leukotrienes, key mediators of inflammation and pain in RA (Wang et al., 2017)

Category	Enriched Term	RA relevance
	VEGF signaling pathway	Promotes synovial angiogenesis and pannus formation (Kim et al., 2020)
	MAPK signaling pathway	Regulates pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) (Xie et al., 2025)
	PI3K-Akt signaling pathway	Controls synovial fibroblast survival, proliferation, and inflammation (Wang et al., 2025)

D. Molecular Docking Study

Based on Table II, we selected two strong candidates for RA targets, PTGS2 (PDB ID: 5KIR) and MAPK14 (PDB ID: 6SFO), which are relevant to the inflammatory process in RA. We did not select ESR1 and HDAC1 as docking targets, even though they were among the top five predicted targets, for several reasons. HDACs significantly impact the accessibility and conformational dynamics of binding pockets as part of large multiprotein complexes, which static docking models do not appropriately capture. These protein–protein interactions also modify the local electrostatic environment and steric accessibility, further limiting the interpretability of in silico affinity predictions (Ghiboub et al., 2021). In addition, ESR1 functions primarily through hormone-dependent immune modulation, characterized by conformational flexibility, co-regulator recruitment, and context-specific transcriptional effects, making it less

suitable as a primary docking target compared with direct inflammatory mediators such as MAPK14 or PTGS2 (Kumar et al., 2011). PTGS2 is an inducible enzyme responsible for prostaglandin E₂ (PGE₂) synthesis, a direct mediator of pain, swelling, and bone resorption in RA. In contrast, MAPK14 is a key signaling kinase activated by pro-inflammatory cytokines such as TNF- α and IL-1 β , driving synovial fibroblast proliferation, matrix metalloproteinase expression, and osteoclastogenesis, all of which contribute to joint inflammation and destruction. Targeting MAPK14 addresses the upstream regulation of inflammatory signaling. (Liu et al., 2021), while inhibiting PTGS2 modulates the downstream effector of inflammation and pain, together providing a mechanistically comprehensive strategy (Li et al., 2021).

These will be docked with three selected compounds: 6-dimethylquinoline, 6-methylquinoline, and myricetin. Before performing molecular docking of the

compounds with the receptor targets, redocking was conducted to validate the binding of these molecules with the native ligands at 5KIR and 6SFO, respectively, RCX and LBE. In 6SFO, there was a missing residue, so it was corrected using <https://swissmodel.expasy.org/>.

The grid box was determined at x, y, z for each of 5KIR and 6SFO, namely: 23.287,

0.712, 34.435, and 0.05, 1.071, -19.152, with x, y, z sizes for both targets being 18.0. The RMSD values obtained for 5KIR and 6SFO, respectively, are < 2.0 Å, with values of 1.489 and 1.571 Å, indicating that these parameter values are valid and can be used for further analysis.

Table IV. Binding Interaction of 3 Selected Compounds of EESP Against PTGS2 and MAPK14

Targets (PDB ID)	PubChem ID	Compounds	Canonical SMILES	Binding affinity	Hydrogen bonding	Hydrophobic interactions
5KIR (PTGS2)		Native ligand		-11.76	ARG513, ILE517, PHE518	VAL349,A LA527, LEU352,V AL523,
	13414	2,6-Dimethylquinoline	<chem>CC1=CC=C2N=C(C)C=CC2=C1</chem>	-7.732	PHE518, GLN192, SER353, GLY526	LEU352,V AL523,
	7059	6-Methylquinoline	<chem>CC1=CC=C2N=CC=CC2=C1</chem>	-7.11	-	SER353, VAL523, ALA516, ILE517, VAL523, PHE518, ALA527
	5281672	Myricetin	<chem>O=C1C(O)=C(C2=CC(O)=C(O)C(=C2)OC2=CC(O)=C(C(O)=C12</chem>	-8.915	TYR385, ILE517, MET522	LEU352, VAL523, LEU352
6SFO (MAPK14)		Native ligand		-11.19	MET110, ASP169, HIS108, TYR36	TYR36, PHE170, LEU75, MET79, ILE142, LEU75, LEU76, ILE85, LEU168, ALA173, ALA52

5A show that the native ligands in both receptors demonstrated nearly identical binding energies, stabilized by several hydrogen bonds. By contrast, the quinoline derivatives 2,6-dimethylquinoline and 6-methylquinoline (Figure 4B–C and 5B–C) did not form hydrogen bonds but maintained relatively high binding affinities, with

hydrophobic interactions contributing to ligand–receptor stability.

Among the tested compounds, myricetin showed the lowest binding free energies for PTGS2 and MAPK14 (Figure 4D and 5D). Multiple hydrogen bonds at the receptor binding sites further stabilized its interactions, suggesting a strong inhibitory potential.

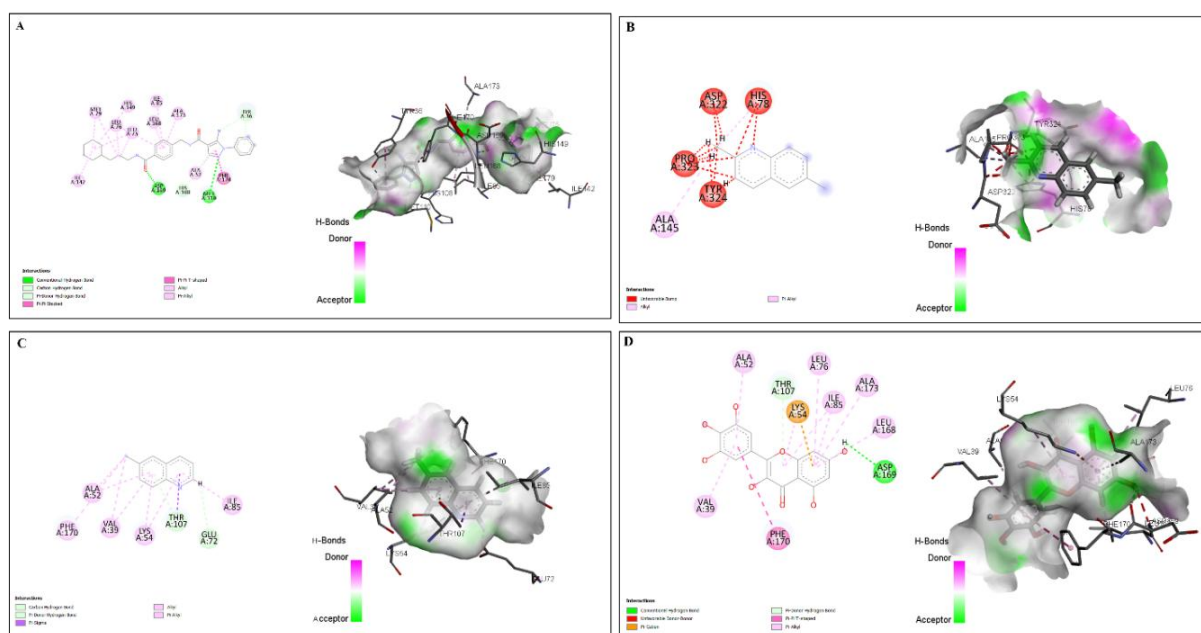


Figure 5. Visualisation of 2D (right) and 3D (H-Bond interaction) between tested ligands and the receptor of MAPK14 (A). Native ligand (B). 2,6- Dimethylquinoline (C). 6-methylquinoline (D). Myricetin

PTGS2, also known as cyclooxygenase-2 (COX-2), plays a pivotal role in the downstream inflammatory processes of RA. As an inducible enzyme, COX-2 is upregulated in response to pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, which are abundant in the RA synovium. Once activated, COX-2 catalyzes the conversion of arachidonic

acid into prostaglandins, particularly prostaglandin E2 (PGE2), a potent mediator of inflammation. Elevated levels of PGE2 contribute to several pathological features of RA, including synovial hyperplasia, pannus formation, cartilage degradation, and osteoclast-mediated bone erosion (Wang et al., 2024).

Moreover, MAPK14 (p38 α) has been widely recognized as a central mediator of RA pathology, as it regulates the production of key pro-inflammatory cytokines such as TNF and IL-1, along with chemokines, COX-2, and matrix metalloproteinases (MMP) that contribute to synovial inflammation, bone erosion, and cartilage degradation. In this study, molecular docking demonstrated that myricetin exhibited the strongest binding affinity to MAPK14, with the most negative binding energy among the tested ligands. This suggests that myricetin may effectively interact with the catalytic domain of p38 α , potentially inhibiting its activation and downstream signaling. Such an effect could attenuate cytokine overproduction, osteoclast differentiation via the RANK–RANKL axis, and cartilage degradation mediated by MMPs. Previous generations of synthetic p38 inhibitors have shown therapeutic promise in reducing inflammation, and the strong interaction observed between myricetin and MAPK14 highlights the potential of this natural compound as a complementary therapeutic candidate for RA. However, despite its biological relevance, clinical trials of synthetic MAPK14 inhibitors have largely failed, mainly due to limited efficacy, pathway redundancy, and adverse effects caused by complete inhibition of the signaling cascade. Despite its biological

relevance, clinical trials of synthetic MAPK14 inhibitors have largely failed, mainly due to limited efficacy, pathway redundancy, and adverse effects caused by complete inhibition of the signaling cascade (Clark & Dean, 2012).

A previous study of Lee et al (2010) reported that myricetin reduced IL-1 β -induced production of IL-6 and MMP-1 in synovial cells by diminishing the phosphorylation of MAPK14 (Lee & Choi, 2010). Moreover, myricetin has also been shown to inhibit cyclooxygenase-2 (COX-2; PTGS2) and suppress several kinases within the MAPK pathway, thereby attenuating inflammatory cytokine expression in RA (Jang et al., 2020).

IV. CONCLUSION

This study integrates network pharmacology and molecular docking to elucidate the potential mechanisms of EESP in treating rheumatoid arthritis. Three compounds—2,6-dimethylquinoline, 6-methylquinoline, and myricetin—were highlighted as key chemical markers from seven LC-HRMS-identified metabolites. Network analysis identified 113 overlapping RA-related targets, with PTGS2 and MAPK14 emerging as inflammation-associated central nodes. Molecular docking demonstrated that all selected compounds exhibited favorable binding affinities, with hydrophobic and

hydrogen bond interactions contributing to ligand–receptor stability. Notably, myricetin showed the strongest binding to PTGS2 and MAPK14, consistent with its reported anti-inflammatory and immunomodulatory activities. These findings suggest that EESP exerts therapeutic effects against RA by targeting critical inflammatory pathways, supporting further in vitro and in vivo validation of these candidate compounds.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this research.

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