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Network Pharmacology Approach to *Acalypha indica* L. and *Plumbago zeylanica* L. As Anti-Rheumatoid Arthritis Candidates

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Abstract
Background*: Rheumatoid arthritis (RA) is a chronic autoimmune disease that can reduce quality of life. Currently, the goal of therapy is to achieve remission and prevent joint damage and disability. Acalypha indica L. and Plumbago zeylanica L. are known to be involved in rheumatoid pathogenesis.* ***Objective****: This study aimed to determine the compounds in Acalypha indica L. and Plumbago zeylanica L. that correlate with target proteins and anti-rheumatoid arthritis mechanisms.* ***Methods****: Plant compound data were collected from the KNApSAcK and IMPPAT databases, target protein data were collected using the KEGG pathway, validated using UniProt, and protein-protein interactions were analyzed using STRING. Target protein prediction using SwissTarget Prediction and SEA. Visualization of network pharmacology profiles using Cytoscape software based on the correlation between plant compounds and target proteins.* ***Results****: Acalypha indica L., which correlates with target proteins, contained quinine, gallotannin, 1,4 benzoquinone, chrysin, and kaempferol. For Plumbago zeylanica L., the compounds were vanillic acid, cinnamic acid, plumbagin, isoaffinetin, isoorientin, isovitexin, methylnaphthazarin, l-tryptophan, beta-sitosterol, stigmasterol, ficusin, suberosin, and quercetin 3-ol-rhamnoside.* ***Conclusion****: Network pharmacology visualization results showed that both Acalypha indica L. and Plumbago zeylanica L. correlated with disease target proteins in their respective rheumatoid arthritis signaling pathways.*

Keywords*: Acalypha indica L. cytoscape, network pharmacology, Plumbago zeylanica L., rheumatoid arthritis*

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INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune disease that causes inflammation in the synovium and cartilage as well as damage to joints and bones through a variety of inflammatory mediators (Ono et al., 2016). Typical symptoms of rheumatoid arthritis include wrist, knee, and finger discomfort and swelling. The typical symptoms of rheumatoid arthritis include wrist, knee, and finger discomfort and swelling. This illness can lower the quality of life and cause death. The incidence increases with age, particularly in women, owing to factors related to hormonal balance. It peaks between 40 and 60 years of age (Amalia et al., 2021). Apart from the aforementioned symptoms, symptoms that are often experienced include stiffness in the morning for >30 min, fatigue, fever, and weight loss (Bullock et al., 2019). (Bullock et al., 2019). The activation of monocyte cells, such as immune cells, macrophages, and synovial fibroblasts, which subsequently generate antigen-activated CD4+ T cells, is one of the many environmental and genetic variables that contribute to the disease (Hu et al., 2019). The primary mediators of rheumatoid arthritis, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α), are subsequently produced as a result of CD4+ T cell activation (Mateen et al., 2016).

Currently, treatment aims to achieve remission or low disease activity; it must also prevent systemic manifestations, joint damage, and disabilities (Burmester & Pope, 2017). Conventional and biological disease-modifying antirheumatic medications (DMARDs) and Janus kinase (JAK) inhibitors are used to treat and halt the progression of rheumatoid arthritis (Schwinghammer et al., 2021). Methotrexate, leflunomide, and sulfasalazine are examples of DMARD that can have harmful adverse effects. It was reported that approximately 20%-30% of rheumatoid arthritis patients stopped using methotrexate within the first year of therapy because they could not tolerate the side effects induced by methorexate, but the potential side effects could persist for 5 years (Huang et al., 2017). The side effects of these drugs are what cause many sufferers to switch to herbal remedies which have fewer side effects (Amalia et al., 2021). Botanical drugs from traditional Chinese medicine have been used to treat rheumatoid arthritis since ancient times. The use of decoction with more than one herb is a common practice, especially in traditional Chinese medicine (Hong et al., 2017)

Acalypha indica L. with the compound kaempferol based on research (Pan et al., 2018), kaempferol suppresses migration, invasion, MMP expression in rheumatoid arthritis FLS (fibroblast-like synovyocites). Kaempferol has been shown to increase the reduction in lipopolysaccharide (LPS) levels (lipopolysaccharide) of chondrogenic markers and reduce the expression levels of MMP3 and MMP13. This shows that kaempferol in rheumatoid arthritis FLS reduced the production of MMP1, MMP3, MMP9, and MMP13.

Plumbago zeylanica L. contain the compound cinnamic acid which is based on research (Zhou et al., 2023) the combination of mangiferin and cinnamic acid reduces joint inflammation and bone erosion by suppressing NLRP3 inflammasome activation by inhibiting NF-κB via TLR4/PI3K/AKT signaling. This results in decreased release of IL1B and IL-18, downregulation of caspase-1, and modulation of pyroptosis GSDMD (Gasdermin D). Vanillic acid compounds, based on research conducted by Thilertdecha et al. (2019), reduced COX-2 expression and NF-κB activation, which in turn led to lower levels of TNF-α and IL-2.

In this case, network pharmacology, which integrates systematic treatment with scientific information, is new in drug discovery. This method incorporates an in silico technique by constructing a network of "protein-active substance/disease-gene" to ascertain the mechanism of the synergistic therapeutic action of traditional medications. Network pharmacology techniques are used to determine active substances, potential targets, and signaling pathways (Noor et al., 2022).

Based on data from previous research, this study was intended to confirm and determine the molecular correlation. This research will carry out an analysis using a network pharmacology approach on *Acalypha indica* L. and *Plumbago zeylanica* L. on rheumatoid arthritis target proteins as anti-rheumatoid arthritis drug candidates. Screening was carried out on *Acalypha indica* L. and *Plumbago zeylanica* L. to determine the compounds found in *Acalypha indica* L. and *Plumbago zeylanica* L. as well as the proteins found in the compounds of *Acalypha indica* L. and *Plumbago zeylanica* L., then continued to look for disease target proteins in the KEGG pathway via the signaling pathway of rheumatoid arthritis in the form of T-cell receptor, Th17 cell differentiation, Toll-like receptor, osteoclast differentiation, VEGF, leukocyte migration, cyclooxygenase, and lipoxygenase which will eventually form network pharmacology visualization.

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MATERIALS AND METHODS

Materials

The materials used in this study was compounds from *Acalypha indica* L. and *Plumbago zeylanica* L. were obtained from KNApSAcK and IMPPAT. The target proteins of RA were obtained from the KEGG database. Protein-protein interactions were obtained from STRING. *Acalypha indica* L. and *Plumbago zeylanica* L. compounds with anti-rheumatoid arthritis activities were obtained from PubChem. Target protein prediction was performed using SwissTargetPrediction and SEA.

Tools

The tools used in this study were a set of ACER Aspire 5 with Intel(R) Core (TM) i3- 1115G4 processor specifications, 8.0 Giga Byte RAM, 233 Giga Byte SSD hard disk, Cytoscape 3.10, KNApSAcK, IMPPAT, PubChem, SwissTargetPrediction, SEA, KEGG, Uniprot, and STRING.

Method

Data collection on plants compounds

Compound data for *Acalypha indica* L. and *Plumbago zeylanica* L. were collected from several databases, including KNApSAcK (http://www.knapsackfamily.com/KNApSAcK_Family/) and IMPPAT (https://cb.imsc.res.in/imppat/). These databases provide smiles from each compound.

Data collection of rheumatoid arthritis target proteins

A rheumatoid arthritis target search was performed using KEGG (KEGG PATHWAY Database (Genome. jp)). Subsequently, target gene names were standardized and invalid targets were eliminated using the UniProt database (https://www.uniprot.org/); only target genes marked as "Reviewed (Swiss-Prot)" and "Homo sapiens" were chosen from UniProt to guarantee prediction accuracy (Deng et al., 2020).

Analysis of protein-protein interactions

STRING database was used to analyze protein-protein interactions (https://STRING-db.org/). “Homo sapiens” was choosen with an interaction score >0.9. (Huang et al., 2020).

Identification of the biological activity of compounds

Identification of biological compound activity data was performed using PubChem (https://pubchem.ncbi.nlm.nih.gov/). The data obtained are sorted based on activity; if it is not active, it is eliminated.

Target proteins prediction via SwissTargetPrediction and SEA

Using the SwissTargetPrediction database (http://www.swisstargetprediction.ch/), SMILES of *Acalypha indica* L. and *Plumbago zeylanica* L. compounds were used to acquire targets using a reverse pharmacophore-matching approach. For this reason, targets with probability ≥0.5 were chosen (Noor et al., 2022b). SwissTragetPrediction accurately predicts bioactive target molecules based on a combination of 2D and 3D similarity measures with known ligands (Gfeller et al., 2014). The function of the Similarity Ensemble Approach (SEA) is to identify pharmacological relationships between molecular targets based on similarity set ligands (Achenbach et al., 2011). In the SEA database, the existing data are sorted by the maximum Tc section, selected by a value ≥ 0.5.

Visualization using cytoscape

Compound-target networks were constructed using the candidate compounds and potential targets. The network was constructed using Cytoscape 3.10. In this bilateral network, the nodes present compounds and potential targets, and the edges present the compound–target or interactions (Huang et al., 2017).

RESULTS AND DISCUSSION

Data collection on plants compounds

Data collection on plant compounds was obtained from several databases including KNApSAcK and IMPPAT, from these two plants, where *Acalypha indica* L. contains 23 compounds, while *Plumbago zeylanica* L. contains 48 compounds.

Data collection of rheumatoid arthritis target proteins

Collection of target proteins involved in the pathophysiology of rheumatoid arthritis was carried out using the KEGG pathway database, there will be a picture of the rheumatoid arthritis signaling pathway. In the picture there are several signaling pathways, namely T-cell receptor, Th17 cell differentiation, Toll-like receptor, Osteoclast differentiation, VEGF, Leukocyte migration. Then in the signaling pathway there is a rheumatoid arthritis target protein. Validation of proteins from the KEGG pathway using UniProt, to obtain universally validated protein names.

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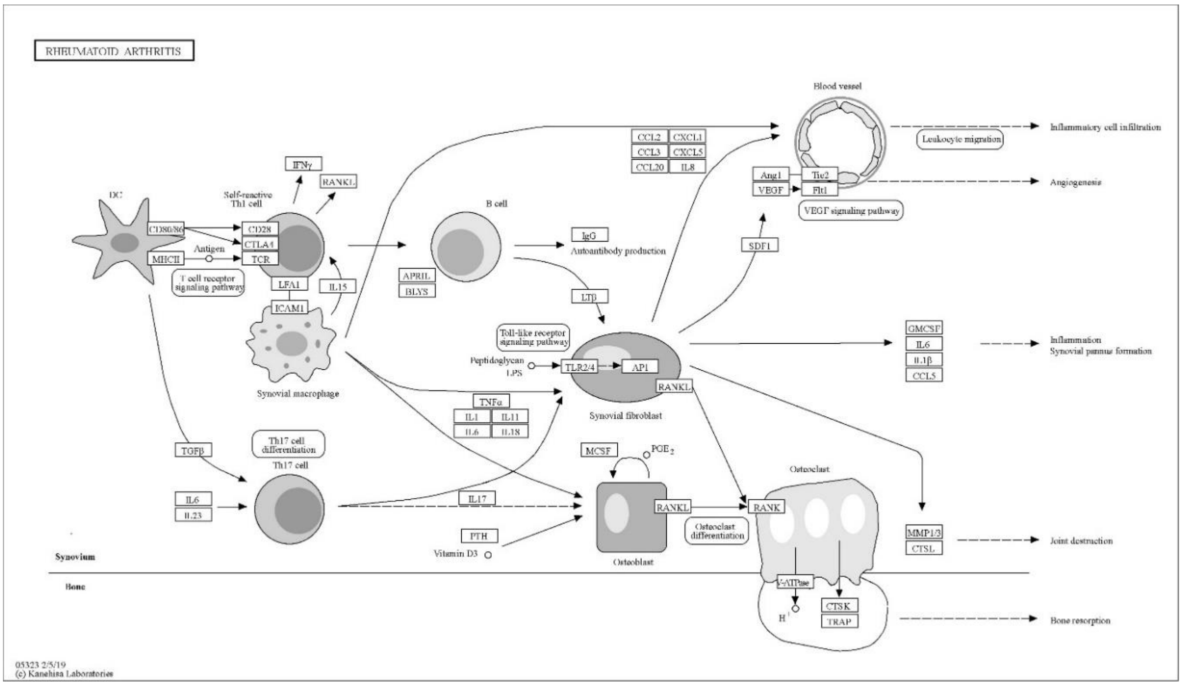

Figure 1. Signaling pathway rheumatoid arthritis from KEGG pathway

Analysis of protein-protein interactions

STRING is an early effort web server that attempts to differentiate protein-protein interactions primarily through wide coverage, user-friendliness, and a constant scoring system (Szklarczyk et al., 2019). Combination scores above 0.9 can be taken, scores below 0.9 will be eliminated. The sorting data obtained 307 protein-protein interactions with a combination score above 0.9. Based on the results of STRING, it was found that proteins from rheumatoid arthritis bind to each other. These proteins have many nodes, which are circle-shaped images that show the proteins present. Apart from that, there are also colorful lines called edges that represent protein-protein associations. It provides a score for every protein-protein interaction that is maintained in STRING. This score (the weight of the edges in each network) represents the trust score and is scaled between zero and one. This is an estimation of the likelihood that in the presence of supporting data, a given interaction is biologically significant, distinct, and repeatable. Authenticity and type of evidence determine which 'evidence channels' the supporting evidence for each encounter. Seven distinct channels were constructed, assessed, and benchmarked (Szklarczyk et al., 2017).

Each interaction is computed as a combination and a final trust score based on the seven channels; this so-called combination score serves as the benchmark when creating networks or organizing and filtering interactions. When there is evidence of many channels contributing to the engagement score, in addition to a high score, it is a positive indication of support (Szklarczyk et al., 2017). The results were sorted based on a combination score ≥ 0.9. This is because the higher the combination score for a protein, the more the interaction between proteins is based on the number of studies that have been conducted.

Identification of the biological activity of compounds

The compound data from KNapSAck and IMPPAT were used to search for biological activity using PubChem. The data obtained is sorted based on activity, if it is not active it will be eliminated. From the identification results using PubChem, we found that the five active compounds in *Acalypha indica* L. were quinine, kaempferol, 1,4-benzoquinone, gallotannin, and chrysin. There are 13 active compounds in Plumbago zeylanica L., including vanillic acid, plumbagin, isoaffinetin, isoorientin, isovitexin, methylnaphthazarin, l-tryptophan, cinnamic acid, beta sitosterol, stigmasterol, ficusin, suberosin, quercetin3-o-l-rhamnoside.

Target proteins prediction via SwissTargetPrediction and SEA

This analysis was used to determine the level of similarity between bioactive compounds and rheumatoid arthritis target proteins. In SwissTargetPrediction, the data that are sorted probability data where only values ≥ 0.5 are taken. In the SEA database, a maximum Tc value ≥ 0.5 is selected.

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Table 1. Prediction of metabolit target proteins in *Acalypha indica* L. with SwissTargetPrediction and SEA

Plant	Compound	Sources	Target Proteins	Prediction		
				PubChem	SwissTargetPrediction	SEA
***Acalypha indica* L.**	*Quinine*	(IMPPAT: Indian Medicinal Plants, 2023a)	IFNG	√		
	Kaempferol	(IMPPAT: Indian Medicinal Plants, 2023a)	NFKB1	√		
			MMP1	√		
			MMP2		√	
			MMP9	√		
			ALOX12	√	√	√
			ALOX15	√	√	√
			ALOX5	√	√	√
			SYK		√	
	1,4 benzoquinone	(IMPPAT: Indian Medicinal Plants, 2023a)	CASP1	√		√
			CCR6	√		
	Gallotannin	(IMPPAT: Indian Medicinal Plants, 2023a)	JUN	√		
			HSPD1	√		
			BCL2L1	√		
			LCK	√		
	Chrysin	(IMPPAT: Indian Medicinal Plants, 2023a)	PGF	√		
			ALOX12	√	√	√
			ALOX15	√	√	√
			CBR1	√	√	

Table 2. Prediction of metabolit target proteins in *Plumbago zeylanica* L. with SwissTargetPrediction and SEA

Plant	Compound	Sources	Target Proteins	Prediction		
				PubChem	SwissTargetPrediction	SEA
***Plumbago zeylanica* L.**	*Vanillic acid*	(IMPPAT: Indian Medicinal Plants, 2023b)	CXCL12			√
			ALOX5		√	√
	Plumbagin	(KNApSAcK Core System, 2023)	EGFR	√		
			EP300	√	√	√
			XPO1	√		
			HSPD1	√		
	Isoaffinetin	(KNApSAcK Core System, 2023)	IL2			√
	Isoorientin	(KNApSAcK Core System, 2023)	IL2			√
			ALOX5	√	√	√
	Isovitexin	(KNApSAcK Core System, 2023)	IL2			√
	Methylnaphthazarin	(IMPPAT: Indian Medicinal Plants, 2023b)	BCL2L1	√		
	L-tryptophan	(KNApSAcK Core System, 2023)	CTSL			√
			MMP1			√
			MMP2			√
			MMP3			√
			MMP9			√
	Cinnamic acid	(IMPPAT: Indian Medicinal Plants, 2023b)	MMP1			√
			MMP2			√
			MMP9			√

Beta sitosterol	(IMPPAT: Indian Medicinal Plants, 2023b)	FGF2			√
Stigmasterol	(KNApSAcK Core System, 2023)	FGF2			√
Suberosin	(KNApSAcK Core System, 2023)	XPO1			√
Ficusin	(KNApSAcK Core System, 2023)	NFKB1	√	√	√
Quercetin 3-o-1 rhamnoside	(KNApSAcK Core System, 2023)	ALOX5		√	√

The results obtained from SwissTargetPrediction and SEA showed that there are several compounds that pass the sorting ≥ 0.5, but some that pass the sorting do not match the rheumatoid arthritis target protein. Only a few compounds from *Acalypha indica* L. and the gout leaf plant are compatible with rheumatoid arthritis target proteins.

Visualization using cytoscape

Network pharmacology visualization was created using Cytoscape software using data from STRING, PubChem, SwissTargetPrediction, and SEA. Pharmacology networks contain nodes and edges. Nodes contain target proteins and interacting compounds that are connected via edges (connecting lines). The visualization of two plants, *Acalypha indica* L. and *Plumbago zeylanica* L., where the nodes were differentiated by color and shape for compounds from *Acalypha indica* L. were yellow with an elliptical shape, while compounds from *Plumbago zeylanica* L. were green and diamond-shaped with orange for target proteins.

As shown in Figure 2, the *Acalypha indica* L. compound, quinine, correlated with the target protein IFNG. This molecule is the primary inflammatory cytokine that marks the Th1 lineage in addition to other CD4+ T subsets. CD8+ T cells secrete IFNG to control infection and are composed of CD4+ T helper 1 (Th1) cells. It is involved in intracellular invasion, inflammation, and autoimmune diseases, suggesting that IFNG produced by Th1 cells is involved in the pathogenesis of rheumatoid arthritis (Peng et al., 2020).

Quinine specifically inhibits autophagy, prevents the activation of MHC II antigens, and increases endosomal pH, which inhibits Toll-like receptors, which are included in the cytokine production pathway (Song & Fields, 2020). IFNG, TNF, IL-1, and IL-6 are examples of pro-inflammatory cytokines that are reduced in production and blocked by suppressing T cell responses (dos Reis Neto et al., 2020). A mechanism that interferes with the production of inflammatory cytokines is the ability to interfere with the synthesis of GMP-AMP signaling (cGAS). cGAS is an important component of the cGAS signaling stimulator of the IFNG gene required for the type I IFN response of immune cells, giving it a critical role in the activation of pro-inflammatory responses in autoimmune diseases (Nirk et al., 2020).

Table 3. Predicted correlation of *Acalypha indica* L. with rheumatoid arthritis signaling pathway based on KEGG

Compound	Target protein code	Name of the main protein	RA Pathway
Quinine	IFNG	CD4 CSF2 IFNG IL1B	*T-cell receptors*
Kaempferol	NFKB1	IL18	*Toll-like receptors*
	MMP1	MMP1 MMP3	*Osteoclast differentiation*
	MMP2	MMP1 VEGFA	*Osteoclast differentiation*
	MMP9	MMP1 MMP3 VEGFA	*Osteoclast differentiation*

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Compound	Target protein code	Name of the main protein	RA Pathway
	ALOX12		*Lipoxygenase*
	ALOX15		*Lipoxygenase*
	ALOX5		*Lipoxygenase*
	SYK	IGH	*Osteoclast differentiation*
1,4-benzoquinone	CASP1	CASP1	*Toll-like receptors*
		IL1B	
		IL18	
	CCR6	CCL20	*Leukocyte migration*
Gallotannin	JUN	JUN	*Toll-like receptors*
	HSPD1	TLR4	*Toll-like receptors*
	BCL2L1	CTSK	*Osteoclast differentiation*
		CTSL	
	LCK	CD28	*T-cell receptors*
Chrysin	PGF	FLT1	*VEGF*
	ALOX12		*Lipoxygenase*
	ALOX15		*Lipoxygenase*
	CBR1		*Cyclooxygenase*

Table 4. Predicted correlation of *Plumbago zeylanica* L. with rheumatoid arthritis signaling pathway based on KEGG

Compound	Target protein code	Name of the main protein	RA Pathway
Vanillic acid	CXCL12	CCL20	*Leukocyte migration*
	ALOX5		*Lipoxygenase*
Plumbagin	EGFR	ANGPT1	*VEGF*
	EP300	JUN	*Toll-like receptors*
	XPO1	TNFSF13	*T-cell receptors*
	HSPD1	TLR4	*Toll-like receptors*
Isoaffinetin	IL2	CSF2	*Th 17 cell differentiation*
		IFNG	
		IL1A	
		IL1B	
		IL15	
		IL16	
		IL17A	
Isoorientine	IL2	CSF2	*Th 17 cell differentiation*
		IFNG	
		IL1A	
		IL1B	
		IL15	
		IL16	
		IL17A	
	ALOX5		*Lipoxygenase*
Isovitexin	IL2	CSF2	*Th 17 cell differentiation*
		IFNG	
		IL1A	
		IL1B	
		IL15	
		IL16	
		IL17A	
Methylnaphthazarin	BCL2L1	CTSK	*Osteoclast differentiation*
		CTSL	
L-tryptophan	CTSL	CTSK	*Osteoclast differentiation*
		CTSL	
	MMP1	MMP1	*Osteoclast differentiation*
		MMP3	
	MMP2	MMP1	*Osteoclast differentiation*
		VEGFA	

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	MMP3	MMP1 MMP3	*Osteoclast differentiation*
	MMP9	MMP1 MMP3 VEGFA	*Osteoclast differentiation*
Cinnamic acid	MMP1	MMP1 MMP3	*Osteoclast differentiation*
	MMP2	MMP1 VEGFA	*Osteoclast differentiation*
	MMP9	MMP1 MMP3 VEGFA	*Osteoclast differentiation*
Beta sitosterol	FGF2	TEK	*VEGF*
Stigmasterol	FGF2	TEK	*VEGF*
Ficusin	NFKB1	TNF IL18	*Toll-like receptors*
Suberosin	XPO1	TNFSF13	*T-cell receptors*
Quercetin 3-ol-rhamnoside	ALOX5		*Lipoxygenase*

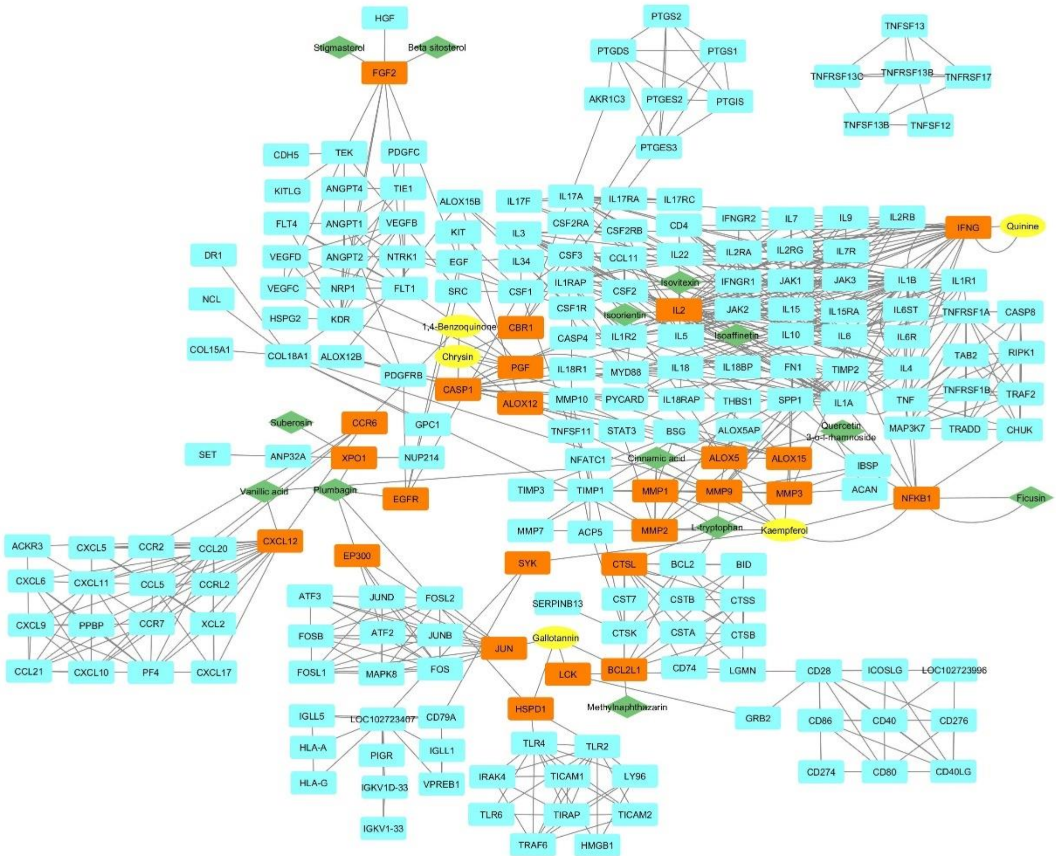

Figure 2. Visualization network pharmacology of *Acalypha indica* L. (yellow) and *Plumbago zeylanica* L. (green) compounds correlated with target proteins (orange)

The above image shows that the target protein NFKB1 correlates with two compounds originating from *Acalypha indica* L. and *Plumbago zeylanica* L.. For *Acalypha indica* L. with the compound kaempferol and *Plumbago zeylanica* L. with the compound ficusin. The family of inducible transcription factors known as NF-κB is involved in several immune system functions (Hayden & Ghosh, 2014).

NF-κB controls the activation, differentiation, and effector activity of inflammatory T-cells. Recent studies have shown that NF-κB plays a role in regulating inflammasome activation. The main inflammatory mediator of rheumatoid arthritis is NF-κB, which has

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been shown to be activated in the synovial tissue of patients with rheumatoid arthritis. The pathogenesis of rheumatoid arthritis involves a variety of cell types, including innate immune cells such as monocytes/macrophages, T cells, B cells, and synovial fibroblasts. NF-κB mediates the activation of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, in monocytes/macrophages. Numerous cytokines can trigger NF-κB in fibroblasts and innate immune cells, which in turn triggers the release of more inflammatory cytokines and chemokines, which in turn promotes the recruitment of more inflammatory immune cells and the expansion of inflammation. Specifically, individuals with rheumatoid arthritis frequently have elevated serum levels of TNF family B cell-activating factors, which are linked to deregulated NF-κB activation. Consequently, NF-κB functions in several cell types to mediate the pathogenesis of rheumatoid arthritis (Liu et al., 2017).

Kaempferol is a flavonoid, and one study examined the possible anti-rheumatoid arthritis effect of kaempferol on synovial tissue after knee arthroplasty. Administration of kaempferol suppressed the expression of NF-κB, MAPK, COX-2, PGE2, MMP3, and MMP1, these results indicating an anti-rheumatic effect of kaempferol on synovial tissue (Behl et al., 2022). Previous studies have shown that ficusin compounds exhibit low cytotoxicity against chondrocytes over a range of doses. Ficusin suppressed chondrocyte proliferation at a concentration of 100 μM. Nevertheless, research on the immunomodulatory effects of these compounds in RA is lacking (Pai et al., 2021).

As shown in Figure 2, kaempferol and cinnamic acid correlated with MMP1, MMP2, and MMP9, and l-tryptophan correlated with MMP1, MMP2, MMP3, and MMP9. Rheumatoid arthritis is one of the diseases for which matrix metalloproteinase (MMP) is implicated in the pathogenesis. MMP is strongly linked to the development of RA because it frequently results from abnormally increased MMP levels, which induce synovial joint lesions. It is also recognized that MMPs cause permanent damage to the tendons, bones, and cartilage in joints. Tissue inhibitors of MMP (TIMP) have been shown to ameliorate rheumatoid arthritis; hence, MMP is a significant therapeutic target for rheumatoid arthritis (Li et al., 2022).

Kaempferol inhibits migration, invasion, and MMP expression in rheumatoid arthritis FLS. Kaempferol has been shown to lower MMP3 and MMP13 expression levels as well as lower LPS levels of chondrogenic markers. This indicates that kaempferol in rheumatoid arthritis FLS reduces the production of MMP1, MMP3, MMP9, and MMP13 (Pan et al., 2018). These chemicals, such as cinnamic acid, have a propensity to displace hydrogen atoms and donate electrons from aromatic phenolic rings to transform them into free radicals. Thus, it absorbs free radicals and functions as a reducing agent. It can activate different endogenous antioxidant pathways, leading to an increase in antioxidant enzyme levels (Behl et al., 2022). Anti-inflammatory role due to inhibitory effect on the NF-κB signaling pathway (Ruwizhi & Aderibigbe, 2020). Previous studies have shown that cell invasion and migration of synovial fibroblasts can be considerably decreased by MMP inhibition, and cinnamic acid suppresses the expression of MMP1, MMP2, and MMP3 (Liu et al., 2020).

Kaempferol was correlated with the target protein SYK. The cytoplasmic protein tyrosine kinase Spleen tyrosine kinase (Syk) is a member of the Src family of non-receptor tyrosine kinases (Deng et al., 2016). Patients with rheumatoid arthritis have increased levels of pSyk in the peripheral blood B cells. In antibody-induced arthritis, depleting Syk from neutrophils was useful in preventing joint inflammation, and injecting Syk siRNA directly into the joint stopped the disease progression (Deng et al., 2016). In another study, kaempferol reduced the increased levels of Syk autophosphorylation induced by Myc-Syk overexpression. Kaempferol also decreased Syk-induced NF-κB-mediated luciferase activity, suggesting that kaempferol can directly suppress Syk at the enzyme and associated functional levels. Kaempferol blocked the catalytic activity of IRAK1 and IRAK4, suggesting that the protein tyrosine kinases Src and Syk were suppressed and that these enzymes were directly targeted (Kim et al., 2015).

Three compounds, isoaffinetin, isoorientin, and isovitexin, were correlated with the IL2 target protein. T-cell activation and proliferation are stimulated by IL-2, an autocrine growth factor, and cytokines generated by Th1 lymphocytes. Clinical research has shown a correlation between serum IL-2 level and RA disease activity. It has been shown that IL-2 has both an indirect suppressive effect and a direct stimulatory effect in the CIA model. As both early and late treatment with IL-2 exacerbated CIA in mice treated with anti-IFNG Ab, it was determined that the suppressive action was not directly mediated by IFNG. It has been discovered that the IL-2/anti-IL-2 monoclonal antibody immune complex inhibits murine CIA. According to current research, CD8+ T cells are the main source of IFNG, which activates monocytes/macrophages, synovial

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fibroblasts, and CD4+ T cells. IFNG, which is produced by monocytes/macrophages, promotes osteoclastogenesis and causes joint damage in rheumatoid arthritis (Kondo et al., 2021).

Isoaffinetin, this compound from *Plumbago zeylanica* L. shows therapeutic activity such as rheumatoid arthritis (Bharadvaja, 2017). In vitro experiments using LPS-stimulated mouse macrophage RAW 264.7, demonstrated the strong anti-inflammatory effects of isoorientin, a specific inhibitor of COX-2. Isoorientin effectively reduced carrageenan-induced inflammatory rat paw edema Inactivation of NF-κB and downregulation of pro-inflammatory gene expression, including COX-2, iNOS, and TNFα, mediates this effect (Anilkumar et al., 2017). Isovitexin exhibits a range of pharmacological properties, including anti-inflammatory, antioxidant, and antineoplastic effects. Isovitexin is known to suppress the NF-κB and MAPK pathways in macrophages (Zhang et al., 2021).

β-Sitosterol and stigmasterol compounds were correlated with FGF2. The only bone-resorptive cytokine that has been shown to be highly expressed in the synovial fluid of patients with rheumatoid arthritis is correlated with the extent of joint destruction is basic FGF2. It is well known that via binding to the receptor (FGFR), FGF2 stimulates osteoclastogenesis and promotes bone resorption by binding to the receptor FGFR (Zhao et al., 2020).

Beta-sitosterol is a bioactive phytosterol having antioxidant and anti-oxidant effects-inflammation. VEGF expression was decreased by beta-sitosterol in kidney tissue. Beta-Sitosterol Inhibits VEGFR2 Production and Activation. Previous research has also shown that beta-sitosterol has an anti-angiogenic function by inhibiting VEGF or inflammatory cytokine expression. This suggests that beta-sitosterol acts on the VEGF pathway to treat rheumatoid arthritis (Qian et al., 2022).

Stigmasterol exerts antipyretic, anticancer, and anti-inflammatory effects. In the research carried out (Ahmad Khan et al., 2020), showed the results that stigmasterol improved clinical severity in CIA mice compared to controls. The therapeutic effect is associated with a reduction in joint destruction and an improvement in histological changes. By downregulating the expression of NF-κB and p38MAPK in joints, stigmasterol treatment also markedly inhibited the expression of pro-inflammatory mediators (TNFα, IL6, IL-1β, iNOS, and COX-2) and boosted the expression of anti-inflammatory cytokines (IL10) (Ahmad Khan et al., 2020).

The compound 1,4-benzoquinone was correlated with the target protein, CASP1. Gasdermin D protein is cleaved by caspase-1, triggering pyroptosis, a pro-inflammatory form of dead cells and pro-IL-1β and pro-IL-18 interleukins in their active cytokine forms (Caruso et al., 2022).

1,4-benzoquinone also known as para-benzoquinone(Jing et al., 2021) showed that celastrol a methylated triterpenoid quinone, has anti-rheumatoid arthritis effects, where the secretion of IL-1β and IL-18 in mouse serum induced by complete Freund's adjuvant (CFA) and THP-1 cell supernatant was decreased (Jing et al., 2021).

This suggests that CCR6 may be downregulated upon effector/memory T cell infiltration because of the inflammatory environment of rheumatoid arthritis joints (Schutyser et al., 2003).

The target protein JUN correlates with gallotannin, and in rheumatoid arthritis, VCAM-1 production is induced by IL-18, which is activated by AP-1. AP-1 functions as a signaling molecule that triggers the production of VCAM-1, mostly through p-38/MAPK, instead of epithelial cell NF-κB. Therapeutically, AP-1 impairs cell migration and invasiveness and prevents pannus development in rheumatoid arthritis joints. Inflammatory disorders, cartilage degradation, leukocyte infiltration, eicosanoid synthesis, and antioxidant effects are all caused by AP-1 inhibition. In addition, AP-1 inhibition can minimize synovial expansion and hyperplasia (Le Rossignol et al., 2018)

Two compounds, gallotannin and plumbagin, correlate with the same target protein, HSPD1. Serum HSP60, also known as HSPD1, is elevated in patients with inflammatory conditions, such as colitis, diabetes, and acute lung injury. According to previous reports, HSP60 antibodies balance cytokines toward anti-inflammatory responses and prevent colitis and arthritis in mice. Furthermore, HSP60 triggers an inflammatory cascade by activating macrophages through TLR4 (Huang et al., 2020).

Gallotannin and methylnaphthazarin correlate with BCL2L1, the BCL-2 family of proteins known to be involved in promoting or inhibiting apoptosis. The mitochondrial apoptotic pathway requires the presence of two important pro-apoptotic multi-domains, BAX and BAK, for its execution phase. Common anti-apoptotic proteins that support cell survival include BCL2, BCL-xL (gene/transcript name BCL2L1), MCL1, BCL2A1, and BCL-W (Loo et al., 2020).

Gallotannin compounds correlated with LCK, Four gene biomarkers (LCK, MS4A1, CXCL13, and IGHM) had good predictive ability for rheumatoid

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arthritis. Studies show that LCK regulates initiation of TCR signaling, T cell development, and homeostasis (Ao et al., 2023)

Chrysin is correlated with PGF target protein. Patients with rheumatoid arthritis have higher levels of VEGF expression in their serum and synovial fluid, which correlates with CRP in connection with radiological abnormalities in the hands and feet. VEGF interacts with one or two receptor tyrosine kinases, VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2). VEGFR-1, also known as fms-related tyrosine kinase 1 (FLT-1), triggers the production of pro-inflammatory cytokines that contribute to inflammation in rheumatoid arthritis patients. VEGFR-1 plays a core role in pathological angiogenesis during rheumatoid arthritis, which is mediated by VEGF and placental growth factor (PGF). Upregulation of FLT-1 expression was positively correlated with VEGF and PGF concentrations. This causes hyper-responsiveness and increased production of specific pro-inflammatory cytokines in rheumatoid arthritis. Animal models of rheumatoid arthritis using antibodies against FLT-1 have shown suppression of angiogenesis and inflammatory joint damage. This suggests that selective reduction of pathological angiogenesis and inflammatory responses in patients with active rheumatoid arthritis may be attainable by suppressing FLT-1 (Paradowska-Gorycka et al., 2017).

Chrysin compounds correlated with two target proteins, namely ALOX12 and ALOX15, also correlated with CBR1, while kaempferol correlated with three target proteins, ALOX5, ALOX12, and ALOX15.

Vanillic acid is associated with CXCL12. One of the primary sources of chemokine motif CXC ligand 12 (CXCL12), which is essential for the migration and activation of inflammatory cells into synovial tissue, is stromal cells. The natural receptor for CXCL12 is CXC receptor 4 (CXCR4). The chemokine CXCL12 mediates T cell and B cell migration and activation in immune cells and may contribute to the immunological response against rheumatoid arthritis. Joint synovial cells produce and secrete CXCL12. Apoptosis and chondrocyte destruction can result from articular chondrocytes secreting different inflammatory agents when CXCR4 and CXCL12 are activated (Peng et al., 2020).

The plumbagin compound correlated with the target protein EGFR. Serum and joint epidermal growth factor receptor (EGFR) concentrations were significantly higher in rheumatoid arthritis. The EGFR inhibitor erlotinib was shown by Swanson et al. to mitigate antigen-induced arthritis in mice and decrease synovitis, pannus development, cartilage loss, and bone erosion, suggesting that EGFR may be a potential target for rheumatoid arthritis treatment (Yuan et al., 2013).

Plumbagin has been linked to the pathogenesis of fibrosis, inflammation, transition from epithelial to mesenchymal, and promotion of extracellular matrix deposition. It is connected to EP300 (Rubio et al., 2023).

Plumbagin and suberosin, which correlate with the same target protein XPO1. XPO1 is a novel candidate for targeted therapy in rheumatoid arthritis. These genes were primarily enriched in intercellular communication and fungal immune-related pathways, including tight junction formation, Th17 cell differentiation, cell-leukocyte adhesion, focal adhesion, cytokine-mediated regulation of signaling pathways, and regulation of interleukin 2 production. This was revealed by GO and KEGG pathway enrichment analyses of HRG (Birga et al., 2022).

l-Tryptophan was correlated with the target protein CTSL. Three compounds correlate to one target protein, namely isoorientin, quercetin 3-ol-rhamnoside, and vanillic acid. These three compounds were correlated with the same target protein, ALOX5. In this study, quercetin 3-o-l-rhamnoside correlated with the inflammatory lipoxygenase signaling pathway.

Quercetin inhibits LPS-induced TNF-α and IL-8 production generated by LPS in macrophages and lung A549 cells. It has been reported to inhibit LPS-induced TNF-α mRNA levels and IL-1α expression. Quercetin also inhibits inflammatory lipoxygenase (LOX) and cyclooxygenase (COX) (Shorobi et al., 2023).

In research conducted by Anilkumar et al., 2017, isoorientin has been shown to decrease inflammation in mice with air sac models. Additionally, Western blot analysis has revealed the expression of inflammatory proteins COX-2, TNFα, IL-1β, iNOS, and 5-LOX. Carrageenan significantly raised the expression of COX-2, TNFα, IL-1β, iNOS, and 5-LOX; however, isoorientin treatment reduced the expression of these proteins.

In the network pharmacology visualization results of the two plants, it was found that several compounds from the two plants had the same correlation with the rheumatoid arthritis target protein. There are also three compounds that correlate with only one target protein and there are compounds that have many correlations with several target proteins. It can be seen that compounds from the two plants correlate with the target proteins of various rheumatoid arthritis signaling pathways. If these plants are used together, it is expected that they will have an effect in accordance with the intended protein target or signaling pathway.

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CONCLUSION

In summary, the network pharmacology results of the two plants Acalypha indica L. and Plumbago zeylanica L. showed a correlation between each compound and the target proteins of rheumatoid arthritis in different signaling pathways. The results of this screening can be used to determine whether compounds from the two plants have a correlation with various signaling pathways in rheumatoid, which can be used for further research.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Achenbach, J., Tiikkainen, P., Franke, L., & Proschak, E. (2011). Computational tools for polypharmacology and repurposing. In *Future Medicinal Chemistry* (Vol. 3, Issue 8, pp. 961–968). https://doi.org/10.4155/fmc.11.62

Ahmad Khan, M., Sarwar, A. H. M. G., Rahat, R., Ahmed, R. S., & Umar, S. (2020). Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines. *International Immunopharmacology*, *85*. https://doi.org/10.1016/j.intimp.2020.106642

Amalia, R., Suyatno, S., & Sabila, F. (2021). *Arthritis Assay on Combination of Red Ginger (Zingiber Officinale) and Secang Wood (Caesalpinia Sappan) Extract Towards Rat Oedema Induced by Complete Freund's Adjuvant*.

Anilkumar, K., Reddy, G. V., Azad, R., Yarla, N. S., Dharmapuri, G., Srivastava, A., Kamal, M. A., & Pallu, R. (2017). Evaluation of Anti-Inflammatory Properties of Isoorientin Isolated from Tubers of Pueraria tuberosa. *Oxidative Medicine and Cellular Longevity*, *2017*. https://doi.org/10.1155/2017/5498054

Ao, Y., Wang, Z., Hu, J., Yao, M., & Zhang, W. (2023). Identification of essential genes and immune cell infiltration in rheumatoid arthritis by bioinformatics analysis. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-29153-3

Behl, T., Mehta, K., Sehgal, A., Singh, S., Sharma, N., Ahmadi, A., Arora, S., & Bungau, S. (2022). Exploring the role of polyphenols in rheumatoid arthritis. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 19, pp. 5372–5393). Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2021.1924613

Bharadvaja, N. (2017). Medicinal Plants in the Management of Cancer: A Review. *International Journal of Complementary & Alternative Medicine*, *9*(2). https://doi.org/10.15406/ijcam.2017.09.00291

Birga, A. M., Ren, L., Luo, H., Zhang, Y., & Huang, J. (2022). Prediction of New Risk Genes and Potential Drugs for Rheumatoid Arthritis from Multiomics Data. *Computational and Mathematical Methods in Medicine*, *2022*. https://doi.org/10.1155/2022/6783659

Bullock, J., Rizvi, S. A. A., Saleh, A. M., Ahmed, S. S., Do, D. P., Ansari, R. A., & Ahmed, J. (2019). Rheumatoid arthritis: A brief overview of the treatment. In *Medical Principles and Practice* (Vol. 27, Issue 6, pp. 501–507). S. Karger AG. https://doi.org/10.1159/000493390

Burmester, G. R., & Pope, J. E. (2017). Novel treatment strategies in rheumatoid arthritis. In *The Lancet* (Vol. 389, Issue 10086, pp. 2338–2348). Lancet Publishing Group. https://doi.org/10.1016/S0140-6736(17)31491-5

Caruso, F., Pedersen, J. Z., Incerpi, S., Kaur, S., Belli, S., Florea, R. M., & Rossi, M. (2022). Mechanism of Caspase-1 Inhibition by Four Anti-inflammatory Drugs Used in COVID-19 Treatment. *International Journal of Molecular Sciences*, *23*(3). https://doi.org/10.3390/ijms23031849

Deng, G. M., Kyttaris, V. C., & Tsokos, G. C. (2016). Targeting syk in autoimmune rheumatic diseases. In *Frontiers in Immunology* (Vol. 7, Issue MAR). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2016.00078

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Deng, Y., Li, Q., Li, M., Han, T., Li, G., & Liu, Q. (2020). Network Pharmacology Identifies the Mechanisms of Sang-Xing-Zhi-Ke-Fang against Pharyngitis. *Evidence-Based Complementary and Alternative Medicine*, *2020*. https://doi.org/10.1155/2020/2421916

dos Reis Neto, E. T., Kakehasi, A. M., de Medeiros Pinheiro, M., Ferreira, G. A., Lopes Marques, C. D., da Mota, L. M. H., dos Santos Paiva, E., Salviato Pileggi, G. C., Sato, E. I., Gomides Reis, A. P. M., Xavier, R. M., & Provenza, J. R. (2020). Revisiting hydroxychloroquine and chloroquine for patients with chronic immunity-mediated inflammatory rheumatic diseases. *Advances in Rheumatology*, *60*(1). https://doi.org/10.1186/s42358-020-00134-8

Gfeller, D., Grosdidier, A., Wirth, M., Daina, A., Michielin, O., & Zoete, V. (2014). SwissTargetPrediction: A web server for target prediction of bioactive small molecules. *Nucleic Acids Research*, *42*(W1). https://doi.org/10.1093/nar/gku293

Hayden, M. S., & Ghosh, S. (2014). Regulation of NF-κB by TNF family cytokines. In *Seminars in Immunology* (Vol. 26, Issue 3, pp. 253–266). Academic Press. https://doi.org/10.1016/j.smim.2014.05.004

Hong, M., Li, S., Tan, H. Y., Cheung, F., Wang, N., Huang, J., & Feng, Y. (2017). A network-based pharmacology study of the herb-induced liver injury potential of traditional hepatoprotective Chinese herbal medicines. *Molecules*, *22*(4). https://doi.org/10.3390/molecules22040632

Hu, X. X., Wu, Y. jing, Zhang, J., & Wei, W. (2019). T-cells interact with B cells, dendritic cells, and fibroblast-like synoviocytes as hub-like key cells in rheumatoid arthritis. In *International Immunopharmacology* (Vol. 70, pp. 428–434). Elsevier B.V. https://doi.org/10.1016/j.intimp.2019.03.008

Huang, J., Li, L., Cheung, F., Wang, N., Li, Y., Fan, Z., Yin, F., Yang, J., Gao, R., He, Y., & Feng, Y. (2017). Network Pharmacology-Based Approach to Investigate the Analgesic Efficacy and Molecular Targets of Xuangui Dropping Pill for Treating Primary Dysmenorrhea. *Evidence-Based Complementary and Alternative Medicine*, *2017*. https://doi.org/10.1155/2017/7525179

Huang, Q., Gao, W., Mu, H., Qin, T., Long, F., Ren, L., Tang, H., Liu, J., & Zeng, M. (2020). HSP60 Regulates Monosodium Urate Crystal-Induced Inflammation by Activating the TLR4-NF- κ B-MyD88 Signaling Pathway and Disrupting Mitochondrial Function. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/10.1155/2020/8706898

Huang, X. F., Zhang, J. L., Huang, D. P., Huang, A. S., Huang, H. T., Liu, Q., Liu, X. H., & Liao, H. L. (2020). A network pharmacology strategy to investigate the anti-inflammatory mechanism of luteolin combined with in vitro transcriptomics and proteomics. *International Immunopharmacology*, *86*. https://doi.org/10.1016/j.intimp.2020.106727

IMPPAT: Indian Medicinal Plants, P. A. T. (2023a). *Acalypha indica L.* IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics. https://cb.imsc.res.in/imppat/phytochemical/Acalypha%20indica

IMPPAT: Indian Medicinal Plants, P. A. T. (2023b). *Plumbago zeylanica L.* IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics. https://cb.imsc.res.in/imppat/phytochemical/Plumbago%20zeylanica

Jing, M., Yang, J., Zhang, L., Liu, J., Xu, S., Wang, M., Zhang, L., Sun, Y., Yan, W., Hou, G., Wang, C., & Xin, W. (2021). Celastrol inhibits rheumatoid arthritis through the ROS-NF-κB-NLRP3 inflammasome axis. *International Immunopharmacology*, *98*. https://doi.org/10.1016/j.intimp.2021.107879

Kim, S. H., Park, J. G., Lee, J., Yang, W. S., Park, G. W., Kim, H. G., Yi, Y. S., Baek, K. S., Sung, N. Y., Hossen, M. J., Lee, M. N., Kim, J. H., & Cho, J. Y. (2015). The dietary flavonoid kaempferol mediates anti-inflammatory responses via the src, syk, IRAK1, and IRAK4 molecular targets. *Mediators of Inflammation*, *2015*. https://doi.org/10.1155/2015/904142

KNApSAcK Core System. (2023). *Plumbago zeylanica L.* KNApSAcK Core System. http://www.knapsackfamily.com/knapsack_core/result.php?sname=all&word=plumbago%20zeylanica

Kondo, N., Kuroda, T., & Kobayashi, D. (2021). Cytokine networks in the pathogenesis of rheumatoid arthritis. In *International Journal of Molecular Sciences* (Vol. 22, Issue 20). MDPI. https://doi.org/10.3390/ijms222010922

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Le Rossignol, S., Ketheesan, N., & Haleagrahara, N. (2018). Redox-sensitive transcription factors play a significant role in the development of rheumatoid arthritis. In *International Reviews of Immunology* (Vol. 37, Issue 3, pp. 129–143). Taylor and Francis Ltd. https://doi.org/10.1080/08830185.2017.1363198

Li, R. L., Duan, H. X., Liang, Q., Huang, Y. L., Wang, L. Y., Zhang, Q., Wu, C. J., Liu, S. Q., & Peng, W. (2022). Targeting matrix metalloproteases: A promising strategy for herbal medicines to treat rheumatoid arthritis. In *Frontiers in Immunology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2022.1046810

Liu, J., Li, R.-L., Wei, S.-J., Jin, W., & Wu, C.-J. (2020). *Cinnamomi ramulus exhibits anti-proliferative and anti-migration effects on MH7A rheumatoid arthritis-derived broblast-like synoviocytes through induction of apoptosis & cell arrest and suppression of matrix metalloproteinase*. https://doi.org/10.21203/rs.2.24246/v1

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. In *Signal Transduction and Targeted Therapy* (Vol. 2). Springer Nature. https://doi.org/10.1038/sigtrans.2017.23

Loo, L. S. W., Soetedjo, A. A. P., Lau, H. H., Ng, N. H. J., Ghosh, S., Nguyen, L., Krishnan, V. G., Choi, H., Roca, X., Hoon, S., & Teo, A. K. K. (2020). BCL-xL/BCL2L1 is a critical anti-apoptotic protein that promotes the survival of differentiating pancreatic cells from human pluripotent stem cells. *Cell Death and Disease*, *11*(5). https://doi.org/10.1038/s41419-020-2589-7

Mateen, S., Zafar, A., Moin, S., Khan, A. Q., & Zubair, S. (2016). Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. In *Clinica Chimica Acta* (Vol. 455, pp. 161–171). Elsevier B.V. https://doi.org/10.1016/j.cca.2016.02.010

Nirk, E. L., Reggiori, F., & Mauthe, M. (2020). Hydroxychloroquine in rheumatic autoimmune disorders and beyond. *EMBO Molecular Medicine*, *12*(8). https://doi.org/10.15252/emmm.202012476

Noor, F., Rehman, A., Ashfaq, U. A., Saleem, M. H., Okla, M. K., Al-Hashimi, A., Abdelgawad, H., & Aslam, S. (2022a). Integrating Network Pharmacology and Molecular Docking Approaches to Decipher the Multi-Target Pharmacological Mechanism of Abrus precatorius L. Acting on Diabetes. *Pharmaceuticals*, *15*(4). https://doi.org/10.3390/ph15040414

Noor, F., Rehman, A., Ashfaq, U. A., Saleem, M. H., Okla, M. K., Al-Hashimi, A., Abdelgawad, H., & Aslam, S. (2022b). Integrating Network Pharmacology and Molecular Docking Approaches to Decipher the Multi-Target Pharmacological Mechanism of Abrus precatorius L. Acting on Diabetes. *Pharmaceuticals*, *15*(4). https://doi.org/10.3390/ph15040414

Ono, T., Okamoto, K., Nakashima, T., Nitta, T., Hori, S., Iwakura, Y., & Takayanagi, H. (2016). IL-17-producing γδT cells enhance bone regeneration. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms10928

Pai, F. T., Lu, C. Y., Lin, C. H., Wang, J., Huang, M. C., Liu, C. T., Song, Y. C., Ku, C. L., & Yen, H. R. (2021). Psoralea corylifolia l. Ameliorates collagen-induced arthritis by reducing proinflammatory cytokines and upregulating myeloid-derived suppressor cells. *Life*, *11*(6). https://doi.org/10.3390/life11060587

Pan, D., Li, N., Liu, Y., Xu, Q., Liu, Q., You, Y., Wei, Z., Jiang, Y., Liu, M., Guo, T., Cai, X., Liu, X., Wang, Q., Liu, M., Lei, X., Zhang, M., Zhao, X., & Lin, C. (2018). Kaempferol inhibits the migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes by blocking activation of the MAPK pathway. *International Immunopharmacology*, *55*, 174–182. https://doi.org/10.1016/j.intimp.2017.12.011

Paradowska-Gorycka, A., Sowinska, A., Pawlik, A., Malinowski, D., Stypinska, B., Haladyj, E., Romanowska-Prochnicka, K., & Olesinska, M. (2017). FLT-1 gene polymorphisms and protein expression profile in rheumatoid arthritis. *PLoS ONE*, *12*(3). https://doi.org/10.1371/journal.pone.0172018

Peng, H., Ren, S., Liu, Y., Zhou, H., Tang, X., Yang, J., Tian, J., Xu, P., Xu, H., & Wang, S. (2020). Elevated Expression of the Long Noncoding RNA IFNG-AS1 in the Peripheral Blood from Patients with Rheumatoid Arthritis. *Journal of Immunology Research*, *2020*. https://doi.org/10.1155/2020/6401978

Peng, L., Zhu, N., Mao, J., Huang, L., Yang, Y., Zhou, Z., Wang, L., & Wu, B. (2020). Expression levels of CXCR4 and CXCL12 in patients with rheumatoid arthritis and its correlation with

P-ISSN: 2406-9388
E-ISSN: 2580-8303

disease activity. *Experimental and Therapeutic Medicine*. https://doi.org/10.3892/etm.2020.8950

Qian, K., Zheng, X. X., Wang, C., Huang, W. G., Liu, X. B., Xu, S. Di, Liu, D. K., Liu, M. Y., & Lin, C. S. (2022). β-Sitosterol Inhibits Rheumatoid Synovial Angiogenesis Through Suppressing VEGF Signaling Pathway. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.816477

Rubio, K., Molina-Herrera, A., Pérez-González, A., Hernández-Galdámez, H. V., Piña-Vázquez, C., Araujo-Ramos, T., & Singh, I. (2023). EP300 as a Molecular Integrator of Fibrotic Transcriptional Programs. In *International Journal of Molecular Sciences* (Vol. 24, Issue 15). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijms241512302

Ruwizhi, N., & Aderibigbe, B. A. (2020). Cinnamic acid derivatives and their biological efficacy. In *International Journal of Molecular Sciences* (Vol. 21, Issue 16, pp. 1–36). MDPI AG. https://doi.org/10.3390/ijms21165712

Schutyser, E., Struyf, S., & Van Damme, J. (2003). The CC chemokine CCL20 and its receptor CCR6. In *Cytokine and Growth Factor Reviews* (Vol. 14, Issue 5, pp. 409–426). Elsevier BV. https://doi.org/10.1016/S1359-6101(03)00049-2

Schwinghammer L. Terry, T. Joseph DiPiro, L.Vicki Ellingrod, & V. Cecily DiPiro. (2021). *Pharmacotherapy Handbook Eleventh Edition* (Vol. 11). https://www.facebook.com/groups/2202763316616203

Shorobi, F. M., Nisa, F. Y., Saha, S., Chowdhury, M. A. H., Srisuphanunt, M., Hossain, K. H., & Rahman, M. A. (2023). Quercetin: A Functional Food-Flavonoid Incredibly Attenuates Emerging and Re-Emerging Viral Infections through Immunomodulatory Actions. In *Molecules* (Vol. 28, Issue 3). MDPI. https://doi.org/10.3390/molecules28030938

Song, Y., & Fields, E. (2020). Pharmacological Advances of Chloroquine and Hydroxychloroquine: From Antimalarials to Investigative Therapies in COVID-19. In *Natural Product Communications* (Vol. 15, Issue 9).

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Von Mering, C. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N. T., Roth, A., Bork, P., Jensen, L. J., & Von Mering, C. (2017). The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Research*, *45*(D1), D362–D368. https://doi.org/10.1093/nar/gkw937

Thitilertdecha, P., Tantithavorn, V., Poungpairoj, P., & Onlamoon, N. (2019). Determination of suppressive effect on human T-cell activation by hispidulin, nepetin, and vanillic acid. *Immunopharmacology and Immunotoxicology*, *41*(6), 591–598. https://doi.org/10.1080/08923973.2019.1675165

Yuan, F. L., Li, X., Lu, W. G., Sun, J. M., Jiang, D. L., & Xu, R. S. (2013). Epidermal growth factor receptor (EGFR) as a therapeutic target in rheumatoid arthritis. In *Clinical Rheumatology* (Vol. 32, Issue 3, pp. 289–292). https://doi.org/10.1007/s10067-012-2119-9

Zhang, Y., Qi, Z., Wang, W., Wang, L., Cao, F., Zhao, L., & Fang, X. (2021). Isovitexin Inhibits Ginkgolic Acids-Induced Inflammation Through Downregulating SHP2 Activation. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.630320

Zhao, S., Wang, Y., Hou, L., Wang, Y., Xu, N., & Zhang, N. (2020). Pentraxin 3 inhibits fibroblast growth factor 2 induced osteoclastogenesis in rheumatoid arthritis. *Biomedicine and Pharmacotherapy*, *131*. https://doi.org/10.1016/j.biopha.2020.110628

Zhou, Q., Li, T., Fang, G., Pang, Y., & Wang, X. (2023). Bioactive Molecules against Rheumatoid Arthritis by Suppressing Pyroptosis. In *Pharmaceuticals* (Vol. 16, Issue 7). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ph16070952

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