NASKAH PUBLIKASI

IDENTIFIKASI TARGET TERAPI KURKUMIN SEBAGAI AGEN HEPAPROTEKTOR PADA SIROSIS HATI BERDASARKAN PENDEKATAN *NETWORK PHAMACOLOGY* DAN *MOLECULAR DOCKING*

IDENTIFICATION OF THE THERAPY TARGET OF CURCUMIN AS HEPAPROTECTOR AGENT IN LIVER CIRRHOSIS BASED ON NETWORK PHAMACOLOGY AND MOLECULAR DOCKING APPROACH

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Naskah Publikasi

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Revealing Curcumin Therapeutic Targets on SRC, PPARG, MAPK8 and HSP90 as Liver Cirrhosis Therapy Based on Comprehensive Bioinformatic Study

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Revealing Curcumin Therapeutic Targets on SRC, PPARG, MAPK8 and HSP90 as Liver Cirrhosis Therapy Based on Comprehensive Bioinformatic Study

Cirrhosis develops naturally in three stages: compensated, decompensated, and late decompensated, increasing the risk of death. According to The Global Burden of Disease (GBD), In 2017, 112 million people worldwide had compensated cirrhosis, and late decompensated cirrhosis only lasted one year. Cirrhosis killed 1.472.000 people in 2019, up 10% from 2010. Curcumin's anti-cirrhosis mechanisms have been studied, but the correlation between upregulated genes and patient survival remains unclear. Network pharmacology uses bioinformatics and high-throughput screening to build a drug-target-disease network. Curcumin-target-liver cirrhosis network has 54 genes and 282 protein-protein interactions. Network centrality analysis identifies crucial genes two-step CytoNCA plugin in Cytoscape software identified eight critical genes. Functional enrichment analysis, including the KEGG pathway, shows that essential genes are involved in cancer, endocrine resistance, estrogen signaling, chemical carcinogenesis - receptor activation, lipid, and atherosclerosis pathways. These gene targets mainly participate in cancer pathways. Hepatocellular carcinoma patients upregulate four genes and downregulate four. Hepatocellular carcinoma, a cirrhosis complication, killed most people. Four upregulated genes—SRC, PPARG, MAPK8, and HSP90AA1—strongly correlated with a lower survival probability of liver hepatocellular carcinoma patients with survival time less than 4000 days (~11 years). Curcumin binds native SRC, PPARG, MAPK8, and HSP90AA1 ligands in molecular docking. Curcumin also binds PPARG better than rosiglitazone. After screening critical target genes and pathways, Network Pharmacology, Gene Expression, Survival Rate, and Molecular Docking Analysis reveal curcumin's potential mechanism in liver cirrhosis. These findings provide a theoretical foundation for pharmacological research on curcumin's mechanism in liver cirrhosis.

Keywords: Liver Cirrhosis Therapy; Curcumin; Network Pharmacology; Gene Expression; Survival Rate; Molecular Docking

1. Introduction

Liver cirrhosis is characterized by the deterioration of the hepatic lobule structures and the failure of blood flow (Sanz-García *et al*., 2021). Several causes of liver diseases lead to cirrhosis, but mainly cirrhosis occurs in the end stage of liver fibrosis (Wiegand *et al*., 2013). Liver fibrosis was defined by the accumulation of quantitative and qualitative alterations in the extracellular matrix (ECM), which included collagens, non-collagen glycoproteins, matrix-bound growth factors, glycosaminoglycans, and matrixcellular proteins produced primarily by stellate cells (Karsdal *et al*., 2015). By degrading the ECM, matrix proteases convert the normal hepatic matrix to scar matrix, resulting in hepatic cell dysfunction. The transformation of vitamin A-rich cells (stellate cells) into specific conditions of proliferation, matrix deterioration, retinoid loss, fibrogenesis, and contractile myofibroblast is referred to as a phenotype feature of HSC activation during a liver injury (Farzaei *et al*., 2018; Friedman, 2017). Various liver diseases such as hepatitis B, hepatitis c, alcoholic liver disease, non-alcoholic fatty liver disease, and toxic and drug-induced hepatotoxicity commonly lead to fibrosis and cirrhosis (Nabavi *et al*., 2014). Cirrhosis develops naturally in three stages: compensated cirrhosis, decompensated cirrhosis, and late decompensated at the end of the state (Paik *et al*., 2020; Pinter *et al*., 2016). These three stages demonstrated an increased risk of death in cirrhosis, the two-year risk analysis of cirrhosis patients revealed that late decompensated patients can only survive within one year (D'Amico *et al*., 2018). The development of cirrhosis complications mainly happened in the transformation of those steps. The difficulty could be various health damage conditions such as hepatorenal syndrome, spontaneous bacterial peritonitis, kidney

failure, renal dysfunction (Perez *et al*., 2021; Solà *et al*., 2015), Cirrhosis hepatogenous-diabetes and Hepatocellular carcinoma (HCC) (Garcia-Compean *et al*., 2009). A 17-year cohort study of 214 cirrhosis patients by Angelo Sangiovanni and the team revealed that HCC was the leading cause of death and the first complication that appeared (Sangiovanni *et al*., 2006).

Curcumin, a primary compound of turmeric, has been extensively studied for its anticarcinogenic, antifibrotic, anti-inflammatory, antihepatotoxic, antioxidant, immunomodulatory effect and anticancer properties (Abo-Zaid *et al*., 2020; Chung *et al*., 2018; Khan *et al*., 2019; Salehi *et al*., 2021). Curcumin is a multitargeting molecule in various hepatoprotective pathways. Curcumin restored the NF-kB, JNK-Smad3, and TGF-Smad3 pathways, reducing activated hepatic stellate cells. Curcumin inhibition in cirrhosis was demonstrated by restoring PON1 expression and activity (Khodarahmi *et al*., 2021). Curcumin is a powerful antioxidant that can upregulate Nrf2 genes, increasing GSH levels, glutathione peroxidase activity, and superoxide dismutase (SOD) (Ashrafizadeh *et al*., 2020). Curcumin also inhibits liver fibrosis by targeting NF-KB, IL-1, IL-10, TGF-, CTGF, Col-I, MMP-13, and Smad7 proteins (Hernández-Aquino *et al*., 2020). Curcumin regulates IFN-gamma, NF-kB, TNF-α, and IL-12 levels, resulting in a decrease in the level of inflammatory mediators (Aggarwal *et al*., 2013). Curcumin also has antifibrotic properties via targeting HCS and Collagen 1cI receptors and upregulating PPARG. Curcumin also has an anticarcinogenic impact via downregulating the p53 and p21 genes (Moghtaderi *et al*., 2017). The role of curcumin compounds has been demonstrated in clinical studies to reduce disease

activity scores and the severity of cirrhosis in patients with cirrhosis (Nouri-Vaskeh *et al*., 2020).

As mentioned, cirrhosis stages development leading to cirrhosis complications and increasing mortality. Although the mechanisms underlying curcumin's anti-cirrhosis and anti-fibrosis effects have been investigated, the correlation of curcumin's action with upregulated genes and low survival of patients is still limited (Kronborg *et al*., 2021). Despite this, the Global Burden of Disease (GBD) study published in 2017 found that 112 million people worldwide suffer from compensated cirrhosis (Sepanlou *et al*., 2020). Cirrhosis was responsible for 1.472.000 deaths worldwide in 2019, an increase of 10% from 2010 (D. Q. Huang *et al*., 2023). As a result, more efforts are needed to promote primary prevention and early treatment

The mechanism of anti-hepatic curcumin cirrhosis based on biological systems, virtual screening of potential pharmacological pathways, and exploration of potential curcumin targets that may inhibit complications and possibly help patients with greater chances of survival are still very scientifically limited. Therefore, an alternative strategy of building drug-target-disease networks using bioinformatics approaches and high-throughput technologies is required for further investigation of the role of curcumin in related diseases. In short, the Network pharmacology approach is part of Bioinformatics by using high-throughput screening to build a network model of disease target compounds and the role of big data as a data repository used to look for linkages of proteins to disease. With this Network pharmacology approach, it is beneficial in determining the protein that correlates with the condition you want to target as a therapy candidate by

understanding the mechanisms underlying the activity of compounds in a biological network (Noor *et al*., 2022). Therefore, Network pharmacology is particularly useful in investigating alternate treatments for some lethal disorders and diseases, especially in preventing liver cirrhosis development. Using the Network Pharmacology method, we hope to understand the mechanism of curcumin in liver cirrhosis and identify the potential targets and related pathways (Mahmoudi *et al*., 2022). These potential cirrhosis targets also further analyzed their expression and their association with the survival rate of patients based on the clinical database (Lei *et al*., 2021). This helps identify potential protein targets that may give a longer life to cirrhosis patients. Molecular docking was also done to analyze the strength of interaction between curcumin and active sites of cirrhosis targets compared to native ligands.

2. Materials and Methods

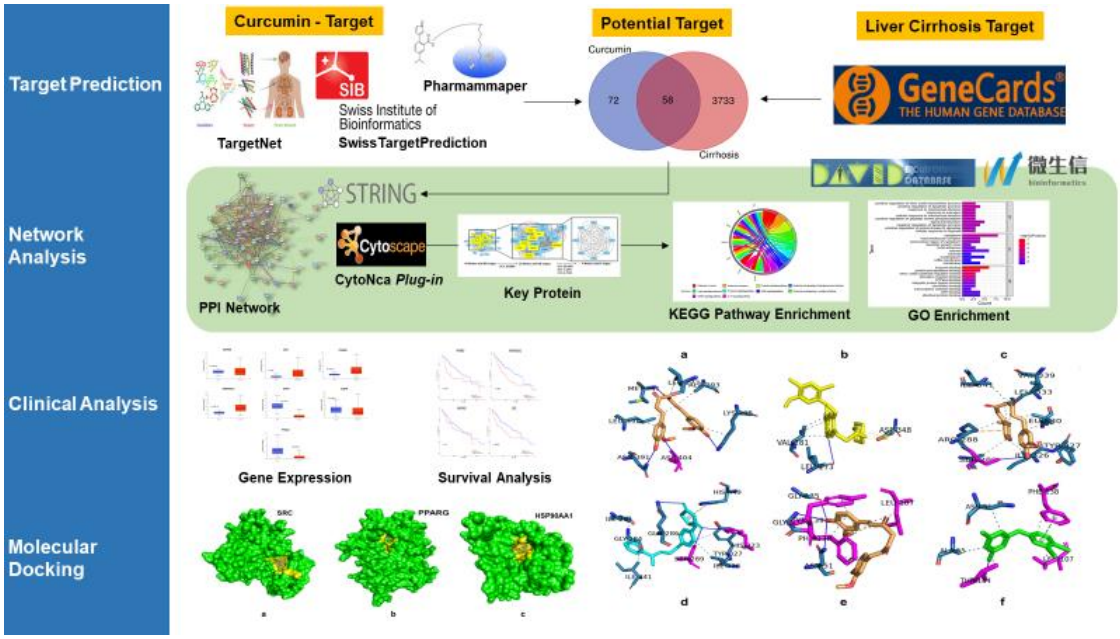

Figure 1. The flowchart of the *in silico* network pharmacological analysis, gene expression, survival analysis and molecular docking approached of curcumin in liver cirrhosis

2. 1 Acquisition of Curcumin-pharmacological target in Liver cirrhosis

All pharmacological targets of Curcumin were screened and collected from accessible online tools: SwissTargetPrediction (http://www.swisstargetprediction.ch/) and PharmaMapper (https://www.lilab-ecust.cn/pharmmapper/) with "Homo Sapiens" and 'Probability 0.6' used in the selection of target (Daina *et al*., 2019; X. Liu *et al*., 2010; Su *et al*., 2019; Yao *et al*., 2016). Furthermore, GeneCards Website Database (https://www.genecards.org/) used to find genes related to liver cirrhosis (Safran *et al*., 2021). The Online Venn Diagram Generator (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to build the intersection between the curcumin target and the target related to Liver Cirrhosis.

2. 2 Identifying Core Targets of Curcumin Against Liver Cirrhosis

The STRING Database Ver.11.5 (https://string-db.org/) network map used to conduct protein-protein interactions (PPI) between Curcumin protein targets and Liver Cirrhosis related protein (Stelzer *et al*., 2016; Szklarczyk *et al*., 2015). The topology parameters were analyzed using the Cytoscape software's Network-Analyzer settings (Shannon *et al*., 2003). To determine the pharmacological mechanism of curcumin against liver cirrhosis, all genes were filtered in Cytoscape software (https://cytoscape.org/, Version 3.7.2) using the *plug-in* CytoNCA based on their topological parameters of

Degree Centrality (DC), Closeness Centrality (CC), and Betweenness Centrality (BC) (Mao *et al*., 2017; Tang *et al*., 2015). The threshold was set as median values of each centralities parameter. Step 1 used only DC, and Step 2 used DC, CC, and BC with the threshold of median values (Niu *et al*., 2018; Yang *et al*., 2021).

2. 3 Functional Enrichment Analysis

In this study, the Database for Annotation, Visualization, and Integrated Discovery (DAVID database, https://david.ncifcrf.gov/) was used to investigate Gene Ontology (GO), including Molecular Function (MF), Cellular Components (CC), Biological Processes (BP), and KEGG enrichment analysis (Kyoto Encyclopedia of Genes and Genomes) (Dennis *et al*., 2003; Kanehisa *et al*., 2016; D. Li *et al*., 2022; Vaghasia *et al*., 2022). Followed by filtering through the results of the parameter (*P-value*) <0.05 to measure statistical differences and potential targets from the effects of protein combinations related to curcumin and Liver Cirrhosis (Chen *et al*., 2015; Kanehisa *et al*., 2017). In addition, the Bioinformatics website platform (http://www.bioinformatics.com.cn) was used to illustrate GO and KEGG target data (Gentleman *et al*., 2004; Shang *et al*., 2023).

2. 4 Prognostic Values of Predicted Core Targets

All mRNA expression levels of hub genes expressed in HCC and corresponding normal liver tissues were obtained using The UALCAN Database (https://ualcan.path.uab.edu/analysis.html) (X. Gao *et al*., 2018; Z. Liu *et al*., 2022). UALCAN is a comprehensive, user-friendly, and

interactive web resource to enable users to identify biomarkers in silico validation of potential genes of interest in cancer OMICS data (Chandrashekar *et al*., 2022; Choy *et al*., 2021). In this study, we want to analyze the expression of critical genes that upregulated and identify the gene associated with prognosis in HCC. The upregulated targets were further analyzed for their association with survival life by Survival analysis in UALCAN tools (Cai *et al*., 2023; L. Yu *et al*., 2023). Then, the results are plotted as forest plots and curves *Kaplan-Meier* (KM) (X. Huang *et al*., 2022). The Molecular Docking study further simulated the selected proteins (Qin *et al*., 2022).

2. 5 Molecular Docking

Molecular docking carried out using Autodock Vina in PyRx software version 0.9.9 (Dallakyan *et al*., 2015). The PDB files containing the crystal structures of the five protein targets were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/) (Burley *et al*., 2021). 4MXO (SRC) (Levinson *et al*., 2014), 1ZGY (PPARG) (Y. Li *et al*., 2005), 2XRW (MAPK8) (Garai *et al*., 2012) and 4BQG are the PDB IDs (HSP90AA1) (Brasca *et al*., 2013) , and the structures of curcumin were acquired from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al*., 2023). All protein structures were prepared in Autodock tools by removing water molecules and heteroatoms, adding hydrogen polar, merging non-polar, and adding gasteiger charges. Then, prepared protein structures were saved in pdqt format file. Curcumin .sdf files from PubChem were converted to .pdb

file and prepared with the same procedure as protein in Autodock tools, then saved in pdbqt format file. Before starting molecular docking, protein structure validation was carried out by redocking the co-crystallized ligand in the binding site of protein using Autodock Vina in PyRx software version 0.9.9. The binding sites were chosen using a grid box (center on ligand) of each protein in Autodock. The grid box coordinates for SRC was X = 12.846 , Y= -36.992, and Z = -6.653 ;coordinate for PPARG were X = 30.231 , Y = 0.048, and Z = 27.89 ; and coordinate for HSP90AA1 was X = 1.828 , Y= 13.488 , and Z = 23.27 (Wargasetia *et al*., 2021). The root mean square deviation between crystallized ligand before and after re-docking were calculated by the LigRMSD web server, and it must be within 2Å (Velázquez-Libera *et al*., 2020). The capacity of a ligand to attach to a receptor is referred to as affinity (Syaifie *et al*., 2022; Widyananda *et al*., 2022). The BIOVIA Discovery Studio Visualizer and PyMOL visualize docking results in 3D (BIOVIA, 2019; Schrödinger, 2023). Moreover, a protein-ligand interaction profiler (PLIP) web server is utilized to investigate protein-ligand interactions in PDB files that encrypt the docking results (Laskowski *et al*., 2011; Salentin *et al*., 2015; Wallace *et al*., 1995).

3. Results

3.1 Acquistion of Curcumin-Pharmacological Target in Liver Cirrhosis

By merging genes from databases (TargetNet, PharmaMapper, SwissTargetPrediction, and GeneCard) and deleting duplicate genes, 4.320 Proteins linked to Liver Cirrhosis were found. Overlaying the Liver Cirrhosis protein with curcumin- targets resulted in 58 proteins associated

with Liver Cirrhosis (Figure. 2).

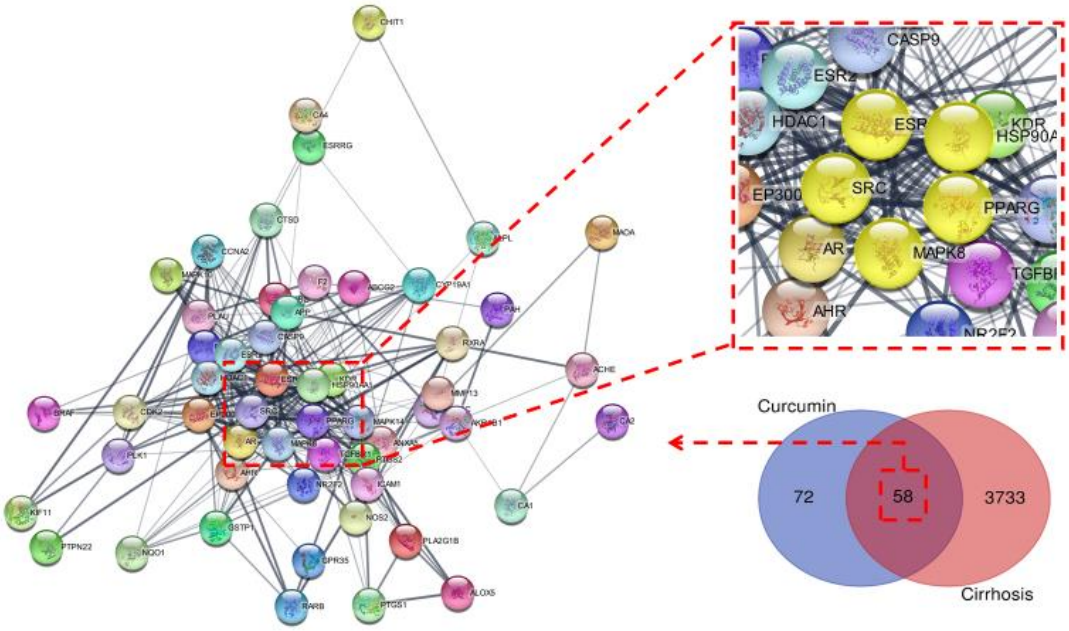

Figure 2. Protein-protein interaction network using STRING. Number of nodes: 54 (After Removing 4 Proteins that have No Interaction Therein), Number of edges: 282, Average node degree: 9.72, Avg. local clustering coefficient: 0.544, Expected number of edges: 108 and PPI enrichment *P-value* : < 1.0e-16

3.2 Identifying Curcumin Targets and Intersection with Liver Cirrhosis

The PPI network of 58 major targets of curcumin-liver cirrhosis was determined using STRING analysis (Figure 2). The topological characteristics of the PPI network curcumin target liver cirrhosis consist of 54 proteins (nodes) and 282 network interactions (edges). They were then ordered based on the median centralities value. With a threshold of DC more than 20.8889, 22 Proteins (Nodes) and 143 Network Interactions appeared as a result of the first screening step. The second step is optimized by using the threshold median values of Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC) of 26.9091, 7.5455, and

0.7512, respectively. The final findings revealed eight proteins (Nodes) and 27 interactions (Figure 3).

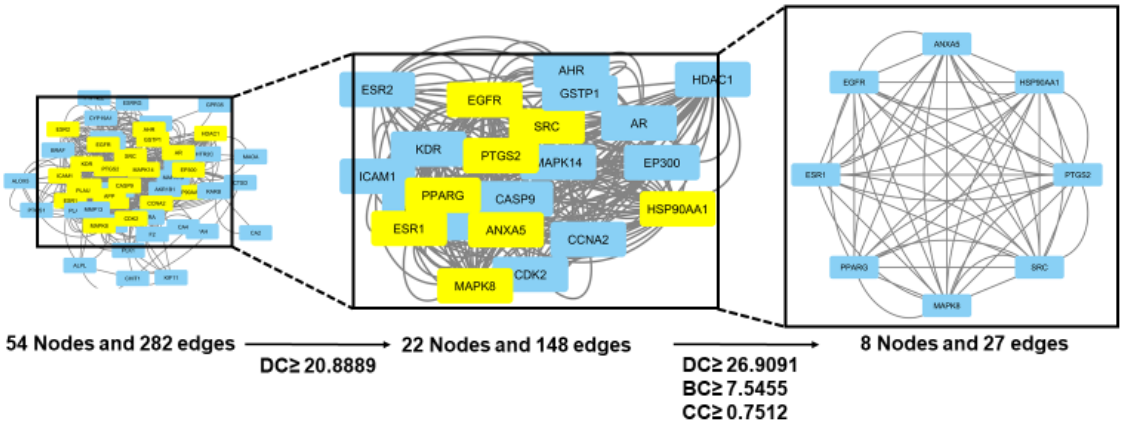

Figure 3. Inter-Protein Interaction Process using CytoNCA plug-in in 2 screening steps, whereas SRC, PPARG, HSP90AA1, ESR1, MAPK8, EGFR, PTGS2, and ANXA5 are present in final results based on three centralities parameters: Degrees Centrality, Betweenness Centrality, and Closeness Centrality

3.3 GO and KEGG Enrichment Analysis

The GO enrichment analysis was carried out by analyzing three ontologies, namely Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Then, data results were chosen with sorting based on statistical significance (*P-value*) < 0.05). This step resulted in 46 BP, 10 CC, and 17 MF data. Furthermore, only the top 10 Gene Ontology and KEGG data *P-value*s were chosen as final results. Table 2 and Figure 4 show the detailed KEGG pathway classification results presented in Table 1. In addition, the ANXA5 Gene Protein did not carry out gene expression and docking validation. The ANXA5 Protein in the KEGG results has no linkages from the Top 10 KEGG pathways. Therefore, stage no further analysis of ANXA5 is carried out on Gene Expression and Molecular

Docking simulation (Figure. 7).

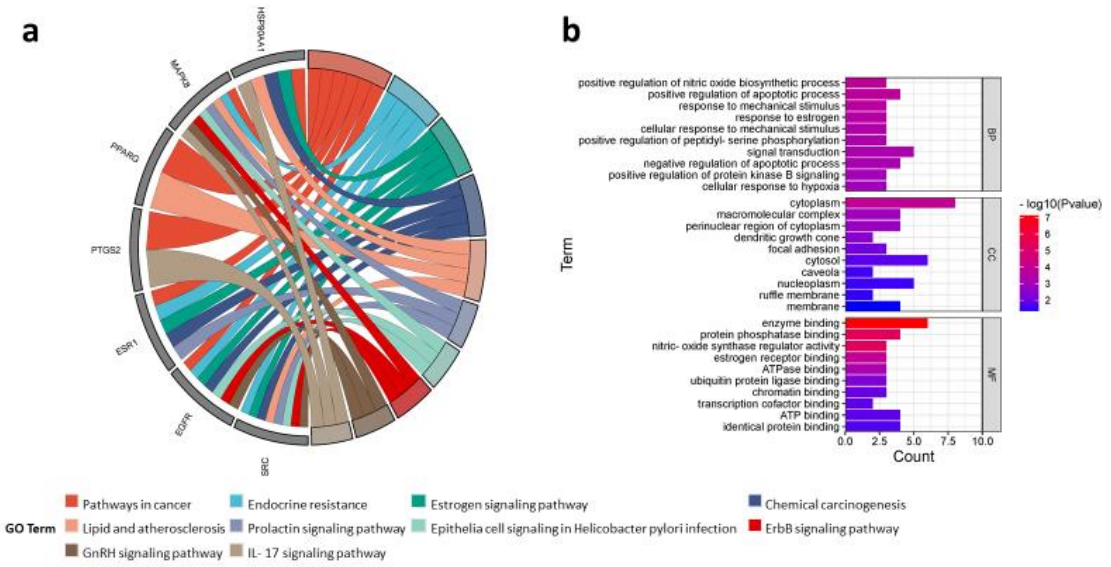

Figure 4. KEGG pathway analysis of target genes shortist by Top 10 representative pathways according to gene count (a), Gene Ontology search results of potential targets of Curcumin on Liver Cirrhosis, including Biological Processes, Cellular Components, and Molecular Functions. Color represents difference -Log10 (b)

Table 1. Top 10 KEGG Pathway Classification of targets

KEGG Pathways	**Count**	**%**	***PValue***	**Genes**
Pathways in cancer	6	85.71429	6.33E-06	HSP90AA1, MAPK8, PPARG, PTGS2, ESR1, EGFR
Endocrine resistance	4	57.14286	3.22E-05	MAPK8, SRC, ESR1, EGFR
Estrogen signaling pathway	4	57.14286	8.97E-05	HSP90AA1, SRC, ESR1, EGFR
Chemical carcinogenesis - receptor activation	4	57.14286	3.21E-04	HSP90AA1, SRC, ESR1, EGFR
Lipid and atherosclerosis	4	57.14286	3.35E-04	HSP90AA1, MAPK8, SRC, PPARG
Epithelial cell signaling in Helicobacter pylori infection	3	42.85714	1.05E-03	MAPK8, SRC, EGFR
Prolactin signaling pathway	3	42.85714	0.001053	MAPK8, SRC, ESR1

ErbB signaling pathway	3	42.85714	0.001549	MAPK8, SRC, EGFR
GnRH signaling pathway	3	42.85714	0.001851	MAPK8, SRC, EGFR
IL-17 signaling pathway	3	42.85714	0.001891	HSP90AA1, MAPK8, PTGS2

Table 2. Top 10 Gene Ontology (Biological Process, Molecullar Function, and Cellular Component)

Gene Ontology	Term	Count	*PValue*	Genes
Biological Process	positive regulation of nitric oxide biosynthetic process	3	1.32E-04	HSP90AA1, PTGS2, ESR1
	positive regulation of apoptotic process	4	1.63E-04	MAPK8, SRC, PPARG, PTGS2
	response to mechanical stimulus	3	2.19E-04	MAPK8, SRC, PPARG
	response to estrogen	3	2.86E-04	HSP90AA1, PPARG, ESR1
	cellular response to mechanical stimulus	3	4.18E-04	MAPK8, PTGS2, EGFR
	positive regulation of peptidyl-serine phosphorylation	3	4.57E-04	HSP90AA1, PTGS2, EGFR
	signal transduction	5	5.56E-04	SRC, ANXA5, PPARG, ESR1, EGFR
	negative regulation of apoptotic process	4	6.53E-04	MAPK8, SRC, ANXA5, EGFR
	positive regulation of protein kinase B signalling	3	9.01E-04	HSP90AA1, SRC, EGFR
	cellular response to hypoxia	3	0.001076	SRC, PPARG, PTGS2

Molecullar Function	enzyme binding	6	7.58E-08	MAPK8, SRC, PPARG, PTGS2, ESR1, EGFR
	protein phosphatase binding	4	4.04E-06	HSP90AA1, MAPK8, PPARG, EGFR
	nitric-oxide synthase regulator activity	3	4.27E-06	HSP90AA1, ESR1, EGFR
	estrogen receptor binding	3	1.17E-04	SRC, PPARG, ESR1
	ATPase binding	3	5.00E-04	SRC, ESR1, EGFR
	ubiquitin protein ligase binding	3	0.005214	HSP90AA1, SRC, EGFR
	chromatin binding	3	0.01189	PPARG, ESR1, EGFR
	transcription cofactor binding	2	0.014057	PPARG, ESR1
	ATP binding	4	0.015017	HSP90AA1, MAPK8, SRC, EGFR
	identical protein binding	4	0.019905	HSP90AA1, PPARG, ESR1, EGFR
Cellular Component	Cytoplasm	8	1.10E-04	HSP90AA1, MAPK8, SRC, ANXA5, PPARG, PTGS2, ESR1, EGFR
	macromolecular complex	4	0.001272	HSP90AA1, PTGS2, ESR1, EGFR
	perinuclear region of cytoplasm	4	0.001613	HSP90AA1, SRC, PPARG, EGFR
	dendritic growth cone	2	0.003415	HSP90AA1, SRC
	focal adhesion	3	0.008388	SRC, ANXA5, EGFR

	Cytosol	6	0.017403	HSP90AA1, MAPK8, SRC, ANXA5, PPARG, ESR1
	Caveola	2	0.026037	SRC, PTGS2
	Nucleoplasm	5	0.029151	HSP90AA1, MAPK8, SRC, PPARG, ESR1
	ruffle membrane	2	0.033369	SRC, EGFR
	Membrane	4	0.045719	HSP90AA1, ANXA5, ESR1, EGFR
	Cytoplasm	8	1.10E-04	HSP90AA1, MAPK8, SRC, ANXA5, PPARG, PTGS2, ESR1, EGFR
	macromolecular complex	4	0.001272	HSP90AA1, PTGS2, ESR1, EGFR
	perinuclear region of cytoplasm	4	0.001613	HSP90AA1, SRC, PPARG, EGFR

3.4 Prognostic Value of The Potential Target Genes of Curcumin

The expression level and survival rate analysis of critical genes were performed in the LIHC (Liver Hepatocellular Carcinoma) context using the TCGA dataset by the UALCAN tool. Hepatocellular carcinoma was the primary liver cancer outcome. Consequently, since this study presents that most critical gene targets have a role in cancer-based on KEGG pathway analysis, LIHC patients in TCGA dataset were used as a focus of this study. The expression level analysis was conducted to identify biomarker genes that seem high when the disease occurs. Figure 5 shows that only SRC, PPARG, MAPK8, and HSP90AA1 have an increased expression in LIHC patients than in normal patients. While ESR1, EGFR, and PTGS2 were

lower in LIIHC patients than normal. Therefore, the higher expression gene in LIHC was further analyzed on their association with the survival rate of LIHC patients.

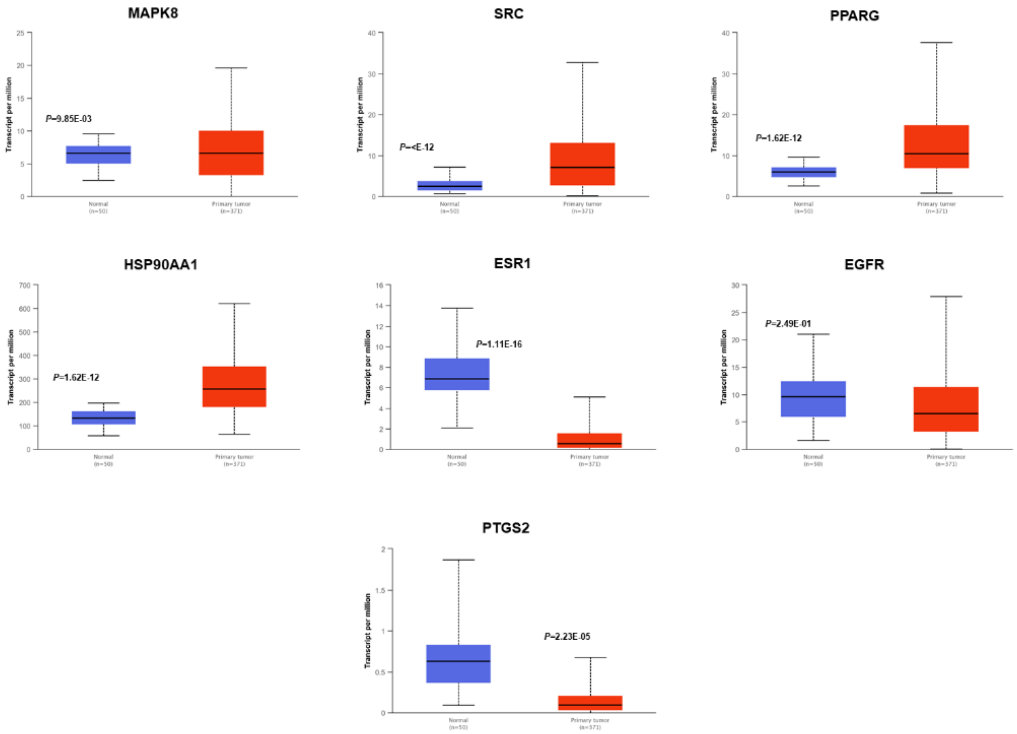

Figure 5. The expression level of crucial genes in normal and LIHC samples. The upregulated genes are shown in red and the downregulated genes are shown in blue.

Over 4000 days (11 years), the overall survival of liver hepatocellular carcinoma patients was analyzed using *Kaplan-Meier* survival. Figure 6 shows the expression of SRC, PPARG, MAPK8, and HSP90AA1 positively associated with the overall survival rate in LIHC patients. While Figure 6 shows that patients with high gene expression had significance poorer survival than patients with low gene expression ($p<0.05$). High SRC levels in patients can survive less than 3000 days (~8 years), while high PPARG, MAPK8, and HSP90AA1 levels can survive less than 4000

days (~11 years). Therefore, these genes were further analyzed in a molecular docking study to identify the molecular interaction with curcumin.

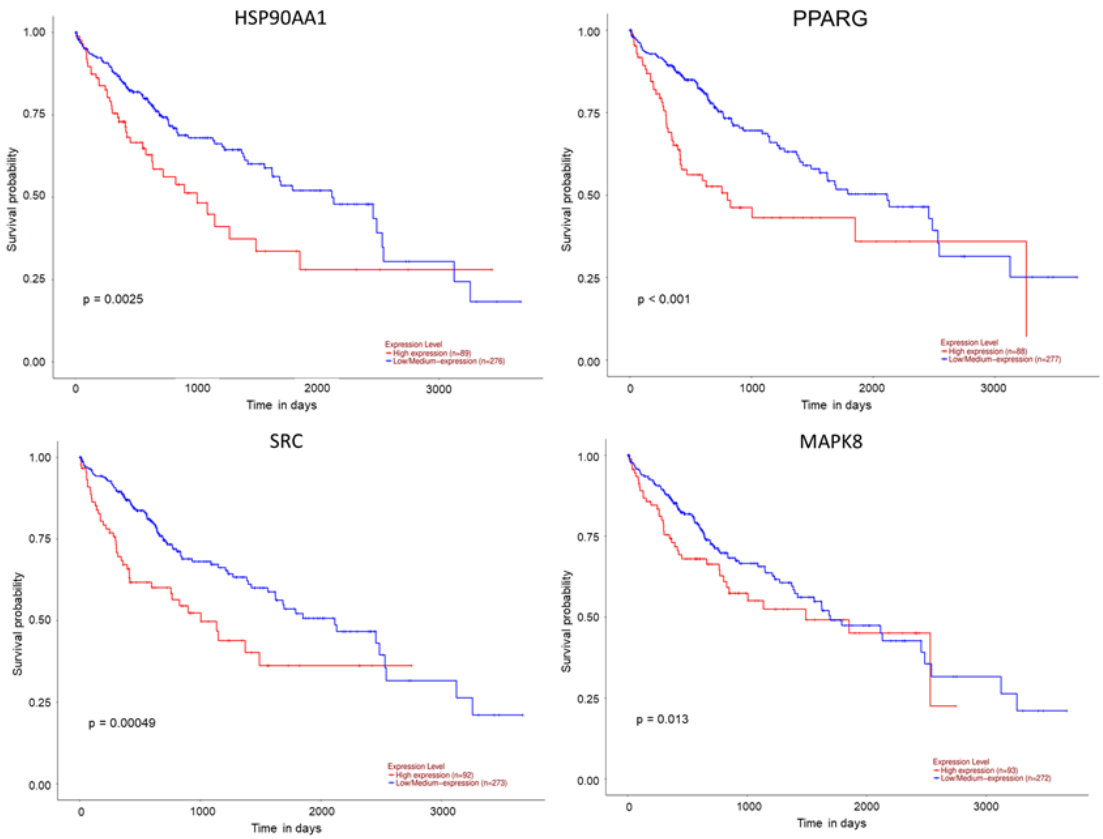

Figure 6. The association of the expression level of crucial genes with LIHC patient survival. The upregulated genes are shown in red and the downregulated genes are shown in blue.

3.5 Molecular Docking analysis and Validation

We used Autodock Vina in PyRx software version 0.9.9 to perform molecular docking of curcumin to protein targets, namely, SRC, PPARG, MAPK8, and HPS90AA1. We use Discovery Visual Studio, PyMOL, and PLIP webserver to visualize and analyze the docking results. Before docking simulation, validation docking was performed in Autodock Vina in PyRx software version 0.9.9 by redocking co-crystallized protein ligands to their binding pocket. The binding pose of the native ligand before and after

docking was compared. The results demonstrated that validation docking was completed successfully, with the Root Mean Square Deviation (RMSD) less than 2Å (Figure 7). RMSD was calculated in the LigRMSD webserver by inputting the native ligand pose in mol format before and after docking (Velázquez-Libera *et al*., 2020).

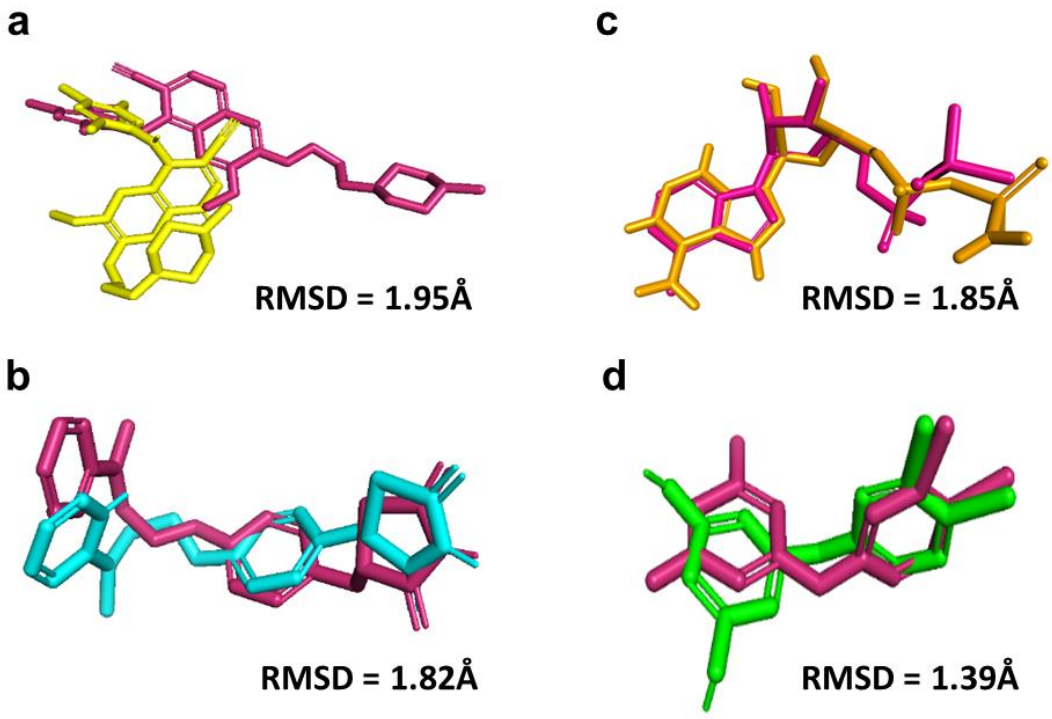

Figure 7. Superimpose of native ligand before and after Re-docking, all native ligand in initial position shown as magenta, Bosutinib (native ligand of SRC PDB ID 4MXO) pose after redocking to SRC shown as yellow (a), 2,4-thiazolidiinedione, 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]- (native ligand of PPARG PDB ID 1ZGY) pose after redocking to PPARG shown as cyan (b), Phosphoaminophosphonic Acid-Adenylate Ester (native ligand of MAPK8 PDB ID 2XRW) pose after redocking to MAPK8 shown as orange and 5-(3,4-dichloro-phenoxy)-benzene-1,3-diol (native ligand of HSP90AA1 PDB ID 4BQG) pose after redocking HSP90AA1 shown as green(d).

In (Table 3.) displayed the docking results for curcumin and the native ligand to

each protein target. Curcumin has a higher affinity for SRC, PPARG, MAPK8 and HSP90AA1 than native ligands for each gene including bosutinib, 2,4-thiazolidiinedione (BRL), Phosphoaminophosphonic Acid-Adenylate Ester and 5-(3,4-dichloro-phenoxy)-benzene-1,3-diol. The interaction of curcumin binding to protein targets was primarily composed of hydrogen bonds and hydrophobic interactions, as shown in Table 3. Key active residues of protein also found in binding site of curcumin, marked in bold.

Table 3. The docking mode between curcumin and the target protein molecule core

n (PDB 0)	Ligand	Binding Affinity (Kcal/Mol)	Interaction		
			Hydrogen Bond	Distance (Å)	Hydrophobic Interaction
C 4MXO)	Curcumin	-7.8	Lys295	3.18	Leu273
			Met341	1.85	Ala293
			Asn391	2.21	Lys295
			Asp404	2.36	Leu393
	Bosutinib*	-7	Leu273	2.91	Val281
RG 1ZGY)	Curcumin	-8.2	**Ser289**	2.58	Arg288 Ala292 Ile326 Tyr327 Leu330 Val339 Ile341
	2,4-thiazolidiinedione (BRL)*	-7.8	Gly284	3.30	Ile281
			Gln286	3.25	Ile326
			Ser289	2.26	Tyr327
			His323	2.06	Ile341
PK8 2XRW)	Curcumin	-8.4	Lys55	2.43	Val40 Ala53 Val158 Leu168
			Arg69	2.83	
			Glu109	2.14	
			Met111	1.92	
			Asp151	2.32	
	Phosphoaminophosphonic Acid-Adenylate Ester*	-6.6	Ala36	2.95	-
			His66	3.61	
			Arg69	2.51	
			Asp151	3.09	
			Lys153	2.45	
			Ser155	3.48	

			Asp169	2.16	
			Arg192	2.24	
	Curcumin	-9.1	Asn51	2.95	**Leu107**
			Gly135	2.24	**Phe138**
			Gly137	3.4	**Tyr139**
)AA1 4BQG)	5-(3,4-dichloro-phenoxy)-benzene-1,3-diol*	-8.3	**Thr184**	3.17	Asn51 Ala55 **Leu107** **Phe138** **Thr184**

(*) means native ligand or natural inhibitor of protein, Bold letter means key active residues

4. Discussion

Liver cirrhosis, the most common type, is caused by alcoholic liver disease, chronic viral hepatitis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, or other factors (J. Li *et al*., 2019; Tsochatzis *et al*., 2014). Despite various treatments, such as dietary changes, drug therapy, and surgical intervention, the treatment effect of cirrhosis remains modest, with high rates of adverse effects and risks of liver function deterioration (Du *et al*., 2023; Ge *et al*., 2016). In previous studies, Curcumin showed beneficial action against Liver cirrhosis (Nouri-Vaskeh *et al*., 2020). Curcumin poses as a hepatoprotector. An earlier in vitro study suggested Curcumin's role in treating liver cirrhosis through its anti-inflammatory effect and suppression of HSC activity, thereby attenuating liver inflammation and fibrogenesis (Efsen, 2001; Kyung *et al*., 2018). Curcumin inhibits liver cirrhosis through its action on multiple pathways, such as a mechanism in antioxidant activity capable of capturing superoxide ions and breaking the chain between superoxide ions (O2-), thereby preventing liver cell damage due to lipid peroxidation (Bao *et al*., 2010; Farombi *et al*., 2008).

By cross-mapping them with Liver Cirrhosis-related targets in TargetNet, PharmaMapper, SwissTargetPrediction, and GeneCard datasets, 58 targets for Curcumin in the therapy of Liver Cirrhosis were found. The "Curcumin target-Liver cirrhosis target" network structure study used network pharmacology and analysis (Figure 1). The PPI network was then built, demonstrating that these target proteins did not operate alone but had relationships with each other. Based on the findings, ESR1, PTGS2, EGFR, MAPK8, PPARG, HSP90AA1, and SRC are proteins that play an important role in liver cirrhosis.

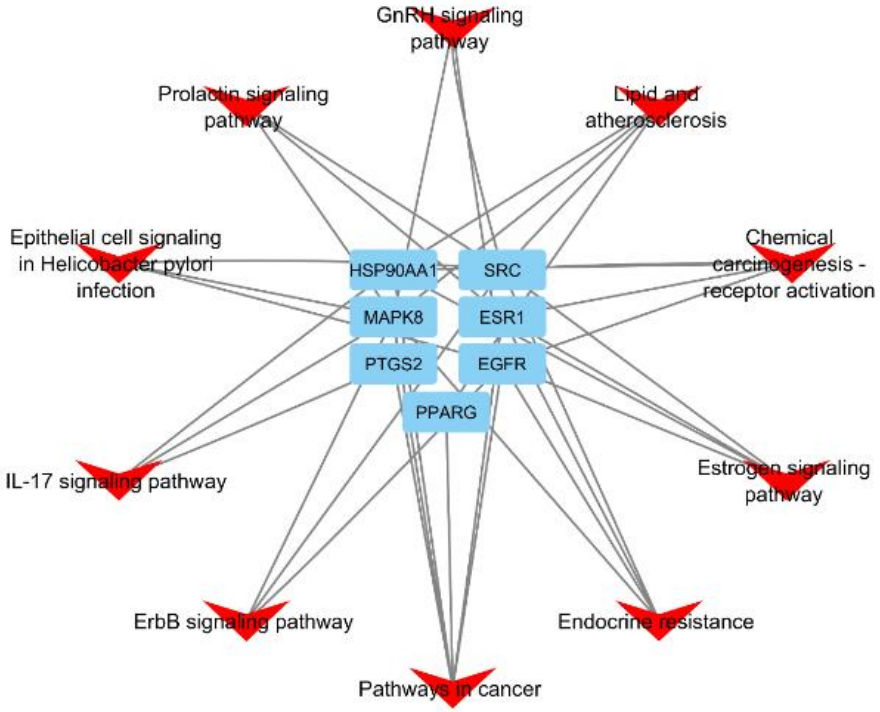

Figure 8. 10 representative pathways according to gene count

Pathway enrichment by KEGG analysis shows that liver cirrhosis is a crucial target of curcumin, mainly related to the pathway in cancer. Since hepatocellular carcinoma was a critical liver cancer outcome, LIHC patients in the TCGA data set were used. Prognostic analysis of critical genes done by UALCAN tools to analyze gene expression level and their association with survival rate in LIHC patients. This study revealed that SRC, PPARG, MAPK8, and HSP90AA1 may be significant curcumin targets in preventing cirrhosis and complications, especially in LIHC patients, and they have a substantial role in the overall survival of life patients.

To confirm the ability of curcumin to target SRC, PPARG, MAPK8, and HSP90AA1, molecular docking simulations were carried out by comparing the binding affinity of curcumin with the native ligand of each target (Zhang *et al.*,

2023). Autodock vina was used to run molecular docking. AutoDock Vina uses an optimization gradient and algorithm method to improve the accuracy of autodock. Trott and Olson discovered that autodock vina had a success rate of 80% in matching the predicted and experimental free binding energies, compared to autodock's success rate of 53% (Trott *et al*., 2009).

In molecular docking, the protein's active site must be identified first before starting the simulation to ensure that the docking molecule travels within the accurate binding pocket. SRC has Glu310, Asp404, and Phe405 catalytic residues. This residue is represented in the surface binding of bosutinib (a kinase inhibitor) to the SRC binding pocket (Levinson *et al*., 2014). Later, the native ligand in PPARG (rosiglitazone) was reported to have active binding interactions with His323, Tyr289, and Tyr473 as active residues of PPARG (Choi *et al*., 2010). Based on the bindindDB database, the A Tiazolidindion structure was reported as a PPARG inhibitor because it has a maximum IC50 value at 298 nM, and this structure is the original PPARG ligand with PDB ID 1ZGY (T. Liu *et al*., 2007).

Third, the binding of active residues to MAPK8 includes Lys55, Glu109, Met111, Ala36, Lys153, Ser155, Arg192, Val40, Ala53, and Val158 based on the literature which simulated protein structure docking simulations in JNK1 and JNK3 (Sailapathi *et al*., 2020). Lastly, HSP90AA1 has an active site with Ser52, Leu48, Asp93, Gly97, Thr 107, Phe138, Tyr 139, Trp162, and Thr184 residues. The PPARG inhibitor, 5-(3,4-dichloro-phenoxy)-benzene-1,3-diol, binds to the HSP90 active site with all the active residues involved (Brasca *et al*., 2013). Thus, in this study, those native ligands and inhibitors were used as references to compare curcumin's binding ability and potency to protein targets in molecular docking

simulation. In the results, molecular docking showed that curcumin was successfully located in the same binding pocket of native ligand to each target, and they bind to the same active residues (Figure 9-10).

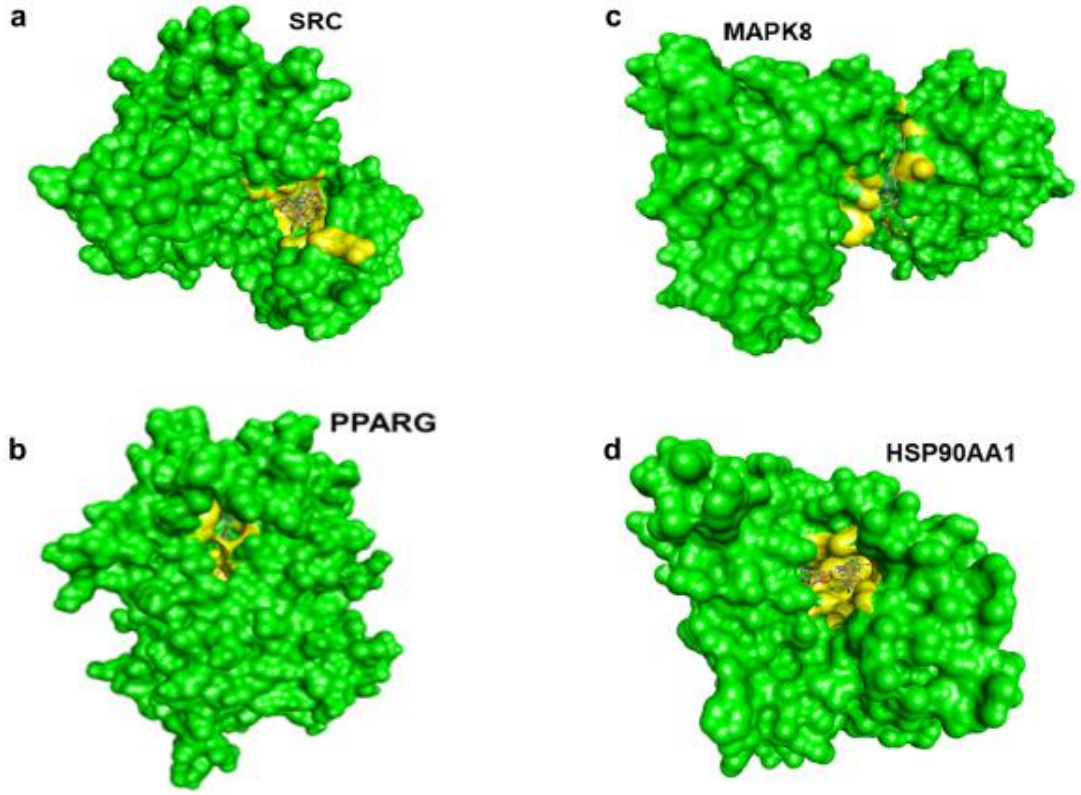

Figure 9. Binding pocket of SRC (a) PPARG (b) MAPK8 (c) and HSP90AA1(d), all ligands bind in the active site of SRC, PPARG, MAPK8 HSP90AA1 (yellow surface represents the active site)

As shown in Table 1, this molecular docking study demonstrated curcumin's ability as a potential inhibitor against SRC, PPARG, MAPK8, and HSP90AA1 because it has a lower binding affinity than the original ligand/inhibitor. The molecular interaction analysis of curcumin binding to protein targets is visualized in Figure 10. Especially hydrogen bonding and hydrophobic interactions are found in curcumin binding. Figure 10a shows that curcumin forms strong hydrogen bonds with Asp404, a catalytic residue that plays an important role in SRC. This interaction has a distance of 2.36Å (Table 1).

Interestingly, this curcumin binding was better than that of bosutinib, a kinase inhibitor (SRC inhibitor) drug (Figure. 10b). Curcumin and 2,4-

thiazolidinedione (the original PPARG ligand) form hydrogen bonds with the same PPARG active residue, namely Ser289. Although the original ligand binds to another PPARG active residue, namely His323 (Figure. 10c–d), the binding energy of curcumin to PPARG is better than that of the original ligand (Table 1). Phosphoaminophosphonic Acid-Adenylate Ester, as a native ligand from MAPK8, forms active residues which are found as a result of interactions with curcumin. Besides that, the similarity was found in hydrogen bonds between the native ligand and curcumin, namely Arg69 and Asp151. Finally, the number of active residues in this protein is also found in the interaction of curcumin with HSP90AA1, forming hydrophobic bonds. These active residues are Leu107, Phe138, and Tyr139. Therefore, our molecular docking study found the inhibitory potential of propolis against SRC, PPARG, MAPK8, and HSP90AA1 and may be important for developing new drugs for liver disease as these proteins play a critical role in liver disease.

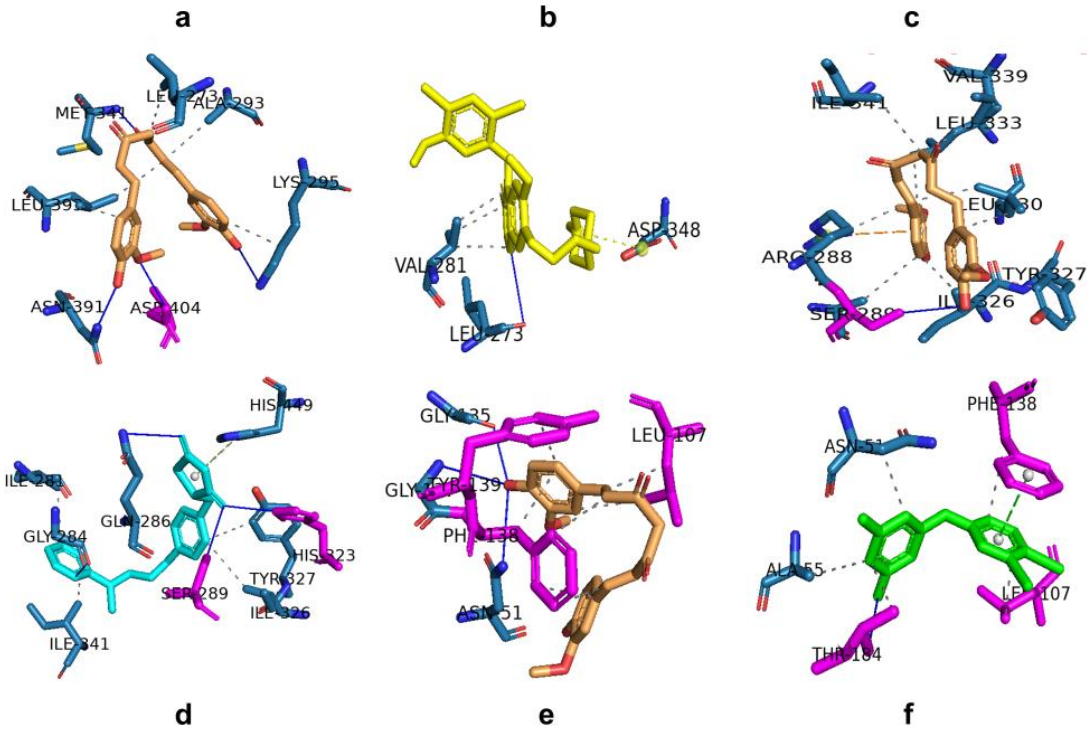

Figure 10. Molecular interaction of (a) Curcumin and (b) Bosutinib to SRC binding site, (c) curcumin and (d) 2,4-thiazolidiinedione (BRL) to PPARG binding site, (e) curcumin and (f) 5-(3,4-dichloro-phenoxy)-benzene-1,3-diol to HSP90 binding site. Orange color show curcumin structure, magenta highlighted the key active residues of protein.

SRC protein is a possible treatment route for liver treatment because it can stop TAA from causing liver fibrosis, stop HSC from activating, and inhibit TGF-b from driving CTGF expression (Seo *et al*., 2020). An earlier study also linked hepatocellular carcinoma risk and SRC expression levels. The development of cancers is aided by the activation of the SRC pathway. Experimental studies of HepG2 cell proliferation found that curcumin can downregulate differential genes commonly related to ferroptosis in liver cancer, whereas SRC is one of them (S. Yu *et al*., 2021). Our study supports previous findings that SRC could be a novel target for new treatment of HCC (Walker *et al*., 2019). Furthermore, based on experimental results in HCC, PPARG expression is higher in hepatocytes and HSCs than in KC and LSEC, although there may be differences in the number of PPARG1 and PPARG2 alleles (Berthier *et al*., 2021; Gonzalez-Sanchez *et al*., 2017; Z. Li *et al*., 2013). According to other studies, PPARG is extensively expressed in the liver and regulates different genes implicated in fatty acid metabolism, and PPARG stimulation increases fatty acid oxidation and beta-oxidation genes (Hardwick *et al*., 2009; Wang *et al*., 2020). Rosiglitazone, a thiazolidinedione drug, acts as a PPARG ligand that can suppress cell proliferation, invasion, migration, and metastasis of hepatoma cells by upregulating E-cadherin expression in HepG2 cells (Chi *et al*., 2014). In this study, curcumin had better binding energy to PPARG than rosiglitazone as a native ligand (Table 1). Figure 9b-c also shows that curcumin shares the same binding pocket of PPARG with rosiglitazone and possesses some matching critical residues. A previous study reported that curcumin inhibits HSC activation by disrupting Shh signaling, DLK expression, and PPARG expression

(Qiu *et al*., 2014). Therefore, curcumin can be suggested as a PPARG ligand for HCC treatment.

To investigate the function of MAPK8 in cirrhosis, several studies have found that MAPK8 plays a significant part in the formation of liver fibrosis, designating it as a profibrotic kinase and a potential cell-directed target for liver fibrosis (Zhao *et al*., 2014). Moreover, according to various research, MAPK8 regulates the growth of human HCC cells by influencing the production of p21 and c-Myc. MAPK8 suppression decreases the development of xenografted human HCC cells and chemically causes rodent liver carcinoma. These results establish a molecular connection between MAPK8 activity and liver cell growth via p21 and c-Myc, implying that blocking MAPK8 could be a novel therapeutic strategy for HCC (Hui *et al*., 2008). In another report, the role of MAPK8 in Cirrhosis is mentioned. The liver plays a pro-inflammatory role in increasing the secretion of inflammatory factors and the activation and infiltration of macrophages into the liver tissue. MAPK8 enhances M1 gene activation and chemokine secretion in macrophages by suppressing M2 genes and encouraging macrophages to infiltrate tissues (Schattenberg *et al*., 2012; Westenberger *et al*., 2021). HSP90AA1 is often raised in various cancer types such as lung, stomach, and breast. A study reported more HSP90AA1 mRNA in late-stage HCC patients than in early-stage HCC patients (Giaginis *et al*., 2009; Pick *et al*., 2007; Rodina *et al*., 2007). Another study showed that HSP90AA1 is more strongly expressed in HCC cell lines than in normal cells. HSP90AA1 also shows HCC patients' life recovery improvement by controlling survivin, cyclin D1, p53, and nuclear factor-B (M. Gao *et al*., 2015; Leng *et al*., 2012; D. Li *et al*., 2016; Sun *et al*., 2010; Toraih *et al*., 2019). Numerous

studies reported the inhibition of HSP90 in various types of cancer by curcumin and its derivatives. Curcumin analog suppresses HSP90 isoform (including HSP90AA1) expression in hepatocellular carcinoma cells, which induces apoptotic pathway activation (Bhullar *et al*., 2015; Forouzanfar *et al*., 2019). As a result, HSP90AA1 could be an appropriate target of curcumin for HCC treatment.

A network pharmacology study followed by gene expression and survival analysis of curcumin results in a better understanding of curcumin pharmacological actions in liver cirrhosis. Target fishing, PPI, functional enrichment analysis including the KEGG pathway, and gene expression and survival rate analysis in UALCAN tools revealed the critical gene targets of curcumin actions in preventing liver cirrhosis. Several previous studies reported that genes have important roles in hepatocellular carcinoma development. This is consistent with the results of KEGG, which placed the pathway in cancer as the top based on the *p-value* and the number of proteins associated with this pathway. SRC, PPARG, MAPK8, and HSP90AA1 expressed high concentrations in primary tumors and strongly correlated with low survival probability of LIHC patients. Molecular docking results also show that curcumin poses better binding affinities to the targets than native ligan s. A previous study revealed that curcumin improves the survival rates of the rat model of TAA-induced hepatotoxicity by targeting NF-KB and INOS protein expression (Vera-Ramirez *et al*., 2013). Our present study discovered new findings of curcumin action in targeting SRC, PPARG, MAPK8, and HSP90AA1 that may improve survival in rats. This finding will support further experimental studies of curcumin's effects on liver disease by targeting SRC, PPARG, MAPK8, and HSP90AA1.

Conclusion

Based on Network Pharmacology, Gene Expression, Survival Rate, and Molecular Docking Analysis, the present study provides insights into the potential mechanism of Curcumin in Liver Cirrhosis after successfully screening for associated critical target genes and pathways. Curcumin has been proposed as a possible ligand for SRC, PPARG, MAPK8, and HSP90AA1. Therefore, curcumin may prevent liver cirrhosis development into liver cancer by inhibiting SRC pathway activation, HSC activation, inducing apoptosis, and suppressing HSP90 isoform expression. These findings further offer a theoretical basis for further pharmacological research into the potential mechanism of Curcumin in Liver Cirrhosis.

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Disclosure statement

Authors declare that there are no conflicts of interest.

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Authors' Contributions

K.A.A.K.S. Writing – Original Draft, Collected Data, Investigation., K.A.A.K.S, and P.H.S. Design Methodology, Data Curation, Validation., P.H.S., M.M.J., A.G.A., and N.T.R., Writing – Review & Editing, Software, Conceptualization.

P.H.S., P.M.K., and E.M: Supervision, Project Administration, Finalized the manuscript. All authors read and approved the final manuscript.

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SURAT KETERANGAN ARTIKEL PUBLIKASI

Assalamu'alaikum Warahmatullahi wabarakatuh

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Menyatakan bahwa artikel yang berjudul "*Revealing Curcumin Therapeutic Targets on SRC, PPARG, MAPK8 and HSP90 as Liver Cirrhosis Therapy Based on Comprehensive Bioinformatic Study*" telah di submit pada Journal of Biomolecular Structure and Dynamics pada tahun 2023.

Demikian surat keterangan ini dibuat untuk dapat dipergunakan sebagai mana mestinya.

Wassalamu'alaikum Warahmatullahi wabarakatuh

Samarinda, Senin 18 Desember 2023

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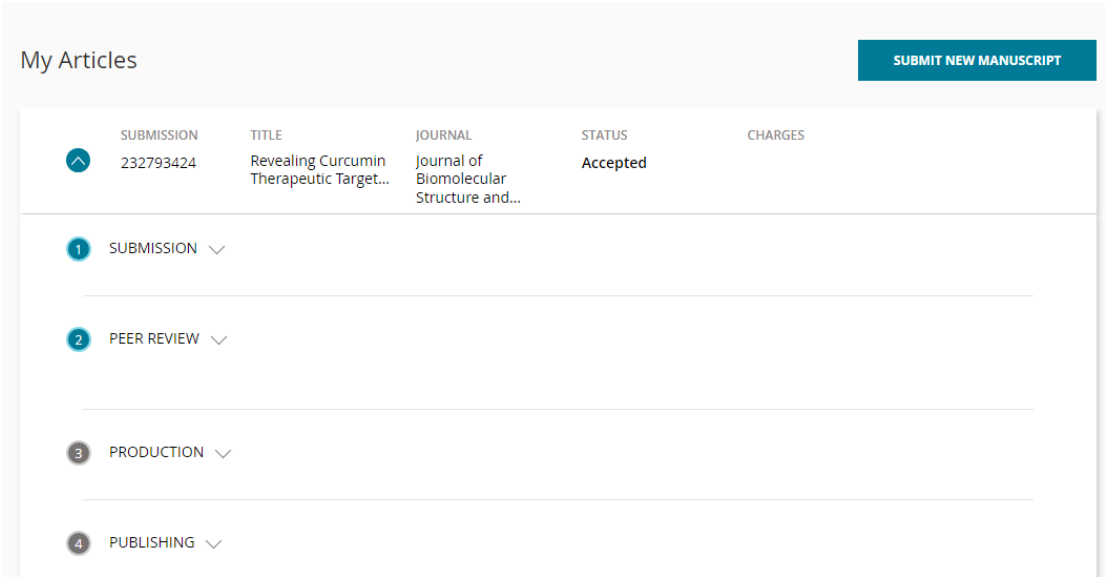
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