Mechanistic insights into the curcumin-mediated neuroprotection in Alzheimer's disease: an integrated System Pharmacology and Molecular Simulation Study

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Abstract: Curcumin is one of the bioactive metabolites of turmeric (*Curcuma longa*), known for its pleiotropic pharmacological actions, including antioxidant and anti-inflammation, anticholinesterase, immunomodulation, and neuroprotection. Substantial evidence suggests the therapeutic benefits of curcumin against neurodegenerative disorders, including Alzheimer's disease (AD), acting on a diverse array of brain targets that make the molecular mechanisms complicated. System biology level-investigation could potentially present a comprehensive molecular mechanism to delineate the neuropharmacological action of curcumin. In this study, we used integrated system pharmacology and molecular simulation analysis to gain insights into the underlying mechanism of curcumin action against AD. Network pharmacology study identified curcumin-targeted potential cellular pathways such as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling, neurotrophin signaling, toll-like receptor (TLR) signaling, and autophagy, and proteins such as tropomyosin receptor kinase B (TrkB), liver X-receptor-beta (LXR-β), estrogen receptor-β (ER-β), mammalian target of rapamycin (mTOR), TLR-2, *N*-methyl-*D*-acetate receptor subunit 2B (GluN2B), *β*-secretase and glycogen synthase kinase-3β (GSK-3β), which are intimately associated with neuronal growth and survival, immune response, and inflammation. Moreover, the molecular modeling further verified that curcumin showed a significant binding affinity to mTOR, TrkB, LXR-β, TLR-2, ER-β, GluN2B, *β*-secretase, and GSK-3β, which are the crucial regulators of molecular and cellular processes associated with AD. Together, the

 doi:10.20944/preprints202001.0109.v1

present system pharmacology and *in silico* findings demonstrate that curcumin might play a significant role in modulating AD-pathobiology, supporting its therapeutic application for the prevention and treatment of AD.

Keywords: Alzheimer's disease; curcumin; network pharmacology; molecular simulation; neurodegeneration; TrkB/PI3K signaling; autophagy.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that impairs memory and cognition. The main pathological features- amyloid plaques and neurofibrillary tangles-define the proteinopathy nature of AD. Neuroinflammation and oxidative stress are the main causal factors that underlie the pathophysiology of AD. The signaling pathways that are severely perturbed in AD include neurotrophin, PI3K/Akt, TNF, and TLR signaling, which are critically associated with the neuronal growth and survival, inflammation, and immune response. Being a multifactorial disorder, AD could suitably be managed through multitargeted approaches. Bioactive secondary metabolites with antioxidant, anti-inflammatory and immunomodulatory potentials targeting various AD-associated cellular signaling pathways are promising candidates against AD pathobiology.

Curcumin, a principal bioactive polyphenol of turmeric *(Curcuma longa*), has been shown to be effective against a plethora of chronic diseases, such as cardiovascular disease [1], Alzheimer's disease [2], diabetes [3], cancer [4], and hepatic disease [5] among many others, which are attributed to its antioxidant, anti-inflammation, immunomodulation, chemosensitizing [6], lipid-modifying [7], and antiatherogenic [8] potentials. Curcumin interacts with a wide range of molecular targets, including those that are potentiality implicated in AD pathophysiology. For instance, curcumin inhibits GSK-3β, a kinase that phosphorylates presenilin 1, thus reduces Aβ production by inhibiting GSK-3β-mediated PS1 activation [9]. Curcumin also elicits heat shock proteins, a molecular chaperone, which is involved in aggregated Tau clearance. Moreover, curcumin reduces existing tangles and can ameliorate Tau-dependent behavioral deficits [10]. Curcumin restrains lipid peroxidation which in turn diminishes amyloid accumulation and oxidative stress-mediated neuro-toxicity [11]. Several in vivo studies also demonstrate curcumin-mediated neuroprotection against amyloid-β-induced neurotoxicity and memory impairment [12, 13]. Curcumin also enhances Aβ uptake by macrophages in AD patients [14]. With these evidence, we can speculate that curcumin could pose a therapeutic promise against AD.

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Although substantial reports present a wide array of brain targets and propose numerous mechanisms of curcumin action against AD, the information is too diverse to draw a precise conclusion and to identify potential molecular targets. A system biology level study, therefore, needs to be employed to elucidate the precise pharmacological mechanism of curcumin action. Here, we employed an integrated system pharmacology approach, an efficient bioinformatics tool for molecular pharmacology study [15-17]to identify a potential pharmacological mechanism of curcumin against AD. First, through multiple bioinformatics tools, we demonstrate that curcumin interacted with signaling pathways related to the development and survival of neuron, inflammation and immune response. Next, we employed *in silico* analysis to further verify the biophysical interaction of curcumin with some potential targets. The present study elucidates the underlying mechanism of molecular neuropharmacology of curcumin and establishes a basis in favor of its therapeutic application against AD.

2. Results

2.1. ADME/T Properties of Curcumin

A diagrammatic overview showing the different steps of network pharmacology was illustrated in Figure 1. The drug-like properties of curcumin was evaluated by QikProp ADME/T prediction tool. Curcumin exhibited drug-like attributes (Table S1) and is more likely to be orally available as it did not violate any of the Lipinski's rule of five [18] (mol_MW < 500, donor HB ≤ 5, and acceptor HB ≤ 10) and Jorgensen's rule of three [19] (QPlogS > −5.7 and QPPCaco > 22 nm/s). Moreover, the predicted brain/blood partition coefficient (QPlogBB) of curcumin was −2.256, which falls within the recommended range (−3.0–1.2), indicating that this polyphenolic might pass the blood–brain barrier.

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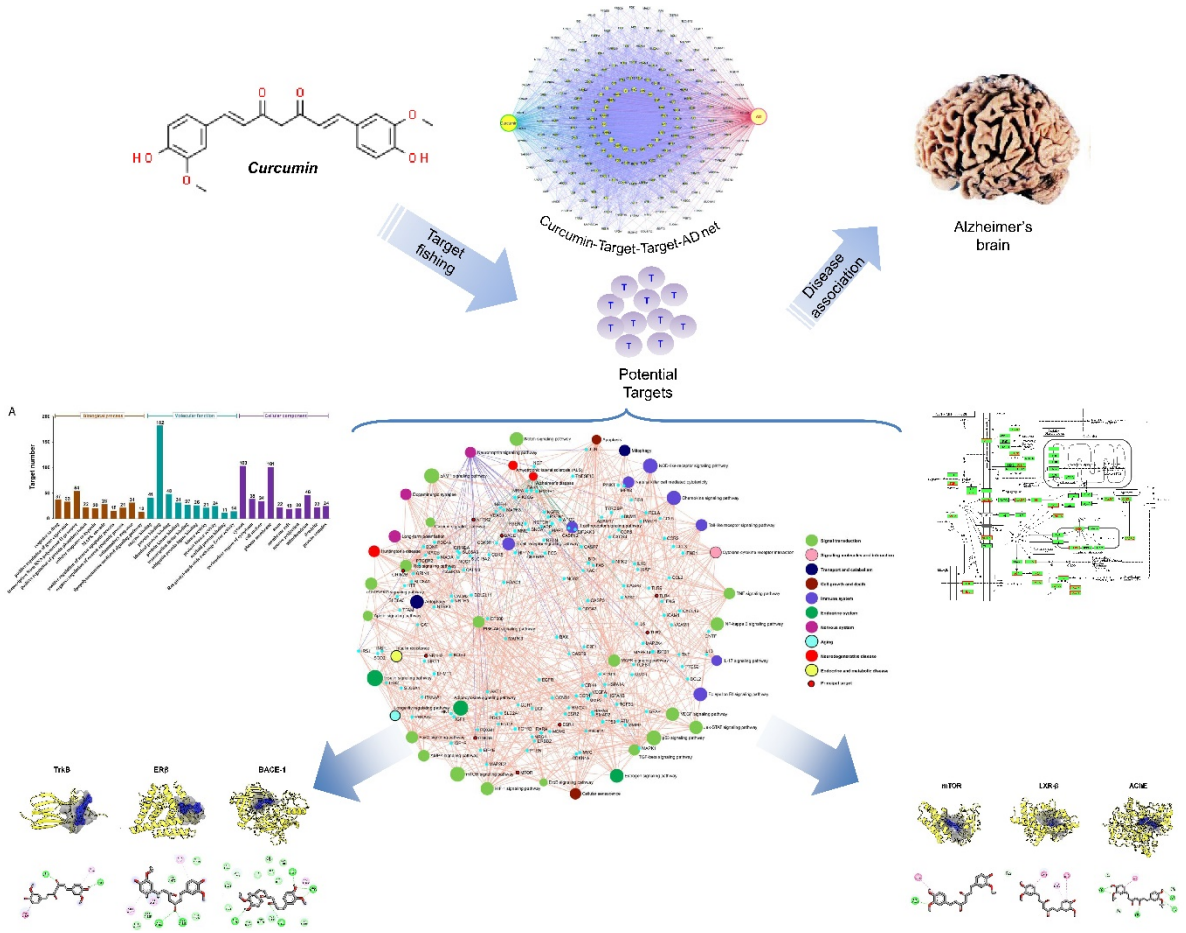

Figure 1. A diagrammatic overview of the system pharmacology-based elucidation of neuropharmacological action mechanism of curcumin in AD therapy.

2.2. Target Fishing

A total of 238 targets of curcumin were retrieved from the PALM-IST database (Table S2) and validated through literature scanning in the PubMed database. Targets that have been reported to be under the regulation of curcumin in AD-associated experimental evidence were primarily considered.

2.3. Network Building

Curcumin–Target–Target–AD (C–T-T–AD) network presented the interactions among the curcumin's targets. A total of 222 targets were associated with AD (Figure 2). The highly connected targets include those that are the members of crucial cellular pathways such as PI3K/AKT pathway (*AKT1, PIK3CA*), MAPK pathway (*MAPK1, MAPK3, MAPK8*), growth factor pathway (*BDNF, NTRK2, EGF, VEGFA, EGFR, NGF*), and toll-like receptor pathway (*TLR2*), indicating the involvement of these targets or others to their upstream signaling as a potential drug-target for curcumin in the AD treatment. Moreover, some

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other AD-associated potential targets, including LXR-β (*NR1H2*), ER-β (*ESR1*), mTOR, β-secretase (*BACE1*), and AChE have appeared.

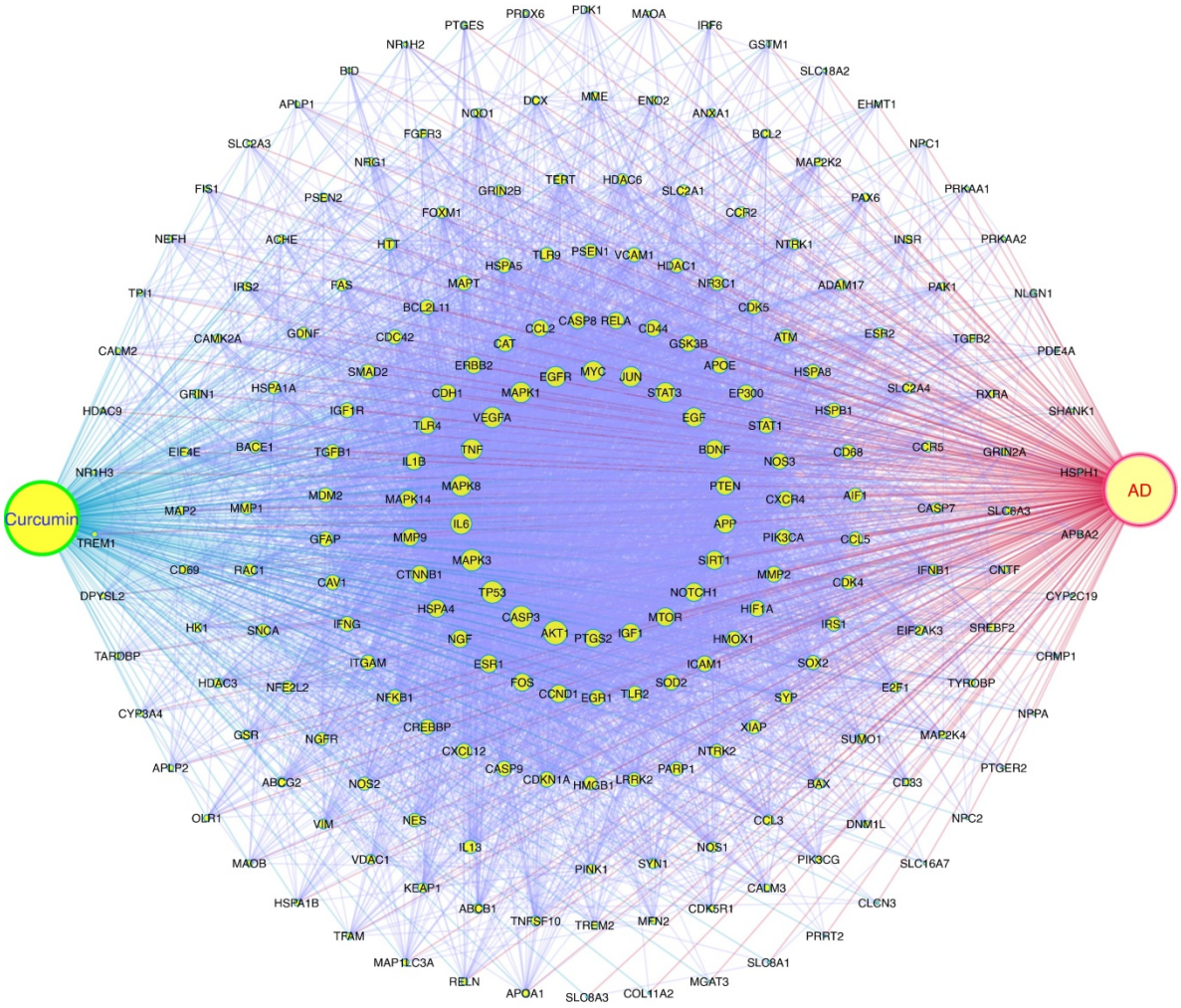

Figure 2. Network analysis: Curcumin-target-target-Alzheimer's disease (C-T-T-AD) network. Small circular nodes represent targets of curcumin. Node size is proportional to its degree. Edges represent interaction among curcumin, targets, and AD.

2.4. Gene Ontology (GO) Analysis

Top 10 highly enriched GO terms under biological process (BP), molecular function (MF), and cellular component (CC) were displayed (Figure 3A). The CC term with the highest gene sequestration was the cytosol (103), followed by the plasma membrane (101). The highly enriched MF term was protein binding (182). The overrepresented biological processes include transcription regulation, inflammatory response, lipopolysaccharide-mediated signaling pathway, MAPK cascade, and cellular response to hypoxia, which are closely associated with the AD pathobiology. Moreover, the target proteins were categorized into 21 different classes based on their cellular function, indicating their functional diversity (Figure 3B).

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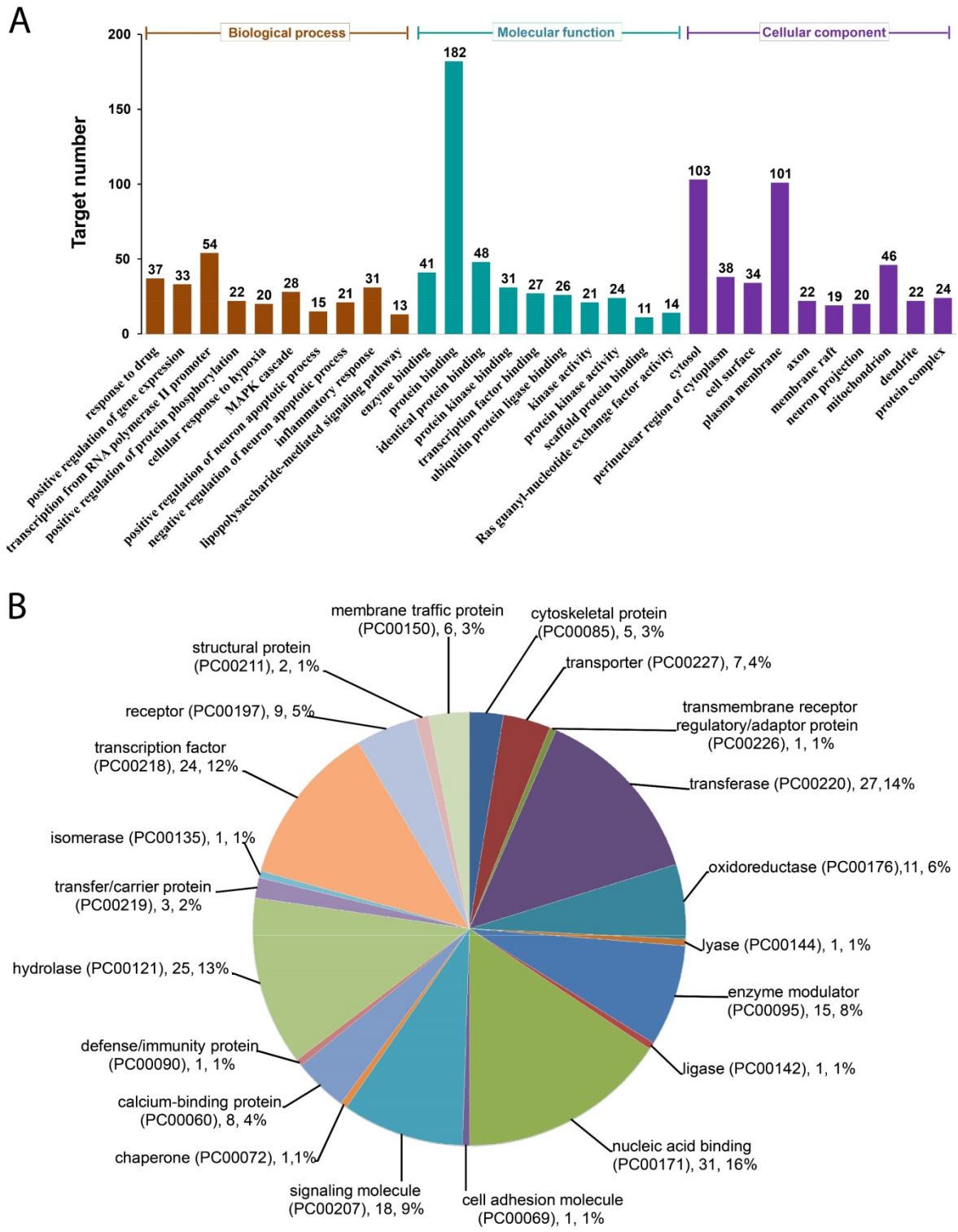

Figure 3. Bioinformatics analysis of curcumin target genes. Gene ontology (GO) analysis by DAVID annotation tool (A): Top 10 GO terms for biological process, molecular function, and cellular component were displayed where the x-axis representing GO terms for the target genes ($p < 0.001$) and y-axis showing gene counts. The number next to the bars indicates the gene number. Panther classification categorized target proteins into 21 classes (B). The figures next to the group in the pie chart indicate the number and percentage of protein in the given functional class.

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2.5. Network and Pathways related to AD Pathophysiology

A comprehensive network visualizes the interactions of curcumin's targets with AD and AD-associated cellular pathways, which were categorized into ten modular systems using KEGG pathway annotation (Figure 4). A total of 18 signaling pathways were enriched (p-value < 0.01) in the "signal transduction" module (Figure 5A). Most overrepresented signal transduction pathways include PI3K/Akt signaling (Figure 5B) and MAPK signaling, which are involved in neuronal growth and survival. TrkB, a curcumin targeted protein, regulates the enriched signaling pathways involved in neuronal growth and survival. Therefore, the interaction of curcumin with TrkB was further verified by *in silico* analysis.

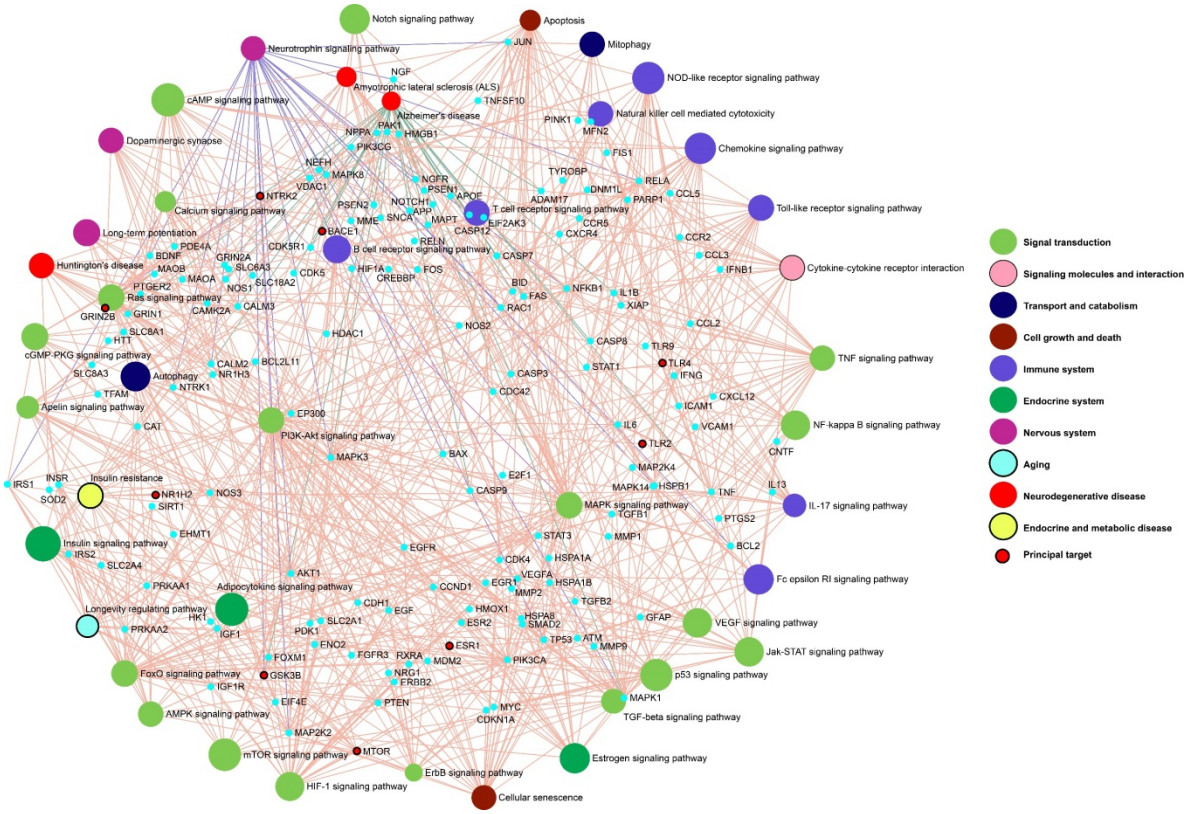

Figure 4. Integrated Disease-Target-Pathway network, a comprehensive network that visualizes the interactions of curcumin's targets with AD and AD-associated cellular pathways which were categorized into ten modular systems (differentiated by color) using KEGG pathway annotation. Small red nodes represent potential druggable targets.

Enriched nervous system-related pathways (Figure 6A) include neurotrophin signaling (Figure 6B), dopaminergic synapse and long-term potentiation, whose functional impairment is associated with AD pathobiology. Several inflammation-related signaling pathways, including NF-κB signaling and TNF signaling, were also enriched (Figure S1). LXR-β, a curcumin targeted proteins involved in the regulation of the enriched

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inflammatory pathways, was further verified for its physical interaction with curcumin by *in silico* analysis. In addition, numerous immune system pathways (Figure 7A), including toll-like receptor signaling (Figure 7B), as the top enriched pathway, appeared in the network. Considering the significance of TLR signaling and the involvement of TLR2 in the immune signal recognition, the interaction of curcumin with this receptor target was further validated through molecular docking.

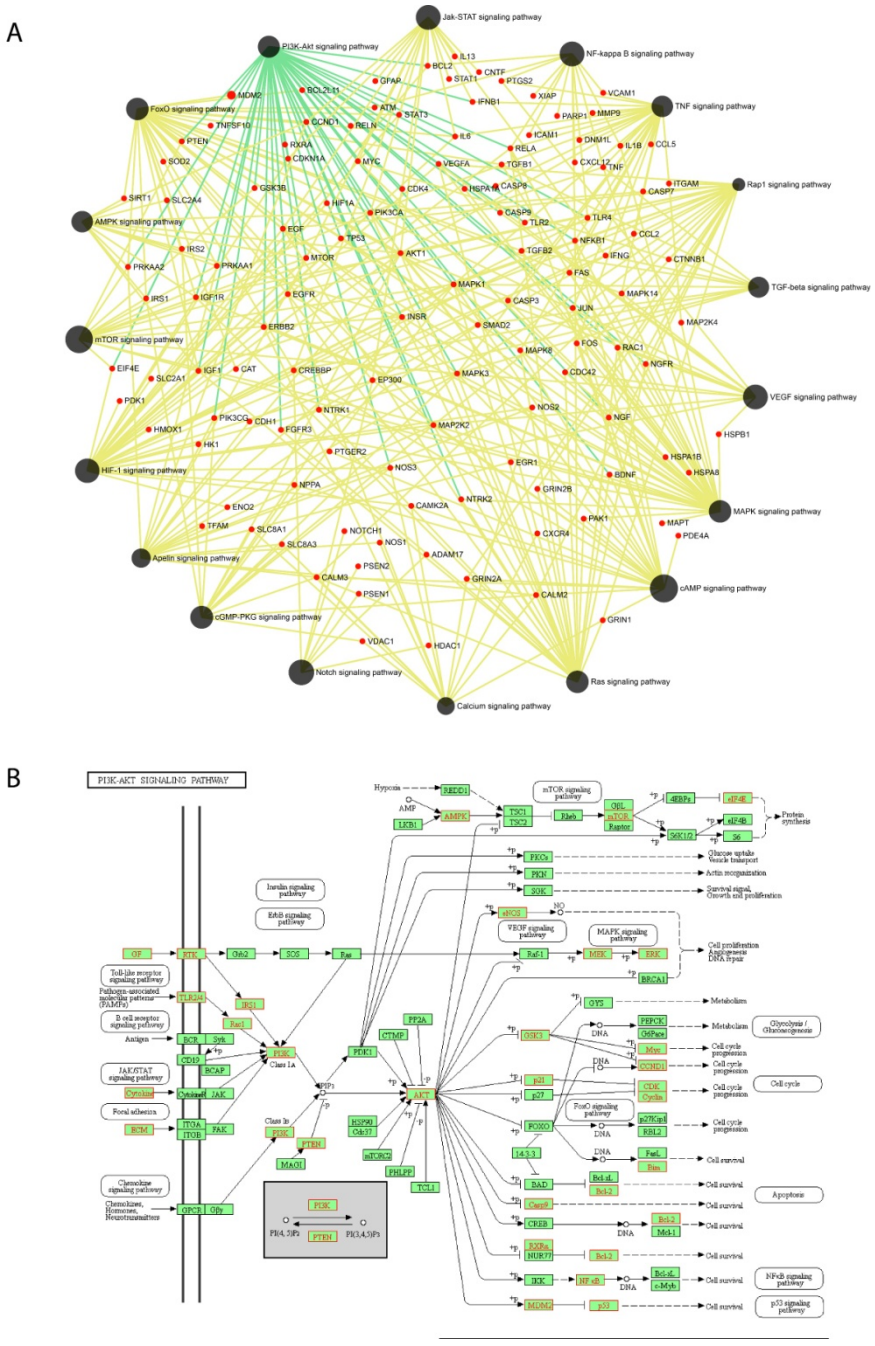

Figure 5. Signal transduction pathways appeared in the Disease-Target-Pathway network. A target-pathway network in the 'signal transduction' module (A). PI3K-Akt pathway (B) is the top signaling pathway. Curcumin targets in the pathway are highlighted in red.

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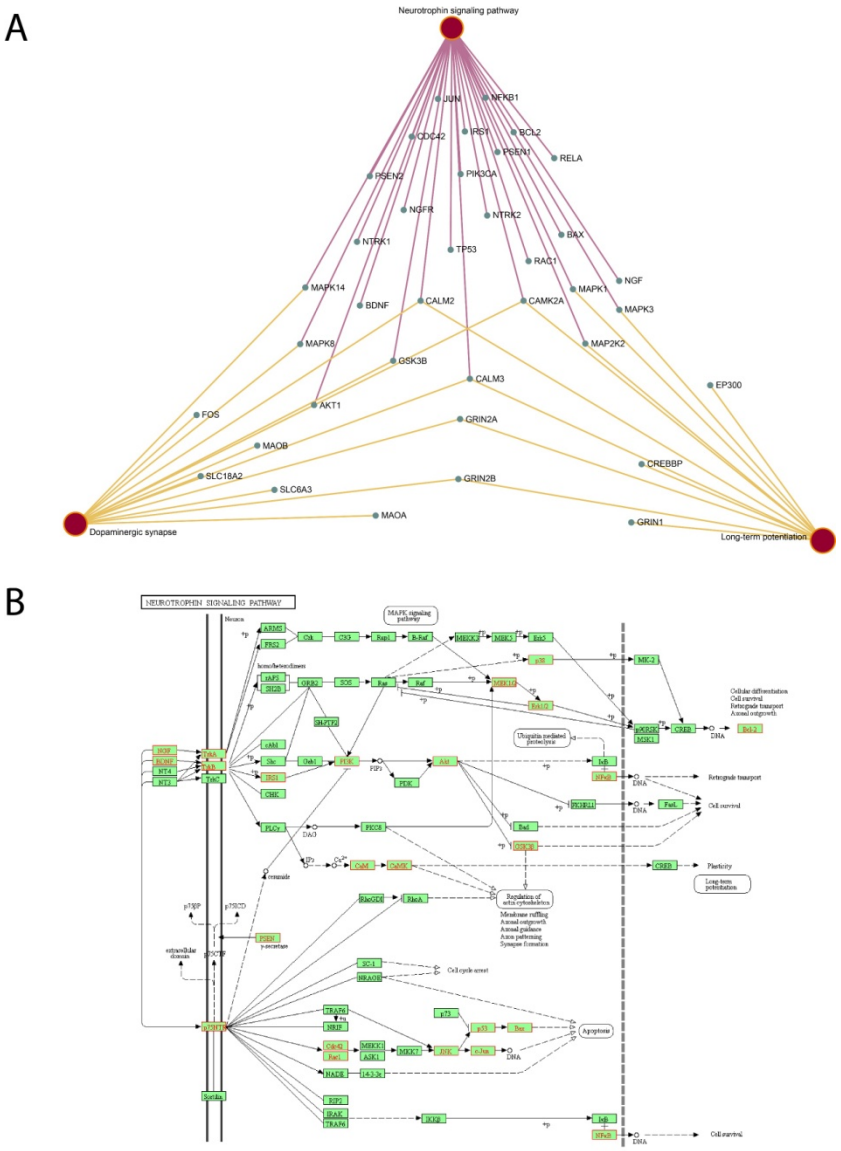

Figure 6. Nervous system pathways appeared in the Disease-Target-Pathway network. A target-pathway network in the 'Nervous system' module (A). The highly enriched pathway includes neurotrophin signaling pathway (B). Curcumin targets in the pathway are highlighted in red.

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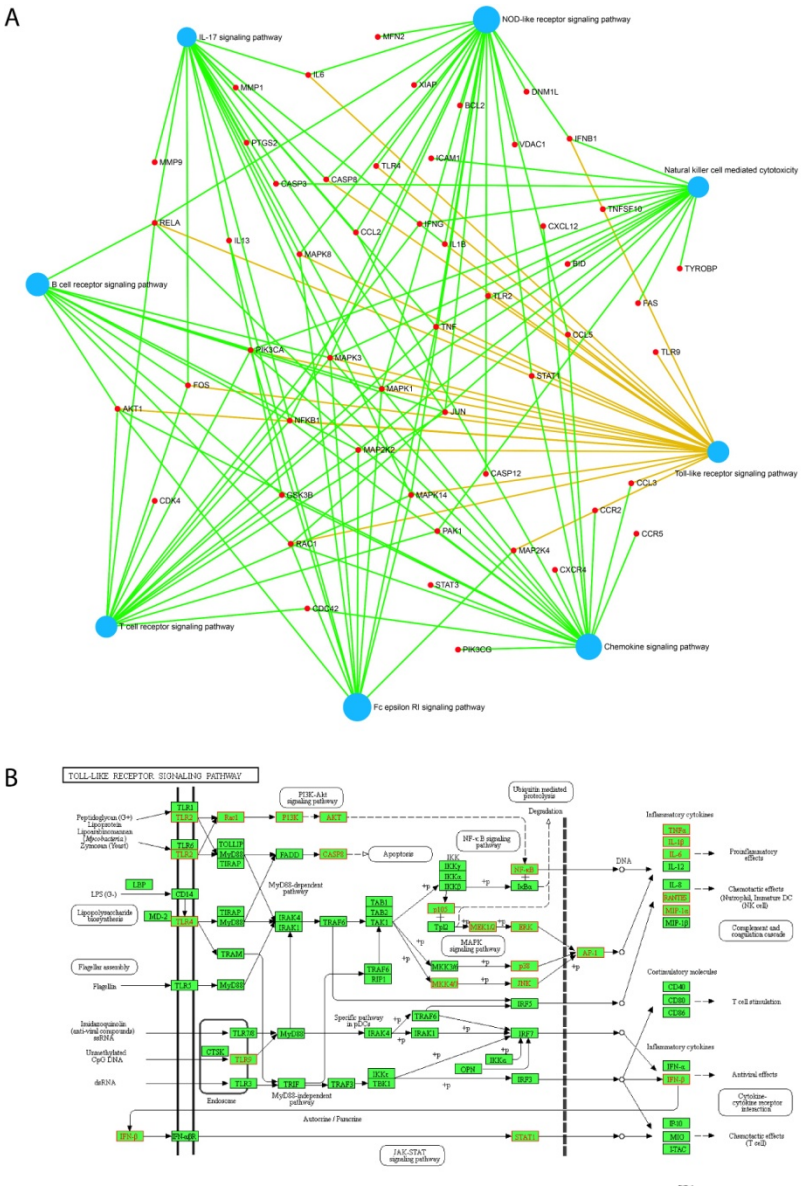

Figure 7. Immune system pathways appeared in the Disease-Target-Pathway network. A target-pathway network in the 'Immune system' module (A). Toll-like receptor signaling pathway (B) is the most over-represented immune-related pathway. Curcumin targets in the pathway are highlighted in red.

Cellular pathways under transport and catabolism include autophagy and mitophagy which are crucially implicated in AD. Several proteins of these cellular pathways were targeted by curcumin (Figure 8). Two curcumin-targeted proteins, such as mTOR, a central regulator of autophagy, and estrogen receptor-β, a nuclear receptor regulating mitophagy, were further validated for their interactions with curcumin by molecular modeling.

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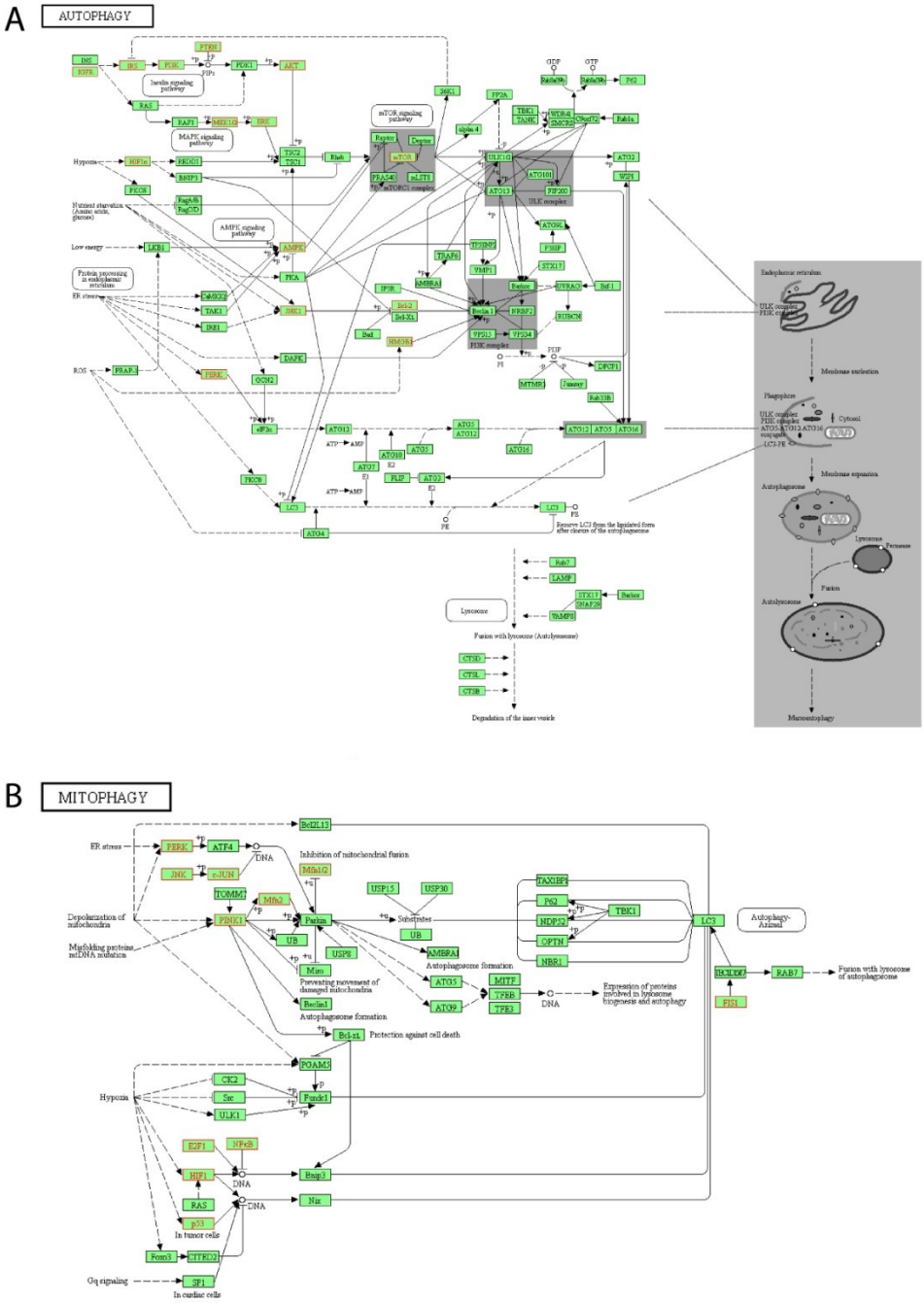

Figure 8. Autophagy (A) and mitophagy (B) appeared in the Disease-Target-Pathway network. Curcumin targets in the pathway are highlighted in red.

Finally, in AD-pathway, a total of 25 target proteins were illustrated including those that are involved in amyloidogenesis (for example, *BACE, APP, PSEN*), Tau aggregation (Tau, GSK-3β, CASP3, CASP7, p53, p25, and cdk5), cholesterol homeostasis and Aβ-clearance (for example, ApoE, NEP), neuronal growth and survival (for example, Erk1/2 or *MAPK1*), synaptic plasticity (for example, GluN2A and GluN2B), inflammation (for example, NOS, *COX2*, TNFR, *TNF*, and *IL1B*), and apoptosis (for example, *CASP3,*

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CASP9, CASP8, CASP12, Fas and CaM) (Figure 9). Of these, the interaction of BACE and AChE with curcumin were further validated through molecular simulation as these enzymes are already potential drug targets for AD.

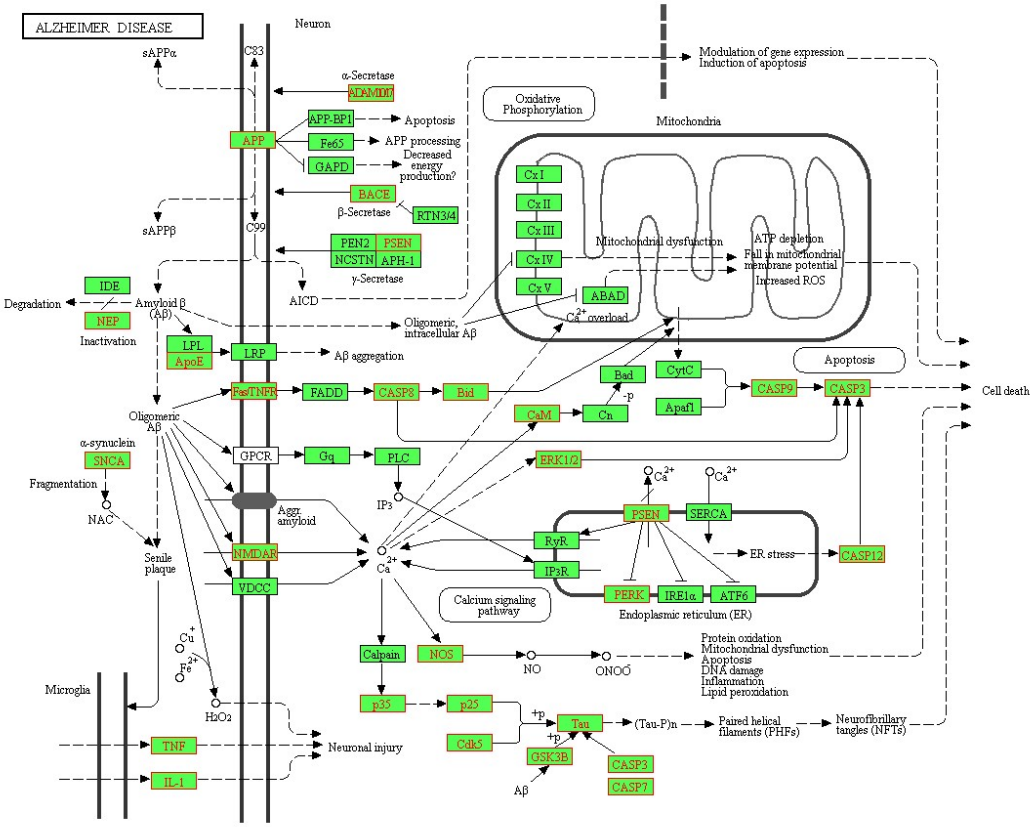

Figure 9. AD pathway in KEGG mapper linked many of the curcumin targets that are highlighted in red.

2.6. Molecular Docking Simulation

Following the network analysis, molecular docking analysis was further incorporated to validate the binding of curcumin to LXR-β, ER-β, TrkB, TLR2, mTOR, BACE1, AChE, GSK-3β and GluN2B, which are crucial regulators of several AD-related molecular and cellular processes. The docking analysis revealed that curcumin interacted with all targets with binding energy, ranging from -39 to -62 kcal/mol, respectively (Table S3). Among these targets, curcumin showed the highest binding energy to mTOR, while the lowest was obtained for BACE-1. The significant binding was also found for LXR-β, AChE, GSK3β, GluN2B and TLR2, followed by the binding energies including -60.14, -55.089, -54.65, -52.00 and-50.52 kcal/mol, respectively.

For a detailed understanding, the intermolecular interaction patterns of all protein-ligand complexes from curcumin docking simulations were revealed. As shown in Figure

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10, curcumin interacted with the mTOR by the hydrogen and hydrophobic interactions with Tyr198 and Trp175 residues through its feruloyl groups. Docking studies with LXR-β receptor showed that curcumin binds in the ligand-binding domain by only hydrophobic interactions with the residues including, Phe329, Leu345 and Trp457. However, curcumin displayed several hydrogen bondings with AChE, where the majority of the bonds were made by feruloyl groups, especially with the Tyr133, Ser293 and Arg296 residues in the active site. The Tyr124 residue was also seen to interact with the beta-diketone group utilizing hydrogen bonding. Curcumin additionally made hydrophobic interaction with the Trp86 residue.

In docking study, curcumin formed electrostatic interaction with the active site residues of GSK3β, where the major interactions were mediated by Asn186, Asp200, Lys85 and Val135 through hydrogen bonding. The feruloyl groups also made salt-bridge interaction with Cys199 residue. The docking scenario in TLR2 represented that curcumin interacts with the active site by utilizing hydrophobic interactions with Leu334, Leu312, Val343, leu355, Val351 and Val348 residues. On the other hand, curcumin showed both hydrogen bonding and hydrophobic interaction with the active site residue of GluN2B receptor, where the interactions were mediated to Tyr198 and Trp175 residue, respectively. However, curcumin showed moderate binding to ERβ and TrkB, having the binding energy to -46.07 and -43.93 kcal/mol, respectively. The compound interacted with ERβ receptor by hydrogen bonding with Val487 and Tyr488 residue, where both interactions were made by beta-diketone group. Additionally, feruloyl groups were involved in the hydrophobic interactions with the Cys481, Leu477 and Met473 residues, respectively. The molecular docking analysis with TrkB revealed that curcumin interacted through hydrogen bonding with Ile323 and Ser327 residues, while hydrophobic interaction was maintained with Tyr329, Leu324 residues at the extracellular domain of TrkB receptor. The minimum binding energy was obtained for BACE-1, although curcumin showed hydrogen bonding with Ile187, Thr293, Trp137 and Arg189 residues, with additional hydrophobic interaction with the Val130.

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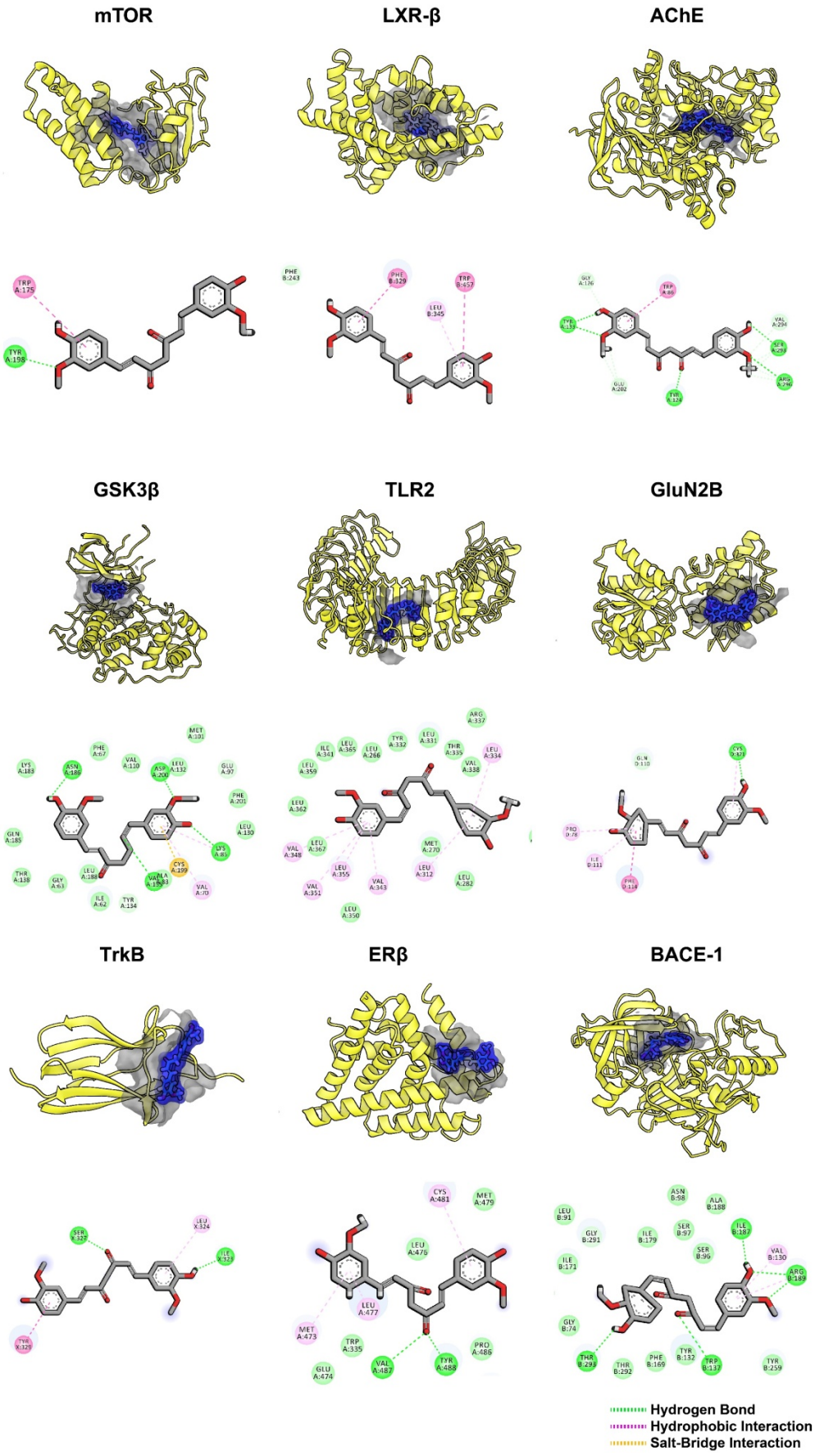

Figure 10. Binding and interaction pattern of curcumin with the mTOR, LXR-β, AChE, GSK-3β, TLR2, GluN2B, TrkB, ER-β and BACE1, respectively. In every protein, the lower panel displays

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the corresponding two-dimensional representation of binding interactions occurred in the respective complex. The curcumin made stable complex through hydrogen bonding (green) and the hydrophobic interactions (pink) and, in some cases, by salt-bridge interaction (orange).

3. Discussion

In this study, we elucidated the molecular mechanism of neuropharmacological action of curcumin against AD using integrated system pharmacology and molecular simulation approach. Initially, we characterized the pharmacokinetic behaviors of curcumin, which supports its drug-like properties and accessibility to the brain tissue. However, numerous studies claimed the poor bioavailability that limits the use of curcumin through oral administration. To overcome this limitation, nanoparticle-mediated drug delivery systems have been proposed for curcumin [20].

Gene ontology-based bioinformatics analysis identified several enriched biological processes, including inflammatory response, MAPK cascade, response to hypoxia, and LPS-mediated signaling, that are linked with the AD pathology, suggesting the possibility that curcumin could intervene the disease progression through modulating these vital biological processes. The system pharmacology information, as revealed from network analysis, indicated that curcumin exhibited interaction with the target proteins of many crucial pathways at the molecular and cellular levels.

In the pathway analysis, signal transduction pathways are predominantly targeted by curcumin. Of these, the PI3K/Akt signaling pathway, along with the MAPK pathway, regulates neuronal growth and survival. Curcumin showed interaction with multiple downstream effectors of these pathways, notably GSK-β and mTOR. Being a broad spectrum phosphorylating enzyme, GSK-β once activated catalyzes the phosphorylation of many substrates, including Tau. Aberrant activation of GSK-3β results in Tau hyperphosphorylation that leads to tangle formation within the neurons [21]. The interaction and binding affinity of curcumin with GSK-3β in this network pharmacology and molecular docking analysis and its inhibition against GSK-3β activity in the previous reports [22] suggest that curcumin could help stabilize Tau-linked microtubules.

The nervous system-related pathways- particularly neurotrophin signaling- are crucial for the maintenance of adult neurons and are closely associated with AD pathobiology. In AD, TrkB signaling, the most dominant neurotrophin signaling pathway in the adult brain, is severely interrupted, primarily due to the insufficient neurotrophic (particularly BDNF) support that causes atrophy and death of the neurons and could aggravate AD pathogenesis [23, 24]. However, pharmacological intervention by BDNF mimetic could help the

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compromised BDNF/TrkB signaling regain its physiological function. Substantial evidence suggests that curcumin either enhances or restores the BDNF level in the brain [25-27] and shows neuroprotective function through activating BDNF/TrkB-dependent MAPK and PI3K/Akt cascades [28, 29]. In line with these evidence, our system pharmacology and molecular docking analysis also revealed that curcumin showed an interaction and a significant binding affinity to TrkB, which suggests that curcumin could function as BDNF-mimetic and modulate neuronal growth and survival through modulating the classical neurotrophin/PI3K/Akt signaling pathway.

Curcumin also showed interaction with multiple synaptic proteins that are often deregulated in AD. For example, the activity of GluN2B, an extrasynaptic glutamate receptor, is critically implicated in the development of AD, whereas GuN2A is clearly involved in the memory. The effect of curcumin on the latter has been well-established [30]; however, the interaction of curcumin with GluN2B might be either agonistic or antagonistic, which is needed to be investigated using a suitable experimental model. Our in silico findings, however, indicated a significant interaction between curcumin and GluN2B. Another curcumin-targeted synaptic protein was AChE that is involved in cholinergic neurotransmission. The cholinergic deficit, one of the pathological consequences in AD, is the primary target of current AD therapy and could be compensated by the AChE inhibitor. Previously reported anticholinesterase activity [31] and our molecular docking results suggest that curcumin might be an alternative to the current anti-AChE agent.

Curcumin's interaction with several proteins of inflammation-related pathways, particularly TNF and NF-κB signaling pathways, suggest that curcumin-mediated anti-inflammatory action could play a significant role in the treatment of AD. Activation of NF-κB pathway triggers inflammation by inducing the release of proinflammatory and inflammatory components like TNF, COX-2, iNOS, IL-1, IL-6 [11]. Deregulation of NF-κB signaling pathway in immune cells induces a detrimental inflammatory response to the neuron, which leads to neuronal death. Evidence suggests that curcumin can alleviate neurodegeneration and neuroinflammation through multiple mechanisms, by reducing inflammatory mediators such as TNF-α, IL-1β, and NF-κB gene expression [32]

Given the key role of cholesterol in brain physiology and function, disturbances in cholesterol homeostasis provoked inflammation and oxidative stress, and thus, have been associated with the onset of AD [33]. LXRs, particularly LXR-β, play a critical role in brain cholesterol homeostasis. Upon ligand activation, LXR-β upregulates the expression of *ApoE, ABCA1,* and *ABCG1*, the key genes in reverse cholesterol transport [34]. The activity

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of ABC transporters mediates the cholesterol efflux to ApoE, which enhances Aβ clearance to blood [34, 35]. An LXR-β dependent expression of ABCA1 and ABCG1 by curcumin has been reported [36]. These findings suggest that curcumin might take part in LXR-β-mediated cholesterol homeostasis in the brain. Moreover, curcumin showed a significant binding affinity to LXR-β in our docking analysis, indicating that this natural molecule could be a potential LXR-β agonist, which might play a significant role against AD pathology through maintaining cholesterol homeostasis and Aβ clearance involving ABC-dependent pathways.

Several immune-related pathways that are intimately associated with AD progression are targeted by curcumin. For instance, toll-like receptor (TLR) signaling is crucially implicated in innate immunity. TLR2, a pattern recognition receptor that senses both endogenous and exogenous antigens, produces an immune response in the cells [37]. The current network pharmacology and *in silico* findings on curcumin-TLR2 interaction and previously reported curcumin-mediated TLR2-downregulation [38] suggest that curcumin could improve inflammation-mediated AD pathobiology through regulating TLR-mediated immune response.

Autophagy is a lysosome-dependent degradation pathway that plays a crucial role in cell survival and homeostasis. The impairment of this surveillance pathway is known to be associated with AD pathogenesis. Inhibition mTOR, a critical regulator of autophagy restores autophagic flow that attenuates neurodegeneration [39]. mTOR inhibitor might, therefore, be a potential therapeutics in AD. Wang and colleagues reported that curcumin induces autophagy by inhibiting PI3K/Akt/mTOR signaling pathway in APP/PS1double transgenic mice [40]. In addition, curcumin might also take part in autophagy regulation through interaction with estrogen receptor-β (ER-β) as it has shown to stimulate transcription of estrogen-responsive genes [41]. Also, it has been established that ER-β induces autophagy via suppressing the PI3K/AKT/mTOR pathway [42]. On the other hand, mitophagy, mitochondria-targeted autophagy that clears abnormal mitochondria, regulates the mitochondrial quality and promotes cell survival in neurodegenerative diseases [43] could represent an attractive drug target for AD therapy. In our network pharmacology analysis, curcumin targeted several molecular markers of both autophagy and mitophagy. Of these, mTOR is of particular interest, showing the highest binding affinity to curcumin

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in molecular docking analysis. The binding affinity of curcumin to ER-β is also significant. Together, these evidence suggest that curcumin could contribute potential pharmacological benefits in AD patients through modulating autophagy.

Amyloid β deposition in the brain installs AD pathogenesis [44]. β-Secretase, an enzyme that initiates Aβ formation in the brain [45], therefore, offers an attractive target for an anti-AD agent. Curcumin has been reported to inhibit β-Secretase activity [46]. Moreover, curcumin has been shown to inhibit the formation of Aβ and reduce the same in an animal model [12]. The present system pharmacology and *in silico* analysis further support the interaction of curcumin with β-Secretase and suggest that curcumin might offer a promising therapeutic lead against AD.

4. Materials and Methods

4.1. ADME/T Analysis of Curcumin

Pharmacokinetic properties such as absorption, distribution, metabolism, and excretion/transport (ADME/T) of curcumin were analyzed through QikProp (Schrödinger Release 2019-3**:** QikProp, Schrödinger, LLC, New York, NY, USA), an ADME/T prediction tool. ADME/T properties forecast the drug-like activity of ligand molecules during clinical trials based on Lipinski's rule of five.

4.2. Data Mining for Target Selection

First, we collected relevant targets information against every selected compound primarily from 'Pathway Assembly from Literature Mining-an Information Search Tool' (PALM-IST) (www.hpppi.iicb.res.in/ctm) [47]. We then cross-checked every target protein on an individual basis using PubMed database links and excluded those targets that had no direct interaction with the curcumin. Additionally, while scanning the PubMed database, we included some curcumin targets that we skipped. A similar approach of target fishing has been employed in our recently published article [48] which identifies real-time targets because the proteins which are under the direct regulation (up or down) of a particular compound are the actual targets and are implicated in the concerned biological effects in experimental models. Finally, we selected only those targets, for further analysis, which were coincided with AD-related genes from DisGeNET database v6.0 (Barcelona, Spain) [49], a database that integrates human gene-disease associations from expert-curated databases and text-mining derived associations. UniProt (http://www.uniprot.org/), a database of protein sequence and functional information, was searched to retrieve the protein information, including protein name, gene ID, and host organism [50].

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4.3. Network Construction for Curcumin–Target–Target-Alzheimer's Disease (C–T–T–AD)

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), an online tool for functional protein association networks, was used to construct the protein–protein interaction (PPI) network among the targets. The curcumin-target and PPI networks were combined through Cytoscape v3.7.1 (Seattle, Washington, USA) [51] to construct the curcumin-target-target network, which was finally merged with the target–AD network to establish C–T–T–AD network by Cytoscape.

4.4. Gene Ontology (GO) Analysis

Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8. was used to perform the functional enrichment analysis of GO for the biological process, molecular function, and cellular components [52] (https://david.ncifcrf.gov/). GO terms were considered as significant at p-value < 0.05. Target proteins were categorized by the Panther classification system [53] (http://pantherdb.org/).

4.5. Network and Pathway Analysis

To extensively understand the molecular mechanisms, NetworkAnalyst, a visual analytics platform for comprehensive gene expression profiling & meta-analysis, was used to construct a comprehensive network [54] (https://www.networkanalyst.ca/). Cellular pathways were categorized into modular systems using the KEGG pathway database (www.genome.jp/kegg/pathway.html). Pathways highlighting curcumin targets were uncovered through the KEGG pathway mapper [55] (https://www.genome.jp/kegg/tool/map_pathway2.html).

4.6. Molecular Docking and Binding Energy Analysis

The three dimensional crystal structure of human LXR-β (PDB ID: 1P8D), ER-β (PDB ID: 1QKM), TrkB (PDB ID: 1HCF), TLR2 (PDB ID: 6NIG), mTOR (PDB ID: 5GPG), BACE1 (PDB ID: 5HDZ), AChE (PDB ID: 4EY7), GSK-3β (PDB ID: 4AFJ), GluN2B (PDB ID: 5EWM) were retrieved from the protein data bank [56] and prepared for molecular docking studies by using protein preparation module of Schrödinger 2017-1 following previously described protocols [57-61]. Briefly, the amino acid orientation in the PDB file was fixed by correcting bond orders, adding charges and hydrogen. Following that the structure was optimized at neutral pH (7.0 ± 2.0), and then minimized by using OPLS 3 force filed limiting maximum heavy atom RMSD to 0.30 Å. The candidate compounds structures were prepared for molecular docking by minimizing with OPLS 3 force field

 doi:10.20944/preprints202001.0109.v1

through Ligprep2.5 in Schrödinger Suite (2017-1). The Epik2.2 module was used to generate the ionization state of each compound at pH 7.0±2.0. For molecular docking analysis, the receptor grids were fixed at the ligand-binding site of the receptor. In both cases, a cubic box of specific dimensions centered on the centroid of residues involved in the ligand-binding site was generated. The bounding box was set to 18 Å × 18 Å × 18 Å for docking experiments, keeping default parameters of glide docking procedure. After that glide docking with extra precision setting (XP) performed with default settings, consisting of Van der Waals scaling factor and partial charge cutoff for ligand atoms of 0.80 and 0.15, respectively. Binding free energy was calculated to rescore and for choosing the top hits from the candidate ligands. In prime MM-GBSA method, the calculation of binding energy was performed by combining OPLSAA molecular mechanics energies (EMM), an SGB solvation model for polar solvation (GSGB) and a non-polar solvation term (GNP) composed of the non-polar solvent accessible surface area and van der Waals interactions [62]. Here, as the source in Prime MM-GBSA simulation, the glide pose viewer file of the best conformation was given. For modeling directionality of hydrogen-bond and π-stacking interactions, the dielectric solvent model VSGB 2.0 [63] was used to apply empirical corrections. Keeping the protein chain flexible [64-68], minimizing approach is applied as sampling methods. The analysis denotes more excellent binding by more negative binding energy. Overall free energy of binding:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand}), \text{ where } G = EMM + GSGB + GNP$$

5. Conclusion

The present system pharmacology-based investigation revealed that the PI3K/Akt signaling, neurotrophin signaling, toll-like receptor signaling, and autophagy are the major cellular signaling pathways that are primary targets of curcumin. In addition, TrkB, LXR-β, mTOR, TLR-2, and β-secretase are the potential druggable targets that could be exploited for future curcumin-based drug designing against AD pathology. Together, our system pharmacology and computational findings, along with the previously described experimental evidence, provide clear insights into the underlying molecular mechanism of neuroprotection of curcumin against AD.

Supplementary Materials: Figure S1: Inflammatory pathways enriched in network analysis, Table S1: ADME/T properties of curcumin, Table S2: Data for the curcumin targets used to construct C–T–T–AD network and disease–target–pathway network, Table S3: Molecular docking simulation findings of curcumin targets.

Author Contributions: Conceptualization, M.A.H. and I.S.M.; Data curation, M.A.H., R.D., M.N.H and A.A.M.S.; Formal analysis, M.A.H. and R.D.; Funding acquisition, I.S.M.; Methodology, M.A.H. and R.D.; Supervision, I.S.M.; Visualization, M.A.H. and R.D.; Writing – original draft, M.A.H. and A.A.M.S.; Writing – review & editing, M.A.H., M.N.H, A.A.M.S. and I.S.M.

Funding: This work was supported by Korea Research Fellowship Program (grant No. 2018H1D3A1A01074712) to MAH, and by the Basic Science Research Program (grant number 2018R1A2B6002232) to ISM through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning.

Conflicts of interest: There is no conflict of interest to declare. The funders had no role in the design of the study; in the acquisition, analyses, or interpretation of data; in drafting the manuscript; or in the decision to publish the findings.

References

1. Qin, S.; Huang, L.; Gong, J.; Shen, S.; Huang, J.; Ren, H.; Hu, H., Efficacy and safety of turmeric and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Nutrition journal* **2017,** 16, (1), 68.
2. Goozee, K. G.; Shah, T. M.; Sohrabi, H. R.; Rainey-Smith, S. R.; Brown, B.; Verdile, G.; Martins, R. N., Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. *The British journal of nutrition* **2016,** 115, (3), 449-65.
3. Nabavi, S. F.; Thiagarajan, R.; Rastrelli, L.; Daglia, M.; Sobarzo-Sanchez, E.; Alinezhad, H.; Nabavi, S. M., Curcumin: a natural product for diabetes and its complications. *Current topics in medicinal chemistry* **2015,** 15, (23), 2445-55.
4. Devassy, J. G.; Nwachukwu, I. D.; Jones, P. J., Curcumin and cancer: barriers to obtaining a health claim. *Nutrition reviews* **2015,** 73, (3), 155-65.
5. Alhusaini, A.; Fadda, L.; Hasan, I. H.; Zakaria, E.; Alenazi, A. M.; Mahmoud, A. M., Curcumin Ameliorates Lead-Induced Hepatotoxicity by Suppressing Oxidative Stress and Inflammation, and Modulating Akt/GSK-3beta Signaling Pathway.

Biomolecules **2019,** 9, (11).

6. Limtrakul, P., Curcumin as chemosensitizer. *Advances in experimental medicine and biology* **2007,** 595, 269-300.
7. Yang, Y. S.; Su, Y. F.; Yang, H. W.; Lee, Y. H.; Chou, J. I.; Ueng, K. C., Lipid-lowering effects of curcumin in patients with metabolic syndrome: a randomized, double-blind, placebo-controlled trial. *Phytotherapy research : PTR* **2014,** 28, (12), 1770-7.
8. Chen, F. Y.; Zhou, J.; Guo, N.; Ma, W. G.; Huang, X.; Wang, H.; Yuan, Z. Y., Curcumin retunes cholesterol transport homeostasis and inflammation response in M1 macrophage to prevent atherosclerosis. *Biochemical and biophysical research communications* **2015,** 467, (4), 872-8.
9. Xiong, Z.; Hongmei, Z.; Lu, S.; Yu, L., Curcumin mediates presenilin-1 activity to reduce beta-amyloid production in a model of Alzheimer's Disease. *Pharmacological reports : PR* **2011,** 63, (5), 1101-8.
10. Ma, Q.-L.; Zuo, X.; Yang, F.; Ubeda, O. J.; Gant, D. J.; Alaverdyan, M.; Teng, E.; Hu, S.; Chen, P.-P.; Maiti, P.; Teter, B.; Cole, G. M.; Frautschy, S. A., Curcumin suppresses soluble tau dimers and corrects molecular chaperone, synaptic, and behavioral deficits in aged human tau transgenic mice. *J Biol Chem* **2013,** 288, (6), 4056-4065.
11. Bhat, A.; Mahalakshmi, A. M.; Ray, B.; Tuladhar, S.; Hediyal, T. A.; Manthiannem, E.; Padamati, J.; Chandra, R.; Chidambaram, S. B.; Sakharkar, M. K., Benefits of curcumin in brain disorders. *BioFactors (Oxford, England)* **2019,** 45, (5), 666-689.
12. Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M., Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J Biol Chem* **2005,** 280, (7), 5892-901.
13. Garcia-Alloza, M.; Borrelli, L. A.; Rozkalne, A.; Hyman, B. T.; Bacskai, B. J., Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. *Journal of neurochemistry* **2007,** 102, (4), 1095-104.
14. Fiala, M.; Liu, P. T.; Espinosa-Jeffrey, A.; Rosenthal, M. J.; Bernard, G.; Ringman, J. M.; Sayre, J.; Zhang, L.; Zaghi, J.; Dejbakhsh, S.; Chiang, B.; Hui, J.; Mahanian, M.; Baghaee, A.; Hong, P.; Cashman, J., Innate immunity and transcription of MGAT-III and Toll-like receptors in Alzheimer's disease patients are improved by bisdemethoxycurcumin. *Proceedings of the National Academy of Sciences of the United States of America* **2007,** 104, (31), 12849-54.
15. Chen, Y.; Chen, D.; Liu, S.; Yuan, T.; Guo, J.; Fang, L.; Du, G., Systematic Elucidation of the Mechanism of Genistein against Pulmonary Hypertension via Network Pharmacology Approach. *Int J Mol Sci* **2019,** 20, (22), 5569.
16. Kim, S.-K.; Lee, S.; Lee, M.-K.; Lee, S., A systems pharmacology approach to investigate the mechanism of Oryeong-san formula for the treatment of hypertension. *J Ethnopharmacol* **2019,** 244, 112129-112129.
17. Song, X.; Zhang, Y.; Dai, E.; Du, H.; Wang, L., Mechanism of action of celastrol

against rheumatoid arthritis: A network pharmacology analysis. *Int Immunopharmacol* **2019,** 74, 105725-105725.

18. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews* **2001,** 46, (1-3), 3-26.
19. Jorgensen, W. L.; Duffy, E. M., Prediction of drug solubility from structure. *Advanced drug delivery reviews* **2002,** 54, (3), 355-66.
20. Del Prado-Audelo, M. L.; Caballero-Floran, I. H.; Meza-Toledo, J. A.; Mendoza-Munoz, N.; Gonzalez-Torres, M.; Floran, B.; Cortes, H.; Leyva-Gomez, G., Formulations of Curcumin Nanoparticles for Brain Diseases. *Biomolecules* **2019,** 9, (2).
21. Llorens-Martín, M.; Jurado, J.; Hernández, F.; Avila, J., GSK-3β, a pivotal kinase in Alzheimer disease. *Front Mol Neurosci* **2014,** 7, 46-46.
22. Bustanji, Y.; Taha, M. O.; Almasri, I. M.; Al-Ghussein, M. A.; Mohammad, M. K.; Alkhatib, H. S., Inhibition of glycogen synthase kinase by curcumin: Investigation by simulated molecular docking and subsequent in vitro/in vivo evaluation. *Journal of enzyme inhibition and medicinal chemistry* **2009,** 24, (3), 771-8.
23. O'Bryant, S. E.; Hobson, V.; Hall, J. R.; Waring, S. C.; Chan, W.; Massman, P.; Lacritz, L.; Cullum, C. M.; Diaz-Arrastia, R., Brain-derived neurotrophic factor levels in Alzheimer's disease. *Journal of Alzheimer's disease : JAD* **2009,** 17, (2), 337-41.
24. Giuffrida, M. L.; Copani, A.; Rizzarelli, E., A promising connection between BDNF and Alzheimer's disease. *Aging (Albany NY)* **2018,** 10, (8), 1791-1792.
25. Liu, D.; Wang, Z.; Gao, Z.; Xie, K.; Zhang, Q.; Jiang, H.; Pang, Q., Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress. *Behavioural brain research* **2014,** 271, 116-21.
26. Franco-Robles, E.; Campos-Cervantes, A.; Murillo-Ortiz, B. O.; Segovia, J.; Lopez-Briones, S.; Vergara, P.; Perez-Vazquez, V.; Solis-Ortiz, M. S.; Ramirez-Emiliano, J., Effects of curcumin on brain-derived neurotrophic factor levels and oxidative damage in obesity and diabetes. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **2014,** 39, (2), 211-8.
27. Xu, Y.; Ku, B.; Tie, L.; Yao, H.; Jiang, W.; Ma, X.; Li, X., Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. *Brain Research* **2006,** 1122, (1), 56-64.
28. Wang, R.; Li, Y. H.; Xu, Y.; Li, Y. B.; Wu, H. L.; Guo, H.; Zhang, J. Z.; Zhang, J. J.; Pan, X. Y.; Li, X. J., Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3K cascades in rodent cortical neurons. *Progress in neuro-psychopharmacology & biological psychiatry* **2010,** 34, (1), 147-53.
29. Wang, R.; Li, Y. B.; Li, Y. H.; Xu, Y.; Wu, H. L.; Li, X. J., Curcumin protects against glutamate excitotoxicity in rat cerebral cortical neurons by increasing brain-derived neurotrophic factor level and activating TrkB. *Brain Res* **2008,** 1210, 84-91.

30. Mallozzi, C.; Parravano, M.; Gaddini, L.; Villa, M.; Pricci, F.; Malchiodi-Albedi, F.; Matteucci, A., Curcumin Modulates the NMDA Receptor Subunit Composition Through a Mechanism Involving CaMKII and Ser/Thr Protein Phosphatases. *Cellular and molecular neurobiology* **2018,** 38, (6), 1315-1320.
31. Ahmed, T.; Gilani, A. H., Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. *Pharmacology, biochemistry, and behavior* **2009,** 91, (4), 554-9.
32. Hatami, M.; Abdolahi, M.; Soveyd, N.; Djalali, M.; Togha, M.; Honarvar, N. M., Molecular Mechanisms of Curcumin in Neuroinflammatory Disorders: A Mini Review of Current Evidences. *Endocrine, metabolic & immune disorders drug targets* **2019,** 19, (3), 247-258.
33. Arenas, F.; Garcia-Ruiz, C.; Fernandez-Checa, J. C., Intracellular Cholesterol Trafficking and Impact in Neurodegeneration. *Frontiers in Molecular Neuroscience* **2017,** 10, (382).
34. Ito, A.; Hong, C.; Rong, X.; Zhu, X.; Tarling, E. J.; Hedde, P. N.; Gratton, E.; Parks, J.; Tontonoz, P., LXRs link metabolism to inflammation through Abca1-dependent regulation of membrane composition and TLR signaling. *eLife* **2015,** 4, e08009.
35. Wolf, A.; Bauer, B.; Hartz, A., ABC Transporters and the Alzheimer's Disease Enigma. *Frontiers in Psychiatry* **2012,** 3, (54).
36. Tian, M.; Zhang, X.; Wang, L.; Li, Y., Curcumin induces ABCA1 expression and apolipoprotein A-I-mediated cholesterol transmembrane in the chronic cerebral hypoperfusion aging rats. *The American journal of Chinese medicine* **2013,** 41, (5), 1027-42.
37. Gambuzza, M. E.; Sofo, V.; Salmeri, F. M.; Soraci, L.; Marino, S.; Bramanti, P., Toll-like receptors in Alzheimer's disease: a therapeutic perspective. *CNS & neurological disorders drug targets* **2014,** 13, (9), 1542-58.
38. Shuto, T.; Ono, T.; Ohira, Y.; Shimasaki, S.; Mizunoe, S.; Watanabe, K.; Suico, M. A.; Koga, T.; Sato, T.; Morino, S.; Sato, K.; Kai, H., Curcumin decreases toll-like receptor-2 gene expression and function in human monocytes and neutrophils. *Biochemical and biophysical research communications* **2010,** 398, (4), 647-652.
39. Ye, B.; Wang, Q.; Hu, H.; Shen, Y.; Fan, C.; Chen, P.; Ma, Y.; Wu, H.; Xiang, M., Restoring autophagic flux attenuates cochlear spiral ganglion neuron degeneration by promoting TFEB nuclear translocation via inhibiting MTOR. *Autophagy* **2019,** 15, (6), 998-1016.
40. Wang, C.; Zhang, X.; Teng, Z.; Zhang, T.; Li, Y., Downregulation of PI3K/Akt/mTOR signaling pathway in curcumin-induced autophagy in APP/PS1 double transgenic mice. *European journal of pharmacology* **2014,** 740, 312-20.
41. Bachmeier, B. E.; Mirisola, V.; Romeo, F.; Generoso, L.; Esposito, A.; Dell'eva, R.; Blengio, F.; Killian, P. H.; Albini, A.; Pfeffer, U., Reference profile correlation reveals estrogen-like trancriptional activity of Curcumin. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* **2010,** 26, (3), 471-82.

42. Yang, Z.-M.; Yang, M.-F.; Yu, W.; Tao, H.-M., Molecular mechanisms of estrogen receptor β-induced apoptosis and autophagy in tumors: implication for treating osteosarcoma. *J Int Med Res* **2019,** 47, (10), 4644-4655.
43. Naoi, M.; Wu, Y.; Shamoto-Nagai, M.; Maruyama, W., Mitochondria in Neuroprotection by Phytochemicals: Bioactive Polyphenols Modulate Mitochondrial Apoptosis System, Function and Structure. *Int J Mol Sci* **2019,** 20, (10), 2451.
44. Hardy, J.; Selkoe, D. J., The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)* **2002,** 297, (5580), 353-6.
45. Vassar, R., BACE1: the beta-secretase enzyme in Alzheimer's disease. *Journal of molecular neuroscience : MN* **2004,** 23, (1-2), 105-14.
46. Murata, K.; Matsumura, S.; Yoshioka, Y.; Ueno, Y.; Matsuda, H., Screening of β-secretase and acetylcholinesterase inhibitors from plant resources. *Journal of Natural Medicines* **2015,** 69, (1), 123-129.
47. Mandloi, S.; Chakrabarti, S., PALM-IST: Pathway Assembly from Literature Mining--an Information Search Tool. *Scientific reports* **2015,** 5, 10021.
48. Hannan, M. A.; Dash, R.; Sohag, A. A. M.; Moon, I. S., Deciphering Molecular Mechanism of the Neuropharmacological Action of Fucosterol through Integrated System Pharmacology and In Silico Analysis. *Marine Drugs* **2019,** 17, (11), 639.
49. Pinero, J.; Bravo, A.; Queralt-Rosinach, N.; Gutierrez-Sacristan, A.; Deu-Pons, J.; Centeno, E.; Garcia-Garcia, J.; Sanz, F.; Furlong, L. I., DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res* **2017,** 45, (D1), D833-d839.
50. Wu, C. H.; Apweiler, R.; Bairoch, A.; Natale, D. A.; Barker, W. C.; Boeckmann, B.; Ferro, S.; Gasteiger, E.; Huang, H.; Lopez, R.; Magrane, M.; Martin, M. J.; Mazumder, R.; O'Donovan, C.; Redaschi, N.; Suzek, B., The Universal Protein Resource (UniProt): an expanding universe of protein information. *Nucleic Acids Res* **2006,** 34, (Database issue), D187-91.
51. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N. S.; Wang, J. T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T., Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **2003,** 13, (11), 2498-504.
52. Dennis, G., Jr.; Sherman, B. T.; Hosack, D. A.; Yang, J.; Gao, W.; Lane, H. C.; Lempicki, R. A., DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* **2003,** 4, (5), P3-P3.
53. Mi, H.; Muruganujan, A.; Casagrande, J. T.; Thomas, P. D., Large-scale gene function analysis with the PANTHER classification system. *Nature Protocols* **2013,** 8, (8), 1551-1566.
54. Zhou, G.; Soufan, O.; Ewald, J.; Hancock, R. E. W.; Basu, N.; Xia, J., NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Research* **2019,** 47, (W1), W234-W241.
55. Kanehisa, M.; Sato, Y., KEGG Mapper for inferring cellular functions from protein sequences. *Protein science : a publication of the Protein Society* **2019**.

56. Wang, Z.; Benoit, G.; Liu, J.; Prasad, S.; Aarnisalo, P.; Liu, X.; Xu, H.; Walker, N. P.; Perlmann, T., Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature* **2003,** 423, (6939), 555.
57. Dash, R.; Arifuzzaman, M.; Mitra, S.; Abdul, M. H.; Absar, N.; Hosen, S., Unveiling the Structural Insights into the Inhibitor Binding Mechanism of Protein Kinase D1. *Current pharmaceutical design* **2019**.
58. Dash, R.; Junaid, M.; Islam, N.; Akash, C.; Forhad, M.; Khan, M.; Arifuzzaman, M.; Khatun, M.; M Zahid Hosen, S., Molecular insight and binding pattern analysis of Shikonin as a potential VEGFR-2 inhibitor. *Current Enzyme Inhibition* **2017,** 13, (3), 235-244.
59. Dash, R.; Junaid, M.; Mitra, S.; Arifuzzaman, M.; Hosen, S. Z., Structure-based identification of potent VEGFR-2 inhibitors from in vivo metabolites of a herbal ingredient. *Journal of molecular modeling* **2019,** 25, (4), 98.
60. Dash, R.; Mitra, S.; Arifuzzaman, M.; Hosen, S. Z., In silico quest of selective naphthyl-based CREBBP bromodomain inhibitor. *In silico pharmacology* **2018,** 6, (1), 1.
61. Mitra, S.; Dash, R., Structural dynamics and quantum mechanical aspects of shikonin derivatives as CREBBP bromodomain inhibitors. *Journal of Molecular Graphics and Modelling* **2018,** 83, 42-52.
62. Vijayakumar, B.; Umamaheswari, A.; Puratchikody, A.; Velmurugan, D., Selection of an improved HDAC8 inhibitor through structure-based drug design. *Bioinformation* **2011,** 7, (3), 134.
63. Li, J.; Abel, R.; Zhu, K.; Cao, Y.; Zhao, S.; Friesner, R. A., The VSGB 2.0 model: a next generation energy model for high resolution protein structure modeling. *Proteins* **2011,** 79, (10), 2794-812.
64. Chen, F.; Liu, H.; Sun, H.; Pan, P.; Li, Y.; Li, D.; Hou, T., Assessing the performance of the MM/PBSA and MM/GBSA methods. 6. Capability to predict protein–protein binding free energies and re-rank binding poses generated by protein–protein docking. *Physical Chemistry Chemical Physics* **2016,** 18, (32), 22129-22139.
65. Xu, L.; Sun, H.; Li, Y.; Wang, J.; Hou, T., Assessing the performance of MM/PBSA and MM/GBSA methods. 3. The impact of force fields and ligand charge models. *The Journal of Physical Chemistry B* **2013,** 117, (28), 8408-8421.
66. Sun, H.; Li, Y.; Tian, S.; Xu, L.; Hou, T., Assessing the performance of MM/PBSA and MM/GBSA methods. 4. Accuracies of MM/PBSA and MM/GBSA methodologies evaluated by various simulation protocols using PDBbind data set. *Physical Chemistry Chemical Physics* **2014,** 16, (31), 16719-16729.
67. Sun, H.; Li, Y.; Shen, M.; Tian, S.; Xu, L.; Pan, P.; Guan, Y.; Hou, T., Assessing the performance of MM/PBSA and MM/GBSA methods. 5. Improved docking performance using high solute dielectric constant MM/GBSA and MM/PBSA rescoring. *Physical Chemistry Chemical Physics* **2014,** 16, (40), 22035-22045.
68. Hou, T.; Li, N.; Li, Y.; Wang, W., Characterization of domain–peptide interaction interface: prediction of SH3 domain-mediated protein–protein interaction network

in yeast by generic structure-based models. *Journal of proteome research* **2012,** 11, (5), 2982-2995.