

Therapeutic Potential of Curcumin on the Cognitive Decline in Alzheimer's Disease: A Study Integrated Meta-Analysis, Network Pharmacology, and Molecular Docking

Zheyu Zhang

Second Xiangya Hospital

Hongli Li

Second Xiangya Hospital

Shan Hui

Hunan Provincial People's Hospital

Min Yi

Second Xiangya Hospital

Xin Chen

Second Xiangya Hospital

Jianhua Huang

Hunan University of Chinese Medicine

Weijun Peng (✉ pengweijun87@csu.edu.cn)

Second Xiangya Hospital <https://orcid.org/0000-0002-4506-0942>

Jingjing Yang

Xiangya Hospital Central South University

Research

Keywords: Curcumin, Alzheimer's disease, cognitive decline, meta-analysis, network pharmacology, molecular docking

Posted Date: June 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-590600/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Curcumin, a polyphenol derived from the herb turmeric, has emerged as a promising potential therapy in the management of Alzheimer's disease (AD). However, the efficacy and potential therapeutic mechanisms remains largely unknown.

Objective: To systematically meta-analysis the effect and to investigate the potential pharmacological mechanisms of curcumin on cognitive deficits in AD.

Methods: A systematic collection of curcumin studies was performed from MEDLINE's database, PubMed, Web of Science, and Google Scholar until October 31th, 2020. Following quality assessment of study eligibility, stratified meta-analysis and meta-regression analyses were undertaken to recognize and control the heterogeneity in meta-analysis. An integrated network pharmacology and molecular docking approach were applied to decipher the potential pharmacological mechanisms of curcumin on AD.

Results: A meta-analysis of 29 publications showed that curcumin exerts significantly positive effects on cognitive performance. For acquisition, the global estimated effect of curcumin was -2.027 (95% CI: -2.435 to -1.619, $p < 0.001$); For retention, the global estimated effect of curcumin was 1.606 (95% CI: 1.101 to 2.111, $p < 0.001$). Stratified meta-analysis demonstrated that an increased effect size depended on various study characteristics. Network pharmacology analysis identified 63 genes targets, and STAT3, CHEK1, AKT1, EGFR, MMP9, hsp90AA1, and EP300 were core target proteins. Molecular docking showed that curcumin can closely bind with these seven targets. Besides, 69 potential pathways of curcumin were identified, like nitrogen metabolism.

Conclusions: Our findings suggested that curcumin may reduce cognitive deficits in AD through multi-target and multi-pathway mechanism, providing a scientific basis for further experimental and clinical application.

1. Introduction

Alzheimer's disease (AD), a neurodegenerative illness, is characterised by deterioration of cognitive functions and behavioural changes that eventually lead to dementia. According to the statistics, an estimated 30 million people have AD today globally, and the number of people living with AD are predicted to increase to 100 million by 2050 [1]. The increasing prevalence of AD has resulted in substantial burdens at the personal, social, and economic levels. However, despite substantial efforts, there are no effective therapeutic strategies currently available for AD [2].

Curcumin, a polyphenol derived from the herb turmeric (*Curcuma longa*), is a multifunctional tissue-protecting agent that exerts anti-inflammatory, antioxidant, antiproliferation, chemo preventive, and chemotherapeutic effects [3]. Indeed, curcumin demonstrated as a promising potential therapy in the treatment of AD. The main mechanism of action by which curcumin modifies AD pathology has been

summarised as follows[4]: curcumin inhibits the formation and ameliorates the cytotoxicity of amyloid- β (A β) fibrils and attenuates the hyperphosphorylation of tau fibrils and enhances their clearance.

However, a thorough review provided evidence that curcumin did not affect the A β aggregation in vitro and inhibit tau fibril formation. According to the study authors, more than 120 clinical trials have not established a clear and direct causal effect of curry consumption on improving cognitive function[5]. Not surprisingly, this manuscript contributed to the major controversy about curcumin in *Nature*. A supportive report by M. Baker defined curcumin as a "chemical liar" under "gold armor"[6]. Then, another short essay co-authored by multiple scientists noted that the researchers missed very important pharmacological or clinical data and that the pharmacological and medicinal values of curcumin were misinterpreted [7].

Systematic reviews and meta-analyses of animal experiments would offer a sensible and rational method for evaluating the translational potential of promising experimental interventions before decisions are made to proceed with clinical trials [8]. Network pharmacology attempts to understand drug actions and interactions with multiple targets in a holistic manner. It helps in systematically explaining the role of a drug or drugs in disease treatment, and possess obvious advantages over conventional methods in the illustration of comprehensive mechanisms. In this study, a meta-analysis conducted here is expected to provide guidance for animal and clinical studies on curcumin intervention in AD. Furtherly, network pharmacology combined with molecular docking were applied to explore the potential molecule targets and mechanism of action, in order to have a deeper understanding of the intervention effect of curcumin in treating AD.

2. Methods

2.1 Meta-analysis

2.1.1 Search strategy

To identify animal studies describing the effect of curcumin in AD, a literature search was conducted based on databases, including MEDLINE's database, PubMed, Web of Science, and Google Scholar until October 31th, 2020. Search terms included (curcumin OR curcuminoids) AND (dementia OR cognitive impairment OR AD OR senile dementia).

2.1.2 Screening criteria

Inclusion criteria: (1) experimental AD was induced in rodents. (2) an AD treatment group was treated with curcumin, and a control group of animals was treated with placebo. (3) cognitive function was measured by the Morris water maze (MWM), and escape latency time was used to represent acquisition (learning) and/or time in the target quadrant in the retention test was used to represent retention (memory).

Exclusion criteria: (1) curcumin was not administered, or the treatment group was administered another neuroprotective agent in addition to curcumin. (2) only biochemical or physiological outcomes of treatment efficacy were assessed. (3) duplicate publications.

2.1.3 Data extraction

Two investigators extracted details of individual study characteristics, including reference details (publication year and name); recipient animal (rat strain, gender); AD model; main experimental groups and quantity; substances used as experimental and control treatments; method/dose/timing of curcumin administration; and time of cognitive outcome assessments. GetData software was used for obtaining data at the final time point in the study of behavioural testing.

2.1.4 Methodological quality

The methodological quality of study was assessed using a 13-point project quality checklist as previously described with minor modifications[9], which was based on the CAMARADES (Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies) quality checklist.

2.1.5 Statistical analysis

The global estimated effect of curcumin on cognitive outcome was determined using the standardised mean difference (SMD) and a 95% confidence interval (CI). Within- and between-study variation or heterogeneity was assessed using Cochran's Q-statistic, with a significant Q-statistic ($p < 0.10$) indicating heterogeneity among studies. Quantification of heterogeneity was calculated using the I^2 metric and was represented on a scale ranging from 0–100%. Funnel plots (visually assessing for asymmetry) and Egger's test ($p < 0.05$ indicating the presence of a small effect size) were selected for the assessment of potential publication bias. STATA version 15.0 was used for statistical analyses.

2.2 Network pharmacology to predict the potential mechanisms

2.2.1 Prediction of targets

The molecular target of curcumin was obtained from The Traditional Chinese medicine system pharmacology technology platform (TCMSP, <http://tcmssp.com/tcmssp.php>). AD targets obtained from the GeneCards (<https://www.genecards.org/>) and Online Mendelian Inheritance in Man (OMIM, <http://www.omim.org/>) databases.

2.2.2 Enrichment analysis

To explore the gene functions, Gene Ontology (GO) and Kyoto Gene and Genomic Encyclopaedia (KEGG) pathway were conducted and visualized using the R software (Version 3.26). Only functional annotations with an enrichment P-value less than 0.05 were chosen for further analysis.

2.2.3 Protein–protein interaction (PPI)

The predicted targets of curcumin toward AD were submitted to the STRING database (<http://stringdb.org/cgi/input.pl>) for PPI analysis. In this study, the obtained target was submitted to STRING, with the species limited to "Homo sapiens" and confidence scores limited to those > 0.4, and the PPI data and network were extracted.

2.2.4 Network Construction

The screened target and pathway files were input into Cytoscape 3.7.2 to construct the "component-target-pathway" network diagram of curcumin. In the network, component, targets and pathway are represented by nodes, and the interaction between two nodes is represented by edges.

2.3 Molecular docking analysis

The protein crystal structure was derived from the Protein Data Bank (PDB, <http://www.rcsb.org/>) and imported into the Molecular Operating Environment software for structure construction, as previously described [10]. The specific steps to construct the protein structure include removal of water, protonation, and energy minimisation, etc. Then, the protein structure was matched to the three-dimensional structure of the curcumin which downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).

3. Results

3.1 Meta-analysis

3.1.1 Study selection and quality evaluation

A total of 29 publications, including 40 comparisons of acquisition and 22 comparisons of retention, were assessed in the meta-analysis (Figure 1). Relevant characteristics of these trials, including recipient animal, AD model, main experimental groups and quantity, experimental and control treatments, method/dose/timing of curcumin administration, and time of cognitive outcome assessments are described in Table S1 [11-16] [17-21] [22-26] [27-30] [31-36] [37, 38]. The quality evaluation of those studies was listed in Table S2. The median quality score was 8 items of 13 (range 5–10).

3.1.2 Meta-analysis and stratification analysis

For acquisition, the global estimated effect of curcumin was -2.027 (95% CI: -2.435 to -1.619, $p < 0.001$), with significant heterogeneity among studies ($\chi^2 = 190.97$, d.f. = 39, $p < 0.001$, $I^2 = 79.6\%$; Figure 2A). For retention, the global estimated effect of curcumin was 1.606 (95% CI: 1.101 to 2.111, $p < 0.001$) with significant heterogeneity among studies ($\chi^2 = 104.22$, d.f. = 21, $p < 0.001$, $I^2 = 79.8\%$; Figure 2B).

Stratification analysis revealed significant differences in effect size as shown in Table 1 and Table 2. For acquisition, the effect size was larger in studies that used species of rat (-2.196; 95% CI: -2.792 to -1.599), the Swiss albino strain (-5.239; 95% CI: -7.187 to -3.29), female gender (-2.728; 95% CI: -7.509 to 2.053), or an aluminium-damaged model of AD (-10.793; 95% CI: -13.935 to -7.651). Intervention was more effective

when studies chose oral administration of curcumin (-2.251; 95% CI: -2.77 to -1.727). For retention, the effect size was comparatively larger in studies that selected mouse species (2.541; 95% CI: 1.727 to 3.354), the Swiss albino strain (3.719; 95% CI: 1.852 to 5.587), or an STZ-injected model of AD (3.262; 95% CI: 1.399 to 5.124). Intervention was more effective when studies chose oral administration of curcumin (1.83; 95% CI: 1.042 to 2.618) than intraperitoneal injection (1.354; 95% CI: 0.709 to 1.998). However, low-quality (score<8) studies were associated with the highest beneficial outcome (2.052; 95% CI: 0.87 to 3.234).

3.1.3 Meta-regression

Meta-regression is an extension of subgroup analysis that allows investigation of the effect of continuous as well as categorical characteristics. Hence, a meta-regression and subgroup analysis by animal model (species, strain, gender, model), study design (quality score), and intervention characteristics (delivery route) was performed. For acquisition, heterogeneities were independent of these factors (Table 1). For retention, species differences were significant sources of heterogeneity ($p < 0.05$) (Table 2).

Table 1. Stratification of estimated effect size for acquisition memory

variable	studies	comparison	SMD (95%)	%Weight	I^2	P*	P**
Species							
rat	15	23	-2.196(-2.792 - -1.599)	54.71	81.8%	0.000	0.582
mouse	12	17	-1.876(-2.430 - -1.321)	45.29	76.4%	0.000	
Strain							
Wistar	6	8	-3.573(-4.903- -2.244)	16.71	81.60%	0.000	0.969
SD	8	11	-0.897(-1.225- -0.57)	32.04	30.40%	0.000	
APP/PS1	5	8	-1.523(-2.235- -0.811)	23.34	78.0%	0.000	
albino	3	6	-5.239(-7.187- -3.29)	9.4	74.80%	0.000	
5×FAD	1	2	-1.234(-2.053- -0.415)	5.53	0.00%	0.003	
SAM	1	2	-1.746(-2.763- -0.73)	5.73	45.30%	0.001	
C57BL/6	2	2	-1.884(-3.123- -0.645)	4.19	.%	0.003	
Kunming	1	1	-2.716(-3.586- -1.846)	3.06	.%	0.000	
Gender							
male	18	25	-1.699(-2.119 - -1.280)	64.50	70.6%	0.000	0.143
mixed	6	12	-2.479 (-3.379 - -1.580)	28.5	84.9%	0.000	
female	2	2	-2.728 (-7.509 - 2.053)	5.03	95.1%	0.000	
not reported	1	1	-5.315(-7.260 - -3.370)	1.97	.%	.	
Model							
ICV-STZ injected	4	4	-4.017 (-6.192 - -1.842)	8.19	83.70%	0.000	0.407
A β infused	5	7	-0.794 (-1.142 - -0.445)	21.18	12.80%	0.000	

APP/PS1	5	8	-1.523(-2.235 - -0.811)	23.34	78.0%	0.000	
Aged	2	3	-2.007(-3.761 - -0.254)	8.34	89.40%	0.025	
SAM	1	2	-1.746(-2.763 - -0.730)	5.73	45.30%	0.001	
ALU-treated	2	2	-10.79(-13.935 - -7.65)	1.36	0.00%	0.000	
COL-injected	1	3	-1.989(-2.700 - -1.278)	8.04	0.00%	0.000	
OKA- injected	2	2	-2.416(-3.705 - -1.127)	4.09	0.00%	0.000	
SCO- induced	1	4	-4.988(-6.881 - -3.094)	5.96	54.70%	0.000	
D-gal- induced	1	1	-1.824(-3.352 - -0.296)	2.37	0.00%	0.019	
mixed	1	1	-1.202(-2.317 - -0.088)	2.81	0.00%	0.034	
5×FAD	1	2	-1.234(-2.053 - -0.415)	5.53	0.00%	0.003	
AlCl3	1	1	-2.716(-3.586 - -1.846)	3.06	0.00%	0.000	
Delivery							
oral	19	30	-2.269(-2.780 - -1.757)	72.21	80.5%	0.000	0.269
i.p.	8	10	-1.496(-2.162 - -0.831)	27.79	77.0%	0.000	
Quality score							
8	9	16	-1.450(-1.856 - -1.043)	44.48	57.7%	0.000	0.404
8	10	14	-3.139(-4.054 - -2.224)	30.10	84.5%	0.000	
8	8	10	-1.954(-2.886 - -1.021)	25.42	84.2%	0.000	

Abbreviations: SD=Sprague Dawley; ICV-STZ=Intracerebroventricular streptozotocin; Aβ= Amyloid beta; SAM=senescence accelerated mouse; ALU=Aluminium; COL=colchicine; SCO=Scopolamine; i.p.=Intraperitoneal injection; OKA=okadaic acid; SMD=standardized mean difference.

Note: *p value for subgroup differences; **p value for heterogeneity between subgroups with meta-regression analysis.

Table 2. Stratification of estimated effect size for retention memory.

variable	studies	comparison	SMD (95%)	%Weight	I ²	P*	P**
Species							
rat	9	12	0.942(0.466 - 1.419)	60.05	70.2%	0.000	0.007
mouse	9	10	2.541(1.727 - 3.354)	39.95	70.2%	0.000	
Strain							
Wistar	4	4	1.620(0.016 - 3.225)	18.63	87.8%	0.048	0.223
SD	5	8	0.646(0.334 - 0.958)	41.43	14.9%	0.000	
APP/PS1	3	3	2.699(1.299 - 4.100)	13.34	75.6%	0.000	
albino	3	3	3.719(1.852 - 5.587)	10.69	71.8%	0.000	
5xFAD	1	2	2.318(1.329 - 3.307)	8.30	0.0%	0.000	
C57BL/6	2	2	0.771(-0.361 - 1.903)	7.61	9.6%	0.182	
Gender							
male	11	15	1.132(0.722 - 1.541)	70.24	58.6%	0.000	0.054
mixed	3	3	2.999(1.218 - 4.780)	12.82	83.3%	0.001	
female	2	2	1.333(-2.327-4.992)	8.99	94.0%	0.475	
not reported	2	2	3.578(1.465 - 5.691)	7.95	74.1%	0.001	
Model							
STZ	3	3	3.262(1.399 - 5.124)	11.95	79.6%	0.007	0.690
Aβ	5	7	0.944(0.377 - 1.511)	34.52	62.4%	0.014	
5xFAD	1	2	2.318(1.329 - 3.307)	8.30	0.0%	0.991	
Aged	2	3	0.271(-0.555 - 1.097)	15.89	69.6%	0.037	

D-gal	2	2	1.495(-0.674 - 3.664)	8.92	83.7%	0.013	
OKA	2	2	2.094(0.884 - 3.305)	7.07	0.0%	0.556	
APP	3	3	2.699(1.299 - 4.100)	13.34	75.6%	0.017	
Delivery							
oral	10	12	1.830(1.042 - 2.618)	53.64	83.4%	0.000	0.522
i.p.	8	10	1.354 (0.709 - 1.998)	46.36	74.5%	0.000	
Quality score							
⊗	5	6	1.329 (0.283 - 2.376)	26.67	83.7%	0.013	0.425
8	8	9	1.535(0.859 - 2.211)	42.59	69.4%	0.000	
⊗	5	7	2.052(0.870 - 3.234)	30.74	88.4%	0.001	

Abbreviations: SD=Sprague Dawley; ICV-STZ=Intracerebroventricular streptozotocin; A β = Amyloid beta ALU=Aluminium; COL=colchicine; SCO=Scopolamine; i.p.=Intraperitoneal injection; OKA=okadaic acid; SMD=standardized mean difference.

Note ⊗*p value for subgroup differences; **p value for heterogeneity between subgroups with meta-regression analysis

3.1.4 Publication bias

The funnel plots were applied to identify whether there was publication bias in these studies. As shown in Figure 3a and Figure 3b, asymmetry was identified in both funnel plots of acquisition and retention functions, suggesting publication bias existed. Furthermore, Egger's tests were created to statistically detect asymmetry, with a null hypothesis denying the existence of small-study effects. A p-value of <0.05 was found in both acquisition and retention data, evidently indicating to reject the null hypothesis in favour of the alternative (i.e. publication bias does exist in these studies).

3.2 Network pharmacology analysis

3.2.1 Predicting the targets of curcumin

A total of 7945 AD-related genes were searched from the disease databases. After matching them with the predicted curcumin target, 63 anti-AD targets were obtained. The PPI network containing 63 nodes and 231 edges were building. In this network (Figure 4), the nodes, including AKT1, EGFR, MMP9, HSP90AA1, STAT3, CHEK1, and EP300 had higher degree. That's means, they had a closer relationship with the more surrounding genes (37 for AKT1, 33 for EGFR, 24 for MMP9, 21 for HSP90AA1, 21 for STAT3, 14 for CHEK1, 14 for EP300). The network results revealed that the above genes may play an important role in the mechanism of curcumin in the treatment of AD.

3.2.2 Function enrichment analysis and compound–target–pathway networks

The bar graph of GO enrichment terms for the identified genes showed that one-carbon metabolic process (GO:0006730), vesicle lumen (GO:0031983), and carbonate dehydratase activity (GO:0004089) were the most highly enriched biological processes, cell component, and molecular functions, respectively. The top twenty entries in each GO category were listed in Figure 5 (A-C). The bar graph of KEGG enrichment terms for the identified genes showed that nitrogen metabolism (hsa00910), prostate cancer (hsa05215), EGFR tyrosine kinase inhibitor resistance (hsa01521) were the most highly enriched signaling pathways. The top 20 pathways were listed in Figure 5D.

3.2.3 Compound–target–pathway network construction

Cytoscape software was used to construct “component-target-pathway” network model for exploring the potential mechanism by which curcumin treats AD. The relationship among the compound, targets, and pathways are shown in Figure 5. The network revealed that curcumin exert its therapeutic effect on AD through multiple targets and multiple pathways.

3.3 Molecular docking analysis

STAT3 (PDB code: 2izv), CHEK1 (PDB code: 2x8e), AKT1 (PDB code: 1q1m), EGFR (PDB code: 3c09), MMP9 (PDB code: 6esm), HSP90AA1 (PDB code: 3o0i) and EP300 (PDB code: 6v90) were chosen as preparations of the receptor protein, and then they were send to molecular dock with curcumin. Figure 6(A–G) are schematic views of curcumin interacting with STAT3, CHEK1, AKT1, EGFR, MMP9, hsp90AA1, and EP300, respectively.

Curcumin formed an arene-H conjugation with Glu316 in STAT3 (Figure 8A). As shown in Figure 8B, curcumin formed one arene-H conjugation and one arene-cation conjugation with Leu15 and Lys132 in CHK1, respectively. As shown in Figure 8C, curcumin formed two hydrogen and arene-arene conjugation with Asp80, Trp80, and Ser205 in EGFR, respectively. As shown in Figure 8D, curcumin formed one hydrogen and two arene-H conjugation with Asp93, Asn51, and Leu107 in HSP90AA1, respectively. As shown in Figure 8E, curcumin formed one hydrogen and one arene-arene conjugation with Lys268 and Asp292 in AKT1, respectively. As shown in Figure 8F, curcumin formed two hydrogen, arene-arene, and arene-H conjugation with Leu188, ZN301, His226, and Tyr248 in MMP9, respectively. As shown in Figure

8G, curcumin formed one hydrogen and arene-H conjugation with Gln1455 and Tyr1414 in EP300, respectively. Energy docking scores, an indicator that reflects the binding ability of compound and proteins, were listed in Table 3. These results indicated that curcumin has a good ability to bind to target genes.

Table 3 The docking score between the curcumin and core targets

Target	STAT3	CHEK1	AKT1	mmp9	EGFR	hsp90AA1	ep300
PDB Code	2izv	2x8e	3o96	6esm	3c09	3o0i	6v90
Score	-7.1121	-7.2906	-8.1215	-8.7935	-7.9268	-7.932	-7.9944

4. Discussion

The natural polyphenol curcumin has been demonstrated as a promising potential therapy to treat AD. However, there have been researches contributed to the major controversy about curcumin in related fields. In these studies, the meta-analysis results revealed that curcumin has a certain treatment effect on AD. Then, the network pharmacology strategy was applied to investigate the mechanisms of curcumin on AD treatment. Furtherly, molecular docking provides reliably molecular binding evidence for network pharmacology research results. The use of meta-analysis, network pharmacology methods in combination with molecular docking for the first time enabled an investigation of the efficacy and potential underlying biological mechanisms of curcumin when being used in the treatment of AD.

In preclinical studies of AD, curcumin was shown to inhibit the formation of tau fibrils and A β fibrils and improve cognitive function in experimental treatment groups. Additionally, phase II clinical trials studying the tolerability and efficacy of oral curcumin were reported [39]. This is the first meta-analysis that systematically collected and evaluated the current preclinical evidence supporting the efficacy of curcumin in experimental AD in relation to improve learning and memory ability. Though small-study effects and statistical heterogeneity among studies exist, these findings revealed a beneficial effect of curcumin in experimental AD and strengthened the evidentiality in terms of supporting curcumin-based therapy for experimental AD.

The stratified analysis detected a significant influence of animal gender, animal strain, animal species, animal model, drug delivery route, and study quality. For example, we found that studies that did not use the most widely used animal models. The results above should be applied to provide a reference for future research when ascertaining the curcumin characteristics for successful outcomes. The major cause of heterogeneity, we found in the treatment effects between the study groups, is the potential bias of studies selected for analysis. The results of meta-regression showed acquisition and retention outcome heterogeneity was lower than would be expected by chance. Moreover, the methodological quality of each study were assessed [40]. Studies with lower scores showed a trend towards better

learning and memory outcomes. The global estimated effect of curcumin on cognition may be overstated in low-quality studies.

The network pharmacology methodology was then used to predict the molecular targets and the potential pathways modulated by curcumin during AD treatment. Specific, key targets, including AKT1, EGFR, MMP9, HSP90AA1, STAT3, CHEK1, and EP300 were found to play potential pivotal roles in the anti-AD action of curcumin. Transcriptional regulation of Akt, related to the protein fragments involved in learning and memory, could directly has led to the impacts upon the central nervous system function, and highly correlated with AD [41]. Moreover, Akt1 has been mapped to be a susceptibility gene on AD, and A β reflects an inhibition-activation effect toward AKT1 in neurons and then accelerate the progression of the disease [42]. Meanwhile, it is revealed that curcumin possesses a protective effect on neurotoxicity and memory deficits and its mechanism is closely linked to the regulation of the JNK/NF- κ B/Akt signalling pathway [43]. Current studies suggested over expression of EGFR in astrocytes [44], and it is closely related to AD and aging nerve metabolic disorders [45]. In addition, EGFR inhibitors play successful therapeutic effects in improving pathologic and behavioural conditions in neurodegenerative diseases, especially in AD [43]. There is a significant higher level of MMP9 expression in plasma and brain tissue in AD patients [46]. MMP9 augmentation in patients with AD may lead to a more vulnerable blood-brain barrier, inhibit the activity of nerve growth factor, and induce neuronal inflammatory reactions, which eventually cause nerve function defect in patients with AD [47]. HSP90AA1 is involved in oxidative stress, cell apoptosis, and mitochondrial dysfunction [48]. Upregulation of its expression can inhibit the aggregation and denaturation of protein [49], increase calcium current and neuronal excitability, inhibit amyloid plaque formation, and then exert an positive effect on spatial learning ability in AD mouse model [50]. Besides, other three targets, especially STAT3, are also closely associated with abnormal synaptic plasticity, neurogenic oxidative damage, neurogenic inflammation, and other typical neurodegenerative diseases pathological features [51]. Subsequently, the recognitions and interactions of curcumin with these seven crucial targets were studied via molecular docking method. The biological activity of curcumin was predicted reasonably well, and it was found that curcumin have a good binding affinity with gene targets (AKT1, EGFR, MMP9, HSP90AA1, STAT3, CHEK1, and EP300). Through network pharmacology and molecular docking, we can screen out the key genes which played an important role in the anti-AD action of curcumin.

The KEGG analysis identified the nitrogen metabolism pathway was the most significant in the potential anti-AD pathway of curcumin. The urea cycle, which converts excess systemic nitrogen from the decomposition of nitrogen-containing metabolites into urea, is strongly implicated in the pathogenesis of AD [52]. According to the requirements of cells for urea cycle intermediates, the body expresses different urea cycle enzymes. Previous studies have confirmed that all the genes encoding for urea cycle enzymes are expressed in the brains of AD patients [53]. Disorders in retrograde messenger nitric oxide (NO) are frequently observed in AD. Beta-amyloid protein can triggers induce a series of NO-mediated reactions, including excitotoxicity, neuroinflammation and oxidative stress [54]. In this study, the GO analysis suggested that the biological processes of potential targets were mainly reflected in responding to amyloid-beta. Growing evidence suggests that NO synthases (NOSs) is closely related to the

pathophysiology of AD. Two constitutive isoforms of NOSs are critically involved in the synaptic plasticity [55], and the pathological role of iNOS in AD immunoreactivity was known by the people [56]. Besides, beta-amyloid protein induce excitotoxicity through increases in intracellular calcium and subsequent activation of nNOS [57]. NOSs substrates, including amino acids L-arginine and L-citrulline, are important cellular metabolites. Their metabolic disorder may seriously affect the pathophysiology of AD [58]. GO analysis results showed that the molecular functions were mainly reflected in the protein serine/threonine kinase activity, which are also associated with nitrogen metabolism to a certain extent. Thus, further pharmacological experiments for verification of the related pathway are needed.

5. Conclusions

The current study combined meta-analysis, network pharmacology, and molecular docking to determine and elucidate the therapeutic mechanism of curcumin in the treatment of AD. The findings suggest that curcumin has promising therapeutic potential on learning and memory recovery in animal models of AD. However, more well-designed and well-reported experimental animal studies are needed. The core genes targeted by curcumin toward AD were: AKT1, EGFR, MMP9, HSP90AA1, STAT3, CHEK1, and EP300. This finding has been proven by the molecular docking analysis. Likewise, curcumin was found to be involved in AD treatment by targeting nitrogen metabolism. Based on the results of network pharmacology and molecular docking, we will further reveal its mechanism of action through in vivo efficacy experiments.

Declarations

Author Contributions

Weijun Peng and Jingjing Yang contributed to the design of this study. Zheyu Zhang conducted the experiments. Zheyu Zhang and Weijun Peng drafted the main manuscript text. Li Hongli, Hui Shan, Min Yi, Xin Chen, and Jianhua Huang contributed to drafting the manuscript and interpreting data. All the authors participated in the interpretation of results. All the authors have read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that they have no competing interests.

Funding Statement

This work was financially supported by the National Natural Science Foundation of China (Nos. 81873169, 81603670) and the Hunan Provincial Natural Science Foundation of China (Nos. 2017JJ3459, 2020JJ4803, 2021192).

References

1. 2021 Alzheimer's disease facts and figures. *Alzheimers Dement* 2021, 17(3):327-406.
2. Du X, Wang X, Geng M: Alzheimer's disease hypothesis and related therapies. *Translational neurodegeneration* 2018, 7:2.
3. Shehzad A, Qureshi M, Anwar MN, Lee YS: Multifunctional Curcumin Mediate Multitherapeutic Effects. *Journal of food science* 2017, 82(9):2006-2015.
4. Tang MX, Taghibiglou C: The Mechanisms of Action of Curcumin in Alzheimer's Disease. *Journal of Alzheimers Disease* 2017, 58(4):1003-1016.
5. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA: The Essential Medicinal Chemistry of Curcumin. *Journal of medicinal chemistry* 2017, 60(5):1620-1637.
6. BAKER M: Chemists warn against deceptive molecules. *Nature* 2017, 541:144-145.
7. Heger M: Don't discount allcurcumin trial data. *Nature* 2017, 543:40.
8. Pandora Pound SE, Peter Sandercock, Michael B Bracken, Ian Roberts: Where is the evidence that animal research benefits humans. *British Medical Journal* 2004, 328:514-517.
9. Wang Z, Peng W, Zhang C, Sheng C, Huang W, Wang Y, Fan R: Effects of stem cell transplantation on cognitive decline in animal models of Alzheimer's disease: A systematic review and meta-analysis. *Scientific reports* 2015, 5(srep12134).
10. Zhang Z, Li B, Huang J, Huang S, He D, Peng W, Zhang S: A Network Pharmacology Analysis of the Active Components of the Traditional Chinese Medicine Zuojinwan in Patients with Gastric Cancer. *Med Sci Monit* 2020, 26:e923327.
11. Ishrat T, Hoda MN, Khan MB, Yousuf S, Ahmad M, Khan MM, Ahmad A, Islam F: Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). *European Neuropsychopharmacology* 2009, 19(9):636-647.
12. Samy DM, Ismail CA, Nassra RA, Zeitoun TM, Nomair AM: Downstream modulation of extrinsic apoptotic pathway in streptozotocin-induced Alzheimer's dementia in rats: Erythropoietin versus curcumin. *European journal of pharmacology* 2016, 770:52-60.
13. Agrawal R, Mishra B, Tyagi E, Nath C, Shukla R: Effect of curcumin on brain insulin receptors and memory functions in STZ (ICV) induced dementia model of rat. *Pharmacological Research* 2010, 61(3):247-252.
14. Awasthi H, Tota S, Hanif K, Nath C, Shukla R: Protective effect of curcumin against intracerebral streptozotocin induced impairment in memory and cerebral blood flow. *Life sciences* 2010, 86:87-94.
15. Rinwa P, Kaur B, Jaggi AS, Singh N: Involvement of PPAR-gamma in curcumin-mediated beneficial effects in experimental dementia. *Naunyn-Schmiedeberg's archives of pharmacology* 2010, 381(6):529-539.
16. Isik AT, Celik T, Ulusoy G, Ongoru O, Elibol B, Doruk H, Bozoglu E, Kayir H, Mas MR, Akman S: Curcumin ameliorates impaired insulin/IGF signalling and memory deficit in a streptozotocin-treated rat model. *Age* 2009, 31(1):39-49.

17. Wang Y, Yin H, Li J, Zhang Y, Han B, Zeng Z, Qiao N, Cui X, Lou J, Li J: Amelioration of beta-amyloid-induced cognitive dysfunction and hippocampal axon degeneration by curcumin is associated with suppression of CRMP-2 hyperphosphorylation. *Neuroscience letters* 2013, 557 112-117.
18. Zhang L, Fang Y, Xu Y, Lian Y, Xie N, Wu T, Zhang H, Sun L, Zhang R, Wang Z: Curcumin Improves Amyloid beta-Peptide (1-42) Induced Spatial Memory Deficits through BDNF-ERK Signaling Pathway. *PLoS One* 2015, 10(6).
19. Wang Y-l, Li J-f, Wang Y-t, Xu C-y, Hua L-l, Yang X-p, Geng S, Wang S-s, Wang Z, Yin H-l: Curcumin reduces hippocampal neuron apoptosis and JNK-3 phosphorylation in rats with A β -induced Alzheimer's disease: protecting spatial learning and memory. *Journal of Neurorestoratology* 2017, 5:117-123.
20. Yin HL, Wang YL, Li JF, Han B, Zhang XX, Wang YT, Geng S: Effects of curcumin on hippocampal expression of NgR and axonal regeneration in Abeta-induced cognitive disorder rats. *Genetics and molecular research : GMR* 2014, 13(1):2039-2047.
21. S.A. Frautschy* WH, P. Kim, S.A. Miller, T. Chu, M.E. Harris-White, G.M. Cole: Phenolic anti-inflammatory antioxidant reversal of A β -induced cognitive deficits and neuropathology. *Neurobiology of Aging* 2001, 22:993-1005.
22. Zheng K, Dai X, Xiao N, Wu X, Wei Z, Fang W, Zhu Y, Zhang J, Chen X: Curcumin Ameliorates Memory Decline via Inhibiting BACE1 Expression and beta-Amyloid Pathology in 5xFAD Transgenic Mice. *Molecular neurobiology* 2017, 54(3):1967-1977.
23. Wang P, Su C, Li R, Wang H, Ren Y, Sun H, Yang J, Sun J, Shi J, Tian J *et al*: Mechanisms and effects of curcumin on spatial learning and memory improvement in APP^{swE}/PS1^{dE9} mice. *J Neurosci Res* 2014, 92(2):218-231.
24. Liu ZJ, Li ZH, Liu L, Tang WX, Wang Y, Dong MR, Xiao C: Curcumin Attenuates Beta-Amyloid-Induced Neuroinflammation via Activation of Peroxisome Proliferator-Activated Receptor-Gamma Function in a Rat Model of Alzheimer's Disease. *Frontiers in pharmacology* 2016, 7(Article261).
25. Zhang L, Fang Y, Cheng X, Lian YJ, Xu HL, Zeng ZS, Zhu HC: Curcumin Exerts Effects on the Pathophysiology of Alzheimer's Disease by Regulating PI(3,5)P2 and Transient Receptor Potential Mucolipin-1 Expression. *Frontiers in neurology* 2017, 8(Article531).
26. 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, 740:312-320.
27. Suzhen Dong QZ, E. Siobhan Mitchell, Jin Xiu, Yale Duan, Chunxia Li, Jyoti K, Tiwari, Yinghe Hu, Xiaohua Cao, Zheng Zhao: Curcumin Enhances Neurogenesis and Cognition in Aged Rats: Implications for Transcriptional Interactions Related to Growth and Synaptic Plasticity. *Plos One* 2012, 7(2):e31211.
28. Sun CY, Qi SS, Zhou P, Cui HR, Chen SX, Dai KY, Tang ML: Neurobiological and pharmacological validity of curcumin in ameliorating memory performance of senescence-accelerated mice. *Pharmacology, biochemistry, and behavior* 2013, 105:76-82.

29. Banji OJ, Banji D, Ch K: Curcumin and hesperidin improve cognition by suppressing mitochondrial dysfunction and apoptosis induced by D-galactose in rat brain. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2014, 74:51-59.
30. Belviranlı M, Okudan N, Atalik KE, Oz M: Curcumin improves spatial memory and decreases oxidative damage in aged female rats. *Biogerontology* 2013, 14(2):187-196.
31. Kumar A, Naidu PS, Seghal N, Padi SS: Effect of curcumin on intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. *J Med Food* 2007, 10(3):486-494.
32. Nam SM, Choi JH, Yoo DY, Kim W, Jung HY, Kim JW, Yoo M, Lee S, Kim CJ, Yoon YS *et al*: Effects of curcumin (*Curcuma longa*) on learning and spatial memory as well as cell proliferation and neuroblast differentiation in adult and aged mice by upregulating brain-derived neurotrophic factor and CREB signaling. *J Med Food* 2014, 17(6):641-649.
33. Rajasekar N, Dwivedi S, Tota SK, Kamat PK, Hanif K, Nath C, Shukla R: Neuroprotective effect of curcumin on okadaic acid induced memory impairment in mice. *European journal of pharmacology* 2013, 715:381-394.
34. Kumar A, Dogra S, Prakash A: Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. *Behav Brain Res* 2009, 205(2):384-390.
35. Ali EH, Arafa NM: Comparative protective action of curcumin, memantine and diclofenac against scopolamine-induced memory dysfunction. *Fitoterapia* 2011, 82(4):601-608.
36. Khurana S, Jain S, Mediratta PK, Banerjee BD, Sharma KK: Protective role of curcumin on colchicine-induced cognitive dysfunction and oxidative stress in rats. *Human & Experimental Toxicology* 2012, 31(7):686-697.
37. Huang HC, Zheng BW, Guo Y, Zhao J, Zhao JY, Ma XW, Jiang ZF: Antioxidative and Neuroprotective Effects of Curcumin in an Alzheimer's Disease Rat Model Co-Treated with Intracerebroventricular Streptozotocin and Subcutaneous D-Galactose. *Journal of Alzheimer's disease : JAD* 2016, 52(3):899-911.
38. Ahmed T, Enam SA, Gilani AH: Curcuminoids enhance memory in an amyloid-infused rat model of Alzheimer's disease. *Neuroscience* 2010, 169(3):1296-1306.
39. Ringman JM, Frautschy SA, Teng E, Begum AN, Bardens J, Beigi M, Gylys KH, Badmaev V, Heath DD, Apostolova LG *et al*: Oral curcumin for Alzheimer's disease: tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. *Alzheimer's research & therapy* 2012, 4(43).
40. Wardlaw JM, Warlow CP, Sandercock PAG, Dennis MS, Lindley RI: Neuroprotection disappointment yet aGAIN. *The Lancet* 2000, 356:597.
41. Ahmad F, Singh K, Das D, Gowaikar R, Shaw E, Ramachandran A, Rupanagudi KV, Kommaddi RP, Bennett DA, Ravindranath V: Reactive Oxygen Species-Mediated Loss of Synaptic Akt1 Signaling Leads to Deficient Activity-Dependent Protein Translation Early in Alzheimer's Disease. *Antioxid Redox Signal* 2017, 27(16):1269-1280.

42. Jo J, Whitcomb DJ, Olsen KM, Kerrigan TL, Lo SC, Bru-Mercier G, Dickinson B, Scullion S, Sheng M, Collingridge G *et al*: A β (1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3 β . *Nat Neurosci* 2011, 14(5):545-547.
43. Khan MS, Muhammad T, Ikram M, Kim MO: Dietary Supplementation of the Antioxidant Curcumin Halts Systemic LPS-Induced Neuroinflammation-Associated Neurodegeneration and Memory/Synaptic Impairment via the JNK/NF- κ B/Akt Signaling Pathway in Adult Rats. *Oxidative medicine and cellular longevity* 2019, 2019:7860650.
44. Choi I, Kim J, Jeong HK, Kim B, Jou I, Park SM, Chen L, Kang UJ, Zhuang X, Joe EH: PINK1 deficiency attenuates astrocyte proliferation through mitochondrial dysfunction, reduced AKT and increased p38 MAPK activation, and downregulation of EGFR. *Glia* 2013, 61(5):800-812.
45. Kargbo RB: Treatment of Cancer and Alzheimer's Disease by PROTAC Degradation of EGFR. *ACS Med Chem Lett* 2019, 10(8):1098-1099.
46. Hernandez-Alejandro M, Montaña S, Harrington CR, Wischik CM, Salas-Casas A, Cortes-Reynosa P, Pérez Salazar E, Cazares-Apatiga J, Apatiga-Perez R, Ontiveros Torres M *et al*: Analysis of the Relationship Between Metalloprotease-9 and Tau Protein in Alzheimer's Disease. *Journal of Alzheimer's disease : JAD* 2020, 76(2):553-569.
47. Weekman EM, Wilcock DM: Matrix Metalloproteinase in Blood-Brain Barrier Breakdown in Dementia. *Journal of Alzheimer's disease : JAD* 2016, 49(4):893-903.
48. Zuehlke AD, Beebe K, Neckers L, Prince T: Regulation and function of the human HSP90AA1 gene. *Gene* 2015, 570(1):8-16.
49. Tóth ME, Szegedi V, Varga E, Juhász G, Horváth J, Borbély E, Csibrány B, Alföldi R, Lénárt N, Penke B *et al*: Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. *Cell Stress Chaperones* 2013, 18(6):759-771.
50. Chen Q, Liang B, Wang Z, Cheng X, Huang Y, Liu Y, Huang Z: Influence of four polymorphisms in ABCA1 and PTGS2 genes on risk of Alzheimer's disease: a meta-analysis. *Neurol Sci* 2016, 37(8):1209-1220.
51. Reichenbach N, Delekate A, Plescher M, Schmitt F, Krauss S, Blank N, Halle A, Petzold GC: Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an Alzheimer's disease model. *EMBO Mol Med* 2019, 11(2).
52. Polis B, Srikanth KD, Elliott E, Gil-Henn H, Samson AO: L-Norvaline Reverses Cognitive Decline and Synaptic Loss in a Murine Model of Alzheimer's Disease. *Neurotherapeutics* 2018, 15(4):1036-1054.
53. Hansmannel F, Sillaire A, Kamboh MI, Lendon C, Pasquier F, Hannequin D, Laumet G, Mounier A, Ayrat AM, DeKosky ST *et al*: Is the urea cycle involved in Alzheimer's disease? *Journal of Alzheimer's disease : JAD* 2010, 21(3):1013-1021.
54. Kaur G, Prakash A: Involvement of the nitric oxide signaling in modulation of naringin against intranasal manganese and intracerebroventricular β -amyloid induced neurotoxicity in rats. *J Nutr Biochem* 2020, 76:108255.

55. Förstermann U, Sessa WC: Nitric oxide synthases: regulation and function. *Eur Heart J* 2012, 33(7):829-837, 837a-837d.
56. Wang SS, Jia J, Wang Z: Mesenchymal Stem Cell-Derived Extracellular Vesicles Suppresses iNOS Expression and Ameliorates Neural Impairment in Alzheimer's Disease Mice. *Journal of Alzheimer's disease : JAD* 2018, 61(3):1005-1013.
57. Li S, Wang W, Wang C, Tang YY: Possible involvement of NO/NOS signaling in hippocampal amyloid-beta production induced by transient focal cerebral ischemia in aged rats. *Neuroscience letters* 2010, 470(2):106-110.
58. Jęśko H, Lukiw WJ, Wilkaniec A, Cieślik M, Gąssowska-Dobrowolska M, Murawska E, Hilgier W, Adamczyk A: Altered Expression of Urea Cycle Enzymes in Amyloid- β Protein Precursor Overexpressing PC12 Cells and in Sporadic Alzheimer's Disease Brain. *Journal of Alzheimer's disease : JAD* 2018, 62(1):279-291.

Figures

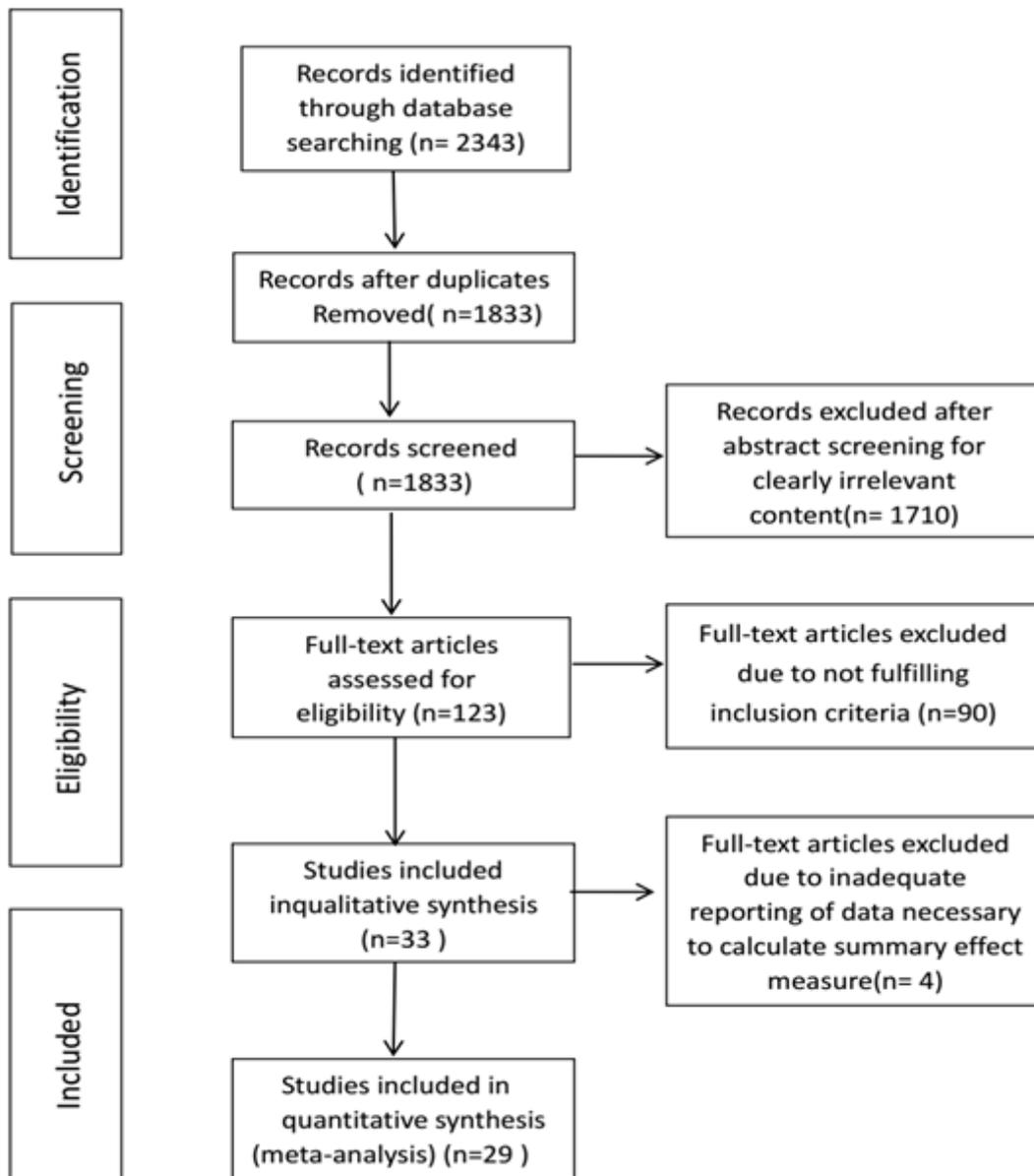
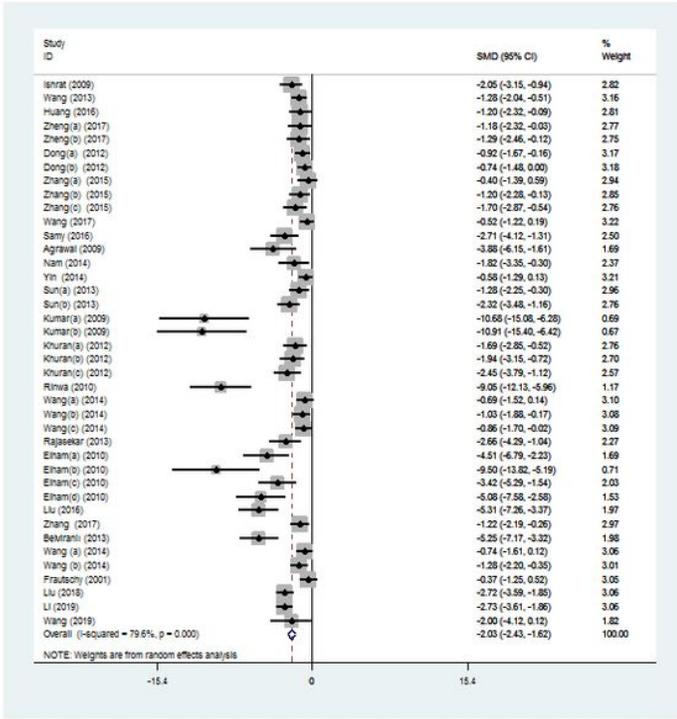
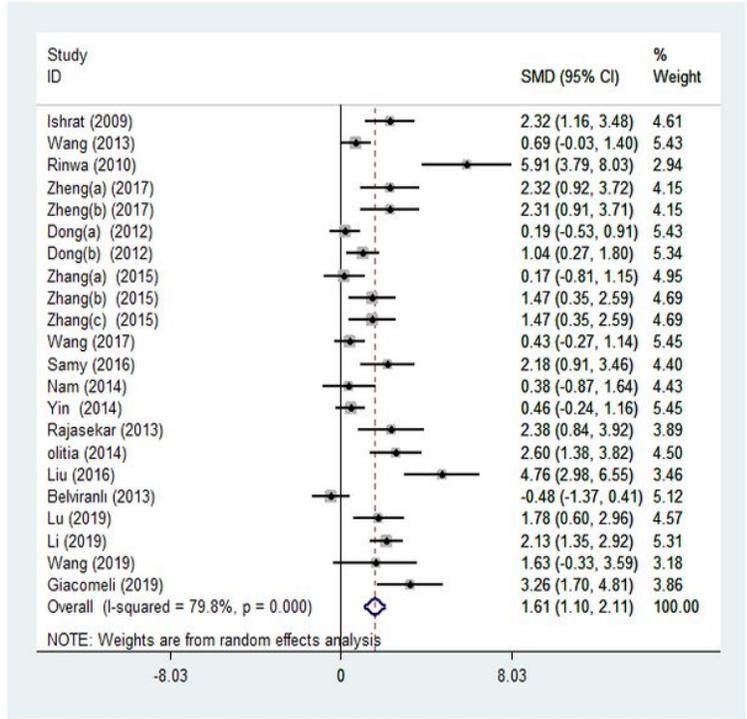


Figure 1

Flow diagram of study search process



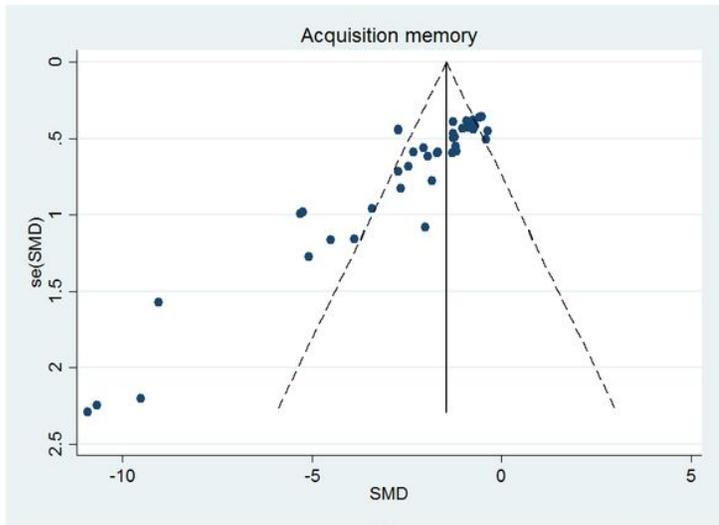
A



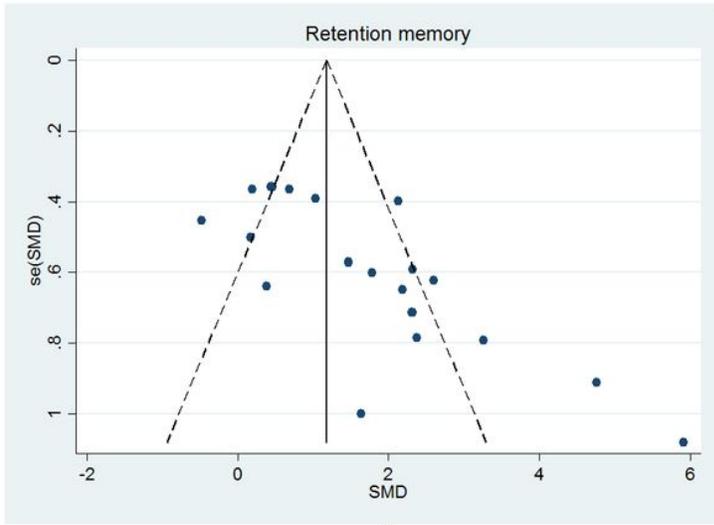
B

Figure 2

Forest plots demonstrating SMD and 95% CI for acquisition memory (A) and retention memory (B)



A



B

Figure 3

Funnel plots for acquisition memory (A) and retention memory (B)

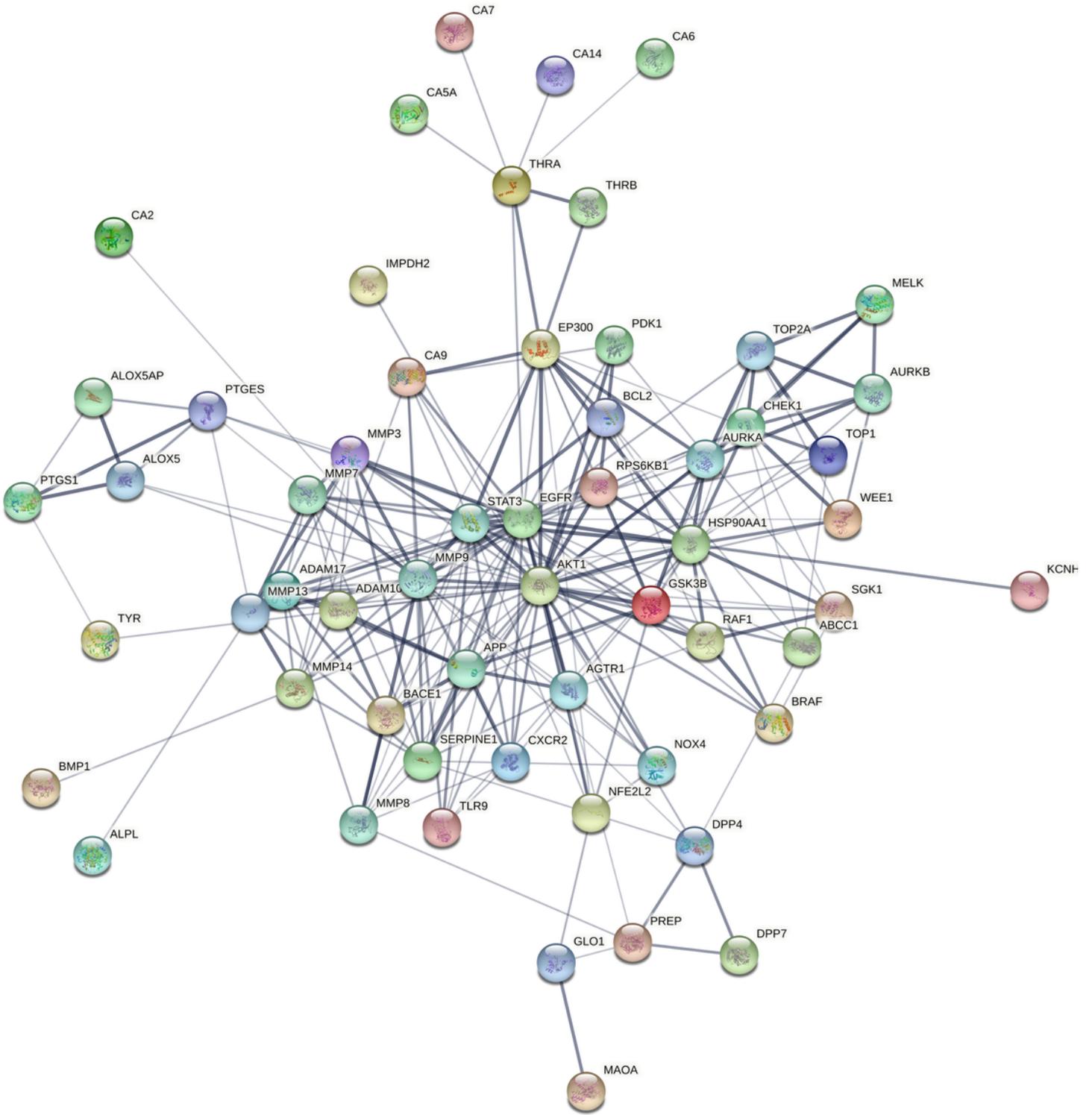


Figure 4

Protein-protein interaction network of curcumin against AD-related targets

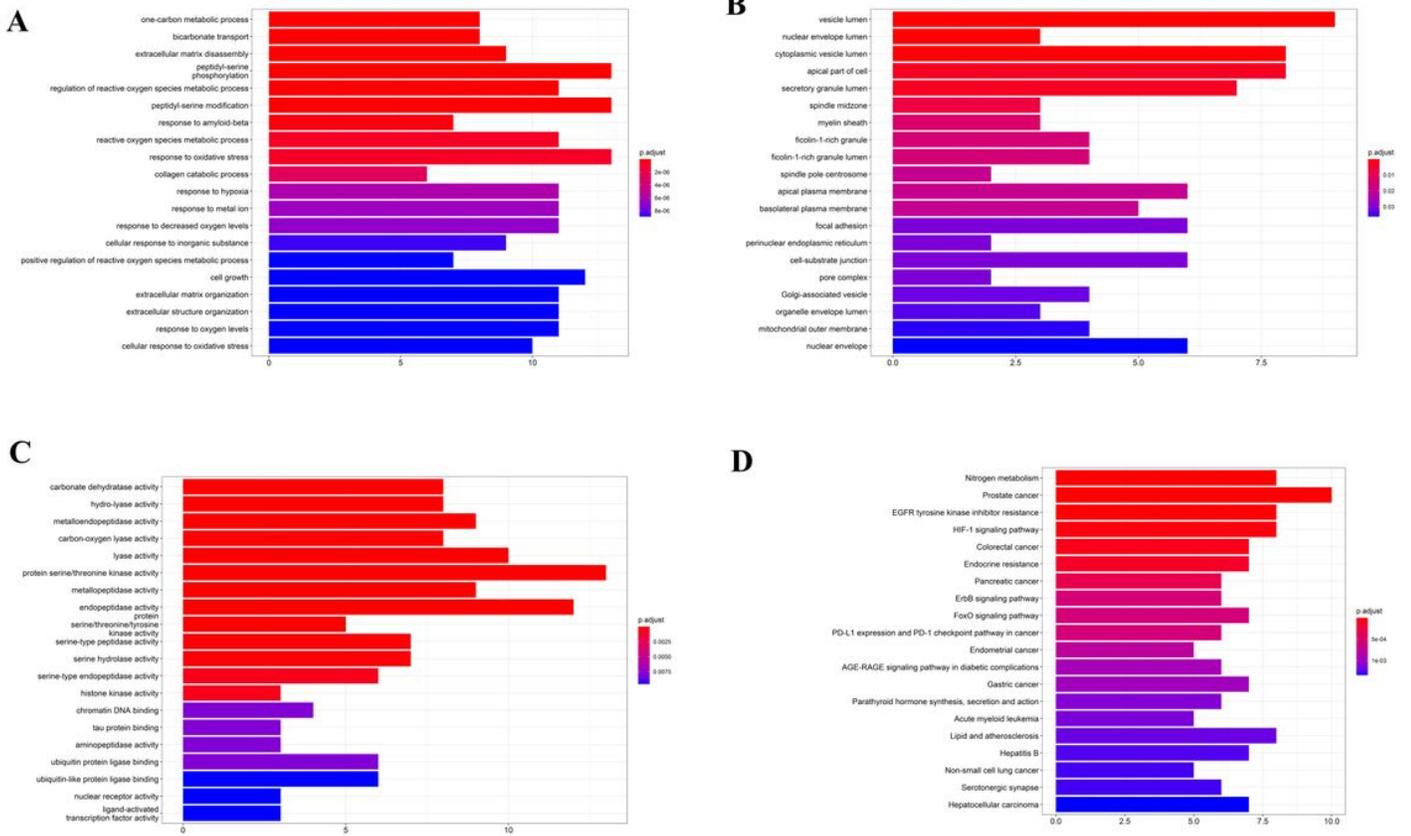


Figure 5

Function enrichment analysis of the identified genes. The ontology covered biological process (A), cellular component (B), and molecular function (C). The KEGG pathway enrichment analysis of gene target(D).

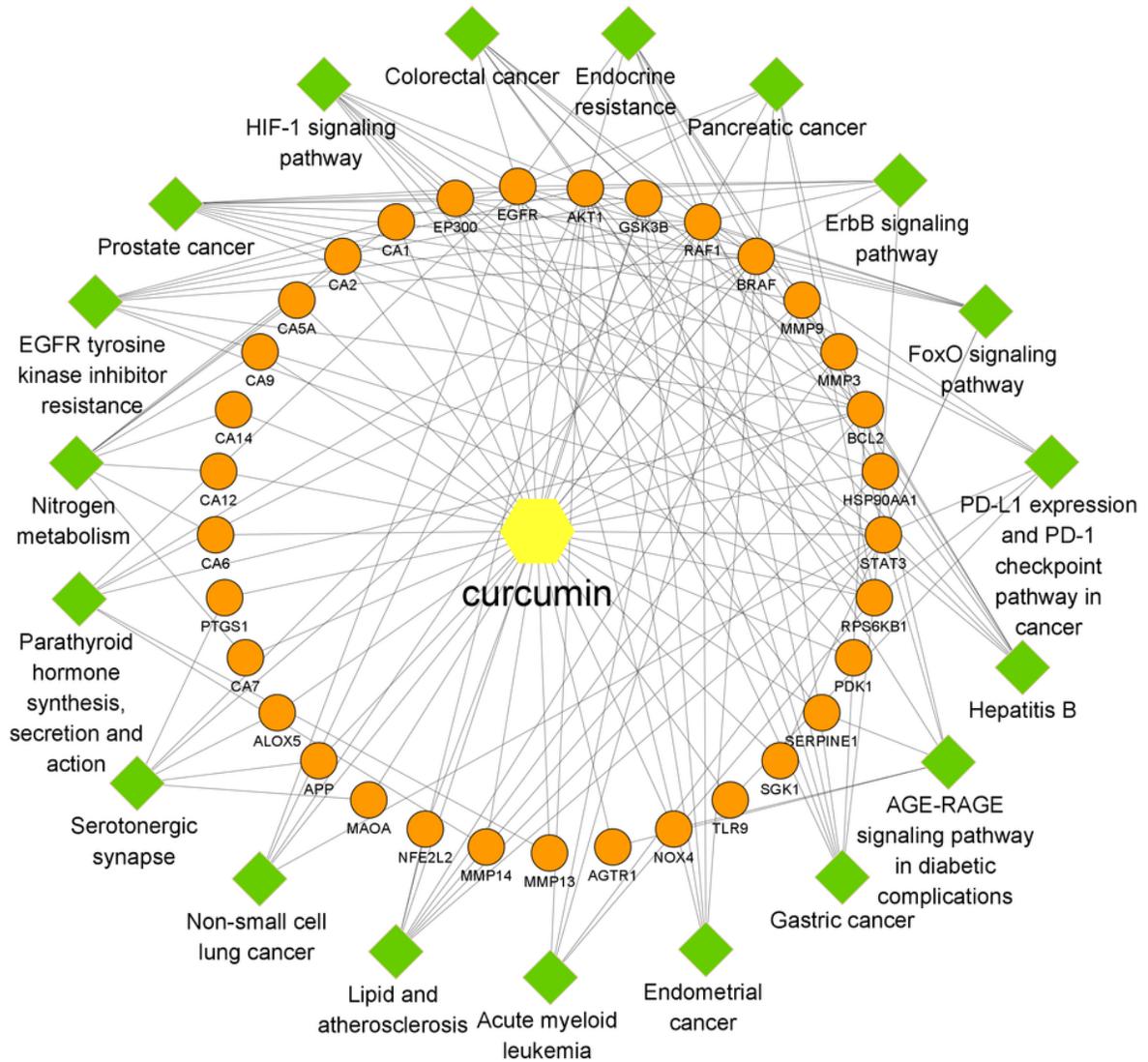


Figure 6

Compound–target–pathway diagram. The yellow diamond represents curcumin, the orange circles represent gene targets, the green squares around each target represent pathways.

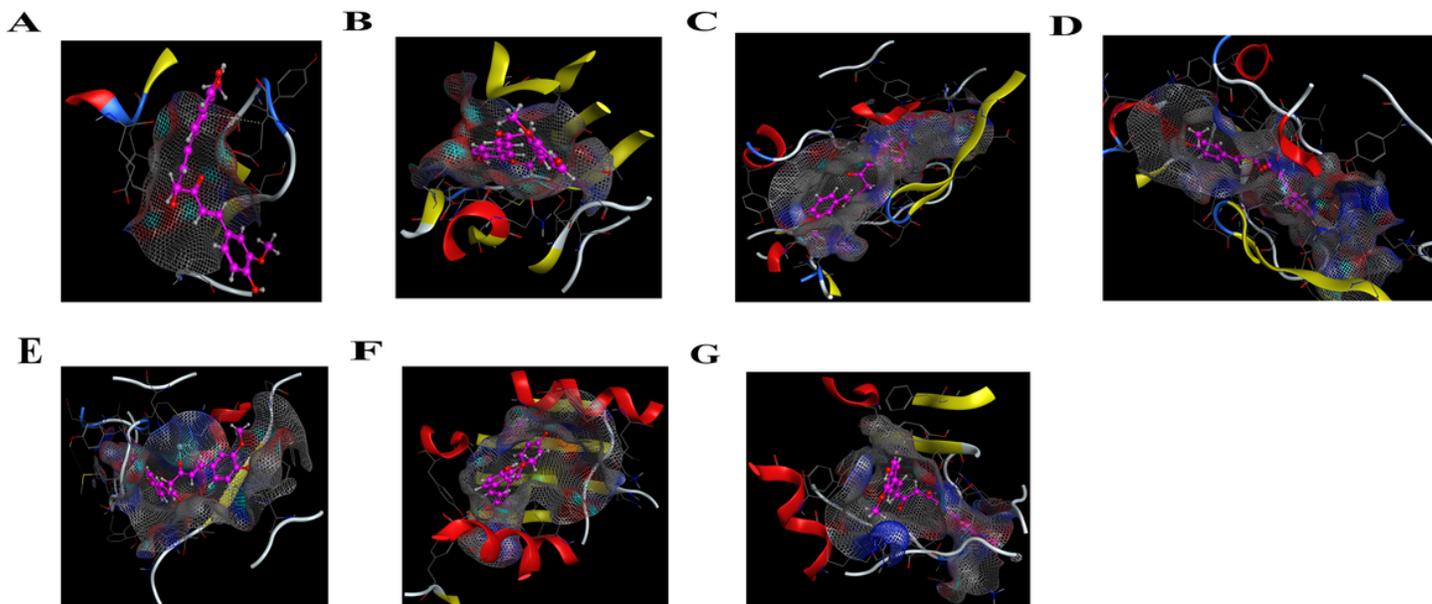


Figure 7

The docking model of curcumin with STAT3, CHEK1, AKT1, EGFR, MMP9, hsp90AA1, and EP300

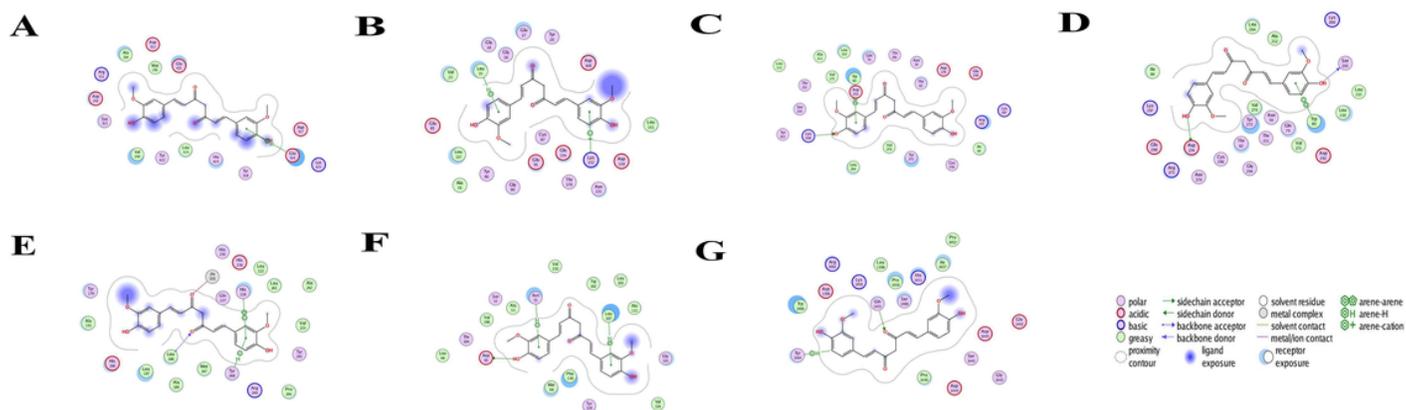


Figure 8

The interaction model of curcumin with STAT3, CHEK1, AKT1, EGFR, MMP9, hsp90AA1, and EP300

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
- [TableS2.xls](#)