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Research Article

JOURNAL OF PHARMACY AND PHARMACOLOGY RESEARCH

ISSN: 2578-1553

A Systems Pharmacology Approach, Using Molecular Docking and Dynamics Simulation, to Unlock Potential New Therapies for Alzheimer's Disease: A Case Study of *Cinnamon* Species

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Abstract

Cinnamon (*Cinnamomum* spp.) is a time-honored species that has been used as a medicine, spice, and flavoring agent for millennia. Some forms of cinnamon are useful in treating neurological disorders including Alzheimer's disease (AD). However, the mechanisms of action are unclear. To elucidate the potential therapeutic value of mechanism, cinnamon ingredients and targets were determined by searching diverse herb databases. AD targets were searched on GeneCards and filtered by reprocessing scRNA-Seq data, which were filtered by STRING to construct the Herb-Ingredient-Target-Disease network. The intersection with AD targets (3445) gave a cinnamon - AD common target set (321). Metascape analysis revealed an enriched pathway/GO process and 123 genes enriched in the Alzheimer's disease pathway were targeted for 78 cinnamon phytochemical components. Effective compounds including eugenol, cinnamaldehyde and coumarin, mainly function against hub targets, for which the binding was validated through molecular docking and dynamics simulation, e.g., cinnamaldehyde against ACHE, PTGS2. Data gathered here suggests that cinnamon may promote anti- Aß aggregation and clearance and modulate the calcium signaling pathway. It provides a therapeutic basis for (hybrid) drug development of cinnamon extracts. Ultimately this study contributes to understanding the mechanism of action of cinnamon constituent metabolites in the treatment of AD.

Keywords: *Cinnamomum* spp., Alzheimer's disease, network analysis, molecular docking, molecular dynamics simulation

Introduction

Alzheimer's Disease (AD) is a progressive and irreversible neurodegenerative disorder. Diverse mechanisms have been proposed for the pathogenesis and progression of AD, including deposition of β-amyloid (Aβ) peptide plaques, a decrease in the concentration of the neurotransmitter acetylcholine (ACh), homeostatic dysregulation of redox metal ions, and prolonged neuronal oxidative stress [1]. A significant understanding of the pathogenesis of AD has been gained, but a limited selection of medicines has been successfully developed to alleviate symptoms and slow down the disease progression of AD, and no therapy can cure AD outright. This is due to the complexity of AD pathology, the simultaneous progress of diverse pathogenetic pathways and the clinical-biological continuum [2].

Cinnamon (*Cinnamomum.* spp) is a time-honored species, that has been used as a medicine and spice for millennia. There are two main species *Cinnamomum cassia* (L.) J.Presl and *Cinnamomum zeylanicum*

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Citation: Wen Qiu, Shouli Yuan, Robbie Kelleher, Lina Sun, Wei Chen, Helen Sheridan, Junying Liu, Weiguang Lv. A Systems Pharmacology Approach, Using Molecular Docking and Dynamics Simulation, to Unlock Potential New Therapies for Alzheimer's Disease: A Case Study of *Cinnamon* Species. Journal of Pharmacy and Pharmacology Research. 7 (2023): 97-132.

Received: June 13, 2023
Accepted: June 22, 2023
Published: June 30, 2023

Qiu W, et al. J Pharm Pharmacol Res 2023
DOI:10.26502/fjppr.074

Blume (Lauraceae family). *C. cassia* Presl, also known as *Cinnamomum arromaticum* (Roxb.) Kuntze. Cinnamon is the raw material incorporated into traditional medicines in Asian countries such as China, India, Indonesia and Vietnam [3]. Cinnamon is also widely used as flavoring agent and a food supplement in America [4] and Europe [5]. The medical usage of cinnamon has been reported against dyspepsia, peptic ulcer disease, ischemic brain injury, and diverse cancers for its anti- inflammatory [6], anti-microbial [7], anti-cancer, anti-oxidant, anti-melanin and anti-diabetic effects [3, 8]. In addition, it exerts a neuroprotective effect against neurological disorders, including AD. Researchers have established that oral administration of an aqueous C. cassia decoction inhibits the formation of Aβ plaques and corrects cognitive impairment in AD animal models (fly and mice) [9].

Furthermore, Kang et al. [10] have demonstrated that aqueous C. cassia extracts significantly inhibit the formation of trademark pathological Aβ polypeptide oligomers in vitro in rat neuronal PC12 cells and in vivo in fly, and murine models, thus demonstrating a neuroprotective effect for C. cassia. Moreover, oral administration of powdered Cinnamonum verum, and its metabolite sodium benzoate (NaB), exerted a positive role in AD mice models, including protection of spatial learning and memory, attenuation of oxidative stress, suppression of neuronal degeneration and tau phosphorylation, and a reduction in amyloid polypeptide charge [11]. Others have reported that cinnamon feeding mitigates the negative effects of a high fat/high fructose diet on signaling and insulin in the brain and alleviates the induced increased expression of AD-associated mRNA, including PTEN, tau and amyloid precursor protein (APP) in vivo in rat models [12]. Peterson and co-authors found that the aqueous extract of C. zeylanicum inhibited the aggregation of human tau in vitro and filament formation, critical processes in AD [13].

George et al. [14] have established that cinnamon extracts play a protective role by preventing oxidation to tau-protein cysteine residues that can lead to dysfunction characteristic of AD. In addition, the extract of Indonesian cassia (Cinnamomum burmannil C. Nees & T. Ness) reduces the production of nitric oxide (NO) and prostaglandin E2 (PGE2), as well as inhibits interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in vitro in LPS-induced RAW264.7 cell line [15]. Metabolites with anti-AD activity provide an advantage over synthetic compounds because they can be consumed as a daily diet. On the other hand, a single compound with therapeutic activity at multiple biological targets might be promising since plant products usually contain phytochemicals that show a synergistic effect.

Therefore, there is heightened awareness of the therapeutic potential of cinnamon in the treatment of AD, an area of unmet clinical need [16, 17]. Recently, more than 160 components were identified within C. cassia [3]. However, all candidate molecular targets of cinnamon, and potential therapeutic mechanisms remain poorly understood. This study explores cinnamon's pharmacological mechanisms of action on AD using the strong combination of network analysis, molecular docking, and molecular dynamics simulation. This approach provides a new, lean strategy to focus the development of cinnamon-based therapeutics.

It will identify key targets and further inform scientists of potential experimental avenues for the therapeutic development of cinnamon extracts or metabolites. The current study confirms the potential therapeutic effects of cinnamon species, in particular C. cassia. The possible mechanisms of action of the cinnamon metabolome and its metabolites were acquired by employing network and molecular computational analyses, which were supported by scRNA-Seq data and experimental reports from the literature. Results here offer perspective options in treating AD, with cinnamon metabolites to promote anti-Aß aggregation and clearance and modulate the calcium signaling pathway. Furthermore, it provides a foundation for novel drug development of cinnamon ingredients. Finally, it supports potential hybrid drug development based on key cinnamon metabolites such as coumarin and cinnamate in the therapeutic treating of AD.

Methods and Materials

Compound Screening

C. cassia was screened to identify its metabolites and their targets. The metabolites of *C. cassia* prediction were investigated using the Encyclopaedia of Traditional Chinese Medicine (ETCM, http://www.nrc.ac.cn:9090/ETCM/) [18], the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://sm.nwsuaf.edu.cn/lsp/tcmsp.php) [19], the TCM Integrated Database (TCMID) [20], the Bioinformatics Analysis Tool for Molecular Mechanism of TCM (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm/) [21], and SymMap (http://www.symmap.org/) [22]. Absorption, distribution, metabolism, and excretion (ADME) was employed as a computational evaluation model in pharmacokinetic research to filter drug-like compounds, for which drug-likeness (DL) > 0.1 and oral bioavailability (OB) > 20% were applied [23].

Target Prediction and Collection

Compounds function by interacting with targets. Canonical SMILES were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Cinnamon targets were obtained either via searching the database: TCMSP, ETCM, BATMAN-TCM, and SymMap, or predicting through a search against TargetNet (http://targetnet.scbdd.com) [24], Swiss Target Prediction (http://www.swisstargetprediction.

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DOI:10.26502/fjppr.074

ch/) [25] and SEA (Similarity ensemble approach, https://sea.bkslab.org/) [26] with the target species setting as *Homo sapiens*, and duplications were removed [18].

Target Screening of Cinnamon (Rougui)

Of the 100 metabolites collected in TCMSP by searching the keywords "Rougui" only 11 were filtered with the criteria of an OB≥20% and a DL≥0.1. Other metabolites were obtained by searching the ETCM, BATMAN-TCM, TCMID, and SymMap databases. And 182 metabolites were established as the most effective components of cinnamon throughout the relevant literature and were included regardless of whether they met the OB and DL criteria. As a result, a total of 265 metabolites were acquired. In addition, canonical SMILES were collected from PubChem for 172 metabolites.

Accordingly, 85 metabolites were filtered based on Lipinski's rules [27]. They met the criteria of drug- likeness (molecular weight, logP, tPSA, RotableB, HBD, HBA, Lipinski violation, oral bioavailability, solubility) for FAFDrugs4 and Target NET, from which the Open Babel logP computation program, drug-like soft in house and published Physchem filters were selected for FAF-Drugs4. Compounds which passed the filtering process were further analyzed. Targets of each active ingredient were identified by target fishing and by integrating the data acquired from the above database and predicted using SEA, Swiss Target Prediction, and TargetNET. The flowchart is described in Fig. 1.

Functional Classification of Cinnamon-AD common Targets

We collected AD-associated genes from the GeneCards database (https://www.genecards.org/) and other references (Table S2). Additionally, genes related to AD were collected and screened from SC2 diseases using single-cell RNA-sequencing (scRNA-seq) technologies [28], differentially expressed genes (DEGs) selection criteria is |logFC| > 0.58 and was summarized using Microsoft Excel software (version 2019, USA). Cinnamon targets interacting with AD-associated targets were considered cinnamon-AD common targets. The functional annotation, classification,

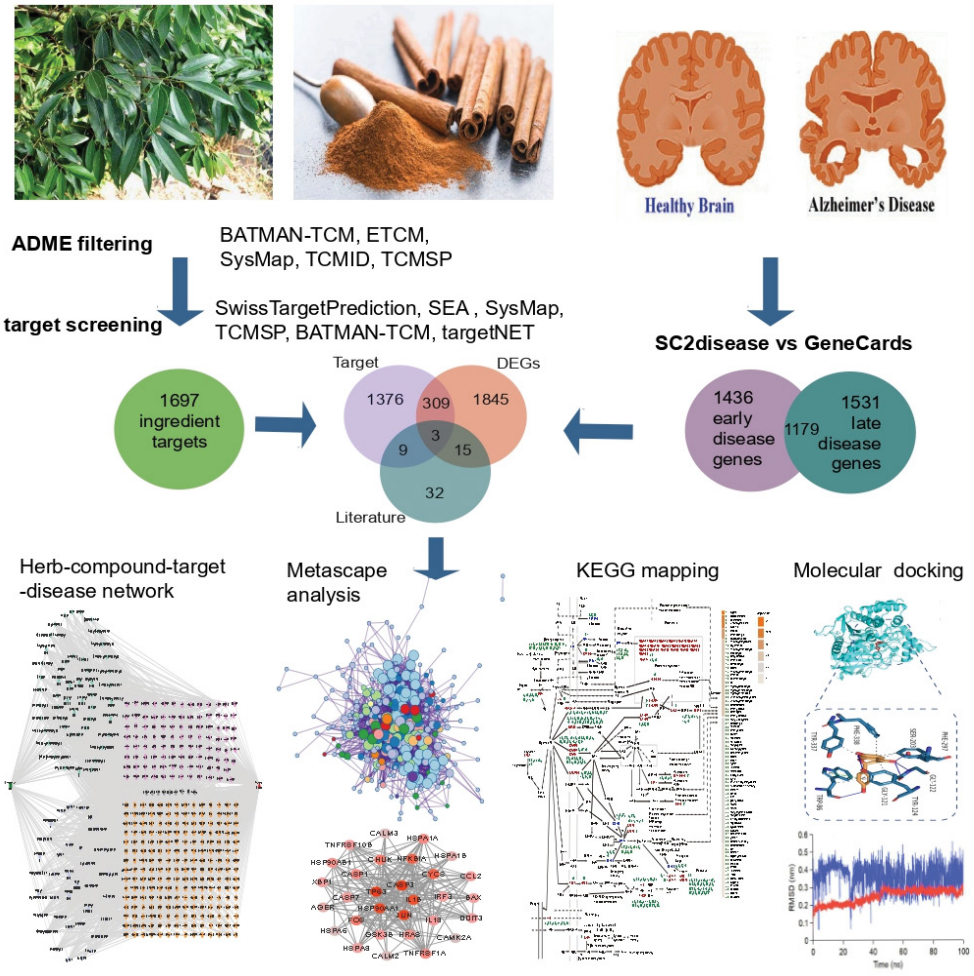

Figure 1: Framework of cinnamon compound (CMP) target screening strategy. Alzheimer brain is modified based on reference [79]

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DOI:10.26502/fjppr.074

and enrichment analysis of common targets were performed using Metascape analysis [29], and the organism was limited to *Homo sapiens*.

Construction of PPI Network and Herb-Metabolite-Target-Disease network

The cinnamon-AD common targets genes were subjected to the STRING platform (version 11.5) with species limited to *Homo sapiens* and the highest confidence score > 0.9. Besides STRING-filtered target genes, cinnamon compounds (herb metabolites) corresponding to AD genes were integrated and subjected to Cytoscape to construct the herb (cinnamon) - metabolite (cinnamon compound) – target (common disease genes)- Alzheimer disease network.

Molecular Docking

The molecular 2D structures for the molecular ligands and active ingredients were downloaded from PubChem to validate the binding of hub genes to their corresponding cinnamon ingredients. The crystal structures of key target proteins were downloaded from RCSB PDB (https://www.wwpdb.org/), respectively. Docking was performed by Autodock Vina 1.1.2, and the molecules with the lowest binding energy in the docking conformation were chosen to observe the binding effect by matching with the original ligands and intermolecular interactions (such as hydrophobic interaction, cation-π, hydrogen bond, anion-π, π-π stacking, salt bridge, metal complexation) [23]. The molecular docking patterns were finally visualized via PyMOL 3.0.

Molecular dynamics simulation

The molecular docking results were further validated by a molecular dynamics simulation strategy using Gromacs 2020.1 [30]. The ligand-receptor complex with the highest binding affinities was taken as an example. The system performed energy minimized calculations by using the steepest descent algorithm with a maximum force of 1000 kJ/mol/nm. Then, it was equilibrated in a constrained NVT (number of particles, volume, temperature) and NPT (number of particles, pressure, temperature) running for 100 ps. The Verlet cut-off scheme and a Leap-frog integrator with a step size of 2 fs were applied. The final analysis of the root-mean-square-deviation (RMSD) and the interaction energy was calculated by Gromacs 2020.1.

Results

Ingredient-target prediction and screening

As a result, 100 ingredients were found in the TCMSP by searching the keywords “Rou Gui,” and only 11 were collected with OB ≥ 20% and DL ≥ 0.1. Other compounds were obtained by searching BATMAN-TCM (score > 10, P < 0.05), ETCM, TCMID, and SymMap (OB > 30), as well as literature searching reported by Zhang et al. [3] and Momtaz et al. [31]. Canonical SMILES for 172 of the 265 compounds were identified from PubChem, which were then subjected to FAFDrugs4 and TargetNET for screening, respectively. The filtered 85 compounds were applied to search potential targets against SwissTargetPrediction (probability > 0.8), SEA (P < 0.01), and the TargetNET database. Together, 1697 targets related to 85 cinnamon metabolites were collected (Table 1), for which 50 were experimentally verified and 35 were identified from literature searching.

Differentially expressed disease gene analysis

By searching keywords “Alzheimer’s Disease” in GeneCards and mapping in STRING limited to *Homo sapiens*, 9327 AD-related genes were acquired. While 2615 DEGs were collected from SC2diseases for AD vs. normal (E2N), 1531 unique DEGs for early-onset vs. late-onset phase (E2L only, and the other 1179 DEGs overlapped with E2N), 59 literature searched AD genes (Fig. 2a and Table S1-S2).

Herb-compound-target-disease network construction

The interaction between cinnamon ingredient targets (1697) and AD-associated disease genes (4146) resulted in 321 cinnamon-AD common target sets: 210 genes for E2N, 202 genes for E2L only, and 12 AD-related genes from literature searching (Fig. 2b and detailed genes in Table S3). These genes maybe critical targets for AD treatment using cinnamon. This common target gene set was applied to Cytoscape v3.6.0 to construct an herb-compound-target-disease network (Fig.3). The cinnamon-AD common genes were imported into STRING (version 11.5), generating a total of 321 nodes and 582 edges with an average node degree 3.63. According to Fig. 3a, most of the individual compounds (82/85) were determined to interact with multiple AD-associated targets (≥ 3 targets) and 76 compounds were found to interact with more than 10 or equal to 10 AD targets. Especially, eugenol has the largest number of targets (102 targets), followed by cinnamic acid, ethylcinnamate and protocatechuic acid (55 targets), cinnamaldehyde and coumarin (54 targets), the other 9 compounds have ≥ 40 targets, indicating that these ingredients are highly likely to become key phytochemicals in AD treatment (Fig. 3b). Then, disconnected nodes were removed. A confidence score higher than 0.9 was applied, resulting in a PPI network in Fig. 3c. Screening of key ingredients and targets results in 33 genes were designed as hub targets with degree > 10, for which degree indicates the number of connections linked to other genes and ClosenessCentrality > 0.45 (Table 2). The expression of 30/33 genes was significant except ACHE, APP, and MAPT according to scRNA-seq data (Fig. 3c, Table S1). Among these, ACHE, ALOX15, DUSP3, PTGS2, RORA, CYP19A1, ESR2, AKR1BA, APP, and ESR1 were

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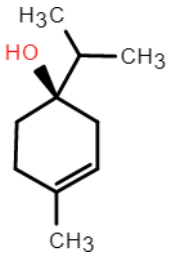
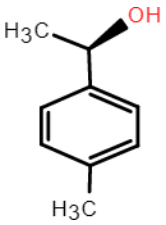
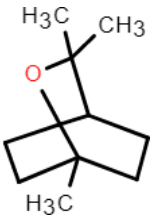
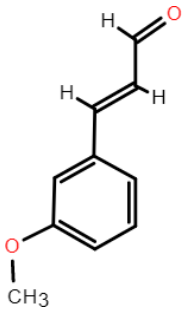
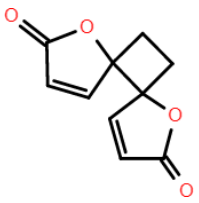
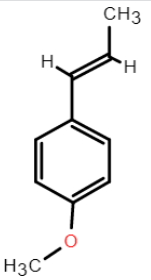
Table 1: Core compounds of cinnamon

No.	name	PubMed CID	Structure
1	()-terpinen-4-ol	5325830	
2	(1R)-1-(4-methylphenyl)ethanol	6999807	
3	1,8-cineole	2758	
4	3-methoxycinnamaldehyd e	5373819	
5	anemonin	10496	
6	anethole	637563	

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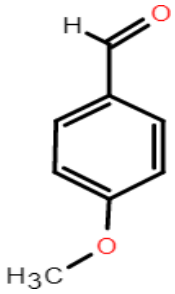
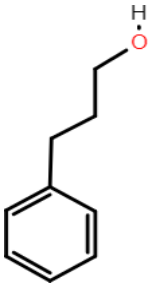
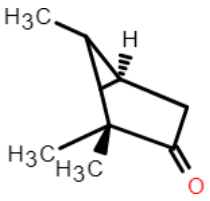
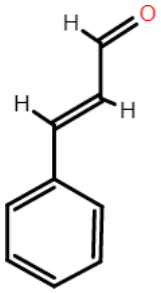
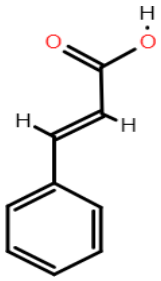
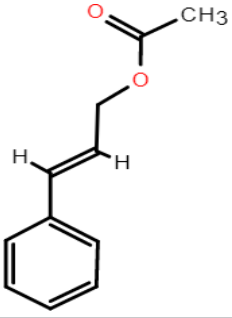
7	anisaldehyde	31244	
8	benzenepropanol	31234	
9	cam	159055	
10	cinnamaldehyde	637511	
11	cinnamic acid	444539	
12	cinnamyl acetate	5282110	

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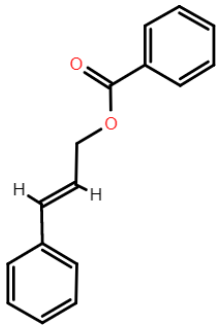
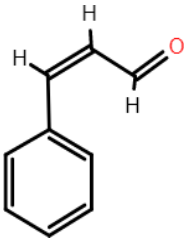
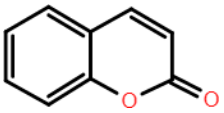
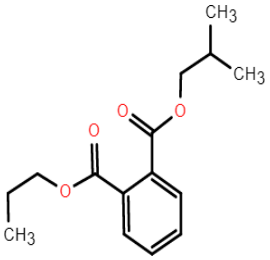
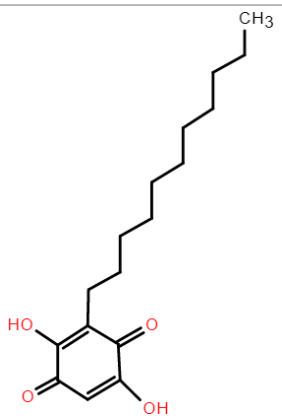
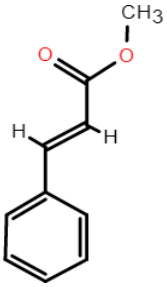
13	cinnamyl benzoate	5705112	
14	cis-cinnamaldehyde	6428995	
15	coumarin	323	
16	DIBP	6782	
17	embelin	3218	
18	ethylcinnamate	637758	

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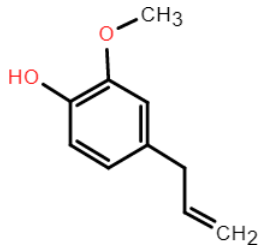
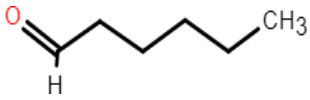
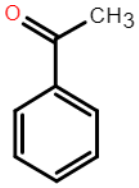
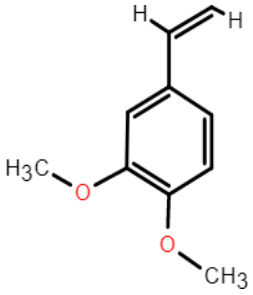
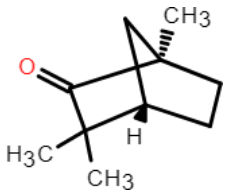
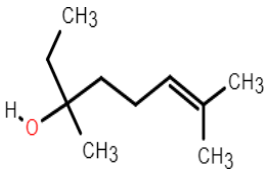
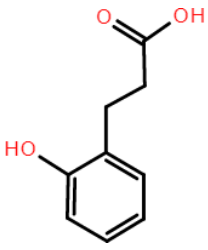
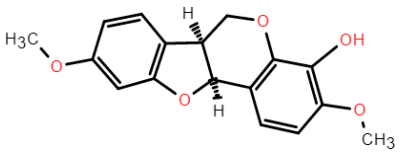
19	eugenol	3314	
20	hexanal	6184	
21	hypnon	7410	
22	isohomogenol	637776	
23	l-alpha-fenchone	82229	
24	linalool	6549	
25	melilotic acid	873	
26	melilotocarpan A	5319349	

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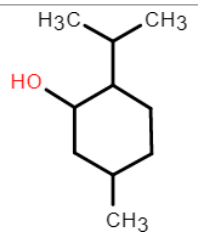
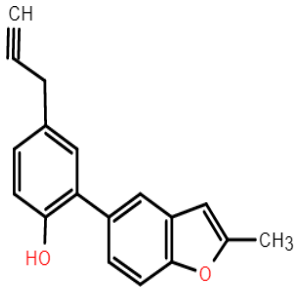
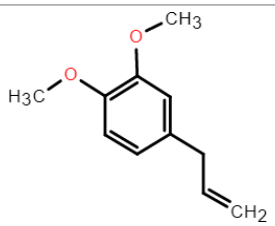
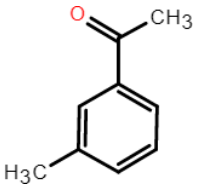
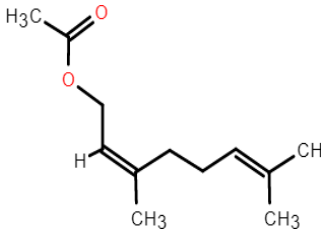
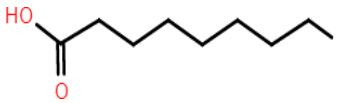
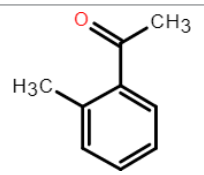
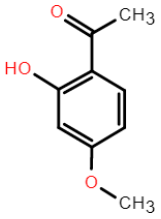
27	menthol	1254	
28	methylbenzofuran	71546085	
29	methyleugenol	7127	
30	M-methylacetophenone	11455	
31	neryl acetate	1549025	
32	nonanoic acid	8158	
33	O-acetyltoluene	11340	
34	paeonol	11092	

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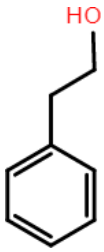
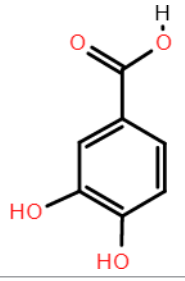
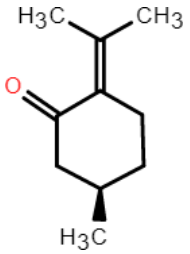
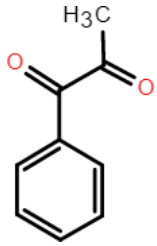
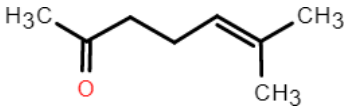
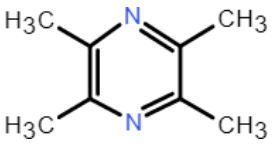
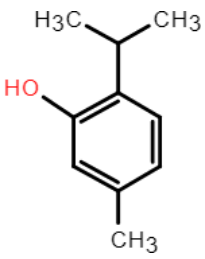
35	pel	6054	
36	protocatechuic acid	72	
37	pulegone	442495	
38	pyruvophenone	11363	
39	sulcatone	9862	
40	tetramethyl11363pyrazi ne	14296	
41	thymol	6989	

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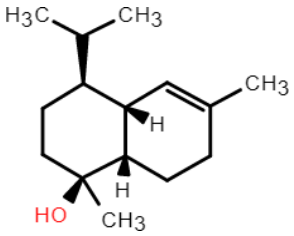
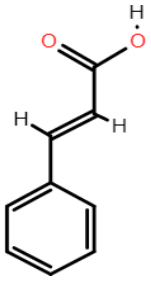
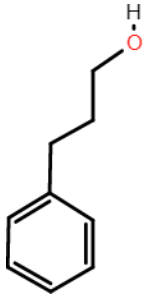
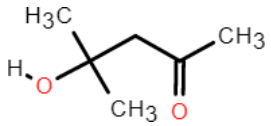
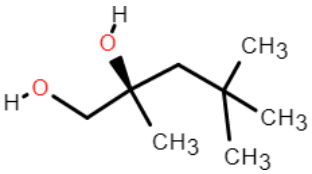
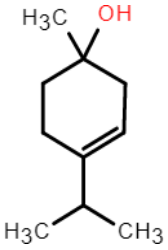
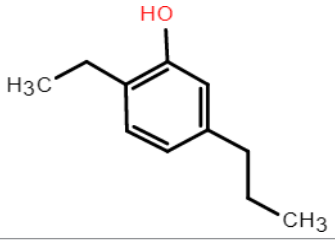
42	T-muurolol	3084331	
43	trans-cinnamic acid	444539	
44	trans-cinnamic alcohol	308	
45	tyranton	31256	
46	1,3-pentanediol,2,2,4- trimethyl	92158843	
47	1-terpineol	11468	
48	2-ethyl-5-propylphenol	522454	

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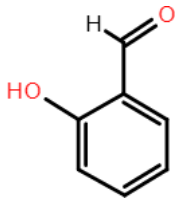
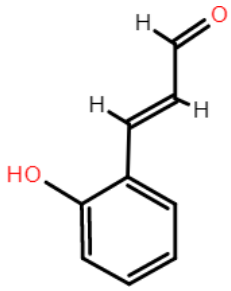
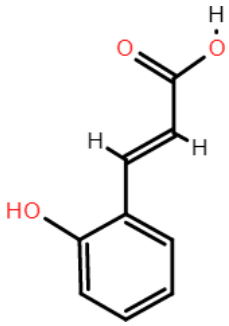
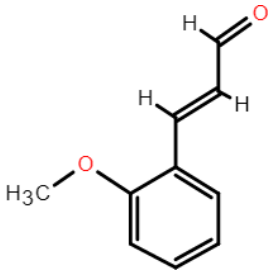
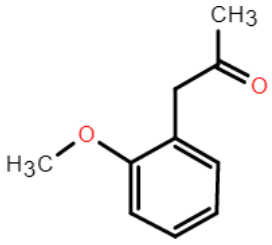
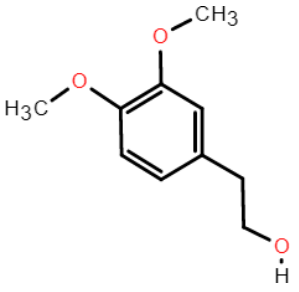
49	2-hydroxybenzaldehyde	6998	
50	2-hydroxycinnamaldehyde	5318169	
51	2-hydroxycinnamic acid	637540	
52	2-methoxycinnamaldehyd e	641298	
53	2-methoxyphenylacetone	78887	
54	3,4-dimethoxyphenethyl alcohol	81911	

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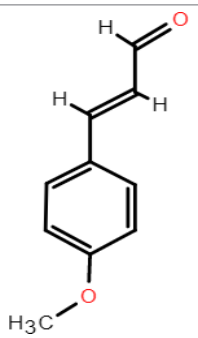
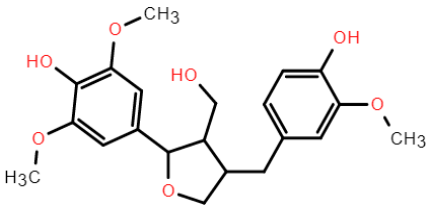
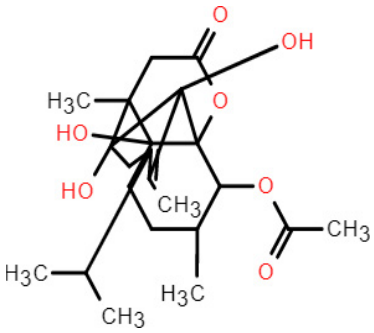
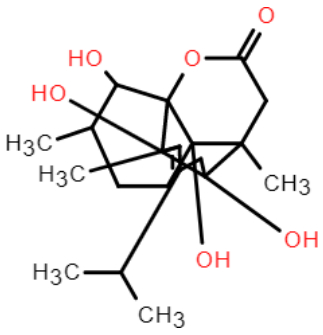
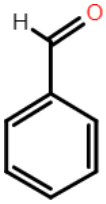
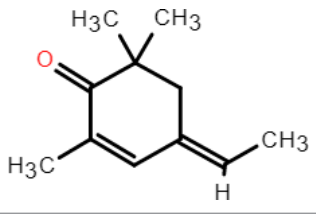
55	4-methoxycinnamaldehyd e	641294	
56	5′-methoxylariciresinol	10340333	
57	anhydrocinnzeylanine	131752069	
58	anhydrocinnzeylanol	73099741	
59	benzaldehyde	240	
60	benzene,1,3-dimethyl	643928	

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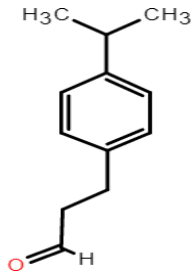
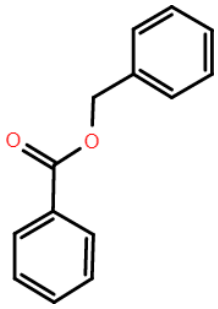
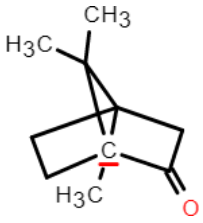
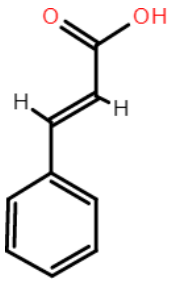
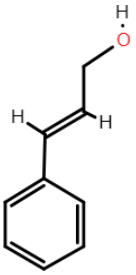
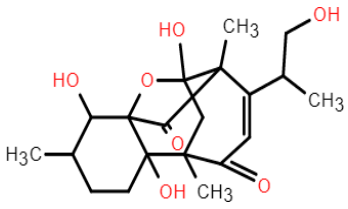
61	benzenepropanal	62654	
62	benzyl benzoate	2345	
63	camphor	2537	
64	cinnamate	5957728	
65	cinnamylalcohol	5315892	
66	cinncassiol C	75144796	

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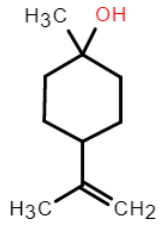
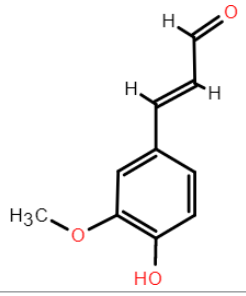
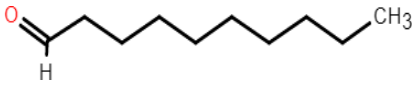
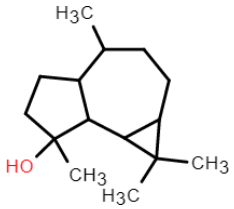
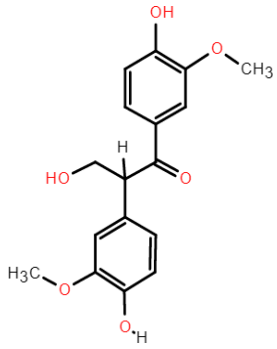
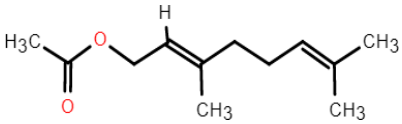
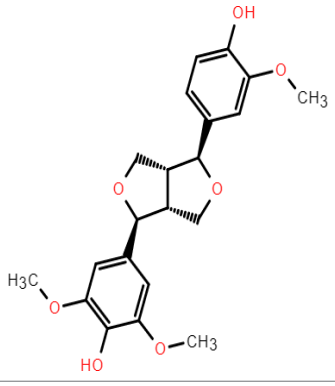
67	cis-β-terpineol	8748	
68	coniferaldehyde	5280536	
69	decanal	8175	
70	espatulenol	6432640	
71	evofolin B	5317306	
72	geranyl acetate	1549026	
73	medioresinol	181681	

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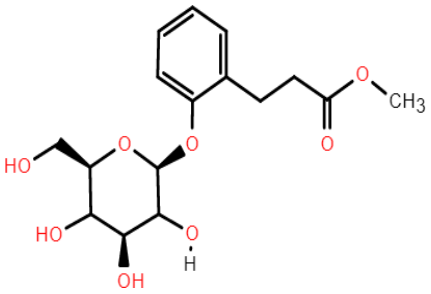
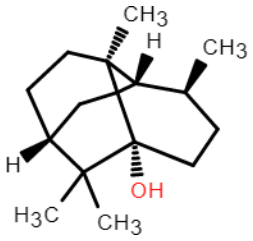
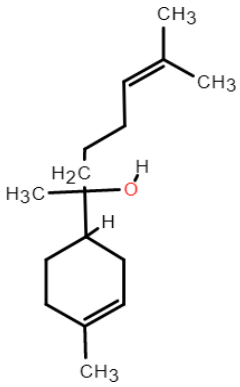
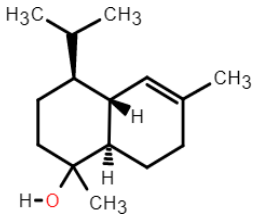
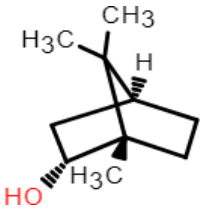
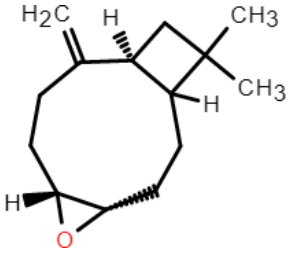
74	methyl dihydromelilotoside	46881368	
75	patchouli alcohol	10955174	
76	α-bisabolol	10586	
77	α-cadinol	10398656	
78	borneol	1201518	
79	caryophyllene oxide	1742210	

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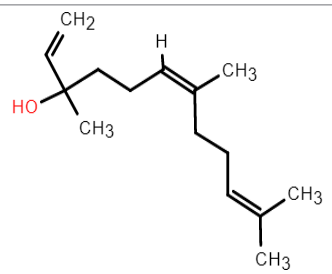
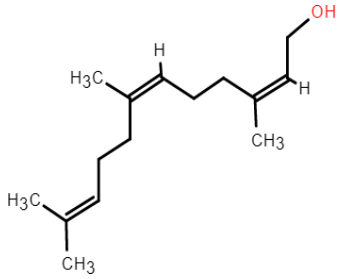
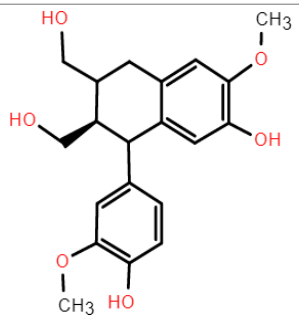
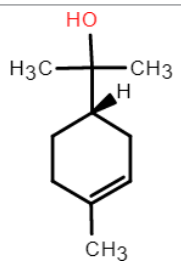
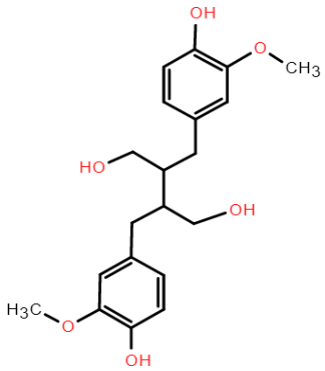
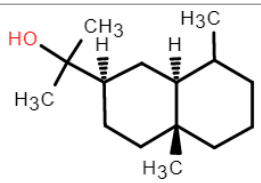
80	d-nerolidol	5356544	
81	farnesol	1549107	
82	isolariciresinol	160521	
83	l-alpha-terpineol	443162	
84	secoisolariciresinol	65373	
85	β-eudesmol	91457	

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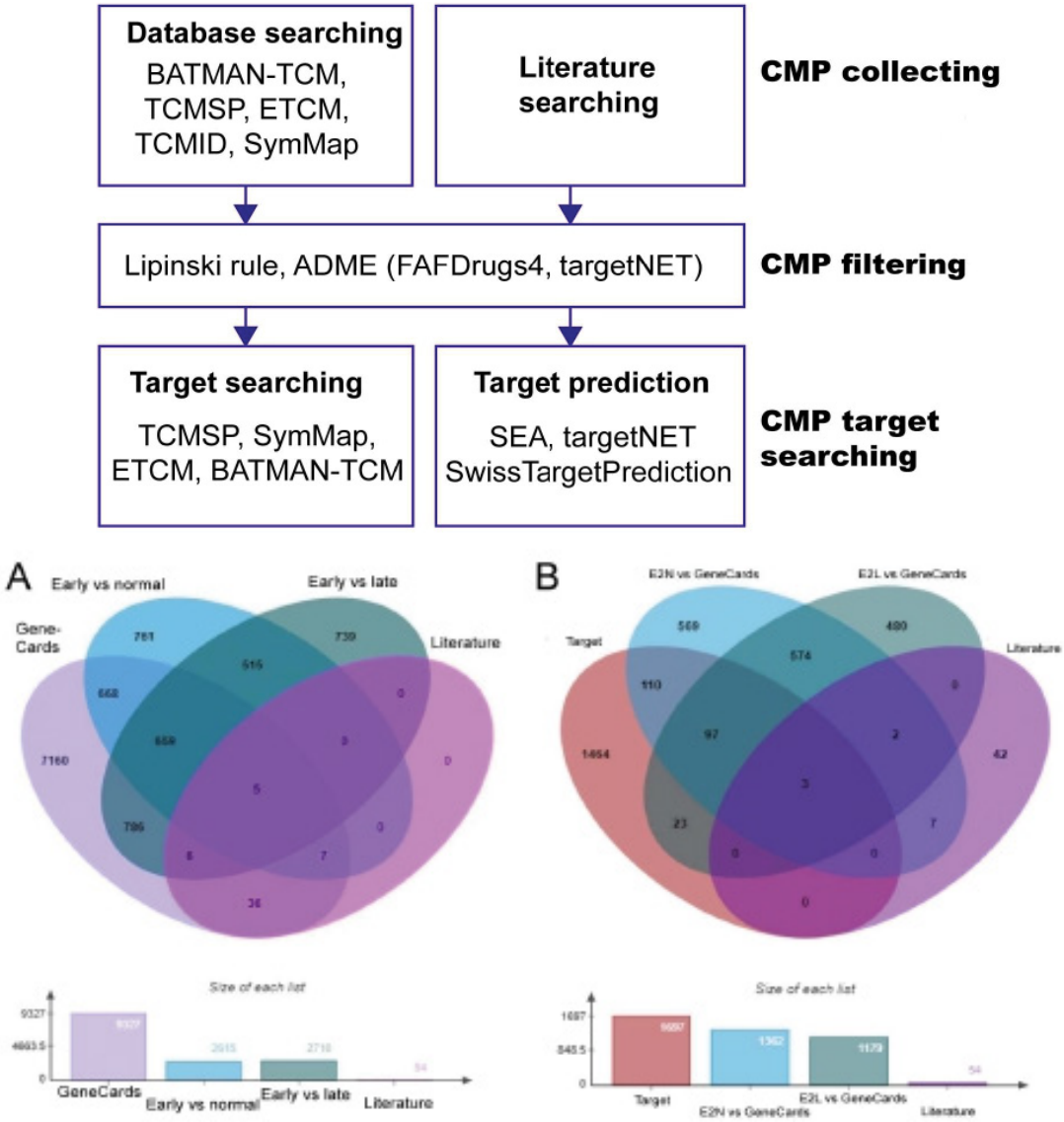

Figure 2: Venn diagram shows the disease gene for Alzheimer's Disease (AD) from database and literature searching. E2N: early-onset vs. normal, E2L only: early-onset vs. late-onset based on SC2diseases database.

the top 10 hub targets for E2N comparison, suggesting that these were the main targets for cinnamon treat against early-onset AD (Fig. 3d). ACHE showed the highest degree (72), followed by ALOX15 (degree 64), DUSP3 (degree 62), PTGS2 (degree 61), RORA (degree 52), CYP19A1 (degree 50), ESR2 (degree 44), AKR1BA (degree 43), APP (degree 41) and ESR1 (degree 40). We further analyzed the number of hub targets and corresponding core targets of cinnamon ingredients. Cinnamic acid, eugenol, coumarin, pel, paeonol, protocatechuic acid, etc. have the largest number of 10 targets, followed by others.

Enrichment analysis of cinnamon-AD common genes

According to the results of Metascape analysis, cinnamon-AD common targets were remarkably enriched in 11 MCODE clusters, which were distributed in multiple biological processes and pathways (Fig. 4a, 4d and table S4). For early-onset *vs.* normal conditions, the top 20 enriched terms ranked by p-value were (Fig. 4b): Alzheimer's disease pathway of KEGG (hsa05010), response to xenobiotic stimulus (GO0009410), response to hormone (GO0009725), regulation of ion transport (GO0043269), nuclear receptors meta-pathway (WP2882), regulation of inflammatory response (GO0050727), response to oxidative stress (GO0006979), trans-synaptic signaling (GO0099537), Alzheimer's Disease (WP5124), and so forth. The Alzheimer's disease pathway is the most significantly enriched (hsa05010, 2.81E-34, 40 targets).

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In contrast, pathway/GO functional categories for early-onset Alzheimer *vs.* late-onset Alzheimer (Fig. 4c) were enriched in the cellular response to organic cyclic compound (GO0071407), regulation of neuron death (GO1901214), nuclear receptors meta-pathway (WP2882), response to xenobiotic stimulus (GO0009410), behavior (GO0007610), regulation of secretion by cell (1903530), lipid and atherosclerosis (hsa05417), and so forth. Nuclear receptors meta-pathway (WP2882), lipid and atherosclerosis (hsa05417), response to xenobiotic stimulus (GO0009410), and cellular response to organic cyclic compound (GO0071407) were enriched in both comparisons.

A PPI network of 42 cinnamon targets was involved in the nuclear receptors meta-pathway (WP2882) and contained 42 nodes and159 edges, in which JUN, EGFR, NQO1, and ESR1 play an important role (Fig. 4e). Lipid and atherosclerosis pathway (hsa05417), which has been linked to AD, is also significantly enriched. The cinnamon targets involved in lipid and atherosclerosis (hsa05417) form a PPI network with 33 nodes and 212 edges. At the same time, TP53, CASP3, JUN, HSP90AA1 and IL1B were core targets (Fig. 4f).

Cellular response to organic cyclic compound (GO0071407) was significantly enriched. As a result, a PPI network containing 44 nodes and 32 edges was formed, for which HSPA8, HSPA90A1 and EGFR were core targets (Fig. 4g). In addition, response to xenobiotic stimulus (GO009410) formed a PPI network with 46 nodes and 192 edges, with TP53, FOS, CASP3, JUN and PTGS2 play a key rule (Fig. 4h). To identify key cinnamon ingredients against AD, target genes involved in the Alzheimer's disease pathway (hsa05010, 7.57E-43, 54 targets, combine early-onset *vs.* normal and early-onset *vs.* late-onset Alzheimer) were visualized using KEGG mapper and corresponding ingredients was mapped in Fig. 5. The red font targets indicate the cinnamon targets. The green numbers 1 to 78 represent metabolites of cinnamon. Senile plaque deposition is a result of imbalanced Aβ production and degradation. Eugenol, benzyl benzoate, pel, benzenepropanol, melilotic acid (3-(2-hydroxyphenyl) propanoic acid), nonanoic acid and diisobutyl phthalate (DIBP) could act on membrane metalloendopeptidase and participate in the degradation of Aβ. The generation of Aβ depends on the cleavage of APP, catalyzed by α-secretase (ADAM17) and β-secretase (BACE1). According to Fig. 5, eugenol, ethylcinnamate, coniferaldehyde, cinnamyl acetate and cinnamyl benzoate are key compounds that modulate the production of Aβ.

Moreover, CSK3B, CASP3 and CASP7 mainly regulate tau protein phosphorylation, another important AD pathological process. Correspondingly, eugenol, isolariciresinol (8-(4-hydroxy- 3-methoxyphenyl)-6,7-bis(hydroxymethyl)-3-methoxy-5,6,7,8-tetrahydronaphthalen-2-ol), cinnamaldehyde, 2-methoxyphenylacetone and other compounds are determined to be key cinnamon ingredients that regulate tau protein phosphorylation. Eugenol, isolariciresinol, cinnamaldehyde and l- alpha-terpineol can interact with GSK3B; eugenol, cinnamaldehyde, protocatechuic acid and the other 5 compounds can interact with CASP3, and 2-methoxyphenylacetone can interact with CASP7.

Furthermore, multiple cinnamon metabolites interact with GRIN1, GRIN2A, GRIN2B, or CACAN1S, which affects the downstream calcium signaling pathway and cell death. In detail, eugenol, secroisolariciresinol, cinnamaldehyde, protocatechuic acid, and the other 17 compounds interact with GRIN2B, cinnamaldehyde, protocatechuic acid, cinnamate, and the other 10 compounds interact with GRIN1, while eugenol, cinnamyl acetate, cinnamyl benzoate and the other 3 compounds interact with CACAN1S. In addition, oligomeric forms of Aβ known to be an important inflammatory stimulus of neuronal cells. For example, benzyl-benzoate can interact with AGER, methyl dihydromelilotoside (2-(beta-D- glucopyranosyloxy)benzenepropionic acid methyl ester) can interact with HRAS, eugenol, anethole, cinnamaldehyde, and the other 5 compounds interact with IL1, eugenol, secroisolariciresinol, isolariciresinol, anethole, cinnamaldehyde, protocatechuic acid and the other 53 compounds interact with PTGS2 to improve the inflammation induced by oligomeric Aβ.

Eugenol can interact with 31 AD pathway targets, secroisolariciresinol can interact with 26 targets, isolariciresinol can interact with 25 targets, and 63 of 78 compounds have more than 2 targets suggesting that cinnamon compounds can act on different targets of the AD pathway; thus cinnamon may play a key role in the treatment of AD.

Molecular validation of compound-target interactions

Molecular docking analysis was carried out to validate the binding of core targets and the corresponding cinnamon metabolites. The 85 active ingredients (Table 1) include cinnamyl benzoate (PubChem CID 5705112), coumarin (CAS no. 91-64-5), DIBP (CAS no. 84-69-5), the para-benzoquinone embelin (CAS no. 550-24-3), melilotocarpan A (PubChem CID 442792), etc. The protein structures of key targets were obtained from RCSB PDB, including ACHE, DUSP3, PTGS2, CYP19A1, APP, ESR1, MAPT, CASP3, HSP90AA1, FOS, TP53 and GAPDH based on the STRING interaction analysis and importance reference. Results here indicated that all the core targets were well combined with the corresponding cinnamon metabolites (Table 3, Table S5).

Furthermore, the binding affinities of all docking patterns were higher than 6 kcal/mol, suggesting stable binding in between. Docking affinities and binding residues for the

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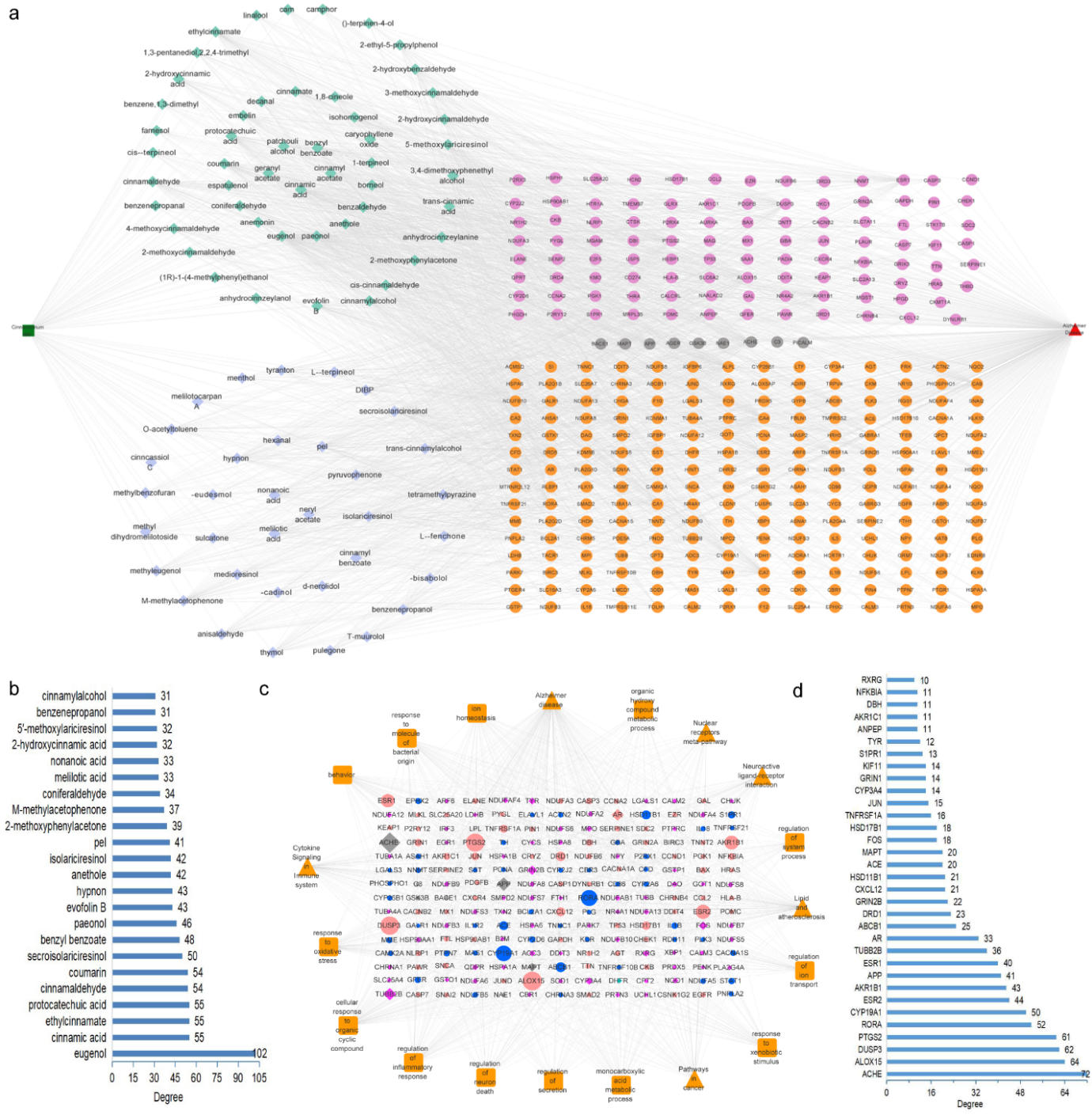

Figure 3: The Herb-Compound-Targets-Disease network of cinnamon (a). The green square represents the herb cinnamon; the diamond represents the active ingredients (colored in light green means determined from cinnamon, in blue purple is searched from literature); the circles are common targets as the result of the intersection of the herb targets and disease targets (colored in cherry red are for E2N, in orange are for E2L only, and in grey are from literature searching); the red triangle is a disease. (b) Key compounds in the PPI network and correlated degree. The Y-axis is the cinnamon compounds of the common targets, the x-axis is the degree of the compounds. (c) Protein-Protein interaction network (PPI) of common targets. The size of the nodes represents the target degree. The diamond means in both comparisons (E2N and E2L), and circles represent in either comparison. Nodes colored in red/blue represent up/down-regulation, grey means the regulation is not significant in SC2disease data, cherry red is downregulated in E2N but upregulated in E2L, and turquoise blue is upregulated in E2N but downregulated in E2L. (d) Core genes in the PPI network and the X-axis indicates the degree.

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Table 2: Top 33 hub genes with high targets connectivity

Name	Annotation	Degree	ClosenessCentrality
ACHE	Acetylcholinesterase (cartwright blood group)	72	0.553
ALOX15	Arachidonate 15-lipoxygenase	64	0.538
DUSP3	Dual specificity protein phosphatase 3	62	0.534
PTGS2	Prostaglandin G/H synthase 2	61	0.532
RORA	Rar-related orphan receptor alpha	52	0.515
CYP19A1	Aromatase	50	0.512
ESR2	Estrogen receptor beta	44	0.502
AKR1B1	Aldose reductase	43	0.5
APP	Amyloid-beta A4 protein	41	0.497
ESR1	Estrogen receptor	40	0.495
TUBB2B	Tubulin beta-2B chain	36	0.489
AR	Androgen receptor	33	0.484
ABCB1	Multidrug resistance protein 1	25	0.472
DRD1	D(1A) dopamine receptor	23	0.469
GRIN2B	Glutamate receptor ionotropic, NMDA 2B	22	0.467
CXCL12	Stromal cell-derived factor 1	21	0.466
HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1	21	0.466
ACE	Angiotensin-converting enzyme	20	0.465
MAPT	Microtubule-associated protein tau	20	0.465
FOS	Proto-oncogene c-Fos	18	0.462
HSD17B1	Estradiol 17-beta-dehydrogenase 1	18	0.462
TNFRSF1A	Tumor necrosis factor receptor superfamily member 1A	16	0.459
JUN	Transcription factor AP-1	15	0.457
CYP3A4	Cytochrome p450 family 3 subfamily a polypeptide 4	14	0.456
GRIN1	Glutamate receptor ionotropic, NMDA 1	14	0.456
KIF11	Kinesin-like protein KIF11	14	0.456
S1PR1	Sphingosine 1-phosphate receptor 1	13	0.455
TYR	Tyrosinase	12	0.453
ANPEP	Alanyl aminopeptidase, membrane	11	0.452
AKR1C1	20alpha/3alpha-hydroxysteroid dehydrogenase / dihydrodiol dehydrogenase	11	0.452
DBH	Dopamine beta-monooxygenase	11	0.452
NFKBIA	NF-kappa-B inhibitor alpha	11	0.452
RXRG	Retinoic acid receptor RXR-gamma	10	0.451

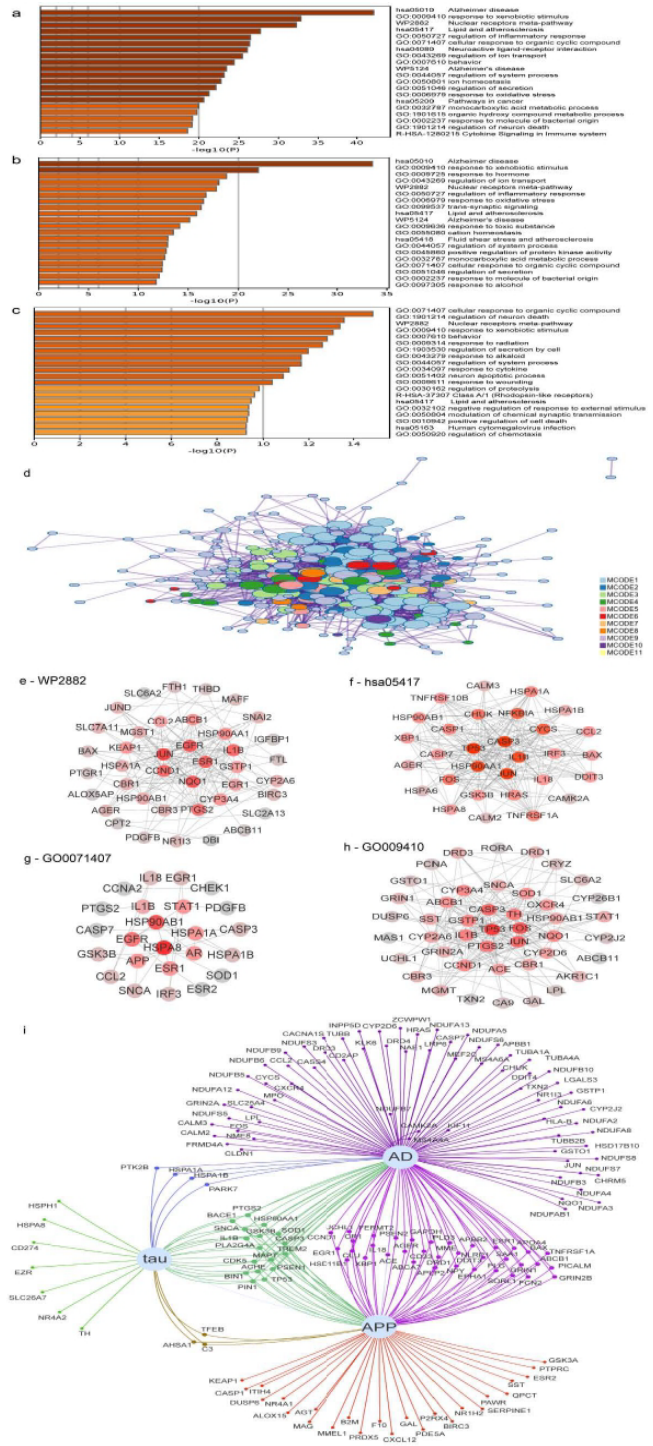

Figure 4: Metascape analyses of the Alzheimer's disease-associated genes target by cinnamon. (a-c) Bar graph of enriched terms colored by p-values to visualize the top 20 clusters for all common genes, early *vs.* normal conditions and early Alzheimer *vs.* late Alzheimer conditions, respectively. (d) Network of enriched terms: colored by cluster-ID and ranked by p-values for the top 20 clusters; (e-h) PPI network construction for nuclear receptors meta-pathway (WP2882), lipid and atherosclerosis (hsa05417), targets involved in response to xenobiotic stimulus (GO009410), and regulation of inflammatory.

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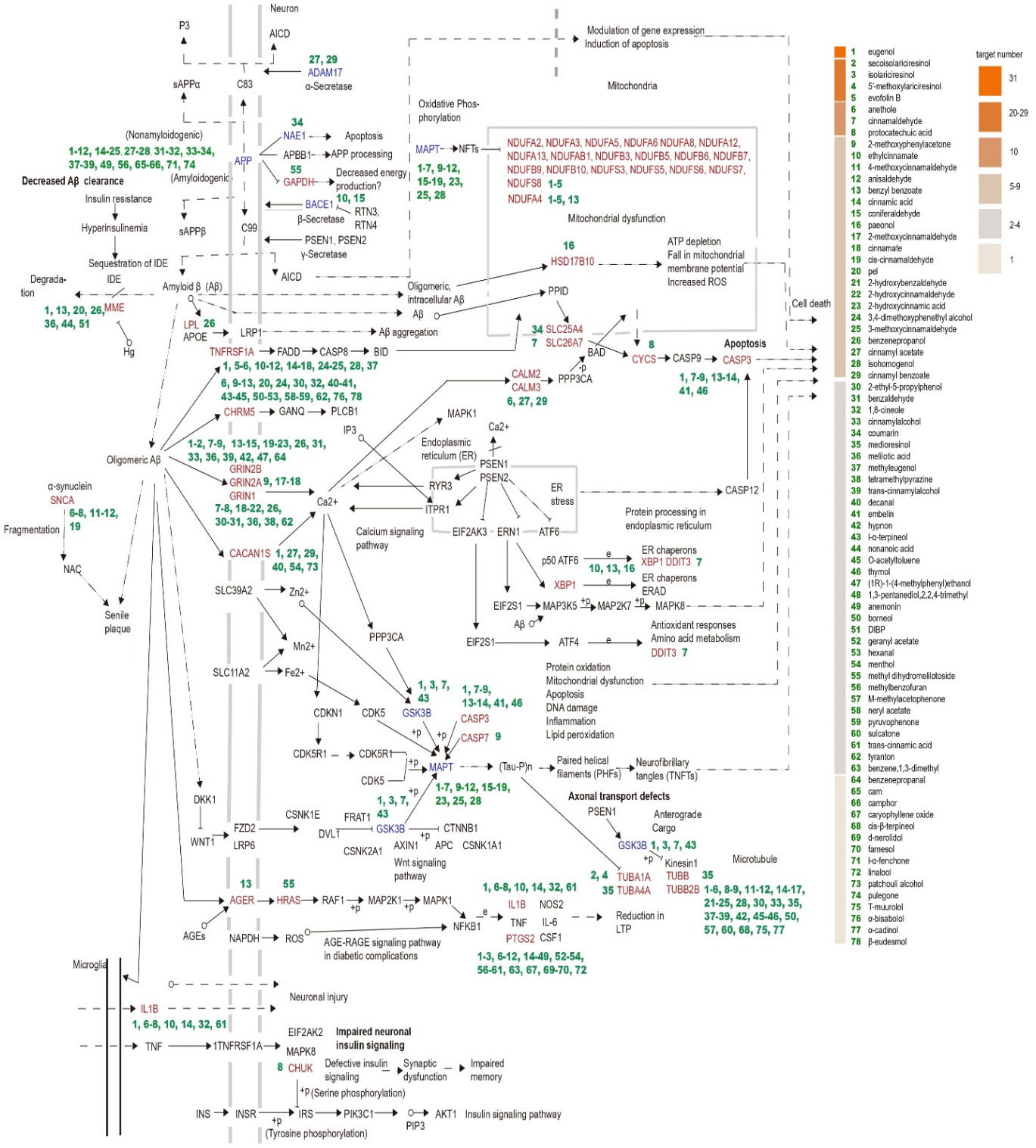

Figure 5: The cinnamon targets associated with the Alzheimer's disease pathway (hsa05010) were constructed using KEGG mapping. A). Red/blue targets represent the cinnamon targets involved in the AD pathway. Regulation is significant for the red font and not the font in blue based on data from the SC2disease database. The green numbers 1 to 78 represent the main cinnamon ingredients. The gradient color on the left represents its related target number. Cinnamon compounds target the number next to the gene.

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Table 3: The lowest 10 binding affinities (kcal/mol) and binding residues of the cinnamon compound and Alzheimer's disease gene.

Compound-tARGet	affinity (kcal/mol)	Binding residues
Cinnamyl benzoate-ACHE	-9.6	TRP86, TRP286, PHE297, TYR337, PHE338, TYR341
Medioresinol-PTGS2	-9.1	ASN34, HIS39, CYS41, CYS47, PRO156
Alpha-cadinol-ACHE	-9.1	TYR72, TRP286, SER293, VAL294, PHE297, TYR337, PHE338, TYR341
Methylbenzofuran-PTGS2	-8.8	ALA202, GLN203, TYR385, TRP387, LEU391, PHE395, PHE407, ILE408, VAL444
Cinnamyl benzoate-PTGS2	-8.6	PHE200, ALA202, VAL295, TYR385, TRP387, HIS388, LEU391, PHE395, TYR404, ILE408
Anemonin-ACHE	-8.2	TRP86, GLY120, TYR124, TYR133, TYR337
Medioresinol-ACHE	-8.2	TYR124, TRP286, PHE297, TYR337, PHE338, TYR341
Medioresinol-CYP19A1	-8.1	THR310, VAL370, MET374
Patchouli alcohol-ESR1	-8.1	ALA350, LEU387, PHE404, LEU525
Secroisolariciresinol-ACHE	-8.1	TYR72, TYR124, TRP286, SER293, ARG296, PHE338, TYR341

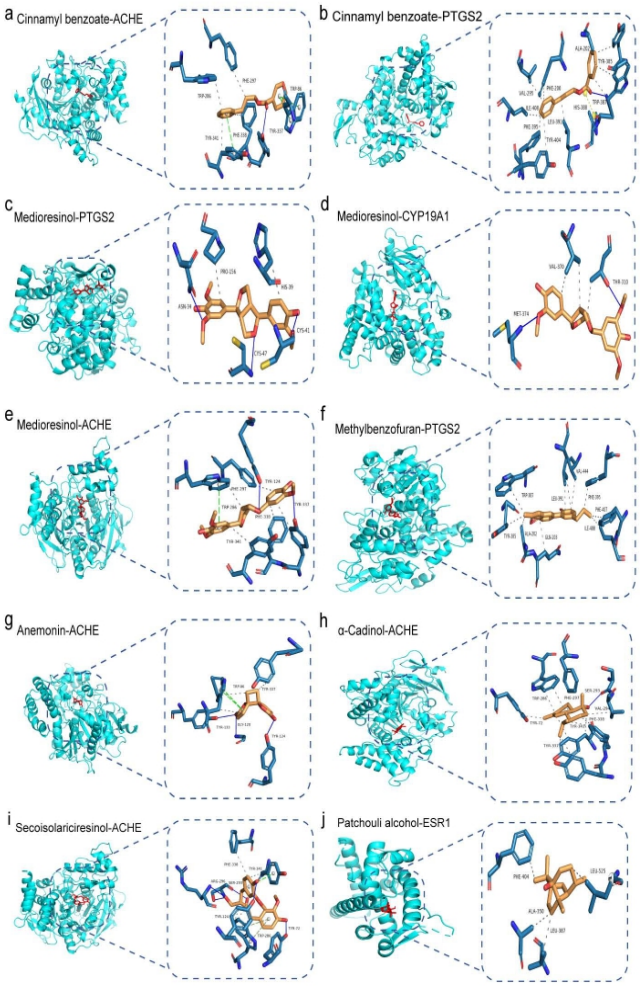

Figure 6: Docking patterns of selected targets according to the lowest binding affinities with corresponding active metabolite a-b) cinnamyl benzoate, c-e) medioresinol, f) methylbenzofuran, g) anemonin, h) α-cadinol, f) secroisolariciresinol, g) patchouli alcohol. The binding affinities (kcal/mol) and binding residues are listed in Table 3. For other docking patterns, see the supplementary Table S4.

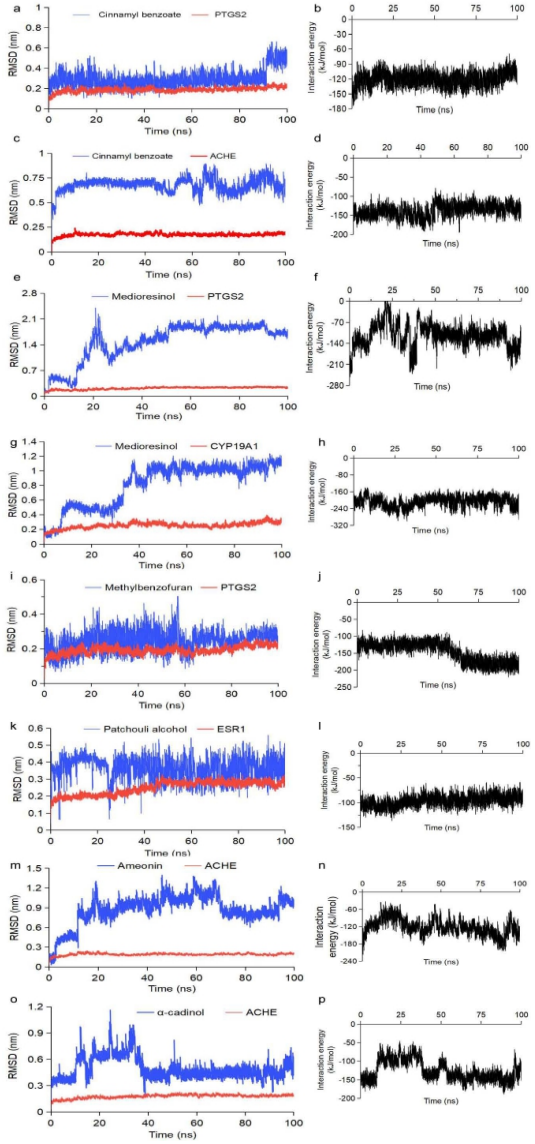

Figure 7: Profiles of molecular dynamics simulation results. (a, c, e, g, i, k, m, o) RMSD analysis for the ligand-receptor complexes. (b, d, f, h, j, l, n, p) Interaction energy for the ligand-receptor complexes.

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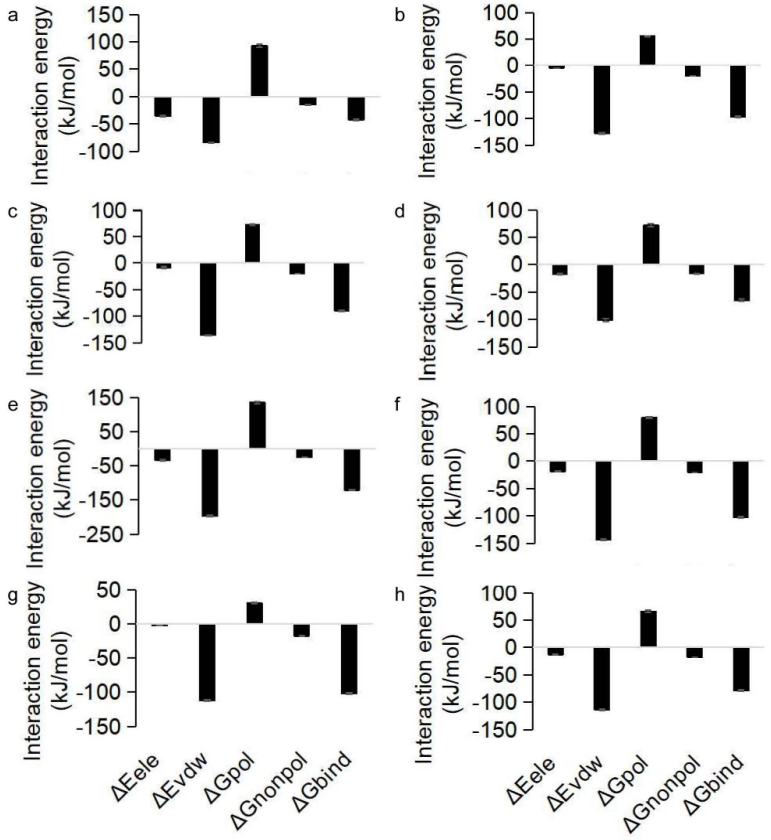

Figure 8: Binding free energy decomposition of cinnamon ingredient and target. ΔEele: Electrostatic interaction energy. ΔEvdw: van der Waals interaction energy. ΔGpolc: Polar contributions to the solvation-free energy. ΔGnonpold: Nonpolar contributions to the solvation-free energy. ΔGbinde: Binding energy.

Table 4: Binding energies for the complex of protein and molecule using MM/PBSA during 0-100 ns of MD simulation trajectory

Protein - molecule	Δeelea (kJ/mol)	Δevdwb (kJ/mol)	Δgpolc (kJ/mol)	Δgnonpold (kJ/mol)	Δgbinde (kJ/mol)
ACHE - Ameonin	-33.75	-83.23	92.54 +2.63	-15.08	-41.19
PTGS2 - Cinnamyl benzoate	-4.44	-127.15	55.42 +0.78	-19.17	-96.02
ACHE - Cinnamyl benzoate	-7.08	-134.45	72.85 +1.28	-19.49	-88.62
PTGS2 -Medioresinol	-16.58	-98.3	71.15 +3.47	-16.74	-63.7
CYP19A1 - Medioresinol	-31.03	-195.56	134.93 + 2.75	-25.66	-119.66
PTGS2 - Methylbenzofuran	-18.1	-141.81	80.00 + 1.46	-20.16	-101.31
ESR1 - Patchouli alcohol	-1.88	-110.9	29.87 + 1.19	-17.65	-100.95
ACHE - α-cadinol	-12.2	-112.33	66.17 + 1.52	-18.09	-77.68

[a]Electrostatic interaction energy.

[b]van der Waals interaction energy.

[c]Polar contributions to the solvation free energy.

[d]Nonpolar contributions to the solvation free energy. e Binding energy.

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DOI:10.26502/fjppr.074

lowest 10 compound-target pairs were listed in Fig. 6, Table 3 and Table S5. Eugenol showed the highest binding score with ACHE and PTGS2, with score values of -6.6 and -6.2 kcal/mol, respectively; cinnamaldehyde showed the highest binding score with ACHE, CYP19A1, TP53 and PTGS2, with score values of -6.5, -6.2, -6.2, and -6.1 kcal/mol, respectively; coumarin showed the highest binding score with ACHE, PTGS2, CYP19A1, ESR1 and APP, with score values of -7.3, -7.2, -6.7, -6.6 and -6.0 kcal/mol, respectively. It suggests that cinnamon metabolites are associated with changes in AD pathology, which may play a key role in treating AD. Molecular dynamic simulations were carried out to evaluate the stability of the interaction between core targets and the cinnamon ingredient predicted by the docking experiments. The RMSD values reflect the fluctuations for the protein-ligand complex. It can be seen that cinnamyl benzoate - PTGS2 (Fig. 7a), methybenzofuran - PTGS2 (Fig. 7c) and cinnamyl benzoate - ACHE (Fig. 7i) systems indicate strong stability. Patchouli alcohol - ESR1 system had a sharp drop within 30 ns and then tended to balance out the last 70 ns (Fig. 7k). The medioresinol (lignan) - PTGS2 (Fig. 7e), medioresinol - CYP19A1 (Fig. 7g) and α - cadinol - ACHE (Fig. 7o) systems had a sharp rise within 40 ns and balanced out the last 60 ns. MM/PBSA (molecular mechanics energies combined with the Poisson- Boltzmann and surface area continuum solvation) calculated the binding free energy and energy components, for which 0 ns to 100 ns RMSD phase trajectory was applied. Binding free energy analysis assessed protein and molecular binding affinity; here, all the complexes and relevant leading molecules' structures are very stable, as shown in Fig. 8 (and detailed in Table 4). It is observed that the van der Waals interaction, SASA energy, and electronic energy promote the binding of the cinnamon component to the target protein. In contrast, the polar solvation energy has the opposite effect. Taken together, all these results confirm the structure stability of ligand-receptor complexes.

Table 5: Literature validation of predicted nature products

CMP	Origin	Result	Ref
Medioresinol, cryptamygin A	*Cinnamomum cassia* Blume (Lauraceae)	Show anti-amyloidogenic effects (inhibits Aβ40 production) through decrease the amount of β- secretase in APP-Chinese hamster ovarian cells	[1]
Cinnamaldehyde	*Cinnamomum zeylanicum*	Aqueous extract of cinnamon inhibits aggregation of tau. Cinnamaldehyde displays tau aggregation inhibitory activity	[2]
Coumarin, Indonesian cassia extract (ICE)	*Cinnamomum burmannil C. Nees & T. Ness*	Both possess anti-inflammatory properties through reducing the production of NO and PGE2, inhibits IL-6, IL-1β and TNF-α in LPS-induced macrophages cell line RAW264.7	[3]
Coumarin	Coumarin-based hybrid drug	Coumarin based conjugates have been described as potential AChE, BuChE, MAO and β- amyloid inhibitors	[4,5]
Aqueous cinnamon extract	*Cinnamomum spp.*	Markedly inhibits the formation of toxic Aβ oligomers and prevents the toxicity of Aβ on neuronal PC12 cells. When administered to an AD *Drosophila melanogaster* (fly) model, CEppt rectified their reduced longevity, fully recovered their locomotion defects and totally abolished tetrameric species of Aβ in their brain. Oral administration of CEppt to an aggressive AD transgenic mice model led to marked decrease in 56 kDa Aβ oligomers, reduction of plaques and improvement in cognitive behavior	[6]
Cinnamon powder and its metabolite sodium benzoate (NaB)	Cinnamonum verum	Oral administration of cinnamon powder to 5XFAD transgenic mice produce NaB in the hippocampus. NaB decreased the production of reactive oxygen species in activated microglia via suppressing the activation of p21rac, a member of the NADPH oxidase complex. Oral administration of both cinnamon and NaB inhibited the activation of p21rac and reduced oxidative stress, protects spatial learning and memory, suppresses neurons degeneration and Tau phosphorylation, reduces Aβ charge	[7]
Cinnamaldehyde	C. cassia	Inhibited NO production, inhibited mRNA expression of pro-inflammatory mediators (cytokines and chemokines) includes TNF-α, IL-1β, IL-6, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α in LPS-activated cells. Decreased expression of inducible enzymes inducible nitric oxide synthase, cyclooxygenase-2, microsomal prostaglandin-E synthase-1. Increased mRNA expression and the production of antiinflammatory cytokines IL-10 and transforming growth factor-β	[8]
Phenylethyl alcohol	C. cassia Presl (Lauraceae)	Essential oil from the twigs significantly reduced oxytocin-induced writhing responses with a maximal inhibition of 66.5%. Decreased the expression levels of PGF2α, COX-2, p-MLC20 in oxytocin-induced mice uterine tissue	[9]

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Methylbenzofuran	Hybrid incorporate benzofuran and chalcone fragments	Decreased Aβ aggregation and increased ACh levels along with the overall availability of Ach at the synaptic junction. decrease AChE levels, reduce oxidative stress in Caenorhabditis elegans, lower lipid content, and to provide protection against chemically induced cholinergic neurodegeneration	[10]
Secoisolariciresinol	Folk medicine *Haplophyllum sahinii* O. Tugay & D. Ulukuş, H. vulcanicum Boiss. & Heldr	Secoisolariciresinol and secoisolariciresinol dimethyl ether were the main lignans in the stems and flowers. The highest inhibition was caused by the stem extract of *H. sahinii* against BChE (IC50 =64.93±1.38 µg/mL). All of the extracts were found to exert a selective inhibition towards BChE to some extent. root extract of *H. vulcanicum* that could inhibit AChE at low level (IC50 =203.18±5.33 µg/mL).	[11]
Patchouli alcohol (PTA)	C. cassia	Antifungal activity against *Aspergillus species* (suppression colony growth)	[12]
Patchouli alcohol (PTA)	Pogostemon cablin	Immunomodulatory, anti-inflammatory, antioxidative, antitumor, antimicrobial, insecticidal, antiatherogenic, antiemetic, whitening, and sedative activity	[13]
β-eudesmol	Folk medicine *Atractylode macrocephala* Koidz (Qi-Zhu)	Show AChE inhibitory activity	[14]
Embelin	Folk medicine Embelia ribes	Stopping the formation of Aβ oligomers (via inhibition of BACE-1), improves cholinergic-transmission (via inhibition of AChE/BChE) and increases Aβ clearance (via P-gp induction).	[15]
Ethylcinnamate	Melatonin– cinnamate hybrids	Showed antioxidant properties and high neuroprotective effect in the rot/olig model. Cinnamate esters were previously reported as moderate Nrf2 inducers, Nrf2 activation in animal models of AD and other neurodegenerative diseases (NDDs) extended survival and reversed cognitive decline. implement the scavenger and neuroprotective effect of melatonin with the tuneable Nrf2 induction properties of cinnamates, to obtain a multi-target compound specifically designed for NDDs	[16]
Isolariciresinol	Folk medicine Crataegus spp.	Showed stronger inhibition of Aβ1-42 aggregation	[17]
Isolariciresinol	Taxus baccata L.	Exhibited a moderate inhibition against both BChE and LOX, whereas they were inactive towards AChE. displayed a great scavenging activity against DPPH especially at 500 and 1000 microg ml(-1). exert noteworthy reducing antioxidant power on ferric ions	[18]
α-Cadinol	Folk medicine Alpinia oxyphylla Miq.	Preventing Aβ(1-42)-induced neuronal apoptosis may be via inhibiting Aβ(1-42)-induced reactive oxygen species (ROS) production, attenuating Aβ(1-42)-induced caspase-3 activation and inhibiting caspase-3 activity in cultured rat hippocampal neurons	[19]
Espatulenol	Folk medicine *Filifolium sibiricum* (L.) Kitam	Significant antibacterial activities against Staphylococcus aureus	[20]
T-muurolol	*Calocedrus macrolepis* var. *formosana* Florin	Antifungal activity, strongly inhibited the growth of Rhizoctonia solani and Fusarium oxysporum	[21, 22]
Melilotocarpan A	Folk medicine Red Mexican Propolis	Pterocarpans constitute the second largest group of natural isoflavonoids, show antifungal and antibacterial activities	[23, 25]
Isohomogenol, eugenol	*C. cassia* Presl	Might affect the key targets of thermogenesis effect of mice model	[26]
Protocatechuic acid	*C. burmannii*	Addition of cinnamon extract to white chocolate result in an end-product rich in polyphenols, whose content and antioxidant activity in food are useful for a preliminary prediction	[27]
Ethyl cinnamate	*C. cassia* Blume	As judged by 24-hour LC50 values, two cassia oils (0.084–0.085 mg/ml) and four cinnamon oils (0.064–0.113 mg/ml) exhibited good nematicidal activity toward adult *Bursaphelenchus* xylophilus	[28]
Benzenepropanol/3-Phenyl-1-propanol	*Liquidamabar orientalis*	Essential oils showed 87.5% mortality against female spotted wing drosophila at 5.0 µg/fly concentration	[29]
Linalool (69.94%), camphor (10.90%), nerolidol (10.92%), safrole (8.24%)	*Cinnamomum camphora* var. linaloofera Fujita	Linalool had bactericidal activity against *Escherichia coli*	[30]

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Cinnamaldehyde	*C. zeylanicum*	Antifungal activity against *Aspergillus ochraceus and Penicillium verrucosum* (inhibit growth and ochratoxin A production)	[31]
α-Terpineol, terpinen-4-ol, δ-terpineol	*Cinnamomum longepaniculatum*	Antimicrobial activity towards Shigella flexneri, increase the membrane permeability, damage cell membranes. The minimum inhibition concentration (MIC) and minimum bactericide concentration (MBC) of α-terpineol and terpinen-4-ol were similar (0.766 mg/mL and 1.531 mg/mL, respectively), while the MIC and MBC values of δ-terpineol were 0.780 mg/mL and 3.125 mg/mL.	[32]
Paeonol	Moutan Cortex of Guizhi Fuling capsule	PGF2α level reduction, intracellular Ca2+ reduction, NO level elevation, suppression of COX-2 and OTR expression in primary dysmenorrhea treatment	[33]
Anethole/trans-anethole, trans -cinnamaldehyde	*C. cassia*	Trans-anethole is effective for fumigant activity, trans-cinnamaldehyde show contact toxicity against *Drosophila suzukii*	[34]
Farnesol	Bee-hive propolis	Antibacterial against *Strptococcus mutans* and *Streptococcus sanguinis*	[35]
Cinnamyl benzoate	Synthesize from benzoyl chloride and cinnamyl alcohol	Inhibited the growth of yeast-phase cells of the pathogenic fungus *Sporothrix schenckii* through inhibit sterol synthesis	[36]
Eugenol	*Ocimum sanctum L.*	Eugenol showed greater inhibitory effect against AChE and BChE activities, and demonstrated higher antioxidant potential compared to butylated hydroxylanisole (BHA) and butylated hydroxyl toluene (BHT)	[37, 38]
Protocatechuic acid (PA)	Commercially purchased PA from *Momordica* charantia	Oral administration of PA for 14 days at 200 mg/kg/day protected against cognitive impairment in Aβ-induced AD mouse model through attenuating Aβ25-35-induced neuroinflammation (downregulating inflammatory mediators, inducible nitric oxide synthase and cyclooxygenase-2 in the brain).	[39]
Eugenol	Commercially purchased	Eugenol bind to different AD targets (BACE1, MAO-B, cholinesterase's, Aβ1-42 fibrils), shows free radical scavenging activity and anti-aggregation activity against Aβ peptides. Pre- treatment with eugenol significant protected PC12 cells against H2O2-induced oxidative stress and Aβ-induced cytotoxicity	[40]
Cinnamic acid	Commercially purchased	As a potent ligand, cinnamic acid activates the nuclear hormone receptor PPARα and indues lysosomal biogenesis in mouse primary brain cells. Oral administration of cinnamic acid significantly attenuated cerebral Aβ plaque burden and improved memory and behavioral performance of male and female 5× Familial Alzheimer's disease mice.	[41]
Patchouli alcohol (PTA)	Commercial purchased	PTA as a selective agonist of ERβ efficiently ameliorated AD-like pathology of APP/PS1 model mice. Reduced amyloid plaque deposition in the hippocampus by promoting microglial phagocytosis. Alleviated o-Aβ25-35-induced oxidative stress in primary neurons through targeting ERβ and increasing catalase expression. PTA administration improved synaptic integrity through enhancing BDNF/TrkB/CREB signaling, ameliorated oxidative stress by Catalase level, and regulated Bcl-2 family proteins in the hippocampus. patchouli alcohol target ER.	[42]
Protocatechuic acid (PA)	Commercial purchased	PA increases PC12 cell viability and significantly decreases the levels of Aβ42, p-tau, β- secretase and Beclin-1, promotes the expression of p-Akt and MEF2D and suppresses the expression of p-GSK-3β. Thus, PA has potent as a drug candidate against AD	[43]
Cinnamaldehyde	Commercial purchased	significantly improved lifespan and healthspan of male AD flies (*Drosophila melanogaster* model) overexpressing the Tau proteins	[44]
Embelin	Commercial purchased	Inhibit AChE, docking prediction for interaction with AChE and amyloid beta (Aβ) binding sites	[45]
Embelin	Commercial purchased	nootropic and anti-amnesic effects on scopolamine-induced amnesia in rats. significantly exhibited a memory-enhancing effect in the absence of scopolamine, besides improving the recognition index when challenged with chronic scopolamine treatment, increase in inflection ratio in nootropic activity. contributed toward the elevated expression of BDNF, CREB1, and scavenger enzymes (SOD1 and CAT) mRNA levels. pretreatment of rats with embelin mitigated scopolamine-induced neurochemical and histological changes	[46]
Paeonol	Commercial purchased	Significantly relieved Aβ deposition and Aβ -mediated neuropathology in the brain of APP/PS1 mice	[47]
Farnesol	Commercial purchased	Neuroprotective role of geranylgeraniol / farnesol in the survival of neuronal cells	[48]
Anemonin Eugenol	Commercial purchased	Suppression of IL-1β/NF-κB pathway for inflammation related osteoarthritis	[49]

Note: nitric oxide (NO), prostaglandin E2 (PGE2), inhibits interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), acetylcholine (ACh)

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Discussion

Background

Previous studies have shown that cinnamon may exert a neuroprotective role through the suppression of neuroinflammation and oxidative injury and thus have a potential therapeutic role due to its pro- cognitive effects in managing AD [16]. Systems-level screening of drug candidates for therapeutic AD treatment from cinnamon is attracting global research attention [1, 2] because cinnamon preparations, extracts, key metabolites [11], and hybridized molecules [32, 33] have made cinnamon an interesting and indispensable candidate for future drug research in the area of AD. Therefore, it is opportune to investigate the detailed mechanisms and apply the pharmacology of the cinnamon -AD network for predictive analysis.

Current study

In this study, we adopted network-pharmacological strategies to screen bioactive metabolites of cinnamon. The analysis incorporates 50 experimentally verified metabolites from cinnamon [3, 31] and 35 components screened using public databases. Targets of the above metabolites were collected by searching TCMSP, ETCM, Batman-TCM, SymMap, and TargetNET and predicting targets using SEA, and SwissTargetPrediction, resulting in 1697 targets related to the above 85 active metabolites. A previous study showed that 121 compounds were identified by HPLC-Q-TOF-MS in a cinnamon extract, including coumarin (1.96 mg/g), cinnamic acid (4.73 mg/g), cinnamaldehyde (8.04 mg/g) and 2-methoxycinnamaldehyde (3.66 mg/g) [34]. The disease targets were acquired by identifying AD- related genes and filtering by processing the expressing profiles of the high-throughput single-cell transcriptome SC2 diseases database. While 4246 DEGs (2615 for E2N and 1531 for E2L only) were obtained by bioinformatics analysis of scRNA-seq data, AD-associated genes from Genecards and DisGeNET resulted in 9327 targets mapped in the STRING database. Together, 321 cinnamon-AD common targets were matched. When we compared early-onset to normal and to. late-onset to normal Alzheimers genes, almost two-thirds (210/321) of the disease genes were unique for early-onset vs. normal conditions, which is attributed to the integration of single-cell technology [35].

General analysis of MOA of cinnamon metabolites

Metascape GO and KEGG enrichment analysis indicate that cinnamon metabolites may have therapeutic effects towards AD via the Alzheimer's pathways, regulation of inflammatory response, regulation of ion transport or ion homeostasis, as well as calcium signaling pathways. Aβ peptides are produced from the cleavage of APP, which is catalyzed by α-secretase, β-secretase (BACE1), and γ- secretase [36]. Prevention of Aβ aggregation to keep a homeostatic balance between Aβ production and clearance is critical to maintaining brain health [37], cinnamon metabolites interfere with Aβ involved in the Alzheimer's pathway and thus could be beneficial to the treatment of AD. For instance, oral administration of the phenolic compound paeonol (20 mg/kg) for three weeks significantly relieved the Aβ deposition burden, which contributes to the microglial inhibition and proinflammatory cytokine downregulation (TNF-α and IL-1β) in the hippocampus, and thus improved cognitive performance of AD model of APP/PS1 mice [38]. Methanol extract of cinnamon bark containing the lignan medioresinol efficiently reduced Aβ40 production (by 50%) via decreased BACE1 in Chinese hamster ovarian cells stably expressing APP [39]. The usage of embelin stops the formation of Aβ oligomers via inhibition of BACE1, improves cholinergic transmission through inhibition of AChE/BChE, and increases Aβ clearance via enhanced P-GP induction in a neuronal cell line [40]. Another study evaluated *in vitro* inhibitory activity of embelin toward AChE of the primary porcine brain endothelial cell model of the blood-brain barrier. It is evidenced that embelin has the potential to degrade mature fibrils of Aβ peptides through molecular binding with Aβ peptides [41]. Isolariciresinol at 20 μm shows inhibitory activity toward self-induced Aβ1–42 aggregation [42]. Treatment with patchouli alcohol (PTA) by administration of PTA (20, 40 $mg/kg^{-1}/d^{-1}$) for 90 days ameliorated AD-like pathology of APP/PS1 model mice through ameliorated o-Aβ25-35-induced reduction of synapse- related proteins via enhancing ERβ/BDNF/TrkB/CREB pathways and relieved oxidative stress in primary neurons through targeting Erβ, thus increasing catalase expression respectively [43]. Mechanistic evidence for activity of cinnamon derived metabolites in AD, has also been demonstrated through experiments using these same metabolites isolated from different sources. The commercial embelin can inhibit AChE and display nootropic as well as anti-amnesic effect in the model rat, while the embelin extract from *Embelia ribes* can stop the formation of Aβ oligomers by inhibiting BACE-1 and improve cholinergic-transmission. Secoisolariciresinol and secoisolariciresinol dimethyl ether are the main lignans in the stem extract of *Haplophyllum sahinii* and the flower extract of *H. vulcanicum*, which can inhibit BChE (IC50 = 65 μg/ml) and AChE (IC50 = 203 μg/ml), respectively.

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DOI:10.26502/fjppr.074

Isolariciresinol from *Taxus baccata* L. inhibited BChE and lipoxygenase with a noteworthy antioxidant activity [32], while those isolated from seeds of hawthorn (*Crataegus spp*.) inhibited Aβ1-42 aggregation [42]. Alpha- cadinol extracted from fruits of *Alpinia oxyphylla* Miquel prevents Aβ1-42-induced neuronal apoptosis [44].

Experimental studies strongly support that protocatechuic acid (PA) prevents the neurodegenerative processes including Alzheimer's diseases, due to its favorable influence on accumulation of the Aβ plaques in brain tissues, hyperphosphorylation of tau protein in neurons, excessive formation of ROS and neuroinflammation [45-47]. Aqueous extract of *C. zeylanicum* (containing cinnamaldehyde) show tau aggregation and filament formation inhibitory activity [13], and pure cinnamaldehyde has been shown to significantly improve lifespan and health-span of male AD flies overexpressing tau proteins in the model of *Drosophila melanogaster* [48]. In addition, cinnamic acid activates the nuclear hormone receptor PPARα and induces lysosomal biogenesis in mouse primary brain cells. Oral administration of cinnamic acid significantly attenuated cerebral Aβ plaque burden and improved memory and behavioral performance of male and female 5× Familial Alzheimer's disease mice [49]. Thus, cinnamon ingredients found in other species show similar effects on AD, which may open up other plant species' potential therapeutic use.

Major cinnamon metabolites with associated targets and pathways

The herb-compound-target network revealed that eugenol, cinnamic acid, cinnamaldehyde, and coumarin are the key cinnamon metabolites that counteract AD core targets, including ACHE, ALOX15, DUSP3, PTGS2, RORA, CYP19A1, ESR2, AKR1BA, APP, ESR1. Eugenol has the largest number of therapeutic targets for AD (degree = 78). Indeed, compared to synthetic phenolic compounds, eugenol is a natural antioxidant with no side effect, indicating a greater inhibitory effect against AChE, BChE, and monoamine oxidase (MAO) dose-dependently [50]. Cassia leaf oil (containing 78.35% of cinnamaldehyde and 4.52% of eugenol) shows a higher inhibition effect than cinnamaldehyde on NO production and iNOS expression cells in LPS-activated J774A.1 [6], since eugenol has been found to inhibit iNOS and NO production in LPS-stimulated human macrophages U937 [51]. Our results agree well with a recent report that eugenol could bind to different AD targets, including BACE1, MAO-B, cholinesterase's, and Aβ1-42 fibrils and shows free radical scavenging activity and anti-aggregation activity against Aβ peptides, while pre-treatment with eugenol significant protected PC12 cells against H2O2-induced oxidative stress and Aβ-induced cytotoxicity [52].

Cinnamaldehyde (3-phenyl-2-propenal) has the 3rd greatest number of AD targets (degree = 54). It displays significant neuroprotective effects in AD animal models by regulating neuroinflammation, suppressing oxidative stress, and improving the synaptic connection. The associated signaling pathways consist of TLR4/NF-κB, NLRP3, ERK1/2-MEK, NO, and Nrf2 pathways [53]. Using network pharmacology, Liu et al., speculated that cinnamaldehyde could play a role in the treatment of breast cancer via the inhibition of the peroxisome proliferator-activated receptor and P13K-Akt pathway [54]. The *in vitro* verification experiments showed that cinnamaldehyde can significantly inhibit cell proliferation, change cell morphology, cell migration, and invasion ability and promote cell apoptosis [23]. Surprisingly, our research group established the relationship between the estrogen receptor-α gene (ESR1) and neuroinflammation through the data mining of the high-throughput dataset of ESR1 knockdown breast cancer samples [55]. In this study, ESR1 is one of the targets of cinnamaldehyde. Therefore, cross-validation demonstrated that ESR1 dysfunction could trigger neuroinflammation or pyroptosis as a causative factor in the AD process and further applications of cinnamaldehyde in treating breast cancer. Pannee and colleagues compared the effects of cassia leaf oil from *C. cassia* and cinnamaldehyde on LPS-activated J774A.1 cells. Both saw remarkable down- regulation of TNF-α, IL-6, and IL-1β and decreased the mRNA expression of chemokines MCP-1 and MCP-1α [6] while stimulating the production of anti-inflammatory mediator cytokines TGF-β and IL- 10 [51], suggesting that the inhibitory effects mainly came from cinnamaldehyde [51]. Another aqueous extract of *C. zeylanicum* (containing cinnamaldehyde) was shown to inhibit the aggregation of human tau *in vitro* and filament formation [13]. On the other hand, cinnamaldehyde significantly improved the lifespan and health span of male AD flies overexpressing the tau proteins in model *Drosophila melanogaster* [48]. Another study showed that cinnamon extracts containing either cinnamaldehyde or epicatechin might play a protective role in preventing oxidation to tau-protein cysteine residues that can lead to dysfunction characteristic of AD [14]. Pre-incubating tau[187] with cinnamaldehyde prevented the formation of tau fibers, critical structures necessary for driving the aggregation of tau tangles [14]. Incubation with *C. verum* extract containing cinnamaldehyde significantly inhibited the formation of fibrillar assemblies of hen egg white lysozyme by disrupting the pi-pi interactions [10]. AD rat model treated with 100 mg/kg of cinnamaldehyde (intraperitoneal) daily for 2 weeks, improved recognition/spatial memory deficits and anxiety-like behavior, negated the effects of streptozotocin on Aβ aggregation and caspase-3 cleavage in the hippocampus through decreasing phosphorylation and modulating the hippocampal IRS-1/AKT/GSK-3β signaling

pathway [56]. Essential oil of *C. verum* (containing 68.23% of (E)-cinnamaldehyde) demonstrated potent activity toward AChE and BChE with IC50 values of 453.7 and 184.7 µg/mL, respectively [57]. However, the clinical application of cinnamaldehyde is limited due to possible allergic reactions [58], developments on cinnamaldehyde based derivatives or hybrid drugs with anti-Alzheimer's potent is promising but needs full exploration [59].

Among the targets revealed by network analysis, drugs targeted for ACHE, APP, 1L1B, ESR2, KDR and PLG have been proven to be successful, MAPT, SNCA, BACE1, DRD1, GRIN1, CHRNB4 and HSD11B1 are under clinical investigation through TTD or DrugBANK searching [60, 61], 9 of 13 genes were targeted by cinnamon metabolites, indicating our data searching strategy is of high quality and cinnamon-based drug development is promising. Molecular docking on 8 (ACHE, DUSP3, PTGS2, CYP19A1, ESR1, APP, MAPT, and FOS) of 33 key and another 4 targets (HSP90AA1, CASP3, GAPDH, TP53) and 77 metabolites were carried out to validate the result of network analysis. The docking within the docking pockets between the target protein and ingredients was visualized by AutoDockVina and PyMOL, resulting in binding affinities ranging from 6.0 to 9.6 kcal/mol, which suggests stable binding. Then, the molecular dynamics simulation was further applied to assess the stability of cinnamyl benzoate, methylbenzofuran, medioresinol and PTGS2 in the binding pocket at 100 ns. Dynamics simulation results consist of molecular docking results. PTGS2, also known as the cyclooxygenase 2 gene (COX2), showed the highest binding affinities with medioresinol (9.1), followed by the other 40 metabolites (range from 6.0 to 8.8). COX-2-induced AD development and COX-2 dysregulation significantly affect abnormal cleavage of Aß, aggregation and deposition of Aß in amyloid plaques and the inclusion of phosphorylated tau in neurofibrillary tangles [62]. Growing evidence suggests that treating patients with AD using a COX-2 selective inhibitor (celecoxib) improves cognitive performance and protects the learning ability losses for patients with AD [63-65]. Together, it indicates that cinnamon may play a protective effect on AD by inhibiting the response of COX-2 [62, 66, 67]. In addition, heat shock proteins as cellular chaperones have also been involved in the pathogenesis of AD [68]. Our results showed that six cinnamon metabolite target HSP90AA1, while two cinnamon compounds (2-hydroxycinnamic acid and methyl dihydromelilotoside) target HSPA8 (also known as HSP70). HSPA8 interacts with tau, and significant downregulation of HSPA8 was observed in AD samples [69]. HSP90 may function by maintaining oligomeric p-tau levels [70], HSPA8 and HSP90 may prevent tau aggregation and neurofibrillary tangles formation [70], and the HSP70/HSP90 balance may be a key factor in modulating p-tau stabilization [71]. These findings imply a therapeutic potential of HSPA8 or HSP90AA1 inhibitors in treating AD [72]. Indeed, HSP90 inhibitors reduced levels of tau phosphorylation [73], while daily administration of HSP90 inhibitor (17-AAG) over 7 d resulted in a significant up-expression of synaptic protein PSD95 in hippocampi [72], and PSD95 reduction degree (both mRNA and protein levels) is correlating with Aß oligomer levels and disease severity [74]. Therefore, the therapeutic effect of cinnamon against AD may be partly associated with Aß and tau phosphorylation. The interaction of cinnamon metabolites with HSPA8 and HSP90AA1 deserves more attentions.

Besides playing a role in APP or tau pathology, cinnamon metabolites may function in other AD pathology-related processes. Insoluble polymeric Aβ cleaved from APP aggregates into amyloid plaques, contributing majorly to AD pathogenesis. The damage to synapses caused by Aβ oligomers triggers microglial and astrocytic activation, leading to an inflammatory response and disrupting neuronal ionic homeostasis. It then causes oxidative injury that contributes to the formation of tau tangles and consequently causes synaptic dysfunction and neuronal loss [1, 2]. Five effective cinnamon metabolites have been widely investigated and confirmed, exerting beneficial effects on AD or AD- related inflammation and immunity. For instance, cinnamaldehyde [13, 14], epicatechin [14], eugenol [51], coumarin [15], have the potential to be used as an anti-inflammatory agent. Coumarin and Indonesian cassia extract exert anti-inflammatory activities by reducing NO and PGE2 production and inhibiting TNF-α, IL-6, and IL-1β production in macrophage cell line RAW264.7 [15]. In addition, cinnamon metabolites target the critical elements of calcium hypothesis of AD: calcium signaling pathways, and oxidative stress/inflammation pathway, offering an opportunity for the development of cinnamon-based AD therapeutic agents [75, 76].

Summary of findings, limitation and prospect

Findings from our network analysis studies have provided valuable information to support the use of cinnamon for developing therapeutic strategies against AD. According to the target distribution of cinnamon metabolites, multiple active ingredients display a synergistic effect in treating AD. The binding affinity results show that various ingredients can bind to the same protein. For instance, AChE is a target for 63 compounds, PTGS2 is the target for 41 compounds, CYP19A1 is the target for 28 metabolites, DUSP3 is the target for 7 compounds, and 8 compounds target APP and MAPT. This suggests systemic regulation of cinnamon for AD targets. On the one hand, it may pose a great challenge for new drug discovery [77]. On the other hand, besides the direct application of cinnamon extracts or purified commercially available compounds, the specific design and development

of multi- target compounds incorporating active cinnamon ingredients, which target AD, are promising therapeutic candidates [32]. One good example is coumarin-based hybrid molecules, which have shown excellent potential for AChE inhibition, MAO-B inhibition, and anti-Aβ aggregation, and have been well discussed [32, 33]. Furthermore, melatonin-cinnamate hybrids show antioxidant properties and the highest neuroprotective effect in the rat/olig model through the implementation of tunable Nrf2 induction properties of cinnamates and the scavenger and neuroprotective effect of melatonin [78]. Another hybrid incorporates benzofuran and chalcone fragments which decrease Aβ aggregation and AChE levels, reduce oxidative stress, and provide protection against chemically induced cholinergic neurodegeneration in the worms *Caenorhabditis elegans* [32].

We propose the mechanism of cinnamon counteracting AD: interference with Aβ involved Alzheimer's pathway, reduces Aβ production, increases Aβ clearance, e.g., degrades mature fibrils of Aβ peptides through molecular binding with Aβ peptides, or relieves oxidative stress, targets critical elements of calcium signaling pathways. This study presents with several limitations, for example, although the molecular docking and molecular dynamics showed positive results, further experimental validation are needed to evaluate the predicted compound–target relationships.

Conclusion

In this case study, we see the significant contribution that a system analysis approach can make to understanding the therapeutic benefits of plant metabolites in AD. The current study using cinnamon as a case study confirms the potent therapeutic effects of cinnamon species, particularly *C. cassia*, which is used globally as raw materials in foods, nutraceuticals, and traditional medicinal use. The possible mechanisms of action of the cinnamon metabolome and its metabolites were acquired by employing network systems pharmacology and molecular computational analyses, which were supported by scRNA-Seq data and experimental reports from the literature. Results here offer perspective options in treating AD, with cinnamon metabolites to promote anti-Aß aggregation and clearance and modulate the calcium signaling pathway. To support these findings, studies will need to be carried out to identify an optimized standardized extract, as will studies to establish the maximum tolerated and optimal dosing for therapeutic effect and to further verify related pathways and targets.

Author Contributions

Wen Qiu: Software, Investigation, Writing - Original Draft, Visualization. Shouli Yuan: Software, Formal analysis. Junying Liu: Methodology, Resources, Formal analysis. Lina Sun, Wei Chen: Project administration. Robbie Kelleher: Writing - Review & Editing. Helen Sheridan: Conceptualization, Writing - Review & Editing. Weiguang Lv: Supervision.

Funding

This work was financially supported by Hu-Nong-Ke-Zhuo (2022) 008, and National Agricultural Experimental Station for Agricultural Environment Program (NAES035AE03).

Conflicting Interests

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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