Original Article

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Agarwood as a potential therapeutic for Alzheimer's disease: Mechanistic insights and target identification

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SUMMARY Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and functional impairments. Despite extensive research, its pathogenesis remains incompletely understood, and effective treatments are limited. This study explored the therapeutic potential of agarwood in AD by integrating network pharmacology, protein-protein interaction (PPI) network analysis, and single-cell expression analysis. The results revealed that agarwood compounds may modulate key inflammatory genes such as *NFKB1*, *STAT1*, and *TLR4*, alleviating neuroinflammation; enhance the expression of *HSP90* and regulate KDR signaling to improve blood-brain barrier (BBB) integrity; and promote the activity of PTPN11 and CXCR4 to support oligodendrocyte precursor cell (OPC) repair and remyelination. Single-cell expression analysis highlighted cell-type-specific expression patterns, particularly in OPCs and endothelial cells, underscoring their relevance in AD pathology. Agarwood's multi-dimensional therapeutic potential positions it as a promising candidate for the development of novel AD treatments.

Keywords Alzheimer's disease, agarwood, network pharmacology, neuroinflammation, blood-brain barrier, oligodendrocyte precursor cells

1. Introduction

Alzheimer's disease (AD) is a multifaceted, progressive neurodegenerative disorder and the most prevalent cause of dementia, characterized by cognitive decline, memory loss, and impaired daily functioning (*1*). In 2018, approximately 50 million people worldwide were affected by AD, with projections indicating that this number may rise to 152 million by 2050 (*2*). The increasing incidence of AD imposes significant social and economic burdens, creating immense challenges for both individuals and society (*3*). Current therapeutic approaches, which include traditional pharmacological treatments and immunotherapies, have shown limited efficacy, as no curative treatment targeting AD has been identified (*4*). This underscores the pressing need for further research into the underlying biological mechanisms of AD and the development of more effective therapeutic strategies.

Traditional Chinese medicine (TCM) has garnered attention in clinical practice due to its notable therapeutic effects and minimal side effects. Among these, agarwood, a valuable tropical plant, is rich in terpenoids, such as agarwood oil and agarol, which possess antioxidant, anti-inflammatory, and neuroprotective properties. Studies have demonstrated that agarwood extracts can reduce inflammation and inhibit cholinesterase activity in mouse models of AD (*5*). Given its potential as a neuroprotective agent, agarwood is an appealing candidate for further research. However, its precise mechanisms of action in AD remain unclear. In recent years, network pharmacology has emerged as an innovative approach for investigating drug-disease interactions, providing a comprehensive understanding of the relationship between therapeutics and their molecular targets (*6*). In this study, we applied network pharmacology to identify the target proteins associated with agarwood and elucidate its potential mechanisms in AD.

2. Materials and Methods

2.1. Prediction of active ingredients and targets of agarwood

The active components of agarwood were identified using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

(TCMSP), applying the screening criteria of oral bioavailability (OB) greater than 30% and drug-likeness (DL) greater than 0.18. The molecular structures and canonical SMILES numbers of the components were obtained from the PubChem database (*http://pubchem.ncbi.nlm.nih.gov*). Target genes of these active components were predicted using the SwissTargetPrediction (*http://swisstargetprediction.ch/*) and SuperPred databases (*https://prediction.charite.de/subpages/target_prediction.php*). Targets were filtered based on a SwissTargetPrediction probability of ≥ 0.60 and a SuperPred probability of ≥ 60%. After compiling the target genes, their IDs were converted using Perl language, and gene symbols were obtained through alignment with the UniProt database.

2.2. Construction of the agarwood active component-target network

A network depicting the interactions between agarwood's active components and their target genes was created using Cytoscape software. The collected data were imported into Cytoscape to visualize the interactions within the network.

2.3. Disease target prediction

Genes associated with AD were identified using "AD" as the keyword, with "Homo sapiens" as the species. These genes were obtained from the GeneCards (*https://www.genecards.org/*), DisGeNET (*http://www.disgenet.org/*), and Online Mendelian Inheritance in Man (OMIM) databases (*http://www.omim.org/*), using a GeneCards score ≥ 50 and a DisGeNET score ≥ 0.10 as the filtering criteria. After eliminating duplicates, the AD-associated genes were consolidated.

2.4. Comparison of drug targets and disease targets

A Venn diagram was used to identify overlapping targets between agarwood's potential therapeutic targets and the disease-related genes associated with AD.

2.5. Biological function and pathway analysis of agarwood-AD common targets

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for the shared targets were performed using the DAVID Bioinformatics Database (*https://david.ncifcrf.gov/home.jsp*). Terms with a *P*-value ≤ 0.05 were collected for GO term clustering, and KEGG pathway enrichment was used to identify statistically significant pathways ($P < 0.05$).

2.6. Construction of the drug-target-disease interaction network

The protein-protein interaction (PPI) data of agarwood's components and their predicted targets were imported into Cytoscape software. Nodes represented drug components and disease-related target genes, with degree values used to filter key components and targets. Additionally, a drug-target-disease interaction network was constructed by importing relevant data into Cytoscape.

2.7. Construction of the PPI network for common targets

The PPI network for the common targets between agarwood and AD was built using the STRING platform (*https://string-db.org/*), with "Homo sapiens" as the species and a minimum interaction confidence of 0.400. The network was then analyzed in Cytoscape, and the main targets were identified based on degree values.

2.8. Expression levels of top ten genes in different brain regions

The AlzData database (*http://www.alzdata.org/*) was utilized to assess the expression levels of the top ten identified genes across various brain regions and individual brain cells.

2.9. Convergent functional genomic (CFG) ranking for target genes

To assess the relevance of the identified target genes for agarwood in AD, we applied the CFG approach. This method integrates data from various sources, including genetic, transcriptomic, and proteomic information, to evaluate the involvement of each gene in AD. The analysis began by selecting the candidate genes identified through network pharmacology. These genes were then cross-referenced with genome-wide association studies (GWAS) and other functional genomics datasets. Each gene was assigned a CFG score, reflecting its cumulative association with AD based on factors such as the number of related genetic variants, known interactions with key proteins, and involvement in inflammatory pathways. This CFG score provided a comprehensive ranking of each gene's potential contribution to AD pathology.

2.10. Expression analysis of hub genes in single cells

Single-cell expression analysis for the hub genes was performed using the GSE67835 dataset from the Gene Expression Omnibus (GEO) database, revealing their expression patterns in different brain cell types.

3. Results

3.1. Screening of active compound targets

A total of eight active compounds from agarwood

were screened from the TCMSP database. The SwissTargetPrediction and SuperPred databases were used to predict the target genes for these compounds. After eliminating duplicates, 511 potential target genes were identified.

3.2. Construction of the target network for agarwood components

A network diagram illustrating the interactions between agarwood's active components and their respective target genes was constructed using Cytoscape software (Figure 1). The network revealed a one-to-many relationship between active compounds and interacting genes, consisting of 52 nodes and 100 edges. DMPEC and norboldine were identified as potential key components in AD treatment.

3.3. Screening of disease targets

Based on the established screening criteria, 123 genes were identified from GeneCards, 268 genes from the OMIM database, and 3,421 genes from the DisGeNET database. After consolidating these data and removing duplicates, 3,611 AD-associated genes were identified.

3.4. Identification of common targets

A Venn diagram comparison revealed 122 overlapping genes between the active components of agarwood and AD-related genes. These common genes represent the potential targets through which agarwood might exert therapeutic effects on AD.

3.5. GO and KEGG enrichment analyses

GO and KEGG enrichment analyses were performed on the 122 common genes identified in the study, using the DAVID database. These analyses revealed significant enrichment in a total of 1,939 GO terms that are relevant to AD treatment.

Among the 176 terms associated with molecular function (MF), the primary activities involved include amide binding, peptide binding, drug binding, protein serine/threonine kinase activity, endopeptidase activity, neurotransmitter receptor activity, protein tyrosine kinase activity, protein phosphatase binding, gated channel activity, and ion channel activity (Figure 2A). These molecular functions highlight the involvement of key signaling and enzyme pathways critical to AD pathology.

In the biological process (BP) category, 1,643 terms

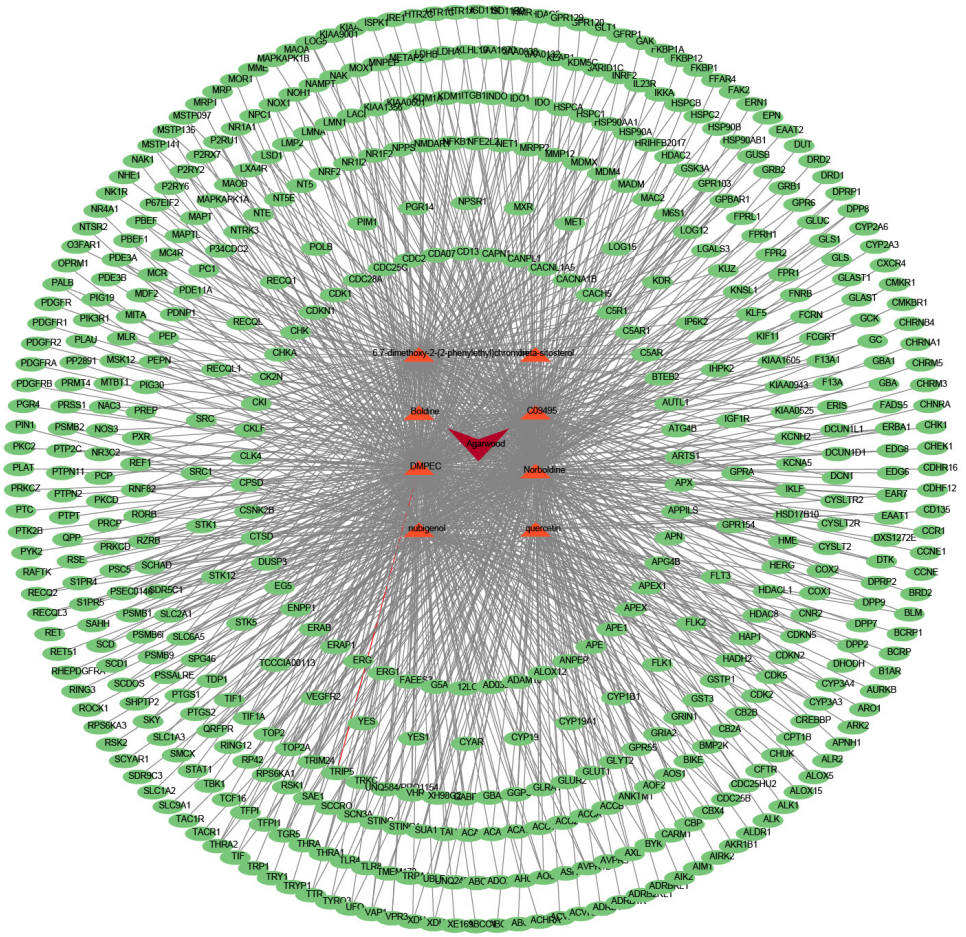

Figure 1. Agarwood components-target network diagram.

were identified. The top 10 processes were responses to drugs, oxygen levels, second-messenger-mediated signaling, oxidative stress, reactive oxygen species (ROS) metabolic processes, radiation, neutrophil degranulation, neutrophil activation involved in immune responses, decreased oxygen levels, and cellular responses to peptides and calcium ion transport (Figure 2B). These processes underscore the role of oxidative stress, immune responses, and calcium signaling in AD pathogenesis, all of which are key areas for potential therapeutic intervention.

For the cellular component (CC) category, 120 terms were identified. The most enriched terms included membrane rafts, membrane microdomains, membrane regions, glutamatergic synapses, secretory granule lumens, cytoplasmic vesicle lumens, vesicle lumens, cell leading edges, dendritic spines, neuron spines, and focal adhesions (Figure 2C). These findings suggest that critical cellular structures involved in signal transduction and synaptic function may play central roles in the progression of AD.

KEGG pathway enrichment analysis revealed 103 associated pathways. The top 10 pathways were neuroactive ligand-receptor interaction, microRNAs in cancer, AD, neurodegeneration pathways common to multiple diseases, the calcium signaling pathway, prostate cancer, the cAMP signaling pathway, the PI3K/Akt signaling pathway, proteoglycans in cancer, Parkinson's disease, and Huntington's disease. These pathways provide further insight into how cellular signaling, neurodegeneration mechanisms, and cancer-related pathways overlap in the context of AD (Figure 2D). In particular, pathways such as the PI3K/Akt and calcium signaling pathways are well-documented in their involvement in neuronal survival, synaptic plasticity, and neuroinflammation, which are critical to the disease process.

By focusing on the most enriched GO terms and KEGG pathways, the analysis reveals how agarwood's active compounds may exert therapeutic effects on AD through modulating biological functions such as protein binding, immune activation, and neuroprotection. These results not only provide potential therapeutic targets for future AD treatments but also highlight the complex interplay between neuroinflammation, oxidative stress, and cellular signaling in AD pathology.

3.6. Drug-component-disease-target network construction

The common target genes between agarwood and AD were imported into Cytoscape software to build a drug-component-disease-target interaction network (Figure 3). This visual model provided insights into how agarwood's

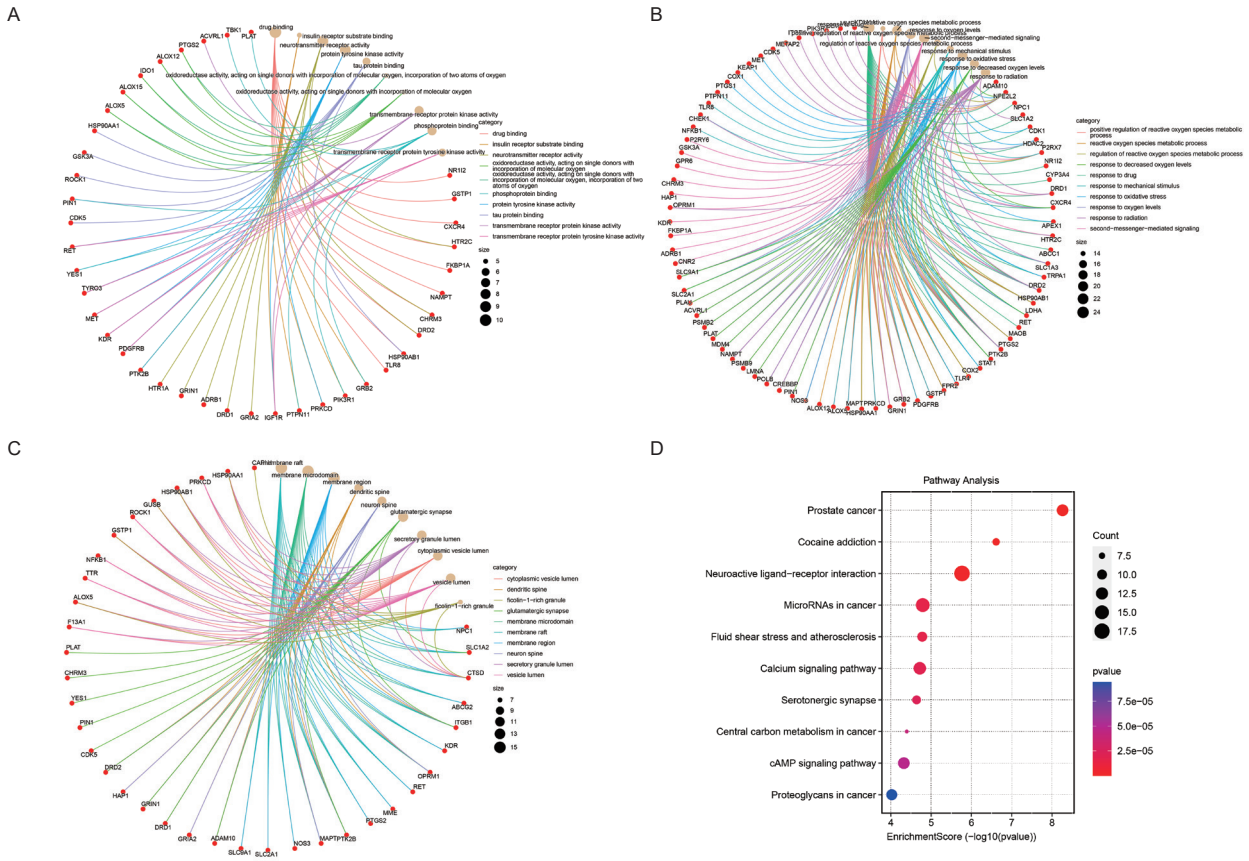

Figure 2. GO and KEGG enrichment analyses of common genes. Significant terms in molecular function **(A)**, biological process **(B)**, cellular component **(C)**, and KEGG pathways **(D)** associated with AD treatment.

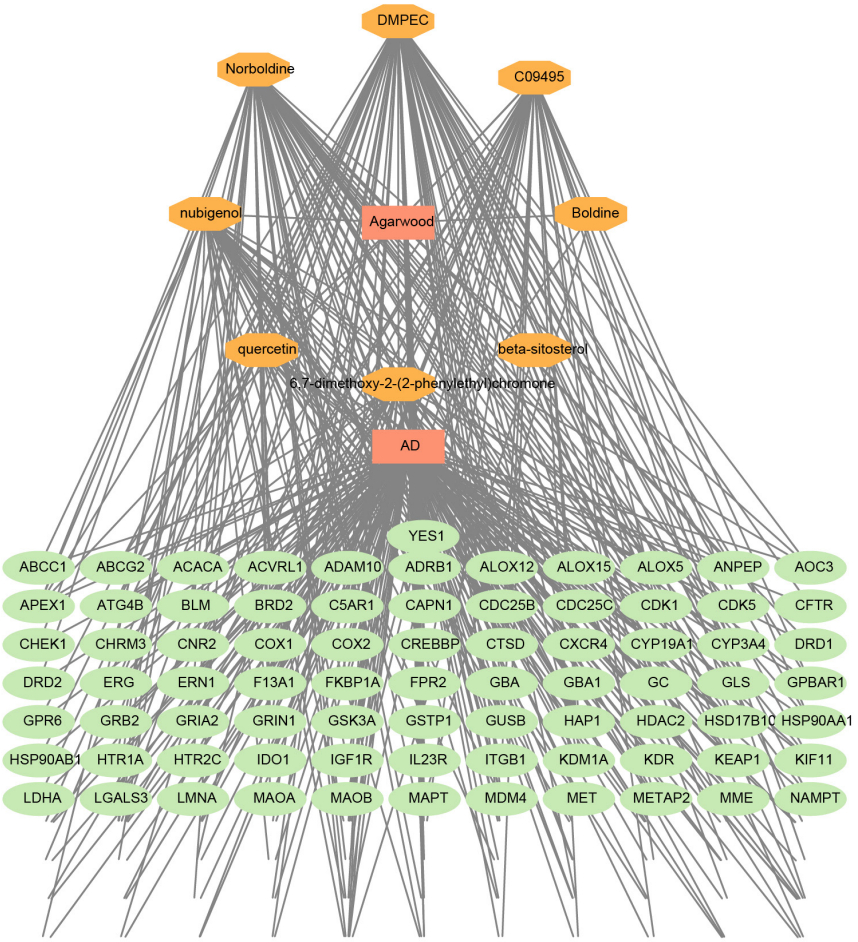

Figure 3. Drug-component-disease-target network diagram.

active components might interact with disease-related targets.

3.7. PPI network analysis

The 122 potential targets of agarwood in AD were further analyzed using the STRING platform, and a PPI network was constructed in Cytoscape after filtering out unconnected targets (Figure 4). The core target interaction network, based on degree values calculated *via* the cytoHubba module, revealed the top 10 hub genes: *HSP90AA1*, *HSP90AB1*, *NFKB1*, *TLR4*, *PTGS2*, *KDR*, *CXCR4*, *IL6*, *EGFR*, and *AKT1*.

3.8. Expression of top 10 genes in different brain regions

To further explore the roles of the ten hub genes (*HSP90AA1*, *HSP90AB1*, *NFKB1*, *TLR4*, *PTGS2*, *KDR*, *CXCR4*, *STAT1*, *PTPN11*, and *GRB2*), we analyzed their expression levels in 1,246 postmortem brain samples, which included 684 from AD patients and 562 from controls. The analysis focused on key brain regions implicated in AD pathology: the entorhinal cortex, hippocampus, temporal cortex, and frontal cortex (Table 1).

In the entorhinal cortex, significant differences were observed in the expression of *GRB2*, *CXCR4*, *KDR*, *TLR4*, *NFKB1* and *HSP90AB1* (Figures 5A, 5D, 5E, 5G-5I). Similarly, in the hippocampus, *PTPN11*, *CXCR4*, *NFKB1* and *HSP90AB1* showed altered expression levels (Figures 5B, 5D, 5H, 5I). In the temporal cortex, genes such as *GRB2*, *PTPN11*, *CXCR4*, *PTGS2*, *TLR4*, *NFKB1*, *HSP90AB1* and *HSP90AA1*, demonstrated differential expression patterns (Figures 5A, 5B, 5D, 5F-5J). Lastly, in the frontal cortex, significant variations were primarily observed for *CXCR4* and *NFKB1* (Figures 5D, 5H).

3.9. CFG ranking for target genes

The CFG analysis ranked the importance of the ten hub genes based on their association with Alzheimer's disease (Table 2). *GRB2* was regulated by five AD-related genetic variants, *HSP90AB1* by four variants, and *CXCR4* by three. According to GWAS, *PTPN11* was associated with three AD variants, while *CXCR4* had one AD-related variant. Additionally, *TLR4* and *PTGS2* were each regulated by two AD variants, and *NFKB1* by one variant.

Further analysis of physical interactions revealed that several of these genes interact with key proteins

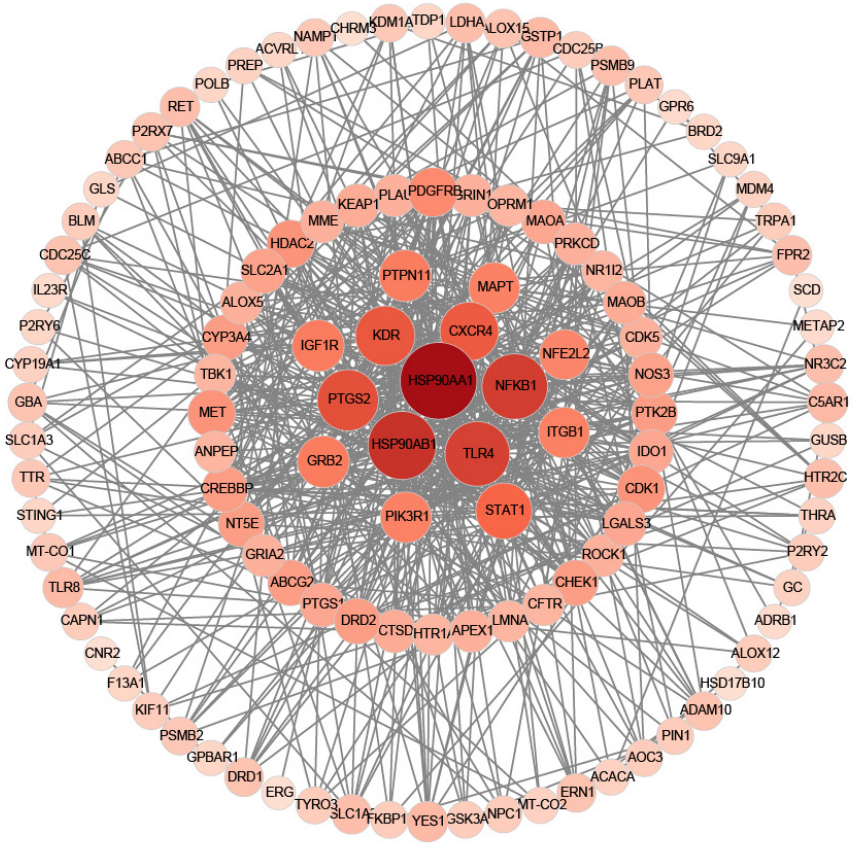

Figure 4. PPI network of agarwood targets in AD.

Table 1. Gene expression in Alzheimer's disease across brain regions

Gene	Entorhinal Cortex			Hippocampus			Temporal Cortex			Frontal Cortex		
	logFC	*P*-value	FDR	logFC	*P*-value	FDR	logFC	*P*-value	FDR	logFC	*P*-value	FDR
HSP90AA1	NA	NA	NA	-0.14	0.096	0.293	-0.36	0.009	0.046	0.11	0.177	0.327
HSP90AB1	-0.6	0.005	0.037	-0.29	0.032	0.155	-0.92	1.75E-06	0.000148231	-0.13	0.095	0.215
NFKB1	0.44	0.002	0.023	0.25	0.039	0.174	0.6	0.001	0.008	0.19	0.002	0.015
TLR4	0.48	0.003	0.027	0.21	0.075	0.256	0.48	0.014	0.063	-0.04	0.708	0.922
PTGS2	-0.07	0.702	0.82	-0.13	0.442	0.679	-0.46	0.01	0.05	-0.19	0.095	0.512
KDR	0.38	0.034	0.125	0.19	0.13	0.349	0.14	0.473	0.682	-0.12	0.458	0.826
CXCR4	0.8	0.0003	0.008	0.56	0.001	0.017	0.55	0.003	0.021	0.5	3.79E-05	0.002
STAT1	-0.17	0.12	0.275	-0.11	0.218	0.465	-0.06	0.61	0.784	0.03	0.631	0.761
PTPN11	NA	NA	NA	0.14	0.029	0.148	-0.31	0.024	0.093	-0.05	0.449	0.61
GRB2	-0.25	0.002	0.02	-0.06	0.308	0.561	-0.26	0.003	0.021	-0.05	0.373	0.537

Notes: logFC: logarithmic fold change, represents the fold change in gene expression, with positive values indicating upregulation and negative values indicating downregulation. FDR: false discovery rate – adjusted *P*-value to account for multiple testing.

implicated in AD pathology, including APP, PSEN1, PSEN2, APOE, and MAPT. For instance: HSP90AA1 interacts with APP, PSEN2, APOE, and MAPT; HSP90AB1 interacts with APP and PSEN1; NFKB1 interacts with APP, PSEN2, and MAPT; TLR4 interacts with PSEN2; PTGS2 interacts with APP, PSEN1, MAPT, and APOE; KDR and STAT1 interact with APOE; CXCR4 interacts with APP and APOE; PTPN11 interacts with APP, PSEN1, MAPT, and APOE; GRB2 interacts with APP, PSEN1, PSEN2, MAPT, and APOE.

The *PTPN11* gene showed differential expression early in the progression of AD, while *NFKB1* and *STAT1* were strongly linked to the development of amyloid-beta (Aβ) pathology. However, none of these hub genes were directly connected to tau pathology. Based on the CFG rankings, *NFKB1*, *CXCR4*, and *PTPN11* were ranked 3, *HSP90AB1*, *STAT1*, *TLR4*, *PTGS2*, and *GRB2* were ranked 2, while *HSP90AA1* and *KDR* were ranked 1. This indicates varying degrees of influence on AD pathology among these target genes.

3.10. Single-cell expression analysis of hub genes

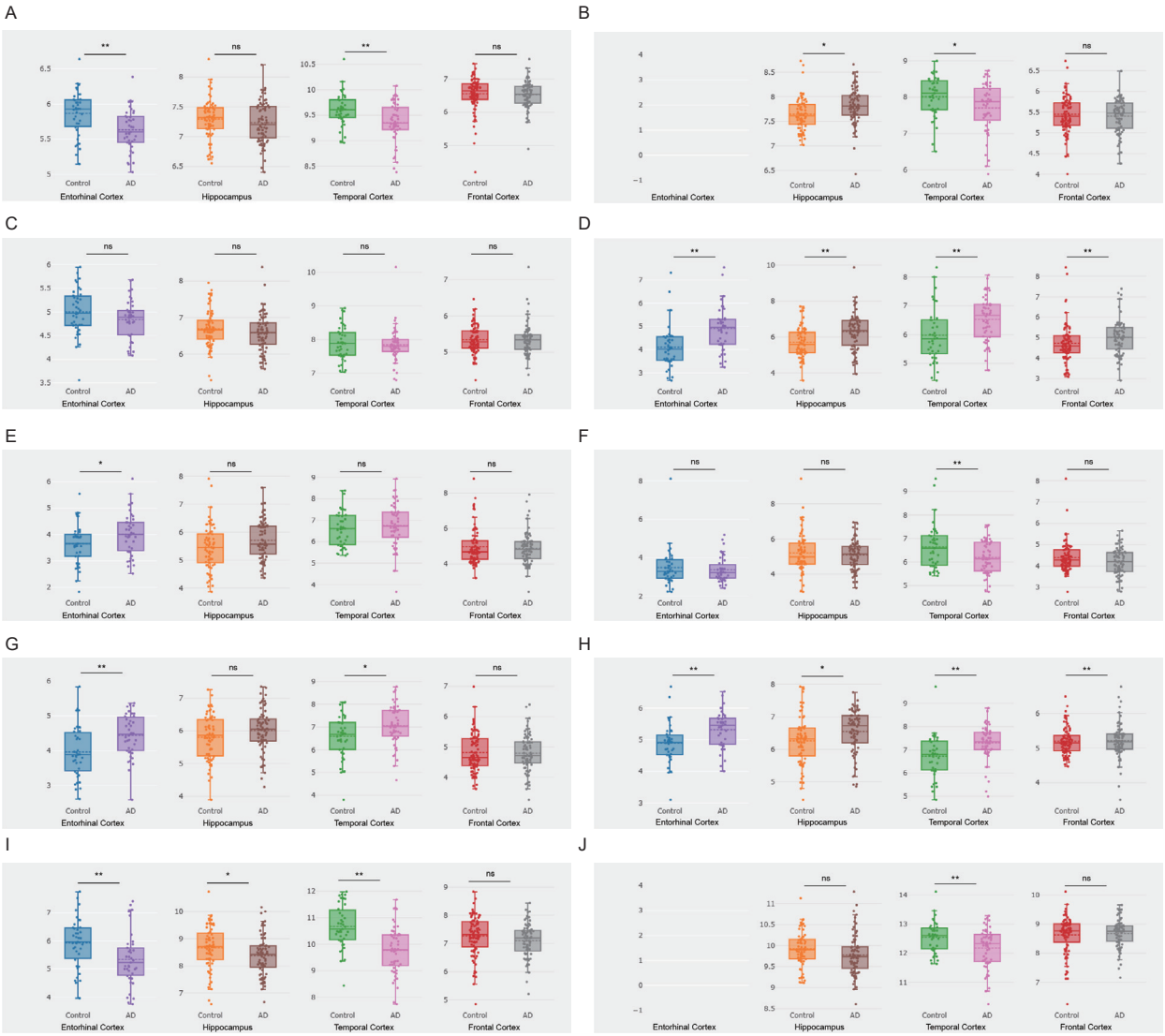

Figure 5. The expression levels of hub genes in different brain regions. Cross-platform nomalized expression level of *GRB2* **(A)**, *PTPN11* **(B)**, *STAT1* **(C)**, *CXCR4* **(D)**, *KDR* **(E)**, *PTGS2* **(F)**, *TLR4* **(G)**, *NFKB1* **(H)**, *HSP90AB1* **(I)**, *HSP90AA1* **(J)**.

Table 2. CFG ranking and evidence for target genes in Alzheimer's disease

Gene	eQTL	GWAS	PPI	Early_DEG	Pathology cor (Aβ)	Pathology cor (tau)	CFG
HSP90AA1	0	0	APP, PSEN2, MAPT, APOE	NA	NA	NA	1
HSP90AB1	4	0	APP, PSEN1	NA	NA	NA	2
NFKB1	1	0	APP, PSEN2, MAPT	NA	0.731,***	0.247, ns	3
TLR4	2	0	PSEN2	NA	NA	NA	2
PTGS2	2	0	APP, PSEN1, MAPT, APOE	NA	NA	NA	2
KDR	0	0	APOE	NA	0.064, ns	0.456, ns	1
CXCR4	3	1	APP, APOE	no	0.104, ns	0.490, ns	3
STAT1	0	0	APOE	NA	0.789,***	0.490, ns	2
PTPN11	0	3	APP, PSEN1, MAPT, APOE	yes	0.175, ns	-0.258, ns	3
GRB2	5	0	APP, PSEN1, PSEN2, MAPT, APOE	NA	0.110, ns	0.138, ns	2

Notes: CFG (convergent functional genomics): The CFG score indicates the functional relevance of a target gene based on various lines of evidence. Each significant piece of evidence contributes one point to the total CFG score, ranging from 0 to 5. Early_DEG: Indicates whether the target gene is differentially expressed in the early stages of AD. "yes" denotes differential expression, "no" indicates no significant differential expression, and "NA" indicates a lack of available data. eQTL: Indicates whether the expression of a target gene is regulated by genetic variants associated with Alzheimer's disease (AD). Genetic variant data are derived from the IGAP GWAS, with significance thresholds set at GWAS $P < 1E-3$ and eQTL $P < 1E-3$. GWAS: Represents a direct association between the target gene and AD in GWAS studies ($P < 0.05$). If a significant association is observed, the value is marked as 1; otherwise, it is 0. Pathology cor (Aβ): Refers to the correlation between target gene expression and AD pathology in Aβ mouse models. "ns" denotes non-significant correlation ($P > 0.05$), ***$P < 0.001$). Pathology cor (tau): Refers to the correlation between target gene expression and AD pathology in tau mouse models. "ns" denotes non-significant correlation ($P > 0.05$). PPI (protein-protein interaction): Indicates significant physical interactions ($P < 0.05$) between the target gene and key AD-related proteins, including APP, PSEN1, PSEN2, APOE, and MAPT. Interacting proteins are listed if a significant interaction is observed.

Figure 6. Expression levels of hub genes in single brain cells. Expression level of *GRB2* **(A)**, *PTPN11* **(B)**, *STAT1* **(C)**, *CXCR4* **(D)**, *KDR* **(E)**, *PTGS2* **(F)**, *TLR4* **(G)**, *NFKB1* **(H)**, *HSP90AB1* **(I)**, *HSP90AA1* **(J)** in single brain cells. OPC, oligodendrocyte precursor cell.

Single-cell expression analysis revealed distinct patterns of hub gene expression across various brain cell types, including neurons, microglia, astrocytes, and oligodendrocytes. These patterns underscore the potential involvement of these genes in AD pathology.

Specifically, the expression levels of different genes in oligodendrocyte precursor cells (OPCs) showed significant variability (Figure 6). Genes such as *HSP90AA1*, *HSP90AB1*, *PTGS2*, and *KDR* exhibited low expression, while *STAT1* and *TLR4* were moderately expressed (Figures 6C, 6E-6G, 6I, 6J). In contrast,

NFKB1, *CXCR4*, and *PTPN11* demonstrated high expression levels in OPCs, suggesting their potential roles in neuroinflammation and immune regulation (Figures 6B, 6D, 6H). Additionally, *GRB2* displayed consistently stable expression levels across OPCs and other cell types, highlighting its ubiquitous regulatory function (Figure 6A).

For endothelial cells, which are critical components of the brain vascular system, gene expression patterns directly influence the functional integrity of the blood-brain barrier (BBB). Analysis revealed high expression levels of *KDR*, *HSP90AA1*, and *HSP90AB1* in endothelial cells (Figures 6E, 6I, 6J). Moderate expression levels were observed for *TLR4*, *CXCR4*, *PTPN11*, and *GRB2*, whereas *NFKB1*, *STAT1*, and *PTGS2* exhibited low expression (Figures 6A-6D, 6F-6H).

4. Discussion

AD is a severe chronic neurodegenerative disorder characterized by memory loss, cognitive decline, behavioral and emotional abnormalities, and ultimately, the inability to perform daily activities independently (*7*). Despite significant advances in AD research, the precise mechanisms underlying its pathogenesis remain elusive. Studies have identified several critical factors contributing to AD progression, including neuroinflammation, Aβ plaque accumulation, tau protein tangles, oxidative stress, and BBB dysfunction (*3*).

Given the complexity of these interconnected pathological mechanisms, traditional single-target therapies have shown limited effectiveness. Consequently, multi-target therapeutic strategies have emerged as a promising approach for combating AD. In this study, we explored the potential mechanisms by which agarwood may modulate key AD-related pathological pathways, particularly in neuroinflammation, neuroprotection, and vascular function regulation.

Neuroinflammation is widely recognized as a central driver of AD onset and progression (*8*). Chronic activation of microglia, astrocytes, and OPCs leads to the release of pro-inflammatory cytokines, chemokines, and ROS. This toxic microenvironment exacerbates neuronal damage and disrupts synaptic function, further contributing to cognitive decline (*9*).

NFKB1 is a pivotal regulator of neuroinflammation (*10*). Studies have shown that its overexpression is closely associated with Aβ accumulation and upregulation of pro-inflammatory genes in AD pathology (*11*). Our findings indicate that NFKB1 is highly expressed in OPCs, suggesting its involvement not only in inflammation signaling but also in amplifying immune responses, which may impact myelin repair processes (*12*). Interestingly, NFKB1 exhibits a dual role: while it contributes to the inflammatory cascade, it may also participate in resolving inflammation by promoting anti-inflammatory pathways. This duality underscores its potential as a therapeutic target. Modulating NFKB1 activity could reduce inflammation while preserving neuronal function, offering a balanced approach to mitigating AD pathology.

STAT1 plays a critical role in promoting the pro-inflammatory (M1) phenotype of microglia and is closely linked to Aβ and tau pathology (*13,14*). In our study, moderate STAT1 expression was observed in both endothelial cells and OPCs, indicating its involvement in immune regulation and vascular function. Agarwood-derived compounds may inhibit excessive STAT1 activation, thereby reducing the expression of iNOS and COX2, key mediators of oxidative stress and neuroinflammation (*15,16*). Moreover, the antioxidant properties of agarwood could further alleviate STAT1-mediated neurotoxicity by mitigating ROS levels. This dual anti-inflammatory and antioxidant effect highlights the potential of agarwood-derived compounds in addressing the multifaceted pathology of AD.

TLR4 is a key pattern recognition receptor that detects damage-associated molecular patterns (DAMPs) such as Aβ (*17,18*). Persistent activation of TLR4 amplifies neuroinflammation and impairs endothelial function, contributing to BBB dysfunction. In our study, moderate TLR4 expression was detected in both OPCs and endothelial cells, suggesting its role as an innate immune sensor involved in amplifying inflammatory signaling (*19*). Agarwood-derived compounds, particularly terpenoids, have demonstrated inhibitory effects on TLR4 signaling (*20*). By suppressing TLR4 activation, these compounds may limit chronic inflammation while preserving the structural and functional integrity of vascular and neuronal systems. This suggests that agarwood has potential as a multi-functional therapeutic agent for mitigating TLR4-driven pathology in AD.

BBB dysfunction is a critical pathological feature of AD. In AD, increased BBB permeability allows peripheral immune cells and toxic molecules to infiltrate the central nervous system (CNS), exacerbating neuroinflammation and neuronal damage. This study identified several key genes, including *KDR* (*VEGFR2*), *HSP90* family proteins, and *TLR4*, that play essential roles in regulating BBB integrity and vascular function.

KDR, a key receptor in the VEGF signaling pathway, promotes angiogenesis and endothelial cell survival under normal conditions(*21*). However, in AD, aberrant VEGF-KDR signaling has been associated with pathological angiogenesis, contributing to BBB dysfunction (*21,22*). This study suggests that agarwood-derived compounds may regulate KDR activity, restoring normal angiogenesis, reducing vascular leakage, and enhancing BBB functional integrity.

HSP90 family proteins, including HSP90AA1 and HSP90AB1, are highly expressed in endothelial cells and play critical roles in mitigating neurotoxicity by

facilitating the autophagic clearance of misfolded proteins such as Aβ and tau (*23,24*). Agarwood's antioxidant and anti-inflammatory properties may synergize with HSP90 family proteins, enhancing its ability to eliminate toxic proteins while minimizing potential adverse effects. This dual action highlights the therapeutic potential of agarwood in protecting endothelial cells from the pathological stressors associated with AD.

Activation of TLR4 in endothelial cells increases the activity of matrix metalloproteinases (MMPs), which degrade tight junction proteins and further compromise BBB integrity (*25*). This process facilitates the infiltration of peripheral inflammatory mediators into the CNS, aggravating neuroinflammation. Agarwood-derived compounds have been shown to inhibit TLR4-related pathways, effectively reducing endothelial inflammation and maintaining BBB barrier function. This suggests that agarwood could play a protective role in preserving vascular and neuronal homeostasis in AD.

OPCs play a pivotal role not only in myelin repair but also in immune regulation (*26,27*). The high expression of *PTPN11* is particularly critical for the differentiation of OPCs into mature oligodendrocytes, a process essential for restoring myelin integrity (*28*). However, in the inflammatory microenvironment of AD, the proliferation, migration, and differentiation of OPCs are significantly impaired (*29*). Agarwood-derived compounds may enhance PTPN11 activity, promoting myelin repair and restoring neuronal signal transmission. Furthermore, OPCs exhibit increased expression of genes involved in antigen processing and presentation through the major histocompatibility complex (MHC)-II pathway. OPCs expressing MHC-II contribute to the activation of memory CD4+ T cells (*30*). In this study, high expression levels of *NFKB1*, *CXCR4*, and *PTPN11* were observed in OPCs, indicating their dual roles in AD-related inflammatory and reparative pathways.

During embryonic development, OPCs are positioned between perivascular cells and neuroglial cells, directly contributing to the formation of the BBB (*31*). Additionally, OPCs regulate the proliferation of perivascular cells and influence the expression of functionally relevant proteins in endothelial cells by releasing regulatory factors (*32*). These findings suggest that OPCs are not only critical for myelin formation and repair but also play a key role in vascular regulation and the maintenance of neurovascular unit homeostasis. This dual functionality underscores the potential of OPCs as therapeutic targets in AD and highlights the importance of agarwood-derived compounds in supporting their protective and reparative roles.

This study, through the construction of drug-target-disease and PPI networks, elucidates the potential mechanisms by which agarwood active compounds may act on multiple targets to influence the progression of AD. The active compounds in agarwood not only target core genes related to neuroinflammation, such as *NFKB1* and *STAT1*, but also demonstrate significant effects in regulating cholinesterase activity and mitigating oxidative stress (*33*). These multi-target actions suggest that agarwood may offer advantages over traditional single-target drugs in AD therapy. By acting through various synergistic mechanisms, agarwood compounds have the potential to alleviate neuroinflammation, inhibit pathological protein aggregation, and improve cognitive function, providing a multidimensional therapeutic strategy for AD.

Despite the promising findings, this study has several limitations that should be acknowledged. First, the study primarily relies on database-driven and bioinformatics analyses, with data sourced from multiple public databases, including TCMSP, GeneCards, STRING, and AlzData. While these databases are supported by extensive data and robust quality control measures, they are still subject to biases and inconsistencies arising from data origin, platform differences, and ongoing updates in gene annotations. Although the databases provide a preliminary framework for exploring the connections between agarwood compounds and AD-related targets, the results lack direct experimental validation. Thus, the reliability of these findings must be further confirmed through *in vitro* and *in vivo* studies.

Second, this study does not fully address the complexity of agarwood components or the pharmacokinetic and pharmacodynamic characteristics of these compounds *in vivo*. Although initial screening of active compounds was conducted using criteria such as OB and DL, these standards may not comprehensively predict the metabolic processes and ultimate biological effects of these compounds in living organisms. Agarwood contains numerous active compounds that may produce metabolic byproducts or interact with each other *in vivo*, and these factors were not extensively examined in this study.

To overcome these limitations, future research should involve animal models and clinical trials to thoroughly investigate the metabolism, bioavailability, and pharmacological effects of individual agarwood compounds as well as their interactions. These studies will provide a more detailed understanding of how agarwood functions as a therapeutic agent and ensure the translatability of these findings to clinical applications.

5. Conclusion

This study, through network pharmacology analysis, PPI network analysis, and single-cell expression analysis, reveals the potential multi-target mechanisms of agarwood in the treatment of AD. Agarwood compounds may modulate key inflammatory factors such as NFKB1, STAT1, and TLR4 to alleviate neuroinflammation; they may enhance expression of HSP90 family proteins and regulate the KDR signaling pathway to improve BBB function; and they may promote the activity of PTPN11

and CXCR4, supporting the repair and remyelination functions of OPCs, providing a multi-dimensional approach for AD therapy. These findings not only offer significant theoretical support for agarwood as a candidate drug for AD treatment but also point the way for the development of novel multi-target therapeutic strategies. With its unique natural medicinal advantages, agarwood has the potential to become an emerging therapy for AD, offering tangible benefits to patients.

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