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Using network pharmacology and molecular docking to investigate the mechanism of action of the active components of agarwood moxibustion therapy for the treatment of chronic atrophic gastritis

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Abstract: Chronic atrophic gastritis (CAG) is a long-term inflammatory condition of the gastric mucosa characterized by glandular atrophy, intestinal metaplasia and reduced acid secretion, often considered a precancerous lesion of the stomach. This study utilized a combined network pharmacology and molecular docking approach to elucidate the mechanisms of moxibustion therapy against CAG. Disease-related targets were retrieved from GeneCards, OMIM, PharmaGkb, TTD and DrugBank, while moxibustion's active components and their targets were sourced from TCMSP database. Overlapping targets between the drug and disease were identified, representing potential therapeutic targets. Cytoscape was employed to construct component-target networks and R was used for GO and KEGG enrichment analyses. STRING database facilitated protein-protein interaction network construction and identification of key targets. Molecular docking, via AutoDock, validated component-target interactions. Eighteen common targets were identified from 379 drug targets and 361 disease targets, linked to 18 active components. Topological analysis pinpointed TP53, IL-1β, PTGS2, CXCL8, CASP8 and STAT1 as crucial targets. KEGG enrichment revealed involvement of TNF-κB, p53, IL-17 and Toll-like receptor signaling pathways. Molecular docking confirmed stable binding between key components and targets. These findings suggest that moxibustion's therapeutic effects on CAG are mediated through modulation of immune, inflammatory and tumor-related pathways.

Keywords: Chronic atrophic gastritis, agarwood moxibustion, agarwood, network pharmacology

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INTRODUCTION

Chronic atrophic gastritis (CAG), is a prevalent gastrointestinal conditions and recognized precancerous lesion for gastric cancer, particularly intestinal adenocarcinoma (Seeneevassen, Bessède, Mégraud, Lehours, Dubus *et al.,* 2021). It is pathologically characterized by the loss of gastric glandular cells, thinning of the gastric mucosa and intestinal metastasis, often resulting from prolonged Helicobacter pylori infection or autoimmune gastritis (Waldum and Fossmark, 2021). Clinically CAG presents with nonspecific and persistent symptoms, including epigastric discomfort, distension, eructation, anorexia and fatigue, which substantially affect patient comfort (Tong *et al*., 2021). Giving its association with high risk of gastric cancer and its chronic progressive nature, early intervention and long term management of CAG are crucial. Emerging evidence has highlighted that the potential role of Traditional Chinese Medicine (TCM) and functional foods could be used as a complementary approach in the management of numerous pathological disorders including CAG and inflammatory conditions (Arain *et al*., 2024; Saeed *et al*., 2021). TMC formulations which typically incorporated multi-component herbal prescriptions, have demonstrated the ability to alleviate gastrointestinal symptoms, modulate gastric inflammation and possibly reverse precancerous lesions by targeting various molecular mechanisms, involving immune regulation, anti-inflammatory effects and modulation of gastric microbiota (Du *et al*., 2024; Rehman *et al.,* 2025; Shahrajabian, 2021). Despite these promising results, the existing research are limited to small sample size, inconsistent methodologies and unpredictable mechanistic exploration. Consequently, large scale randomize clinical trials with standardized diagnostic therapeutic criteria are essential to validate the clinical efficacy of TCM and uncover its underlying biological mechanisms in the treatment of CAG (Ho, 2022).

Agarwood moxibustion therapy is a traditional folk therapeutic practice rooted in the cultural heritage of the Li ethnic group in China. As a specialized form of moxibustion, this therapy primarily incorporates two traditional Chinese medicine groups including agarwood (*Aquilaria sinensis*) and mugwort (*Artemisia argyi*), also

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known as Ai Ye. Agarwood a rare and valuable resinous product formed in response to fungal or microbial infection of *Aquilaria* tree, is recognized not only as traditional medicine, but also used as a classic aromatic spice in culinary tradition. According to the *Pharmacopoeia* of the People's Republic of China (2020 edition), agarwood is traditionally used to regulate qi, alleviate asthma, control vomiting and relieve pain (Alamil *et al*., 2022). It is frequently prescribed for conditions such as hiccups, epigastric discomfort, stomach cold-induced gastrointestinal distress, vomiting and distension of the chest and abdomen (Wang *et al.,* 2021). Recent pharmacological studies support these traditional uses and have further identified multiple active constituents in agarwood, including sesquiterpenes, chromones and flavonoids, which exert therapeutic effects against several pathophysiological conditions (Huo *et al*., 2017). Furthermore, agarwood exhibits a wide range of pharmacological properties, including anti-inflammatory, analgesic, antitumor and antibacterial, making it a promising candidate for treating chronic and inflammatory disease. Mugwort, a member of the Asteraceae family, complement agarwood in this therapeutic modality. In TCM mugwort is characterized by its warm and arid nature with therapeutic potential against cold congestion, restrict bleeding and relieve pain (Xu *et al*., 2021). Given the complementary of agarwood and mugwort, represents a holistic therapeutic strategy, that integrate the warming and anti-inflammatory properties of both herbs. This therapy hold potential for the management of chronic inflammatory disease like CAG. However, despite its historical use the underlying molecular mechanism and pharmacodynamics basis of agarwood moxibustion in the treatment of CAG remain poorly understood.

Network pharmacology is an emerging discipline in system biology that integrates concepts and methodologies from computer science, molecular biology, pharmacology and bioinformatics to explore the complex interactions among drug targets and disease pathways (Arain *et al.,* 2024; Safdar *et al*., 2024). This holistic approach shifts the traditional "one-drug one-target" paradigm toward a multi-component multi-target and multi-pathway" model, which better reflects the multifactorial nature of disease processes and the pharmacodynamics complexity of traditional medicine (Guo *et al.,* 2020; Yuan *et al*., 2022). This study, aimed to employ the principles and methodologies of network pharmacology to investigate the therapeutic mechanisms of agarwood moxibustion therapy in the treatment of CAG. Specifically, we aim to identify and evaluate the active chemical constituents and their corresponding molecular targets, as well as to delineate the signaling pathways potentially involved in mediating the therapeutic effects. By constructing and analyzing the interaction networks, this research seeks to provide mechanistic insights and a scientific foundation for the clinical application and further development of agarwood moxibustion-based therapies for CAG. Ultimately, our findings may contribute to the advancement of integrative medicine and the rational design of novel therapeutic strategies rooted in traditional practices.

MATERIALS AND METHODS

Screening active agarwood moxibustion compound and therapeutic targets

For systematic evaluation of bioactive constituents of agarwood and mugwort we utilized Traditional Chinese Medicine System Pharmacology database and analysis platform (TCMSP https://tcmspw.com/tcmsp.php). The search terms agarwood and mugwort were used to retrieved the comprehensive chemical composition of both herbs. In accordance with standard pharmacokinetics screening criteria, potential active compounds were filtered based on oral bioavailability (OB) and drug-likeness (DL), two key parameters commonly employed in network pharmacological studies to assess the likelihood and in vivo efficacy and drug ability. Specific compounds with OB $\geq$ 30% and DL $\geq$ 0.18 were retained for further analysis consistent with previous methodological framework used to identify pharmacological relevant ingredients in TCM (Zhang *et al*., 2020). All known and predicted targets of the selected compounds were obtained from the TCMSP database. The drug target associations were processed and filtered using R programing scripts to refine the list based on relevance and redundancy. To facilitate downstream functional annotation and enrichment analysis, the target proteins were then standardized to recognize the targeted gene by using UniProt Database (http://www.uniprot.org/).

Identification of chronic atrophic gastritis associated targets

To identify potential therapeutic targets associated with CAG, we conducted extensive search across multiple reputable disease related database including GeneCards (https://www.genecards.org/), Online Median Inheritance in Man (OMIM) (https://omim.org/), DrugBank (https://www.drugbank.cn/), Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) and PharmGkb (https://www.pharmgkb.org/) (Stelzer *et al*., 2016; Wishart *et al*., 2018). After collecting the dataset, all retrieved targets were processed using the R language "Venn" package to integrate data from multiple sources, thereby enhancing reliability and minimizing the false positives. Duplicate entries were removed to ensure the non-redundant list of CAG-related targets. Additionally, chemical components that did not match any disease associated targets were excluded to refine the dataset and focused on biological relevant interactions (Raj, 2019).

Forecasting the mechanisms of action of active ingredients in agarwood moxibustion therapy for the treatment of CAG

To elucidate the potential molecular mechanism through which agarwood moxibustio therapy exerts therapeutic effects against chronic CAG, a network pharmacological approach was employed. Initially, disease related genes

associated with CAG were retrieved from publicly available biomedical databases such as GeneCards, OMIM and DisGeNET, ensuring comprehensive coverage of the genetic landscape underlying CAG pathology. To visualize and analyze the complex interrelationships among traditional Chinese medicines, their active compounds and potential therapeutic targets, a "Traditional Chinese Medicine-Active Ingredients-Target Genes" network was constructed. This was performed using R statistical software (version 4.3.1), enabling efficient data integration and preprocessing (Hopkins, 2008; Li Shao and Zhang Bo, 2013).

Construction of protein-protein interaction networks and core target identification

To elucidate the molecular interactions among the predicted therapeutic targets a protein-protein interaction (PPI) network was constructed. The targeted genes were submitted to the STRING database (https://string-db.org/cgi/input.pl), a widely used platform for predicting and visualizing protein associations based on experimental data, computational prediction and public text collection (Szklarczyk *et al*., 2019). The species was sent to *Homo Sapiens* with a confidence score threshold at the default medium level (0.4). The resulting PPI network was imported into cytoscape version (version 3.9.1). To identify key regulatory proteins within the network, topological parameters including degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), network centrality (NAC) and local average connectivity (LAC), were calculated using the CytoNCA plugin. These parameters help to quantify the topological importance of individual protein nodes within the network (Tang *et al.,* 2015).

Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment and gene ontology analyses

CAG pathway enrichment analysis were performed using R software (version 4.3.1). The analysis employed several widely used bioinformatics packages including cluster Profiler, org. Hs.eg.db, enrichplot, ggplot2 and pathview (Luo and Brouwer, 2013; Yu *et al.,* 2012). Gene ontology enrichment analysis was carried out to categorize the gene targets into three functional domains: biological processes (BPs), cellular components (CCs) and molecular functions (MFs). The top ten significantly enriched items in each category were visualized using bar plots to highlight the key functional role of the targeted genes. In parallel KEGG pathway enrichment analysis was carried out to identify the main signaling pathways, potentially modulated by the active compounds in agarwood and mugwort. The top 30 significantly enriched KEGG pathways were visualized using bulb plots to illustrate the degree of enrichment and the number of associated genes.

Molecular docking

The primary molecular structures of the active constituents of agarwood moxibustion therapy were procured from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). These structures were subsequently imported into Chem3D software, for energy minimization using the MM2 force field, ensuring that the molecular confirmation were optimized to their lowest energy state for improved docking efficacy. The three-dimensional (3D) structure of the targeted protein were obtained from the AlphaFold protein structure database (https://AlphaFold.ebi.ac.uk/) (Jumper *et al*., 2021). Furthermore, for the examination of connection between the key targets' and the active ingredients' actions, Autodock Vina software was used to carry out molecularly docking (Trott and Olson, 2010).

RESULTS

Active ingredients and their potential targets in agarwood moxibustion therapy

To identify the bioactive compounds of agarwood moxibustion therapy, a comprehensive screening was conducted using TCMSP database. The screening criteria were set to OB ≥ 30% and DL ≥ 0.18, as these thresholds are commonly used to ensure pharmacokinetic relevance and structural suitability for drug development table 1. This screening yield a total of 18 candidates' active ingredients, comprising 9 compounds derived from mugwort leaf and 9 from agarwood. The details of these bioactive substances are shown in table 2. Subsequently, target prediction analysis identified a total of 379 putative protein targets associated with these active ingredients. Specifically, 192 targets were linked to agarwood derived compounds, while 188 were associated with constituents from mugwort leaf. After removing duplicates and standardizing gene nomenclature using the UniProt Knowledgebase, a final list of 139 unique human target genes was obtained.

Chronic atrophic gastritis disease-related targets

Identification of potential therapeutic targets associated with cag, five authoritative disease related databases, were used, including genecards, omim, pharmgkb, drugbank and ttd. The data retrieval process yielded 294 from genecards, 51 from omim, 25 from pharmgkb, 18 from drugbank and 1 from ttd. After integrating the dataset duplicate entries were systematically removed using the R software (version 4.3.1) and retained only gene targets. Following this deduplication and integration process, a total of 361 non-redundant genes assosiated with cag were identified. The distribution of these targets across five databases and their interaction are visually represented in the venn diagram (fig. 1), highlighting both database-specific and overlapping targets.

Analysis of the active ingredients in agarwood moxibustion therapy and network construction for the treatment of CAG

To understand the molecular mechanism of action underlying the agarwood moxibustion therapy against CAG, a network pharmacology approach was employed,

Table 1: Identification of active ingredients of the agarwood and mugwort using TCMSP database

Traditional Chinese Medicine	Molecule Number	Active ingredient	OB (%)	DL
Agarwood	MOL010495	6,7-dimethoxy-2-(2-phenylethyl)chromone	31.93	0.30
Agarwood	MOL000358	Beta-sitosterol	36.91	0.75
Agarwood	MOL010917	Boldine	31.18	0.51
Agarwood	MOL010913	C09495	77.09	0.25
Agarwood	MOL010496	DMPEC	32.38	0.39
Agarwood	MOL010907	Norboldine	40.92	0.46
Agarwood	MOL010916	Nubigenol	42.55	0.19
Agarwood	MOL000098	Quercetin	46.43	0.28
Agarwood	MOL000359	Sitosterol	36.91	0.75
Mugwort	MOL000098	Quercetin	46.43	0.28
Mugwort	MOL005735	Dammaradienyl acetate	44.83	0.83
Mugwort	MOL000449	Stigmasterol	43.83	0.76
Mugwort	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
Mugwort	MOL001494	Mandenol	42.00	0.19
Mugwort	MOL005720	24-Methylenecyloartanone	41.11	0.79
Mugwort	MOL005741	Cycloartenol acetate	41.11	0.8
Mugwort	MOL000358	Beta-sitosterol	36.91	0.75
Mugwort	MOL002883	Ethyl oleate(NF)	32.40	0.19

Table 2: Using active molecules to molecularly dock prospective core targets

Core Targets	Active ingredient	Source of Ingredients	Binding energy (kcal/moL)
TP53	Quercetin	Agarwood/ Mugwort	-5.3
STAT1	Quercetin	Agarwood/ Mugwort	-3.65
IL-1β	Quercetin	Agarwood/ Mugwort	-4.56
CXCL8	Quercetin	Agarwood/ Mugwort	-5.65
CASP8	Beta-sitosterol	Agarwood/ Mugwort	-7.7
CASP8	Quercetin	Agarwood/ Mugwort	-5.67
PTGS2	Quercetin	Agarwood/ Mugwort	-6.87
PTGS2	Beta-sitosterol	Agarwood/ Mugwort	-6.83
PTGS2	6,7-dimethoxy-2-(2-phenylethyl)	Agarwood	-6.81
PTGS2	Boldine	Agarwood	-9.09
PTGS2	DMPEC	Agarwood	-7.39
PTGS2	Norboldine	Agarwood	-5.63
PTGS2	Nubigenol	Agarwood	-5.12
PTGS2	Stigmasterol	Mugwort	-6.12
PTGS2	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)	Mugwort	-6.88
PTGS2	Mandenol	Mugwort	-4.59

by using R software (version 4.3.1). In this technique we identified and integrate the predicted target genes associated with both CAG and active compounds derived from agarwood and mugwort. Comparative analysis revealed a total of 19 overlapping targets that may serve as common molecular mediators. These targets include NOS2, PTGS1, CHRM5, PTGS2, CASP8, EGF, TP53, XDH, SOD1, STAT1, ERBB2, CYP1A2, IL1B, CXCL8, CHEK2, CRP, SPP1, CD40LG and IRF1.

Furthermore, a total of 11 active ingredients were identified as potentially effective constituents in the treatment of CAG, via modulation of these shared targets. To visualized these interactions, a relationship file was assembled and imported into the Cytoscape software (version 3.7.2), which was then used to generate the Traditional Chinese Medicine-Active Ingredient-Target interaction network (fig. 2). In the network diagram, blue nodes represent active ingredients from agarwood and purple nodes denote the target genes involved in the pathogenesis of CAG. Each node corresponds to either an active ingredient or a molecular target, with the size of the node reflecting its degree value of the number of connections present within the network. Higher degree value indicating the key role in the therapeutic network.

Among the identified active ingredients, the following compounds demonstrated a degree value greater than 3, indicating prominent involvement in targeted interactions; mol000098 (quercetin, degree: 17), mol010495 (6,7-

dimethoxy-2-(2-phenylethyl) chromone, degree: 4), mol000358 (beta-sitosterol, degree: 3) and mol010917 (boldine, degree: 3). Notably, quercetin (mol000098) emerged as the most significant compound within the network, exhibiting interaction with 17 targets, underscoring its potential as a key multi-target therapeutic agent. Additionally, both quercetin beta-sitosterol were found to be common constituents of agarwood and mugwort, indicating potential synergistic effects between two herbs in agarwood moxibustion therapy.

Building the ppi network and identifying important targets

The protein-protein interaction network of the possible targets for the treatment of cag with the active ingredients of agarwood moxibustion therapy was obtained by importing the shared targets into the string database, setting the protein species to "*homo sapiens*," and leaving the other parameter values as default, as shown in fig. 3. Subsequently the topological analysis of the ppi network was conducted using the cytonca plugin in cytoscape to assess key network metrics including degree centrality, betweenness centrality and closeness centrality. Based on these criteria, six core hub targets with high centrality scores were identified: tp53, il-1β, ptgs2, cxcl8, casp8 and stat1. These targets are highlighted as yellow color nodes in fig. 4 and are considered to play pivotal role in the therapeutic action of agarwood moxibustion therapy against cag.

GO enrichment and KEGG pathway enrichment analyses

Gene Ontology and KEGG pathway enrichment analyses were conducted to investigate the biological relevance of the active constituents of agarwood and mugwort therapy against CAG. The overlapping target genes were identified based on their interaction with CAG-related targets and GO enrichment was performed using the enrichGO function, by using R software (version 4.3.1) with a significant threshold of $P < 0.01$. A total of 19 overlapping target genes, were enriched across 191 biological processes, 21 molecular functions and no cellular components, implicating involvement in 212 signaling pathways. The top ten GO terms were visualized in a bar graph with the pathway names on the T-axes and the number genes on the X-axis. Key enriched biological processes included response to xenobiotic stimulus, positive regulation of cytokine production, reactive oxygen species (ROS) metabolic processes, lipid export from cells, mononuclear cell proliferation and cellular responses to abiotic stimuli (fig. 5). These functions suggest that the therapeutic mechanism may involve modulation of oxidative stress, immune regulation and cellular adaptation to environmental stimuli. In terms of molecular functions, significant enrichment was observed in tumor necrosis factor receptor superfamily binding, protein phosphatase binding, heme binding, cytokine receptor binding and tumor necrosis factor receptor binding, indicating potential regulation of inflammatory signaling pathways.

A total of 36 significantly enriched signaling pathways were identified through kegg pathway enrichment analysis used for the treatment of cag. The analysis was performed with a significance threshold of $p < 0.01$ and the top 30 pathways were visualized (fig. 6). The x-axis represents the ratio of target genes enriched in each pathway relative to the total number of input genes, while the y-axis lists the pathway names. Notably, key inflammation- and immune-related pathways were prominently enriched, including the p53 signaling pathway, nf-κb signaling pathway, tnf signaling pathway, IL-17 signaling pathway and the toll-like receptor signaling pathway. These pathways are known to regulate cell apoptosis, immune responses and chronic inflammation, all of which are central to the pathophysiology of cag. The results suggest that agarwood-mugwort therapy may exert its therapeutic effects in cag through the coordinated modulation of these critical molecular pathways and target specific proteins fig.7.

DISCUSSIONS

Chronic atrophic gastritis (CAG) is a progressive inflammatory condition of the gastric mucosa characterized by the loss of gastric glandular cells, leading to mucosal atrophy and intestinal metaplasia, which may increase the risk of gastric carcinoma (Wang *et al.,* 2021). It is often associated with *Helicobacter pylori* infection, autoimmune factors and environmental influences contributing to its pathogenesis (Rugge *et al*., 2023). Epidemiological data highlights the significant public health burden of gastritis, with prevalence rate approximately 10% in individuals having age in between 20-50, while 50% prevalence was noted in 51-65 year old population (Weck and Brenner, 2006). While the pathophysiology of CAG remains incompletely elucidated in contemporary medicine, owing to the contributing factors including advanced aged, immune dysregulation, duodenal reflexes and genetic predictions, with *Helicobacter pylori* infection and aging are identifies as primary risk factors (Adamu *et al.,* 2011). In contrast, traditional Chinese medicine (TCM) offers a holistic approach with potentially fewer adverse effects compared to the often singular therapeutic targets and associated toxicities of Western pharmaceuticals (Oh, Adnan and Cho, 2021; Saeed, *et al*., 2024). Agarwood mugwort therapy is an integrative therapeutic approach that synergistically combines the principles of moxibustion, pharmacological effects of medicinal herbs. Moxibustion, a heat-based therapy rooted in traditional Chinese medicine (TCM), has been shown to alleviate clinical symptoms in patients with various chronic conditions, including cancer-related fatigue and gastrointestinal disturbances, by modulating immune responses and enhancing local circulation (Huang *et al*., 2020).

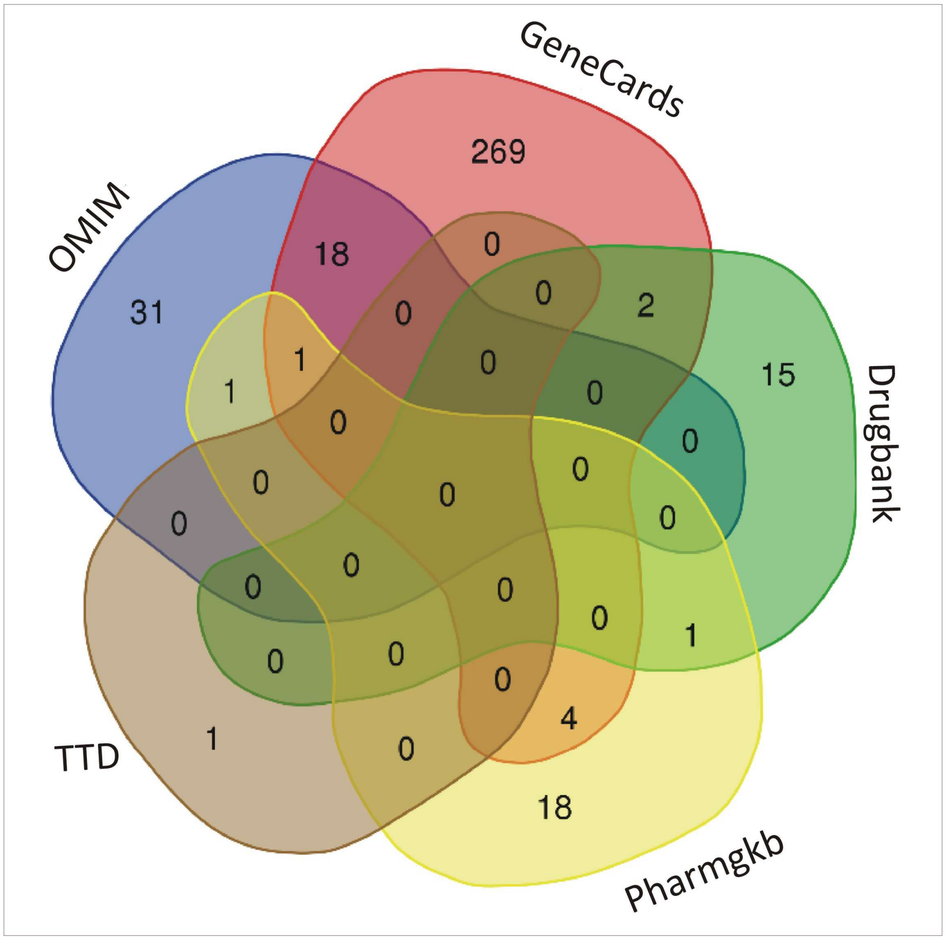

Fig. 1: Venn diagram of CAG-related targets taken and set from 5 disease databases

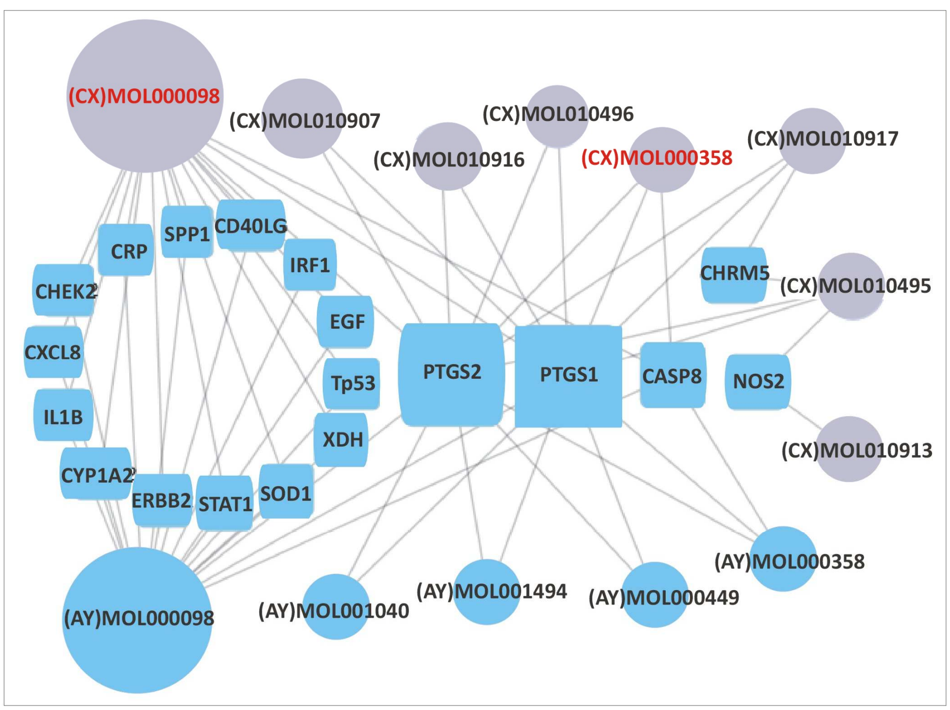

Fig. 2: Interaction network of active compounds of agarwood and mugwort with therapeutic targets of chronic atrophic gastritis.

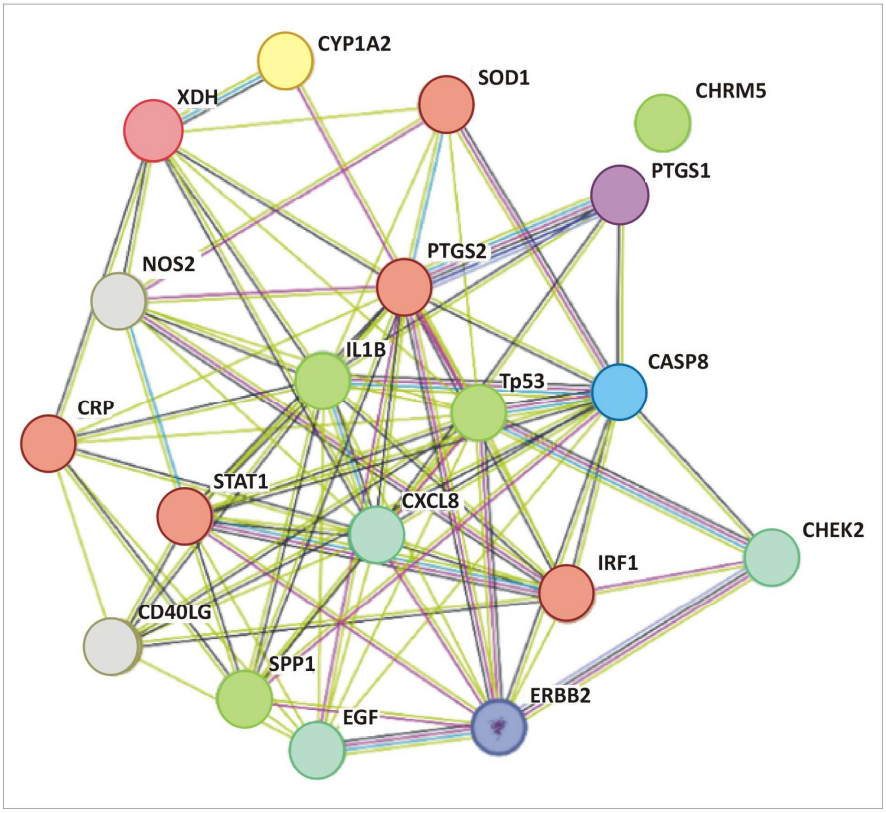

Fig. 3: Protein-protein interaction (PPI) network of potential chronic atrophic gastritis (CAG) targets modulated by the active components of agarwood-mugwort moxibustion therapy.

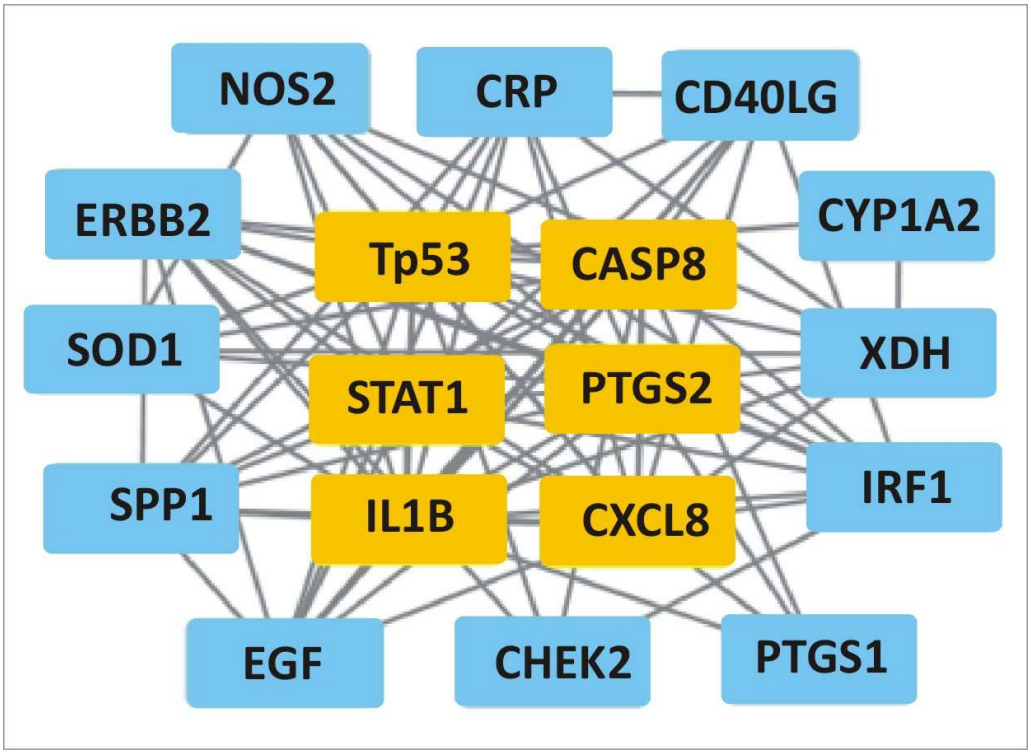

Fig. 4: Network diagram illustrating the interactions among active components, CAG-related targets and signaling pathways involved in agarwood mugwort moxibustion therapy.

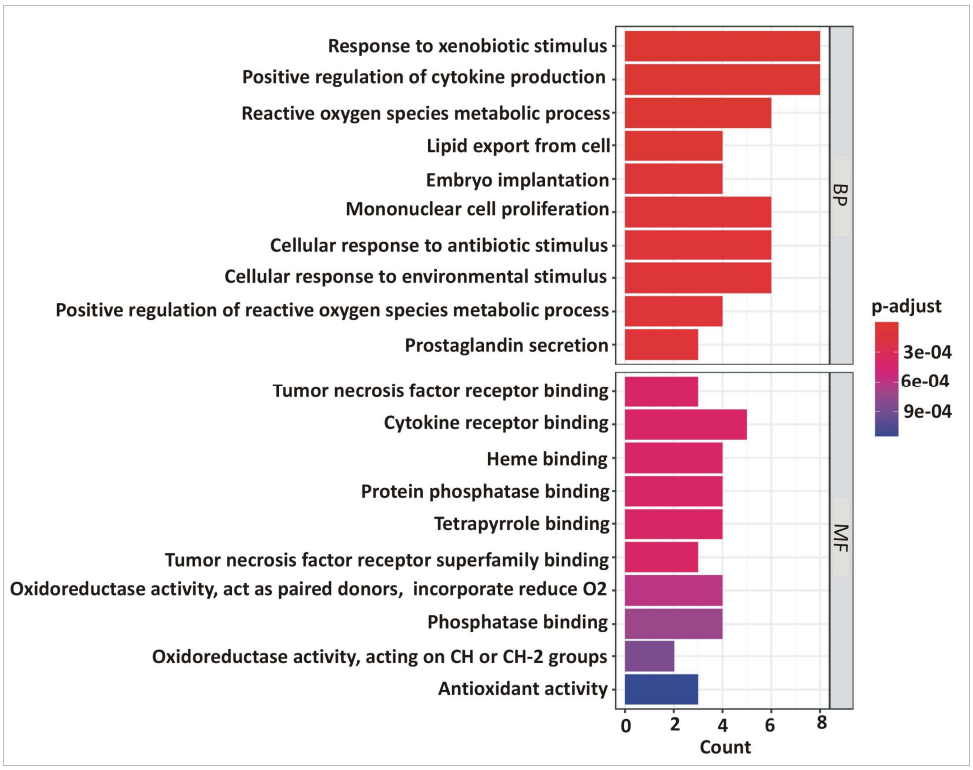

Fig. 5: Gene ontology enrichment analysis of the active components in agarwood-mugwort moxibustion therapy, highlighting potential molecular targets associated with the treatment of chronic atrophic gastritis.

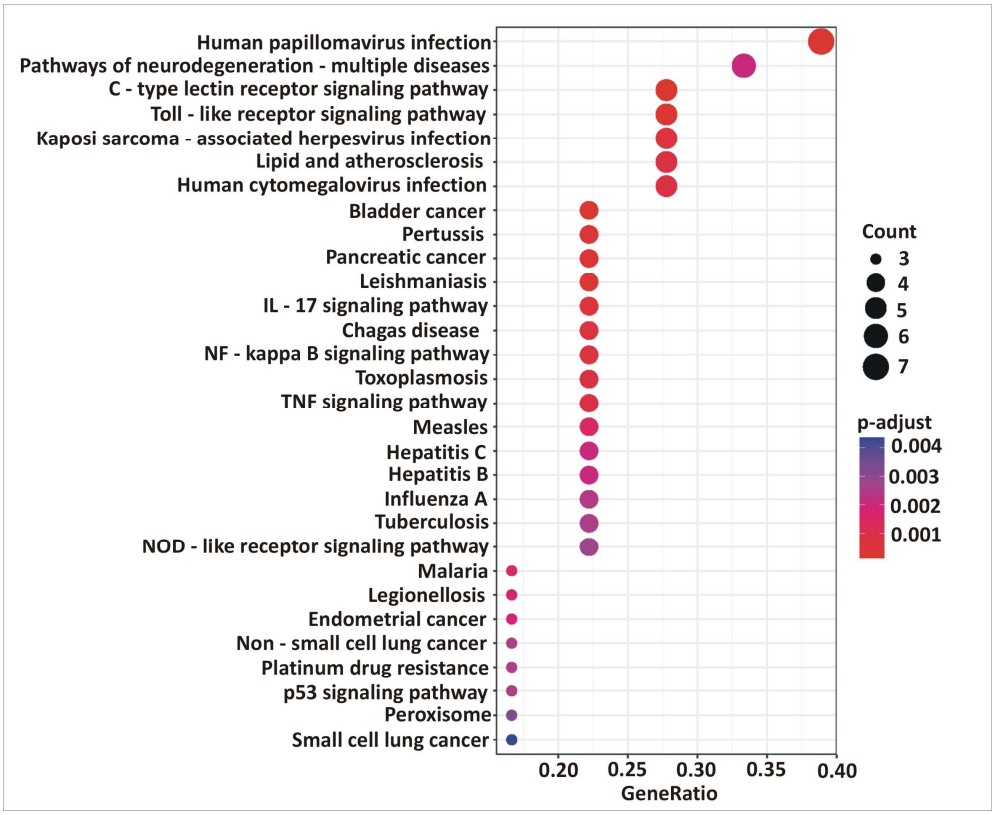

Fig. 6: Active Components and Therapeutic Targets of Agarwood-Mugwort Moxibustion Therapy in the Treatment of Chronic Atrophic Gastritis

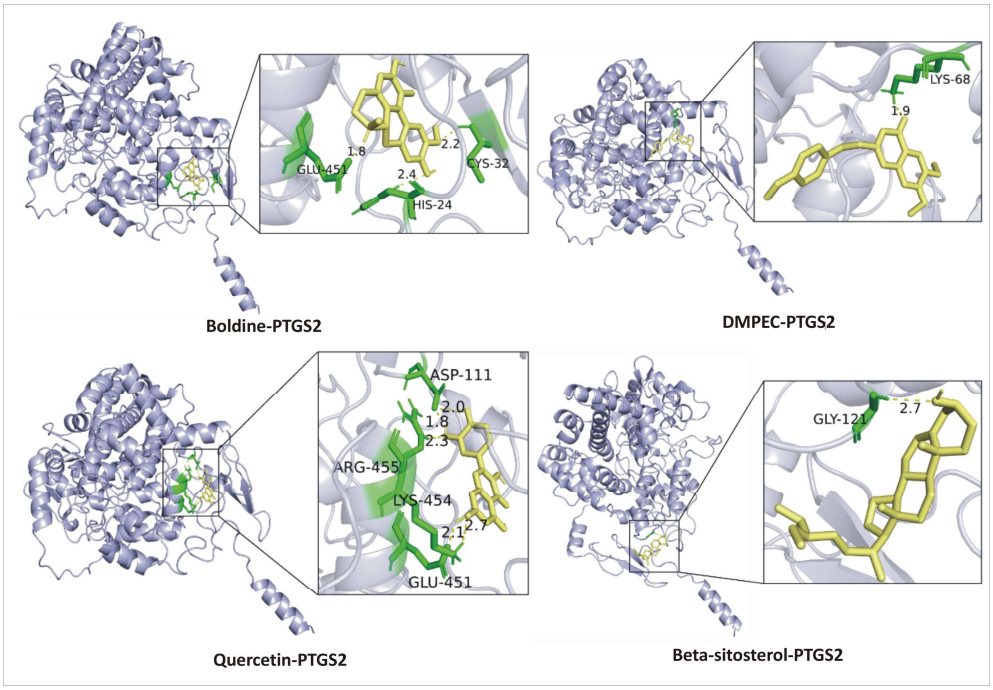

Fig. 7: Top four molecular docking score profiles of key active compounds from the agarwood–mugwort combination targeting the PTGS2 protein.

Present study focused solely on evaluating the therapeutic effects of this traditional treatment in the context of CAG, the findings indicate that the active components in agarwood moxibustion may exert beneficial effects through anti-inflammatory, analgesic and immunomodulatory pathways, as supported by previous pharmacological studies on agarwood and mugwort constituents (Liu *et al*., 2021; Sharifi-Rad, *et al*., 2022). However, important mechanistic aspects such as the specific warming effect, the bioactive composition of moxibustion smoke and the physiological relevance of targeted acupoints were not addressed in this study. These factors are critical in understanding the holistic efficacy of moxibustion, as thermal stimulation and volatile compounds have been shown to influence local circulation, nerve signaling and immune responses (Ding *et al.,* 2021).

Results of current study, demonstrated a total of 18 bioactive compounds 9 derived from agarwood and 9 from mugwort were identified as potentially active agents in the treatment of CAG, via agarwood moxibustion therapy. For this purpose, using network pharmacology and molecular docking approach, 379 putative CAG-related targets were mapped, revealing several core constituents with therapeutic potential. Notably, quercetin, β-sitosterol, 6,7-dimethoxy-2-(2-phenylethyl) chromone and boldine emerged as key compounds. Quercetin, a well-established flavonoid, exhibits broad-spectrum biological activities, including anti-inflammatory, antioxidant, anti-*H. pylori* and gastric mucosal protective effects and has been reported to significantly reduce the risk of gastric adenocarcinoma in epidemiological studies (Lee and Park, 2019; Miao *et al*., 2019; Saeed *et al*., 2017). β-Sitosterol, a phytosterol with proven gastroprotective, anti-inflammatory and anti-tumor effects, enhances gastric juice secretion, scavenges reactive oxygen species and inhibits gastric cancer cell proliferation (Nattagh-Eshtivani *et al*., 2022; Ponnulakshmi *et al.,* 2019). 6,7-Dimethoxy-2-(2-phenylethyl) chromone, a key chromone compound in agarwood, has been shown to attenuate inflammation and oxidative stress and its derivatives are known to modulate immune responses in inflammatory diseases, including gastrointestinal disorders (He *et al.,* 2024). Boldine, another potent alkaloid, has been demonstrated to reduce oxidative stress, inhibit inflammatory mediators and strengthen gastric mucosal defenses, protecting against ethanol and NSAID-induced damage (Boeing *et al*., 2020; O'Brien *et al.,* 2006).

Target analysis identified TP53, IL-1β, PTGS2, CXCL8, CASP8 and STAT1 as critical molecular nodes. TP53, a pivotal tumor suppressor gene, regulates DNA repair, apoptosis and cell cycle arrest and its mutations are early drivers of gastric carcinogenesis (Sahgal *et al*., 2021). IL-1β, a key inflammatory cytokine, activates multiple immune pathways, promotes angiogenesis and contributes to tumor progression by inducing COX-2 and IL-8 expression (Kang *et al*., 2009; Sánchez-Zauco *et al*., 2017). PTGS2 (COX-2), a major regulator of prostaglandin synthesis, plays a critical role in inflammation and is frequently overexpressed in gastrointestinal cancers (Zhu *et al*., 2024). CXCL8 (IL-8), an inflammatory chemokine,

facilitates tumor invasion, metastasis and angiogenesis and its persistent expression correlates with poor gastric cancer prognosis (Piao *et al*., 2022). CASP8, a cysteine protease involved in apoptotic signaling, is associated with malignancy development through abnormal activation in gastric and hepatic carcinomas (Jiang *et al.,* 2021). STAT1, a transcription factor essential for immune response modulation and apoptosis, is implicated in both tumor suppression and progression in gastric malignancies (Li *et al.,* 2022).

Gene Ontology enrichment analysis linked the therapeutic targets to biological processes such as response to environmental stimuli, oxidative stress modulation and monocyte proliferation. Kyoto Encyclopedia of Genes and Genomes pathway enrichment identified 36 significant pathways, including Toll-like receptor, IL-17, TNF, NF-κB and p53 signaling. Toll-like receptors, especially TLR2, TLR4 and TLR5, are crucial in innate immunity and are increasingly expressed in stages of gastric disease, indicating their involvement in gastric inflammation and cancer progression (Lu *et al.,* 2022; Pimentel-Nunes *et al*., 2013). The p53 pathway is central to maintaining genomic stability and preventing malignant transformation in gastric mucosa (Busuttil *et al*., 2014). IL-17 signaling, predominantly activated in *H. pylori* infections, contributes to chronic gastritis through neutrophil recruitment and mucosal inflammation (Dewayani *et al*., 2021). NF-κB is a master regulator of inflammatory gene transcription and a key driver of gastric epithelial transformation under chronic inflammation. TNF-α contributes to gastric pathogenesis by modulating cytokine networks, cell proliferation and tumor angiogenesis (Nabi *et al*., 2020). Collectively, these findings suggest that agarwood-mugwort moxibustion therapy may exert therapeutic effects in CAG by modulating inflammation, enhancing immune defense, preventing oxidative damage and inducing apoptosis via a multi-target, multi-pathway approach. Nevertheless, further validation through *in vitro*, *in vivo* and clinical studies is essential to elucidate the precise pharmacodynamics and therapeutic efficacy of these bioactive components in CAG management.

Despite the comprehensive use of network pharmacology and molecular docking, this study primarily offers a predictive framework, underscoring the need for empirical validation. The identified bioactive compounds and targets were based on in silico analysis, which, while valuable for hypothesis generation, lack direct experimental support. Future studies should prioritize *in vitro* and *in vivo* validation to confirm therapeutic effects on CAG. Additionally, pharmacokinetic and toxicological assessments are essential to establish safety and dosage parameters. Clinical trials, alongside transcriptomic, proteomic and metabolomic profiling, can elucidate underlying mechanisms. Evaluating thermal stimulation and transdermal absorption may further enhance mechanistic understanding.

CONCLUSION

In conclusion, quercetin, β-sitosterol, 6,7-dimethoxy-2-(2-phenylethyl) chromone and bordetine are proposed as the main bioactive compounds in agarwood-mugwort therapy for treating CAG. These compounds target key molecules including, TP53, IL-1β, PTGS2, CXCL8, CASP8, STAT1 and modulate critical signaling pathways, including Toll-like receptor, IL-17, TNF, NF-κB and p53. Through immunomodulation, anti-inflammatory activity and apoptosis regulation, they may exert therapeutic effects in CAG. Further mechanistic studies and clinical validations are necessary to substantiate these findings.

Ethics approval and consent to participate
Not applicable.

Human and animal rights
No animals / humans were used in the studies that formed the basis of this research.

Availability of data and materials
The data used supports the findings of this study and are available from the corresponding author, upon request.

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Conflict of interest
The authors declare no conflict of interest, financial or otherwise.

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