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Case Study

Revealing the antimicrobial potential of traditional Chinese medicine through text mining and molecular computation

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Abstract

Traditional Chinese Medicine (TCM), with its extensive knowledge base documented in ancient texts, offers a unique resource for contemporary drug discovery, particularly in combatting microbial infections. The success of antimalarial drugs like artemisinin and artesunate, derived from the TCM herb *Artemisia annua L.*, exemplifies the potential of TCM-derived small molecules. This rich repository of natural products and intricate molecular structures could reveal novel compounds with unexplored mechanisms of action. Our study employs a multifaceted approach that combines text mining, detailed textual analysis, and modern antibacterial molecular prediction methodologies to unlock the potential of ancient TCM remedies. We use external knowledge maps, which include databases of known bioactive compounds and their targets, to identify promising TCM candidates. This approach leverages both historical texts and contemporary scientific data to explore the therapeutic potential of TCM. We discovered that herb patterns DiYu→ZeXie and Kushen→ShengJiang potentially combat both Grams-positive and Grams-negative bacteria. We utilized the AntiBac-Pred online tool to identify and analyze the chemical components of herbs, integrating data from ancient texts and TCMDB@Taiwan external knowledge graph. The DiYu→ZeXie groups showed antimicrobial potential against resistant *Staphylococcus simulans*, while the Kushen→ShengJiang groups exhibited dual antimicrobial effects against *Bacillus subtilis*. Exploring TCM's extensive repository offers numerous opportunities for discovering therapeutically active compounds. Our synergistic approach, which combines ancient wisdom with modern science, holds significant promise for enhancing our ability to combat infectious diseases. This method could pave the way for a new era of personalized medicine, addressing the urgent need for innovative treatments against multidrug-resistant bacteria and viruses.

Keywords: text mining; drug discovery; anti-microbial TCM; TCM; association rule

Introduction

The global antimicrobial crisis presents an urgent challenge, making the potential of Traditional Chinese Medicine (TCM) in addressing this issue increasingly significant [1]. Excessive antibiotic use (e.g. penicillin, tetracyclines, methicillin, vancomycin, and erythromycin) and stagnated drug development have led to rising drug-resistant infections, threatening public health and economy [2, 3]. Accelerating antimicrobial discovery and policy development is urgent. TCM relies significantly on herbal remedies derived from natural plants, playing a crucial role in its historical and detailed application within treatment methods [4–7]. Recent advancements in modern science and technology have increasingly focused global attention on Chinese medicinal drugs, highlighting their promising applications in antimicrobial, antibacterial, and antiviral therapies [5, 7–9]. Artemisinin, extracted from *Artemisia annua*, an ancient component of Chinese medicine, is renowned for its high effectiveness and low toxicity in treating malaria. The discovery of artemisinin represents a pivotal achievement in Chinese medicine, playing a crucial role in global efforts to prevent and treat malaria [10–12]. Additionally, researchers utilized *A. annua L.* to combat various other microorganisms [13]. This information suggests that herbs still possess significant potential for exploring antibacterial and antiviral agents.

Ancient Chinese medical literature holds a wealth of knowledge for discovering Chinese medicine. These texts document numerous formulas and their therapeutic effects, providing valuable insights for modern antimicrobial drug discovery [5]. However, the archaic language used in these documents often impedes understanding, thereby restricting them from realizing their full potential [14].

In our investigation of potential antibacterial herbal patterns in ancient Chinese medical literature, we propose an approach

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centered on ancient texts. This method integrates text mining, thorough text analysis, and association techniques. Our objective is to discover commonly used combinations of Chinese herbal medicines in traditional texts, potentially unveiling new mechanisms or patterns of herbal co-use. Text mining facilitates extracting information from unstructured or semi-structured data, specifically within ancient Chinese medicine, to identify medicinal formulas and their documented therapeutic effects. Text mining techniques encompass natural language processing (NLP) [15], which analyses human language to identify medicine names, ingredients, and therapeutic indications; information retrieval (IR) [16] which retrieves literature from databases to explore specific formulations or therapeutic effects in TCM; and external knowledge mapping (EKM) [17], which integrates external knowledge maps to elucidate potential mechanisms by defining relationships among herbal patterns, drug components, and target entities. While text mining can pinpoint promising herbal pairs, it does not delve deeply into the biological mechanisms of these combinations. Therefore, comprehensive insights require in-depth textual analyses via integrating EKM [17, 18].

In-depth textual analyses encompass examining modern herbal literature to comprehend traditional theories and practices, thereby elucidating the mechanisms of action of herbal formulations. Furthermore, conducting experimental case studies validates the therapeutic effects of identified herbal pairs and explores their underlying mechanisms.

In this study, we utilized text-mining techniques to extract co-occurring herbal pairs from ancient Chinese medical literature that involve infections or infection-induced diseases. Subsequently, we conducted a detailed bioactive analysis using external databases to identify potential antimicrobial candidates among these herbal pair patterns. Finally, we reviewed relevant literature on antimicrobial experimental research to validate the efficacy of these herbal pairs. Discovering antimicrobial properties in Chinese medicinal herbs poses a significant challenge in TCM, with broad applications. Our comprehensive approach to drug discovery in Chinese medicine aims to uncover potential antimicrobial herbal patterns from ancient texts, providing innovative strategies for combating infections.

Materials and methods

Preparation of infection-related text datasets

Ancient texts often describe using specific herbs and natural products for centuries to treat infections. These traditional remedies may contain compounds with antimicrobial activity that have yet to be fully explored by modern science. Systematically investigating the formulas and treatments documented in *Pu-Ji Fang* to uncover leads for novel antimicrobial agents. This approach is particularly relevant given the increasing prevalence of drug-resistant pathogens and the urgent need for new antimicrobial therapies.

This study investigates the formulation and therapeutic strategies derived from the Ju Chong phylum (諸蟲門) of the ancient Chinese medical text *Pu-Ji Fang*. The Ju Chong phylum encompasses many diseases caused by parasitic worms. In ancient times, healers believed these parasites infected humans and caused issues such as the unfavorable treatment of primary symptoms according to TCM theory, with intestinal parasites being the most prevalent. However, modern literature has demonstrated that parasitic infections predispose hosts to compromised immunity, increasing susceptibility to co-infections with other pathogens [19]. Exploring the historical knowledge contained within the Ju Chong phylum can yield valuable insights for identifying bioactive compounds with potential antimicrobial properties.

To ensure a comprehensive analysis, we examine *Pu-Ji Fang*'s relevant sections on Ju Chong, using original texts, translations, and scholarly commentaries to understand the historical and medicinal context. The integration of modern biomedical resources, including antimicrobial journals and databases such as the Encyclopedia of Traditional Chinese Medicine (ETCM) [17], ChEMBL [20], PASS Online [21], and TCMDB@Taiwan [22], validates the contemporary antimicrobial potential of herbs referenced in ancient Chinese medicine. This multidisciplinary approach bridges ancient knowledge with current biomedical research.

Exploratory analysis of herbal pair combinations using the Apriori algorithm

Expanding on our previous research using the Apriori algorithm to extract association rules from *Pu-Ji Fang* [23], this study delves deeper. To evaluate the quality and relevance of the extracted rules, we employed a three-pronged approach utilizing support, confidence, and lift metrics. This method enabled us to identify statistically significant antimicrobial patterns and relationships within the dataset. We implemented an iterative process to achieve this goal. Initially, we identified frequent herbal itemsets. Then, we evaluated the co-occurrence and independence between the antecedent and consequent elements within the extracted rules. The iterative process continued until the identification of frequent itemsets reached saturation.

Grid search for optimal minimum support threshold in Apriori algorithm

The Apriori algorithm, a fundamental technique in frequent itemset mining, leverages a minimum support threshold to identify frequently co-occurring item combinations within a dataset. However, effectively setting this threshold is critical for optimal pattern discovery; this is where the Grid Search method proves advantageous.

Grid Search systematically evaluates the Apriori algorithm across a predefined range of minimum support values. It achieves this by constructing a grid of candidate values, typically starting from a high threshold and progressively decreasing it. The Grid Search method applies the Apriori algorithm to each value within the grid, allowing it to identify frequent itemsets that meet or exceed the specified minimum support threshold.

Analysing herbal patterns at each threshold level, we observe how the number and characteristics of frequent itemsets evolve, helping us determine the optimal minimum support value. Ideally, this value should filter out uninteresting patterns while capturing the most relevant and frequent item combinations within the *Pu-Ji Fang* dataset.

The Grid Search method adopts a data-driven approach to ascertain the optimal minimum support threshold, ensuring effective extraction of valuable insights by the Apriori algorithm from the dataset. In our research, this method facilitates the identification of optimal patterns involving herbal pairs, determining the minimum support parameter necessary for the most frequent items while maintaining a confidence level of 1.

Systematic quantification of research trends on antibacterial herbs using PubMed E-utilities analysis

We systematically searched PubMed E-utilities to evaluate the level of research interest in antibacterial herbs. Initially, antibacterial herbs were primarily identified through a comprehensive review of relevant literature [9, 24, 25]. These references provided a list of herbs traditionally recognized for their antimicrobial properties. We extracted herb names from these studies and compiled them as search terms.

We then utilized the PubMed E-utilities platform to quantify the number of studies focused on each herbal antibacterial effect. We combined each herbal name with the keyword ‘antibacterial’ to form the search query. This strategy enabled us to identify research articles addressing the herb’s antibacterial properties. By E-utilities API, we automated the search process to ensure consistent results across all herbs. We documented the total number of publications for each herb-antibacterial query, representing the research attention each herb has received. These results were then analyzed to assess the relative research focus and to highlight trends in antibacterial herb studies. This method offers insights into interest and research gaps over recent years.

Mapping potential components

Utilizing herbs identified in the ETCM database, we constructed a chemical target gene network to link these herbs with their respective constituent molecules [17]. Leveraging a network analysis graph implemented in Python, our investigation utilized chemical constituents from the ETCM database to identify potential target genes [26].

Utilizing TCMDB@Taiwan for compound retrieval

We utilized the TCM Database@Taiwan (TCMDB@Taiwan) to obtain a comprehensive list of compounds associated with Chinese herbal medicine [22]. TCMDB@Taiwan is a widely recognized database that curates detailed information on the chemical constituents of Chinese medicinal herbs.

We began by accessing the database and employing its search functionality to query our findings based on *Pu-Ji Fang*’s results of herbal pairs of the Apriori. Each herb’s entry within TCMDB@Taiwan provides a detailed profile, including the identified chemical compounds, their molecular structures, pharmacological activities, and relevant literature references. We extracted these profiles in a standardized format, ensuring all pertinent compound information.

Subsequently, we systematically organized the retrieved chemical names and retrieved the canonical SMILES chemical formulae through the ChEMBL database and stored them for AntiBac-Pred predictive analysis [20, 21]. This structured approach enabled us to comprehensively catalog the compounds, forming the foundation for subsequent research phases, including bioactivity screening and antimicrobial efficacy assessment. Thus, utilizing TCMDB@Taiwan facilitated a robust and methodical gathering of essential phytochemical data for studying Chinese herbal medicine’s therapeutic potential.

Herbal pair identification and functional enrichment analysis

The analysis identified herbal pairs using the Apriori algorithm. The chemical-gene target network derived from the ETCM database established gene sets linking these herbal pairs to their respective components. Functional enrichment analysis in the database for annotation, visualization, and integrated discovery (DAVID) bioinformatics resources utilized these gene sets as the reference background.

The calculation of *P*-values and fold enrichment (FE) scores in DAVID employed over-representation analysis (ORA) and gene set enrichment analysis [18, 27]. For *P*-values, DAVID compares user-supplied differentially expressed genes against a background set, typically all expressed genes, using statistical methods like Fisher’s exact test to assess the significance of enrichment in functional categories [28]. The smaller *P*-value indicates a stronger association between the gene set and the biological process.

Specifically, a smaller *P*-value (e.g. < 0.05) suggests that the observed genes are significantly concentrated in a specific biological process, meaning the association between the gene set and the process is unlikely to be random and is statistically significant. Therefore, a smaller *P*-value implies that the gene set likely plays a critical key role in the biological process, with higher biological relevance.

Additionally, we employed FE as a primary analytical metric in our investigation. FE measures the extent to which a specific gene set is over-represented among differentially expressed genes relative to what would be expected by random chance.

The significance of FE lies in its capacity to reveal biologically relevant gene sets that may play crucial roles in specific pathways or biological processes. By comparing the observed occurrences of genes within a particular set against a null distribution generated through permutation testing, we can evaluate the statistical significance of the enrichment. This methodology enables the identification of gene sets that are both statistically significant and biologically informative, thereby enhancing our understanding of the underlying biological mechanisms.

Case studies

Jin et al. isolated a new triterpenoid [29], alisol Q 23-acetate, along with 14 known terpenes from the rhizomes of Alisma orientale. Using 1D and 2D NMR techniques, they identified the structures of these compounds. Among them, four compounds appealed to antibacterial activity. These four Compounds showed remarkable effectiveness against eight antibiotic-resistant strains, with MIC values of 5–10 μg/ml, which were lower than those of the positive controls, chloramphenicol, and ampicillin (MICs 5–80 μg/ml). Thus, the study highlights the significant antibacterial properties of these compounds.

A study comparing the antibacterial activity of two *Sanguisorba* species, *Sanguisorba officinalis L.* and *Sanguisorba minor Scop.*, from Romanian flora [30]. They utilized HPLC-DAD-MS (ESI+) to analyze the ethanol extracts of these plants. Research indicated that *S. minor Scop.* leaves have a higher phenolic content and demonstrate superior antibacterial activity, particularly against *Staphylococcus aureus*. In contrast, *S. officinalis* L. exhibited lower phenolic content and antibacterial efficacy. The study concludes that the *Sanguisorba* species are more effective in antibacterial applications and may serve as a valuable food supplement.

Results

Exploring herbal pattern relationships in antimicrobial ancient manuscripts of the Ju Chong phylum: Insights from Apriori algorithm results

In Table 1, the Apriori algorithm reveals frequent co-occurrences of herbal pairs, highlighting their relationships in classical Chinese medicinal texts. The support value indicates the proportion of records in which these pairs appear together. Simultaneously, a confidence value consistently equal to 1 indicates a robust association; selecting one herb guarantees the presence of the other. Additionally, the lift metric quantifies the strength of this association, surpassing random chance.

DiYu and ZeXie exhibit the highest lift value of 95.5, underscoring a significant synergistic relationship. Similarly, YiYiZen and ShiLiuZhi follow with a lift of 63.67, further emphasizing their

Table 1. Exploration of ancient texts reveals associative results of herbal patterns in Ju Chong phylum

Antecedents	Consequents	Antecedent support	Consequent support	Support	Confidence	Lift	Leverage	Conviction
DiYu	ZeXie	0.010471204	0.010471204	0.010471204	1	95.5	0.010361558	inf
ZeXie	DiYu	0.010471204	0.010471204	0.010471204	1	95.5	0.010361558	inf
YiYiZen	ShiLiuZhi	0.010471204	0.015706806	0.010471204	1	63.66666667	0.010306735	inf
KuShen	ShengJiang	0.010471204	0.020942408	0.010471204	1	47.75	0.010251912	inf
BaiJiangCan	HouPu	0.010471204	0.02617801	0.010471204	1	38.2	0.010197089	inf
GanJiang	FuZi	0.015706806	0.02617801	0.015706806	1	38.2	0.015295633	inf
KuShen	AiYe	0.010471204	0.02617801	0.010471204	1	38.2	0.010197089	inf
DiYu	ZaoJiao	0.010471204	0.031413613	0.010471204	1	31.83333333	0.010142266	inf
ZhiShi	BaiZhu	0.010471204	0.031413613	0.010471204	1	31.83333333	0.010142266	inf
ZeXie	ZaoJiao	0.010471204	0.031413613	0.010471204	1	31.83333333	0.010142266	inf
KuShen	HuangLian	0.010471204	0.031413613	0.010471204	1	31.83333333	0.010142266	inf
CaiHu	BaiShao	0.010471204	0.036649215	0.010471204	1	27.28571429	0.010087443	inf
ShiHui	QingFen	0.010471204	0.041884817	0.010471204	1	23.875	0.01003262	inf
TaoHua	GouJi	0.010471204	0.047120419	0.010471204	1	21.22222222	0.009977797	inf
CaiHu	DanGui	0.010471204	0.057591623	0.010471204	1	17.36363636	0.009868151	inf

strong co-use. Other pairs like KuShen and ShengJiang (47.75 lift) or GanJiang and FuZi (38.2 lift) demonstrate substantial associations, possibly indicating their complementary therapeutic effects.

Herbal pairs, such as CaiHu and BaiShao (lift = 27.29), as well as ShiHui and QingFen (lift = 23.88), demonstrate moderate yet statistically significant associations. The Apriori analysis provides valuable insights into these co-prescription patterns, facilitating a deeper understanding of traditional formulation structures. By revealing potential synergistic interactions, this analysis offers a foundation for further exploration of the therapeutic strategies employed in classical Chinese medicine.

In the subsequent analysis, we focused on the top three herbal combinations found in the TCM database, excluding unlocatable in TCMDB items such as ShiLiuZhi (pomegranate branch, 石榴枝) and non-herbal materials such as BaiJiangCan (white zombie silkworm, 白殭蠶) from further consideration. This deliberate narrowing of scope enabled a more in-depth and focused investigation of vital herb interactions, ensuring that the analysis remained centered on the most relevant combinations.

Heatmap of antibacterial herbs and Apriori analysis results from PubMed reference counts

Figure 1 presents a heatmap of research references, highlighting common antibacterial herbs alongside the results of our Apriori analysis. This visualization integrates established research with our findings to provide a comprehensive overview of antibacterial herbs and their associations. By mapping the cross-searching herbal keywords with 'antibacterial' words and the strength of herb associations, the heatmap illustrates the alignment between widely recognized antibacterial herbs and the patterns identified through our Apriori algorithm. This integrated approach facilitates a clearer understanding of the relationship between existing research and novel insights derived from our analysis, offering valuable context for evaluating the effectiveness and potential of various herbal remedies.

Exploring the efficacy of Chinese herbal medicine against antibiotic-resistant pathogens using Louvain community detection

Figure 2A uses the Louvain community detection method to cluster pathogens based on AntiBac-Pred predictions and their corresponding Chinese herbal pairs, aiming to forecast antimicrobial effects. Higher-resolution images are available in the Supplementary Information. The Louvain algorithm, which is modularity-based, analyzes relationships between pathogens and herbal medicines. Figure 2B-D display histograms of herb classes and bacterial types within the dataset as analyzed by the community algorithm. The figure distinguishes between herbs' associations with Gram-negative and Gram-positive bacteria, identifying two primary communities. Each community links specific pathogens with herbal treatments, allowing us to predict the potential efficacy of these Chinese herbal therapies against resistant strains.

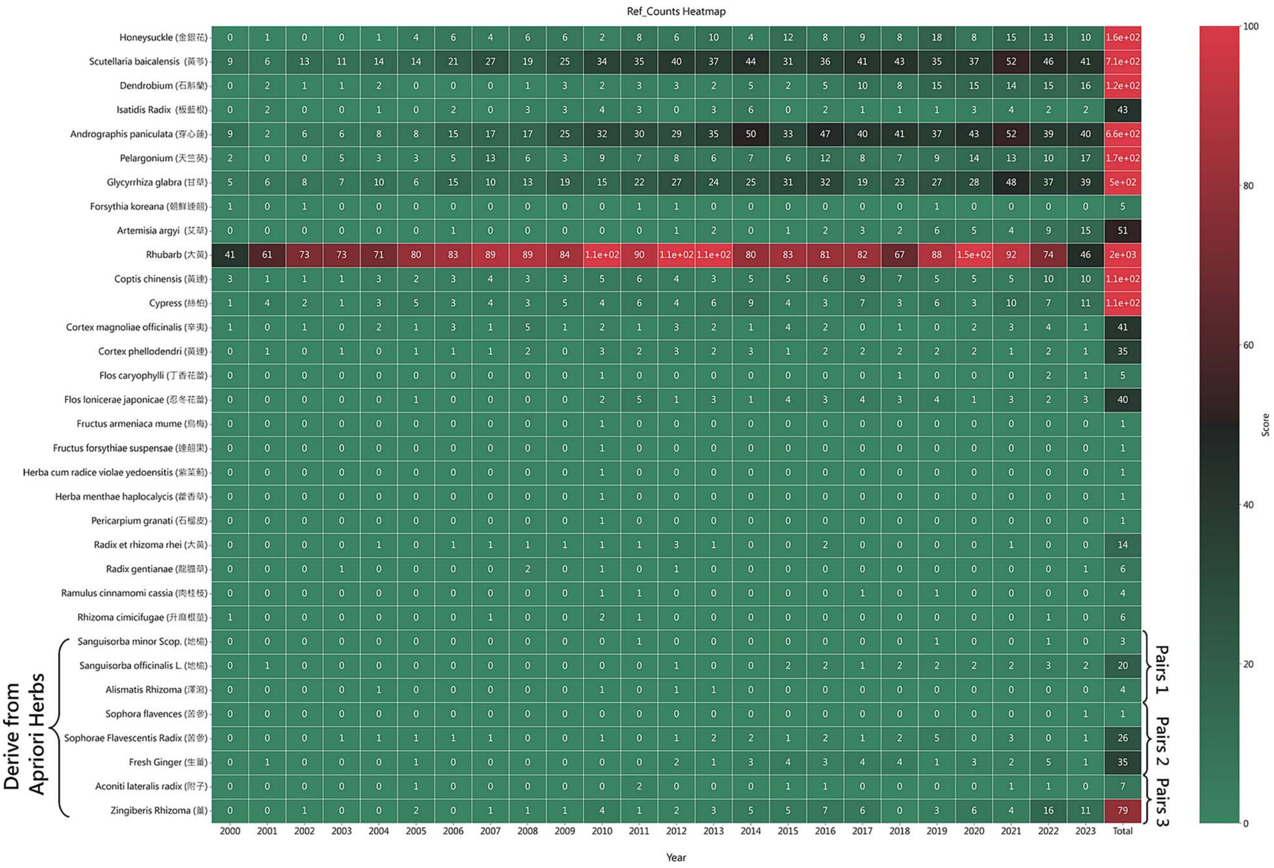
The 'DiYu' Community includes a diverse group of pathogens, many of which are known to exhibit resistance to conventional antibiotics, such as *Propionibacterium acnes*, *Clostridium difficile*, *Acinetobacter calcoaceticus*, *S. aureus subsp. aureus RN4220*, *Salmonella typhi, Klebsiella spp.*, *Escherichia coli DH5alpha*, and *Enterobacter cloacae*. These pathogens are associated with various infections ranging from skin conditions to severe gastrointestinal diseases. Including the 'DiYu' suggests potential broad-spectrum antimicrobial properties, warranting further investigation into its active components and mechanisms of action.

The 'ZeXie' Community also contains several resistant strains, including *Mycobacterium ulcerans*, *Chlamydia trachomatis*, *Bacteroides thetaiotaomicron*, *Bacillus anthracis*, *Streptococcus pyogenes*, and *Providencia rettgeri*. This community includes pathogens responsible for various infections from systemic infections. The ZeXie indicates its potential effectiveness against a spectrum of resistant bacterial strains, highlighting the importance of further phytochemical and pharmacological studies.

Applying the Louvain community detection method in this study provides a novel approach to predicting the antimicrobial potential of Chinese herbal medicines. Identifying distinct pathogen clusters associated with specific herbal treatments underscores the need for further research to validate these findings and explore the underlying mechanisms. This approach holds promise for developing alternative antimicrobial therapies in the face of rising antibiotic resistance.

Quantitative visualization of antimicrobial indicators in herbal modalities using bubble plots

Figure 3 employs bubble plots to visualize antimicrobial indicators for various herbal modalities, using AntiBac-Pred scores



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total
Honeysuckle (金銀花)	0	1	0	0	1	4	6	4	6	6	2	8	6	10	4	12	8	9	8	18	8	15	13	10	1.6e+02
Scutellaria baicalensis (黃芩)	9	6	13	11	14	14	21	27	19	25	34	35	40	37	44	31	36	41	43	35	37	52	46	41	7.1e+02
Dendrobium (石斛蘭)	0	2	1	1	2	0	0	0	1	3	2	3	3	2	5	2	5	10	8	15	15	14	15	16	1.2e+02
Isatidis Radix (板藍根)	0	2	0	0	1	0	2	0	3	3	4	3	0	3	6	0	2	1	1	1	3	4	2	2	43
Andrographis paniculata (穿心蓮)	9	2	6	6	8	8	15	17	17	25	32	30	29	35	50	33	47	40	41	37	43	52	39	40	6.6e+02
Pelargonium (天竺葵)	2	0	0	5	3	3	5	13	6	3	9	7	8	6	7	6	12	8	7	9	14	13	10	17	1.7e+02
Glycyrrhiza glabra (甘草)	5	6	8	7	10	6	15	10	13	19	15	22	27	24	25	31	32	19	23	27	28	48	37	39	5e+02
Forsythia koreana (朝鮮連翹)	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	5
Artemisia argyi (艾草)	0	0	0	0	0	0	1	0	0	0	0	0	1	2	0	1	2	3	2	6	5	4	9	15	51
Rhubarb (大黃)	41	61	73	73	71	80	83	89	89	84	1.1e+02	90	1.1e+02	1.1e+02	80	83	81	82	67	88	1.5e+02	92	74	46	2e+03
Coptis chinensis (黃連)	3	1	1	1	3	2	3	4	3	3	5	6	4	3	5	5	6	9	7	5	5	5	10	10	1.1e+02
Cypress (絲柏)	1	4	2	1	3	5	3	4	3	5	4	6	4	6	9	4	3	7	3	6	3	10	7	11	1.1e+02
Cortex magnoliae officinalis (辛夷)	1	0	1	0	2	1	3	1	5	1	2	1	3	2	1	4	2	0	1	0	2	3	4	1	41
Cortex phellodendri (黃連)	0	1	0	1	0	1	1	1	2	0	3	2	3	2	3	1	2	2	2	2	2	1	2	1	35
Flos caryophylli (丁香花蕾)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2	1	5
Flos lonicerae japonicae (忍冬花蕾)	0	0	0	0	0	1	0	0	0	0	2	5	1	3	1	4	3	4	3	4	1	3	2	3	40
Fructus armeniaca mume (烏梅)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fructus forsythiae suspensae (連翹果)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Herba cum radice violae yedoensitis (紫菜薊)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Herba menthae haplocalycis (藿香草)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pericarpium granati (石榴皮)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Radix et rhizoma rhei (大黃)	0	0	0	0	1	0	1	1	1	1	1	1	3	1	0	0	2	0	0	0	0	1	0	0	14
Radix gentianae (龍膽草)	0	0	0	1	0	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	6
Ramulus cinnamomi cassia (肉桂枝)	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	4
Rhizoma cimicifugae (升麻根莖)	1	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	1	0	6
Sanguisorba minor Scop. (地榆)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	3
Sanguisorba officinalis L. (地榆)	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2	2	1	2	2	2	2	3	2	20
Alismatis Rhizoma (澤瀉)	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	4
Sophora flavences (苦參)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Sophorae Flavescentis Radix (苦參)	0	0	0	1	1	1	1	1	0	0	1	0	1	2	2	1	2	1	2	5	0	3	0	1	26
Fresh Ginger (生薑)	0	1	0	0	0	1	0	0	0	0	0	0	2	1	3	4	3	4	4	1	3	2	5	1	35
Aconiti lateralis radix (附子)	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	1	1	0	0	0	0	1	1	0	7
Zingiberis Rhizoma (薑)	0	0	1	0	0	2	0	1	1	1	4	1	2	3	5	5	7	6	0	3	6	4	16	11	79

Figure 1. Heatmap shows PubMed publication numbers, reflecting herbal antimicrobial research popularity.

median values. Distinct color coding—blue dots for antecedent items and red dots for consequent items—facilitate comparison between these sets. The size of the bubbles reflects the strength of antimicrobial activity, with large bubbles signifying more robust evidence of effectiveness. Overlapping bubbles are depicted as double dots, indicating simultaneous antimicrobial activity and suggesting potential synergistic effects or a broader spectrum of activity. This visualization method effectively conveys complex data, highlighting the individual antimicrobial properties and possible interactions among different herbal modalities. By enhancing data clarity, bubble plots aid in identifying promising combinations that could possess antimicrobial efficacy.

Figure 3A presents predicted antimicrobial confidence scores for Herb A and Herb B against various Gram-positive bacteria. DiYu demonstrates higher confidence scores against *Bacillus subtilis subsp. spizizenii* ATCC 6633 (0.26525) and resistant *S. simulans* (0.4537), indicating its potential effectiveness against these bacteria. Conversely, ZeXie shows significant antimicrobial potential against *B. subtilis subsp. subtilis* str. 168 (0.2841), resistant *S. aureus subsp. aureus* MW2 (0.1409), resistant *S. aureus subsp. aureus* RN4220 (0.34375), and resistant *S. simulans* (0.2744). Some bacteria, such as *Enterococcus faecalis* ATCC 29212, resistant *B. subtilis subsp. spizizenii* ATCC 6633, resistant *Streptococcus pneumoniae*, and *B. subtilis subsp. spizizenii* ATCC 66330, exhibits zero confidence scores for both herbs, suggesting no predicted antimicrobial activity. Notably, ZeXie appears to have broader spectrum activity across multiple bacteria compared to DiYu, which has zero confidence scores for several bacteria.

Figure 3B presents the predicted antimicrobial efficacy scores of DiYu and ZeXie against various gram-negative bacterial strains. DiYu demonstrates antimicrobial activity against most bacterial strains, with confidence scores ranging from 0.0041 to 0.2017. The highest score for DiYu is observed against *E. coli* (0.2017), indicating a potentially strong antibacterial effect. Conversely, ZeXie shows selective antimicrobial activity, with notable efficacy against resistant *Burkholderia pseudomallei* (0.44285) and resistant *E. coli* K12 (0.2609), among others. The confidence scores for ZeXie are generally lower, except for these few instances where the values are relatively high; this suggests that while DiYu exhibits broad-spectrum activity, ZeXie is effective against specific bacterial strains.

Detailed antimicrobial values are provided in Supplementary Table S1 in the Supplementary Information.

Gene ontology enrichment analysis of DiYu and ZeXie interaction highlights potential antimicrobial properties in human immunology

As illustrated in Fig. 4, gene ontology (GO) term analysis of the interaction between DiYu and ZeXie identifies significant enrichment in several biological processes, indicating their potential antimicrobial properties.

Terms related to neurological system processes and synaptic transmission, such as ‘Neurological System Process’ (17 genes, P-value = 6.24×10^{-23}, FE = 48.11) and ‘Synaptic Transmission, Gabaergic’ (13 genes, P-value = 2.9×10^{-20}, FE = 76.41), suggest a potential connection between neural activities and immune responses. The substantial enrichment observed in ‘Regulation of Postsynaptic Membrane Potential’ (15 genes, P-value = 1.38×10^{-22}, FE = 67.42) and ‘Gamma-Aminobutyric Acid Signaling Pathway’ (14 genes, P-value = 1.28×10^{-21}, FE = 73.78)

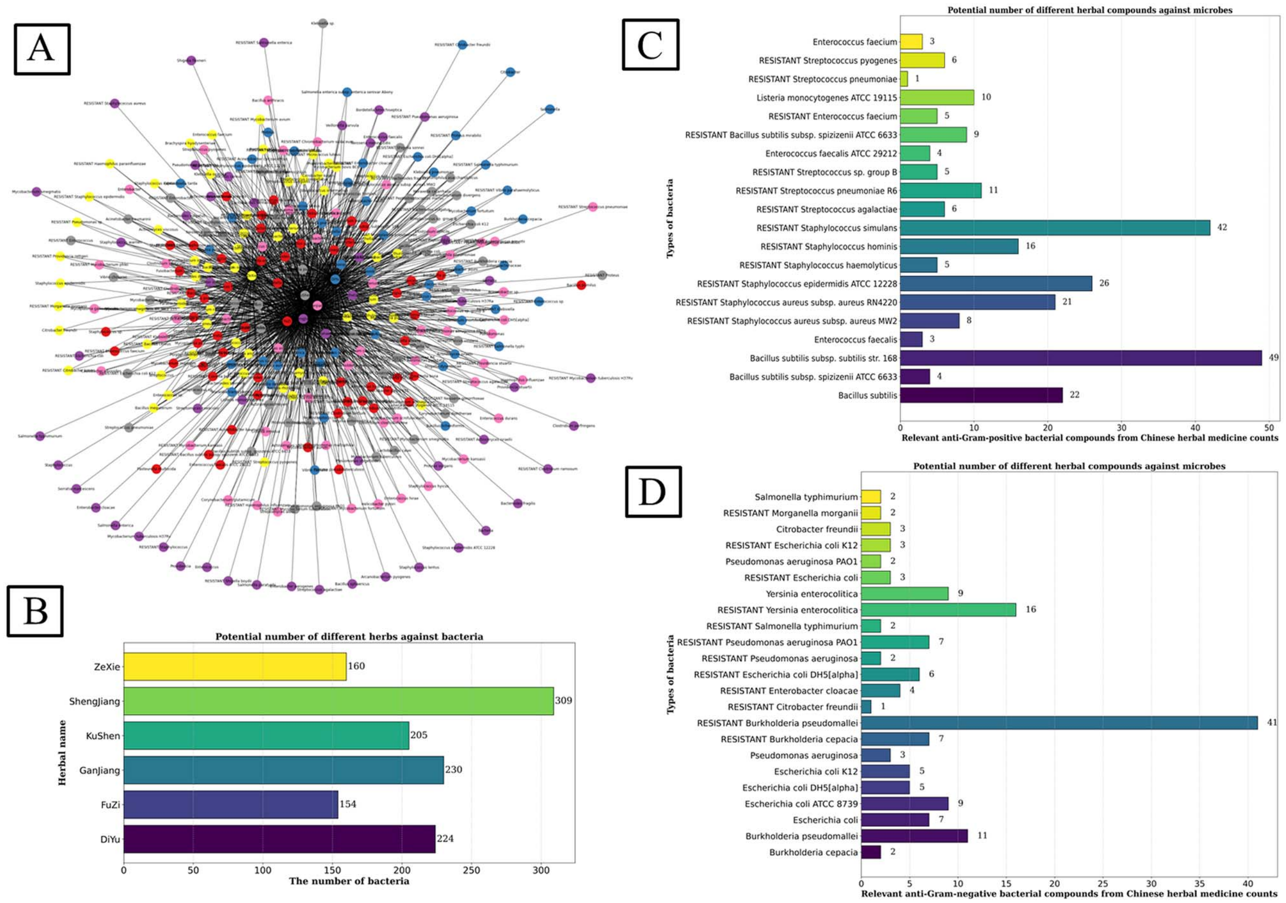


Figure 2. Assessing antimicrobial efficacy and identifying active compounds in herbal medicines via community algorithms.

indicates that these pathways may modulate immune functions and enhance antimicrobial defenses.

These suggest a potential link between neural signaling pathways and immune system regulation, providing a framework for understanding how neural activity may influence immune responses.

Zhang et al. previously demonstrated that splenic innervation is crucial for plasma cell formation [31]. They showed that alcohol-induced denervation of the splenic plexus disrupts plasma cell generation during T cell-dependent immune responses while leaving T cell-independent responses intact. This finding underscores the importance of splenic neural activity in regulating specific immune responses [31].

Their study also revealed that developing splenic plasma cells (SPPCs) interact with acetylcholine (ACh) and are directly influenced by it. Splenic neural activity facilitates SPPC formation through ACh-induced mechanisms in B cells [31].

Additionally, research has demonstrated that acetylcholine receptor stimulation regulates partial lipopolysaccharide-induced pro-inflammatory cytokine secretion, highlighting the parasympathetic nervous system's role in modulating inflammation through neuro-immune interactions [32].

Reports indicate that *Sanguisorba officinalis L.*, a variant of DiYu, possesses significant anxiolytic and neuroprotective properties [33]. Ethanol extracts of *Sanguisorba minor* (also a type of DiYu) exhibit strong inhibitory effects on acetylcholinesterase (AChE) [34], while components of *Alisma Rhizomes* (ZeXie) regulate the activity of $\alpha 3\beta 4$ nicotinic acetylcholine receptors [35]. These findings suggest that herbal extracts have the potential to modulate human immune responses and neural activity.

Our study builds on these findings, providing a foundation for exploring the potential of herbal pairs to enhance human resistance to drug-resistant microorganisms by leveraging their neural-immune communication properties and direct antimicrobial effects.

Other pathways, such as mitochondrial activity plays a pivotal role in immune modulation, as highlighted by the significant enrichment of pathways such as 'Mitochondrial Electron Transport, Cytochrome C to Oxygen' (13 genes, P-value = 1.57×10^{-20}, FE = 79.47) and 'Cellular Respiration' (11 genes, P-value = 8.71×10^{-14}, FE = 41.00). The mitochondrial electron transport chain is a key regulator of macrophage metabolism and innate immunity, influencing macrophage polarization, antibacterial responses, and tissue repair through mitochondrial bioenergetics and reactive oxygen species generation [36]. These findings suggest that herbs may leverage these pathways to enhance immune function and antimicrobial activity.

Moreover, the enrichment of 'Chloride Transmembrane Transport' (18 genes, P-value = 4.04×10^{-19}, FE = 25.24) and 'Hydrogen Ion Transmembrane Transport' (12 genes, P-value = 1.71×10^{-9}, FE = 13.19) points to the role of ion or pH transport in maintaining cellular homeostasis and potentially combating infections [37].

Our results indicate that the interaction between DiYu and ZeXie may enhance antimicrobial activity by integrating neural regulation, energy metabolism, and ion transport mechanisms.

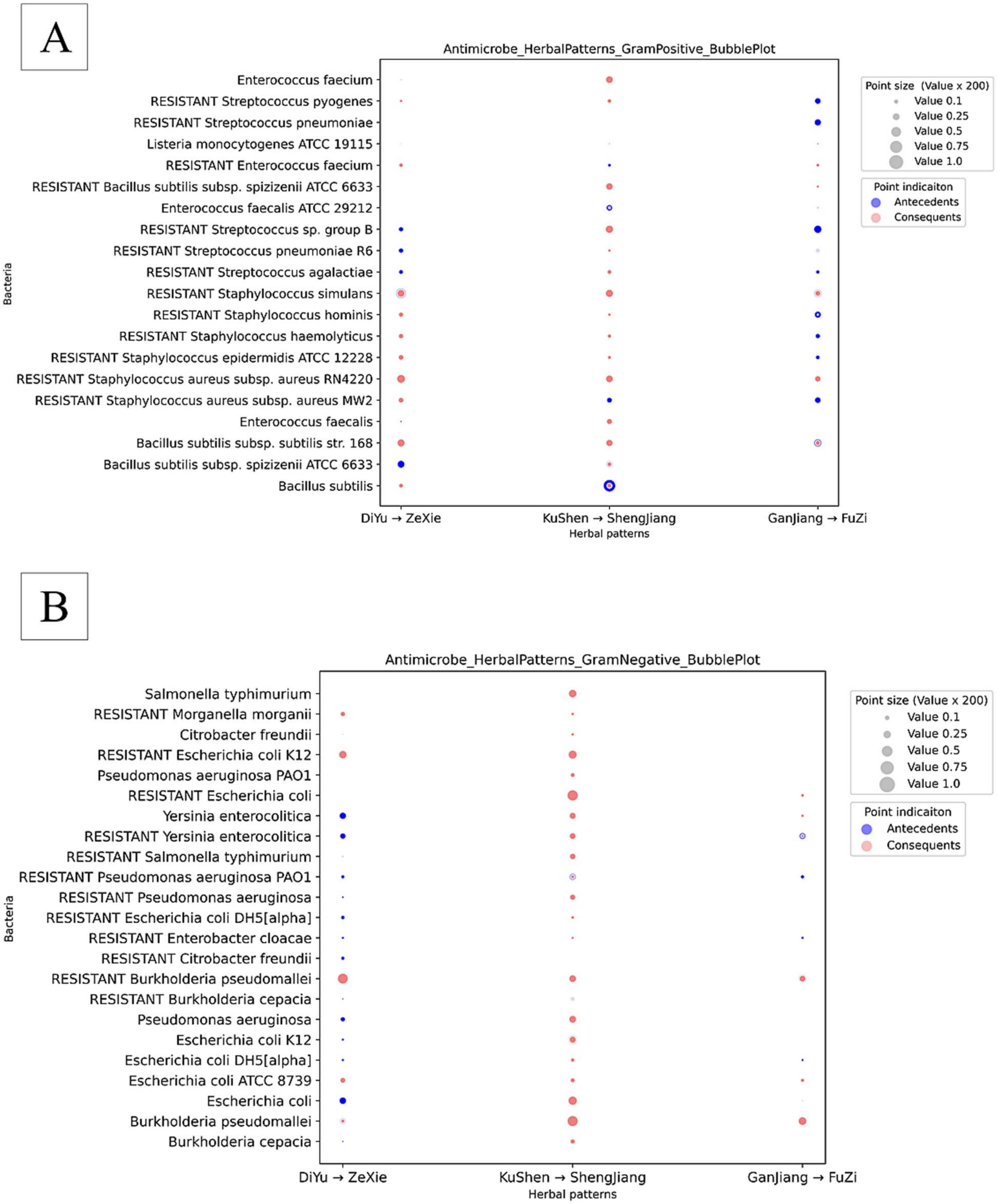


Figure 3. Bubble diagram illustrating the antimicrobial fractions of herbs in the antecedent and consequent items.

We emphasized that further investigation is warranted to elucidate these complex interactions.

Statistically significant biological processes and pathways in the antimicrobial impact of KuShen to ShengJiang

Figure 5 demonstrates the application of FE to identify several statistically significant biological processes and pathways related to the 'KuShen' and 'ShengJiang' herbal pairs. This analysis elucidates the potential mechanisms by which this group of herbs exerts its involved effects on various compounds.

The results were similar to DiYu to ZeXie indicating that the 'Neurological System Process' exhibits a strong association (P-value = 3.12×10^{-30}), suggesting its potential role in modulating neural responses that may influence immune function against microbial defense mechanisms [38]. The FE of this mechanism

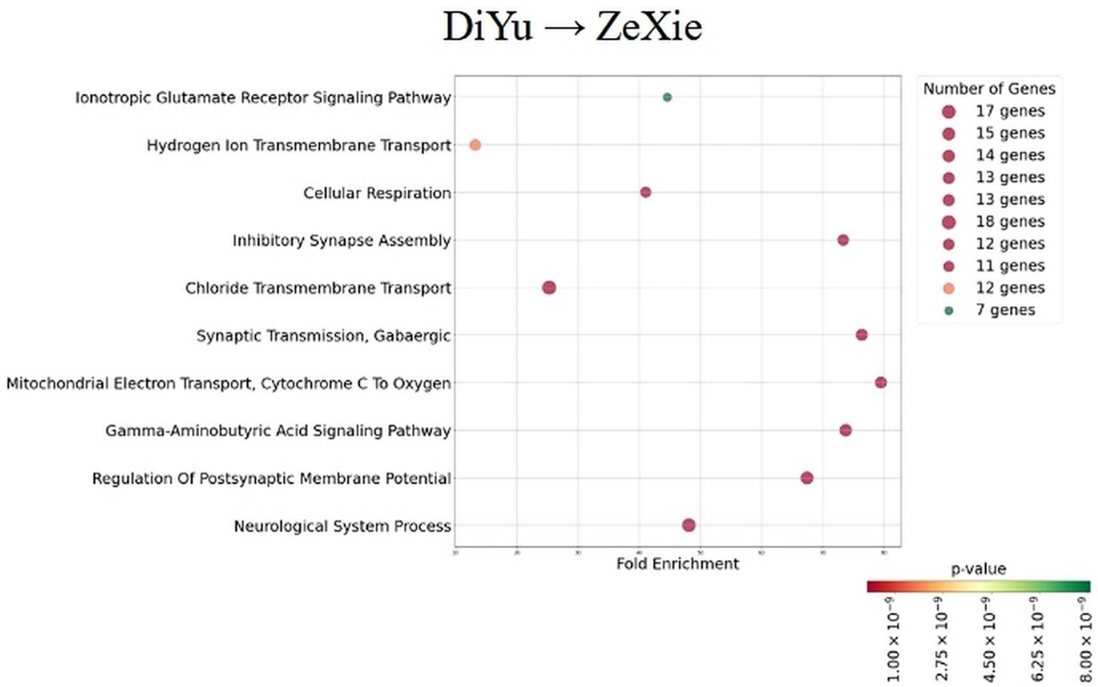


Figure 4. Based on the traditional association of DiYu and ZeXie, as identified through the DAVID ontological analysis.

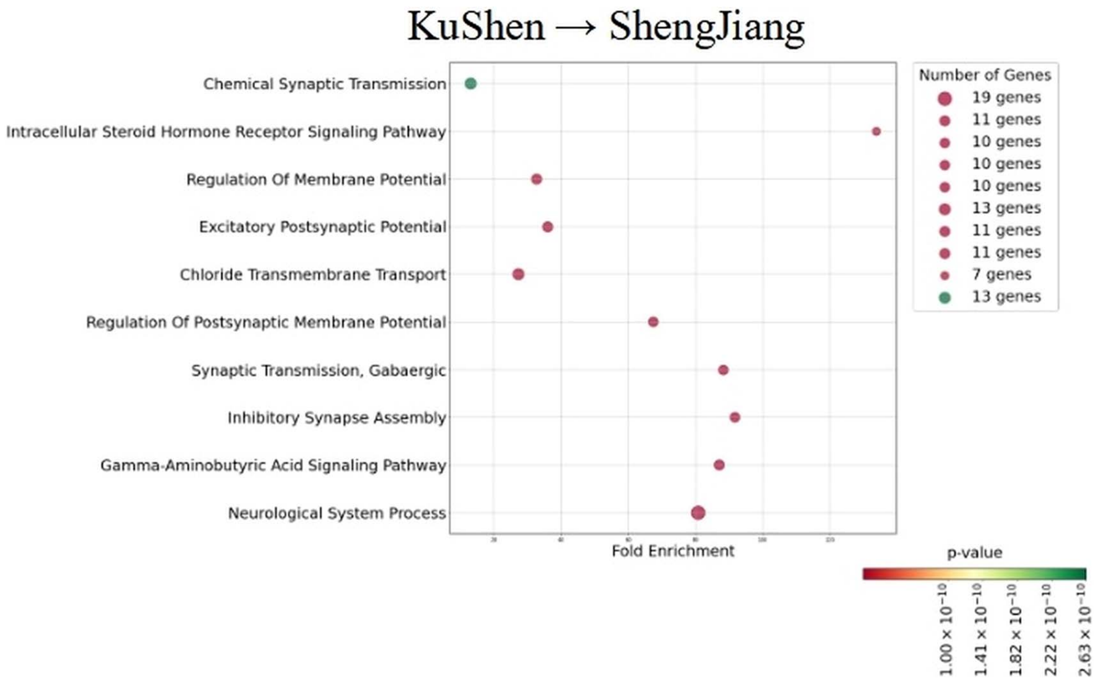


Figure 5. Based on the traditional association of KuShen and ShengJiang, as identified through the DAVID ontological analysis.

is 80.66, highlighting the significant overexpression of genes related to the nervous system among the gene group affected by the herb treatment. Especially, Xu et al. indicate that Matrine (Mat), an alkaloid from *Sophora flavescens* (Kushan), demonstrates neuroprotective effects against focal cerebral ischemia by inhibiting the NF-κB signaling pathway, significantly reducing infarction volume and neurological deficits in a dose-dependent manner [39]; this suggests a potential neural protection mechanism for involvement in neuroimmune that differs from the possible signal pathway of neural activity in DiYu to ZeXie groups.

The ‘Gamma-Aminobutyric Acid (GABA) Signaling Pathway’ (*P*-value = 2.35×10^{-17}) and ‘Inhibitory Synapse Assembly’ (*P*-value = 6.30×10^{-16}) also emerge as significant; this is potentially related to earlier reports that components of *S. flavescens* (KuShen) enhance the influx of GABA-induced chloride ions in *Xenopus* oocytes [40] or have a sedative effect [41].

Moreover, ‘Chloride Transmembrane Transport’ (*P*-value = 4.03×10^{-14}) and ‘Chemical Synaptic Transmission’ (*P*-value = 2.63×10^{-10}) highlight processes involved in ion transport and synaptic signaling, respectively. Guo et al. demonstrated that matrine directly targets calcium-activated chloride channels and exerts inhibitory effects on their function [42]. Furthermore, Bai et al. reported that inflammatory mediators and pathogens actively upregulate TMEM16A expression and enhance activity [43]. TMEM16A, or anoctamin-1, is an essential component of neuronal cells that functions as a Ca^{2+}-activated Cl^- channel; this channel regulates sensory neuronal excitability and maintains ion transport and membrane potential stability [44, 45]. Additionally, some studies indicate that S. *flavescens* and fresh ginger possess antimicrobial effects [46–48]. These mechanisms are fundamental in cellular communication and homeostasis, potentially influencing microbial membrane stability and susceptibility to antimicrobial agents [46].

The enrichment analysis suggests that ‘KuShen to ShengJiang’ treatment may affect broader biological systems beyond direct antimicrobial pathways, potentially enhancing host defense mechanisms or directly inhibiting microbial growth. These findings advance our understanding of herbal interventions in antimicrobial therapy, revealing potential new strategies that integrate traditional herbal medicine with modern approaches.

The study highlights the complex impact of ‘KuShen to ShengJiang’ on biological processes related to antimicrobial defense and suggests potential synergies between herbal therapies and conventional treatments. Further research on specific molecular targets and clinical outcomes will be essential for translating these findings into effective therapeutic applications.

Conclusions

Integrating classical literature with modern molecular biotechnology enhances the development of antibacterial herbal medicines. Ancient Chinese texts identify plants with antibacterial potential, guiding contemporary research, while biotechnology studies reveal active ingredients and their mechanisms. This interdisciplinary approach, incorporating botany, biochemistry, bioinformatics, and pharmacology, strengthens research and improves the efficiency of herbal formulation discoveries. By merging ancient knowledge with modern science, this method offers innovative solutions to antimicrobial resistance and fosters practical applications in biotechnology and drug development.

Key Points

- Integrating ancient texts with biotechnology databases advances antibacterial herbal medicine by identifying active compounds and their mechanisms.
- Interdisciplinary exploration strengthens research, combining botany, biochemistry, bioinformatics, and pharmacology for improved herbal formulations.
- Merging classical knowledge with modern science offers innovative solutions to antimicrobial resistance and advances drug discovery applications.
- Combining computational modeling with ancient herbal medicine enhances the precision of identifying effective antimicrobial agents and accelerates drug development.

Author contributions

Meng-Chi Chung (Writing–original draft, Methodology, Formal analysis, Validation, Investigation, Funding acquisition, Conceptualization), Li-Jen Su (Writing – review and editing, Investigation, Conceptualization), Chien-Lin Chen (Writing – review and editing, Conceptualization), and Li-Ching Wu (Writing – review and editing, Supervision, Methodology, Funding acquisition).

Supplementary data

Supplementary data is available at *Briefings in Bioinformatics* online.

Conflict of interest: The authors declare no conflicts of interest related to commercial or financial relationships in this research.

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Case Study