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Article

Exploring the therapeutic mechanism of the Qing Palace Summer-avoiding Pearl based on network pharmacology and molecular dynamics simulation

Haotian Li[1,†], Meixin Zhu[1,†], Sile Hu[2], Yuewei Song[1], Yanjun Liu[2,*], Yuping Zhao[3,*]

[1] Beijing University of Chinese Medicine, Beijing 100013, China
[2] Institute of Basic Theory for Chinese Medicine, China Academy of Chinese Medical Sciences, Beijing 100010, China
[3] Experimental research center, China Academy of Chinese Medical Sciences, Beijing 100010, China
*** Corresponding authors:** Yanjun Liu, yanjundoctor@126.com; Yuping Zhao, 18810084632@163.com
[†] These authors contribute equally.

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Abstract: Heatstroke is a thermal injury disease resulting from excessive water and electrolyte loss, as well as impaired heat dissipation, in hot and humid conditions. Modern medicine typically focuses on physical measures for early heatstroke intervention and prevention, with drug-related research being somewhat limited in scale and scope. In Chinese contexts, heatstroke is often referred to as “Zhongshu”, encompassing symptoms like nausea, vomiting, loss of appetite, emotional fluctuations and agitation, and headaches due to elevated body temperature. Traditional Chinese medicine boasts a long history and extensive literature on treating heatstroke. The Qing Palace Summer-avoiding Pearl, a treasured medicine used by ancient Chinese royalty for “Zhongshu” treatment and prevention, is of particular interest. This study aims to explore a new approach for early heatstroke prevention and intervention using the Qing Palace Summer-avoiding Pearl. We identified the disease types associated with this medicine through disease enrichment analysis and pinpointed the most likely therapeutic targets and effective substances via network pharmacology and molecular docking techniques. Furthermore, we conducted molecular thermodynamic analyses on six target Plant Extracts (PEs) using molecular dynamics simulations, examining parameters such as Root Mean Square Displacement (RMSD), Radius of gyration (Rg), and hydrogen bonds. The results indicated that the complexes exhibited favorable binding performance, which may facilitate further research on the Qing Palace Summer-avoiding Pearl.

Keywords: heatstroke; complementary and alternative medicine; traditional Chinese medicine; gene enrichment; disease enrichment

1. Introduction

Heatstroke typically denotes a thermal injury disease stemming from excessive water and electrolyte loss, coupled with impaired heat dissipation in high temperature and humidity settings [1], primarily manifesting as central nervous system and cardiovascular dysfunction. Clinically, symptoms encompass dizziness, headache, flushing, thirst, profuse sweating, general weakness, palpitations, rapid pulse, lack of concentration, and uncoordinated movements [2].

Owing to global warming, abnormal temperature surges, and inadequate power supply, heatstroke incidence has risen in recent years [3–5]. The mortality rate can soar to 80%, often resulting in death due to multiple organ failure [6]. Heatstroke can also trigger various complications, with many patients contracting infections upon intensive care unit admission. Notably, high temperature exposure can elevate the incidence of cerebrovascular diseases [7].

Common heatstroke interventions involve physical methods like cooling and hydration, while drug research for this condition has long faced numerous challenges, exhibiting limitations in scale and scope [8]. Hence, early heatstroke prevention is crucial to curb disease progression. For instance, the Australian Recovery Commission recommends cooling measures such as wearing loose, lightweight clothing, preventing sunburn, and drinking ample water [9]. The thermal insulation of protective gear and prolonged high temperature exposure during multiple shifts heighten risks, making physically demanding workers another vulnerable group to heat-related issues [10]. However, for these individuals, basic measures like avoiding high-temperature work are insufficient.

Traditional Chinese medicine has a long-standing history in treating heatstroke, with numerous Chinese medicines like Huoxiang Zhengqi Water proven to alleviate nausea and vomiting induced by high temperatures [11]. The Qing Palace Summer-avoiding Pearl was a medicine utilized by ancient Chinese royalty for heat-related disease treatment and prevention [12]. Extensive literature and archives document that Qing emperors frequently distributed this medicine to court officials and the military, with its efficacy being safe and stable. Its ingredients comprise cattail, chrysanthemum, cork tree, golden thread, forsythia suspensa capsules, shrub fruit, white atractylodes, cinnabar, Indian sandalwood, dolomite, Sichuan lovage root, sodium sulfate, borneol, rose, and realgar [13]. Although the formula and production process of the Qing Palace Summer-avoiding Pearl are detailed in ancient texts and believed to offer health benefits and heatstroke prevention and cooling effects, there is a dearth of modern in-depth research and evidence on its specific mechanism of action to verify its efficacy, with related studies being scarce. In recent years, the research and application of artemisinin have underscored the vast potential of traditional Chinese medicine [14–16]. We are confident that studying the Qing Palace Summer-avoiding Pearl can systematically predict its potential disease treatment range.

This study pioneers the use of network pharmacology and molecular docking techniques to predict specific diseases treated with the Qing Palace Summer-avoiding Pearl via component-targeted disease enrichment analysis, validating results through molecular dynamics. It aims to provide effective medication for early heatstroke prevention and treatment, thereby preventing and controlling high temperature-induced injuries.

2. Methods

2.1. The Qing Palace Summer-avoiding Pearl's ingredients and target screening

In the Chinese context, "Zhongshu" symptoms include sweating, fatigue, weakness, dizziness, headache, nausea, vomiting, muscle spasms, significantly increased heart rate, orthostatic hypotension, or fainting [17]. These symptoms encompass multiple systemic diseases in modern medicine and differ from the symptoms of heatstroke as defined in modern medical terms. There is a lack of documentation in the literature regarding the specific diseases treated by the Qing

Palace Summer-avoiding Pearl. By searching in the Traditional Chinese Medicine Database, which includes the National Medical Products Administration (http://www.nmpa.gov.cn/datasearch/home-index.html#category=yp), Traditional Chinese Medicines Integrated Database (TCMID) [18], and Rheumatism Knowledge Map (http://tcmrd-kg.cn/), no similar prescriptions were found. The Qing Palace Summer-avoiding Pearl is highly likely a secret formula from the Qing Dynasty court, which was highly valued by the emperor and kept strictly confidential, thus leading to a severe lack of relevant literature. According to the literature records, its therapeutic effect is stable, and further research is necessary to expand the application scope of this drug. This not only helps to explore the potential value of the prescriptions contained in ancient Chinese medicine books but also revitalizes the wisdom crystallization that has been passed down for thousands of years, and cleverly integrates it into the contemporary clinical medical system. This can not only provide clinical doctors with more diverse and distinctive treatment options, enrich treatment methods, but also contribute to the construction of a more complete and efficient modern heatstroke diagnosis and treatment system. The treatment plan of traditional Chinese medicine is to formulate herbal prescriptions based on the patient's clinical manifestations and symptoms [19,20]. By analyzing typical diseases related to potential pharmacological targets of traditional Chinese medicine, utilizing information on these targets and genes associated with these diseases, we can build a bridge between traditional Chinese medicine and modern medicine. This method points the way for exploring the potential of Chinese herbal medicine in treating other possible diseases. The purpose of this study is to predict the specific diseases treated by the Qing Palace Summer-avoiding Pearl through component targeted disease enrichment analysis.

In the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database, input the names of herbs sequentially and filter for active ingredients with an Oral Bioavailability (OB) ≥ 30% and Drug-likeness (DL) ≥ 0.18 [21]. And search for potential targets in databases such as Herb [22], Symmap [23], Batman [24], a total of 151 active ingredients and 458 potential targets were identified.

2.2. Disease target acquisition

Common disease targets were extracted from the Therapeutic Target Database (TTD) [25] and subjected to disease enrichment analysis.

2.3. PEs and nausea and vomiting targets analysis

Targets related to nausea and vomiting were obtained from the TTD database. The intersection of these targets with the previously obtained targets was considered as potential targets. Cytoscape 3.9.1 software was used to construct the active ingredient-potential target-disease network for effective PEs in the treatment of nausea and vomiting.

2.4. Protein–Protein interaction (PPI) network construction

All targets of Plant Extract (PE) and those related to nausea and vomiting were input into the STRING database (https://cn.string-db.org/) [26] to build the PPI

network, and Cytoscape 3.9.1 software was used for network visualization.

2.5. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment

To further explore the mechanism of key targets of effective PEs in treating nausea and vomiting, key targets were introduced into the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/home.jsp) [27]. Homo sapiens was selected for GO function and KEGG pathway enrichment and analysis. GO enrichment analysis included molecular function (MF), biological process (BP), and cellular component (CC), with the top 10 enriched items displayed.

2.6. Molecular docking

Molecular docking was performed on the main active ingredients and related protein targets in the drug-active ingredient-target-pathway-disease network. The Protein Data Bank (PDB) files of core target proteins were downloaded from the PDB database (https://www.rcsb.org/) [28]. AutoDock Vina 1.2.2 was used for dehydration, hydrogenation, and charge setting, saving files in pdbqt format for use as docking receptors. Active ingredients were downloaded from the TCMSP database in Mol2 format. AutoDock Vina 1.2.2 was used for ligand docking. After docking with AutoDock 4 software, the lowest binding energy model was selected as the best, and results were visualized with PyMOL software. A binding energy less than -9 kJ·mol^{-1} indicated strong binding between the protein target and compound, with lower energy signifying more stable and reliable structures.

2.7. Molecular dynamics simulation

GROMACS 2022.3 software was used for molecular dynamics simulations [29]. For small molecule preprocessing [30], AmberTools 22 added the General Amber Force Field (GAFF) force field to small molecules, while Gaussian 16 hydrogenated small molecules and calculated Restrained Electrostatic Potential (RESP) potential. This potential data was added to the molecular dynamics system's topology file. Simulations were conducted at 300 K and 1 Bar pressure using Amber99sb-ildn force field and Tip3p water model solvent, with the system charge neutralized by adding Na+ ions. The simulation system underwent energy minimization using the steepest descent method, followed by Number of particles, Volume, Temperature (NVT) and Number of particles, Pressure, Temperature (NPT) equilibration for 10,0000 steps each, with a 0.1 ps coupling constant and 100 ps duration. Finally, free molecular dynamics simulation was performed, consisting of 5,000,000 steps, a 2 fs step length, and a total duration of 100 ns. Post-simulation, the software's built-in tools analyzed the trajectory, calculating RMSD, Root Mean Square Fluctuation (RMSF), protein rotation radius, and combining Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) and free energy topography data.

3. Result

3.1. Disease enrichment

Disease enrichment analysis identified 26 significantly relevant diseases ($P < 0.05$), as shown in **Figure 1**, with heatstroke-related diseases listed in **Table 1**.

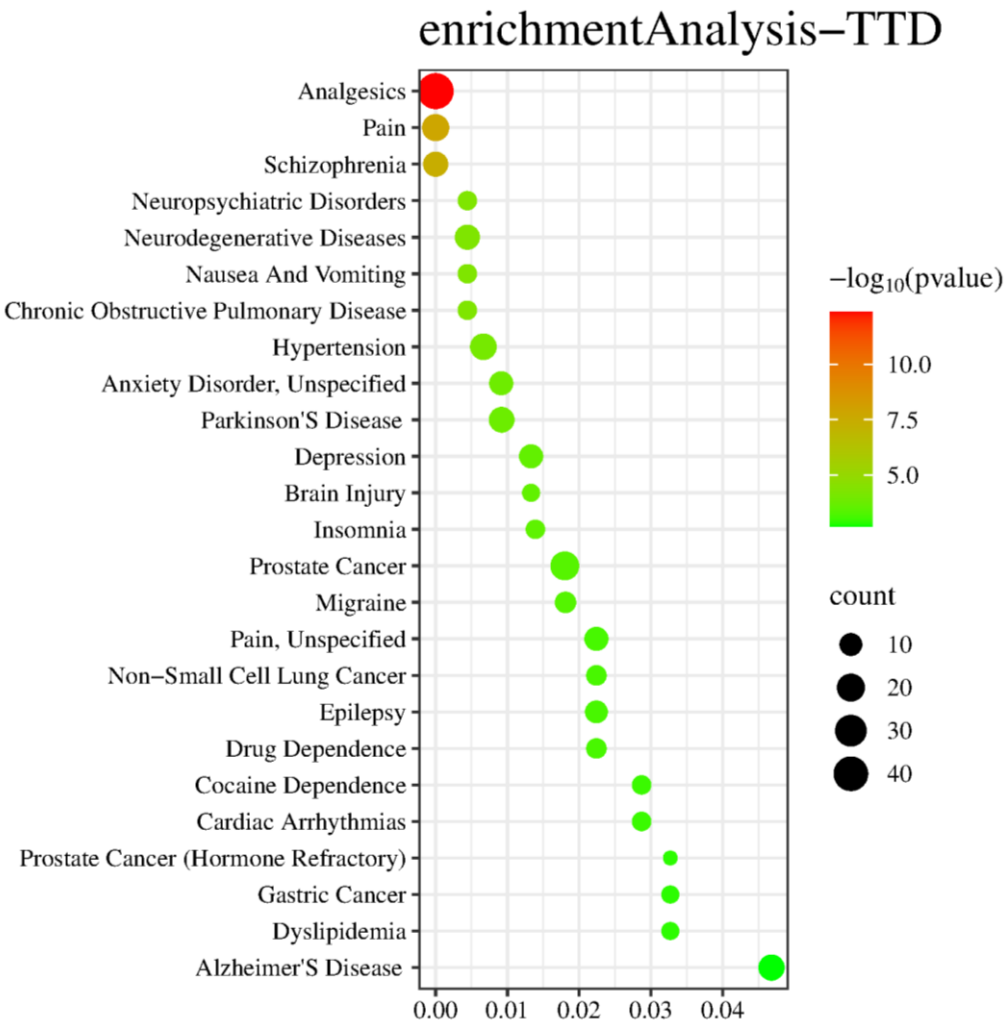

Figure 1. Disease enrichment analysis results.

Table 1. Disease enrichment analysis results related to heatstroke.

Disease	***P*-value**
Anxiety Disorder, Unspecified	1.48×10^{-4}
Insomnia	3.25×10^{-4}
Migraine	4.89×10^{-4}
Cardiac Arrhythmias	1.08×10^{-3}
Nausea and vomiting	5.57×10^{-5}

3.2. Construction of a drug-active ingredient-target network

The active ingredients in the PEs and their corresponding targets were imported into Cytoscape 3.9.1 to construct a drug-active ingredient-target network as shown in **Figure 2**. The six drug-active ingredients are wogonin, quercetin, berberine, luteolin,

kaempferol, and vitamin E, with corresponding targets being the Androgen Receptor (AR), Estrogen Receptor 1 (ESR1), Prostaglandin-endoperoxide synthase 2 (PTGS2), Nitric oxide synthase 2 (NOS2), Dipeptidyl peptidase 4 (DPP4), and Glycogen synthase kinase 3 beta (GSK3B).

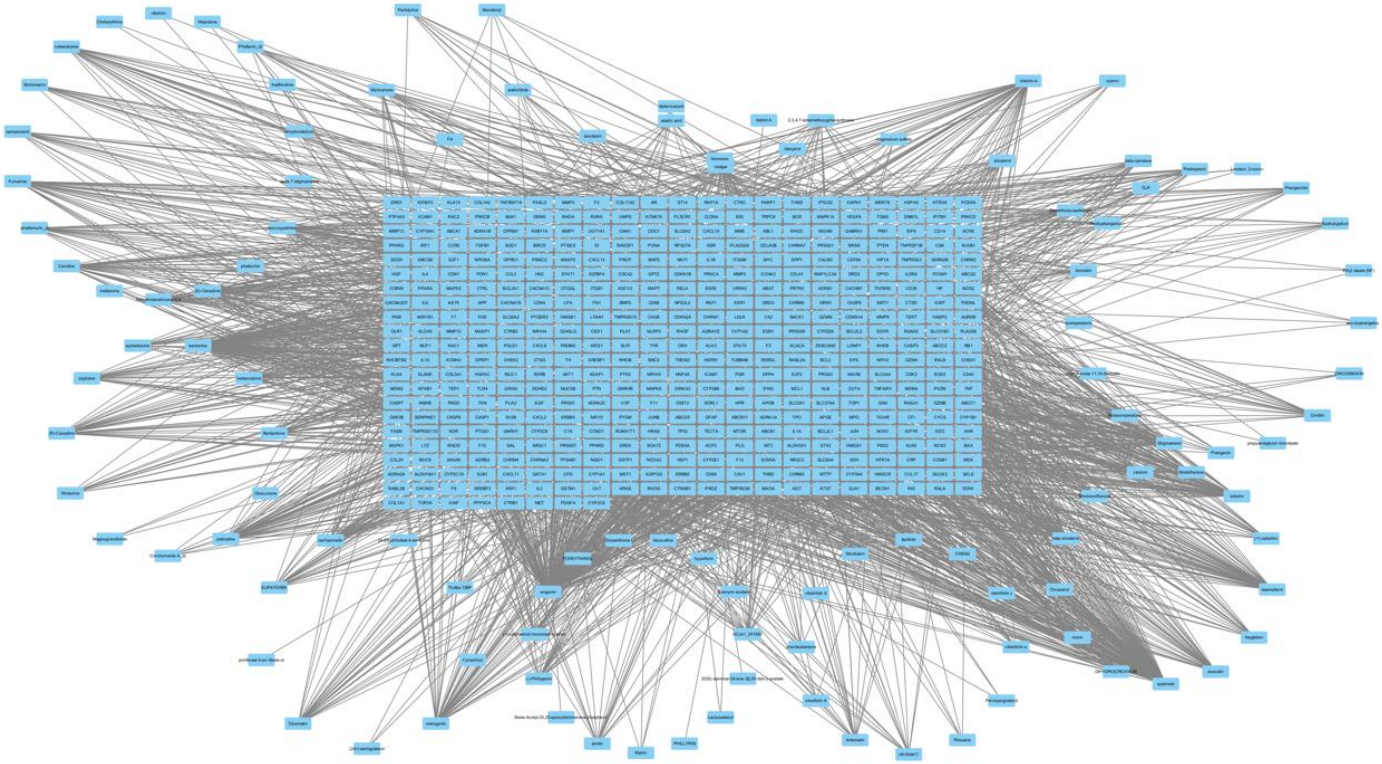

Figure 2. The drug-active ingredient-target network.

3.3. The PPI network of PEs and nausea and vomiting common targets

To elucidate the interaction between PEs and nausea and vomiting targets, 94 intersection targets were imported into the STRING database. Key targets in the PPI network model, as shown in **Figure 3**, included Caspase-3 (CASP3), Caspase-9 (CASP9), Caspase-7 (CASP7), Intercellular cell adhesion molecule-1 (ICAM1), and Integrin beta (IGTB1). These genes are crucial in the PPI network and may play a significant role in nausea and vomiting.

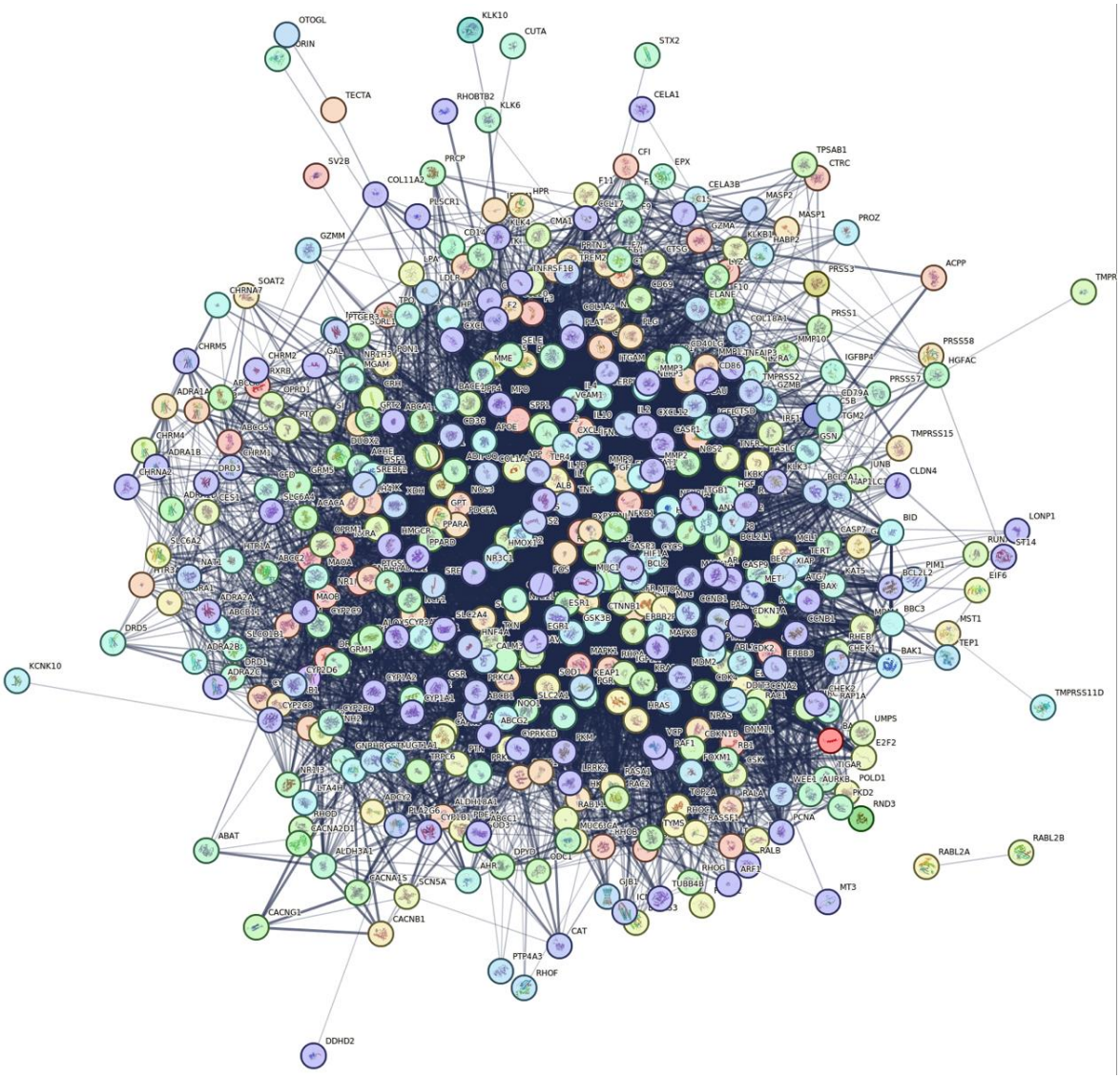

Figure 3. Identification of candidate targets via PPI analysis.

3.4. GO analysis and KEGG pathway enrichment analysis

Intersection targets were submitted to the DAVID database for GO and KEGG enrichment analysis. A total of 3391 BP, 198 CC, and 308 MF GO items ($p < 0.01$) were screened, with the top 10 entries in each category shown in **Figure 4**. Additionally, 205 KEGG items were identified, with the top 10 pathways illustrated in **Figure 5**.

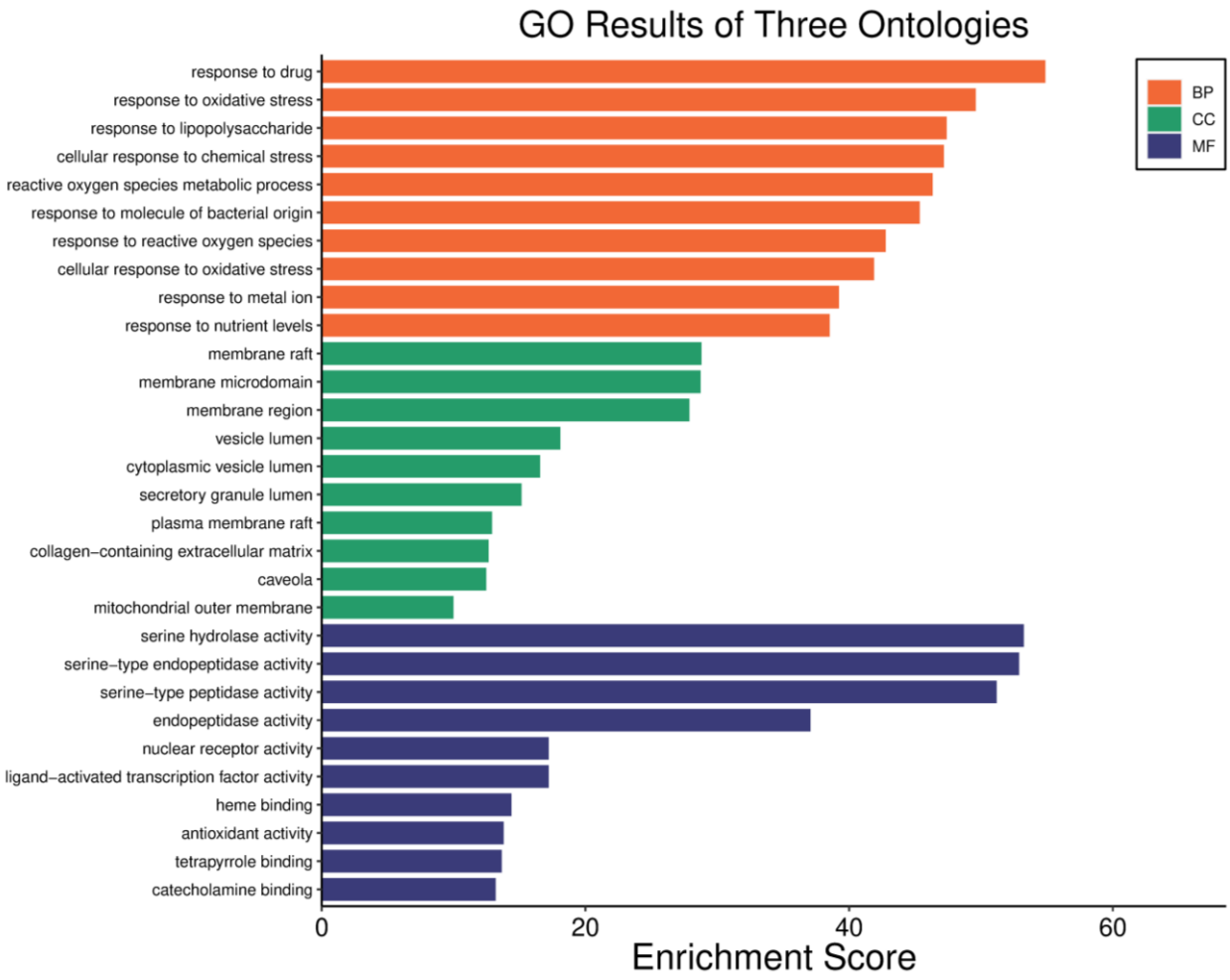

Figure 4. Results of the top 10 GO entries in the three categories.

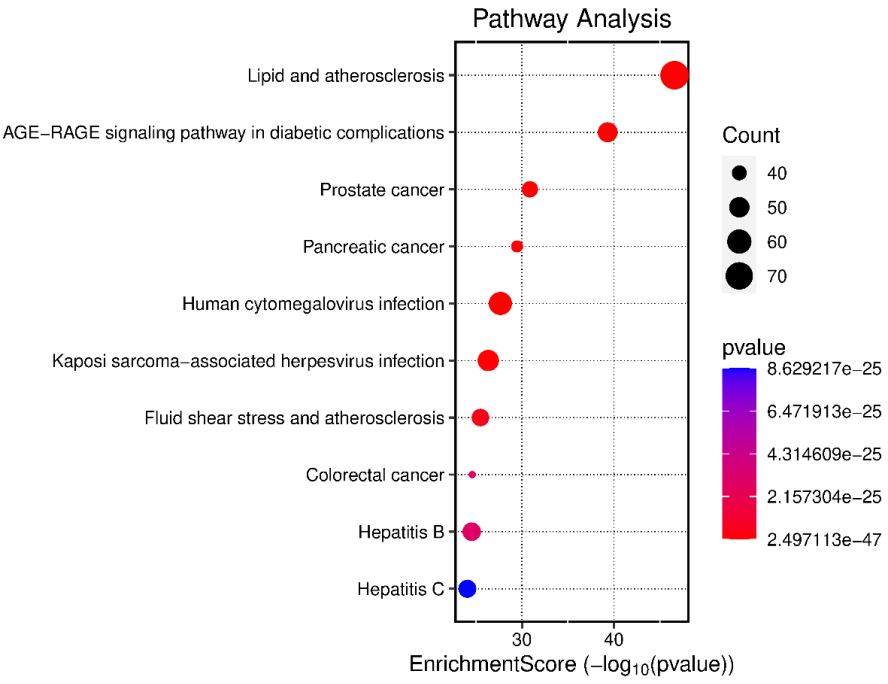

Figure 5. Bubble chart of the top 10 KEGG pathways.

3.5. Molecular docking

Molecular docking revealed that the selected targets exhibited good binding activities with the core active ingredients, further validating the network pharmacology analysis. The results suggested the presence of intermolecular binding forces such as hydrogen bonds, π-π stacking, and hydrophobic interactions between the active ingredients and key targets, as shown in **Figures 6** and **7**.

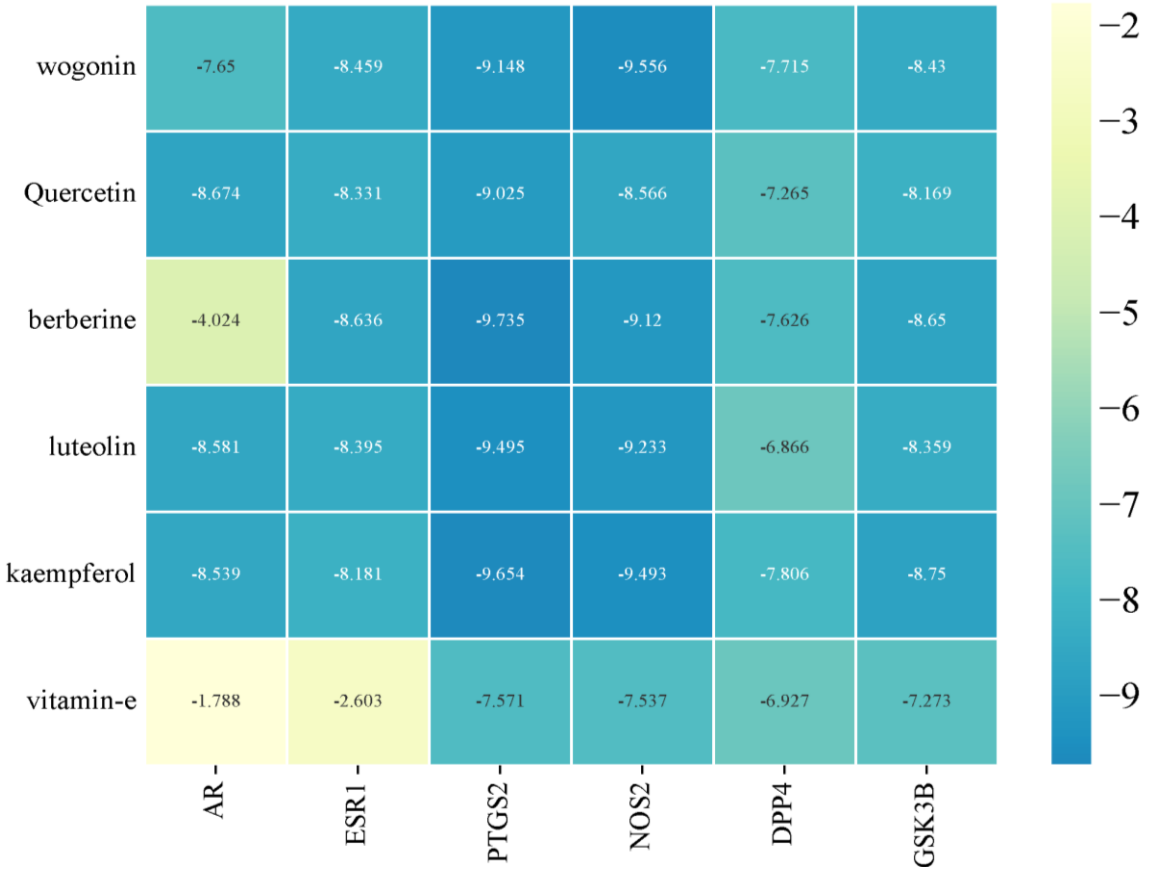

Figure 6. Heat map of molecular docking score. Binding energy (kcal/mol) of the core targets and active ingredients.

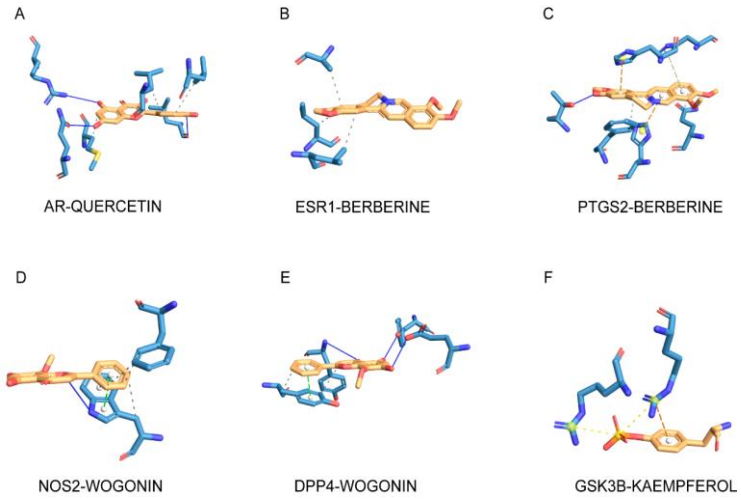

Figure 7. AR-QUE of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

3.6. RMSD

The RMSD plot (**Figure 8**) offers insights into the stability and conformational changes of the six complexes compared to the target alone and solvent system. Initial fluctuations in all systems during the first ~20 ns indicate system equilibration under Molecular Dynamics (MD) conditions. Target-PEs complexes (black) show higher fluctuations than targets (red), likely due to induced conformational changes. This pattern suggests that PE binding influences the target protein's structural stability, achieving a relatively stable conformation post 20 ns, indicating an equilibrated complex.

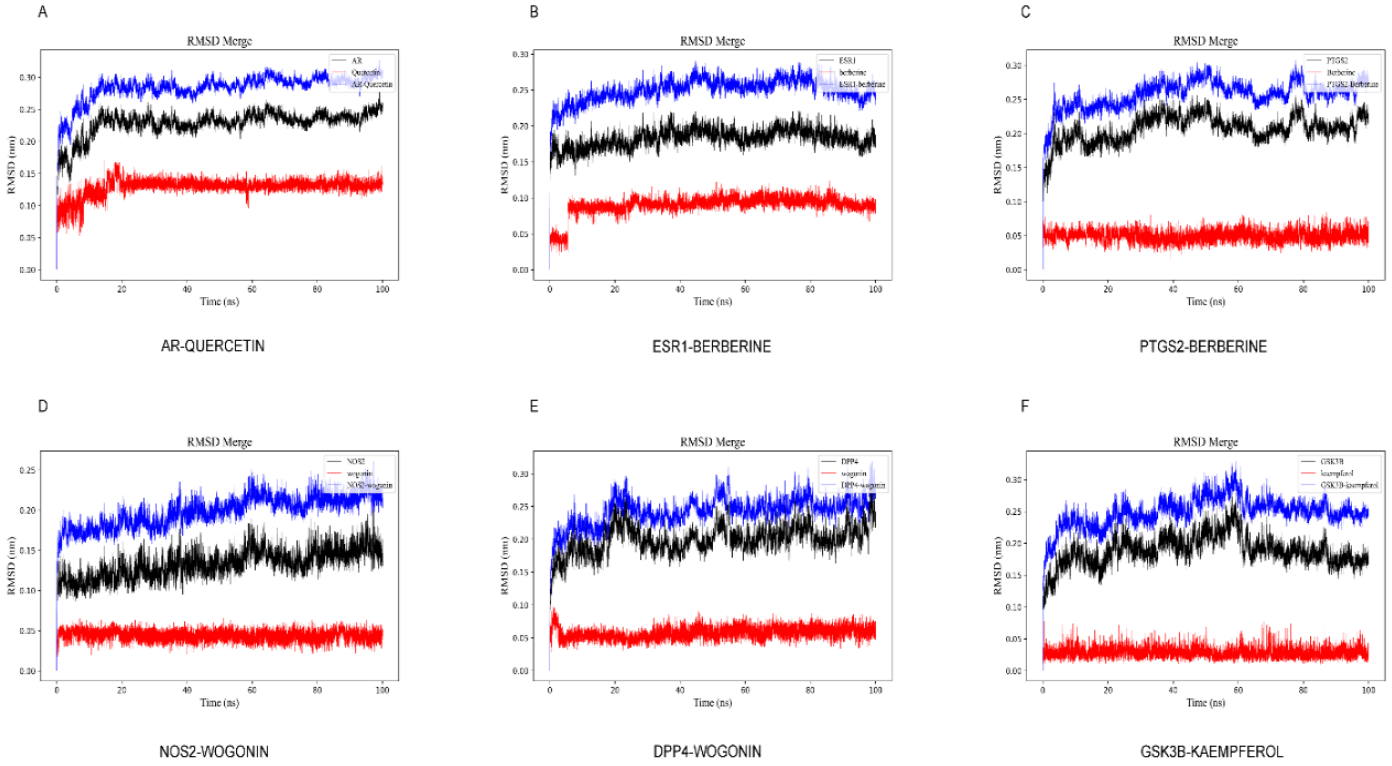

Figure 8. RMSD of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

3.7. Rg

The Rg plot (**Figure 9**) assesses the compactness of the Target-PE complexes. Stable Rg values over time suggest that the complex maintains a consistent tertiary structure with minimal expansion or contraction, indicating structural stability. Slight fluctuations are expected in MD simulations and do not signify significant unfolding or aggregation, reinforcing the compact nature of the Target-PE complexes.

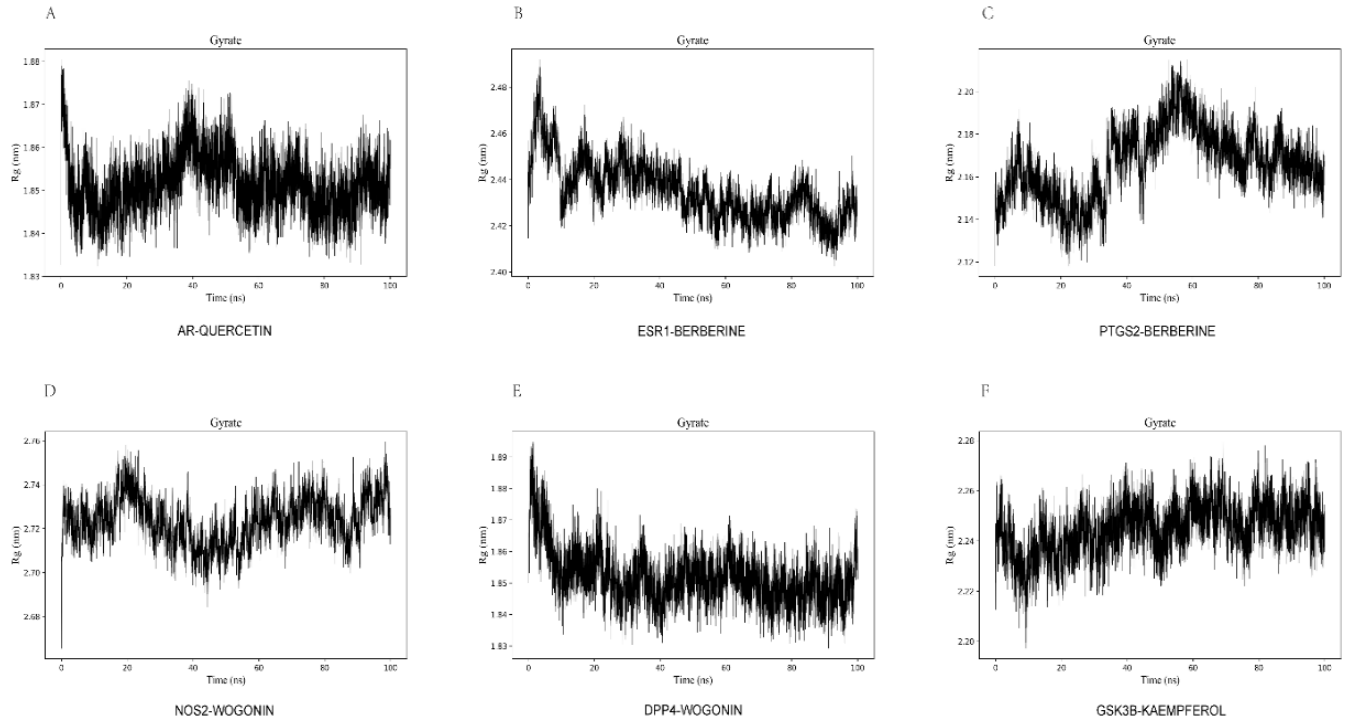

Figure 9. Rg of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

3.8. Solvent accessible surface area (SASA)

The SASA plot (**Figure 10**) tracks the solvent exposure of the Target-PE complexes. Stable SASA values with minor fluctuations suggest that the complex's solvent exposure remains relatively constant. This consistency implies that binding does not significantly alter the solvent-exposed regions of targets, correlating with maintained functionality and stability of the enzyme in its bound form.

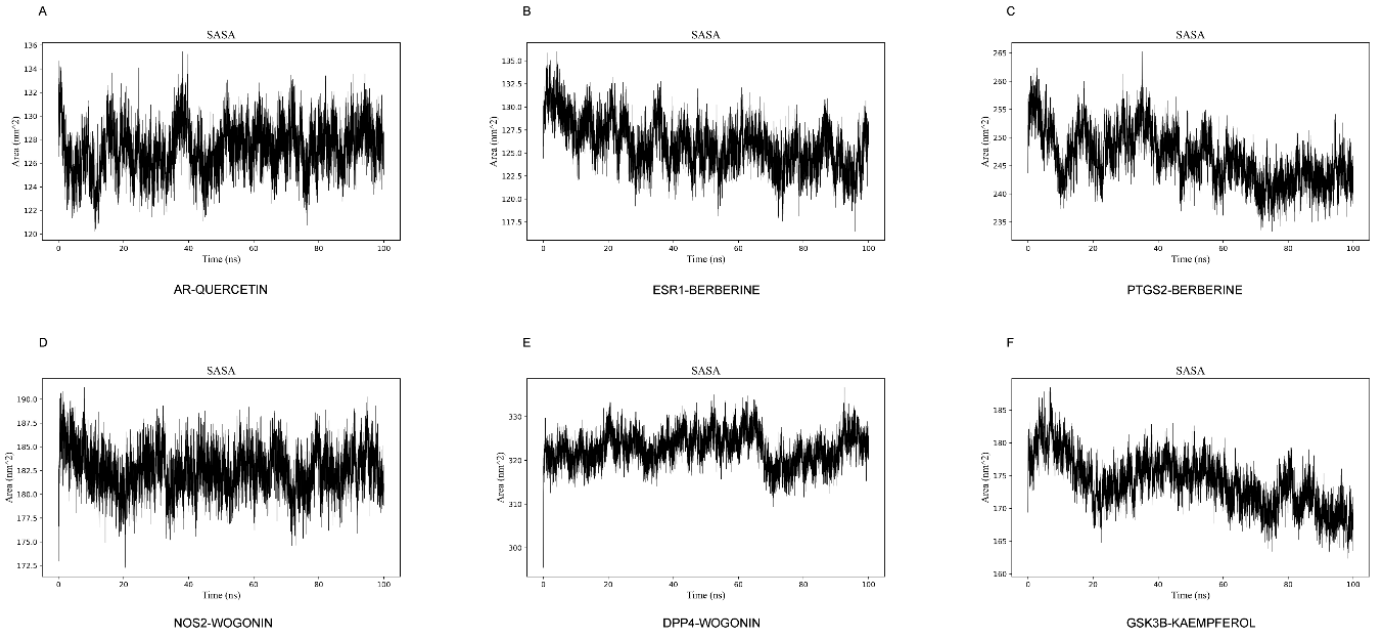

Figure 10. SASA of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

3.9. H-bond number (HN)

The hydrogen bond analysis (**Figure 11**) shows the dynamic hydrogen bonding interactions between Target-PE complexes over the simulation time. Fluctuations in the number of hydrogen bonds throughout the simulation indicate a transient but sustained interaction profile between Target-PE complexes (except for ESR1-berberine). The stability of these interactions is crucial as hydrogen bonds maintain ligand-enzyme complex integrity and affect binding affinity and specificity.

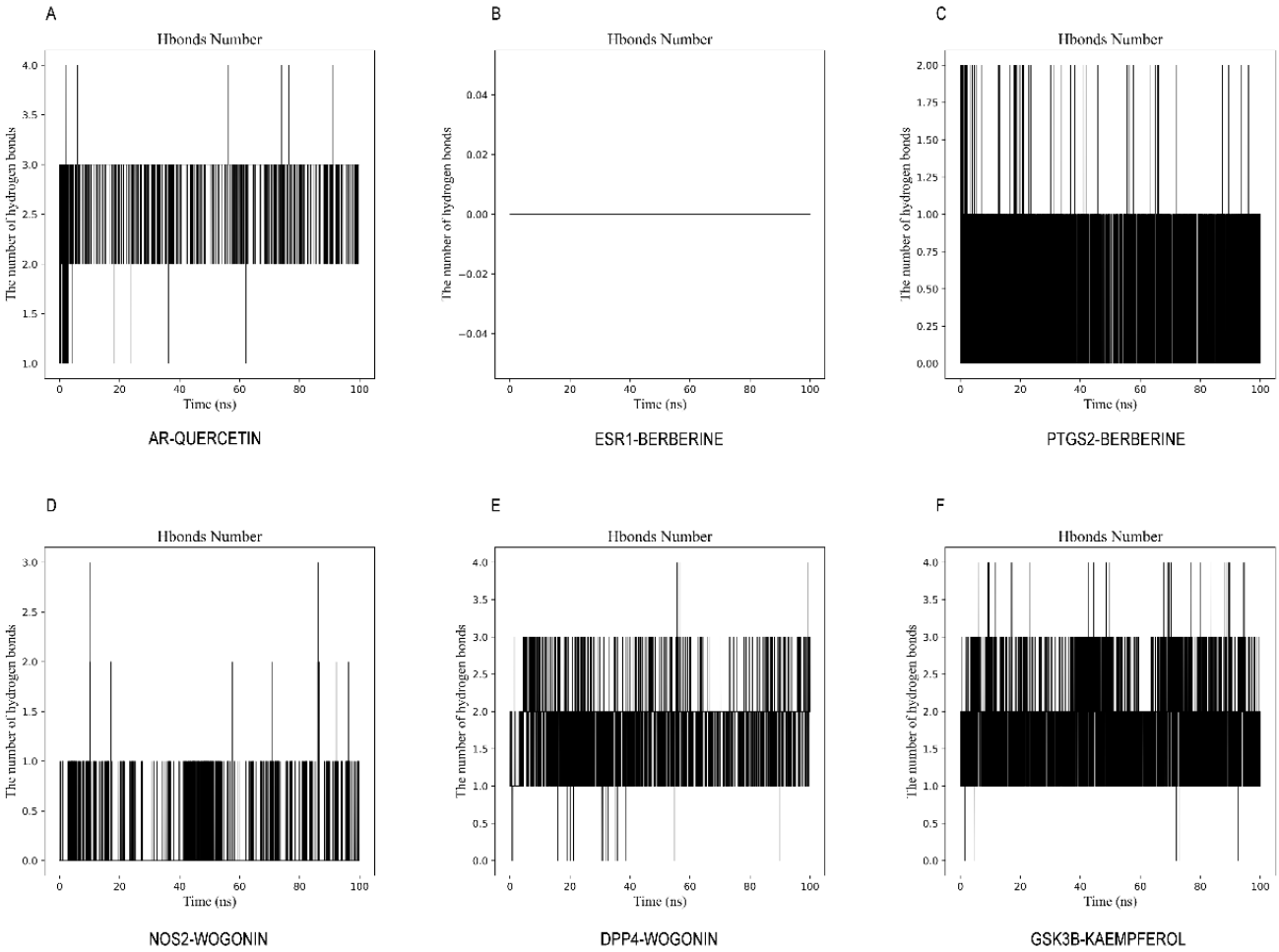

Figure 11. HN of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

3.10. Root mean square fluctuation (RMSF)

The RMSF plot (**Figure 12**) highlights the flexibility of residues within targets. Peaks around residues 180 and 240 suggest highly flexible regions, potentially loops or active-site-adjacent areas involved in substrate binding. Increased flexibility in these areas may facilitate ligand interaction, contributing to functional adaptability. Lower RMSF values in other regions indicate rigid, structurally stable segments within targets, maintaining the enzyme structure's integrity in the complex.

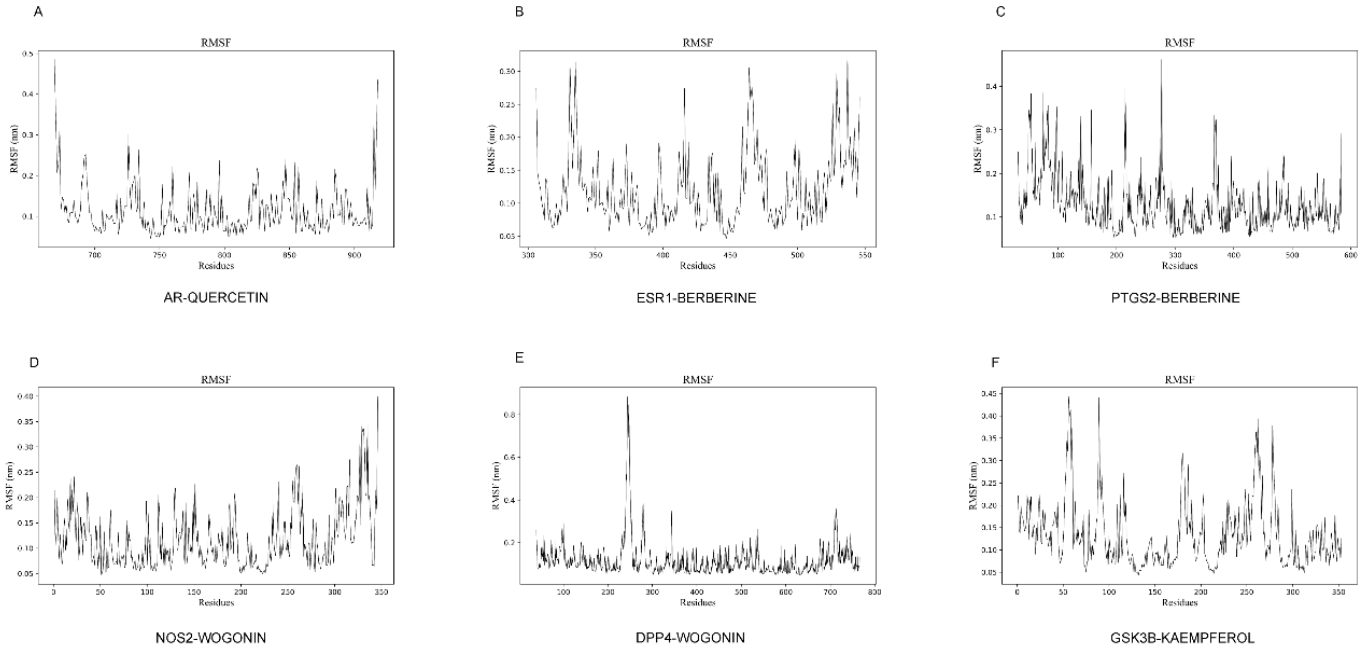

Figure 12. RMSF of the six complexes, **(A)** AR and QUERCETIN; **(B)** ESR1 and BERBERINE; **(C)** PTGS2 and BERBERINE; **(D)** NOS2 and WOGONIN; **(E)** DPP4 and WOGONIN; **(F)** GSK3B and KAEMPFEROL.

4. Conclusion

The enrichment analysis results of this study clearly indicate that the primary therapeutic effects of summer fragrant beads are concentrated in the early symptoms of heatstroke, such as anxiety, insomnia, migraine, arrhythmia, nausea, and vomiting. This finding not only strongly supports the efficacy records of summer fragrant beads in the Qing Palace archives but also highlights their significant potential in treating early heatstroke symptoms. Clinically, the presence of these symptoms often serves as a precursor to heatstroke [31,32], making the application of summer fragrant beads crucial for early intervention.

Through network pharmacology analysis, we successfully identified six potential targets and six key substances that interact with them. To further validate these interactions, we employed molecular docking technology, which showed good binding performance between the key substances and targets. Subsequent MD simulations revealed that the binding of six target proteins with PE complexes significantly affected the complexes' stability and flexibility, particularly in regions critical for functional adaptability. Stable RMSD, Rg, and SASA values observed during simulation indicated that the target-PE complexes maintained good structural integrity. Additionally, energy landscape analysis revealed the preferred low-energy conformation of the complexes, suggesting stable binding modes between targets.

These findings provide a deep understanding of the structural and dynamic characteristics of the interactions between target-PE complexes, guiding future research on how small molecules regulate biological activity. This information not only enhances our understanding of the therapeutic mechanism of summer fragrant beads but also offers valuable structural biology data for new drug development, laying a theoretical foundation for subsequent drug design and optimization.

5. Discussion

From the perspective of traditional Chinese medicine, it may trigger a re-examination and in-depth exploration of the application scope of traditional Chinese medicine. In the field of modern medicine, it may provide inspiration for the research ideas of heatstroke in modern medicine, prompting researchers to think about the mechanisms, treatment targets, and drug development directions of heatstroke from a new perspective.

In summary, this study not only provides a solid scientific foundation for further exploring the mechanism of action and clinical application prospects of the Qing Palace Summer-avoiding Pearl, but also, under the research paradigm of modern network pharmacology, molecular docking, and molecular dynamics simulation, drugs like the Qing Palace Summer-avoiding Pearl that have definite therapeutic effects but are only recorded in archives due to history or other reasons now have the possibility of revitalizing themselves in the contemporary research system. This means that we can leverage the power of modern scientific research to uncover the potential value of more ancient medicines.

It should be emphasized that our research requires further basic experiments and clinical observations to verify the effectiveness and reliability of the Qing Palace Summer-avoiding Pearl for all the results of this study. We hope with great anticipation that through these follow-up studies, we can gain a deeper and more comprehensive understanding of the mechanism of action of the Qing Palace Summer-avoiding Pearl and their clinical application in diseases such as anxiety, migraine, nausea and vomiting, and arrhythmia.

We look forward to this research arousing high attention from the academic and medical communities on early prevention and treatment of heatstroke, while also providing a new exploration path for the modernization of traditional Chinese medicine research. Through these efforts, we are expected to provide more comprehensive and effective protective measures for public health, reducing the impact of heatstroke on people’s lives. From the perspective of traditional Chinese medicine, it may trigger a re-examination and in-depth exploration of the application scope of traditional Chinese medicine. In the field of modern medicine, it may provide inspiration for the research ideas of heatstroke in modern medicine, prompting researchers to think about the mechanisms, treatment targets, and drug development directions of heatstroke from a new perspective.

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Author contributions: Writing—original draft preparation, HL and MZ; writing—review and editing, HL, MZ, SH, YS, YZ and YL; funding acquisition, YL; supervision, YL. All authors have read and agreed to the published version of the manuscript.

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Data availability statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethical approval: Not applicable.

Conflict of interest: The authors declare no conflict of interest.

Abbreviations

PEs	Plant Extracts
RMSD	Root Mean Square Displacement
Rg	Radius of gyration
TCMID	Traditional Chinese Medicines Integrated Database
TCMSP	Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform
OB	Oral Bioavailability
DL	Drug-likeness
TTD	Therapeutic Target Database
PPI	Protein–Protein Interaction
PE	Plant Extract
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes

MF	molecular function
BP	biological process
CC	cellular component
DAVID	Database for Annotation, Visualization and Integrated Discovery
PDB	Protein Data Bank File
GAFF	General Amber Force Field
RESP	Restrained Electrostatic Potential
NVT	Number of particles, Volume, Temperature
NPT	Number of particles, Pressure, Temperature
RMSF	Root Mean Square Fluctuation
MM/GBSA	Molecular Mechanics/Generalized Born Surface Area
AR	Androgen Receptor
ESR1	Estrogen Receptor 1
PTGS2	Prostaglandin-endoperoxide synthase 2
NOS2	Nitric oxide synthase 2
DPP4	Dipeptidyl peptidase 4
GSK3B	Glycogen synthase kinase 3 beta
CASP3	Caspase-3
CASP9	Caspase-9
CASP7	Caspase-7
ICAM1	Intercellular cell adhesion molecule-1
IGTB1	Integrin beta
MD	Molecular Dynamics
SASA	Solvent Accessible Surface Area
HN	H-bond Number

References

1. Gauer R, Meyers BK. Heat-Related Illnesses. Am. Fam. Physician. 2019; 99(8): 482–489.
2. Shimazaki J, Hifumi T, Shimizu K, et al. Clinical characteristics, prognostic factors, and outcomes of heat-related illness (Heatstroke Study 2017–2018). Acute. Med. Surg. 2020; 7(1): e516.
3. Oka K, Honda Y, Phung VLH, Hijioka Y. Prediction of climate change impacts on heatstroke cases in Japan's 47 prefectures with the effect of long-term heat adaptation. Environmental Research. 2023; 232: 116390. doi: 10.1016/j.envres.2023.116390
4. Shi L, Wang B, Wu Q, et al. Heatstroke: A multicenter study in Southwestern China. Frontiers in Public Health. 2024; 12: 1349753. doi: 10.3389/fpubh.2024.1349753
5. Lancet T. Heatwaves and health. Lancet. 2018; 392(10145): 359. doi:10.1016/S0140-6736(18)30434-3
6. Zhu X, Chen R, Yuan J, et al. Hourly Heat Exposure and Acute Ischemic Stroke. JAMA Netw. Open. 2024; 7(2): e240627.
7. Bi P, Williams S, Loughnan M, et al. The effects of extreme heat on human mortality and morbidity in Australia: Implications for public health. Asia Pac. J. Public Health. 2011; 23(2): 27–36.
8. Liu S, Rao Z, Huang P, et al. The role of cGAS STING pathway in myocardial injury caused by exertional heat stroke. Sports Science. 2024; 4: 80–88. doi: 10.16469/j.css.202404008.
9. Australian Resuscitation Council. ANZCOR Guideline 9.3.4. Heat Induced Illness (Hyperthermia): First Aid Management. Available online: https://resus.org.au/wpfb-file/anzcor-guideline-9-3-4-hyperthermia-jan16-pdf/ (accessed on 2 December 2018).

10. Brearley MB. Are Recommended Heat Stroke Treatments Adequate for Australian Workers? Annals of Work Exposures and Health. 2019; 63: 263–266.
11. Wu M, Luo SS, Yin J, et al. Latent class analysis of therapeutic effects of Huoxiang Zhengqi Oral Liquid on patients with cold due to wind-cold and dampness stagnation. Zhongguo Zhong Yao Za Zhi. 2024; 49(21): 5944–5950.
12. Sun L. Research on the History of Mingqing Fragrance Medicine [PhD thesis]. Chinese Academy of Traditional Chinese Medicine; 2015.
13. Guan X. Qing Dynasty Court Medicine and Medical Relics. Forbidden City Press; 2008. pp. 180–181.
14. Huang, Yangmu et al. "New clinical application prospects of artemisinin and its derivatives: a scoping review." Infectious diseases of poverty vol. 12,1 115. 11 Dec. 2023, doi:10.1186/s40249-023-01152-6
15. Li, Ying et al. "Research Progress on Artemisinin and Its Derivatives against Hematological Malignancies." Chinese journal of integrative medicine vol. 26,12 (2020): 947-955. doi:10.1007/s11655-019-3207-3
16. Tu, Youyou. "Artemisinin-A Gift from Traditional Chinese Medicine to the World (Nobel Lecture)." Angewandte Chemie (International ed. in English) vol. 55,35 (2016): 10210-26. doi:10.1002/anie.201601967
17. Li L. Wang M, Chen J, et al. Therapeutic potential of traditional Chinese medicine on heat stroke. Frontiers in Pharmacology. 2023; 14: 1228943. doi: 10.3389/fphar.2023.1228943
18. Chen X, Zhou H, Liu YB, et al. Database of traditional Chinese medicine and its application to studies of mechanism and to prescription validation. British Journal of Pharmacology. 2006; 149(8): 1092–1103. doi: 10.1038/sj.bjp.0706945
19. Huang L, Wang Q, Duan Q, et al. TCMSSD: A comprehensive database focused on syndrome standardization. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology. 2024; 128: 155486. doi: 10.1016/j.phymed.2024.155486
20. Liu JY, Li X. Standardization, objectification, and essence research of traditional Chinese medicine syndrome: A 15-year bibliometric and content analysis from 2006 to 2020 in Web of Science database. Anatomical Record. 2023; 306(12): 2974–2983. doi: 10.1002/ar.24821
21. Ru J, Li P, Wang J, et al. TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. Journal of Cheminformatics. 2014; 6: 13. doi: 10.1186/1758-2946-6-13
22. Fang SS, Dong L, Liu L, et al. HERB: A high-throughput experiment- and reference-guided database of traditional Chinese medicine. Nucleic Acids Research. 2021; 49(D1): D1197–D1206. doi: 10.1093/nar/gkaa1063
23. Wu Y, Zhang F, Yang K, et al. SymMap: An integrative database of traditional Chinese medicine enhanced by symptom mapping. Nucleic Acids Research. 2019; 47(D1): D1110–D1117.
24. Kong X, Liu C, Zhang Z, et al. BATMAN-TCM 2.0: An enhanced integrative database for known and predicted interactions between traditional Chinese medicine ingredients and target proteins. Nucleic Acids Research. 2024; 52(D1): D1110–D1120.
25. Zhou Y, Zhang Y, Zhao D, et al. TTD: Therapeutic Target Database describing target druggability information. Nucleic Acids Research. 2024; 52(D1): D1465–D1477.
26. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021; 49(1): D605–D612.
27. Sherman BT, Hao M, Qiu J, et al. DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 2022; 50(W1): W216–W221.
28. Burley SK, Bhikadiya C, Bi C, et al. RCSB Protein Data Bank (RCSB.org): Delivery of experimentally-determined PDB structures alongside one million computed structure models of proteins from artificial intelligence/machine learning. Nucleic Acids Res. 2023; 51(D1): D488–D508.
29. Abraham MJ, Murtola T, Schulz R, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 2015;1: 19–25.
30. Van Der Spoel D, Lindahl E, Hess B, et al. GROMACS: Fast, flexible, and free. Journal of Computational Chemistry. 2005; 26(16): 1701–1718.
31. Zhang Z, Wu X, Zou Z, et al. Heat stroke: Pathogenesis, diagnosis, and current treatment. Ageing Research Reviews. 2024; 100: 102409. doi: 10.1016/j.arr.2024.102409
32. Ouyang L, Ji J, Liu Z, et al. Research progress of heatstroke warning system. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2022; 34(7): 777–781. doi: 10.3760/cma.j.cn121430-20210831-01297