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Original Research Article

Network pharmacology and molecular docking studies on the mechanism of action of moist exposed burn ointment for treatment of diabetic foot ulcer

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Abstract

Purpose: *To investigate the bioactive components and mechanism of action of moist exposed burn ointment (MEBO) for treatment of diabetic foot ulcers using network pharmacology (NP) and molecular docking technology*

Methods: *Through pharmacology database of traditional Chinese medicine (TCM) systems (TCMSP), analysis platform and symptom mapping (SymMap) database, the bioactive components of MEBO were screened for and protein binding to bioactive components was predicted. Proteins related to Diabetic foot ulcer (DFU) were collected from GeneCards, OMIM, PharmGkb and TTD disease databases. With R language software, the binding proteins of bioactive components of MEBO intersected with the proteins related to occurrence of DFU. Proteins of DFU linked to treatment with MEBO were subjected to analysis using gene ontology (GO) and KEGG with R language software. AutoDock and PyMOL software were employed to dock active components of MEBO and major proteins of DFU. Targeted binding potential of bioactive compounds in MEBO to core proteins of DFU was analyzed.*

Results: *One hundred and five (105) bioactive ingredients and 246 likely therapeutic proteins were obtained. Proteins were mainly involved in biological processes in wound healing, oxidative stress, and lipopolysaccharide. The enriched signaling pathway focused on lipid metabolism and the process of atherosclerosis which involved PI3K and Akt, advanced glycation end-products (AGE)-receptor (RAGE) and mitogen-activated protein kinase (MAPK). The absolute values of these proteins were screened out with a docking score greater than 5 kcal/mol.*

Conclusion: *The biological effects of MEBO are related to multiple proteins and multiple signaling pathways. Therefore, healing effect of MEBO on DFU occurs via multiple pathways. The biological characteristics of MEBO need to be fully elucidated in further studies.*

Keywords: *Moist exposed burn ointment, Diabetic foot ulcer, Network pharmacology, Molecular docking, Proteins*

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INTRODUCTION

Diabetic foot ulcer (DFU) is a common complication of diabetes mellitus which occurs in 19 – 34 % of all diabetic patients in their lifetime [1]. More than 50 % of these patients require amputation [2,3]. Moreover, DFU is characterized by a high recurrence rate, a long treatment cycle, and a heavy economic burden. As the number of diabetic patients continues to rise worldwide, DFU has increasingly become a major problem that endangers human health and social development. The treatment of DFU is still a difficult problem for clinicians. Many new therapeutic methods aimed at abnormal inflammatory reactions and repair of cell dysfunction and free radical-induced DFU wounds have been explored and developed. These include autogenous skin transplantation, vascular reconstruction, negative pressure suction, growth factor therapy, and stem cell therapy, amongst others [4]. Some of the treatment methods have been used in clinics [5]. However, the major challenges are the high cost of the treatment methods and concerns about the effectiveness and safety of some of the emerging treatment methods.

Moist-exposed burn ointment (MEBO) is formulated from HuangLian (*Coptidis rhizoma*), HuangBo (Phellodendri chinensis Cortex), HuangQin (*Scutellariae radix*), YingSuQiao (*Papaveris pericarpium*) and DiLong (*Pheretima*). It is currently widely used in clinical treatment of DFU, pressure ulcers, perianal diseases, burns and other wounds [6-8]. Topical application of MEBO improves blood circulation in wounds and surrounding tissues, stimulates cell proliferation and tissue repair, controls infection, and promotes wound healing [9,10]. It is very convenient to use. The only necessity is to apply the ointment gently and evenly to the wound surface, 1 to 2 times a day [11].

Network pharmacology (NP) has gradually become a new strategy for studying the mechanisms of drug effects in biological systems. Drug side effects and interactions may be predicted using NP research such that adverse drug reactions are avoided. Moreover, the new drug ingredients and proteins obtained will be used to develop new drugs. Furthermore, NP may be applied to drug repurposing and exploration of new uses.

In the current study, NP methods and molecular docking technology were used to construct a network to confirm the proteins of MEBO involved in treatment of DFU. The aim was to determine the mechanism through which the active ingredients contained in MEBO treat DFU. It is hoped that the results might provide further theoretical basis for DFU therapy using TCM.

EXPERIMENTAL

Screening of the active ingredients and proteins of MEBO

The main active ingredients of MEBO were retrieved from the TCMSP and inputted one after the other as "HuangLian, HuangBo, HuangQin and YingSuQiao" in sequence of the query interface. Drug-likeness (DL) is usually a term used to describe the similarity between a drug and another drug in pharmaceutical chemistry or drug development. Drug-like properties are evaluated based on the chemical structures, mechanisms of action, pharmacological properties, and side effects of drugs, to determine the degree of similarity between a new chemical composition and a known drug, thereby providing a reference for selection, optimization, and evaluation during drug development [12]. It is generally believed that when DL property is greater or equal to 0.18, the new chemical composition is similar to the structure of the drug; this has certain research value [13]. The active ingredients were screened based on DL properties greater than or equal to 0.18, and the corresponding target proteins were queried based on the results. The SymMap database was used to locate the active ingredients of DiLong which may have been lost during the search on the TCSMP database. Then, all drug ingredients and their related proteins were summarized in order to obtain the target corresponding proteins of the active ingredients of MEBO after removing the repeated parts.

Screening of DFU proteins

Pharmacology Database of TCM Systems (TCMSP), Analysis Platform (https://old.tcmsp-e.com/tcmsp.php/), and Symptom Mapping (SymMap) database (http://www.symmap.org/) were used to screen for the bioactive components of MEBO and proteins binding to bioactive components were predicted. The databases were used to search the keywords "diabetic foot ulcer". All related target proteins were summarized, and the DFU proteins were obtained after removing the duplicated parts.

Screening of proteins of MEBO for treating DFU

To obtain the potential therapeutic proteins of MEBO involved in treatment of DFU, Proteins related to occurrence of Diabetic foot ulcer (DFU)

were collected from GeneCards (https://www.uniprot.org/), OMIM (https://omim.org/); PharmGkb (PharmGkb; https://www.pharmgkb.org/), and TTD, (https://db.idrblab.net/ttd/) disease databases. With R language software (version 4.3.1, (https://www.r-project.org/), the binding proteins of bioactive components of MEBO were intersected with the proteins related to occurrence of DFU. Proteins of DFU linked to treatment with MEBO were subjected to analysis using GO and KEGG with R language software. The R software was used to match and map the active ingredient-related proteins of MEBO and the related proteins of DFU.

Functional and pathway analysis

The R software was used to conduct GO function analysis and KEGG pathway enrichment analysis ($p < 0.05$) on the obtained MEBO proteins for DFU treatment. AutoDock (https://autodock.scripps.edu/) and PyMOL (https://autodock.scripps.edu/) software were employed to dock active components of MEBO and major proteins of DFU, and the targeted binding potential of bioactive compounds in MEBO to core proteins of DFU was analyzed. The results are displayed in the form of dot diagrams. Values of *p* were used to indicate that the observed results were statistically significant. In this case, $p < 0.05$ was considered a threshold, and if the value was less than this threshold, the difference or association was considered significant.

Protein-protein interaction (PPI) meshwork of MEBO target proteins

The relevant proteins of MEBO for treating DFU were entered into STRING database (https://cn.string-db.org/). A PPI network of the corresponding proteins was constructed, and "highest confidence" was set at > 0.95. A frequency map was made of the interaction between the DFU target protein and proteins using R language software, and the pictures and TSV files were saved.

Construction of a network on MEBO proteins involved in DFU treatment

The Cytoscape vers. 3.8.0 software package was plugged into CytoNCA and employed to score the proteins of DFU based on the methods of Betweenness Centrality, Closeness Centrality, Eigenvector Centrality, Local Average Connectivity and Network Connectivity. Then, 5 proteins with scores greater than the median were kept. Then, the R software was used to identify proteins related to the bioactive components of the drug's corresponding points. An interaction network diagram of bioactive components of MEBO acting on disease targets for DFU treatment was constructed with Cytoscape 3.8.0 software.

Molecular docking

To elucidate the mode of action of the compounds at the molecular level, the active ingredients were docked in protein pocket. Structure data format (SDF) 2D structure of the active ingredients was downloaded from the PubChem website. The top four target 3D structures in the histogram were downloaded from RCSB PDB database (https://www.rcsb.org/). Autodockvina was used for molecular docking, while the compounds showing the least docking energy conformations were used for binding mode diagram plotted using PyMOL software.

RESULTS

Active ingredients and proteins

The bioactive components of *HuangLian, HuangBo, HuangQin, YingSuQiao,* and *DiLong* obtained from the TCMSP database, and screened were a total of 105 MEBO potential bioactive ingredients while 246 linked targeted proteins were identified.

Proteins for DFU

The proteins obtained from searching the indicated databases searched with the keyword " diabetic foot ulcer" were 2647, 167, 19, and 8, respectively. The four databases screened out 2841 targets, while 2690 target proteins were selected after de-duplication.

MEBO proteins involved in treatment of DFU

Using the R language software, 246 target proteins related to the bioactive ingredients of MEBO and 2690 targets related to DFU were matched and mapped, as shown in Figure 1. After intersection of the two search results, the potential therapeutic effect of MEBO on DFU was related to 155 proteins.

Functional and pathway analyses

Results of R language software for GO function and KEGG route enrichment analysis of therapeutic targets showed that dots were involved in biological processes which include peptide binding, wound healing, oxidative stress,

and lipopolysaccharide-binding. Moreover, dots were enriched mainly in PI3K-Akt, AGE-RAGE and MAPK signaling pathways, as shown in Figure 2. These results provide evidence to support the fact that MEBO might bring about DFU therapy through several biological processes and pathways. The area of dots was positively correlated with the number of enriched proteins, while the blue dots changed to red ones, indicating *p* values ranging from large to small. Therefore, the larger the area of the red dots, the higher the significance of the proteins.

Target PPI network of MEBO

Target PPI network corresponding to 155 proteins was established using STRING database, and the discrete proteins were removed. As shown in Figure 3, the PPI network of 113 proteins contained 113 intrinsic and 364 edge components.

Frequency map of proteins of DFU

The R software was used to calculate the frequencies of target interaction and to draw a histogram, as shown in Figure 4. Proteins with higher frequencies of interaction were heat shock protein 90 kDa Alpha class A member 1 (HSP90AA1), threonine kinase 1 (AKT1), tumor protein 53 (TP53), tumor necrosis factor (TNF), estrogen receptor 1 (ESR1), and interleukin 6 (IL6). These data indicate that these proteins are highly related to DFU, and could be used as the key target MEBO proteins for treating DFU.

Network model of "MEBO-active ingredients-proteins-DFU"

A total of 29 disease proteins and 75 drug bioactive ingredients were obtained (Table 1, Table 2). The bioactive components of MEBO and the potential MEBO proteins related to DFU treatment were inputted into the Cytoscape 3.8.0 software. The interaction network diagram of "MEBO-active ingredients-proteins-DFU" is shown in Figure 5.

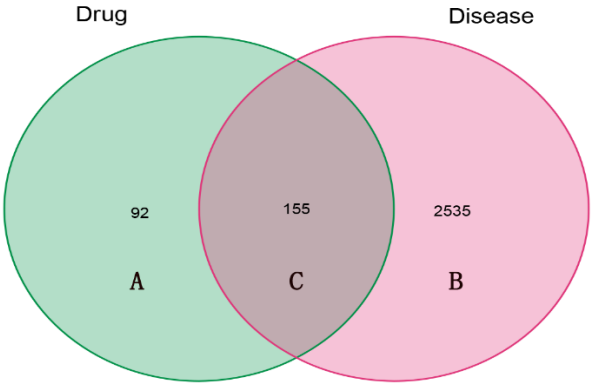

Figure 1: The intersection of targets of MEBO and DFU disease. (A) Predicted proteins of active ingredients of MEBO, (B) Proteins of DFU, (C) Intersection proteins of two search results

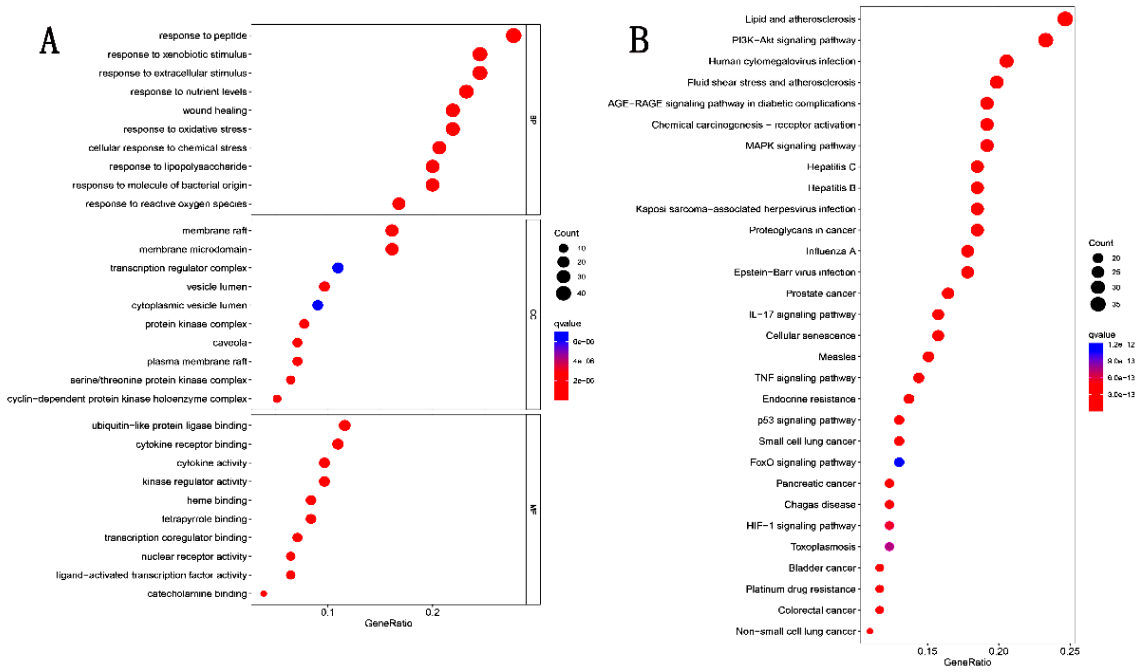

Figure 2: GO function and KEGG pathway enrichment analysis. (A) GO function analysis on MEBO for treating DFU. (B) KEGG pathway enrichment analysis on MEBO for treating DFU

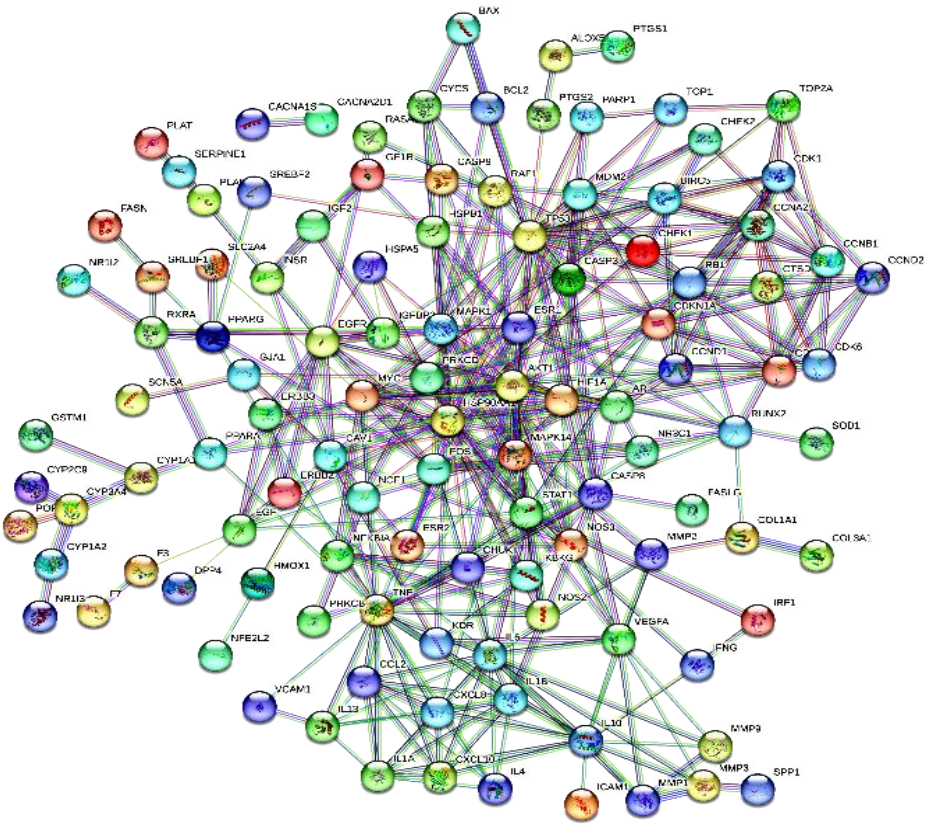

Figure 3: PPI Network of MEBO proteins for treating DFU

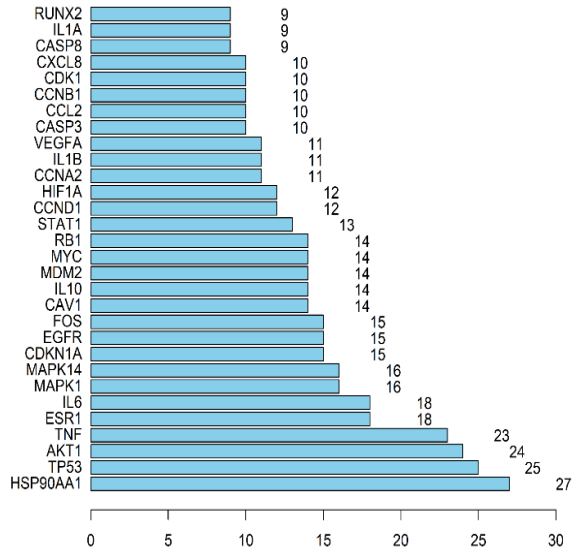

Figure 4: Interaction frequency map of MEBO proteins linked to DFU treatment. ***Note:*** The combination of acronyms and numbers on the left represent the MEBO proteins related to treatment of DFU, while the numbers on the right represent interaction between a single protein and other proteins in PPI. The larger the number, the more complex the relationship between the protein and other proteins, suggesting that it is very important in multiple proteins, and has high research value.

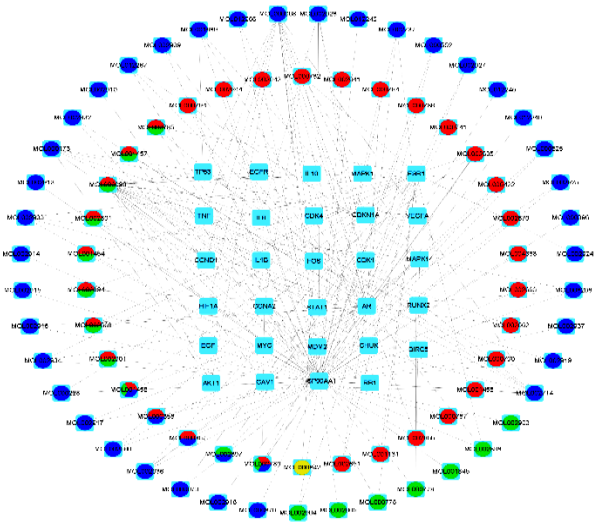

Figure 5: "MEBO bioactive ingredient – DFU protein" interaction network diagram. The squares in the middle are the proteins. The circular dots are active ingredients of drugs. The blue dots represent genes related to HuangQin. The green dots represent genes related to HuangLian. The red ones represent genes related to HuangBo. The yellow ones represent genes related to DiLong. The different colors indicate genes related to different drugs while corresponding colors contain similar components.

Table 1: Main active ingredients information of MEBO

*TCMSP no.	Molecular name	Absorbance	Contained in
MOL002904	Berlambine	0.82	HuangLian
MOL002905	Zosimin	0.36	HuangLian
MOL000779	6-O-E-Feruloylajugol	0.43	HuangLian
MOL002903	(R)-Canadine	0.77	HuangLian
MOL002898	Groenlandicine	0.72	HuangLian
MOL001845	Clemastanin B	0.38	HuangLian
MOL000778	6-O-E-Feruloylajugol	0.85	HuangLian
MOL000525	Norwogonin	0.21	HuangQin
MOL002937	Dihydrooroxylin	0.23	" "
MOL002925	5,7,2',6'-Tetrahydroxyflavone	0.24	" "
MOL012240	2',3',5,7-tetrahydroxyflavone	0.24	" "
MOL002928	Oroxylin A	0.23	" "
MOL002560	Chrysin	0.18	" "
MOL000396	(+)-Syringaresinol	0.72	" "
MOL012266	Rivularin	0.37	" "
MOL002932	Panicolin	0.29	" "
MOL002924	Darendoside B	0.22	" "
MOL002927	Skullcapflavone II	0.44	" "
MOL002913	Dihydrobaicalin	0.21	" "
MOL002934	Neobaicalein	0.44	" "
MOL000073	Ent-Epicatechin	0.24	" "
MOL000552	5,2'-Di (OH)-6,7,8-trimethoxyflavo-ne	0.35	" "
MOL002737	Scutellarein	0.24	" "
MOL000870	Hexatriacontane	0.41	" "
MOL002916	2-(2,6-Dihydroxyphenyl)-3,5,7-trihydroxy-chromone	0.27	" "
MOL012245	5,7,4'-Tri(OH)-6-methoxyflavanone	0.27	" "
MOL002909	5,7,2,5-Tetra (OH)-8,6-dimethoxyflavone	0.45	" "
MOL002914	Eriodyctiol (flavanone)	0.24	" "
MOL002918	Ganhuangenin	0.37	" "
MOL000008	Apigenin	0.21	" "
MOL002915	Salvigenin	0.33	" "
MOL012246	5,7,4'-Tri (OH)-8-methoxyflavanone	0.26	" "
MOL002933	5,7,4'-Tri (OH)-8-methoxyflavone	0.27	" "
MOL000228	(2R)-7-Hydroxy-5-methoxy-2-phenylchroman-4-one	0.20	" "
MOL002917	5,2',6'-Tri (OH)-7,8-dimethoxyflavone	0.33	" "
MOL012267	Scutevulin	0.27	" "
MOL002936	5,8-Di (OH)-6,7-dimethoxyflavone	0.29	" "
MOL002714	Baicalein	0.21	" "
MOL000173	Wogonin	0.23	" "
MOL002910	Carthamidin	0.24	" "

Molecular docking results

The top 4 proteins in PPI, which were HSP90AA1, AKT1, TP53 and TNF, were selected as key proteins. Through R language software, it was found that apigenin, baicalein, quercetin and wogonin were the active ingredients of drugs that could be targeted. The interactive association between apigenin and TNF was verified using molecular docking. The binding relationships between apigenin and TNF, baicalein and HSP90AA1, quercetin and AKT1, and between wogonin and TP53, were also verified using molecular docking, which verification showed absolute docking fraction energy higher than 5 kcal/mol, as shown in Figure 6. This meant that the binding activity between each protein target and the component was good. The docking scores of apigenin and TNF, baicalein and HSP90AA1, quercetin and AKT1, and wogonin and TP53 were -8.5, -7.8, -6.3 and -8.0 kcal/mol, respectively. These values indicate that the active ingredients of MEBO were well bound to DFU-related proteins.

DISCUSSION

In this study, the network construction and analysis of "MEBO-active ingredients- proteins-DFU" was carried out with NP. The results showed that MEBO contained a variety of active ingredients for treating DFU. To our knowledge, this study is the first research with NP screening on the biological characteristics of MEBO for treating DFU.

Table 2: Main active ingredients information of MEBO (*contd.*)

*TCMSP no.	Molecular name	Absorbance	Contained in
MOL002919	Viscidulin III	0.37	“ “
MOL001689	Acacetin	0.24	“ “
MOL008206	Moslosooflavone	0.25	“ “
MOL002644	Phellopterin	0.28	HuangBo
MOL002635	(±)-Lyoniresinol	0.54	“ “
MOL002642	Phellodendrine	0.58	“ “
MOL002663	Skimmianin	0.20	“ “
MOL002670	Cavidine	0.81	“ “
MOL000764	Magnoflorine	0.55	“ “
MOL001131	Phellamurin	0.39	“ “
MOL000787	Fumarine	0.83	“ “
MOL002655	Amurensin	0.44	“ “
MOL000741	(2S,3S)-3,5,7-trihydroxy-2-(4-(OH)Phe-chroman-4-one	0.24	“ “
MOL000782	Menisporphine	0.52	“ “
MOL006422	Thalifendine	0.73	“ “
MOL000794	Menisperine	0.59	“ “
MOL000786	D-Tetrahydropalmatine	0.64	“ “
MOL002662	Rutaecarpine	0.60	“ “
MOL002651	Dehydrotanshinone II A	0.40	“ “
MOL004368	Hyperin	0.77	“ “
MOL001455	(S)-Canadine	0.77	“ “
MOL002641	Phellavin	0.44	“ “
MOL000790	Isocorypalmine	0.59	“ “
MOL000842	Sucrose	0.23	DiLong
MOL002891	Magnoflorine	0.55	HuangLian, HuangBo
MOL000785	Palmatine	0.65	“ “
MOL000098	Quercetin	0.28	“ “
MOL002668	Worenine	0.87	“ “
MOL002894	Berberrubine	0.73	“ “
MOL001454	Berberine	0.78	“ “
MOL002901	Phellodendrine	0.58	“ “
MOL001457	Columbamine	0.59	“ “
MOL000358	Beta-sitosterol	0.75	HuanQin, HuangBo
MOL000357	Sitogluside	0.62	“ “
MOL002897	Epiberberine	0.78	HuangLian, HuangQin
MOL000789	Jatrorrizine	0.59	“ “
MOL001458	Coptisine	0.86	HuangLian, HuangQin, HuangBo

*MOLxxxxxx is the number of the active ingredient in the TCMSP database.

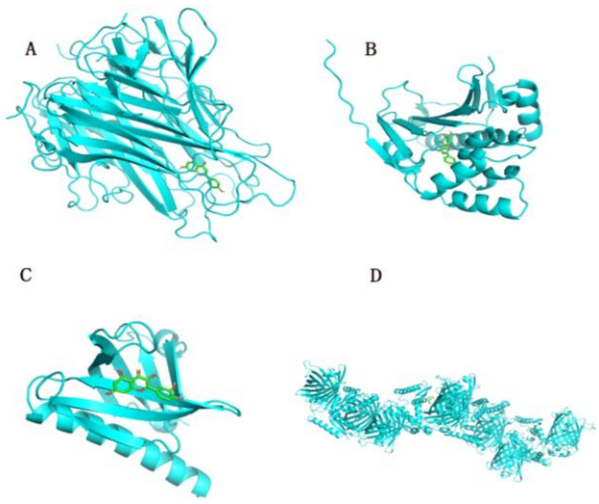

Figure 6: Molecular docking results. (A) Computer simulations of molecular binding of apigenin and TNF, (B) baicalein and HSP90AA1. C: quercetin and AKT1, and D: wogonin and TP53

Firstly, target proteins of MEBO used for treating DFU were identified. A total of 155 proteins were found to be correlated to the predicted proteins of the active ingredients of MEBO and the proteins of DFU. Then, GO function and KEGG pathway enrichment analysis were used to select the intersecting signaling pathways. It was observed that PI3K/AKT, AGE/RAGE, and MARK signaling pathways were highly related to the mechanism underlying the use of MEBO for treating DFU. These signaling pathways were involved in cell proliferation, insulin resistance and inflammation. Therefore, the results indicate that multiple mechanisms underlie the use of MEBO for treating DFU. Thus, it may be assumed that the clinical outcomes of MEBO are an integrated effect via multiple signaling pathways.

Next, the proteins with higher frequencies in DFU therapy were screened. A total of 155 proteins

were obtained from the screening. Then, the roles of proteins in the process of DFU therapy were analyzed. It was observed that HSP90AA1, AKT1, TP53, TNF, ESR1 and IL6 were highly related to DFU. Furthermore, genes related to the 75 active ingredients of MEBO that might be associated with the previous proteins were screened. In summary, the results showed that the therapeutic effect of MEBO is a comprehensive integrated outcome involving multiple proteins and multiple signaling pathways. Then, the association between proteins obtained and the active ingredients of MEBO was predicted. Quercetin plays an anti-diabetic role by increasing insulin secretion, reducing insulin resistance, maintaining glucose homeostasis, blocking inflammation and oxidative stress, and enhancing apoptosis [14]. Topical application of quercetin promotes wound healing by increasing the expressions of vascular endothelial growth factor (VEGF) and TGF-β1. Quercetin reduces wound inflammatory cells, promotes fibroblast proliferation, and increases microvascular density [15]. Moreover, baicalein and wogonin have been shown to suppress high-glucose-mediated vascular inflammatory changes in mice as well as in endothelial cells of human umbilical vein [16]. Wogonin also directly acts on a variety of immune cells and inhibits the production of the inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α. It inhibits the production of inflammatory mediators such as NO prostaglandins, and ROS [17]. Apigenin, another discovery, promotes angio-proteins, reduces endothelial cell apoptosis, alleviates oxidative stress, and activates autophagy [18].

Lastly, the association between the identified genes and DFU was predicted. *Staphylococcus aureus* significantly stimulates HSP90AA1 gene expression in keratinocytes [19]. Wound healing may be negatively affected by HSP90AA1 via its participation in the process of inflammation in DFU. It has been shown that MEBO participates in the repair and healing of diabetic ulcer wounds by regulating the PI3K-Akt-mTOR signal route [20]. The gene TP53 is highly expressed under diabetic conditions, and inhibition of TP53 attenuates hyperglycemia-induced inflammation and oxidative stress in endothelial cells, thereby ameliorating aortic endothelial dysfunction [21]. Serum ESR1 is highly expressed in patients with DFU, and ESR1 inhibits human skin fibroblasts [22]. Indeed, TNF and IL6 are commonly used inflammatory markers that are closely related to the healing of diabetic wounds.

Limitation of study

The databases used have limited research data on traditional Chinese medicine. Secondly, the search was limited to two drug databases (TCMSP and SymMap) and four disease databases, i.e., GeneCards, OMIM, PharmGkb, and TTD. Thus, the results obtained may not fully reflect the biological characteristics of MEBO. Furthermore, a lot of research on traditional Chinese medicine is ongoing.

CONCLUSION

The bioactive ingredients of MEBO used in DFU treatment are mainly apigenin, baicalein, quercetin and wogonin while MEBO proteins involved are TNF, HSP90AA1, AKT1 and TP53. The main active ingredients of MEBO had good targeted binding potential to key proteins. Target genes of MEBO participate in biological processes such as promoting wound healing, inhibiting oxidative stress and lipopolysaccharide formation, through PI3K/AKT, AGE-RAGE and MAPK signaling routes.

DECLARATIONS

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Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yu Zhou and Yi Li conceived and designed the study, and drafted the manuscript. Tianqi Zhang and Ting Luo collected, analyzed and interpreted the experimental data. Ying Liu and Biaoliang Wu

revised the manuscript for important intellectual content. All authors read and approved the final manuscript for publication.

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