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Research Article

Hypericum aqueous extract promotes skin wound healing by modulating the PI3K/Akt signaling pathway

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Abstract: This study investigated the promotional effect of Hypericum aqueous extract on the healing of rabbit skin wounds and its possible mechanism. Sixteen New Zealand rabbits were modelled as skin defect trauma, and each rabbit had three wounds on the back skin, which were saline (Control), Hypericum aqueous extract (HAE), and Yunnan Baiyao (YNBY), and the wounds were cleaned and changed daily. The therapeutic effects were evaluated by wound gross observation, wound healing rate, and wound healing time, respectively, and we found that HAE could promote the wound healing rate. Histopathological staining observations showed that HAE improved the histologic changes of the wound and reduced the amount of inflammation. In addition, through the network pharmacology study, we obtained that the target of action was mainly focused on biological processes such as epithelial cell proliferation and signaling pathways such as PI3K-Akt, and the core targets included VEGFA, etc.; the validation results demonstrated that HAE significantly inhibited the expression of PI3K, P-PI3K, Akt and P-Akt, and it could increase the expression of VEGF, which helped to restore the vascularization of the wounds. In conclusion, Hypericum aqueous extract may promote wound healing by regulating the PI3K/Akt signaling pathway.

Keywords: Hypericum aqueous extract; wound healing; network pharmacology; PI3K/Akt signaling pathway

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1 Introduction

Hypericum monogynum L, for the Garcinia Cambogia, *Hypericum monogynum*, summer and fall when the flowering is harvested, shade-dried or low-temperature drying, its medicinal parts are mainly for the above-ground parts of the plant, is Hypericum monogynum genus with the prospect of development of the wild gardens, medicinal resources plants, in the folk medicine has been used for 2,400 years of history. Its chemical constituents include flavonoids, steroids, triterpenoids, saturated fatty acid esters, xanthones, phenols, etc.[1-3], and it has pharmacological properties such as antidepressant, hypolipidemic blood glucose, sedative, antibacterial and anti-inflammatory, and trauma astringent[4-7].

It has been reported that the volatile constituents of Hypericum have significant wound healing potential, with accelerated wound healing, a significant increase in angiogenesis, and promotion of fibroblast proliferation, maturation, and subsequent collagen deposition in Hypericum perforatum-treated animals as compared to the control group, which showed its role in regulating the inflammatory and proliferative phases of the healing cascade, with Hypericum being the more active in the various wound healing phases of the compounds, and its anti-inflammatory activity may play a role in the wound healing action of the plant[8-10]. Although the effect of nonvolatile components of Hypericum on wound healing has been reported in the literature, the study of its mechanism of action has yet to be in-depth. Therefore, this study is intended to observe the therapeutic effect of Hypericum extracts on wound healing based on the establishment of a skin defect model, to explore the relationship between the influence of each other and the mechanism of wound healing, and to provide a theoretical basis for the selection of topical drugs for wounds.

The integrity of healthy skin plays a vital role in maintaining the physiological homeostasis of the body[11, 12]. Trauma is defined as damage to normal skin (tissues) caused by traumatic factors such as surgery, external forces, heat, electrical currents, chemicals, and low temperatures, as well as internal body factors such as localized blood supply disorders, which are often accompanied by a disruption of the skin's integrity and loss of a certain amount of normal tissue. At the same time, the normal function of the skin is impaired, also known as trauma or traumatic injury. Wound healing is an important physiological process, and until now, various causes of skin trauma and its healing scars have had a

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great impact on human health and quality of life. Therefore, the development of drugs and their corresponding preparations or dressings that can accelerate wound healing, promote the neogenesis of local hair follicles, sweat glands, and other accessory organs, and help the traumatized tissues to be repaired both structurally and functionally is a difficult and key issue in the field of trauma therapy, which is of great significance for research. Traditional Chinese medicine is the traditional medicine of China, which has a wide range of sources and has accumulated rich experience in the treatment of trauma.

2 Materials and methods

2.1 Experimental subjects

Sixteen New Zealand Large White rabbits weighing 1.5-3.0 kg were purchased from Beijing Huazhokang Biotechnology Co. All rabbits were acclimatized for 7 d and fed food and water ad libitum, and drinking water was treated with autoclaved distilled water. The temperature was 25 ± 2 ℃ and the relative humidity was 50 ± 10%. The use of animals in this study was reviewed and approved by the Experimental Animal Ethics Committee of Guizhou Medical University (No. 2103259).

2.2 Preparation of aqueous extract of Hypericum and identification of its LC-MS composition

Clean Hypericum branches were collected, dried, and crushed; 200 g of Hypericum crushed material was weighed into a beaker, pure water was added to the beaker in a ratio of 1:10, and the extract was extracted by soaking, heating in a water bath and ultrasonic extraction for 30 min each, and then pumped for filtration. The filtrate was repeated three times, and the filtrate was put into a rotary evaporator at 55 ℃ for reflux extraction to obtain the flow of ointment. The Hypericum aqueous extract (HAE) was obtained by vacuum freeze-drying. The HAE was put into sterile bottles and stored in a sealed container, and the yield was 11.9% (200 g of dried Hypericum was extracted to obtain 23.8 g of HAE).

2.3 Animal experiments

16 New Zealand rabbits were divided into two groups, 8 rabbits in each group. One group was used to record the wound healing time; the other group was used to record the wound healing rate. The patient was anaesthetized by 10% chloral hydrate (35 mg/kg) intravenous injection at the ear edge. A hair removal machine removed the back. After the skin was disinfected with alcohol, the round full-layer skin with a diameter of about 1.5 cm was surgically cut off to create a full-skin defect trauma model. There were 3 wounds on the skin of each rabbit, which were the control group, YNBY group, and HAE group. After dressing, all wounds were covered with Vaseline gauze and then bandaged with dry gauze; the wounds were replaced every day. The wound was observed for inflammation, exudation, purulent secretion, wound area, contraction of skin tissue around the wound, and new growth of wound margin on the 7th, 14th, and 21st day after administration. Record wound healing time, wound healing area and wound healing rate, The New Zealand rabbits in each group were fixed on the animal table at the time of trauma preparation (0 d), 4 d, 8 d, and 12 d, respectively, and the traumas were covered with transparent films and traced, and the films were drawn along the edges of the traumas with a marker pen, and then the transparent films were pasted on grid paper for grid counting, and the actual areas of traumas in each group were measured, and then the healing rates of traumas in each group were calculated at different time points. Calculate the wound healing rate: (original wound area - unhealed wound area) / original wound area × 100%.

2.4 Collection and processing of tissue specimens

At 21 days after administration, 8 New Zealand rabbits were anesthetized with 10% chloral hydrate, and the wound granulation and surrounding skin tissues were incised on the back of the rabbits under aseptic conditions. The incision was made approximately 0.3 cm beyond the wound edge, perpendicular to the dorsal skin surface of the rabbit and deep to the muscle layer. The skin tissue was immediately fixed with 4% paraformaldehyde and then dehydrated in an automatic tissue dehydrator for 24 h. Successive paraffin sections of 4 μm thickness were cut and stored in a refrigerator at 4 ℃. Some of the sections were stained with conventional HE histology, and the epidermal and dermal structures, tissue necrosis and inflammation were observed under light microscope. The remaining sections were subjected to SP immunohistochemical staining, CD34-labeled microvessel density (MVD) counting, and vascular endothelial growth factor (VEGF) measured by SP immunohistochemical method using Image-Pro Plus image analysis software.

2.5 Screening of active ingredients and prediction of target

The components of HAE obtained by LC-MS were predicted by the SwissTargetPrediction database. Using "wound healing" as the keyword, the gene related to wound healing was retrieved from the GeneCards database. The intersection of the two is the potential target of HAE affecting wound healing.

2.6 Construction of protein interaction network

Through the STRING database, the species was set as "Human (Homo sapiens)", the highest confidence level was selected (0.9, the remaining parameters were kept at the default values), the PPI network of potential targets of hypericum water extract affecting wound healing was constructed, and the interaction data was saved. Targets are scored using the MCC algorithm of Cytoscape software's CytoHubba plugin to screen for core targets.

2.7 Gene ontology (GO) analysis and KEGG pathway enrichment analysis

"enrichplot" and "ggplot2" packages are used to convert gene names into Entrez gene IDs using the "org.Hs.eg.db" package in the R language to remove genes without IDs. The species was set as human, and the P-value screening condition was pvalueFilter < 0.05. After correction, the P-value screening condition was qvalueFilter < 0.05. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and GO molecular function (MF), biological process (BP), and cell component (CC) analysis; the result is plotted as a bubble diagram for visualization.

2.8 Construction of drug-component-target-disease-pathway network

Drug, active ingredient, potential target, their interaction relationship, and enrichment analysis results were imported into Cytoscape 3.7.1 software to output the drug-components-target-disease-pathway network diagram, which was then used to edit

and visualize the network diagram for analysis.

2.9 Western Blotting

The IP lysate (containing 1% PMSF) was added to the skin tissue to prepare about 10% homogenate. The homogenate was frozen at 220 W for 5 min. After standing on the ice for 30 min, the homogenate was put into a high-speed refrigerated centrifuge at the speed of 12,000 rpm, 4 ℃, for 10 min. The supernatant was separated into a new EP tube to extract the total protein in the skin tissue. Put the prepared SDS-PAGE glue into an electric swimming pool, boil the protein in a metal bath for 10 min, and then mix. Mark 5 ul and 20 μg sample protein were added to the sample holes, transferred to PVDF membrane, sealed with 5% BSA, washed the membrane, added the corresponding primary antibody, and incubated at 4 ℃ overnight. TBST solution was washed, diluted secondary antibody was added, and incubated for 120 min in a horizontal shaker. Wash TBST solution and develop with dark room exposure. ImageJ software analyzes protein bands and corrects them.

2.10 Statistical analysis

Statistical software Excel and SPSS 23.0 were used to analyze the measurement data, and the measurement data were expressed as mean ± standard deviation (x ± s). The LSD-t-test method in one-way ANOVA was used to analyze the significance of differences between groups, with $P < 0.05$ and $P < 0.01$ as statistically significant differences.

3 Results

3.1 Compositional characterization of aqueous extracts of Hypericum

According to the LC-MS detection spectrum analysis (Fig. 1), the mass spectrum of each component was retrieved by computer and compared with the standard spectrum, and a total of 23 chemical active components in the HAE were identified. The specific information on the compounds is shown in Table 1. Follow-up network pharmacological studies took these 23 components as research objects.

3.2 Effects of aqueous extracts of Hypericum on a skin trauma model

3.2.1 *Macroscopic Observation of Wound Surface*

As depicted in the figure, following 7 days of treatment, all three

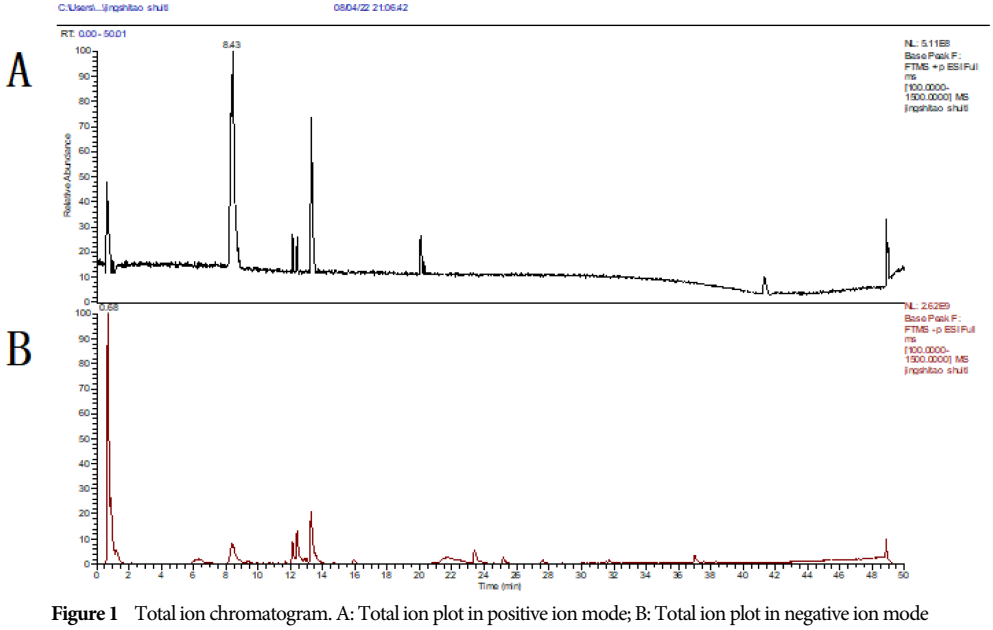

Figure 1 Total ion chromatogram. A: Total ion plot in positive ion mode; B: Total ion plot in negative ion mode

Table 1 Identification of HAE chinensis

Compound name	Formula	molecular weight	Compound name	Formula	molecular weight
Quinic acid	$C_7H_{12}O_6$	192.062,42	Rutin	$C_{27}H_{30}O_{16}$	610.152,39
Shikimic acid	$C_7H_{10}O_5$	174.051,66	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.094,87
2-Pyrrolidinecarboxylic acid	$C_5H_9NO_2$	115.063,40	Astilbin	$C_{21}H_{22}O_{11}$	450.115,50
			Atractyloside A	$C_{21}H_{36}O_{10}$	494.235,58
Trigonelline HCl	$C_7H_7NO_2$	137.047,43	Taxifolin	$C_{15}H_{12}O_7$	304.057,58
Citric acid	$C_6H_8O_7$	192.026,09	Astragalin	$C_{21}H_{20}O_{11}$	448.099,78
L-Leucine	$C_6H_{13}NO_2$	131.094,47	Quercitrin	$C_{21}H_{20}O_{11}$	448.099,96
L-Phenylalanine	$C_9H_{11}NO_2$	165.078,68	Tetrahydroxyxanthone	$C_{13}H_8O_6$	260.031,45
Protocatechuic acid	$C_7H_6O_4$	154.025,45	Morin	$C_{15}H_{10}O_7$	302.042,11
Protocatechualdehyde	$C_7H_6O_3$	138.030,42	Demethylwedelolactone	$C_{15}H_8O_7$	300.026,47
Procyanidin B1	$C_{30}H_{26}O_{12}$	578.141,17	Kaempferol	$C_{15}H_{10}O_6$	286.047,21
(+)-Catechin hydrate	$C_{15}H_{14}O_6$	290.078,49	Amentoflavone	$C_{30}H_{18}O_{10}$	538.088,94

groups exhibited scab formation on their skin wounds, devoid of redness, swelling, exudation, or overt infection. Each group initiated scab formation, causing the surrounding skin to contract into folds. By day 14 of treatment, all three groups had developed scabs with deepening color and a transition to a brownish hue. The drug-treated groups' scabs firmly adhered to the base with noticeable edge contraction and new skin formation; however, the control group still displayed varying degrees of wound edge reaction with less epithelial growth and contraction compared to the other two groups. After 21 days of treatment, the two drug-treated groups had almost entirely been covered by new epithelial tissue, with only a small portion of the scabbed wound remaining. The control group exhibited significantly reduced wound size compared to before but featured some areas covered by thin and fragile new epithelial tissue; nevertheless, its wound healing was notably inferior to that observed in the other two groups (Figs. 2A-2B).

3.2.2 *Effect of aqueous extract of Hypericum on the healing time of rabbit wounds*

Time from the day of surgery to complete epithelialization of the wound as healing in the three groups of wounds, all wounds in each group healed completely after treatment. The healing time of the control group was 30.63 ± 3.54 days, with the longest duration being 33 days; for the HAE group, it was 26.38 ± 2.90 days, and for the YNBY group, it was 26.87 ± 1.53 days, with the shortest duration being 24 days (Fig. 2C). The drug-treated groups exhibited significantly faster wound healing compared to the saline group.

3.2.3 *Effect of aqueous extract of Hypericum on the healing rate of rabbit wounds*

After treatment with three different medication modes, the traumatic area of each group was gradually reduced with the extension of time, and the results of the experimental study showed that after 7d of medication, there was no statistically significant difference in the comparison of the wound healing rate of the three groups. At 14d and 21d, the wound healing rate of the two groups was higher than that of the saline group, and the difference was statistically significant (Fig. 2D).

3.2.4 *Pathological changes of traumatic granulation tissue in various groups of New Zealand rabbits*

Wound healing is a complex biological process involving the regeneration of various tissues, wound epithelial formation, granulation tissue formation, etc. Therefore, the pathological changes of the skin wound tissue in each group could be observed by HE staining. On 21 days after the operation, specimens were taken from the wounds of each group respectively, and HE staining was performed for microscopic observation. As seen in Fig. 3A, the granulation tissue of the wounds of the two groups of drug administration was structurally intact, the granulation tissue had been formed, and there were fewer inflammatory cells. In the control group, the granulation tissue structure was disordered, and more inflammatory cell infiltration was seen.

3.2.5 *Comparison of collagen fibers in the trabeculae*

The control group can be seen as a little collagen fiber generation, partially broken, disorderly arrangement; HAE composed of collagen fiber density is still available, and the arrangement is more regular and orderly than the control group; YNBY collagen fiber density is significantly increased, and the arrangement is orderly (Fig. 3B).

3.2.6 *Immunohistochemical observation of microvessel density of granulation tissue*

CD34 antibody labeling showed vascular endothelial cells, which were stained brownish-yellow under the light microscope. Applying Image image analysis software, 5 fields of view were selected for each section to be observed and counted under a 400 × microscope, and the average of the microvessel counts of the 5 fields of view was taken. Microvessel counts were performed on the wounds of each group after 21 days of administration; as shown in Figs. 4C-4D, the number of microvessels in the wounds

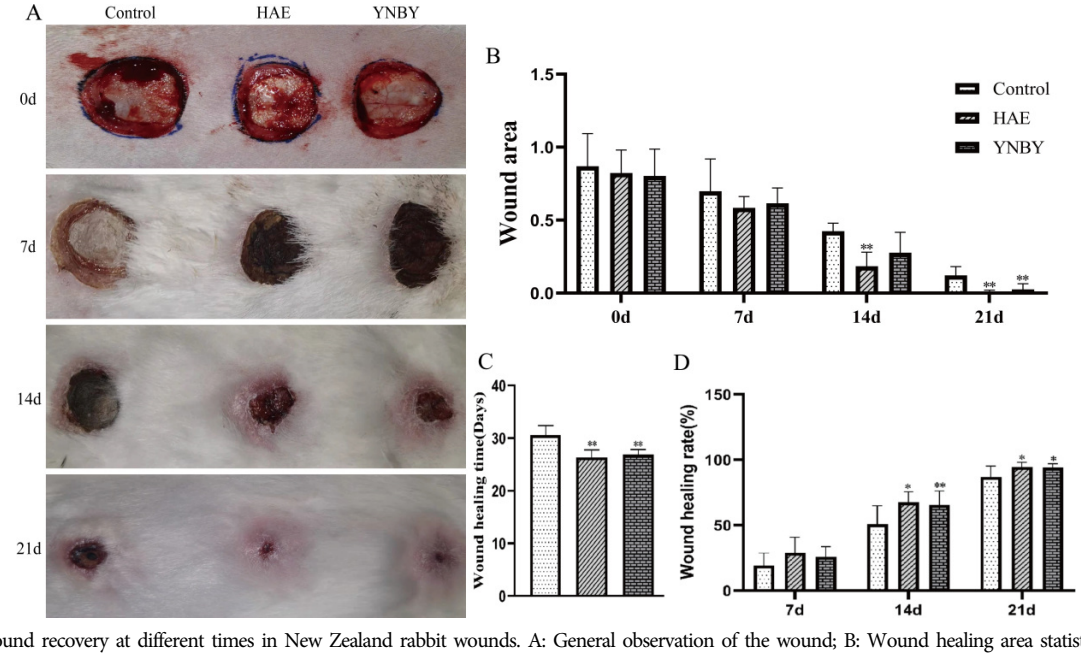

Figure 2 Wound recovery at different times in New Zealand rabbit wounds. A: General observation of the wound; B: Wound healing area statistics; C: Wound healing time; D: Wound healing rate (Compared to the control group, * means the difference is significant at the 0.05 level, ** means the difference is significant at the 0.01 level in other figures.)

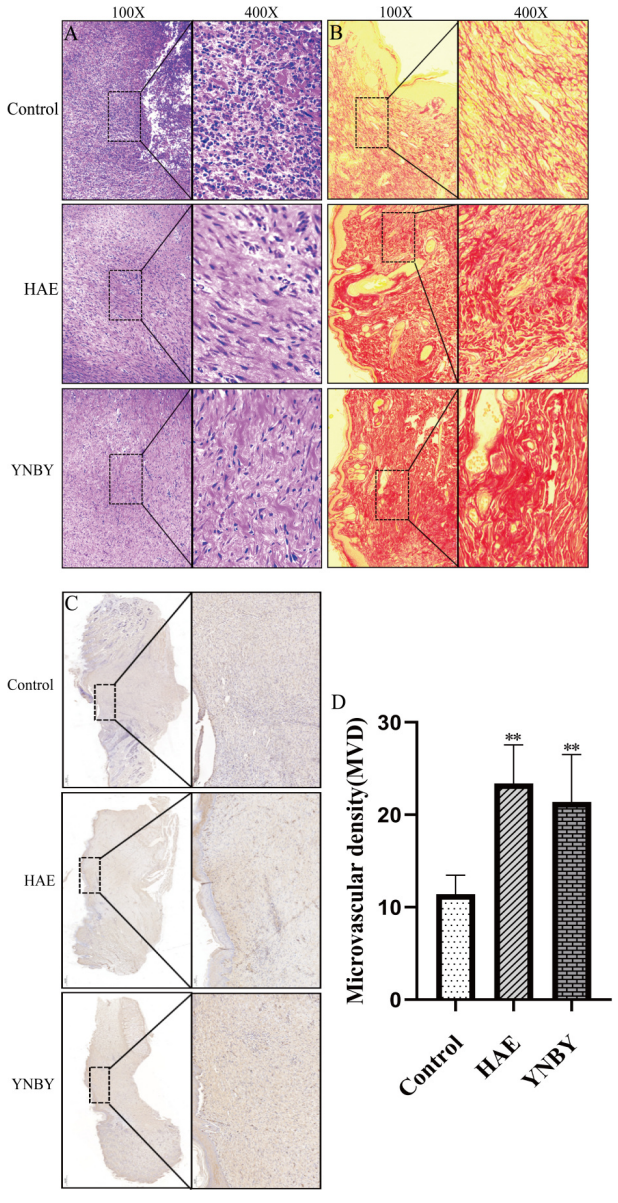

Figure 3 Pathological changes of traumatic granulation tissue in each group. A: HE stains of traumatic granulation tissue; B: Sirius scarlet staining of traumatic granulation tissue; C: CD34 immunohistochemical staining of traumatic granulation tissue; D: microvessel density statistic (Compared to the control group, ** means the difference is significant at the 0.01 level in other figures.)

of the HAE and YNBY groups increased significantly compared with that of the control group, and the counts of the microvessels of the two groups of the administered drugs were significantly higher than those of the control group.

3.3 Network Pharmacology Research

3.3.1 Screening of active ingredients and disease targets of Hypericum aqueous extracts

Based on the results of LC-MS analysis, 316 potential action targets of HAE were predicted and obtained using the SwissTargetPrediction database, 5,412 trauma healing-related genes were obtained from the GeneCards database. 316 potential targets of HAE were localized to 5,412 trauma healing targets, and a total of 199 gene potential targets for ontology analysis (Fig. 4A).

3.3.2 Component-target-disease network construction

Based on the intersection of disease targets and potential drug targets, the active ingredient-target-disease network of HAE was visualized and analyzed using Cytoscape 3.7.1 software (Fig. 4B), with the orange squares as the active ingredient of HAE, the green squares as the potential target of its action on wound healing, and the rest as the pathways obtained from the enrichment analysis. The results suggest that the mechanism by which HAE stimulates skin wound healing is jointly regulated by multi-target complex pathways.

3.3.3 GO and KEGG enrichment analysis

The 199 potential targets of action obtained above for the active ingredients of HAE affecting wound healing were subjected to GO and KEGG enrichment analyses for a better understanding of the mechanism of action of these targets. The results of GO enrichment analysis showed that the 199 potential targets affected 207 molecular functions, 2,461 biological processes, and 110 cellular components. The pathways with the top ten enriched numbers of MF, BP, and CC genes were selected and plotted as bubble diagrams, respectively (Fig. 4C). The results of GO analysis showed that the active components of the HAE were mainly involved in biological processes such as epithelial cell proliferation, etc., molecular functions such as protein serine/threonine kinase activity, etc., and the cellular components that acted mainly on the lumen of the vesicles, the lumen of the cytoplasmic vesicles, the rafts of the membranes, the membrane microregions, and the lumen of secretory granules. The results of KEGG pathway enrichment analysis showed that 199 potential targets affected 148 biological processes. The top thirty biological pathways with the number of enriched genes were selected and plotted as bubble diagrams (Fig. 4D). The results showed that the biological pathways mainly involved in the HAE were the PI3K/Akt signalling pathway and so on.

3.3.4 PPI network construction and topology analysis

Disease and drug core targets were applied to the STRING database to construct target PPI networks (Fig. 4E). The targets were scored using the MCC algorithm of the CytoHubba plugin of Cytoscape software, and 15 core targets such as VEGFA and PI3KR1 were screened out (Fig. 4F). The darker the graph color the higher the score, indicating that its corresponding node is more important in the network.

3.3.5 Effect of Hypericum aqueous extract on the protein expression levels of PI3K, P-PI3K, Akt, and P-Akt in traumatized tissues

Although the PI3K/AKT signaling pathway is related to the development of cancer, it is also crucial in the process of normal tissue repair and plays a key role in the regeneration, remodeling, and re-epithelialization of damaged tissues. Dysregulation of the PI3K/AKT signaling pathway has a significant effect on wound healing. The results of the Western blotting assay showed that the relative expression of PI3K, P-PI3K, Akt, and P-Akt proteins in the trabecular granulation tissues of the administered two groups was significantly lower compared with the control group (Fig. 5A and Fig. 4B).

3.3.6 Effect of aqueous extract of Hypericum on the expression level of VEGF in wound tissue

VEGF is a functional glycoprotein with high biological activity. It is a specific and physiologically potent endothelial cell mitogen

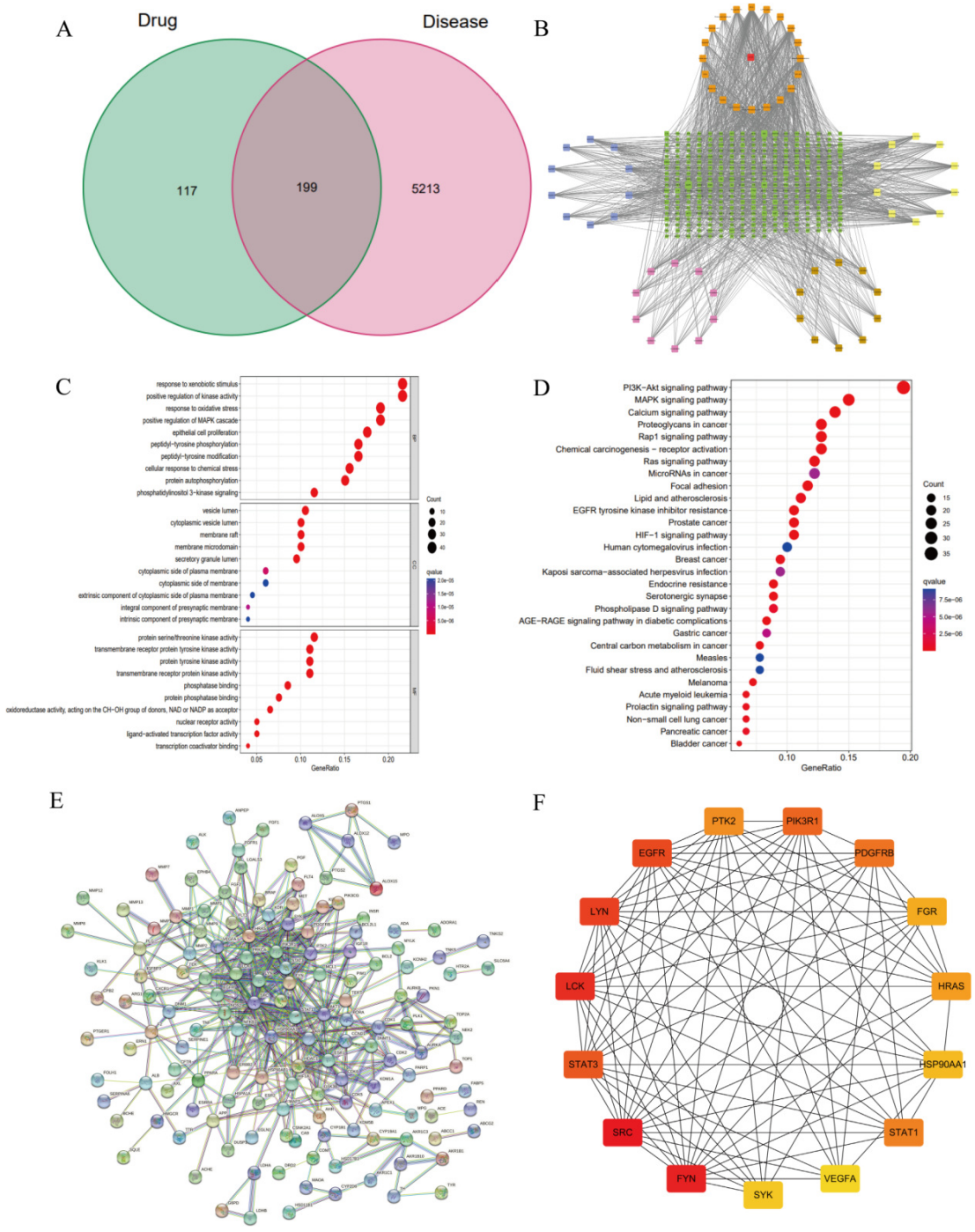

Figure 4 Network pharmacology of HAE for wound healing. A: Potential targets of HAE in wound healing; B: Drug-component-target-disease network diagram; C: GO enrichment analysis of key targets; D: KEGG pathway enrichment analysis; E: PPI network of potential targets of H for the treatment of wounds; F: Visualization of the PPI network.

that acts on vascular endothelial cells, causing their proliferation, migration, lumen formation, participation in angiogenesis, and increased capillary permeability. The expression of VEGF in the newborn granulation tissue was detected by immunohistochemistry, and the positive cells were those with brown colour in the cytoplasm or nucleus of vascular endothelial cells, fibroblasts, and epithelial basal cells, and the experimental data were analyzed statistically. The results of the VEGF showed that (Figs. 5C-5D): the number of positive cells in the HAE and YNBY groups was significantly higher than that of the control group, and the difference was statistically significant.

4 Discussion

Wound healing is a series of pathophysiological processes that repair local tissues through regeneration, repair, and reconstruction after tissue damage caused by traumatic factors. Essentially, it is an inherent defensive adaptive response of the organism to tissue and cell damage caused by various harmful factors. This regenerative repair manifests itself in the restoration of tissue structure and can also restore its function to varying degrees. Hypericum is a well-known medicinal herb with multiple therapeutic properties. Different extracts and many compounds

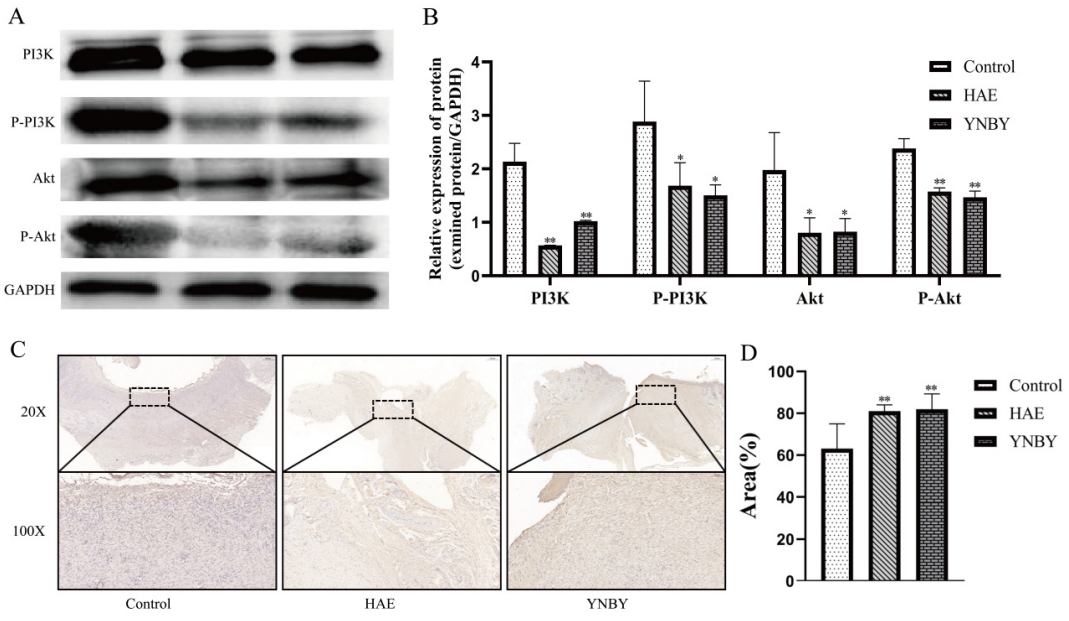

Figure 5 Effect of Hypericum perforatum aqueous extract on protein expression levels in traumatized tissues. A: Changes of PI3K, P-PI3K, Akt, P-Akt protein expression in each trauma tissue; B: Statistics of relative expression of PI3K, P-PI3K, Akt, P-Akt proteins; C: Immunohistochemical staining of VEGF proteins; D: Statistics of VEGF-positive cell rate. (Compared to the control group,* means the difference is significant at the 0.05 level, ** means the difference is significant at the 0.01 level in other figures.)

from this species have been reported to be biologically active, such as the methanolic extract of Hypericum has a wound healing potential in rats comparable to that of the standard drug nitrofurazone ointment; and flavonoids and flavonoids Hypericum, which have been shown to have antiviral and antimicrobial properties against Gram-positive bacteria, and have wound healing potential[13]. This study aimed to investigate the study of the wound-healing effect of aqueous extracts of Hypericum on the skin of New Zealand rabbits and to lay a theoretical foundation for the research and development of traditional Chinese medicine.

Wound healing rate and wound healing time are the most intuitive evidence reflecting the fast and slow efficacy of skin wound healing[14, 15]. The results of this study showed that compared with the control group, the skin wound healing time was shortened, and the wound healing rate was increased in the YNBY and HAE groups, and the HAE group was higher than the YNBY group, suggesting that the HAE could promote the healing rate of skin wounds in New Zealand rabbits.

Wound healing is generally categorized into the coagulation phase, the inflammatory phase, the repair phase, and the maturation phase. The inflammatory phase is the preliminary stage of wound healing; when the skin is damaged, a large number of inflammatory cells are activated, leading to the uncontrolled release of inflammatory cytokines, which results in wound inflammation. Reducing inflammation during wound management is important to prevent tissue damage and fibrosis[16]. Wound injury is also associated with vascular loss, which can lead to hypoxia and reactive oxygen species accumulation in the wound, causing oxidative stress and increased protease levels, leading to inadequate neovascularization and granulation tissue formation in the wound. Wound angiogenesis plays a key role in wound healing and tissue regeneration. It is the material basis for tissue repair and growth. Angiogenesis includes the proliferation, migration, and differentiation of endothelial cells and vascular smooth muscle cells, which promotes blood flow and the formation of capillary networks, as well as accelerating the formation of new blood vessels by promoting the proliferation and migration of vascular endothelial cells, thus accelerating the rate of wound healing[17-19]. The results of HE staining and CD34 immunohistochemistry in this study showed that compared with the control group, the YNBY and HAE group could reduce the inflammatory reaction at the wounds and accelerate the growth of granulation tissues and the formation of neovascularization, thus promoting the healing of the wounds.

The results of the study showed that the HAE was effective in promoting wound healing, but the mechanism of action needs to be clarified. Cyberpharmacology is an excellent tool for studying the active compounds and mechanisms of traditional therapies, including medicinal plants[20]. Therefore, we utilized network pharmacology to predict the molecular mechanism of Hypericum perforatum aqueous extract for promoting wound healing. We constructed a component-target-disease pathway network of HAE and skin wound healing, and our results showed that the mechanism of HAE stimulation of skin wound healing is jointly regulated by multi-target complex pathways, thus further evaluation of biological processes and signaling pathways using GO and KEGG databases is needed, and analysis of these databases showed that HAE regulates angiogenesis, collagen biosynthesis, tissue remodeling and other biological processes related to wound healing. In addition, HAE activates various signaling pathways, such as the PI3K/Akt signaling pathway, the MAPK signaling pathway, etc., and PI3K and its downstream target Akt play a central role in many cellular functions, such as cell proliferation, cellular transformation, paracrine function, and angiogenesis, etc[21-23]. We examined the expression of PI3K, Akt, P-PI3K, and P-Akt in wound tissues taken after 21 days of drug administration and found that the expression of these four proteins decreased after drug administration, which, combined with the previous results of the wound healing observation, indicated that the wounds in the control group might be in the inflammatory or repair phase of the healing stage of inflammation or repair, while the two groups administered with the drug had entered the maturation stage, so it led to a decrease in the

expression of PI3K, Akt and P-PI3K, P-Akt proteins, so it can be shown that the HAE can promote the healing of New Zealand rabbit wounds by regulating the PI3K/Akt signaling pathway. VEGF, as an important biological factor for cell growth, plays an important role in damaged tissues. Some studies have confirmed that VEGF promotes the division and proliferation of vascular endothelial cells, provides a material basis for the migration of vascular endothelium and angiogenesis, stimulates wound healing through angiogenesis, and promotes collagen deposition and epithelial formation. Immunohistochemical assays showed that the expression of VEGF was up-regulated after administration of VEGF, suggesting that its high expression could help restore the vascularization of the wounds and promote the healing of the wounds.

The process of wound healing goes through four different stages. Although in this study, the HAE could promote wound healing, the exact stages at which the HAE acts to promote wound healing are not clear, and therefore, further research is needed. The mechanism of action needs to be investigated in greater depth.

5 Conclusion

In summary, we conclude that the aqueous extract of Hypericum promotes the healing process of skin wounds by regulating the PI3K-Akt signaling pathway, and this finding provides a scientific basis for the application of an aqueous extract of Hypericum in the healing of skin wounds, as well as laying the groundwork for further research on its potential in clinical applications.

Data availability

Data will be made available on request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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References

[1] Tanaka, N., Kashiwada, Y. Characteristic metabolites of *Hypericum* plants: their chemical structures and biological activities. *Journal of natural medicines*, **2021**, 75: 423–433. https://doi.org/10.1007/s11418-021-01489-y

[2] Tian, W. J., Qiu, Y. Q., Jin, X. J., et al. Novel polycyclic polyprenylated acylphloroglucinols from *Hypericum sampsonii*. *Tetrahedron*, **2014**, 70: 7912–7916. https://doi.org/10.1016/j.tet.2014.08.062

[3] Zhao, J., Liu, W., Wang, J. C. Recent advances regarding constituents and bioactivities of plants from the genus *Hypericum*. *Chemistry & biodiversity*, **2015**, 12: 309–349. https://doi.org/10.1002/cbdv.201300304

[4] Ma, J., Zang, Y. D., Zhang, J. J., et al. Nine prenylated acylphloroglucinols with potential anti-depressive and hepatoprotective activities from *Hypericum scabrum*. *Bioorganic Chemistry*, **2021**, 107: 104529. https://doi.org/10.1016/j.bioorg.2020.104529

[5] Faraji, N., Ganji, A., Heshami, N., et al. Hypolipidemic effects of *Hypericum Scabrum* extract on the serum lipid profile and obesity in high-fat diet fed rats. *Human Antibodies*, **2021**, 29: 55–61. https://doi.org/10.3233/HAB-200430

[6] Jin, D. X., He, J. F., Luo, X. G., et al. Hypoglycemic effect of *Hypericum attenuatum* Choisy extracts on type 2 diabetes by regulating glucolipid metabolism and modulating gut microbiota. *Journal of Functional Foods*, **2019**, 52: 479–491. https://doi.org/10.1016/j.jff.2018.11.031

[7] Keser, S., Keser, F., Kaygili, O., et al. Phytochemical compounds and antiradical, antimicrobial, and cytotoxic activities of the extracts from *Hypericum scabrum* L. Flowers. *Natural product research*, **2020**, 34: 714–719. https://doi.org/10.1080/14786419.2018.1493735

[8] Süntar, I. P., Akkol, E. K., Yilmazer, D., et al. Investigations on the *in vivo* wound healing potential of *Hypericum perforatum* L. *Journal of ethnopharmacology*, **2010**, 127: 468–477. https://doi.org/10.1016/j.jep.2009.10.011

[9] Grafakou, M. E., Diamanti, A., Simirioti, E., et al. Wound healing effects from 3 *Hypericum* spp. essential oils. *Planta Medica International Open*, **2021**, 8: e69–e77. https://doi.org/10.1055/a-1492-3634

[10] Nayak, S. B., Isik, K., Marshall, J. R. Wound-Healing potential of oil of *Hypericum perforatum* in excision wounds of male sprague dawley rats. *Advances in Wound Care*, **2017**, 6: 401–406. https://doi.org/10.1089/wound.2017.0746

[11] Sorg, H., Tilkorn, D. J., Hager, S. Skin wound healing: an update on the current knowledge and concepts. *European surgical research*, **2017**, 58: 81–94. https://doi.org/10.1159/000454919

[12] Reinke, J. M., Sorg, H. Wound repair and regeneration. *European surgical research*, **2012**, 49: 35–43. https://doi.org/10.1159/000339613

[13] Mukherjee, P. K., Verpoorte, R., Suresh, B. Evaluation of in-vivo wound healing activity of *Hypericum patulum* (Family: Hypericaceae) leaf extract on different wound model in rats. *Journal of ethnopharmacology*, **2000**, 70: 315–321. https://doi.org/10.1016/s0378-8741(99)00172-5

[14] Khan, A. D., Singh, M. K., Lavhale, P. M., et al. Exploring the wound healing activity of phytosomal gel of *Annona squamosa* and *Cinnamomum tamala* leaves ethanolic extracts with antioxidant and antimicrobial activities in *S aureus* infected excision wound model. *Journal of Biomaterials Science, Polymer Edition*, **2024**, 35: 2447–2468. https://doi.org/10.1080/09205063.2024.2382540

[15] Liu, Y. F., Wei, Y. Z., Xiong, Q. Q., et al. Hollow porous PLA/PBAT composite microfibers with enhanced toughness and prolonged drug release for wound healing. *Materials & Design*, **2024**, 237: 112624. https://doi.org/10.1016/j.matdes.2023.112624

[16] Takahama, M., Akira, S., Saitoh, T. Autophagy limits activation of the inflammasomes. *Immunological reviews*, **2018**, 281: 62–73. https://doi.org/10.1111/imr.12613

[17] Li, R., Tian, J., Du, J. H., et al. Manipulation of autophagy: a novelly potential therapeutic strategy for retinal neovascularization. *BMC ophthalmology*, **2018**, 18: 1–12. https://doi.org/10.1186/s12886-018-0774-6

[18] Bazigou, E., Makinen, T. Flow control in our vessels: vascular valves make sure there is no way back. *Cellular and Molecular Life Sciences*, **2013**, 70: 1055–1066. https://doi.org/10.1007/s00018-012-1110-6

[19] Tonnesen, M. G., Feng, X., Clark, R. A. Angiogenesis in wound healing. *The journal of investigative dermatology. Symposium proceedings*, **2000**, 5: 40–46. https://doi.org/10.1046/j.1087-0024.2000.00014.x

[20] Zhang, H., Li, W., Zhang, Q. Identification of active compounds and molecular mechanisms of *Dalbergia tsoi* Merr. et Chun to accelerate wound healing. *Biomedicine & Pharmacotherapy*, **2022**, 150: 112990. https://doi.org/10.1016/j.biopha.2022.112990

[21] Cantley, L. C. The phosphoinositide 3-kinase pathway. *Science*,

2002, 296: 1655–1657. https://doi.org/10.1126/science.296.5573.1655

[22] Zhang, Z., Zhao, C. X., Liu, B., et al. Inositol pyrophosphates mediate the effects of aging on bone marrow mesenchymal stem cells by inhibiting Akt signaling. *Stem cell research & therapy*, **2014**, 5: 1–12. https://doi.org/10.1186/scrt431

[23] Shi, J. H., Li, J., Guan, H., et al. Anti-fibrotic actions of interleukin-10 against hypertrophic scarring by activation of PI3K/AKT and STAT3 signaling pathways in scar-forming fibroblasts. *PloS one*, **2014**, 9: e98228. https://doi.org/10.1371/journal.pone.0098228