An Exploration of the Active Ingredients of *Cortex phellodendri* in the Treatment of Hemorrhoids and Its Mechanism Based on Network Pharmacology

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Abstract: Background: Huangbo, also known as *Cortex phellodendri* in Latin, is an important drug which has the pharmacological action of clearing heat, drying dampness, purging fire and detoxifying. In recent years, it has been reported that Huangbo also has the activity of treating hemorrhoids. Objective: The aim of this study was to explore the Active Ingredients of *Cortex phellodendri* in the Treatment of hemorrhoids and Its Mechanism. Study Design: We analyzed Active components and target genes of *Cortex phellodendri* in the Traditional Chinese Medicine System Pharmacology (TCMSP) database and analysis platform. We then searched the GeneCards database for target genes related to hemorrhoids and the intersection of these genes with the active components of *Cortex phellodendri*. Target genes related to hemorrhoids were taken as common potential target genes of *Cortex phellodendri*, which could act on hemorrhoids. Using the R programming language, we drew a Venn map of these common potential target genes. The "component–target gene–disease" network of *Cortex phellodendri* in the treatment of hemorrhoids was established using Cytoscape software version 3.7.1; the protein–protein interaction (PPI) network was constructed in the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. With the help of R and Perl languages, we performed gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of potential target genes of *Cortex phellodendri* in the treatment of hemorrhoids. Results: We extracted a total of 10 active components from *Cortex phellodendri*, including quercetin and rutaecarpine and so on, as well as 29 potential target genes for hemorrhoids. According to the Degree ranking in Cytoscape3.7.1 software, the top 10 potential target genes were interleukin-6 (IL-6), CCL2, CXCL8, MMP9, vascular endothelial growth factor A (VEGFA), Myc, IL-10, ICAM1, MMP2, and MMP3. Pathway enrichment mainly involved signaling pathways such as advanced glycation end products and receptor for advanced glycation end products (AGE-RAGE) signaling pathway in diabetic complications, hypoxia-inducible factor 1 (HIF-1), IL-17, and Relaxin. Conclusion: Based on network pharmacology, *Cortex phellodendri* is expected to be mined as a candidate Traditional Chinese Medicine (TCM) for the treatment of hemorrhoids. Its mechanism for treating this disease operates via multiple components and pathways. This study provides the basic theory and the basis for further research.

Keywords: *Cortex phellodendri*, Hemorrhoids, Network Pharmacology, Target Gene, GO Function Enrichment Analysis, KEGG Pathway Enrichment Analysis

1. Introduction

Hemorrhoids is one of the common anorectal diseases [1]. The incidence of hemorrhoids varies from region to region [2, 3]. According to statistics, the incidence of hemorrhoids in the United States population is 53-68% [4]; whereas in China, it's 55 to 75% [5]. Hemorrhoids are currently treated in a variety of ways, each with its own advantages, but postoperative recurrence, bleeding, anal edema and other symptoms are still common [6].

Traditional Chinese Medicine (TCM) treatment of hemorrhoids has a long history and rich theoretical basis [7]. In clinical practice, TCM can effectively improve the clinical symptoms of hemorrhoids patients and reduce the incidence of serious hemorrhoids symptoms [8]. TCM has an overall regulatory effect on the body through multiple components and targets [9]. Cortex phellodendri is one of the common Chinese medicines for hemorrhoids. The medicine of Cortex phellodendri is cold and bitter. It has the effect of clearing heat, drying and dampness, relieving fire and detoxifying [10]. The theory of TCM has proved that hemorrhoids are caused by dampness and heat [11]. Therefore, with the effect of clearing heat and dampness, Cortex phellodendri has a unique important role in the treatment of hemorrhoids [12]. However, the active ingredients and mechanism of Cortex phellodendri in the treatment of hemorrhoids are still unclear.

By integrating computer technology and bioinformatics technology, network pharmacology systematically explores the regulatory pathway of TCM on the signal pathway and selects effective targets, which can systematize, informationize and scientize the mechanism of TCM in the treatment of hemorrhoids, and has become one of the most effective methods to study the mechanism of hemorrhoids in the treatment of traditional Chinese medicine [13]. Therefore, based on network pharmacology, this study explored the effective components and mechanism of Phellodendron bark in the treatment of hemorrhoids, and preliminarily obtained a good discovery. Now the summary report is as follows.

2. Materials and Methods

All researches were approved by the Research Committee of Zhuhai People's Hospital (Zhuhai Hospital Affiliated with Jinan University), and carried out in accordance with the approved guidelines.

2.1. Searching Active Components of Cortex phellodendri

We input the keyword "*Cortex phellodendri*" into the Traditional Chinese Medicine System Pharmacology (TCMSP) database and analysis platform and retrieved *Cortex phellodendri*'s active ingredients. According to recommendations in the literature, we regarded compounds with oral bioavailability (OB) >30% and drug-likeness (DL) >0.18 as active components.

2.2. Searching Potential Target Genes of Cortex phellodendri

We searched all target genes of *Cortex phellodendri* and then extracted the target genes of its active components. We imported 29 potential target genes of *Cortex phellodendri*'s active components into the UniProt database (https://www.uniprot.org/), selected "human" as the species, and obtained names of target genes and their corresponding acronyms.

2.3. Searching for Hemorrhoids–related Genes

The GeneCards database (http://www.genecards.org/) is a 1-stop database for human-gene annotation that covers nearly 90% of human protein-coding genes. We input the keyword "hemorrhoids" into GeneCards and then exported the retrieval results.

2.4. Intersection of Potential Target Genes of Cortex phellodendri and Hemorrhoids–related Genes

We considered genes that were both potential target genes of *Cortex phellodendri* and hemorrhoids -related genes to be the genes of interaction between *Cortex phellodendri* and hemorrhoids.

2.5. Constructing a Component–target Gene–disease Network

We deleted active components that could not act on hemorrhoids–related target genes. Then we imported the remaining active components and potential target genes for hemorrhoids treatment into Cytoscape software version 3.7.1 (US National Institute of General Medical Sciences [NIGMS], Bethesda, Maryland, US; https://cytoscape.org/) to build an active component–target gene–disease network.

2.6. Constructing a Protein–protein Interaction (PPI) Network

We input potential target genes of *Cortex phellodendri* in the treatment of hemorrhoids into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) and selected "*Homo sapiens*" as the species. We obtained the PPI network in tab-separated values (.tsv) file format; the PPI network picture was saved in Portable Network Graphics (.png) format.

2.7. Screening Core Genes of PPI Network

Using the R programming language, we calculated a genes.tsv file and selected the Top 20 target genes of degree value as the core genes.

2.8. Gene Ontology (GO) Function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway Enrichment Analyses

Using R language, we analyzed our GO function enrichment analysis of potential target genes and our KEGG pathway enrichment analysis, including biological processes, molecular functions, and cell components. We selected the path whose kinase insert domain receptor (KDR) value (i.e., *Q*-value) was <0.05, and then we ranked the paths with the 20 highest KDR values in order to draw a histogram and bubble chart.

3. Results

3.1. Acquisition of Active Components and Potential Target Genes of Cortex phellodendri

From the TCMSP database we obtained 10 active components of *Cortex phellodendri*, such as quercetin and rutaecarpine and so on, using the screening conditions of OB >30% and DL >0.18 (Table 1). At the same time, we converted target genes corresponding to active components of *Cortex phellodendri* in the UniProt database.

Table 1. *Active components of Cortex phellodendri.*

No.	molID	molName	MW	OB (%)	DL
01	MOL001454	berberine	336.39	36.86	0.78
02	MOL001458	coptisine	320.34	30.67	0.86
03	MOL002662	rutaecarpine	287.34	40.3	0.6
04	MOL002668	Worenine	334.37	45.83	0.87
05	MOL000358	beta-sitosterol	414.79	36.91	0.75
06	MOL000785	palmatine	352.44	64.6	0.65
07	MOL000787	Fumarine	353.4	59.26	0.83
08	MOL000098	quercetin	302.25	46.43	0.28
09	MOL002894	berberrubine	322.36	35.74	0.73
10	MOL006422	thalifendine	322.36	44.41	0.73

There are 10 active components of *Cortex phellodendri*. molID=molecular identification number; molName=molecular name; MW=molecular weight; OB=oral bioavailability; DL=drug-likeness.

3.2. Predicting Potential Target Genes of Cortex phellodendri in the Treatment of Hemorrhoids

We retrieved a total of 144 hemorrhoids-related genes from the GeneCards database and compared them with target genes corresponding to each active component of *Cortex phellodendri*. We selected 29 common target genes (Table 2) as potential target genes of *Cortex phellodendri* in the treatment of hemorrhoids (Figure 1).
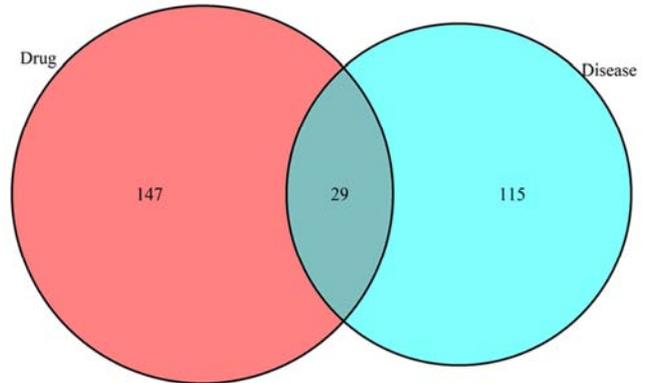

Figure 1. *The target genes in common between Cortex phellodendri and hemorrhoids.*

Table 2. *Potential target information of Cortex phellodendri.*

No.	Uniprotkb	Target	No.	Uniprotkb	Target	No.	Uniprotkb	Target
1	P35228	NOS2	11	P15692	VEGFA	21	P49908	SELE
2	Q8N119	MMP2	12	P22301	IL10	22	P10145	CXCL8
3	P14780	MMP9	13	P05231	IL6	23	O15392	BIRC5
4	P10415	BCL2	14	C9JW72	TP63	24	P60568	IL2
5	Q07812	BAX	15	P03956	MMP1	25	P08514	PLAT
6	P55211	CASP9	16	Q16665	HIF1A	26	P07204	THBD
7	P27169	PON1	17	P01106	MYC	27	P02461	COL3A1
8	Q9NTG1	KDR	18	P04798	CYP1A1	28	P02741	CRP
9	P08254	MMP3	19	P05362	ICAM1	29	P29965	CD40LG
10	P00533	EGFR	20	P13500	CCL2			

There are 29 target genes corresponding to active components of *Cortex phellodendri* in the UniProt database.
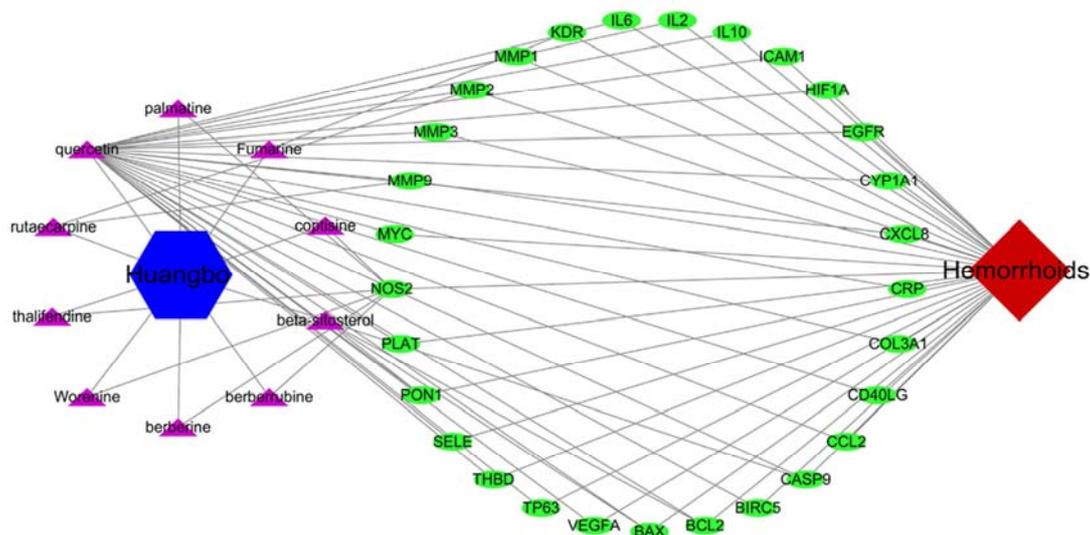
Figure 2. *Construction of component–target gene–disease network.*

3.3. Constructing a Component–target Gene–disease Network

We introduced potential target genes of *Cortex phellodendri* for hemorrhoids treatment into Cytoscape to construct a component–target gene–disease network (Figure 2). The network had 29 nodes and 1190 strip edges. In Figure 2, the blue octagonal shape represents *Cortex phellodendri*, the purple triangle represents the active-component molecule, the green circle represents the target gene, and the red diamond represents the disease. As the figure shows, the same active ingredient can correspond to different target genes, and different active ingredients can correspond to the same target gene. This suggested that the potential mechanism of *Cortex phellodendri* in the treatment of hemorrhoids is mediated by multi-component and multi-target genes.

3.4. Constructing a PPI Network and Screening Core Target Genes

We imported potential target genes of *Cortex phellodendri* in hemorrhoids treatment into the STRING database and obtained a PPI network (Figure 3) and TSV file. With the help of R language, we calculated a genes. tsv file and obtained the Top 20 target genes of degree value (Figure 4).
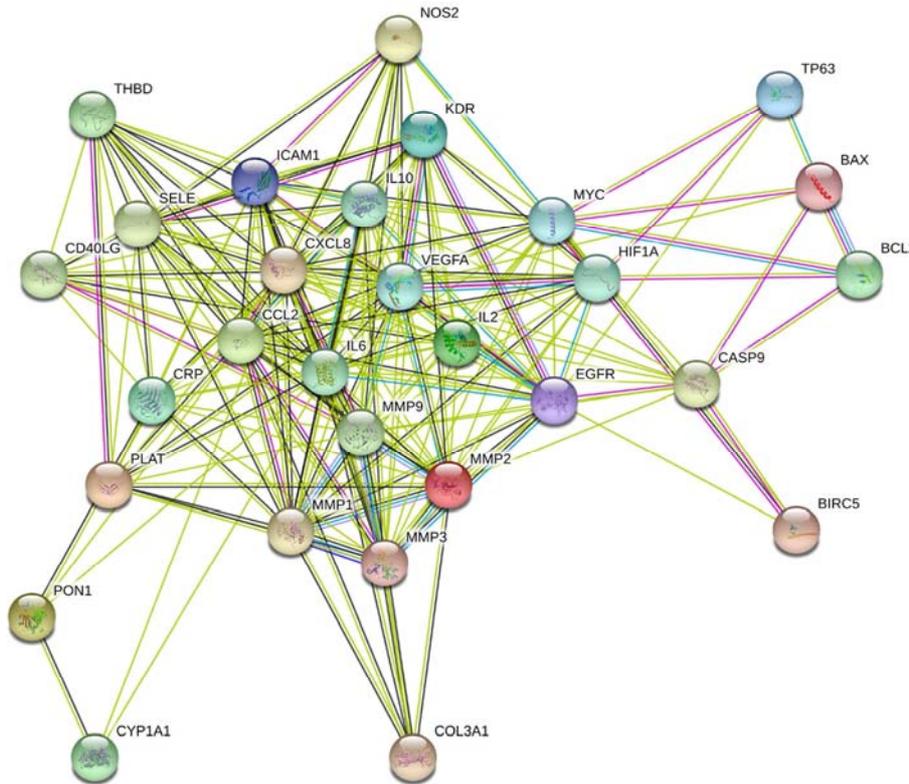

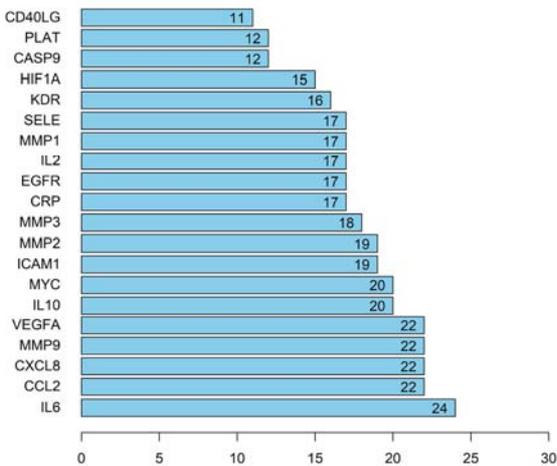**Figure 3.** *Construction of PPI network.*

Figure 4. *Top 20 core target genes.*

3.5. GO Function and KEGG Pathway Enrichment Analyses

Using R language, we performed GO function and KEGG pathway enrichment analyses of potential target genes of *Cortex phellodendri* in hemorrhoids treatment. The results of GO function enrichment analysis (Figures 5 and 6) showed that the biological processes were mainly those such as serine-type endopeptidase activity, serine hydrodase activity, and so on. The cell components were mainly those such as cytokine receptor binding, heat shock protein binding, Hsp90 protein binding, and so on. The results of KEGG pathway enrichment analysis (Figures 7 and 8) showed that potential target genes of *Cortex phellodendri* in the treatment of hemorrhoids were mainly enriched in signaling pathways such as advanced glycation end products and receptor for advanced glycation end products (AGE-RAGE) signaling

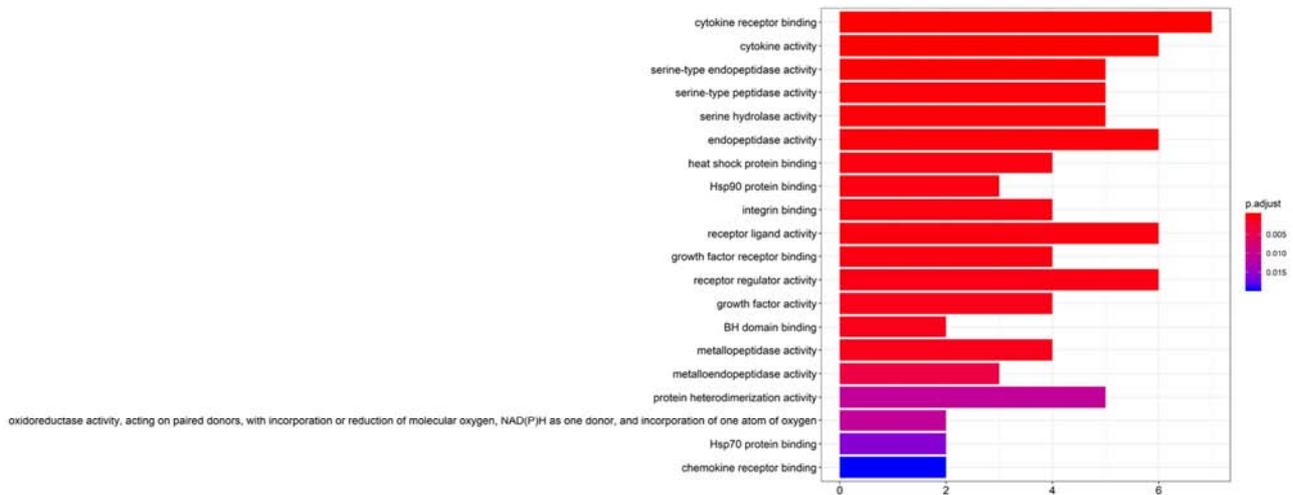pathway in diabetic complications, hypoxia-inducible factor   1 (HIF-1), IL-17, and Relaxin.

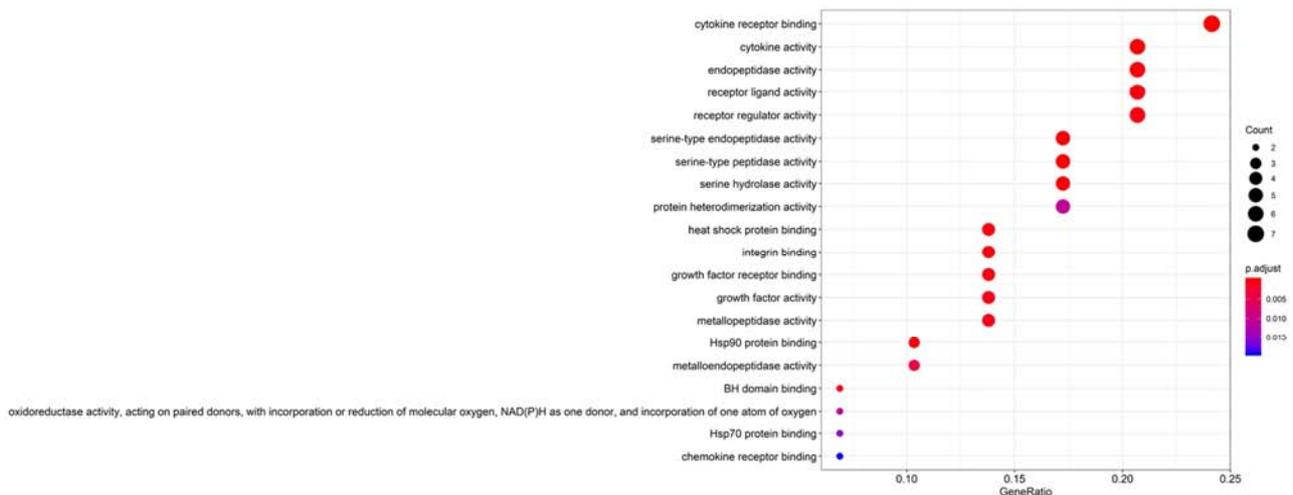**Figure 5.** *GO functional enrichment analysis (histogram).*

Figure 6. *GO functional enrichment analysis (bubble chart).*

4. Discussion

According to TCM, hemorrhoids are caused by eating disorders, dampness and heat blocking the intestines, and stasis over time [14]. Cortex phellodendri is a common medicine for clearing away heat and removing fire and blood stasis [15]. This indicates that Cortex phellodendri has the theoretical basis of TCM in the treatment of hemorrhoids. In fact, various preparations made by Cortex phellodendri have been widely used in clinical practice to treat anal diseases including hemorrhoids.

From Table 1 and Figure 2, we learn that the active ingredients of Cortex phellodendri are quercetin and rutaecarpine and berberine. Pharmacological studies have shown that quercetin can relieve smooth muscle spasm and improve skin edema [16]. rutaecarpine can improve vascular permeability and inhibit microthrombus formation [17]. berberine can accelerate wound healing by promoting angiogenesis and granulation growth of wound [18]; Quercetin

can help to eliminate edema and improve local blood circulation so as to achieve the effect of reducing swelling [19]. Therefore, we hypothesized that the pharmacological action of Cortex phellodendri in the treatment of hemorrhoids may be achieved through the anti-inflammatory and hemostatic effect of quercetin, promoting angiogenesis, promoting the growth of granulation of wound of rutaecarpine, eliminating edema, improving local blood circulation of berberine, and so on.

It is not difficult to conclude from Figure 4 that IL-6, IL-8, CCL2, EGFR and IL-10 are the core genes of Cortex phellodendri in the treatment of hemorrhoids. IL-6, IL-8 and CCL2 are pro-inflammatory factors and IL-10 is anti-inflammatory factor, according to literature studies [20-23]. IL-6 and CCL2 are important factors involved in immune response and inflammatory response [24]. Increased levels of IL-6, IL-8, CCL2, and IL-10 indicate injury and inflammation [25]. When hemorrhoids present symptoms, such as hemorrhoid prolapses, pain, bleeding, etc., IL-6, IL-8,

CCL2, and IL-10 participate in the body's anti-inflammatory response and immune response under the feedback of the neurohumoral system, and their levels are correspondingly increased. The active ingredient of Cortex phellodendri may be to reduce inflammation, promote wound healing and relieve pain by inhibiting IL-6, IL-8, CCL2 and enhancing IL-10 and other cytokines.

Recent studies have found that the interaction between the receptor for advanced glycation end products (RAGE) and its ligand advanced glycation end products (AGE) is associated with vascular diseases [26, 27]. Hemorrhoids is believed to

be caused by tortuous, dilated, hyperemia and other pathological changes in the anorectal vein. Therefore, RAGE-AGE may be related to the pathological changes of hemorrhoids. Increased AGE concentration in the human body may cause pathological changes in the anorectal vein [28, 29], thereby inducing symptoms of hemorrhoids. figures 7 and 8 show that the KEGG pathway of Cortex phellodendri in the treatment of hemorrhoids mainly involves RAGE-AGE, etc. Cortex phellodendri may inhibit RAGE-AGE to reverse hemorrhoid vascular lesions, which may lead to remission or reduction of hemorrhoids symptoms.
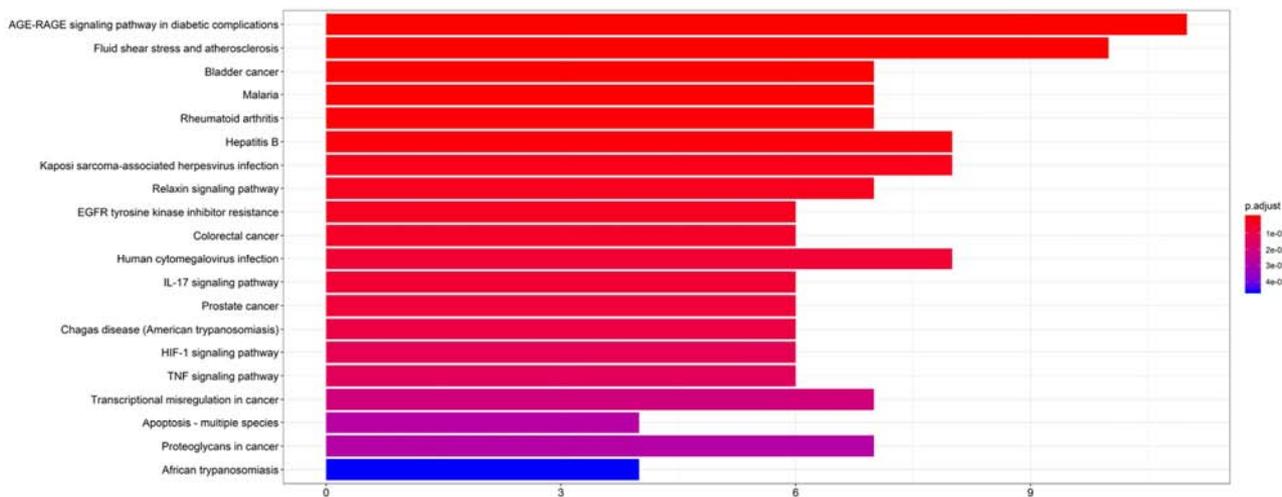

Figure 7. Results of KEGG pathway enrichment analysis (histogram).

To sum up, the effective components of *Cortex phellodendri* were quercetin and rutaecarpine and berberine. These active components may play a pharmacological role through the core gene IL-6, IL-8, CCL2, EGFR and IL-10 and the RAGE-AGE signaling pathway, thus achieving the

therapeutic effect of hemorrhoids. The purpose of this study is to lay the foundation for the further study of the mechanism of action, and provide a new idea for the subsequent clinical improvement of acute hemorrhoid attack symptoms such as prolapse, pain, bleeding, etc.
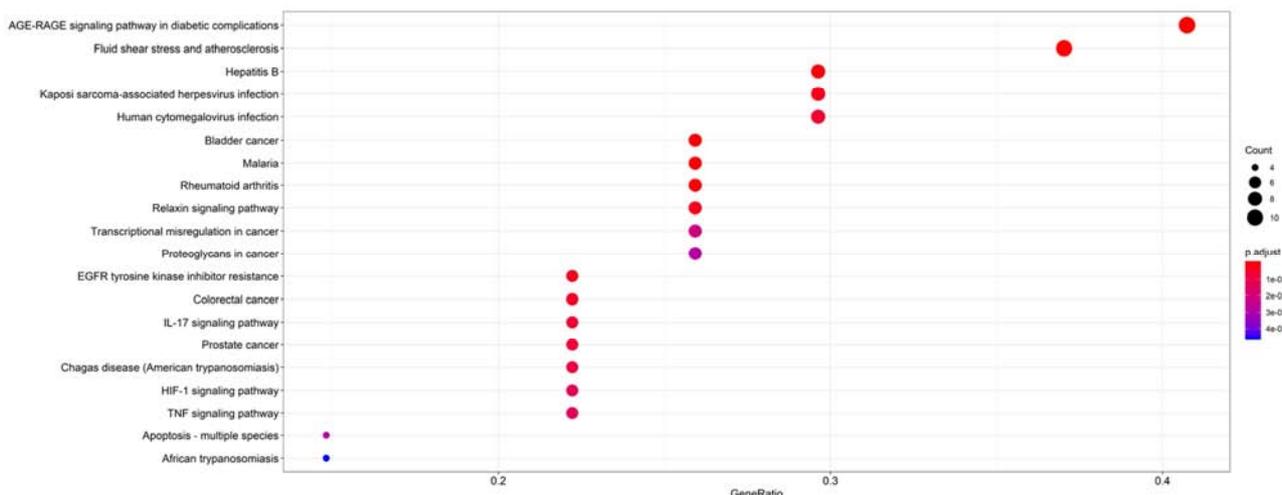

Figure 8. Results of KEGG pathway enrichment analysis (bubble chart).

5. Conclusion

Based on network pharmacology, *Cortex phellodendri* is

expected to be mined as a candidate Traditional Chinese Medicine (TCM) for the treatment of hemorrhoids. Its mechanism for treating this disease operates via multiple components and pathways. This study provides the basic

theory and the basis for further research.

Data Availability

The original data used to support this study can be available from the corresponding author upon request.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

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