Molecular mechanism of Epicedium treatment for depression based on network pharmacology and molecular docking technology

Yankai Dong1, Bo Tao2†, Xing Xue1, Caixia Feng1, Yating Ren1, Hengyu Ma1, Junli Zhang1, Yufang Si1, Sisi Zhang1, Si Liu1, Hui Li3, Jiahao Zhou1, Ge Li1, Zhifei Wang1, Juanping Xie4† and Zhongliang Zhu1∗

Abstract

Background: Increasing attention has been paid to the effect of Epimedium on the nervous system, particularly anti-depression function. In the present study, we applied network pharmacology to introduce a testable hypothesis on the multi-target mechanisms of Epicedium against depression.

Methods: By reconstructing the network of protein–protein interaction and drug–component–target, we predicted the key protein targets of Epicedium for the treatment of depression. Then, through molecular docking, the interaction of the main active components of Epicedium and predicted candidate targets were verified.

Results: Nineteen active compounds were selected from Epicedium. There were 200 targets associated with Epicedium and 537 targets related to depression. The key targets of Epicedium for treating depression were IL6, VEGFA, AKT1, and EGF. According to gene ontology functional enrichment analysis, 22 items of biological process (BP), 13 items of cell composition (CC) and 9 items of molecular function (MF) were obtained. A total of 56 signaling pathways (P < 0.05) were identified by Kyoto Encyclopedia of Genes and Genomes analysis, mainly involving depression-related pathways such as dopaminergic synapse, TNF signaling pathway, and prolactin signaling pathway. The results of molecular docking showed that the most important activity components, including luteoklin, quercetin and kaempferol, were well combined with the key targets.

Conclusions: Luteoklin, quercetin, kaempferol and other active compounds in Epicedium can regulate multiple signaling pathways and targets such as IL6, AKT1, and EGF, therefore playing therapeutic roles in depression.

Keywords: Epimedium, depression, network pharmacology, molecular docking, pathway analysis

* Correspondence: xjp_731205@163.com; zlzhu@nwu.edu.cn
†Yankai Dong and Bo Tao contributed equally to this work.

1Qinba Chinese Medicine Resources R&D Center, School of Medicine, Ankang University, Ankang 710069, Shaanxi Province, China
2Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Institute of Maternal and Infant health, Northwest University, Xian 710069, Shaanxi Province, China

Full list of author information is available at the end of the article

© The Author(s). 2021 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background
Depression is a common chronic and highly disabling disorder with high level of treatment resistance [1]. Depression has affected more than 350 million people worldwide [2–4], and it is characterized by weight loss, low energy, loss of appetite, and insomnia [5, 6]. In recent years, most antidepressants have become NMDA receptor antagonists, which can produce antidepressant effects quickly, but these drugs are not used as first-line antidepressants because of their side effects, such as sensory agitation, cognitive impairment, addiction and hallucination [7, 8]. In addition, the pathogenesis of depression has been partly elucidated based molecular and genetic studies, but the potential mechanism of depression needs to be determined [9].

The primary task of prevention and treatment of depression is the development of new antidepressant therapeutic targets and therapeutic drugs with short onset latency and less side effects. Considering the improved safety and fewer side effects, traditional Chinese medicines (TCMs) performs an important role in the prevention and treatment of diseases [10], and have increasing attention among scientists worldwide. TCMs have multiple ingredients, targets and ways to be effective [11]. As a Chinese herbal medicine, Epicedium is well-known for wide-ranging effects, such as treatment of cancer, cardiovascular diseases, and immune suppression [12]. The extract of Epicedium and its component icariin can effectively promote the regeneration of peripheral nerve and improve the damaged nerve function [13]. Epicedium ethanol extraction exerts an anti-inflammatory effect by inhibiting the production of tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 in lipopolysaccharide(LPS)-induced peritonitis [14]. Icariin can ameliorate depressive behavior of male offspring with prenatal stress [15]. In addition, Epicedium is rich in chemical composition, including flavonoids, lignans, and polysaccharides [12]. However, the therapeutic mechanism of Epicedium on depression is not clear.

With the rapid progress of bioinformatics, systems biology, and poly-pharmacology, network-based drug discovery has become a promising approach for the development of effective drugs. In 2007, Hopkins et al. first proposed the concept “network pharmacology”. This method analyzes the intervention of drugs and potential treated targets of diseases based on system biology [16, 17]. Network pharmacology highlights a paradigm shift from the current “one target, one drug” strategy to a novel version of the “network target, multi-component” strategy [18]. In TCM research, it is widely used because of the integrity and system consistent with the TCM prescription’s principles [19, 20]. As a computer-aided drug design method that depending on the interaction and affinity between the target and active compound, molecular docking has been widely used in the material basis of TCMs [21].

In the present study, we applied network pharmacology and molecular docking to clarify the mechanism of Epicedium in the treatment of depression. We systematically analyzed the active ingredients, potential targets, pathways and networks affected by Epicedium for the treatment of depression. We also performed molecular docking studies to predict the interactions that allow important compounds to bind to its predicted targets. Our results may help clarify how Epicedium can be effective against depression and facilitate the development of novel drugs.

Methods
Prediction of target genes associated with depression
Genes related to depression were obtained from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) through searching “depression”. After filtering with the term “Homo sapiens”, 537 genes associated with depression were identified in the Gene Bank database.

Collection of component targets of Epicedium
The compounds and targets of Epicedium were mainly collected from TCMSp, a natural product database for Chinese herbal medicines (http://ibs.hkbu.edu.hk/LSP/tcmsp.php, updated on May 31, 2014 )[22]. In clinical treatment, TCMs are often used via oral administration. Oral bioavailability (OB) [23] and drug-likeness (DL) [24], which are ADME-related models, mainly affect the absorption of drugs by the gastrointestinal tract. Therefore, we screened bioactive components under the standards of OB≥30% and DL≥0.18 [25], and obtained the related targets of each components. The common targets of Epicedium and the depression related genes were intersected with the Venn map. The intersected genes were the target genes of Epicedium for the treatment of depression.

Protein–protein Interaction (PPI) Data
String database (https://string-db.org/cgi/input.pl) [26] contains known and predicted protein-protein interaction. A large number of PPIs were collected involving 9643763 proteins and 138083840 interactions, including data obtained from experimental detection and bioinformatics prediction. The intersected genes were imported into the string database. The species was defined as “Homo sapiens”. The PPI data was obtained. The results were saved in TSV format. The information of node1, node2 and combined score in the file were retained. The interaction network was drawn and the network was analyzed by cytoscape.
Network Construction
The active components of Epicedium and intersected genes were imported into Cytoscape version 3.6.0 to construct the compound-target network of Epicedium.

Gene Ontology (GO) and Pathway Analysis
We used the Database for Annotation, Visualization, and Integrated Discovery [27] (DAVID, https://david.ncifcrf.gov/, v6.8) database for GO enrichment analysis and accomplished pathway enrichment analysis by using Kyoto Encyclopedia of Genes and Genomes [28](KEGG, http://www.kegg.jp/). Biological information annotation database (David, https://david.ncifcrf.gov/, version 6.8) provides a systematic and comprehensive annotation information on the biological functions of large-scale genes or proteins, and it can determine the most considerably enriched biological annotation. The intersected genes were imported into the David database. The selected identifier was set to official gene symbol. The list type was set to gene list, and the species was limited to “Homo sapiens”. Go analysis and KEGG pathway analysis were performed on the intersected genes.

Molecular docking
The crystal structures of the candidate protein targets of Epicedium were downloaded from the RCSB Protein Data Bank (http://www.pdb.org/) and modified using the Autodock tools 1.5.6 software. The four targets were IL6 (PDB ID: 5fuc) [29], VEGFA (PDB ID: 6d3o; https://www1.rcsb.org/structure/6d3o), AKT1 (PDB ID: 6s9x) [30], and EGF (PDB ID:1jl9) [31]; these targets include ligand and water removal, hydrogen addition, and amino acid optimization and patching. The files were saved in pdbqt format. ChemBioDraw 3D was used to create the 3D chemical structures and minimize their energy. Results were saved in MOL2 format. The compounds were imported into Autodock tools 1.5.6, and all flexible keys were rotatable by default and saved in pdbqt format, as docking ligand. Autodock Vina 1.1.2 was used for docking, while Discovery Studio 3.5 was used to visualize the docking results.

Results
Active compounds of Epicedium
TCMSP database (http://ibts.hkbu.edu.hk/LSPtcmsp.php) is a unique pharmacological platform of TCM system, and it can calculate absorption, distribution, metabolism and excretion (ADME)-related characteristics of natural compounds [22]. A total of 130 components of Epicedium were identified from TCMSP. The components were screened with the criteria of OB≥30% and DL≥0.18. A total of 23 bioactive components of Epicedium were screened, in which four had no targets. Finally, 19 compounds were collected from TCMSP database (Fig. 1A, B; Table 1).

Depression—Epicedium PPI network
By using depression as keyword in NCBI database, 537 genes related to depression were retrieved, and 200 target genes of Epicedium were collected from the TCMSP database. After intersecting the target genes of Epicedium and depression, 53 common genes were found (Fig. 2A). These genes could be the target genes of Epicedium in the treatment of depression. Then, we built a visualized Epicedium–component–targets network by using Cytoscape 3.6. The nodes of different colors and shapes represented the
potential active components and targets of *Epicedium*. The blue nodes represented *Epicedium*, the yellow nodes represented the active components of *Epicedium*, and the red nodes represented the potential antidepressant targets of *Epicedium*. The edges represented the correlation between the active components and the targets (Fig. 2B), confirming the multi-component and multi-target characteristics of *Epicedium*. A total of 199 nodes and 3,302 edges were observed in Fig. 2C, the average node degree was 33.2, and the local clustering coefficient was 0.574. A total of 53 nodes and 449 edges were observed in Fig. 2D, the average node degree was 16.9, and the local clustering coefficient was 0.662. The size of the node represented the degree value of targets. The larger the node was, the greater the degree value was. The thickness of the edge indicated the combination score. The coarser the edge was, the greater the combination score value was. Data for the degree of each target was shown in Table 2.

**Table 1 Basic information about the active compounds in *Epimedium* (OB≥30%, DL≥0.18)**

| N.O. | MolId   | MolName                                      | OB(%) | DL   |
|------|---------|----------------------------------------------|-------|------|
| 1    | MOL001645 | Linoleyl acetate                            | 42.10 | 0.20 |
| 2    | MOL001792 | DFV                                          | 32.76 | 0.18 |
| 3    | MOL003044 | Chryseriol                                   | 35.85 | 0.27 |
| 4    | MOL003542 | 8-Isopentenyl-kaempferol                     | 38.04 | 0.39 |
| 5    | MOL000359 | sitosterol                                    | 36.91 | 0.75 |
| 6    | MOL000422 | kaempferol                                   | 41.88 | 0.24 |
| 7    | MOL004367 | olivil                                       | 62.23 | 0.41 |
| 8    | MOL004373 | Anhydroicaritin                               | 45.41 | 0.44 |
| 9    | MOL004380 | C-Homonythran, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta)-| 39.14 | 0.49 |
| 10   | MOL004382 | Yinyanghuo A                                 | 56.96 | 0.77 |
| 11   | MOL004384 | Yinyanghuo C                                 | 45.67 | 0.50 |
| 12   | MOL004388 | 6-hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-diaza-2,3,4,8-tetrahydro-1H-isochromeno[3,4-hisoquinolin-2-ium | 60.64 | 0.66 |
| 13   | MOL004391 | 8-(3-methylbut-2-ethyl)-2-phenyl-chromone      | 48.54 | 0.25 |
| 14   | MOL004396 | 1,2-bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol | 52.31 | 0.22 |
| 15   | MOL004427 | Icariside A7                                  | 31.91 | 0.86 |
| 16   | MOL000006 | luteolin                                     | 36.16 | 0.25 |
| 17   | MOL000622 | Magnograndiolide                             | 63.01 | 0.19 |
| 18   | MOL000098 | quercetin                                    | 46.43 | 0.28 |
| 19   | MOL004386 | Yinyanghuo E                                 | 51.63 | 0.55 |

**GO analysis of genes targeted by *Epicedium***

David database was applied to analyze the related targets of *Epicedium* for the treatment of depression. In BP analysis, the top ranked targets were distributed in the process of cell division (5 targets/5.9%), positive regulation of sequence-specific DNA binding transcription factor activity (7 targets/8.3%) and transcription from RNA polymerase II promoter (13 targets/15.3%), response to hypoxia (6 targets/7.1%), and immune response (7 targets/8.3%) (Fig. 3A). In CC analysis (Fig. 3B), cell components such as extracellular space (18 targets/21.3%), cytosol (11 targets/13.0%), extracellular matrix (4 targets/4.7%), neuron projection (4 targets/4.7%), integral component of plasma membrane (8 targets/9.4%), and extracellular space (18 targets/21.3%) were at the top. In MF analysis (Fig 3C), molecular functions such as cytokine activity (6 targets/12.8%), dopamine (3 targets/6.4%), IL-1 receptor (3 targets/6.4%), steroid (3 targets/6.4%), and drug binding (3 targets/6.4%), and cytokine activity (6 targets/12.8%) were at the top.

**KEGG analysis of genes encoding proteins targeted by *Epicedium***

David database was used to analyze the KEGG pathway of 53 targets of *Epicedium* for the treatment of depression. According to the p value, the top 20 pathways were selected and shown in Fig. 3D. The color of the nodes in the figure from green to red reflected the p value from large to small. The nodes from small to large reflected the number of related genes in increasing trend. The top ranked pathways were dopaminergic synapse, measles pathways in cancer, inflammatory bowel disease, leishmaniasis, and chagas disease (American trypanosomiasis).
Molecular docking
The results are shown in Fig. 5 and supplementary Figure1-2. The 2D and 3D structures of the ligands are shown in Fig. 4. The estimated free energy of binding and RMSD was summarized in Table 3. The interactions between ligands and target proteins is shown in Table 4. Electrostatic force and van der Waals force are the main forces between ligand and target protein. The binding energies of ligand and receptor were less than -1.19cal / mol, and RMSD were less than 2, indicating that the docking results were good. Fluoxetine was used as positive control, and the binding energies of kaempferol,
quercetin and luteolin were lower than that of fluoxetine, indicating that the binding stability of kaempferol, quercetin and luteolin was better than that of fluoxetine.

Discussion
Depression is a common mental disorder with a high incidence, recurrence rate, and mortality. Depression not only seriously endangers the lives and health of the people but also causes heavy mental and economic burden to the society, patients, and families. The pathogenesis of depression is very complex. Depression has become a prevalent worldwide health concern. It is still mainly treated with drugs.

Epicedium has different effects, such as anti-inflammatory, anti-aging, anti-tumor, anti-oxidation, and anti-inflammatory effects, improves immunization, and acts as antidepressants. In the present study, the main research idea was based on the perspective of disease-target-drug, and we applied the network pharmacology approach to evaluate the anti-depression effects and underlying mechanism of Epicedium. These results provide an important reference for the further study of the pathogenesis of depression and the treatment of depression.

By constructing the "drug–target–disease" network, novel drugs and treatment targets could be found [32]. TCMs are difficult to study because of their complex components and huge system. Network pharmacology provides a new idea and perspective for the study of complex TCMs. We integrated information from open databases to predict the interaction between Epicedium and its potential protein targets in depression. In the present study, Epicedium works in various ways through the effects of multiple compounds and may provide advantages by reducing drug resistance. The results of PPI network show a relationship between the target proteins of Epicedium, which was a complex interaction network rather than acting alone. Nineteen active components were obtained by screening, and the three active components with more targets were luteolin (15 targets), quercetin (34 targets), and kaempferol (14 targets). The results are similar to Naijun Yuan’s results, confirming that flavonoids have obvious antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33]. Many studies have demonstrated their neuroprotective effects in vitro and in vivo. For example, luteolin-regulated genes may act as receptors in the central nervous system to produce antidepressant effect. These flavonoids are common components in many herbal medicines. Their specific roles in the treatment of depression should be clarified [33].

Table 2 Information about potential antidepressant targets from Epimedium

| serial number | name  | degree | serial number | name  | degree |
|---------------|-------|--------|---------------|-------|--------|
| 1             | AKT1  | 41     | 27            | OPRM1| 15     |
| 2             | IL6   | 38     | 28            | IGF2 | 14     |
| 3             | VEGFA | 34     | 29            | ADRB2| 14     |
| 4             | EGF   | 31     | 30            | DRD2 | 13     |
| 5             | IL1B  | 29     | 31            | IF1  | 13     |
| 6             | CCL2  | 29     | 32            | COL1A1|13     |
| 7             | MMP9  | 29     | 33            | SLC6A4|13     |
| 8             | PTGS2 | 28     | 34            | GJA1 | 13     |
| 9             | SERPIN1|25     | 35            | PLAT | 12     |
| 10            | MMP2  | 25     | 36            | HTR3A| 12     |
| 11            | PPARG | 25     | 37            | DPP4 | 12     |
| 12            | CCND1 | 24     | 38            | PRKCB| 11     |
| 13            | APP   | 24     | 39            | ADRA2A|11    |
| 14            | ICAM1 | 23     | 40            | DRD5 | 11     |
| 15            | ESR1  | 22     | 41            | DRD1 | 11     |
| 16            | IL4   | 21     | 42            | SLC6A3|9      |
| 17            | HMOX1 | 21     | 43            | ESR2 | 9      |
| 18            | HIF1A | 20     | 44            | ADRB1| 9      |
| 19            | IL2   | 20     | 45            | SLC6A2|9      |
| 20            | CRP   | 20     | 46            | MAOB | 9      |
| 21            | STAT1 | 19     | 47            | GRIA2| 7      |
| 22            | NOS2  | 18     | 48            | CHRM2| 7      |
| 23            | IL1A  | 17     | 49            | PON1 | 7      |
| 24            | SOD1  | 17     | 50            | PPP3CA|5     |
| 25            | AR    | 16     | 51            | RASA1|4      |
| 26            | GSK3B | 15     | 52            | NR3C2|4      |

Quercetin and luteolin were lower than that of fluoxetine, indicating that the binding stability of kaempferol, quercetin and luteolin was better than that of fluoxetine.
tautomers is 435, the topological molecular polar surface area is 127, the number of heavy atoms is 22, the complexity is 488, and the number of covalent bond units is 1. The kinetic process of quercetin in rats is a two compartment open model with absorption, t1/2(α)=0.19h, t1/2(β)=1.22h, tmax=0.333h [41]. Quercetin was quickly distributed to tissues after it was orally given to rats. The highest concentration of quercetin was observed in stomach in rats, and in order of plasma, liver, kidney, heart, lung, spleen. However, the quercetin concentration in muscle and brain couldn’t be detected by HPLC [42].

The molecular weight of kaempferol is 286.236 and the melting point is 276 °C. The solubility in ethanol is 20 mg/ml. The number of hydrogen bond donors is 4, the number of hydrogen bond acceptors is 6, the number of rotatable chemical bonds is 1, the number of tautomers is 126, the polar surface area of topological

![Fig. 3 Enrichment of gene ontology (GO) and KEGG pathway of Epimedium in the treatment of depression. (A) Enriched GO terms for biological process (BP) of potential antidepressant targets from main active ingredients of Epimedium. (B) Enriched GO terms for the cellular component of potential antidepressant targets from main active ingredients of Epimedium. (C) Enriched GO terms for molecular function of potential antidepressant targets from main active ingredients of Epimedium. (D) Enriched KEGG pathways of potential antidepressant targets from main active ingredients of Epimedium](image-url)
molecules is 107, the number of heavy atoms is 21, the complexity is 451, the number of covalent bond units is 1. The pharmacokinetic behavior of kaempferol conformed to the two compartment model, $t_{1/2}(\alpha) = 0.957 \pm 0.172 \text{min}$, $t_{1/2}(\beta) = 6.409 \pm 1.584$. Kaempferol has the characteristics of slow absorption, wide distribution and rapid elimination [43].

The molecular weight of luteolin is 286.236 and the melting point is 330 °C. The number of hydrogen bond donors is 4, the number of hydrogen bond acceptors is 6, the number of rotatable chemical bonds is 1, the number of tautomers is 162, the topological molecular polar surface area is 107, the number of heavy atoms is 21, the surface charge is 0, the complexity is 447, the number of isotopic atoms is 0, the number of covalent bond units is 1, and the solubility is not determined, $t_{1/2}(\alpha) = 0.27 \pm 0.18 \text{h}$, $t_{1/2}(\beta) = 1.95 \pm 0.54 \text{h}$, $t_{\text{max}} = 0.64 \pm 0.13 \text{h}$ [44]. Luteolin and its metabolites preferred to distribute in the gastrointestinal, liver, kidney and lung. Biliary excretion dominated the elimination pathways of the conjugated luteolin [45].

The four targets with high degree were AKT1(15), IL6(14), VEGFA(14), and EGF(13). IL-6 is a multifunctional cytokine, which is the basis for various immune responses and acute phase reactions, and it is related to depression and autonomic nervous system symptoms. IL-6 is closely related to hypothalamus pituitary adrenal axis hyperactivity, serotonin metabolism disorder, fatigue, anorexia, depression, and autonomic nervous system symptoms [46]. Akt is a serine/threonine protein kinase, which is an important target of PI3K downstream. Akt has three subtypes (AKT1, AKT2, and Akt3), which play an important role in depression. The activity of Akt protein in the brain tissue of patients with severe depression has been significantly reduced, and Akt can enhance the function of hippocampal stem cells and promote the efficacy of antidepressants [47]. AKT1 has attracted much attention in the study of depression, and it is associated with depression, anxiety symptoms, work, activity, and suicidal tendency of patients with depression [48]. Generally, that AKT1 can regulate the

![Fig. 4 2D and 3D structures of ligands. (A) 2D structure of kaempferol. (B) 2D structure of luteolin. (C) 2D structure of quercetin. (D) 3D structure of kaempferol. (E) 3D structure of luteolin. (F) 3D structure of quercetin](image)

| parameter | Estimated Free Energy of Binding | RMSD |
|-----------|----------------------------------|------|
| receptor  | Free | VEGFA | AKT1 | EGF | IL6 | VEGFA | AKT1 | EGF |
| ligand luteolin | -5.48 | -6.74 | -6.95 | -7.33 | 0.062 | 0.063 | 0.067 | 0.066 |
| quercetin | -5.2 | -7.31 | -6.95 | -6.3 | 1.165 | 1.166 | 1.17 | 1.17 |
| kaempferol | -5.9 | -6.85 | -6.82 | -6.49 | 0.064 | 0.066 | 0.069 | 0.069 |
| fluoxetine | -4.77 | -5.11 | -5.35 | -5.71 | 0.653 | 0.632 | 0.643 | 0.627 |
Dopamine, as a monoamine transmitter, is a key neurotransmitter in hypothalamus and pituitary gland. Pathway analysis suggested that *Epicedium* may play an antidepressant role by regulating TNF signaling pathway, dopaminergic synapse, and prolactin signaling pathway. TNF, dopamine, calcium signaling pathway, prolactin, and 1-kappaB kinase/NF-kappaB signaling play important roles in the pathogenesis of depression [50–52]. As a messenger, Ca\(^{2+}\) is involved in the regulation of various neural cell functions, such as the release of neurotransmitters, the construction of cells, and the activation of enzyme system. In the central nervous system, slight changes in Ca\(^{2+}\) can lead to significant changes in the function of nerve cells. Therefore, Ca\(^{2+}\) balance is an important factor to maintain the structural integrity and normal function of nerve cells. The increase of Ca\(^{2+}\) in hippocampal neurons can lead to neuronal apoptosis. The possible key components and targets of antidepressive potential of *Epicedium* are listed above.

The results of molecular docking showed a good binding activity between the three most important components (luteolin, quercetin, and kaempferol) and the four important target proteins (IL6, VEGFA, AKT1, EGF), and the main forms of interaction between components and targets are electrostatic and van der Waals force. Quercetin, kaempferol, and luteolin possess anti-depression effects, indicating that the research method is reasonable and feasible.
Fig. 5 (See legend on next page.)

Luteolin

Quercetin

Kaempferol
The synthesis, release, reuptake, or metabolic disorders of dopamine can lead to depression. Dopamine deficiency can downregulate the dopamine transporter but upregulate the concentration of D2/3 receptor. The decrease of dopamine transporter in amygdala and the increase of dopamine D2/3 receptor in patients with depression indicate that depression may be related to dopamine deficiency in brain. Hence, dopamine plays an important role in the pathophysiological process of depression. The decrease of dopaminergic neurons and the dysfunction of dopaminergic receptors are risk factors for depression. Tumor necrosis factor has two forms, namely, TNF-α and TNF-β. Its physiological effects are mediated by TNF-R1 and TNF-R2. Serum TNF-α levels in patients with depression significantly increase [53]. In an animal experiment, significant depressive behavior was induced by injecting TNF-α or LPS into the lateral ventricle, Reichenberg [54] found that TNF-α can induce depression and cognitive function changes in humans. In addition, various antidepressants can reduce the level of TNF-α in the peripheral blood of patients with depression. The expression of TNF-α in the dorsolateral prefrontal cortex of patients with severe depression is significantly increased [55]. In addition, Pandey GN found that the expression of TNF-α mRNA and protein in the prefrontal cortex of patients with depression has increased. The serum TNF-α levels can also assess the severity of depression. Therefore, the increase of TNF-α level can lead to the occurrence of depressive symptoms.

Furthermore, more genes were involved in the formation of nerves and synapses. Synapses are the basic structure of information transmission between neurons. They adapt to stimuli by constantly modifying neural connections and circuits. Therefore, synaptic plasticity is the main manifestation of neural plasticity. Depression and other psychological disorders are usually associated with decreased synaptic plasticity in the hippocampus.

Estrogen can affect brain function directly through the estrogen receptor in the brain region, and it plays an important role in depression. It can increase the concentration of 5-hydroxytryptamine, dopamine, norepinephrine, and other neurotransmitters in synapses and affect their release and reuptake. Aged female mice show increased anti-anxiety and antidepressant effects after administration of estrogen [56]. In addition, ketamine and its active metabolites have similar affinity to ERα [57]. Ovariectomy can induce depression-like behavior in rats. The implantation of estradiol pellets into both sides of medial amygdala of ovariectomized rats can significantly reduce depressive behavior in rats. This effect may be achieved by activating Erβ, because Erβ agonist can also reverse the depression-like behavior of ovariectomized rats [58]. IL-1 can initiate various immune responses, such as fever, prostaglandin synthesis, neutrophil aggregation and activation, activation of T cells and B cells, and production of cytokines [59]. Animal studies on depression have found that the spleen cell of depression rats induced by chronic mild stimulation have increased the production of IL-1. IL includes 11 factors such as IL-1 α and IL-1 β, among which IL-1α is widely expressed in many kinds of cells and can be activated without any processing [60]. IL-1β is mainly expressed in bone marrow cells. The activation of pattern recognition receptor may be a biological target for innovative treatment of depression. Dahl [59] found that serum IL-1β in patients with depression is significantly higher than that in healthy control group. Huang [60] found that behavioral depression is induced in rats after the intraventricular injection of IL-1 β. The possible mechanism is that IL-1β activates IL-1 receptor type I in hippocampal neural stem cells, which affect nuclear factor kappaB signal transduction pathway, reduce the proliferation of hippocampal cells, and then lead to depression [61]. The loss and overexpression of IL-1β receptor antagonist in the brain of mice can resist the decrease of hippocampal nerve regeneration related to stress, making it less prone to depression [62].

In this study, we applied the method of network pharmacology to explore the antidepressant mechanism of Epimedium for the first time, and docking verification were added to the network pharmacology as complement to predict the drug targets. Protein-protein interaction and KEGG enrichment analysis results show that prolactin signaling pathway and EGF play an important role in the treatment of depression. There are few studies on this aspect. According to the results of this study, we can carry out research on prolactin signaling pathway and EGF in the future depression research.

**Conclusion**

System prediction was used to identify *Epimedium* with the potential effect on anti-depression. The network pharmacology, a multi-component and multi-targets analysis fit to the TCM treatment, predicted the potential targets and mechanism of formula, and our previous research shows that *Epimedium* extract has good
antidepressant effect. This research indicated that prolactin signaling pathway and EGF play an important role in the treatment of depression. The follow-up study could discuss through the regulation of prolactin signaling pathway and EGF in the treatment of depression, which must be thoroughly tested in vivo and in vitro.

Abbreviations
PPI: protein-protein interaction; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; SPT: Sucrose Preference Test; FST: Forced Swimming Test; TCMS: Traditional Chinese Medicine Systems Pharmacology; LPS: Lipopolysaccharide; MDD: Major Depressive Disorder; EGF: Epidermal Growth Factor; VEGFA: Vascular endothelial growth factor A; IL6: Interleukin-6; AKT1: RAC-alpha serine/threonine-protein kinase

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12906-021-03389-w.

Additional file 1: Supplementary Figure 1. The four targets were IL6 (PDB ID: 5fuc), VEGFA (PDB ID: 6d3c), AKT1(PDB ID: 69x1), and EGF (PDB ID:1f9). The top three compounds were leteolin, quercetin, and kaempferol. The binding energies of ligand compounds and protein receptors were as follows. The binding energy range from −7.33 kcal/mol to −5.20 kcal/mol. Structural model of active ingredients with hub targets. (A) Structural model of IL6 with luteolin, quercetin, and kaempferol. (B) Structural model of VEGFA with luteolin, quercetin, and kaempferol. (C) Structural model of AKT1 with luteolin, quercetin, and kaempferol. (D) Structural model of EGF with luteolin, quercetin, and kaempferol. Supplementary Figure 2. Binding site of active ingredients with hub targets. (A) Binding site of IL6 with luteolin, quercetin, and kaempferol. (B) Binding site of VEGFA with luteolin, quercetin, and kaempferol. (C) Binding site of AKT1 with luteolin, quercetin, and kaempferol. (D) Binding site of EGF with luteolin, quercetin, and kaempferol.

Acknowledgements
The authors acknowledge gratitude to all the staff who participated in the study.

Authors’ contributions
YKD and BT participated in the design of the study and wrote the manuscript. XX, CXF, YTR and HYM collected and analyzed the data. JLZ, FYS, SSZ, SL and JPX did the experiment. HL and ZLZ edited the manuscript. JHZ, HY and AL participated in the design of the study and wrote the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China(Grants 81873805).

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no competing of interest.

Author details
1Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Institute of Maternal and Infant Health, Northwest University, Xi’an 710069, Shaanxi Province, China. 2Department of Orthopaedic, Tianjin Medical University General Hospital, Anhuan Road No.154, Tianjin 300052, Heping District, China. 3Department of Neonatology, The First Affiliated Hospital of Medical College/Xian Jiaotong University, Xian 710069, Shaanxi Province, China. 4Qinba Chinese Medicine Resources R&D Center, School of Medicine, Ankang University, Ankang 710069, Shanxi Province, China.

Received: 4 November 2020 Accepted: 10 August 2021
Published online: 03 September 2021

References
1. Scangos KW, Makhoul GS, Sugrue UP, Chang EF, Krystal AD. State-dependent responses to intracranial brain stimulation in a patient with depression. Nature Medicine. Feb 2021;27(2):229–+. 2. Wigner P, Czarny P, Galeyk P, Su K-P, Slivinski T. The molecular aspects of oxidative & nitrative stress and the tryptophan catabolites pathway (TRYC ATs) as potential causes of depression. Psychiatry Research. 2018;26566–74. 3. Cui Y, Cao K, Lin H, et al. Early-Life Stress Induces Depression-Like Behavior and Synaptic-Plasticity Changes in a Maternal Separation Rat Model. Gender Difference and Metabolomics Study. Frontiers in Pharmacology. Feb 26 2020;11. 4. Du H, Zhao H, Lai X, et al. Analysis of Antidepressant Activity of Huangliang-Lian Jie-Du Decoction Through Network Pharmacology and Metabonomics. Frontiers in Pharmacology. May 4 2021;12. 5. Du H, Zhao H, Lai X, et al. Metabolic profiles revealed synergistically antidepressant effects of ilies and Rhizoma Anemarrhenae in a rat model of depression. Biomedical Chromatography. Jul 2017;31(7). 6. Short B, Feng J, Gavel V, Sheller W, Loo CK. Side-effects associated with ketamine use in depression: a systematic review. Lancet Psychiatry. Jan 2018;5(1):65–78. 7. Andrade C. Ketamine for Depression, 1: Clinical Summary of Issues Related to Efficacy, Adverse Effects, and Mechanism of Action. Journal of Clinical Psychiatry. Apr 2017;78(4):E415–9. 8. Cao H, Zuo C, Huang Y, et al. Hippocampal proteomic analysis reveals activation of necroptosis and ferroptosis in a mouse model of chronic unpredictable mild stress-induced depression. Behavioural Brain Research. Jun 11 2021;407. 9. Xiong Z, Yang J, Huang Y, et al. Serum metabonomics study of anti-inflammatory activity of Huangliao 710069, Shanxi Province, China. 3Department of Neonatology, The First Affiliated Hospital of Medical College/Xian Jiaotong University, Xian 710069, Shaanxi Province, China. 4Qinba Chinese Medicine Resources R&D Center, School of Medicine, Ankang University, Ankang 710069, Shanxi Province, China.

Received: 4 November 2020 Accepted: 10 August 2021
Published online: 03 September 2021

References
1. Scangos KW, Makhoul GS, Sugrue UP, Chang EF, Krystal AD. State-dependent responses to intracranial brain stimulation in a patient with depression. Nature Medicine. Feb 2021;27(2):229–+. 2. Wigner P, Czarny P, Galeyk P, Su K-P, Slivinski T. The molecular aspects of oxidative & nitrative stress and the tryptophan catabolites pathway (TRYC ATs) as potential causes of depression. Psychiatry Research. 2018;26566–74. 3. Cui Y, Cao K, Lin H, et al. Early-Life Stress Induces Depression-Like Behavior and Synaptic-Plasticity Changes in a Maternal Separation Rat Model. Gender Difference and Metabolomics Study. Frontiers in Pharmacology. Feb 26 2020;11. 4. Du H, Zhao H, Lai X, et al. Analysis of Antidepressant Activity of Huangliang-Lian Jie-Du Decoction Through Network Pharmacology and Metabolomics. Frontiers in Pharmacology. May 4 2021;12. 5. Du H, Zhao H, Lai X, et al. Metabolic profiles revealed synergistically antidepressant effects of ilies and Rhizoma Anemarrhenae in a rat model of depression. Biomedical Chromatography. Jul 2017;31(7). 6. Short B, Feng J, Gavel V, Sheller W, Loo CK. Side-effects associated with ketamine use in depression: a systematic review. Lancet Psychiatry. Jan 2018;5(1):65–78. 7. Andrade C. Ketamine for Depression, 1: Clinical Summary of Issues Related to Efficacy, Adverse Effects, and Mechanism of Action. Journal of Clinical Psychiatry. Apr 2017;78(4):E415–9. 8. Cao H, Zuo C, Huang Y, et al. Hippocampal proteomic analysis reveals activation of necroptosis and ferroptosis in a mouse model of chronic unpredictable mild stress-induced depression. Behavioural Brain Research. Jun 11 2021;407. 9. Xiong Z, Yang J, Huang Y, et al. Serum metabonomics study of anti-depressive effect of Xiao-Chai-Hu-Tang on rat model of chronic unpredictable mild stress-induced depression. Clinical and Translational Research. Jul 2020;6. 10. Xiong Z, Yang J, Huang Y, et al. Serum metabonomics study of anti-depressive effect of Xiao-Chai-Hu-Tang on rat model of chronic unpredictable mild stress-induced depression. Clinical and Translational Research. Jul 2020;6. 11. Huang S, Meng N, Chang B, Quan X, Yuan R, Li B. Anti-inflammatory Activity of Epimedium brevicornu Maxim Ethanol Extract. Journal of Medicinal Food. Jul 2017;21(7):726–E29. 12. Zhao Y, Chen S, Wang Y, Lv C, Wang J, Du J. Effect of drying processes on prenylflavonoid content and antioxidant activity of Epimedium koreanum Nakai. Journal of Food and Drug Analysis. Apr 2018;26(2):796–806. 13. Kou Y, Wang Z, Wu Z, et al. Epimedium Extract Promotes Peripheral Nerve Regeneration in Rats. Evidence-Based Complementary and Alternative Medicine. 2013;2013. 14. Zhang X, Sun H, Su Q, et al. Antidepressant-like activity of icarin mediated by group I mGluRs in prenatally stressed offspring. Brain & Development. Aug 2017;39(7):593–600. 15. Song X, Zhang Y, Yang N, Dai E, Wang L, Du H. Molecular mechanism of salvistro in the treatment of systemic lupus erythematosus based on network pharmacology and molecular docking technology. Life Sciences. Jan 1 2020;240. 16. Li T, Zhang W, Hu E, et al. Integrated metabolomics and network pharmacology to reveal the mechanisms of hydroxysafflor yellow A against acute traumatic brain injury. Computational and Structural Biotechnology Journal. 2021;2021:19:1002–1013. 17. Zhong Y, Luo J, Tang T, et al. Exploring Pharmacological Mechanisms of Xuefu Zhiyuan Decoction in the Treatment of Traumatic Brain Injury via a Network Pharmacology Approach. Evidence-Based Complementary and Alternative Medicine. 2018;2018:2018.
18. Ma C, Xu T, Sun X, et al. Network Pharmacology and Bioinformatics. Approach Reveals the Therapeutic Mechanism of Action of Baiacalain in Hepatocellular Carcinoma. Evidence-Based Complementary and Alternative Medicine. 2019;2019.

19. Zhu W, Fan X, Wei H, et al. Mechanism Research of Apatinib-Treated Breast Cancer Based on Network Pharmacology. Chinese Pharmaceutical Journal. 2016;61(11):1569-1573.

20. Liu Y, Ju Y, Qin X. Studies on the compatibility mechanism and material basis of Danshui Bukue Decoction against anemia mice using metabonomics and network pharmacology. The Journal of pharmacy and pharmacology. 2021-Apr-27 2021;73(6):767-777.

21. Gong P, Guo Y, Li X, Wang N, Gu J. Exploring active compounds of Jinhua Qionggen Granules for prevention of COVID-19 based on network pharmacology and molecular docking. Chinese Traditional and Herbal Drugs. 2020 2020;51(7):1685-1693.

22. Ru J, Li P, Wang J, et al. TCMSp: a database of systems pharmacology for drug discovery from herbal medicines. Journal of Cheminformatics. Apr 2014;6.

23. Xu X, Zhang W, Huang C, et al. International Journal of Molecular Sciences. Jun 2012;13(6):255-271.

24. Walters WP, Murcko MA. Prediction of ‘drug-likeness’. Advanced Drug Delivery Reviews. Mar 31 2002;54(3):255-271.

25. Feng W, Ao H, Yue S, Peng C. Systems pharmacology reveals the unique mechanism features of Shenzhu Capsule for treatment of ulcerative colitis in comparison with synthetic drugs. Scientific Reports. Nov 1 2018;

26. von Mering C, Jensen LJ, Snel B, et al. STRING: known and predicted protein-protein associations, integrated and transferred across organisms. Nucleic Acids Research. Jan 1 2005;33:D43-D7.

27. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols. 2009;4(1):444-57.

28. Chen L, Zhang Y-H, Wang S, Zhang Y, Huang T, Cai Y-D. Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. PLoS One. Sep 5 2017;12(9).

29. Adams R, Bumley RJ, Valenzano CR, et al. Discovery of a functional epitope antibody that stabilizes IL-6 and gp80 protein: protein interaction and modulates its downstream signaling. Scientific Reports. Jan 30 2017;7.

30. Quanbrusch L, Landel I, Depa L, et al. Covalent-Antilostic Inhibitors to Achieve Akt Isom-Selectivity. Angewandte Chemie-International Edition. Dec 19 2019;58(52):18829-18839.

31. Lu HS, Chai JI, Li M, Huang BR, He CH, Bi RC. Crystal structure of human epidermal growth factor and its dimerization. Journal of Biological Chemistry. Sep 14 2001;276(37):34913-34917.

32. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nature Chemical Biology. Nov 2008;4(11):682-687.

33. Heinrich M, Appignano G, Effert T, et al. Best practice in research - Overcoming common challenges in phytopharmacological research. Journal of Ethnopharmacology. Jan 10 2020;246.

34. Sasaki K, El Omri A, Kordo S, Han J, Isoda H. Rosmarinus officinalis polyphenols produce anti-oxidant like effect through monoaminergic and cholinergic functions modulation. Behavioural Brain Research. Feb 1 2013;238:86-94.

35. Akinindse AS, Adeybi OE. Neuroprotection by luteolin and gallic acid against cobalt chloride-induced behavioural, morphological and neurochemical alterations in Wistar rats. Neurotoxicology. Sep 2019;74:252-263.

36. Mehta V, Parashar A, Udayabanu M. Quercetin prevents chronic unpredictable stress induced behavioral dysfunction in mice by alleviating hippocampal oxidative and inflammatory stress. Physiology & Behavior. Mar 15 2017;171:69-78.

37. Khan A, Ali T, Rehman SU, et al. Neuroprotective Effect of Quercetin Against the Detrimental Effects of LPS in the Adult Mouse Brain. Frontiers in Pharmacology. Dec 11 2018;9.

38. Liang Y, Tan Y, Zhang S, Wang S, Wang L, Yang Y. Effect and mechanism of kaempferol on depression-like behavior in elderly rats with chronic stress depression. The Chinese Journal of Clinical Pharmacology. 2020 2020;36(24):4028-4039.

39. Zhu S, Lei S, Zhou S, et al. Luteolin shows antidepressant-like effect by inhibiting and downregulating plasma membrane monoamine transporter (PMAT, SLC9A4). Journal of Functional Foods. Mar 2019;54:440-8.

40. Samad N, Saleem A, Yamin F, Shehzaad MA. Quercetin Protects Against Stress-Induced Anxiety- and Depression-Like Behavior and Improves Memory in Male Mice. Physiological Research. 2018 2018;67(5):795-808.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.