Active components of *Bupleuri Radix* in the treatment of schizophrenia analyzed by network pharmacology and clinical verification

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Author contributions
Li L and Yang P designed the research. Xiao J and Guo J performed the network pharmacology analysis. Zheng XY collected the clinical samples. Sun W and Ning QX conducted the clinical validation. Tang L and Xiao JY analyzed the data. Xiao J drafted the manuscript. Li L and Yang P revised the manuscript. All authors contributed to the article and approved the final version of the manuscript for submission.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
DEGs, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; PANS, positive and negative symptom scale; TCM, traditional Chinese medicine; TCMS, traditional Chinese medicine systems pharmacology database and analysis platform.

Citation

Abstract

**Background:** *Bupleuri Radix* is a common Chinese medicinal material in traditional Chinese medicine. Currently, the therapeutic effect of treating schizophrenia is relatively well understood. However, there are fewer studies examining the underlying mechanisms of its treatment. The objective of the study was to investigate the primary mechanisms of *Bupleuri Radix* in treating schizophrenia through network pharmacology and clinical validation.

**Method:** Network pharmacology revealed possible molecular mechanisms, followed by clinical verification. Sixty-seven schizophrenia patients undergoing treatment at the Hunan Brain Hospital between October and November 2022 were recruited and randomly divided into the olanzapine group and the olanzapine + *Bupleuri Radix* group. Additionally, 32 healthy people undergoing physical examinations during the same period were included as the control group. The patient’s positive and negative symptom scale scores were compared. qPCR was used to detect the mRNA expression levels of ESR1, mTOR, EIF4E, and SMAD4 in peripheral blood.

**Results:** Through network pharmacological analysis, it was concluded in this study that *Bupleuri Radix* might regulate the mTOR, PI3K-Akt, and HIF-1 signaling pathways. Clinical experiments indicated that compared with before treatment, the positive and negative symptom scale scores and total scores of the two treatment groups were significantly decreased after treatment (*P* < 0.01). In addition, the positive and negative symptom scale scores and total scores in the olanzapine group were significantly decreased (*P* < 0.01) compared to the control group after treatment. Before treatment, ESR1 mRNA expression levels in peripheral blood were significantly higher in the two treatment groups than in the control group, whereas the mRNA expression levels of mTOR, EIF4E, and SMAD4 in peripheral blood were significantly lower (*P* < 0.01). The mRNA expression levels of mTOR, EIF4E, and SMAD4 in peripheral blood were significantly higher after therapy than before treatment, whereas the mRNA expression levels of ESR1 in peripheral blood were significantly lower (*P* < 0.01). After therapy, the olanzapine + *Bupleuri Radix* group’s mRNA expression levels of mTOR, EIF4E, and SMAD4 were significantly higher than those of the olanzapine group, whereas the mRNA expression levels of ESR1 were significantly lower (*P* < 0.01).

**Conclusion:** The mechanism of *Bupleuri Radix*’s therapeutic efficacy in schizophrenia may involve the up-regulation of mTOR, EIF4E, and SMAD4 mRNA expression and the down-regulation of ESR1 mRNA expression in peripheral blood.

**Keywords:** schizophrenia; *Bupleuri Radix*; network pharmacology; clinical verification; active components
Medical history of objective

Bupleuri Radix, a traditional Chinese medicinal herb, is principally acknowledged for its effects of soothing the liver (regulates and balances liver function) and relieving depression, as well as its heat-clearing and detoxifying properties. Its therapeutic uses were first chronicled in the "Shen Nong Benca acs ing" (Sheng Nong’s herbal classic, 25 C.E.–220 C.E.). In ancient times, Bupleuri Radix was widely administered for its antipyretic, sedative, bile secretion-promoting, and detoxifying effects. Contemporary pharmacological research has revealed that Bupleuri Radix exhibits pharmacological activities such as anti-cancer, anti-depressant, anti-inflammatory, cardioprotective, hepatoprotective, and nephroprotective effects.

Background

Schizophrenia is a common and severe mental disorder [1]. It is characterized by delusions, hallucinations, cognitive impairments, and loss of emotions [2]. Traditional Chinese medicine (TCM) classifies schizophrenia as “madness, dementia, and depression.” Most patients are significantly disabled, creating substantial burdens for their families and society [3-5]. Chaihu Longgu Muli Decotion associated with risperidone in the treatment of patients with schizophrenia can reduce the positive and negative symptom scale (PANSS) scores of patients and effectively treat schizophrenia [6]. Bupleuri Radix is a common drug in TCM treatment, and many reports have been drafted regarding treating mental diseases such as schizophrenia [7, 8]. The functions of central dopamine and serotonin in patients with schizophrenia are abnormal, and Bupleuri Radix can also regulate the metabolic process of dopamine and serotonin [9]. It has achieved good results in treating depression, insomnia, and other neurological conditions. The aforementioned studies demonstrate that Bupleuri Radix plays an essential role in treating mental diseases. Numerous studies on the treatment of mental disorders such as schizophrenia have been reported, but none of them have systematically investigated the interconnections between its components, targets, and pathways. Network pharmacology can connect the biological characteristics of diseases and the chemical characteristics of drugs, build a visual network diagram of main active components-targets-pathway, predict the target through the biologically active components of TCM decoction, and reveal the role of their components and targets. This study, therefore, used the network pharmacology method to investigate the specific mechanism of Bupleuri Radix in the treatment of schizophrenia before confirming its efficacy through clinical trials, thereby establishing a scientific foundation for the future clinical treatment of schizophrenia with TCM.

Materials and methods

Network pharmacology

Screening of active components and targets in Bupleuri Radix. The main active components and their drug target genes were retrieved through the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php) with Bupleurum (<Bupleuri Radix>) as the keyword [10]. The oral bioavailability was set at ≥30%, and drug-likeness ≥ 0.2 [11]. The active components with drug target genes in Bupleuri Radix and the human target information of the active components were screened out.

Screening of schizophrenia-related genes. The keyword “schizophrenia” from the Gene Cards database (https://www.genecards.org/) was entered to yield genes associated with schizophrenia [12].

Differentially expressed genes (DEGs) in schizophrenia. The microarray expression data have been deposited in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) with accession numbers GSE17612 [13, 14]. The GSE17612 dataset includes the gene expression profiling of post-mortem tissues from BA10 regional tissue of 28 schizophrenia patients and 23 normal samples. Microarray GSE17612 was analyzed based on the platform GPL570. The Wilcoxon test and Limma package were used for data annotation, and the Fold Change difference analysis log|FC| > 0.5, P < 0.05 was used as the screening criterion to screen DEGs.

Gene ontology (GO) functional enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and protein-protein interaction (PPI) network analysis of DEGs. In this study, GO function analysis and KEGG pathway analysis were performed on the screened DEGs by the Metascape online analysis tool (http://metascape.org), and thresholds were set at P < 0.05 [15-17]. The STRING database (https://string-db.org/) lists protein interactions, including direct physical interactions between proteins and indirect functional correlations [18]. Based on the STRING database, the interaction network of the intersection of schizophrenia-related genes and the therapeutic target genes related to the active components of Bupleuri Radix was constructed. The “biological species” criteria were set as “Homo sapiens,” and the minimum interaction threshold was set to a medium confidence level of 0.4. The rest of the settings were default values to obtain a PPI network.

Main active components-target-pathway visualization network diagram of Bupleuri Radix. The TCMSP platform was used to explore potential targets and pathways related to the Bupleuri Radix’s active components. The predicted active components, targets, and pathways were imported into Cytoscape software to construct a “drug-target-pathway” interaction visualization network diagram [19].

Clinical verification

General information. Inclusion criteria: ICD-10 [20] criteria for schizophrenia; not taking antipsychotics one month before onset; age 18-60 years old; able to cooperate in completing all scales; written informed consent from legal guardians. Exclusion criteria: patients with mental retardation or organic brain diseases and other serious physical conditions such as heart, liver, and kidney diseases; patients with severe social function decline or unable to cooperate due to psychotic symptoms; patients undergoing psychotherapy. According to the treatment method, 67 schizophrenia patients undergoing treatment in the Hunan Brain Hospital during October and November 2022 were divided into the olanzapine group (N = 35) and the olanzapine + Bupleuri Radix group (N = 32). Thirty-two healthy subjects undergoing physical examination during the same period were selected as the control group. There were no significant differences in basic data such as age, gender, education credentials, disease course, and incidence of the three groups of subjects (P > 0.05, Table 1). The protocol of this study was approved by the medical ethical committee of Hunan Brain Hospital (approval No. 2022K17), complying with the rules of Clinical Trial Registration in China (approval No. ChiCTR2200064709). All the participants signed an informed consent form at the beginning of the study.

Treatment methods. The olanzapine group was treated with olanzapine 10 mg/d (Cat. No. 121221002; Jiangsu Haoseng, Lianyungang, China) by oral administration, and the olanzapine +
**Bupleuri Radix** group was treated with olanzapine 10 mg/d and *Bupleuri Radix* TCM ultraline granules 20g/d (Cat. No. 211201; Hunan Rongkang Pharmaceutical, Zhuzhou, China) with warm water by oral administration. The treatment group took the drug for 21 consecutive days.

**Evaluation of clinical efficacy.** The PANSS score was used to evaluate the therapeutic efficacy of the two groups of patients. PANSS [21] consists of 7 positive scales, 7 negative scales, and 16 general psychopathological scales, each of which can be divided into 7 different levels (1-7 points). The patient score is directly proportional to schizophrenia severity.

**Specimen collection.** Fasting blood was collected in the olanzapine and the olanzapine + *Bupleuri Radix* groups before and after 21 days of treatment. The venous blood of the research subjects was drawn on an empty stomach in the morning, placed in an anticoagulant tube, and stored in a −80 °C refrigerator pending experimentation.

**qPCR detection** [22]. Total RNA from peripheral blood was extracted through the Trizol method, and the concentration and purity of RNA were detected by a UV spectrophotometer. The corresponding cDNA was yielded from the reverse transcription of RNA and then added to the Syber mix system, followed by adding primers and PCR amplification. The relative expression levels were calculated using the 2^ΔΔCt method taking GAPDH as an internal reference. See Table 2 for primer sequence information. Partial melting curves of genes are detailed in the Supplementary Figures.

**Statistical analysis.** The data of this experiment were processed by GraphPad, and the final data were expressed as the mean ± standard deviation (x ± s) of the results of three independent experiments. Statistical significance testing was performed using the LSD-t test, and P < 0.05 was considered statistically significant.

**Results**

**Screening of active components and targets in *Bupleuri Radix***

The main active components and drug target genes of *Bupleuri Radix* were analyzed online by TCSP, and 4022 drug target genes were found. Among *Bupleuri Radix*, 5 active components with drug target genes (oral bioavailability ≥ 30%, drug-likeness ≥ 0.2) were found, respectively. They are quercetin, stigmastol, kaemferol, isorhamnetin and Baicalin, which are listed in Table 3.

**Identification of schizophrenia targets**

**Genes associated with schizophrenia.** A search of the Gene Cards database for “schizophrenia” returned 10,005 genes related to schizophrenia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N = 32)</th>
<th>Olanzapine (N = 35)</th>
<th>Olanzapine + <em>Bupleuri Radix</em> (N = 32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>28.97 ± 7.78</td>
<td>29.63 ± 7.81</td>
<td>28.81 ± 8.36</td>
<td>0.6794</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>25</td>
<td>23</td>
<td>0.9677</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3887</td>
</tr>
<tr>
<td>Secondary</td>
<td>20</td>
<td>25</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Postgraduate</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Course (years)</td>
<td></td>
<td></td>
<td>2.99 ± 2.35</td>
<td>0.5634</td>
</tr>
<tr>
<td>Number of episodes</td>
<td></td>
<td></td>
<td>2.69 ± 1.70</td>
<td>0.9017</td>
</tr>
</tbody>
</table>

For analysis of difference between Olanzapine and Olanzapine + *Bupleuri Radix* group, unpaired student’s t-test and pearson’s chi-square test were used. Results were considered statistically significant at P < 0.05.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>MTOR-173</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>ATGCTTGGGAAAGGGGAGCTG</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>TCTTGACTCATCTCTCGGAGTT</td>
</tr>
<tr>
<td>ESR1-158</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>GGGGAAGTATGCTATGGAACTTG</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>TGGCTGGACACATATATCGTGT</td>
</tr>
<tr>
<td>EIF4E-118</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>GAAACACCTCCCTCTCCATTACCC</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>AGAGTGCCCATGTCCTTCGTA</td>
</tr>
<tr>
<td>SMAD4-173</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>AGCAAGAGATGGTATCTACCTG</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>TGCAGATTACCTGGTGATG</td>
</tr>
<tr>
<td>GAPDH-104</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>ACACGCCCTAAGATCATCGC</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>GGTCTAGAGCTCTCTCCACGAT</td>
</tr>
</tbody>
</table>

| Table 3 Screening of active components and targets in *Bupleuri Radix***

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>OB (%)</th>
<th>DL</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>quercetin</td>
<td>302.25</td>
<td>46.43</td>
<td>0.28</td>
<td>3973</td>
</tr>
<tr>
<td>stigmastol</td>
<td>412.77</td>
<td>43.83</td>
<td>0.76</td>
<td>41</td>
</tr>
<tr>
<td>kaemferol</td>
<td>286.25</td>
<td>41.88</td>
<td>0.24</td>
<td>193</td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>316.28</td>
<td>49.60</td>
<td>0.31</td>
<td>56</td>
</tr>
<tr>
<td>baicalin</td>
<td>446.39</td>
<td>40.12</td>
<td>0.75</td>
<td>43</td>
</tr>
</tbody>
</table>

OB, oral bioavailability; DL, drug-likeness; MW, molecular weight.

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Intersection of schizophrenia DEGs and active components of Bupleuri Radix. A total of 520 schizophrenia-related DEGs were screened from the schizophrenia microarray GSE17612; among these, 43 were identified as schizophrenia-related target genes of Bupleuri Radix, including 23 up-regulated and 20 down-regulated (Table 4, Figure 1A). The Venn diagram (Figure 1B) was constructed by intersecting the targets of schizophrenia with the targets of the active ingredients identified in Bupleuri Radix.

GO functional enrichment of DEGs, KEGG pathway analysis, and PPI network analysis. The Metascape online analysis tool was utilized to conduct GO function enrichment and KEGG pathway analyses on the 43 DEGs identified to further investigate the mechanisms of these DEGs in schizophrenia. The results of GO analysis indicated that DEGs were mainly associated with

Table 4 The intersection of schizophrenia DEGs-targets and active components of Bupleuri Radix

<table>
<thead>
<tr>
<th>Gene</th>
<th>logFC</th>
<th>Regulation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMAIP1</td>
<td>-1.96</td>
<td>Down</td>
<td>1.79E-05</td>
</tr>
<tr>
<td>COL3A1</td>
<td>-0.93</td>
<td>Down</td>
<td>5.66E-03</td>
</tr>
<tr>
<td>CREBBP</td>
<td>-0.64</td>
<td>Down</td>
<td>5.85E-03</td>
</tr>
<tr>
<td>CCL3</td>
<td>-0.68</td>
<td>Down</td>
<td>7.29E-03</td>
</tr>
<tr>
<td>SLC22A6</td>
<td>-0.8</td>
<td>Down</td>
<td>9.58E-03</td>
</tr>
<tr>
<td>CNNM4</td>
<td>-0.57</td>
<td>Down</td>
<td>1.31E-02</td>
</tr>
<tr>
<td>NR4A3</td>
<td>-0.79</td>
<td>Down</td>
<td>1.33E-02</td>
</tr>
<tr>
<td>CEP128</td>
<td>-0.74</td>
<td>Down</td>
<td>1.43E-02</td>
</tr>
<tr>
<td>HNMT</td>
<td>-0.51</td>
<td>Down</td>
<td>1.49E-02</td>
</tr>
<tr>
<td>EGR1</td>
<td>-0.6</td>
<td>Down</td>
<td>1.68E-02</td>
</tr>
<tr>
<td>GPNMB</td>
<td>-0.6</td>
<td>Down</td>
<td>1.83E-02</td>
</tr>
<tr>
<td>ARHGEF28</td>
<td>-0.56</td>
<td>Down</td>
<td>1.91E-02</td>
</tr>
<tr>
<td>PTGS1</td>
<td>-0.53</td>
<td>Down</td>
<td>2.33E-02</td>
</tr>
<tr>
<td>EIF4E</td>
<td>-0.92</td>
<td>Down</td>
<td>2.50E-02</td>
</tr>
<tr>
<td>MTOR</td>
<td>-0.62</td>
<td>Down</td>
<td>2.66E-02</td>
</tr>
<tr>
<td>NT5E</td>
<td>-0.57</td>
<td>Down</td>
<td>3.46E-02</td>
</tr>
<tr>
<td>STMN1</td>
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<td>Down</td>
<td>3.65E-02</td>
</tr>
<tr>
<td>THBS1</td>
<td>-0.62</td>
<td>Down</td>
<td>3.97E-02</td>
</tr>
<tr>
<td>PPARA</td>
<td>-0.62</td>
<td>Down</td>
<td>4.08E-02</td>
</tr>
<tr>
<td>SMAD4</td>
<td>-0.55</td>
<td>Down</td>
<td>4.19E-02</td>
</tr>
<tr>
<td>IL1RN</td>
<td>0.87</td>
<td>Up</td>
<td>2.73E-03</td>
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<tr>
<td>ATP2A1</td>
<td>0.52</td>
<td>Up</td>
<td>3.24E-03</td>
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<tr>
<td>ALOX5AP</td>
<td>0.53</td>
<td>Up</td>
<td>3.88E-03</td>
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<tr>
<td>FGF2</td>
<td>0.8</td>
<td>Up</td>
<td>5.14E-03</td>
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<tr>
<td>NTS</td>
<td>0.98</td>
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<td>6.50E-03</td>
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<td>SYK</td>
<td>0.59</td>
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<td>8.14E-03</td>
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<td>ITGB2</td>
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<td>IL1R2</td>
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<td>HSPB1</td>
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<td>EXOC4</td>
<td>0.6</td>
<td>Up</td>
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<td>FNIP2</td>
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<tr>
<td>CD14</td>
<td>0.54</td>
<td>Up</td>
<td>2.46E-02</td>
</tr>
<tr>
<td>CP</td>
<td>0.73</td>
<td>Up</td>
<td>2.56E-02</td>
</tr>
<tr>
<td>BCL2A1</td>
<td>0.91</td>
<td>Up</td>
<td>2.81E-02</td>
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<tr>
<td>TBX3</td>
<td>0.62</td>
<td>Up</td>
<td>2.98E-02</td>
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<tr>
<td>ESR1</td>
<td>0.63</td>
<td>Up</td>
<td>3.32E-02</td>
</tr>
<tr>
<td>BBS9</td>
<td>0.54</td>
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<td>3.84E-02</td>
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<td>AIF1</td>
<td>0.53</td>
<td>Up</td>
<td>3.87E-02</td>
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<td>SERPINC1</td>
<td>0.54</td>
<td>Up</td>
<td>3.91E-02</td>
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<tr>
<td>MYH11</td>
<td>0.71</td>
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<td>4.11E-02</td>
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<tr>
<td>METTL21A</td>
<td>0.6</td>
<td>Up</td>
<td>4.46E-02</td>
</tr>
<tr>
<td>SETD82</td>
<td>0.73</td>
<td>Up</td>
<td>5.00E-02</td>
</tr>
</tbody>
</table>

DEGs, differentially expressed genes.
Figure 1 The intersection of DEGs in schizophrenia and the targets of active components of *Bupleuri Radix*. (A) Volcano diagram; (B) Venn diagram. DEGs, differentially expressed genes.

Figure 2 GO functional enrichment, KEGG pathway analysis, and PPI network analysis of DEGs. (A) GO functional enrichment; (B) KEGG pathway analysis; bubble color indicates P-value, and bubble size indicates gene number. (C, D) PPI network analysis; the nodes in the network diagram represent proteins, and the lines indicate an association between two proteins. DEGs, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction.
biological processes such as inflammatory response, cellular component movement, hormone response, tumor necrosis factor regulation, interleukin-1 regulation, regulation of intracellular signal transduction, fatty acid transport, and metal ion homeostasis (Figure 2A); KEGG analysis showed that DEGs were mainly centered on phagosomes, PI3K-Akt, Rap1, MAPK, HIF-1, mTOR, platelet activation, EGFR tyrosine kinase inhibitor resistance, Cytokine- cytokine receptor interaction and other signaling pathways (Figure 2B). The PPI network of schizophrenia-related genes was displayed (Figure 2C). Among them, four therapeutic target genes (ESR1, mTOR, EIF4E, and SMAD4) can be clustered into a functional cluster related to hypoxia response, leptin signaling pathway, and head and neck squamous cell carcinoma (Figure 2D).

Main active components-target-pathway visualization network diagram of Bupleuri Radix

In this study, a network depicting the relationship between the major active components of the Bupleuri Radix-drug target genes-signaling pathway was established. The bioactive components in Bupleuri Radix include quercetin, stigmasterol, kaempferol, isorhamnetin, and baicalin. The active components of schizophrenia-related targets and pathways are quercetin, stigmasterol, kaempferol, and isorhamnetin. Quercetin acts on ESR1, mTOR, EIF4E, SMAD4. Stigmasterol, kaempferol, and isorhamnetin all act on ESR1. The main active components of Bupleuri Radix are involved in multiple signaling pathways, such as the mTOR signaling pathway, PI3K-Akt signaling pathway, and HIF-1. The results show that Bupleuri Radix treats schizophrenia through multiple targets, components, and pathways (Figure 3).

Clinical verification

Comparison of PANSS score changes before and after treatment. Prior to treatment, negative symptom score, positive symptom score, general psychopathological scale score, and PANSS total score did not change significantly between the two treatment groups (P > 0.05). The PANSS scores and total scores of the two groups exhibited a significant decrease after treatment (P < 0.01) compared to the scores prior to treatment. Moreover, the PANSS scores and total scores of the patients in the olanzapine + Bupleuri Radix group significantly decreased after treatment (P < 0.01) (Figure 4).

The mRNA expression levels of ESR1, mTOR, EIF4E and SMAD4. Compared with the control group, the mRNA expression level of ESR1 in the peripheral blood of the two groups of patients before treatment significantly increased (P < 0.01) while the mRNA expression levels of mTOR, EIF4E, and SMAD4 were distinctly decreased (P < 0.01). Compared with the data before treatment, the mRNA expression level of ESR1 in the two groups after treatment significantly decreased (P < 0.01), and the mRNA expression levels of mTOR, EIF4E, and SMAD4 remarkably increased (P < 0.01). Compared with the olanzapine group after treatment, the mRNA expression level of ESR1 in the olanzapine + Bupleuri Radix group significantly decreased (P < 0.01), and the mRNA expression levels of mTOR, EIF4E, and SMAD4 were significantly increased (P < 0.01) (Figure 5).

Correlation analysis. The results of the bivariate Pearson test showed that the EIF4E level negatively correlated with the PASSN general symptom score (r = −0.376, P < 0.05); ESR1 level negatively correlated with the PASSN negative symptom score (r = −0.354, P < 0.05); mTOR levels negatively correlated with the PASSN negative symptom scores (r = −0.402, P < 0.05); no correlation was observed between the levels of SMAD4 and all clinical symptom scores.

Discussion

In this study, 10,005 genes related to schizophrenia were screened, and by analyzing the main active components of Bupleuri Radix and its drug target genes, quercetin, stigmasterol, kaempferol, and isorhamnetin were identified; Baicalin acts on 4022 drug target genes. By suppressing cholinesterase, quercetin has neuroprotective functions against neurodegenerative Alzheimer's [23, 24]. Quercetin can also exert anti-depressant effects by regulating brain oxidative stress and monoamine levels in chronic, mildly stressed mice [25].

Figure 3 Bupleuri Radix’s main active ingredient-target-pathway visualization network diagram. Yellow represents drug active components, green represents target genes, and purple represents pathways. Baicalin was not shown in the figure because it was not enriched.
Figure 4 PANSS scores before and after treatment in the two treatment groups. **P < 0.01, compared with the data before treatment; ***P < 0.01, compared with the olanzapine group after treatment. PANSS, positive and negative symptom scale.

Figure 5 The mRNA expression levels of ESR1, mTOR, EIF4E, and SMAD4. △△P < 0.01, compared with the control group; ††P < 0.01, compared with the data before treatment; **P < 0.01, compared with the olanzapine group after treatment.

Stigmasterol has a positive regulatory effect on γ-aminobutyric acid, which can effectively combat anxiety and convulsions, and is a potent steroid drug for treating neurological diseases [26]. Stigmasterol also exerts neuroprotective effects against ischemia/reperfusion injury by reducing oxidative stress [27]. Kaempferol, an antioxidant flavonoid found in fruits and vegetables, has multifaceted neuroprotective effects in disorders of the central nervous system [28], and it is also an active anxiolytic component in cabbage, which possesses anxiolytic effects [29]. Isorhamnetin is a 3′-O-methylated metabolite of quercetin with potent anti-inflammatory, antioxidative, and
neuroprotective properties. Studies have concluded that isorhamnetin may possess cognitive and memory-enhancing properties by enhancing antioxidant defense systems, cholinergic signaling, and synaptic plasticity [30]. Baicalin is a naturally occurring flavonoid whose anxiolytic effects have been demonstrated in animal studies, alone and in combination with other anxiolytics [31]. Studies have demonstrated that Bupleuri Radix has active components that are beneficial in treating neuropsychiatric disorders.

By analyzing the schizophrenia microarray, a total of 520 differential genes were yielded, including 43 schizophrenia-related Bupleuri Radix target genes. Further online analysis was performed using Metascape. GO analysis showed that the above 43 differential genes were mainly related to inflammatory response, cellular component movement, hormone response, tumor necrosis factor regulation, interleukin-1 regulation, intracellular signal transduction regulation, fatty acid transport, metal ion homeostasis, and other biological activities; KEGG analysis showed that the above 43 differential genes were mainly associated with the phagosome, PI3K-Akt, Rap1, MAPK, HIF-1, mTOR, platelet activation, and EGFR tyrosine kinase inhibitor resistance. Signal pathways such as sex and Cytokine-cytokine receptor interaction were significantly correlated. The PPI analysis showed that the above 43 differential genes could be clustered into a functional cluster consisting of 4 genes, namely ESR1, mTOR, EIF4E, and SMAD4. Regulation of ESR1 might be crucial in managing complex obsessive-compulsive disorder in patients with schizophrenia; therefore, drugs targeting ESR1 could be considered in the treatment strategy for schizophrenia, regardless of whether it is accompanied by obsessive-compulsive disorder [32]. mTOR has a critical role in managing neurotransmitter signaling and fostering cell growth and survival under normal physiological conditions. Thus, targeting mTOR with novel or existing medications may alleviate some symptoms of schizophrenia, thereby paving the way for the development of innovative treatment regimens [33]. The involvement of EIF4E in cell-specific translation can boost ketamine's anti-depressant effect [34]. Studies have concluded that the expression of SMAD4 protein in the dorsolateral prefrontal cortex and anterior cingulate cortex of patients with schizophrenia is reduced, potentially leading to abnormal signaling of the transforming growth factor superfamily and promoting schizophrenia [35].

In this study, using network pharmacology research methods, a network depicting the relationship between the main active components, targets, and pathways of Bupleuri Radix was constructed. Active components found to act on schizophrenia-related targets and pathways include quercetin, stigmasterol, kaempferol, and isorhamnetin. Quercetin can target ESR1, mTOR, EIF4E, and SMAD4 to play a role in treating schizophrenia. Stigmasterol, kaempferol, and isorhamnetin can target ESR1 to play a role in treating schizophrenia. The main active components of Bupleuri Radix are involved in multiple signaling pathways, such as the mTOR signaling pathway, PI3K-Akt signaling pathway, and HIF-1. The effect of Bupleuri Radix in the treatment of schizophrenia was subsequently further explored by giving different treatment methods. The results showed that after treatment, the PANSS scores and total scores before treatment were distinctly higher than those of the two groups of patients, and the PANSS scores and total scores in the olanzapine group showed significantly lower than those in the olanzapine group. Therefore, Bupleuri Radix could effectively treat schizophrenia patients.

In this study, peripheral blood was collected, and qPCR was used to monitor changes in the mRNA expression levels of ESR1, mTOR, EIF4E, and SMAD4. The expression of ESR1 mRNA increased in patients with schizophrenia, while the levels of the other three decreased. However, treatment with olanzapine, or a combination of olanzapine and Bupleuri Radix, decreased the expression levels of ESR1 and increased the expression of mTOR, EIF4E, and SMAD4, with the intervention of olanzapine combined with Bupleuri Radix showing a particularly notable effect. These qPCR detection results were consistent with the bioinformatics analysis results, supporting the hypothesis that the active components in Bupleuri Radix might play a significant role in treating schizophrenia by regulating the levels of ESR1, mTOR, EIF4E, and SMAD4.

A limitation of this study is that for assessing the impact of Bupleuri Radix while considering disease prognosis and treatment efficacy, patients receiving Bupleuri Radix were also administered olanzapine. This concomitant use of olanzapine potentially confounds the isolated effects of Bupleuri Radix. Therefore, it remains to be determined whether Bupleuri Radix acts alone or interacts with olanzapine to treat schizophrenia. Despite the findings of this study aligning with previous reports, additional experiments are necessary to reach definitive conclusions. Moreover, because it is impossible to get brain tissues from living schizophrenic patients, the mTOR, EIF4E, SMAD4, and ESR1 mRNA expression levels were only detected in the peripheral blood of schizophrenia patients and healthy subjects.

Conclusion

This study, which combined network pharmacology analysis and clinical validation, ascertained that Bupleuri Radix could exert a therapeutic effect on schizophrenia. The mechanism underlying this effect might involve the up-regulation of mTOR, EIF4E, and SMAD4 mRNA expression, and the down-regulation of ESR1 mRNA expression.

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