

1 Potential Pharmacodynamic Mechanism of the Main ingredients in
2 Licorice for Chronic Obstructive Pulmonary Disease

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17

18 **Abstract**

19

20 Purpose: This study aimed to investigate the effect of essential ingredients of
21 licorice on the chronic obstructive pulmonary disease (COPD). Method: The
22 ingredients information were obtained from *PubChem*
23 (<https://pubchem.ncbi.nlm.nih.gov/>), related genes about COPD was collected from
24 *geneCards* (<http://www.genecards.org/>). Network pharmacology was utilized in this
25 study. Result: The intersection data set contains 20 molecular targets between COPD
26 and licorice. Protein-protein interaction network showed that there are a total of 58
27 nodes and 137 edges involved. The link number of AKT1 in PPI network was 39,
28 which is the highest level of interaction. MAPK1 is an important target of Licorice on
29 COPD. Conclusion: MAPK signaling pathway could be the important key target of
30 main ingredients of licorice on COPD.

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32 **Keyword:** COPD, licorice, Gene Ontology, Kyoto Encyclopedia of Genes
33 and Genomes, network pharmacology

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37 **I. Introduction**

38 Chronic obstructive pulmonary disease (COPD) is one of the common preventable
39 and treatable disease, with the emblematic symptom of persistent airflow restriction,
40 which is associated with an increased chronic inflammatory response of the airways
41 and lungs to toxic particles or gases[1-4]. According to the World Health Organization,
42 approximately 3 million people in the world dead as a consequence of COPD each
43 year. One meta-analysis of global prevalence of COPD, which purposed to examine
44 the global prevalence of COPD in men and women, revealed that prevalence rate of
45 COPD was 9.23% (95% CI:8.16%-10.36%) among male group and 6.16% (95%
46 CI:5.41%-6.95%) among female group[5]. Moreover, there were some complications
47 of COPD, such as cardiovascular disease, anxiety and depression, lung cancer [6-8],
48 which leads to the lower living quality and much more burden of patient's life[9, 10].

49

50 Liquorice, widely distributed among Asia, Europe, America, etc., has been used
51 as medicinal material for long years[11-13]. Some researches have proved that
52 liquorice has the function of anti-inflammation, preventing coughing, antianaphylaxis
53 and so on[14, 15]. It was reported that the essential ingredients of liquorice are
54 glycyrrhizic acid, glycyrrhizin, quercetin, and formononetin[16, 17]. Generally,
55 liquorice, the whole plant is utilized to made as pills or decoction. Consequently, the
56 mechanism and molecular pathways for every component was not clear. Up to now,
57 much researches concentrated on the biological effect of liquorice as a whole, while
58 the molecular effect of its every component hasn't been figured out. Considering its
59 bargain price, widespread distribution and unique biological effect, this research
60 purposed to investigate the mechanism of essential component's signaling pathways
61 (formononetin, liquiritin, glycyrrhizic acid, glabridin, quercetin and isoliquiritigenin)
62 based on network pharmacology.

63

64 II. Method and Materials

65 The ingredients of liquorice involved in this research were formononetin,
66 liquiritin, glycyrrhizic acid, glabridin, quercetin and isoliquiritigenin, respectively.
67 Their two-dimension and three-dimension struction were from public database,
68 *PubChem* (<https://pubchem.ncbi.nlm.nih.gov/>). Pharmacokinetic information of these
69 compound was obtained from *Traditional Chinese Medicine Systems Pharmacology*
70 (*TCMSP*), including drug-likeness (DL), oral bioavailability (OB), intestinal epithelial
71 permeability (Caco-2) and number of H-bond donor/acceptor (Hdon/Hacc).

72

73 The target molecule of the Liquorice ingredients were attained from public
74 database *Swisstargetprediction* (<http://www.swisstargetprediction.ch/>). The potential
75 molecular target was selected when the probability was above zero. The related genes
76 about COPD was collected from *geneCards* (<http://www.genecards.org/>), and the
77 genes were used in this research when *relevance score* was more than 30.

78

79 Construction and analysis of component-action target network was based on
80 Cytoscape software (version 3.7.2). Six compound and common target between
81 COPD and Liquorice was imported to Cytoscape, after position adjustment network
82 was finished.

83

84 The String database (<https://string-db.org/>) is one database containing known and
85 predicted protein-protein interactions, in which a large number of protein-protein
86 interactions were collected, involving a total of 9643763 proteins and 138838440
87 interactions, including data detected experimentally and predicted by bio-information
88 methods. The common targets between disease and Liquorice were imported into the
89 String database to define human species and obtain the protein interaction relationship.

90 The results were saved in TSV format. The node1, node2 and combined score
91 information in the file was retained and imported into Cytoscape software to draw the
92 interaction network. The core of protein-protein interaction network was calculated in
93 R software. Then GO enrichment analysis and KEGG pathway annotation analysis
94 were finished in *Rstudio* with the p-value <0.05 as well.

95 III. Result

96 **Figure 1** and **Figure 2** showed the two and three dimension structure of
97 formononetin, liquiritin, glycyrrhizin, glabridin, quercetin and isoliquiritigenin,
98 respectively. Pharmacokinetic information was illustrated in **Table 1**. The number of
99 H-bond donor/acceptor for formononetin, liquiritin, glycyrrhizin, glabridin, quercetin
100 and isoliquiritigenin were 1/4, 8/16, 5/9, 2/4, 5/7 and 6/9, respectively. The oral
101 bioavailability of formononetin, liquiritin, glycyrrhizin, glabridin, quercetin and
102 isoliquiritigenin were 69.67, 9.06, 65.69, 53.25, 46.43 and 8.61, respectively.

103

104 There were 417 target molecule for COPD according to relevance score
105 mentioned above and 64 target molecule for Licorice (**Figure 3**). The intersection
106 data set contains 20 molecular targets between COPD and licorice. The information
107 of the six active ingredients and molecular targets of Licorice was introduced into
108 Cytoscape to construct the network, as shown in **Figure 4**. There are a total of 58
109 nodes and 137 edges involved. The black type indicates the six ingredients of Licorice,
110 blue ellipse represented the potential target. It can be seen from the **Figure 4** that the
111 same target could be corresponding to different active ingredients or the same active
112 ingredient, which fully reflects the multi-component and multi-target action
113 characteristics of Licorice.

114

115 **Figure 5** was the result after the process of String database. Color 'green' is the
116 gene neighborhood, 'black' represents the co-expression, 'blue' is gene co-occurrence,

117 'red' is the gene fusions. As shown in **Figure 6**, the link number of AKT1 was 39,
118 which is the highest level of interaction. **Figure 7** presented the top ten key targets.
119 GO enrichment analysis is a finite acyclic graph that counts the number or
120 composition of proteins or genes at a functional level. The generatio of endopeptidase
121 activity and phosphatase were greater than the others. Nuclear receptor activity and
122 transcription factor activity, direct legend regulated sequence specific DNA binding
123 were least (**Figure 8**, **Table 2**). The results of KEGG analysis are shown in **Figure 9**.
124 The counts of proteoglycans in cancer, endocrine resistance, MAPK signaling
125 pathway, EGFR tyrosine kinase inhibitor resistance, relaxin signaling pathway, Rap1
126 signaling pathway was 14,12, 12,10,10 and 10, respectively.

127 IV. Discussion

128 COPD is a chronic bronchitis and/or emphysema characterized by airflow
129 obstruction that could progress to pulmonary-heart disease and respiratory failure as a
130 common chronic disease[2, 4, 18]. It has been formed a broad consensus that COPD
131 is associated with abnormal inflammatory reactions with high morbidity and
132 mortality[19]. Licorice has been internationally utilized for medicinal herb, while its
133 mechanism of some main ingredients for COPD still kept unclear. The purpose of the
134 present study was to investigate the effect of main ingredients on COPD according to
135 network pharmacology.

136
137 In this study, it revealed that Akt1 is the common target between COPD and
138 licorice. AKT1, also named as protein kinase B, is one of 3 closely related
139 serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase,
140 and which regulates many processes including metabolism, proliferation, cell survival,
141 growth and angiogenesis[20-22]. It appeared as one key node in PI3K-AKT signaling,
142 protects against acute lung injury[23]. Qu's experiment indicated that glycyrrhizic
143 acid inhibited the production of inflammatory factors in LPS-induced ALI by

144 regulating the PI3K/AKT/mTOR pathway related autophagy[24]. Vito Lorusso and
145 Ilaria Marech summarized that isoliquiritigenin could suppress HIF-1 α level, VEGF
146 expression and secretion, cell migration and to reduce the expression and secretion of
147 MMP-9/-2 and these effects might be mediated through inhibition of p38, PI3K/Akt
148 and NF- κ B signaling pathways[25].

149

150 As shown in **Figure 8**, MAPK1 was one of critical targets, which is an important
151 transmitter of signals from the cell surface to the inside of the nucleus. The result of
152 KEGG enrichment analysis also illustrated that licorice relieves COPD symptoms via
153 MAPK signaling pathway. It has been reported that MAPK pathway is one of the
154 common intersection pathways of signal transduction pathways including stress,
155 inflammation, cell proliferation, differentiation, functional synchronization,
156 transformation, apoptosis and so on. Previous studies demonstrated that MAPK signal
157 pathway was involved in the inflammation reaction and oxidative stress[26, 27].
158 Some researchers also found that the ingredients of licorice has the function to
159 mediate the expression of MAPK signaling pathway[28, 29].

160

161 The limitation of the present study was that this study was performed abstractly,
162 in the future study, we will operate animal experiment to prove it with ethical
163 approval.

164

165 V. Conclusion

166 MAPK signaling pathway could be the important key target of main ingredients
167 of licorice on COPD.

168 VI. Availability of data and materials

169 The ingredients of liquorice involved were attained from *PubChem*
170 (<https://pubchem.ncbi.nlm.nih.gov/>). Pharmacokinetic information of these compound
171 was obtained from *Traditional Chinese Medicine Systems Pharmacology (TCMSP)*.

172 The target molecule of the Liquorice ingredients were attained from public
173 database *Swisstargetprediction* (<http://www.swisstargetprediction.ch/>). The related
174 genes about COPD was collected from *geneCards* (<http://www.genecards.org/>).

175 VII. Consent to Publish

176 All author consent to publish this article in this article.

177 Conflict of Interest

178 The authors declare that they have no conflict of interest.

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- 266

267

Table 1 Pharmacokinetic information about six compounds

268

	Hdon	Hacc	OB (%)	Caco-2	DL
Formononetin	1	4	69.67	0.78	0.21
Liquiritin	8	16	9.06	-2.23	0.11
Glycyrrhizin	5	9	65.69	-1.06	0.74
Glabridin	2	4	53.25	0.97	0.47
Quercetin	5	7	46.43	0.05	0.28
Isoliquiritigenin	6	9	8.61	-1.36	0.6

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Table 2 GO enrichment analysis result

ID	Description	pvalue	Count
GO:0019902	phosphatase binding	2.12E-09	9
GO:0004713	protein tyrosine kinase activity	3.60E-09	8
GO:0019903	protein phosphatase binding	5.09E-09	8
GO:0004252	serine-type endopeptidase activity	1.46E-08	8
GO:0004714	transmembrane receptor protein tyrosine kinase activity	2.03E-08	6
GO:0008236	serine-type peptidase activity	3.98E-08	8
GO:0017171	serine hydrolase activity	4.72E-08	8
GO:0019199	transmembrane receptor protein kinase activity	8.87E-08	6
GO:0004175	endopeptidase activity	2.61E-07	10
GO:0004222	metalloendopeptidase activity	4.33E-07	6

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Figure 1 The two and three demension structure compounds (A: formononetin; B: liquiritin; C: glycyrrhizin)

Figure 2 The two and three demension structure of conpounds

Figure 3 The target molecule of COPD and liquorice

Figure 4 The interaction network COPD, liquorice and molecular targets

Figure 5 The structure of protain-protain interaction network

Figure 6 PPI network link number

Figure 7 The top ten targets in the PPI network

Figure 8 The result of GO enrichment analysis

Figure 9 The result of KEGG enrichment analysis

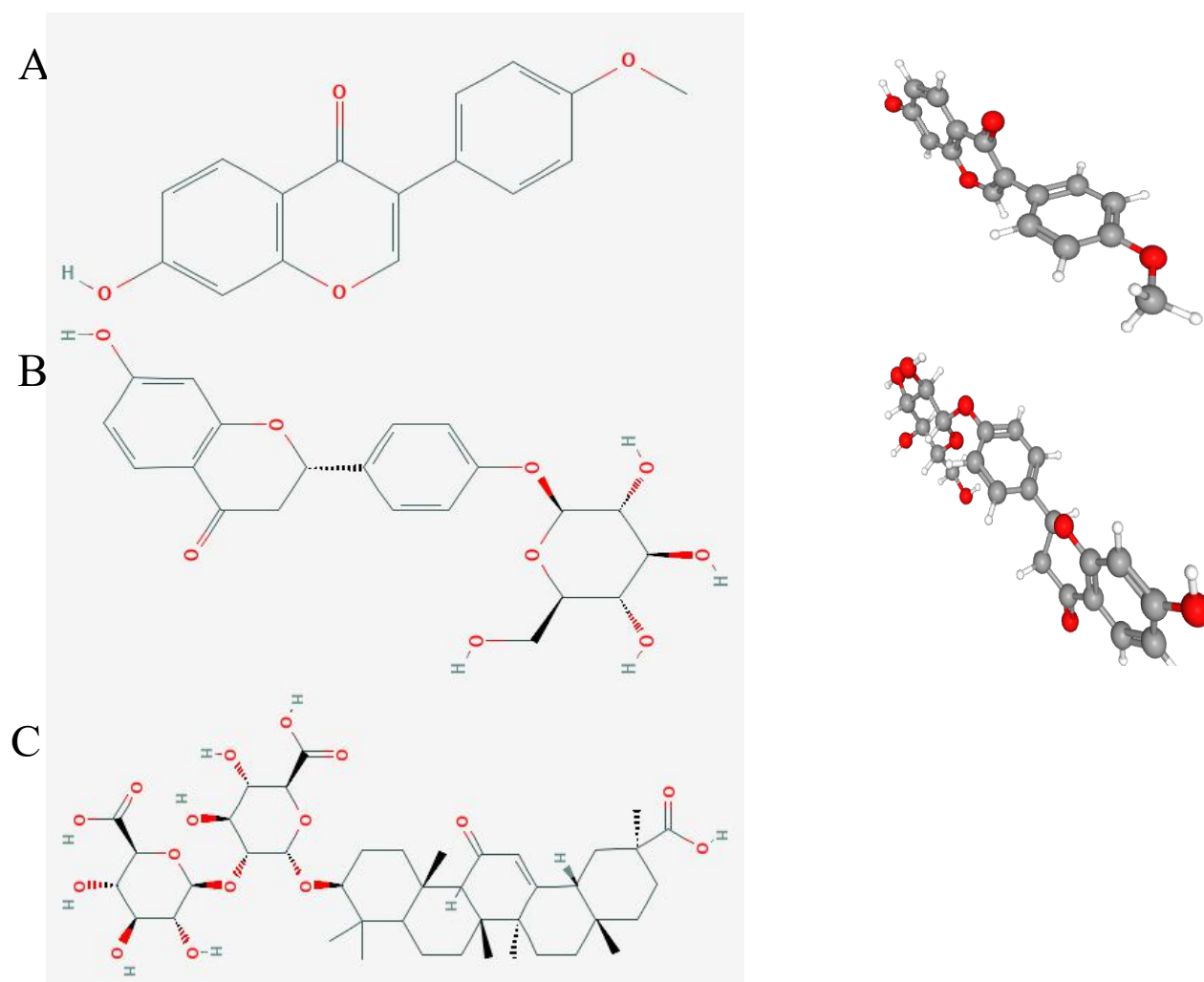


Figure 1 The two and three demension structure compounds (A: formononetin; B: liquiritin; C: glycyrrhizin)

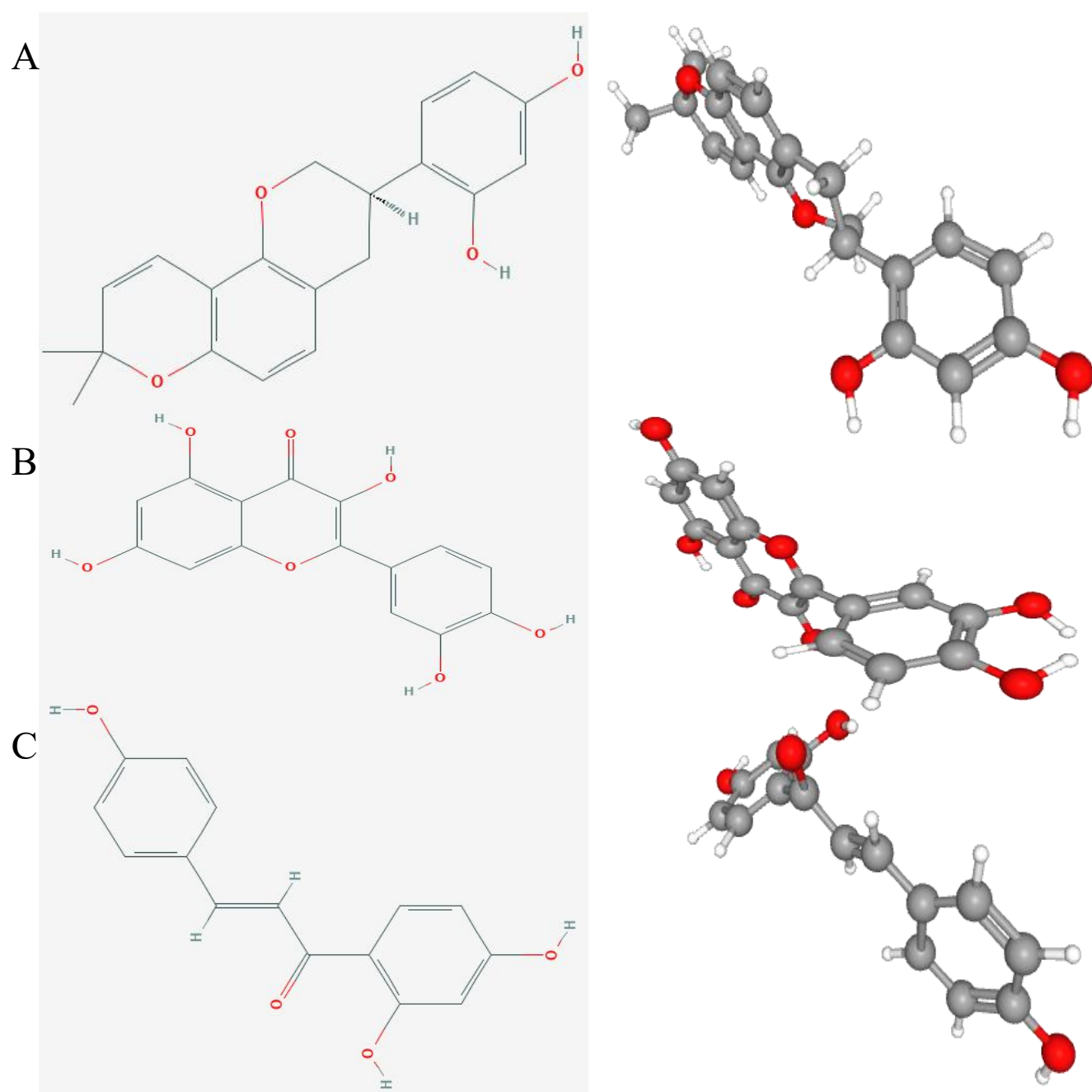


Figure 2 The two and three demension structure of compounds (A: glabridin; B: quercetin; C: isoliquiritigenin)

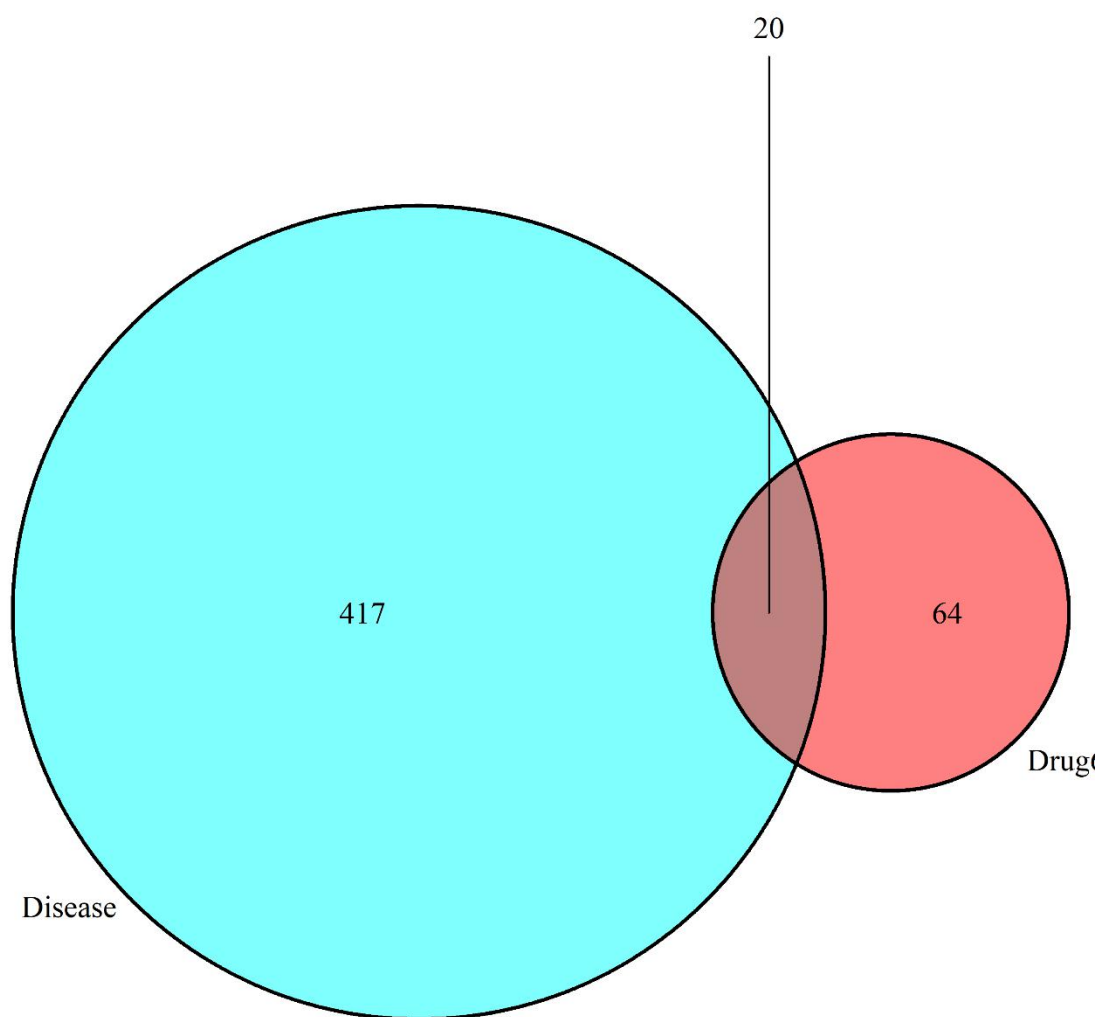


Figure 3 The target molecule of COPD and liquorice

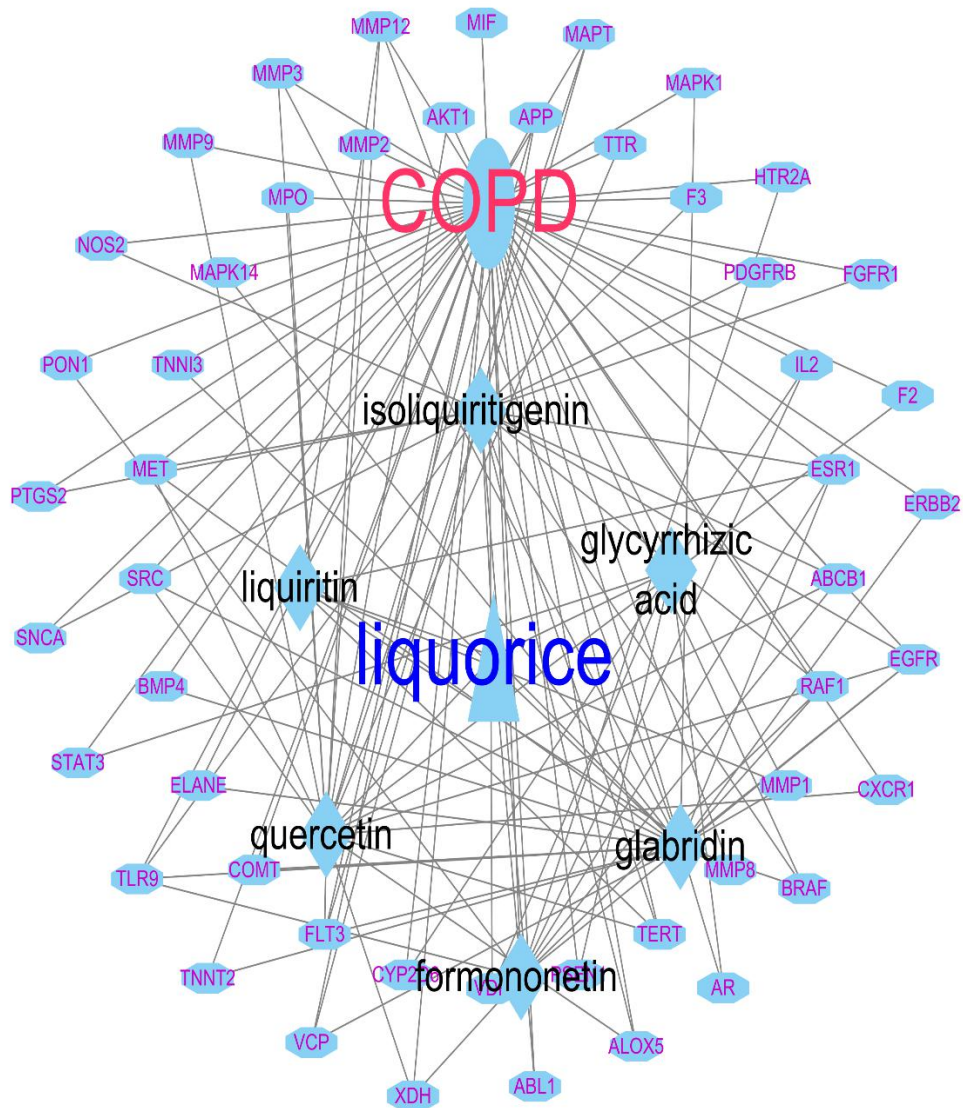


Figure 4 The interaction network COPD, liquorice and molecular targets

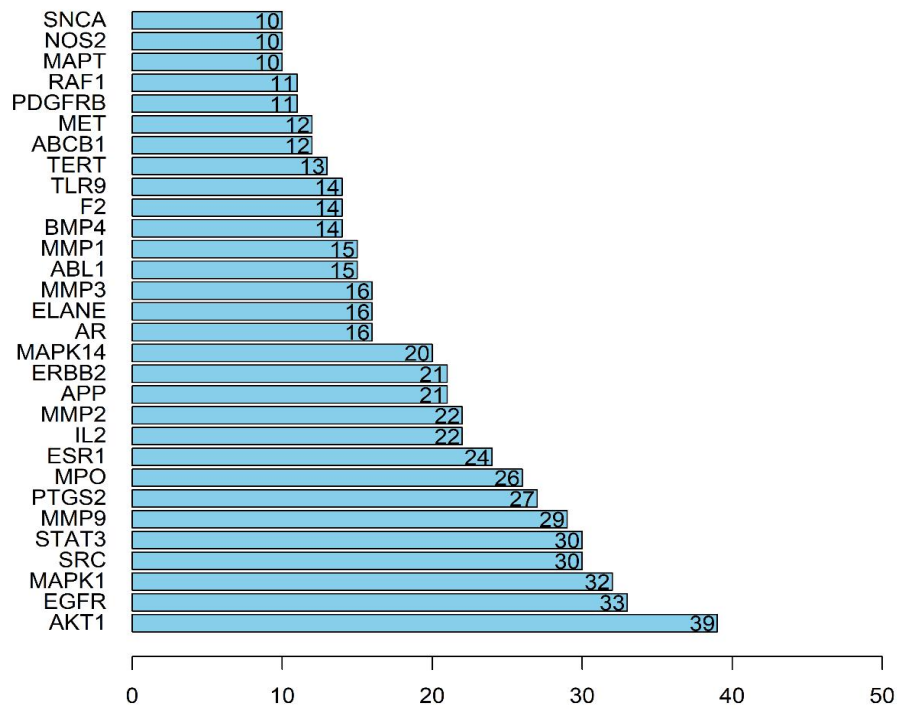


Figure 6 PPI network link number

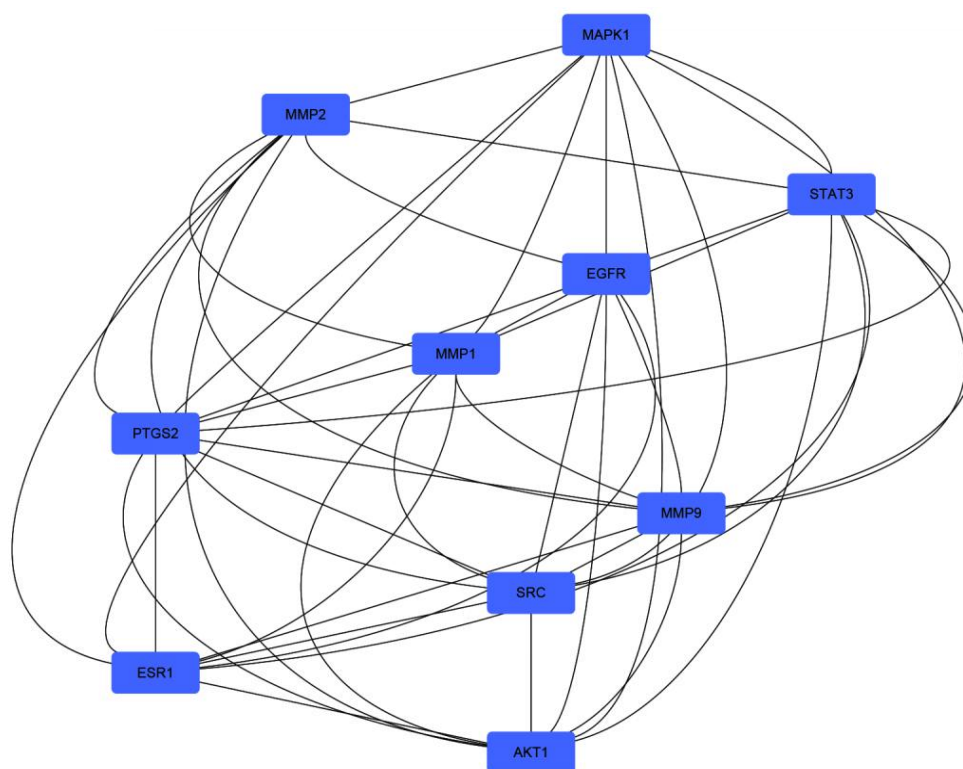
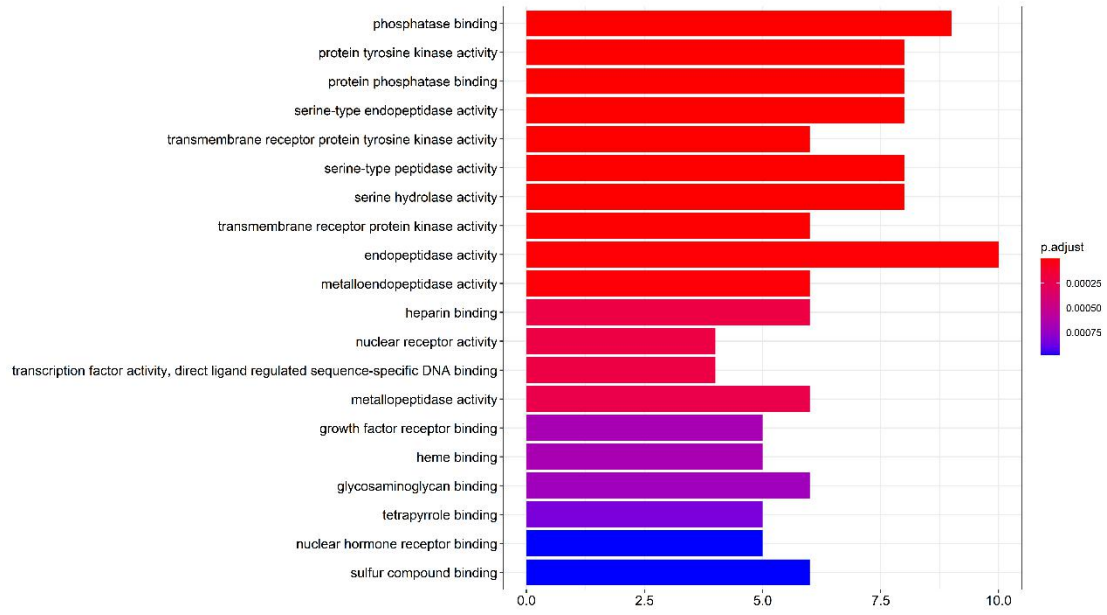
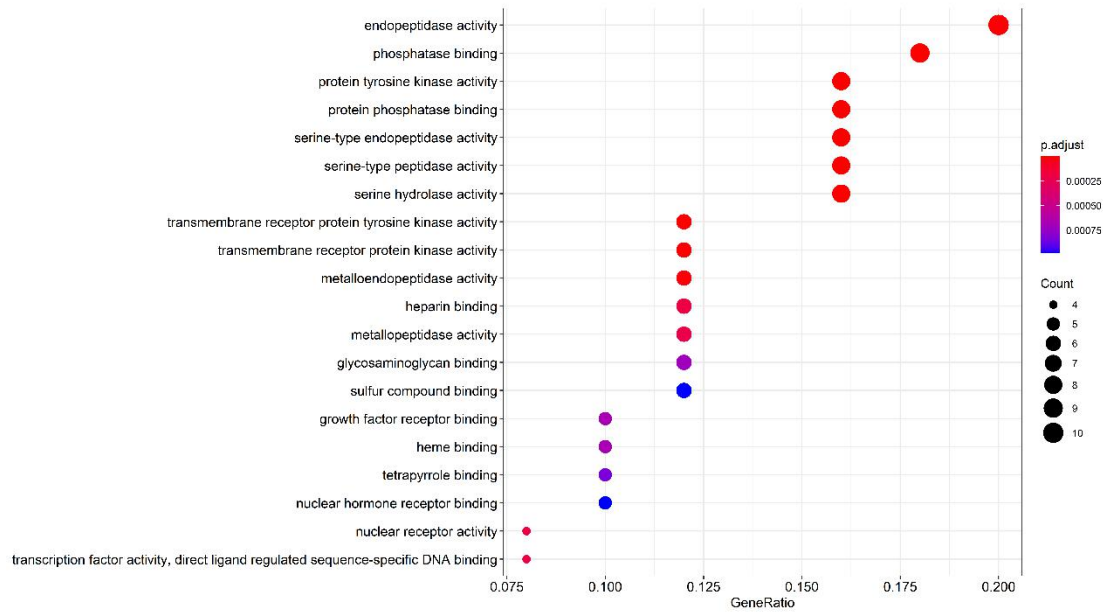


Figure 7 The top ten targets in the PPI network



A



B

Figure 8 The result of GO enrichment analysis

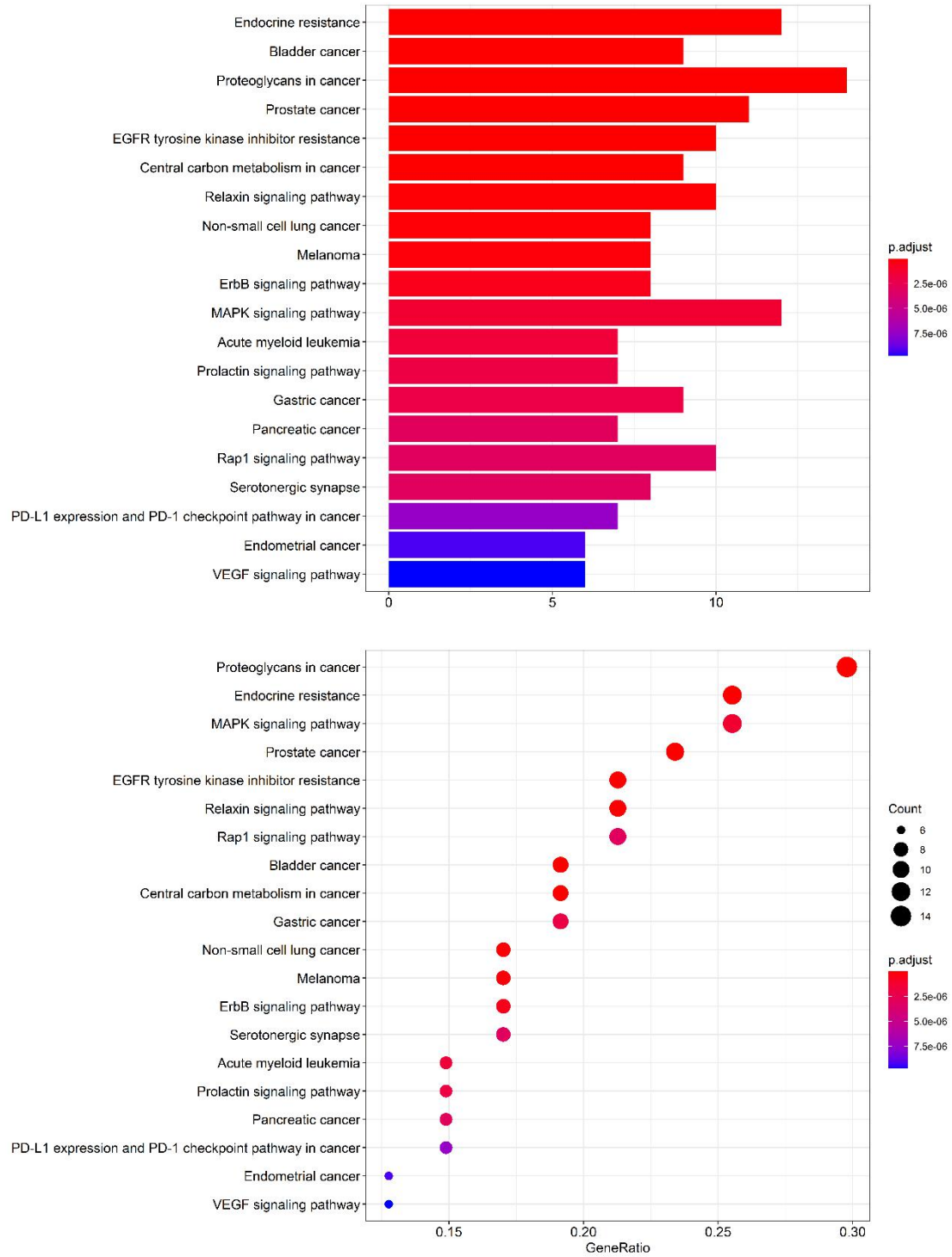


Figure 9 The result of KEGG enrichment analysis