doi.org/10.36721/PJPS.2021.34.2.REG.537-544.1

# Antidepressant mechanism and active compounds of saffron from network pharmacology study

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**Abstract**: Saffron has been applied in depression treatment, but its antidepressant compounds and mechanisms are unclear. In this research, a network pharmacology-based method was proposed to screen the active compounds and the potential mechanisms of saffron for depression treatment. Firstly, the chemical compounds of saffron were collected from literature and filtered by drug-like prediction. Secondly, common targets, by comparing the targets of saffron predicted by Pharm Mapper server with targets associated with depression collected from Genecards, were regarded as the antidepressant targets of saffron. Thirdly, common targets were mapped to KEGG pathways, considered as the pathways related with the antidepressant effects of saffron. Finally, the network of compounds-targets-pathways was constructed and analyzed by cytoscape 3.4.0. Ten compounds including crocetin, picrocrocin, (1R, 5S, 6R)-5-(hydroxymethyl)- 4, 4, 6-trimethyl-7-Oxabicyclo[4.1.0]heptan-2-one and its glycoside were screened as the main antidepressant compounds, some of which were reported for the first time. They might have effective treatment for depression by acting on targets, such as MAP2K1, MAPK1, HRAS, PIK3R1, ALB and AKT1 and pathways related with immune system, signal transduction and so on. This study provided a new insight into the antidepressant mechanism and active compounds of saffron, which also had a guiding effect on later experiments.

**Keywords**: Depression, saffron, network pharmacology.

## INTRODUCTION

Depression, a common mental disorder with a lifetime prevalence of 15-20% (Richards, 2001), has over 300 million patients in the world. It is also a leading cause of disability worldwide and a major contributor to the global burden of disease. However, antidepressant drugs, such as citalopram, duloxetine, fluoxetine, imipramine, sertraline, are limited to use because of their side-effects including suicide risk (Cipriani *et al*., 2016). Complementary and alternative medicines (CAM) are also widely used in the prevention and treatment of depression. According to a 2016 survey study, CAM was used in nearly 40% of adults with moderate mental distress in past one year (Rhee *et al*., 2017).

Saffron, the stigma of *Crocus sativus* L., is a commonly used spice of CAM for depression treatment in China (Yeun *et al*., 2014) and Iran (Mollazadeh *et al*., 2015), which was supported by increasing evidence from clinical researches (Hausenblas *et al*., 2013; Lopresti AL and Drummond PD, 2014). The results of these clinical researches demonstrated that the antidepressant effect of saffron was significantly higher than placebo (Mazidi *et al*., 2016) and was not significantly different with fluoxetine (Noorbala *et al*., 2005; Shahmansouri *et al*., 2014; Kashani *et al*., 2017) or imipramine (Amir-Hossein *et al*., 2004). Compounds from saffron, such as crocin (Talaei *et al*., 2015; Amin *et al*., 2015), crocetin (Amin *et al*., 2015), safranal (Hosseinzadeh *et al*., 2015) and kaempfero (Hosseinzadeh *et al*., 2007), were also turned out to have antidepressant activity from clinical trials or animal studies. In our previous review article (Ping *et al*., 2014), we found that at least 53 compounds had been isolated from saffron. In addition to the above four compounds, which compounds also have antidepressant activity? It is not yet known. At present, there are few studies on the antidepressant mechanism of saffron. As far as we know, only two studies were carried out to demonstrate that saffron could be used for depression treatment by increasing the levels of brain-derived neurotrophic factor (BDNF), VGF (non-acronymic), cAMP response element binding protein (CREB) and phosphorylated CREB (P-CREB) in hippocampus (Hassani *et al*., 2014; Ghasemi *et al*., 2015). However, there has been no study to explore the reasons why saffron can increase the levels of BDNF, VGF, CREB and P-CREB in hippocampus. We think the exact antidepressant mechanisms of saffron remain to be investigated.

The conventional method of discovering the active ingredients from CAM and studying their mechanism of action is to extract and isolate different compounds in CAM, then to test the biological activity in cell or animal models, finally, to elucidate the mechanism of each compound according to the research experience or literatures. This method was time-consuming and costly in separation and screening research process because of the numerous chemical compounds in CAM. In addition, most of the active chemical compounds in CAM may only have weak or moderate activity on multiple cellular

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targets and its pharmacological effects may be mainly due to the synergistic effect of different chemical composition. It is likely to fail in screening the main active ingredients in CAM by isolating the compounds with synergistic effects.

With the rapid development of bioinformatics, the concept of network pharmacology was put forward in 2007 (Hopkins, 2007) and was applied in drug discovery in 2008 (Hopkins, 2008) by Hopkins. From then on, it attracted great interest in CAM research because it can reveal the intricate relationships between muti-compounds, muti-targets and diseases (Qi *et al*., 2016; Fang *et al*., 2017; Zeng and Yang, 2017). It has been used in discovering active compounds in CAM and clarifying its mechanism. As far as we know, there was no report about saffron research on network pharmacology. To obtain a better understanding of which are the main active compounds in saffron for depression treatment and how they affect biological processes, network pharmacology technologies were proposed in our work.

## MATERIALS AND METHODS

### *Database construction of chemical compounds in saffron*

Chemical compounds in saffron were collected from literatures. After removing the repeated compounds, 53 compounds were obtained. Deglycosylation by enteric bacteria would appear in oral administrated drugs, thus compounds with glycosyl were further deglycosylated based on the rule of glycosidase hydrolysis reaction and other 6 compounds without overlap were obtained. The above 59 compounds were drawn using Chem Bio Draw Ultra 14.0 and saved as sdf format for further analysis.

### *Chemical space and drug-likeness prediction*

In this article, the following 8 physicochemical properties were calculated using discovery studio 2.5: Octanol-water partition coefficient (AlogP), molecular weight (MW), number of H acceptors (ON), number of H donors (OHNH), number of rotatable bonds (n rotb), number of rings, number of aromatic rings (n atoms) and molecular fractional polar surface area (TPSA). Four of these properties, including OHNH, ON, MW and AlogP, were used to filter the drug-like compounds in saffron according to Lipinski's 'rule of five' (Lipinski, 2004).

### *Target prediction of saffron compounds*

The saffron compounds met the Lipinski's 'rule of five' were submitted to the PharmMapper server (http://59.78.96.61/pharmmapper) for target prediction (Liu *et al*., 2010; Wang *et al*., 2016). Before the tools running, the targets were chosen as 'Human Protein Targets Only', the maximum generated conformations and the number of reserved matched targets was set as 100 and 300, respectively. After the results given by the PharmMapper server, we chose the targets which the fit score was more than 3.0 as the main targets of the saffron compounds.

### *Collection of potential compounds in saffron and their targets for depression treatment*

The targets related with depression were collected from GeneCards (http://www.genecards.org/) (Rebhan *et al*., 1997). From this database, relevance score was set more than 100 and 164 targets were collected. Then the targets of depression collected from Gene Cards were compared with the targets of saffron compounds predicted by PharmMapper server, and the common targets were regarded as targets of saffron compounds for anti-depression.

### *Pathway enrichment analysis*

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for predicted antidepressant targets of saffron compounds was carried out by Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang, 2009). Only KEGG pathways with P-values<0.01 were regarded as the main pathways interacted with antidepressant compounds of saffron.

### *Network construction and analysis*

The potential compounds-targets-pathways network was constructed by Cytoscape 3.4.0 (Shannon *et al*., 2003; Smoot *et al*., 2010). In this network, the active compounds, targets and pathways were represented as nodes, the active-targets and targets-pathways were connected by edges. If a target was mapped to a pathway, compounds linked to this target were also connected with this pathway by edges. The degree of each node was calculated to measure its importance in the network.

## RESULTS

### *Drug-likeness filtering*

According to the Lipinski's 'rule of five', 41 compounds out of 59 compounds in saffron were identified as absorbable components. The average of number of H donors (OHNH), number of H acceptors (ON), molecular weight (MW) and octanol-water partition coefficient (AlogP) of 41 absorbable components were 2.02, 3.93, 226.17 and 1.05, respectively.

### *The antidepressant targets of saffron compounds*

From the GeneCards database (http://www.genecards.org/), 164 targets of depression were collected. The 5-Hydroxytryptamine Receptor 2A (HTR2A), Dopamine Receptor D3 (DRD3), which were the main targets of FDA-approved antidepressants, were all included. But there were only 14 common targets by comparison of the targets of above 41 absorbable compounds predicted by Pharm Mapper server (http://59.78.96.61/pharmmapper). These 14 common targets were shown in table 1 and identified as the potential antidepressant targets of saffron compounds.

**Table 1**: The potential antidepressant targets of saffron compounds

| Targets | Description | Relevance score |
|---|---|---|
| FGFR1 | Fibroblast Growth Factor Receptor 1 | 213.43 |
| NR3C1 | Nuclear Receptor Subfamily 3 Group C Member 1 | 193.6 |
| MAP2K1 | Dual specificity mitogen-activated protein kinase kinase 1 | 153.02 |
| AKT1 | AKT Serine/Threonine Kinase 1 | 147.69 |
| HRAS | HRas Proto-Oncogene, GTPase | 130.25 |
| PTPN11 | Protein Tyrosine Phosphatase, Non-Receptor Type 11 | 130.25 |
| ALB | Albumin | 120.58 |
| MAPK1 | Mitogen-Activated Protein Kinase 1 | 120.58 |
| DPP4 | Dipeptidyl Peptidase 4 | 112.87 |
| BMP2 | Bone Morphogenetic Protein 2 | 110.08 |
| PDE4D | Phosphodiesterase 4D | 110.08 |
| PIK3R1 | Phosphoinositide-3-Kinase Regulatory Subunit 1 | 110.08 |
| ESR1 | Estrogen Receptor 1 | 106.72 |
| MAOB | Monoamine Oxidase B | 100.13 |

**Table 2**: Enriched KEGG pathways of saffron selected targets

| Pathway class | Term | Count | P Value |
|---|---|---|---|
| Cancers | map05205 | Proteoglycans in cancer | 1.02E-08 |
| | map05230 | Central carbon metabolism in cancer | 4.09E-08 |
| | map05211 | Renal cell carcinoma | 5.21E-08 |
| | map05218 | Melanoma | 8.16E-08 |
| | map05220 | Chronic myeloid leukemia | 8.77E-08 |
| | map05215 | Prostate cancer | 2.29E-07 |
| | map05213 | Endometrial cancer | 1.57E-06 |
| | map05221 | Acute myeloid leukemia | 1.97E-06 |
| | map05223 | Non-small cell lung cancer | 2.11E-06 |
| | map05214 | Glioma | 3.59E-06 |
| | map05231 | Choline metabolism in cancer | 1.93E-05 |
| | map05200 | Pathways in cancer | 2.46E-05 |
| | map05210 | Colorectal cancer | 1.44E-04 |
| | map05212 | Pancreatic cancer | 1.66E-04 |
| | map05216 | Thyroid cancer | 0.001215003 |
| | map05219 | Bladder cancer | 0.002265706 |
| Cell motility | map04810 | Regulation of actin cytoskeleton | 3.64E-04 |
| Cellular Community - eukaryotes | map04550 | Signaling pathways regulating pluripotency of stem cells | 5.10E-08 |
| | map04510 | Focal adhesion | 3.44E-04 |
| Development | map04380 | Osteoclast differentiation | 0.001247367 |
| Endocrine system | map04917 | Prolactin signaling pathway | 8.77E-08 |
| | map04915 | Estrogen signaling pathway | 3.75E-07 |
| | map04919 | Thyroid hormone signaling pathway | 8.49E-07 |
| | map04910 | Insulin signaling pathway | 6.76E-05 |
| | map04914 | Progesterone-mediated oocyte maturation | 3.99E-04 |
| | map04921 | Oxytocin signaling pathway | 0.002214394 |
| | map04912 | GnRH signaling pathway | 0.009022359 |
| Immune system | map04664 | Fc epsilon RI signaling pathway | 3.38E-06 |
| | map04662 | B cell receptor signaling pathway | 4.31E-06 |
| | map04650 | Natural killer cell mediated cytotoxicity | 1.86E-05 |
| | map04660 | T cell receptor signaling pathway | 2.26E-05 |
| | map04062 | Chemokine signaling pathway | 1.58E-04 |
| | map04666 | Fc gamma R-mediated phagocytosis | 3.72E-04 |
| | map04620 | Toll-like receptor signaling pathway | 5.51E-04 |

*Continue…*

| | | | |
|---|---|---|---|
| Infectious diseases | map05161 | Hepatitis B | 8.01E-05 |
| | map05160 | Hepatitis C | 0.001276024 |
| | map05164 | Influenza A | 0.002255549 |
| Nervous system | map04722 | Neurotrophin signaling pathway | 1.10E-06 |
| | map04725 | Cholinergic synapse | 3.15E-05 |
| | map04726 | Serotonergic synapse | 8.43E-04 |
| | map04730 | Long-term depression | 0.004800092 |
| | map04720 | Long-term potentiation | 0.005613127 |
| Signal transduction | map04014 | Ras signaling pathway | 8.10E-07 |
| | map04370 | VEGF signaling pathway | 2.43E-06 |
| | map04012 | ErbB signaling pathway | 1.16E-05 |
| | map04015 | Rap1 signaling pathway | 1.68E-05 |
| | map04071 | Sphingolipid signaling pathway | 4.28E-05 |
| | map04068 | FoxO signaling pathway | 5.84E-05 |
| | map04151 | PI3K-Akt signaling pathway | 1.75E-04 |
| | map04024 | cAMP signaling pathway | 2.63E-04 |
| | map04066 | HIF-1 signaling pathway | 6.59E-04 |
| | map04668 | TNF signaling pathway | 6.78E-04 |
| | map04010 | MAPK signaling pathway | 7.07E-04 |
| | map04022 | cGMP-PKG signaling pathway | 0.002647682 |
| | map04150 | mTOR signaling pathway | 0.004644653 |
| Substance dependence | map05034 | Alcoholism | 0.002381886 |

**Table 3**: The degree of each node in the C-T-P network

| Node | Degree | Node | Degree | Node | Degree | Node | Degree |
|---|---|---|---|---|---|---|---|
| MAP2K1 | 72 | S30 | 60 | map04917 | 26 | map05221 | 25 |
| MAPK1 | 65 | S34 | 60 | map04919 | 26 | map05223 | 25 |
| HRAS | 62 | S44 | 60 | map05211 | 26 | map05231 | 25 |
| PIK3R1 | 51 | S43 | 58 | map05215 | 26 | map04726 | 24 |
| ALB | 49 | S42 | 57 | map05218 | 26 | map04921 | 24 |
| AKT1 | 48 | S38 | 55 | map05220 | 26 | map05034 | 24 |
| DPP4 | 37 | S50 | 55 | map05230 | 26 | map04720 | 23 |
| PDE4D | 26 | S27 | 54 | map04010 | 25 | map04730 | 23 |
| NR3C1 | 22 | S46 | 47 | map04012 | 25 | map04912 | 23 |
| FGFR1 | 21 | S4 | 3 | map04024 | 25 | map05216 | 23 |
| ESR1 | 15 | S39 | 2 | map04062 | 25 | map05219 | 23 |
| PTPN11 | 9 | S51 | 2 | map04068 | 25 | map04022 | 22 |
| BMP2 | 4 | S1 | 1 | map04071 | 25 | map04066 | 22 |
| MAOB | 3 | S2 | 1 | map04370 | 25 | map04380 | 22 |
| S59 | 78 | S37 | 1 | map04510 | 25 | map04620 | 22 |
| S33 | 77 | S6 | 1 | map04650 | 25 | map04666 | 22 |
| S19 | 76 | S60 | 1 | map04660 | 25 | map04668 | 22 |
| S36 | 75 | map05205 | 28 | map04662 | 25 | map04914 | 22 |
| S13 | 73 | map04014 | 27 | map04664 | 25 | map05164 | 22 |
| S31 | 73 | map04550 | 27 | map04725 | 25 | map05210 | 22 |
| S55 | 71 | map05200 | 27 | map04810 | 25 | map05212 | 22 |
| S25 | 66 | map04015 | 26 | map04910 | 25 | map05160 | 19 |
| S52 | 65 | map04151 | 26 | map05161 | 25 | map04150 | 14 |
| S26 | 63 | map04722 | 26 | map05213 | 25 | | |
| S32 | 61 | map04915 | 26 | map05214 | 25 | | |

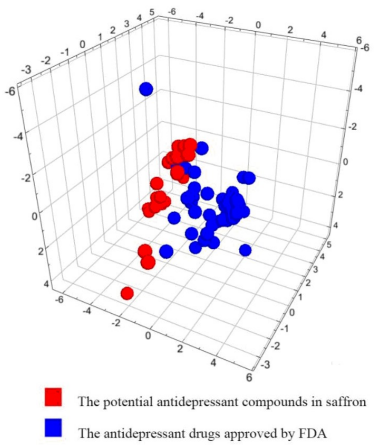


**Fig. 1**: The comparison in physicochemical properties of potential antidepressant compounds of saffron and FDA-approved antidepressants

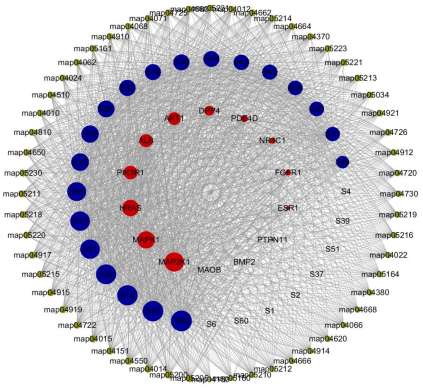

**Fig. 2**: The compounds-targets-pathways network of saffron for depression treatment

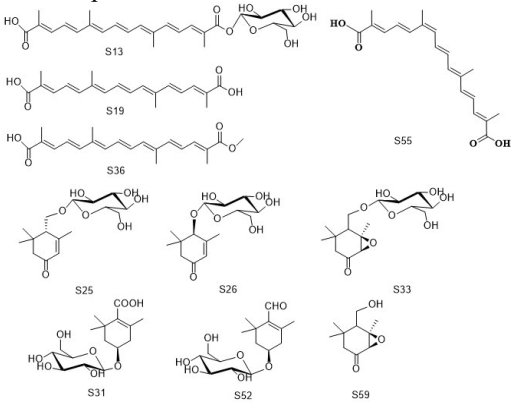


**Fig. 3**: Main active compounds of saffron for depression treatment

***The differences in physicochemical properties of potential antidepressant compounds of saffron and FDA-approved antidepressants***

Of the above 41 absorbable compounds, 28 compounds, which may interact with one or more of the targets in table 1, were considered to be the potential antidepressant compounds of saffron. In order to compare the physicochemical properties of these 28 compounds and FDA-approved antidepressants, 49 antidepressants were collected from drugbank database (https://www.drugbank.ca/). In these antidepressants, there were 47 drugs and 2 nutraceuticals (Oxitriptan and St. John's Wort). Because the St John's Wort was a plant extract with a variety of compounds and Olanzapine/fluoxetine was a mixture of two drugs, both of these two antidepressants were excluded from physicochemical properties analysis.

In addition to number of H donors (OHNH), number of H acceptors (ON), molecular weight (MW) and octanol-water partition coefficient (AlogP), other five physicochemical properties of compounds including number of rota table bonds (n rotb), number of rings, number of aromatic rings (n atoms) and molecular fractional polar surface area (TPSA) were also analyzed. Based on the data of above 8 physicochemical properties, the 28 potential antidepressant compounds of saffron and 47 FDA-approved antidepressants were compared by PCA method. As showed in fig. 1, the physicochemical properties of two groups were quite different.

***Pathway enrichment analysis of 14 targets***

In order to elucidate the biological pathways that antidepressant compounds of saffron might impact, the above 14 targets were mapped to KEGG pathways and showed in table 2. These pathways could be divided into 10 classes, including Cancers, Cell motility, Cellular community-eukaryotes, Development, Endocrine system, Immune system, Infectious diseases, Nervous system, Signal transduction and Substance dependence.

***Compounds-targets-pathways(C-T-P) network analysis***

Network Analyzer, a cytoscape plugin, was used to analyze the C-T-P network. As shown in fig. 2, the C-T-P network was consisted of 98 nodes (including 28 compounds, 14 targets and 56 pathways) and 1570 edges. The degree of each node in C-T-P network was listed in table 3.

The degrees of top 10 compounds, including S59, S33, S19, S36, S13, S31, S55, S25 and S52, were all larger than 65. These compounds shown in fig. 3 were regarded as the main antidepressant compounds of saffron. Dual specificity mitogen-activated protein kinase kinase 1 (MAP2K1), Mitogen-Activated Protein Kinase 1 (MAPK1), HRas Proto-Oncogene, GTPase (HRAS), Phosphoinositide-3-Kinase Regulatory Subunit 1 (PIK3R1), Albumin (ALB) and AKT Serine/Threonine Kinase 1 (AKT1) were the main targets of antidepressant compounds of saffron. The degree of above 6 targets was 72, 65, 62, 51, 49 and 48, respectively. The potential 14 antidepressant targets of saffron were mapped to 56 pathways. Except for map 05160 and map04150, the degrees of all other pathways were ranged from 22 to 28. The results might indicate that the active compounds of saffron may exert antidepressant activity by affecting multiple signalling pathways.

## DISCUSSION

In our study, the ten compounds in Figure 3 were considered to be the main antidepressant ingredients of saffron. Among them, S19 (degree=76), named *trans*-crocetin, was reported to have antidepressant activity (Amin *et al*., 2015). The structures of S13, S36 and S55 were similar to S19. Others in these ten compounds were found to have antidepressant effects for the first time, including picrocrocin (S52, degree=65), which was the most important bitter compound in saffron. But unlike the reported results, the degree of safranal (S34), which can reduce immobility time, increase swimming time and climbing time by using forced swimming test in mice and was considered to be another important antidepressant compound in saffron (Hosseinzadeh *et al*., 2015), was only 60 and ranked 11$^{th}$. Therefore, we think safranal might be an active compound in saffron for depression but it was less important in the antidepressant network of saffron compounds than the 10 compounds in fig. 3. Through the comparison of antidepressant components of saffron with FDA-approved antidepressants, the chemical structure of the two groups was quite different. Therefore, the antidepressant mechanism of saffron might be different from that of FDA-approved antidepressants, which is further confirmed by the results of C-T-P network analysis. From the results of C-T-P network analysis, we found that the main antidepressant targets for the active ingredient of saffron were MAP2K1, MAPK1, HRAS, PIK3R1, ALB and AKT1, rather than targets for FDA-approved antidepressant drugs such as HTR2A, DRD3 and so on. MAP2K1, also named MEK1 and PRKMK1, was an essential component of the MAP kinase signal transduction pathway. In 2014, Wu *et al* found that the MAP2K1 mRNA levels were significantly reduced in depression patients when compared with healthy controls (Hong *et al*., 2014). A recent study of Hu *et al* including 425 depression patients showed that three SNPs (rs1549854, rs1432441 and rs7182853) in the MAP2K1 gene had significantly different distributions in the depression patients than in the healthy volunteers. These results demonstrate that the MAP2K1 gene may be a risk factor for depression (Hu *et al*., 2017). Hu *et al* also found MAP2K1 was associated with the BDNF/MARK pathway in depressive disorder (Hu *et al*., 2017). And as we mentioned in this article, saffron and its main compound can significantly increase the levels of BDNF in hippocampus of rats in the forced swimming test (FST) (Hassani *et al*., 2014; Ghasemi *et al*., 2015), we believe MAP2K1 may be involved in mechanisms of increasing the levels of BDNF in hippocampus by saffron. In addition to MAP2K1, previous researches had demonstrated that the targets of MAPK1 (Calati *et al*., 2013; Garbett *et al*., 2015), HRAS (Garbett *et al*., 2015), PIK3R1 (Garbett *et al*., 2015), ALB (Maes *et al*., 1995) and AKT1 (Yang *et al*., 2012) were associated with depression.

The degree of pathways involved in saffron for depression was almost the same. In fact, some of these pathways had been shown to be closely related to the pathogenesis of depression. For example, recent data had elucidated that inflammation in brain could drive the development of depression and targeting the immune system was a potential way to depression treatment (Miller and Raison, 2016). Therefore, the pathways related with immune system, including Fc epsilon RI signaling pathway (map04664), B cell receptor signaling pathway (map04662), Natural killer cell mediated cytotoxicity (map04650), T cell receptor signalling pathway (map04660), Chemokine signalling pathway (map04062), Fc gamma R-mediated phagocytosis (map04666) and Toll-like receptor signaling pathway (map04620), might be the important pathways for the antidepressant effect of the active compounds from saffron. Some pathways related signal transduction might also be involved in depression treatment. Such as MAPK signalling pathway (map04010) (Malki *et al*., 2015), it might also be activated by ROS to exert a significant effect on inflammatory pathways of depression (Bakunina *et al*., 2015). Except for MAPK signalling pathway, other pathway related with signal transduction, including Ras signalling pathway (map04014) (Pace *et al*., 2007), ErbB signalling pathway (map04012) (Roy *et al*., 2007), VEGF signalling pathway (map04370) (Warner-Schmidtl and Duman, 2008), had also been shown to be associated with depression. In addition, 16 types of cancers associated pathways had been observed from table 2, which may be caused by the close relationship between depression and many types of cancer (Raison and Miller, 2003; Bortolato *et al*., 2017).

## CONCLUSION

In summary, there were ten main active compounds of saffron screened by network pharmacology study, including crocetin, picrocrocin, (1R, 5S, 6R)-5-(hydroxy-methyl)-4, 4, 6-trimethyl-7-Oxabicyclo [4.1.0]heptan-2-one and its glycoside. Meanwhile, the antidepressant mechanism of saffron might be different from that of FDA-approved antidepressants according to the C-T-P network analysis, resulting from the major targets for the active ingredient were MAP2K1, MAPK1, HRAS, PIK3R1, ALB and AKT1, other than targets for FDA-approved antidepressant drugs. Given the results of this study, more studies are needed in the future to further validate the results of this study.

## ACKNOWLEDGEMENTS

This work was supported by Natural Science Foundation of China (81703688), Zhejiang Province International Science and Technology Cooperation Belt and Road Special Project (2017C04009) and Key Special Projects of Intergovernmental International Science and Technology Innovation Cooperation (2017YFE0130100).

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