Chemical Constituents of *Tagetes patula* and Their Neuroprotecting Action

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**Abstract**

Two novel flavonoids (1, 2) and 3 known compounds (3-5) were isolated from the flowers and whole plant of *Tagetes patula* L., and their structures were elucidated by means of ultra-high performance liquid chromatography with electrospray ionization, coupled to quadrupole-time-of-flight/mass spectrometry, ¹H and ¹³C-nuclear magnetic resonance (NMR), as well as 2-dimensional-NMR (heteronuclear single quantum correlation and heteronuclear multiple bond correlation) and chemical methods. In addition, all the compounds were examined for their neuroprotective action on the injury of SH-SY5Y cells induced by glutamate, indicating that the protective effect of these compounds on glutamate-induced SH-SY5Y cell was marigold biflavone > patuletin > quercetin > kaempferol-3-O-β-D-glucoside > patuletin-3-O-α-L-arabinopyranoside. Thus, it could be concluded that flavonoids played a key role in the neuroprotective action of *T. patula*.

**Keywords**

*Tagetes patula* L., flavonoids, depression, isolation, neurological

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*Tagetes patula* L. (French marigold), family Asteraceae, is widely known for its phytochemical and medicinal properties. It is native to Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics. Currently, in China, *T. patula* is a common ornamental cultivated throughout the country. Traditionally, *T. patula* was used to treat various diseases, such as cough, colic, constipation, diarrhea, rheumatism, and eye problems. Today, the plant is specially used as an antimicrobial, antiseptic, hepatoprotective, blood purifying, and diuretic agent. Previous phytochemical investigations of *T. patula* have resulted in the isolation of carotenoids, flavonoids, and monoterpenoids. In our previous studies, we reported the obvious antidepressive effects of *T. patula*, and, in this article, we report 2 new and 3 known flavonoids isolated from *T. patula* by various chromatographic techniques. Furthermore, the injury of human neuroblastoma SH-SY5Y cells induced by glutamate was examined to determine the neuroprotective action of these flavonoids.

**Results and Discussion**

**Structural Elucidation of the Isolated Compounds**

Compound 1 was isolated as a yellow powder that was easily soluble in pyridine but quite sparingly in methanol. The molecular formula was determined as C₃₃H₂₄O₁₆ from a pseudo molecular ion peak at *m/z* 675.1040 [M − H]⁻ (calculated as 675.0992) (Supplemental Figure S1) in the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) of compound 1. In the ¹H-nuclear magnetic resonance (NMR) spectrum of compound 1, an ABX spin system was observed at δH 6.80 (1H, d, *J* = 8.5 Hz, H-5′), 7.81 (1H, d, *J* = 2.2 Hz, H-2′) and 7.51 (1H, dd, *J* = 2.1 and *J* = 8.5 Hz, H-6′), attributable to a trisubstituted B-ring of a flavonoid. The signal at δH 3.70 (3H, s, H-12) indicated the presence of a methoxy group. The ¹³C-NMR spectrum of compound 1 gave 17 carbon signals, 16 of which were assigned to a flavonoid similar to 6-methoxy quercetin (patuletin), except for the C-8 resonance that was shifted downfield to δ 104.6, suggesting that H-8 was substituted by an aliphatic moiety. The ¹³C-NMR spectrum of compound 1 gave 17 carbon signals, 16 of which were assigned to a flavonoid similar to 6-methoxy quercetin (patuletin), except for the C-8 resonance that was shifted downfield to δ 104.6, suggesting that H-8 was substituted by an aliphatic moiety. The ¹¹NMR spectrum of compound 1 showed 17 carbon signals, 16 of which were assigned to a flavonoid similar to 6-methoxy quercetin (patuletin), except for the C-8 resonance that was shifted downfield to δ 104.6, suggesting that H-8 was substituted by an aliphatic moiety. The ¹¹NMR spectrum of compound 1 showed 17 carbon signals, 16 of which were assigned to a flavonoid similar to 6-methoxy quercetin (patuletin), except for the C-8 resonance that was shifted downfield to δ 104.6, suggesting that H-8 was substituted by an aliphatic moiety.
methoxy-4H-chromen-4-one), which is a new compound and was determined to be C_{21}H_{20}O_{12} by HR-ESI-MS showing a pseudomolecular ion peak at m/z 465.1030 [M + H]^+ (calculated as 465.1028) (Supplemental Figure S6). The presence of an ABX system at δ_{H} 6.84 (1H, d, J = 8.5 Hz, H-5'), 7.50 (1H, d, J = 2.3 Hz, H-2'), and 7.65 (1H, dd, J = 2.2 and J = 8.5 Hz, H-6'), and a singlet signal at δ_{H} 11.5 (1H, s, H-8) in the 1H-NMR spectrum of compound 2 indicated that it is a derivative of 6-methoxyquercetin (patuletin). The 13C-NMR signals at δ_{C} 101.4 (C-1), 70.69 (C-2'), 71.63 (C-3'), 66.02 (C-4'), and 64.21 (C-5') assignable to α-L-arabinopyranosyl were observed in the 13C-NMR spectrum of compound 2\(^ {11}\); this was also confirmed by acid hydrolysis of compound 2 to yield l-arabinose, detected by high-performance liquid chromatography (HPLC) (Supplemental Figure S7-S8).

The assignment of 13C-NMR signals of compound 1 was performed by analysis of its heteronuclear single quantum correlation (HSQC) and HMBC spectra (Supplemental Figure S9-S10). The HMBC between the signals at δ_{H} 6.49 (1H, s, H-8) and δ_{C} 130.2 in compound 1 as compared with the known compound at δ_{C} 130.2 in compound 1 closely related to the known compound, 3,5,7,3',4',3'',5'',7''',3''',4'''-decahydroxyl-[8-CH_2-8']-biflavone, except that the C-6 resonance was shifted highfield to δ_{C} 130.2 in compound 1 as compared with the known compound at δ_{C} 97.8, revealing that H-8 was substituted by a methoxy group.\(^ {10}\)

Thus, the structure of compound 1 was elucidated as 8,8'-methylenbis-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-4H-chromen-4-one), which is a new compound and named as mangold biflavone.

Compound 2 was isolated as a yellow amorphous powder and reacted positively to hydrochloric acid-magnesium and Molish reagents. The molecular formula of compound 2 was determined to be C_{20}H_{19}O_{13} by HR-ESI-MS showing a pseudomolecular ion peak at m/z 465.1030 [M + H]^+ (calculated as 465.1028)
methoxy-3-(((2S,3R,4S,5S)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one. This is a new compound and named as patuletin-3-O-α-L-arabinopyranoside.

The spectral properties of the known compounds we isolated were compared with published values, and the compounds were identified as patuletin (3),13,14 quercetin (4),15 and kaempferol-3-O-β-D-glucoside (5)16 (Figure 1, Supplemental Figure S11-13).

**Table 2.** 
1H- Nuclear Magnetic Resonance (NMR) and 13C NMR Data for Compound 2.

| Position | δ_H (J in Hz) | δ_C | Position | δ_H (J in Hz) | δ_C |
|----------|---------------|-----|----------|---------------|-----|
| 1        | 6.49 (s, 1H)  | 93.8| 3        | 3.78-3.76 (m, 1H) | 70.7|
| 2        | 156.2         | 3'  | 4        | 7.65 (dd, J = 8.5, 2.2 Hz, 1H) | 122.0|
| 3        | 133.4         | 4'  | 5        | 6.84 (d, J = 2.3 Hz, 1H) | 115.7|
| 4        | 177.4         | 5'  | 6        | 115.5         | 145.0|
| 5        | 152.3         | 6'  | 7        | 7.50 (d, J = 2.3 Hz, 1H) | 115.4|
| 6        | 131.4         | 1'' | 8        | 3.52 (m, 1H) | 71.6|
| 7        | 151.5         | 2'' | 9        | 5.27 (d, J = 5.2 Hz, 1H) | 101.4|
| 8        | 104.0         | 5'' | 10       | 3.22 (dd, J = 11.5, 2.6 Hz, 1H), 3.61 (dd, J = 11.5, 5.4 Hz, 1H) | 64.2|

**Cytotoxic Activity Assay**

Compared with the negative control group, the compounds at concentrations of 50-100 µmol/L significantly decreased the cell viability, whereas concentrations between 1 µmol/L and 10 µmol/L only slightly affected the viability of cells. Concentrations lower than 10 µmol/L was selected for this study (Supplemental Table S1).
Depression has become the most prevalent mental health problem in developing countries, especially among adolescents. Injury of the human neuroblastoma SH-SY5Y cell line with a low degree of differentiation and metabotropic glutamate receptors induced by glutamate has been applied for the screening of antidepressive active natural products. Here, the isolated compounds were examined by this method.

The isolated compounds were tested for their protective action on neuronal cells. The cells treated with marigold biflavone at a concentration of 0.1 µmol/L produced maximal cell viability of 86.0% ± 0.6%, and at concentrations of 0.01-1 µmol/L significantly increased cell viability (Figure 3). Treatment with patuletin-3-O-α-L-arabinopyranoside at concentrations of 0.1-5 µmol/L significantly increased the cell viability in a dose-dependent manner, with the maximum effect at 5 µmol/L (77.6% ± 0.7% cell viability). Patuletin at concentrations of 0.1-5 µmol/L significantly increased the cell viability and at concentrations of 0.5 µmol/L, reached maximal cell viability of 82.0% ± 0.5%. We found that the viability of cells was maximal when treated with quercetin at concentrations of 0.5 µmol/L (81.2% ± 1.2% cell viability) and at concentrations of 0.1-10 µmol/L significantly increased cell viability. Kaempferol-3-O-β-D-glucoside at concentrations of 0.5-10 µmol/L also showed certain action, with the maximum cell viability found at 1 µmol/L (78.3% ± 0.5%). In this study, we found that the protective effect of flavonoids on glutamate-induced SH-SY5Y cells was in the order marigold biflavone > patuletin > quercetin > kaempferol-3-O-β-D-glucoside > patuletin-3-O-α-L-arabinopyranoside. These results suggested that the aglycone might decrease the protective effect on glutamate-induced SH-SY5Y cells. In addition, with compounds 1 and 3, the electronic effects of oxygen from the methoxy group might play an important role in the protective effect on glutamate-induced SH-SY5Y cells.

Materials and Methods

**Materials and Reagents**

*Tagetes patula* was provided by Dalian Wuzhou Holy Herb Scientific and Technological Co. Ltd., China) and identified by Professor Bing Wang of the Liaoning University of Traditional Chinese Medicine; the voucher specimens (No. 20160911) were deposited in the specimen herbarium, Liaoning University of Traditional Chinese Medicine. Optical rotations were measured with a Jasco P-2000 polarimeter; for HPLC, an Agilent Technology Co, Ltd model was used; semipreparative HPLC was performed using a HITACHI 7100 instrument (Hitachi Corporation, Tokyo, Japan); column chromatography was performed using silica gel (200-300 mesh, Marine Chemical Factory, Qingdao, China) and polyamide resin (200-300 mesh, Marine Chemical Factory, Qingdao, China; 80-100 mesh, Taizhou Luqiao Sijia Biochemical Plastic Factory, Taizhou, Zhejiang, China). Thin-layer chromatography (TLC) was carried out on silica gel GF 254 plates (Qingdao Marine Chemical Co., Qingdao, China).

*δ*-arabinose was obtained from Sigma (St. Louis., MO, USA), as was *α*-glutamate (No. SLBZ3006). The microplate reader was from Shenzhen Kitt Co., the Dulbecco’s modified Eagle medium (DMEM)/F12 medium from Gibco Co. (No. 8119172), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Solarbio Co., No.32LE0518) and SH-SY5Y (TCHu 97) from Shanghai Cell Bank, Chinese Academy of Sciences. All the other chemicals

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**Figure 2.** Heteronuclear multiple bond correlations of compounds 1 and 2.
The dried stems and leaves (15 kg) of *T. patula* were extracted with 60% ethanol (EtOH) at low temperature (45 °C; 3 × 120 L, 2 hours each). After filtration, the combined extracts were concentrated in a vacuum to furnish the crude extract. This was extracted with petroleum and ethyl acetate successively several times to give the ethyl acetate layer and water (H₂O) layer, which was subjected to column chromatography over AB-8 macroporous adsorptive resins using EtOH/H₂O (0:100, 60:40, and 95:5) as eluent. The 60% EtOH eluate and ethyl acetate layer (total 300 g) were combined and treated by column chromatography on silica gel, eluting with dichloromethane (CH₂Cl₂)/methanol (MeOH), then with increasing amounts of MeOH to afford 6 fractions (Fr.A1-A6). Fr. A4 was passed over a silica gel column, using CH₂Cl₂/MeOH as...
eluent, with increasing amount of MeOH to give 6 fractions (Fr.B1-B6). Then, B4 was purified on an ODS column equipped with HPLC eluting with MeOH/H2O. Compounds 5 (34 mg, MeOH/H2O, 45:55) and 1 (14.8 mg, MeOH/H2O, 40:60) were obtained.

The dried flowers (8 kg) of _T. patula_ were extracted 3 times with 95% EtOH for 6 hours each time. After filtration, the residues were extracted with 60% EtOH again and the combined extracts concentrated under reduced pressure. Then, the dry residue was extracted with butyl alcohol and methylene chloride to give butyl alcohol and methylene chloride layers. The butyl alcohol layer was subjected to silica gel column chromatography using CH2Cl2/CH3OH (100:0→50:1→30:1→20:1→10:1→5:1→0:1) as eluent to give 6 fractions (Fr.C1-C6). Fr.C2 was subjected to polyamide column chromatography, using MeOH/H2O as eluent, with increasing amounts of MeOH to give 5 fractions (Fr.C2.1-C2.5). Compound 3 (11 mg) was obtained from Fr.C2.4 (CH3OH/H2O, 75:25). Fr.C2.5, after column chromatography on Sephadex LH-20, using MeOH as eluent, gave 7 fractions (Fr.C2.5.1-C2.5.7). Fr.C2.5.4 when examined by TLC was shown to be a single compound (2; 16 mg). The methylene chloride layer, on silica gel column chromatography using CH2Cl2/CH3OH (100:0→50:1→30:1→20:1→10:1→5:1→0:1) as eluent, gave 7 fractions (Fr.D1-D7). Fr.D4 was fractionated on a silica gel column, using CH3COOC2H5/CH2Cl2/CH3OH/H2O (80:40:10:2) as eluent, to give 5 fractions (Fr.D4.1-D4.5). Fr.D4.4 after silica gel column chromatography, using CH2Cl2/CH3OH (40:1→30:1→20:1→10:1→5:1→0:1) as eluent, gave 5 fractions (Fr.D4.4.1-D4.4.5), including compound 4 (11 mg, CH2Cl2/CH3OH, 30:1).

### Identification of compound 1
Yellow powder; 1H-NMR (dimethyl sulfoxide [DMSO]-d6, 500 MHz) and 13C NMR (DMSO-d6, 125 MHz), see Table 1; HR-ESI-MS m/z 675.1040 [M - H]-, calculated as 675.0992.

### Identification of compound 2
Yellow amorphous powder; [α]D20 -67.88 (c:0.165, MeOH), 1H-NMR (DMSO-d6, 500 MHz) and 13C NMR (DMSO-d6, 125 MHz), see Table 2; HR-ESI-MS m/z 465.1030 [M + H]+, calculated as 465.1028; m/z 487.0850 [M + Na]+, calculated as 487.0847; m/z 333.0606 [M + H-C5H8O4]±, calculated as 333.0605.

### Acid Hydrolysis of Compound 2
Acid hydrolysis was performed using 0.1 mol/L sulfuric acid at 100 °C for 1 hour. The mixture was neutralized with silver carbonate and extracted with ethyl acetate. After the aqueous layer was condensed, both of them were examined by HPLC and compared with authentic samples. Analytical HPLC was performed on an Agilent ZORBAX NH2 column (5 m, 4.6 mm 150 mm) with isocratic elution using acetonitrile-H2O (80:20) for 40 minutes at a flow rate of 1 mL/min. The peaks were detected with an evaporative light scattering detector.

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**Assay Method for Cytotoxic Activity**

SH-SYS5Y cells were cultured in a 96-well plate at a density of 5 × 10^3 cells per well and incubated in a humid atmosphere of 5% carbon dioxide (CO2) and 95% air at 37 °C. Following 24 hours of incubation, the cells were completely detached from the well bottom. The cells were randomly divided into 6 groups (n = 3 per group): the cells of the blank group only had DMEM/F-12. The cells of the administration group were treated with various concentrations of flavonoids (marigold biflavone, patuletin, quercetin, kaempferol-3-O-β-D-glucoside, patuletin-3-O-α-L-arabinopyranoside) (1, 5, 10, 50, 100 μmol/mL). After 24 hours of incubation, 20 μL MTT was added to each well and incubated for 4 hours; the supernatants were aspirated, after which 100 μL of DMSO was added to each well. After oscillation for 10 minutes at 37 °C, absorbance was measured at 492 nm with a Microplate Reader. Cell viability was calculated as below:

\[
\text{cell viability(\%) = } \frac{A(A) - A(B)}{A(N) - A(B)}
\]

where A refers to the administration group, B is the blank group, and N is the negative control group.

**Study on the Protective Effect of Flavonoids on Glutamate-Induced SH-SY5Y Cells**

Glutamate, a major excitatory amino acid neurotransmitter in the central nervous system mediates several physiological processes. An increasing body of evidence indicates the important role of the glutamatergic system in the pathophysiology of depression. First, depressed patients exhibit elevated levels of glutamate both in plasma and the limbic brain areas, which are believed to be involved in mood disorders. Additionally, it has been shown that chronic treatment with antidepressants of different mechanisms reduces the glutamate release in rats. The isolated compounds from _T. patula_ were evaluated for their protective effect on glutamate-induced SH-SYS5 cells. The protective effect of glutamate-induced SH-SYS5Y cells was performed using the MTT assay method.

SH-SYSY cells were cultured in a 96-well plate at a density of 5 × 10^5 cells per well and incubated in a humid atmosphere of 5% CO2 and 95% air at 37 °C. Following 24 hours of incubation, the cells were completely detached to the well bottom. The cells were randomly divided into 9 groups (n = 3 per group): the cells of the model group were treated with L-glutamate (15 mmol/L in DMEM/F-12). The cells of the positive control group were given desipramine, while the blank group only had DMEM/F-12. The cells of the administration group were treated with various concentrations of flavonoids (marigold biflavone, patuletin, quercetin, kaempferol-3-O-β-D-glucoside, patuletin-3-O-α-L-arabinopyranoside) (0.01, 0.1, 0.5, 1, 5, and 10 μmol/L). After 24 hours of incubation, the cells were exposed to L-glutamate. Glutamate was dissolved in...
DMEM/F-12 before addition to the cells. Desipramine (5 μmol/L), as a positive drug, was given to the positive group. Following 24 hours of incubation, 20 μL of MTT stock solution (0.2 mg/mL in phosphate-buffered saline) was added to each well. After 4 hours of incubation, the supernatants were aspirated, and 100 μL of DMSO was added to each well. After oscillation for 10 minutes at 37 °C, the absorbance was measured at 492 nm by a microplate reader. Data were normalized as a percent of vehicle-treated cells (100%) in 3 independent experiments and are presented as the mean ± SEM. The cell viability was calculated using the formula:

\[
\text{cell viability(\%)} = \frac{A(A)-A(B)}{A(N)-A(B)}
\]

where A refers to the administration group, B is the blank group, and N is the negative control group.

Conclusions

In this work, 2 novel (1 and 2) and 3 known compounds were isolated from Tagetes patula for the first time, and these compounds presented a protective effect on glutamate-induced SH-SY5Y cells. This can also provide evidence for the antidepressant effect of flavonoids. The protective effect of flavonoids on glutamate-induced SH-SY5Y cells was in the order marigold biflavone > patuletin > quercetin > kaempferol-3-O-α-l-arabinopyranoside > patuletin-3-O-a-l-arabinopyranoside.

Declaration of Conflicting Interests

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Supplemental Material

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References

1. Vasudevan P, Kashyap S, Sharma S. Tagetes: a multipurpose plant. Bioresour Technol. 1997;62(1-2):29-35. doi:10.1016/S0960-8524(97)00101-6
2. Krzyzaniski LM, Antonelli-Ushirobira TM, Panizzon G, et al. Larvicidal activity against Aedes aegypti and chemical characterization of the inflorescences of Tagetes patula. Evid-Based Compl Alt. 2017;2017(5):1-8. doi:10.1155/2017/9602368
3. Yasukawa K, Kasahara Y. Effects of flavonoids from French (florets of Tagetes patula L.) on acute inflammation model. Int J Inflamm. 2013;2013:493-495. doi:10.1155/2013/309493
4. Quackenbush FW, Miller SL. Composition and analysis of the carotenoids in marigold petals. J Assoc Off Anal Chem. 1972;55(3):617-621. doi:10.1093/jaoac/55.3.617
5. Quackenbush FW. Use of heat to saponify xanthophyll esters and speed analysis for carotenoids in feed materials: collaborative study. J Assoc Off Anal Chem. 1973;56(3):748-753. doi:10.1093/jaoac/56.3.748
6. Bhardwaj DK, Bisht MS, Uain SC, Mehta CK, Sharma GC. Quercetagetin 5-methyl ether from the petals of Tagetes patula. Phytochemistry. 1980;19(4):713-714. doi:10.1016/0031-9422(80)87053-1
7. Ivancheva S, Zdravkova M. Flavonoids in Tagetes patula. Fitoterpia. 1993;64(6).
8. Garg SN, Charles R, Kumar S. A new acyclic monoterpenic glucoside from the capitula of Tagetes patula. Fitoterapia. 1999;70(5):472-474. doi:10.1016/S0367-326X(99)00044-1
9. Liu LL, Wang YM, Zhang X, et al. Study on the antidepressant effect and mechanism of maidenhair decoction on mice. J Tradit Chin Med. 2019;21(05):26-29.
10. Zhao C-C, Shao J-H, Li X, et al. Flavonoids from Galium virum L. J Asian Nat Prod Res. 2008;10(7-8):611-615. doi:10.1080/1028602080233217
11. Schoenborn R, Mues R. Flavone di-C-glycosides from Plagiochila jamesonii and Plagiochasma rupestre. Phytochemistry. 1993;34(4):1143-1145. doi:10.1016/S0031-9422(00)90732-5
12. Bylka W. A new acylated flavonol diglycoside from Atriplex littoralis. Acta Physiol Plant. 2004;26(4):393-398. doi:10.1007/s11738-004-0028-5
13. Becchi M, Carrier M. 6-Methoxy flavones of Santolina chamaecyparissus. Planta Med. 1980;38(3):267-268. doi:10.1055/s-2008-1074873
14. Abdel-Wahhab MA, Said A, Huefner A. NMR and radical scavenging activities of Patuletin from Urtica urens. Against Aflatoxin B1. Pharm Biol. 2005;43(6):515-525. doi:10.1080/1388020050020730
15. Ge I, Li J, Wan H, et al. Novel flavonoids from Lonicera japonica flower buds and validation of their anti-hepatoma and hepatoprotective activity in vitro studies. Ind Crop Prod. 2018;125:114-122. doi:10.1016/j.indcrop.2018.08.073
16. Ibrahim A, Khalfa SI, Khafagi I, et al. Microbial metabolism of biologically active secondary metabolites from Nerium oleander L. Chem Pharm Bull. 2008;56(9):1253-1258. doi:10.1248/cpb.56.1253
17. Nair VD, Niznik HB, Mishra RK. Interaction of NMDA and dopamine D2L receptors in human neuroblastoma SH-SY5Y cells. J Neurochem. 1996;66(6):2390-2393. doi:10.1046/j.1471-4159.1996.66062390.x
18. Kendell SF, Krystal JH, Sanacora G. Gaba and glutamate systems as therapeutic targets in depression and mood disorders. Expert Opin Ther Tar. 2005;9(1):153-168. doi:10.1517/14728222.9.1.153
19. Bonanno G, Giambelli R, Rafteri I, et al. Chronic antidepressants reduce depolarization-evoked glutamate release and protein
interactions favoring formation of SNARE complex in hippocampus. *J Neurosci*. 2005;25(13):3270-3279. doi:10.1523/JNEUROSCI.5033-04.2005

20. Golembiowska K, Dziubina A. Effect of acute and chronic administration of citalopram on glutamate and aspartate release in the rat prefrontal cortex. *Pol J Pharmacol*. 2000;52(6):441-448.

21. Min Z. Research progress of therapeutic effects of flavonoids in Chinese medicine on depression. *Journal of Huna University (Nat Sci)*. 2015;16(05):617-620. doi:10.11713/j.issn.1009-4822.2015.05.013

22. Gong JY, XQ W, Mao JW, Zhang Y. Advanced in studies on antidepressant effect of flavonoids. *Chinese Traditional & Herbal Drugs*. 2011;42(1):195-200.