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# Structure-Activity Relationship for Antidepressant Effect of Luteolin and Its Related Derivatives Isolated from *Taraxacum mongolicum*

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**Abstract** – The inhibitory effect and the structure-activity relationships of luteolin and its related derivatives isolated from *Taraxacum mongolicum* against MAO activities were investigated. The activity-guided isolation of extract from *Taraxacum mongolicum* led to the isolation of three flavonoids, luteolin, diosmetin, and luteolin-7-glucoside, a polyphenol, chlorogenic acid, a tyrosine and a uridine. The inhibitory activities of luteolin and its related derivatives against MAOs activities are dependent on their molecular structures. The presence of the phenolic hydroxy group at *para*-position is the active site for MAO-A inhibition as well as of MAO-B. The methoxy group has no potential on MAO-A inhibition. An additional phenolic hydroxy group at the *ortho*-position alleviates about 4-fold MAO-A inhibitory activity of phenolic hydroxy group at *para*-position. A carboxylic group seems to be critical for DBH inhibition and has no effects on MAO.
**Keywords** – *Taraxacum mongolicum*, Monoamine oxidase inhibitor, Luteolin, Luteolin derivatives, Structure-activity relationship

## Introduction

Monoamine oxidase (MAO) inhibitors were the first antidepressants introduced, but their use has dwindled because of their reported side effects, their food and drug interactions, and the introduction of other classes of agents. However, there has been renewed interest in MAO inhibitors (Molly *et al*., 2010). Recently, Jeffrey group reported the relationship between MAO-A levels and selective serotonin reuptake inhibitor (SSRI) treatment, recovery, and recurrence in major depressive disorder (MDD). They concluded that from the perspective of monoamine theory, SSRI raise serotonin levels vigorously whereas elevated MAO-A levels would be expected to metabolize serotonin, norepinephrine, and dopamine excessively. The mismatch between monoamine levels raised by treatment and monoamine levels lowered by disease processes might, at times, contribute to lack of response to SSRI treatment (Jeffrey *et al*., 2009). Flavonoids are naturally occurring polyphenolic compounds with a wide distribution in the plant kingdom. They play a significant role in plant metabolism and are considered relatively non-toxic bio-active substances. These plant flavonoids are reported to have therapeutic potentials because of their scavenging, antioxidant (Chen *et al*., 1990), anti-inflammatory (Ferrandiz and Alcaraz, 1991), anticancer (Menon *et al*., 1995), antimutagenic (Edenhardder *et al*., 1993), and antispasmodic properties (Duarte *et al*., 1993). Also, flavonoids have been reported to inhibit xanthine oxidase (Hayashi *et al*., 1988), protein kinase (Ferriola *et al*., 1989), and PDE (Kuppusamy and Das, 1992; Ko *et al*., 2004). However little is known about the structure-activity relationship between flavonoids and their inhibitory effects on MAOs.

The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae and widely distributed in the warmer temperate zones of the Northern Hemisphere. The perennial weed has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments such as dyspepsia, heartburn, spleen and liver complaints, hepatitis, and anorexia. Particular attention has been given to diuretic, choleretic, anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, antihyperglycemic, anti-coagulatory, and prebiotic effects (Katrin *et al*., 2006).

In the present study, we attempted to determine the structure-activity relationships of luteolin and its related derivatives isolated from *T. mongolicum* against MAOs activities, which may be helpful for understanding the

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active moieties of MAOs.

## Experimental

**Plant materials** – Fresh leaves and stems of *T. mongolicum* were collected from Eumsoung Korea, identified by Dr. J.H. Lee, botanist at the Korea National Institute of Horticultural & Herbal Science. The voucher specimen (NP20-039) has been deposited in the specimen room of Duksung Women's university, Seoul, Korea.

**Chemicals and instruments** – NMR experiments were performed on a Bruker/Advance-600 (600 MHz). The chemical shifts were reported in parts per million, and the coupling constants (*J* values) were in Hertz. Exact masses were measured by a Hewlett Packard 5890 series II (EI-MS) or Jeol JMSAX505WA Mass spectrometer. Column chromatography was carried out on silica gel 60 (0.063~0.200 μm; Merck 7734) and Lichroprep RP-18 (particle size 40~60 μm, Merck). TLC analyses were carried out on silicagel 60 F254 (Merck 7734) and RP-18 F254s (Merck 15685) plates. In bioassay experiments, UV absorbance was measured by a UVIKON XS UV/Vis spectrometer of SECOMAN. Serotonin creatinine sulfate, Iproniazid, deprenyl Tyramine-HCl, Dowex 50W 8 and Amberlite CG50 were purchased from Sigma Co., USA, and Benzylamine-HCl from Tokyo Kasei Co., Japan.

**Animals** – Male Sprague-Dawley rats weighing 180~200 g were obtained from the Orient Laboratoty Animal (Seoul, Korea) and were maintained on a 12 h light-dark cycle (light phase: 06:30~18:30) in a temperature-controlled environment (22 ± 1 ºC) with free access to food and water. Experiment began after 10 day period of acclimatization. All procedures were approved by the Kunkuk University Animal Care and Use Committee. They complied with the US Guidelines for the Care and Use of Laboratory Animals (NIH publication #85-23, revices in 1985).

**MAO-A assay in vitro** – Rat brain mitochondrial MAO was prepared by Zeller's method (Zeller, 1951). The activity of MAO-A was measured using serotonin as the substrate (Han *et al*., 2001). The reaction mixture containing 0.5 mL of enzyme solution in 10 mM phosphate buffered saline (pH 7.1) and 1 mL of test solution was preincubated at 37 ºC for 15 min, after which 0.5 mL of 1 mM solution of buffered serotonin creatinine sulfate (Sigma, USA) was added. Following incubation at 37 ºC for 90 min, the enzyme reaction was terminated by heating the reaction mixture for 3 min in boiling water bath. After being centrifuged, 1.6 mL of the supernatant was applied to an Amberlite CG50 column (0.8 × 3 cm). The column was washed with 40 mL of distilled water and eluted with 3 mL of 4 N acetic acid. The absorbance of the metabolite that produced after being reacted with MAO-A was measured at 277 nm. Purely isolated compounds were dissolved in DMSO and then suspended in water for testing their inhibitory activities on MAO-A. Final concentration of DMSO in enzymatic reaction mixture was below 5%. Iproniazid was used as positive control for nonselective inhibitor.

**MAO-B assay in vitro** – Rat liver mitochondrial MAO was prepared by Zeller's method (Zeller, 1951). The activity of MAO-B was measured according to Han *et al.,* (2001), using benzylamine as the substrate. The reaction mixture containing 0.5 mL of enzyme solution in 10 mM phosphate buffered saline (pH 7.1) and 1 mL of test solution was preincubated at 37 ºC for 15 min and then cooled in an ice bath. 0.5 mL of 4 mM benzylamine HCl (Tokyo Kasei Co., Japan) in buffer was added to it. This mixture was further incubated at 37 ºC for 90 min in a shaking water bath. The reaction terminated by addition of 0.2 mL of 60% perchloric acid. After extraction with 3 mL of cyclohexane, the organic layer was taken and the absorbance of benzaldehyde produced was measured at 242 nm. Purely isolated compounds were dissolved in DMSO and then suspended in water for testing their inhibitory activities on MAO-A. Final concentration of DMSO in enzymatic reaction mixture was below 5%. Deprenyl was used as positive control for selective MAO-B inhibitor.

**DBH assay in vitro** – The enzyme activity of DBH was determined according to Han *et al*. using tyramine as the substrate (Han *et al*., 1997). The following were sequentially added to 0.3 mL of enzyme solution in 0.25 M sucrose: 1 mL of test solution; 0.2 mL of 3 mg/mL catalase; 0.5 mL of 1 M acetate buffer (pH 5.0); and 0.5 mL of a reaction aid, prepared by dissolving fumaric acid, N-ethylmaleimide, iproniazide phosphate and ascorbic acid to concentrations of 0.06, 0.06, 0.006, and 0.06 M, respectively, in distilled water. The solution was allowed to stir at 37 ºC for 15 min, and then 0.5 mL of 0.12 M tyramine HCl solution was added and the resulting mixture was allowed to stir for 90 min. Next, 0.4 mL of 3 M solution of trichloroacetic acid was added to the reaction mixture to terminate the enzyme reaction. Immediately thereafter, the solution was centrifuged and 3 mL of the supernatant was poured onto a Dowex 50W × 8 column (0.8 i.d. × 3 cm, $H^+$ form, 200~400 mesh) and the column was washed with 30 mL of distilled water. Three milliliters of 4 N $NH_4OH$ solutions were then added to the column. The eluate was collected in test tube

and 0.2 mL of 4% sodium metaperiodate solution was added. The test tube was allowed to stand for 10 min and before 0.2 mL of 20% sodium metabisulfite solution was added. The absorbance of the resulting mixture was measured at 330 nm.

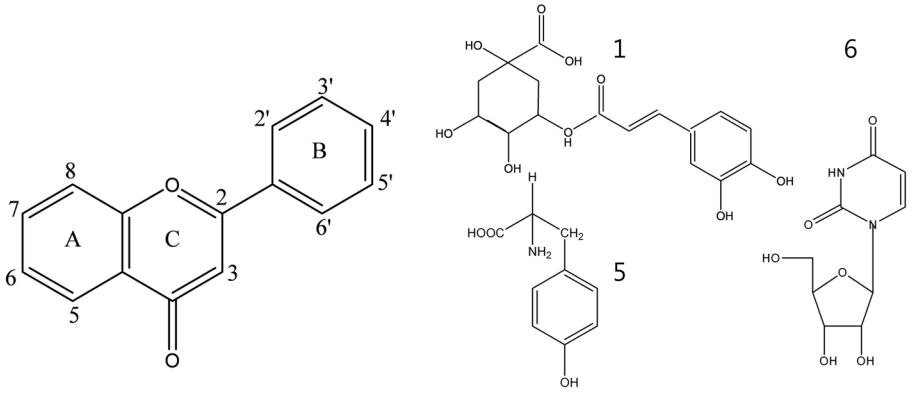
**Isolation of active compounds from *T. mongolicum*** – The dried aerial parts (10 kg) of *T. mongolicum* were extracted three times with a mixture of methanol and distilled water (7 : 3, v/v: 25 L) at 80 °C for 4 hr. The combined MeOH extract was concentrated *in vacuo* at 45 °C to give 2.8 kg residue. The purification and isolation of three flavonoids from the $CHCl_3$ and n-BuOH extract were performed according to general chromatographic methods. Briefly, the $CHCl_3$ extract was chromatographed on a silica gel column (Silica gel 60, 70~230 mesh: Merck Japan, Tokyo, Japan) using stepwise gradient elution with the hexane-ethylacetate, ethylacetate-methanol to yield fraction I to VI. The chlorogenic acid (**1**) (1.2 g, 99% purity, pale yellow powder) was finally purified with a LiChroprep RP-18 Lobar column (Merck, Japan) with 50% methanol from the fraction 1 (5 g). The luteolin (**2**) (300 mg, 99% purity, pale yellow powder) was finally purified with a LiChroprep RP-18 Lobar column (Merck, Japan) with 50% methanol from the fraction 2 (15 g). The diosmetin (**3**) (400 mg, 98% purity, pale yellow powders) was finally purified with a LiChroprep RP-18 Lobar column with 50% methanol from the fraction 2 (6 g). The fraction 5 (13 g) was subjected to silica gel column chromatography using dicloromethane-methanol (5 : 1) to afford luteolin-7-O-glucoside (**4**) (300 mg, 99% purity, yellow needles).

The BuOH extract was chromatographed on a silica gel column (Silica gel 60, 70~230 mesh: Merck Japan, Tokyo, Japan) using stepwise gradient elution with the hexane-ethylacetate, ethylacetate-methanol to yield fraction I to V. The tyrosine (30 mg, 99% purity, white powder) was finally purified with a LiChroprep RP-18 Lobar column (Merck, Japan) with 50% methanol from the fraction 2 (15 g). The uridine (400 mg, 98% purity, white powders) was finally purified with a LiChroprep RP-18 Lobar column with 50% methanol from the fraction 3 (6 g). These six compounds were identified by direct comparison with an authentic sample (Chen *et al*., 2010; Oshima *et al*., 1989; Park *et al*., 1995; Torizawa *et al*., 2005). The structures of the isolated compounds are given in Fig. 1.

**Statistical analysis** – All data were presented as mean ± standard deviation (SD). Statistical analysis was carried out by SPSS 17.0 (SPSS Inc., Chicago, IL, USA) program by analysis of variance (ANOVA) followed by Duncan's multiple range test, considering $P < 0.05$ as statistical significance.

## Results and Discussion

**Inhibitory effects of flavonoids on MAOs and DBH** – All the three flavonoids at 25, 50, 100, and 1000 μg/mL in 5% DMSO were tested for their inhibitory activities against MAO-A and MAO-B (Table 1). The




| Compound No. | Name | Substitution | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 7 | 3' | 4' | 5' |
| 2 | Luteolin | OH | OH | OH | OH | |
| 3 | Diosmetin | OH | OH | OH | OCH3 | |
| 4 | Luteolin-7-glucoside | OH | O-glu | OH | OH | |

**Fig. 1.** The Structures of the three flavonoids, luteolin (**2**), diosmetin (**3**) and luteolin-7-glucoside (**4**) and chlorogenic acid (**1**), tyrosine (**5**), uridine (**6**) isolated from *T. mongolicum*.

**Table 1.** The $IC_{50}$ values of the isolated compounds from *T. mongolicumi* against MAO-A, MAO-B and DBH activities

| Compounds | $IC_{50}$ values (micro mole) | | |
|---|---|---|---|
| | MAO-A | MAO-B | DBH |
| Luteolin | 0.31 | – | – |
| Diosmetin | – | 211 | – |
| Luteolin-7-glucoside | 400 | 268 | 0.041 |
| Iproniazid[1] | 37 | 42.5 | – |
| Deprenyl[2] | 3.3 | 0.046 | – |

[1]Used as a positive control drugs for nonselective MAO inhibitor
[2]Used as a positive control drug for selective MAI-O-B inhibitor

inhibitory activity of luteolin (which contains four hydroxyls at 5, 7, 3', 4' positions) was greater than others on MAO-A selectively. A phenolic hydroxy groups at *para*-position, is considered as the active site for MAO-A inhibition as well as MAO-B. An additional phenolic hydroxy group at the *ortho*-position alleviates about 4-fold MAO-A inhibitory activity of phenolic hydroxy group at *para*-position. Its $IC_{50}$ value was 0.31 ìmole/L for MAO-A. As the report by Ryu *et al*. (1988), in the case of piceid and rhaponticin having the structure modified on only a hydroxy group of resveratrol and rhapontigenin to *O*-glucose exhibited the alleviated effect, as well as 3,5-dihydroxy-4'methoxystilbene which have the structure modified on a hydroxy group of resveratrol to a methoxy group showed less inhibition on MAO-A than resveratrol. In particular, luteolin did not exhibit inhibitory activities against MAO-B and DBH. Diosmetin (which contains three hydroxy at 5, 7, 3' positions, and a methoxy at 4' position) did not exhibit inhibitory activities on MAO-A and DBH. It showed only mild inhibitory activity on MAO-B.

The activity of luteolin-7-glucoside (the hydroxy group at the position 7 is substituted by sugar and the *meta* dihydroxy of the ring A are lost) was considered reduced in comparison with luteolin on MAO-A inhibitory activity. The luteolin-7-glucoside also showed moderate inhibition on MAO-B. The substitution of one hydroxy to glucosyl residue at the position 7 caused the reducing of the inhibitory activity on MAO-A and enhancing of the inhibitory activity on MAO-B. Han *et al*. also reported that masking of hydroxy proton with methyl and glycosyl groups diminished antioxidant activity (Han *et al*., 1990). Thus, MAO-A inhibition that was reduced in luteolin-7-glucoside having a phenolic hydroxy group at *para*-position seems to be because of O-glucose moiety, and the sugar group has no potential of MAO-A inhibition. The results demonstrated that glycosylation of the proton of the hydroxy group at 7 of the ring A influenced their inhibition of MAO-A and MAO-B. The marked decrease of the inhibitory activity against MAO-A can be explained by the absence of *meta* dihydroxy in the A ring of luteolin-7-glucoside, which reveals that both *meta* dihydroxy on the A and *ortho* dihydroxy on the B ring play a significant role in the inhibition on MAO-A.

**The selectivities on inhibitory activities of the isolated compounds** – As shown in Fig. 2, the degree and the way of inhibition against each enzyme of the isolated compound were different. Luteolin was selective MAO-A inhibitor. Its inhibitory potentials ($IC_{50}$ value, total activity, and specific activity) on MAO-A were about 10 times more than those on MAO-B and DBH (Table 1). In addition, its $IC_{50}$ value on MAO-A was at a rate comparable with that of iproniazid used as positive control. Diosmetin was the strongest selective MAO B inhibitor among the isolated compounds. Its inhibitory potentials on MAO-B were more than that of luteolin and luteolin-7-glucoside. In addition, it exhibited about 1000 times less $IC_{50}$ value on MAO-B than that on MAO-A, deprenyl showing about 70 times less that on MAO-B than that on MAO-A. It has been reported that the methoxy group in citric acid methylesters seems to be critical for the inhibitory activity on MAO-B (Han *et al*., 2001). Luteolin-7-glucoside was a selective DBH inhibitor. It exhibited inhibitory activity against DBH at concentrations below 0.9 μM, but did not on MAO-A and MAO-B. These results strongly suppose that the isolated flavonoids are the major active components of *T. mongolicum* against MAOs and DBH.

The activity-guided fractionation of extracts from *T. mongolicum* led to the isolation of three flavonoids, luteolin, luteolin-7-glucoside, and diosmetin. Three compounds were exhibited the inhibitory activities against MAO-A, MAO-B and DBH respectively. As described in the introduction, its use has mainly been based on empirical findings. This contribution provides a comprehensive review of the pharmacologically relevant compounds of *Taraxacum* characterized so far and of the studies supporting its use as a medicinal plant. Particular attention has been given to diuretic, choleretic, anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, anti-hyperglycemic, anti-coagulatory and prebiotic effects (Katrin *et al*., 2006).

In the present study, we attempted to determine the structure-activity relationships of luteolin and its related derivatives isolated from *Taraxacum mongolicum* against MAOs activities, which may be helpful for understanding the active moieties of MAOs. In this study, we can find

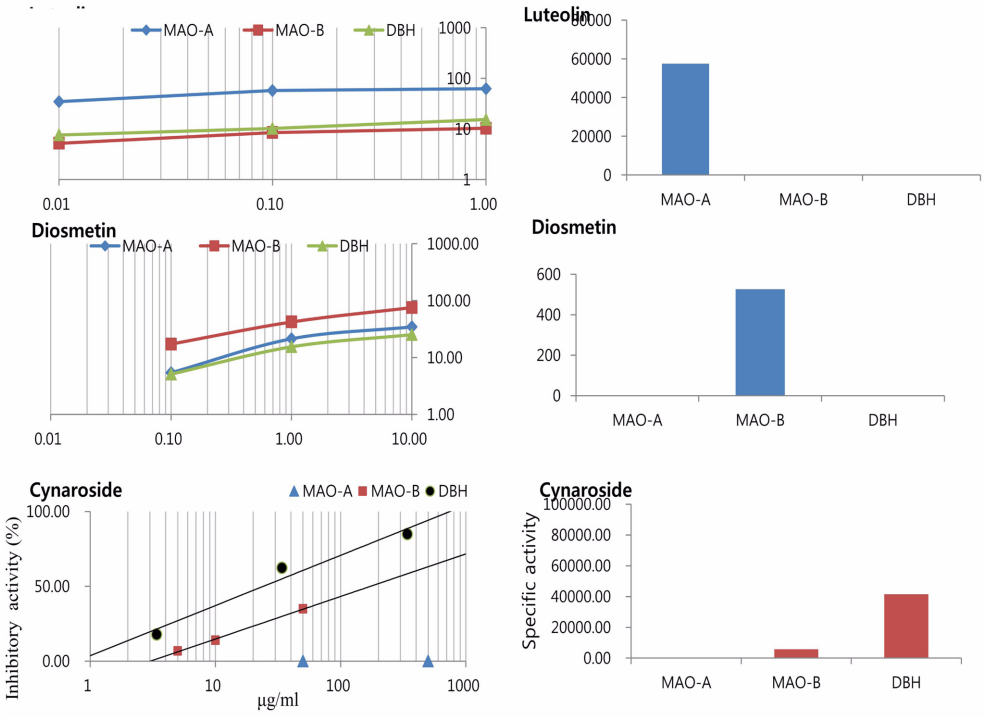



**Fig. 2.** Inhibitory activities on MAO-A, MAO-B and DBH of the three flavonoids luteolin (**2**) , diosmetin (**3**) and luteolin-7-glucoside (**4**) isolated from *T. mongolicum*. *concentrations of serotonin, benzylamine and tyramine as substrates of three enzymes were 0.25, 1.0, and 0.02 mM, respectively.

out that this plant has also antidepressant effect. Each isolated compound was showed different inhibitory patterns against MAO-A, MAO-B and DBH, each other. One of the isolated compounds, luteolin was find out as a selective MAO inhibitor, because of its inhibitory activities on MAO-A was most potent selectively and did not exhibited MAO-B and DBH inhibitory activities. A phenolic hydroxy groups at *para*-position is considered as the active site for MAO-A inhibition in luteolin. An additional phenolic hydroxy group at the *ortho*-position alleviates about 4-fold MAO-A inhibitory activity of phenolic hydroxy group at *para*-position. Although its activity was not so great, diosmetin inhibited the MAO-B activity. A methoxy group at *para*-position is considered as the active site for MAO-B inhibition. In the previous report, acacetin and diosmetin containing a methoxy group showed inhibition of MAO-B, but apigenin and luteolin, which have the structure modified on only the methoxy group of acacetin and diosmetin to a hydroxy group, did not exhibit the effect (Han *et al*., 1987). Moreover, it was reported that the methoxy group in citric acid methylesters seems to be critical for the inhibitory activity on MAO-B (Han *et al*., 2001).

Several reports have described the MAO-A inhibition contributes to the mechanism of antidepressant effects of MAO inhibitors more than MAO-B inhibition (Lipper *et al*., 1979; Mann *et al*., 1989). Larsen *et al*. reported that reversible monoamine oxidase inhibitor (RIMA) has equal antidepressant effects to those of irreversible MAO inhibitors (1991). However, Lotufo-Neto *et al*. examined antidepressant effects of MAO inhibitors in a meta-analysis and described the possibility that non-selective MAO inhibitors are more effective than RIMA(1999). Consequently, it is likely that MAO-B inhibition also contributes to an antidepressant effects. Kitaichi *et al*. measured extracellular noradrenaline and serotonin levels after administration of RIMA and reversible MAO-B inhibitor in the medial prefrontal cortec (PFC) of rats using the *in vivo* micro dialysis method (2010). And they suggested that the combined treatment of MAO-A and MAO-B inhibitors strengthens antidepressant effects because the combined treatment increases extracellular noradrenaline levels more than a MAO-A inhibitor alone through increases in â-phenylethylamine. According to the results of this study, *T. mongolicum* is an excellent combined MAO inhibitor to treatment for depressant, because its major bioactive compounds, luteolin, diosmetin, and luteolin-7-glucoside were selective, nonselective MAO, and also DBH inhibitors, respectively. Luteolin is a selective MAO-A inhibitor, diosmetin is a selective MAO-

B inhibitor, and cynaroside is a selective DBH inhibitor.

There are more MAO-B than MAO-A in human brain, but more MAO-A than MAO-B in the rat brain (Rigal and Zarifian, 1983). The distribution of MAO-A and MAO-B is different between the human brain and the rat brain. The role of MAO-B in antidepressant effects might be greater in human than in rat; stronger antidepressant effects of combined treatment with a MAO-A inhibitor and a MAO-B inhibitor might be likely to be induced in humans (Kitaichi *et al*., 2010). Results of this study suggest that luteolin, as a selective MAO inhibitor; can be potentially used as drug candidates for depressant. Diosmetin, as a selective MAO-B inhibitor, can be potentially used as drug candidates for this kind of disease. Cynaroside, as selective DBH inhibitor, can effectively elevates the level of released dopamine (DA) by preventing DBH from converting DA to norepinephrine and being destroyed by oxidative deamination effect of MAO. Thus, that seems to be helpful materials for antidepressant drug for human.

## Conclusions

Three flavonoids, luteolin, diosmetin, and luteolin-7-glucoside isolated from *T. mongolicum* were major active principles of this plant. Results of this study suggest that the reactivities of luteolin and its related derivatives against MAOs activities are dependent on their molecular structures. The presence of the phenolic hydroxy group at *para*-position is the active site for MAO-A inhibition as well as of MAO-B. The methoxy group has no potential on MAO-A inhibition. An additional phenolic hydroxy group at the *ortho*-position alleviates about 4-fold MAO-A inhibitory activity of phenolic hydroxy group at *para*-position. A carboxylic group seems to be critical for DBH inhibition and have no effects on MAO.

The possibility exists that these three compounds isolated from *T. mongolicum* are expected for potent candidates for development of combined antidepressant drug, and the plant *T. mongolicum* contained luteolin, diosmetin, and luteolin-7-glucoside, as major active components will be an excellent functional food material for combined antidepressant effect.

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