# ผลของน้ำคั้นส้มโอต่อการยับยั้งการทำงานของเอนไซม์โมโนเอมีนออกซิเดส และการประเมินพฤติกรรมซึมเศร้าในหนูไมซ์

จุฑามาศ มณีเนตร[1] อรชุมา วิภัชภาคไพบูลย์[2] อรวรรณ มณฑการิรัตน์[3*] เยาวเรศ ชูลิขิต[4]

**บทคัดย่อ**

**บทนำ:** เอนไซม์โมโนเอมีนออกซิเดส (MAO) เป็นเอนไซม์ที่อยู่บริเวณสมอง ตับและสารสื่อประสาทชนิดโมโนเอมีน MAO มีสองไอโซเอนไซม์ ได้แก่ โมโนเอมีนออกซิเดสเอ (MAO-A) และ โมโนเอมีนออกซิเดสบี (MAO-B) MAO-A มีบทบาทสำคัญในโรคความผิดปกติทางจิตเวช เช่น โรคซึมเศร้า และ MAO-B มีส่วนเกี่ยวข้องกับความผิดปกติของเส้นประสาท เช่น พาร์กินสัน และ อัลไซม์เมอร์ สมมติฐาน MAO เป็นหนึ่งในสาเหตุสำคัญในโรคซึมเศร้าและตัวยับยั้ง MAO คือ ยาต้านซึมเศร้า ซึ่งส่วนใหญ่ของยาต้านซึมเศร้านั้นมีผลข้างเคียงค่อนข้างมาก ปัจจุบันจึงได้ทำการศึกษายาต้านซึมเศร้าที่มาจากผลิตภัณฑ์จากธรรมชาติเพื่อที่จะลดผลข้างเคียงอันไม่พึงประสงค์ และเพื่อความปลอดภัยในระยะยาว **วัตถุประสงค์:** การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของยาต้านซึมเศร้าของพืชที่เป็นผลไม้เศรษฐกิจของประเทศไทย คือ ส้มโอ โดยการทดสอบการยับยั้งเอนไซม์ MAO ในหนูไมซ์และการใช้ 2 แบบจำลอง ได้แก่ Forced swimming test (FST) และ Tail suspension test (TST) **วิธีดำเนินการวิจัย:** ส้มโอ 3 สายพันธุ์ ได้แก่ ทับทิมสยาม ทองดี และขาวน้ำผึ้ง ถูกนำมาใช้ในการทดลอง โดยวิธีการยับยั้งเอนไซม์ MAO และหนูไมซ์จะถูกให้สารสกัดหยาบจากส้มโอขนาด 250 และ 500 มิลลิกรัมต่อน้ำหนักตัว (กิโลกรัม) และยาต้านซึมเศร้ามาตรฐาน imipramine ขนาด 20 มิลลิกรัมต่อน้ำหนักตัว (กิโลกรัม) เป็นเวลา 1 ชั่วโมงก่อนทำการทดสอบโดยแบบจำลอง FST และ TSTเพื่อทำการประเมินพฤติกรรมซึมเศร้าในหนูไมซ์ สถิติที่ใช้ One-Way ANOVA ตามด้วย Student-Newman-Keulstest **ผลการวิจัย:** ผลการศึกษาพบว่าส้มโอ 3 สายพันธุ์ ได้แก่ ทับทิมสยาม ทองดี และขาวน้ำผึ้ง มีผลต่อการยับยั้งเอนไซม์ MAO-A ($IC_{50}$= 721.20±0.26, 426.68±0.33 และ 1080±1.01 µg/ml) และ MAO-B ($IC_{50}$=68.310±0.97, 144.51±0.31 และ 749.31±1.12 µg/ml) ตามลำดับ และมีเพียงส้มโอสายพันธุ์ทับทิมสยามเท่านั้นที่สามารถลด immobility time ในหนูไมซ์ได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ทั้งในแบบทดสอบFST และ TST **สรุปผลการวิจัย:** จากผลการศึกษาชี้ให้เห็นว่าส้มโอทับทิมสยามน่าจะมีประสิทธิภาพดีที่สุดในการลดอาการซึมเศร้าเมื่อเปรียบเทียบกับอีก 2 สายพันธุ์ โดยกลไกการออกฤทธิ์น่าจะเกี่ยวข้องกับการที่สามารถยับยั้งการทำงานของเอนไซม์โมโนเอมีนออกซิเดสได้ จึงอาจมีประโยชน์ในการพัฒนาหรือต่อยอดการศึกษาต่อไปในอนาคต

**คำสำคัญ:** โมโนเอมีนออกซิเดสเอ (MAO-A), โมโนเอมีนออกซิเดสบี (MAO-B), โรคซึมเศร้า, ส้มโอ, พฤติกรรมซึมเศร้า

**วารสารเภสัชศาสตร์อีสาน 2559; 11(ฉบับพิเศษ): 92-100**

---

[1]นักศึกษาระดับบัณฑิตศึกษา คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[2]นักศึกษาระดับปริญญาตรี คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[3]Ph.D. อาจารย์ประจำสาขาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[4]Ph.D. ผู้ช่วยศาสตราจารย์, อาจารย์ประจำสาขาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[*]**ติดต่อผู้นิพนธ์:** โทรศัพท์: +66-43-202378, โทรสาร: +66-43-202379; E-mail: oramon@kku.ac.th

# Effect of *Citrus grandis* [L.] Osbeck on monoamine oxidase inhibitory activities and depressive behavioral assessment in mice

Juthamart Maneenet[1], Onchuma Vipatpakpaiboon[2], Orawan Monthakantirat[3*], Yaowared Chulikhit[4]

**Abstract**

**Introduction:** Monoamine oxidase (MAO) is an enzyme that located in brain, liver at the outer membranes and oxidized monoamine neurotransmitters. MAO has two isoenzymes, MAO-A and MAO-B. MAO-A plays an important role in psychiatric disorder such as depression and MAO-B involved in neuronal diseases such as Parkinson's disease (PD) and Alzheimer's diseases (AD). Monoamine hypothesis, one of the most important causes in depressive disorder and MAO inhibitor is an antidepressant, which have many side effects. Nowadays we look for antidepressant from natural product in order to reducing the unwanted side effects and safety for long term treatment. **Objective:** This study aimed to investigate antidepressant-like effect of the native economic fruit in Thailand, pomelo (*Citrus grandis* (L.) Osbeck), on monoamine oxidase (MAO) inhibitory effect and using two acute animal models of depression, forced swimming test (FST) and tail suspension test (TST). **Materials and Methods:** Three cultivars of pomelo, Tubtim Siam (TS), Thong-dee (TD) and Kao-Nampueng (KN) were used in this study as monoamine oxidase (MAO) inhibitors. Mice were administered the pomelo extract (250, 500 mg/kg, p.o.) and a reference antidepressant, imipramine (IMP, 20 mg/kg, i.p.) 1 hour before starting FST and TST to analyze depression-like behavior in ICR mice (Statistic: One-Way ANOVA followed by the Student-Newman-Keulstest). **Results:** The results showed that 3 cultivars of pomelo including TS, TD and KN inhibited the MAO-A activity with the $IC_{50}$ values of 721.20±0.26, 426.68±0.33 and 1080±1.01 µg/ml and MAO-B activity with the $IC_{50}$ values of 68.31±0.97, 144.51±0.31 and 749.31±1.12 µg/ml, respectively. In addition, only TS significantly decreased the immobility time in mice when compare with control group in both TST and FST. **Conclusion:** The results of this study suggest that TS ameliorated depressive-like symptom and the possible mechanism may be involve in its inhibitory effect on monoamine oxidase enzymes.

**Keywords:** MAO-A, MAO-B, depression, *Citrus grandis* [L.] Osbeck, depression-like behavior
**IJPS 2016; 11(Supplement): 92-100**

---

[1]Graduate student, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen 40002, Thailand.

[2]Undergaduate student, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen 40002, Thailand.

[3]Ph.D. Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen 40002, Thailand.

[4]Ph.D.Assistant Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen 40002, Thailand.

***Corresponding author:** Orawan Monthakantirat Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen 40002, Thailand. Tel. +66-43-202378 Fax: +66-43-202379; E-mail: oramon@kku.ac.th

## INTRODUCTION

Depression is a mood disorder that complex characterized by depressed mood, sadness, hopelessness, loss of pleasure or interest, disturbance of sleep or appetite (WHO, 2012). By the year 2020, depression is the second source of global burden of disease and leading to the cause of death. Depressive symptom are relieved by antidepressant. Although antidepressant medicines are available to treat depression, most of drugs are not without side effects.In addition, antidepressant herbal medicines such as St. John's wort (*Hypericum perforatum*) have been reported that it has some potential for depression treatment but disturb drug metabolizing systems (Sakakibara et al., 2008). Therefore, this study ∧濕med to investigate an effective herbal drug without side effects. Pomelo (*Citrus grandis* [L.] Osbeck), belongs to the family Rutaceae and the native economic fruit in Thailand. Since ancient times, pulp of pomelo has been used as appetizer, antitoxic and cardiac stimulant. The reports have shown that the major flavanoid of pomelo are neohesperidin, hesperidin, dihydrochalcone, hesperidin, hesperitin, naringenin and naringin, which are high in fruit juice and pulp (Mäkynen et al., 2013). The report showed that pomelo had high flavonoids as compared to other Thai fruits (Toh et al., 2013). Extensive studies of pomelo extract have revealed its favourable antioxidant properties (Guo et al., 2003). Hesperidin in citrus fruit has been reported to use to antioxidant, neuroprotective and antidepressant-like effect (Donato et al., 2014). Monoamine oxidase (MAO) is the enzyme responsible for metabolism of monoamine neurotransmitter (Norepinephrine (NE), Epinephrine (EP), Serotonin (5-HT) and Dopamine (Da)). They play the important role in the brain development. The monoamine hypothesis postulated that depression is due to deficiency of monoamine neurotransmitter, NE and 5-HT at key site in the brain (FIŠAR et al., 2010). Furthermore, there has been no report on the possible antidepressant activity of pomelo cultivars. So, the main objectives of this study were to investigate the antidepressant-like effect of pomelo by measurement monoamine oxidase (MAO) enzyme activity.

## Materials and Methods

### Preparation of plant extract

Three pomelo cultivars (Tubtim Siam, Thong-dee, and Kao-Numpueng) were obtained from a local market and harvested at the mature stage. The pulp of pomelo was collected by manual peeling and then homogenized using a commercial blender. The pulp was dried at 50°C and pomelo juice was freeze dried. Both two parts were collected and macerated with 95% ethanol for 7 consecutive days. The supernatant was evaporated in a rotary evaporator at 60 °C and keep at -20 °C prior to analysis.

### Animals

Male ICR mice (20-30 g about 7 weeks old) were obtained from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). Mice were housed on wood chip bedding in stainless steel cages with free

access to food and water. Housing was thermostatically maintained at 22 ± 2 ºC with constant humidity (45% ± 2%) and a 12-hlight–dark cycle (lights on: 07:00–19:00). The experimental protocols for the present studies were approved by Animal Ethics Committee of Khon Kaen University.

**Drugs and administration**

Imipramine (NacalaiTesque, Inc.) 20 mg/kg (i.p.) was dissolved in 0.9% normal saline solution (NSS), and then injected at a volume of 5 ml/kg of body weight. Mice were randomly divided into 8 groups (n=8): four treatment received imipramine (20 mg/kg) are as following, TD (250 and 500 mg/kg), TS (250 and 500 mg/kg), KN (250 and 500 mg/kg). All drugs were administered 1 hour before start the FST, TST and locomotor acitivity.

**Measurement MAO-A and MAO-B activities**

To determine the effect of inhibitory MAO-A and MAO-B enzyme activity by use kynuramine determination assay. This study uses recombinant human MAO-A and MAO-B, source of enzyme. The reaction included kynuramine (45 and 30 µM for MAO-A and MAO-B), substrate of MAO-A and MAO-B, and the pomelo extracts. Then dissolve in DMSO (0-10 mg/ml of extract), add MAO enzyme 0.0075 mg/ml in potassium phosphate buffer (100 mM, pH 7.4). Incubate at 37°C, 20 minutes, add 400 µl 2N NaOH and1000 µl distilled water for stop reaction. MAO-A and MAO-B changed kynuramine to 4-hydroxyquinoline, measure the fluorescence at $\lambda_{ex}$ = 310 nm and $\lambda_{em}$ = 400 nm.

**Standard preparation**

The preparation 4-hydroxyquinoline (0.025-2.00 µM) in potassium phosphate buffer (pH 7.4) 500 µl were mixed with 400 µl 2N NaOH and 1000 µl distilled water before measure the fluorescence.

**Forced swimming test**

Forced swimming test (FST) is one of the most commonly used animal models for assessing antidepressant-like behavior (Moallem et al, 2007). FST involves the scoring of active (swimming and climbing) or passive (immobility) behavior when mice are forced to swim in a cylinder which there is no escape. Mice were placed individually in glass cylinder (12 cm in diameter, height 25 cm) which was filled with water to a height of 10 cm of water at 25ºC (Chatterjee et al., 2012). The experiment was divided into 2 periods. First, mice were placed in glass cylinder for 15 min before test 24 h. Then the test mice were considered to the immobile when they were floating motionless. The duration of FST was recorded for 5 min (Sakakibara et al., 2008).

**Tail suspension test**

Tail suspension test (TST) is that suspending mice suspended upside down leads to characteristic behavior immobility which resembles to human depression (Steru et al., 1985). The mice were individually suspended of the tail suspension test box, 60 cm above the surface of table (Chatterjee et al, 2012). After the administration of respective sample, mice were suspended on the edge of the table 60 above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded for the last 4 min during 6 min period. Mice were considered immobile posture when they hanged passively (Mizuki et al., 2014).

**Open-field test**

Locomotor activity is a simple performed measurement of behavior in mice. To assesses the effects of the pomelo extract on locomotor activity. The open-field apparatus was Y-maze, enclosed of three arms which long 40 cm, high 18 cm and wide 3 cm. Mice were placed on one arm in Y-maze apparatus after the drug administration 1 hour. Measurement the number of arm entries over 5 minute (Monthakantirat et al., 2014).

**Statistical analysis**

The data are expressed as the mean ± SEM. The data were analyze by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons.

**Results**

**Effect of pomelo 3 cultivars on MAO activity**

Figure 1 showed the standard curve of 4-hydroxyquinoline, equation of standard was y = 408.8x+49.01 and coefficient of determination ($R^2$) = 0.999. Effect of *C. grandis* on MAO-A activity showed $IC_{50}$ of TS, TD and KN was 721.20±0.26, 426.68±0.33 and 1080.91±1.01 µg/ml, respectively, $IC_{50}$ of TS, TD and KN on MAO-B activity was 68.31±0.97, 144.51±0.31 and 749.31±1.12 µg/ml, respectively. The result shows the selectivity to MAO-A and MAO-B (Table 1).

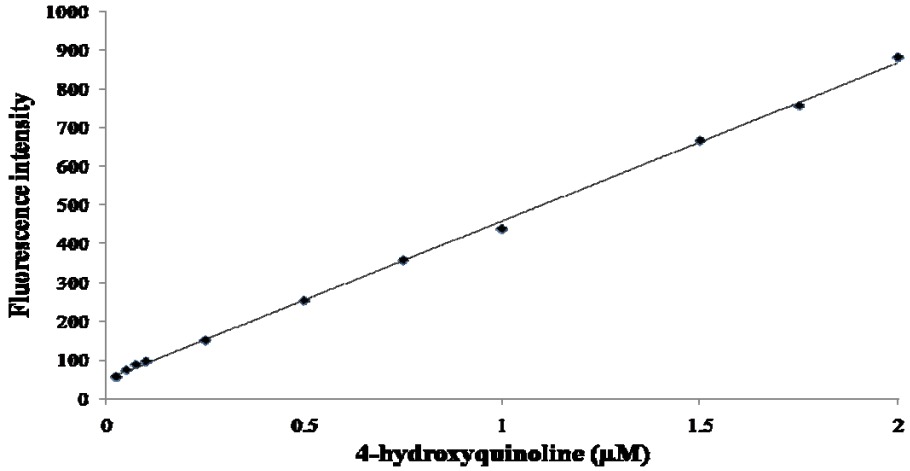



**Figure 1**. Linearity curve of 4-hydroxyquinoline (linear ranges of 0 – 2 µM; $R^2$ = 0.999).

**Table 1**. The $IC_{50}$ values for inhibiton of MAO-A and MAO-B by *C.* and selectivity index.

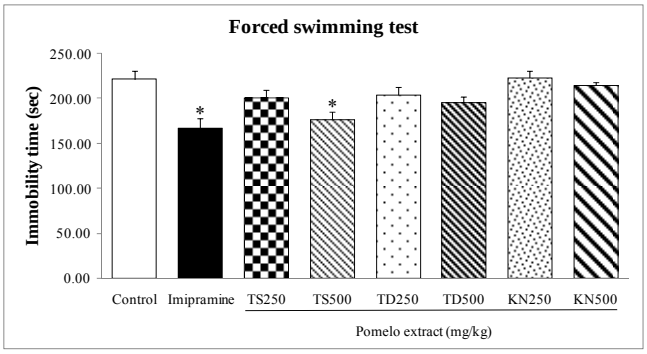
| Plant | $IC_{50}$ (μg/mL) | | SI* for | SI* for |
|---|---|---|---|---|
| | MAO-A | MAO-B | **MAO-A** | **MAO-B** |
| *C.grandis* 'Tubtim Siam' | 721.20±0.26 | 68.31±0.97 | 10.56 | 0.09 |
| *C. grandis* 'Thong Dee' | 426.68±0.33 | 144.51±0.31 | 2.95 | 0.34 |
| *C. grandis* 'Kao-Numpueng' | 1080.91±1.01 | 749.31±1.12 | 1.44 | 0.69 |

*The selectivity index is the selectivity for the MAO-A and MAO-B isoform and is given as the ratio of $IC_{50}$ (MAO-A)/ $IC_{50}$ (MAO-B)

**FST and TST with mice**

*C. grandis* TS, TD and KN (250 or 500 mg/kg of body weight) or imipramine (20 mg/kg of body weight) was orally or intraperitoneal administered to the mice one time before FST and TST. Figure 2 shows the effect of *C. grandis* extract on FST and TST in mice. Once treatment with TS 500 mg/kg was significantly decreased the immobility time in FST and the TST, TS and TD was significantly decreased immobility time when compared with control group. There was no effect of *C. grandis* extract on locomotor activity (Figure 3).

**A)**




**B)**

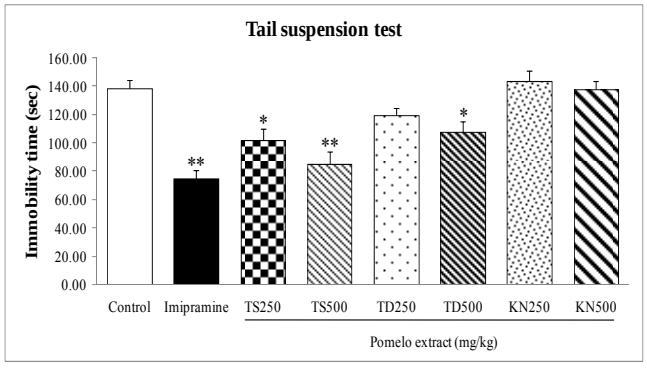



**Figure 2.** Effect of *C. grandis* on animal model A) FST and B) TST. Data expressed as mean ± S.E.M. (n=8). *P<0.05 compared with control group; **P<0.001 compared with control group.

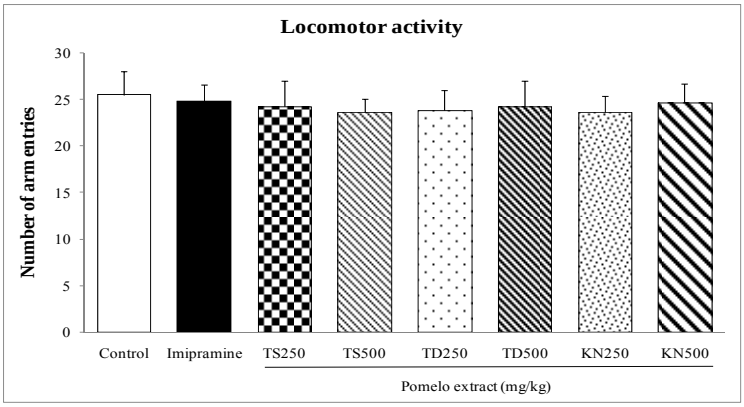



**Figure 3.** The effect of *C. grandis* on locomotor activity in Y-Maze test in mice. Each column represents mean ± S.E.M. (n=8).

**Discussion and Conclusion**

Depression is a common, chronic disease and several characteristics (Donato et al., 2014). The mechanism underlying the pathophysiology of depression is not yet well understood. One of hypothesis of depression is monoamine oxidase which the enzyme responsible for metabolism of monoamine neurotransmitter (Norepinephrine (NE), Epinephrine (EP), Serotonin (5-HT) and Dopamine (Da)), has an important in the brain development. Citrus fruits juices contain mainly naringin, naringenin and hesperidin. The hesperidin ingested with citrus juices is metabolized by human intestinal bacterial microflora to aglycones hesperetin (Gardana et al., 2007). Naringin and naringenin can be absorbed from the human gastrointestinal tract (Kanaze et al., 2006). The present study demonstrates that pharmacological of pomelo (*Citrus grandis* (L.) Osbeck) three cultivars including Tubtim Siam (TS), Thong-dee (TD) and Kao-Nampueng (KN) on antidepressants-like effect. For the screening of monoamine oxidase activity in three cultivars of pomelo extract, they showed the inhibitory effect on the MAO-A activity with the $IC_{50}$ values of 721.20±0.26, 426.68±0.33 and 1080±1.01 µg/ml and MAO-B activity with the $IC_{50}$ values of 68.31±0.97, 144.51±0.31 and 749.31±1.12 µg/ml, respectively. In addition, only TS significantly decreased the immobility time in mice when compare with control group in both TST and FST as same as imipramine. The results from opened filed test exhibited that the pomelo extract did not have any effect on locomotor activity and confirmed the antidepressant activity of pomelo. The previous study by Kittana and co-worker reported that the amounts of naringin and neohesperidinin TS (26.31 ± 0.44 and 29.92 ± 0.18, respectively) were higher than TD (8.13 ± 0.13 and 10.76 ± 0.03, respectively) and KN (2.34 ± 0.11 and 14.76 ± 0.15, respectively) (Mäkynen et al., 2013). So, pomelo has greater flavonoids contents, better effect on improvement of depressive-like behavior in animal model of depression. Therefore, the antidepressant-like effects and the mechanism of action of TS will be further investigated in the other model of depression. TS may have a benefit in the future

and cannot be neglected. The present study suggested that TS ameliorated depressive-like symptom and the possible mechanism may be involved in its inhibitory effect on monoamine oxidase enzymes.

**Acknowledgement**

This research was granted by Thailand research fund (Agriculture), RDG5720007.

**References**

Chatterjee M, Jaiswal M, Palit G. Comparative Evaluation of Forced Swim Test and Tail Suspension Test as Models of Negative Symptom of Schizophrenia in Rodents. International Scholarly Research Network. 2012.1-5.

Donato F, Gomes MG, Goes ATR, et al. Hesperidin exerts antidepressant-like effects in acute and chronic treatment in mice: Possible role of L-arginine-NO-cGMP pathway and BDNF levels. *Brain Research Bulletin.*2014. 104, 19-26.

FIŠAR Z, HROUDOVÁ J, RABOCH J. Inhibition of monoamine oxidase activity by antidepressants and mood stabilizers. *Neuroendocrinol Lett.* 2010. 31(5), 645-656.

Gardana C, Gharnieri S, Riso P, Simonetti P, Porrini M. Flavanone plasma pharmacokinetics from blood orange juice in human. *British Journal of Nutrition.* 2007. 98, 165-172.

Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research.* 2003. 23, 1719-1726.

Kanaze F, Bounartzi M, Georgarakis M, Niopas I. Pharmacokinetics of citrus flavononeaglyconeshesperetin and naringenin after single oral administration in human subjects. *European Journal of Chinical Nutrition.* 2006. 61, 472-477.

Mäkynen K, Jitsaardkul S, Tachasamran P, et al. Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (*Citrus (Burm.) Merrill*) in Thailand. *Food Chemistry.* 2013. 139, 735-743.

Mizuki D, Matsomoto K, Tanaka K, et al. Antidepressant-like effect of Butea superba in mice exposed to chronic mild stress and its possible mechanism of action. *Journal of Ethnopharmacology.* 2014. 156, 16-25.

Moallem SA, Hosscinzadeh H, Ghoncheh F. Evaluation of antidepressant effect of aerial parts of *Echium vulgare* on mice. *Iran J Basic Med Sci.*2007. 10, 189-196.

Monthakantirat O, Sukano W, Umehara K, Noguchi H, Chulikhit Y, Matsumoto K. Effect of miroestrol on ovariectomy-induced cognitive impairment and lipid peroxidation in mouse brain. *Phytomedine.* 2014. 21, 1249-1255.

Sakakibara H, Yoshino S, Kawai Y, Terao J. Antidepressant-like effect of onion (*Allium cepa*L.) powder in a rat behavior model of depression. *Biosci. Biotechnol. Biochem.* 2008. 72(1), 94-100.

Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology.* 1985. 85, 367-370.

World Health Organization, World suicide prevention day 2012. http://www.who.int/mediacentre/events/annual/world_suicide_prevention_day/en/ Accessed 25.11.201