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Abstract of thesis

Depression is one of the most prevalent forms of mental illness, which is characterized by several physical and emotional symptoms including sleep disturbances fatigue and energy level dysregulation. Antidepressant drugs have been prescribed to patients to attenuate their symptoms, however, these drugs have limitation such as serious side effects with low efficacy. Identification of new treatments with higher efficacy and low health risks is of great importance. The author focused on natural plant resources as new candidates for anti-depressant agent as they possess high potential of containing molecules efficient in depression therapy with less side effects. *Lippiacitriodora* (Lam.), commonly called lemon verbena or Louisa, is an aromatic and a medicinal plant rich in terpens and polyphenols used in folk medicine to cure illnesses such as gastrointestinal disorders, fever and headaches and also for its anxiolytic and hypnotic effects. The chemical analysis of verbena extract has been shown to contain different compounds, with higher amount of verbascoside (Vs), a phenypropanoid glycoside. Taking into account that commercial antidepressants can be prescribed in case of anxiety and sleep disorder,the author hypothesized that verbena and Vs might have potential as antidepressants.The author investigated the molecular mechanism underlying the relaxant effect induced by verbena ethanolicextract

(VEE) and the antidepressant-like activity of Vs *in vivo* and *in vitro*.

Initially, the author performedthe tail suspension test using ICR mice in order to investigate the relaxant effect of lemon verbena. The author showed that the treatment of VEE in mice at a concentration of 100 mg/kg/day showed an increase of immobility time compared to control groups. Microarray analysis revealed that treatment of VEE regulate the expression of several genes, which play key roles in calcium homeostasis (calcium channels), cAMP production, and energy metabolism. VEE and Vs enhanced the cell viability and mitochondrial activity in SH-SY5Y cells, human neuroblastoma line. These results suggested the relaxant effect of lemon verbena, which is induced through modulation of cAMP production and energy metabolism.

Next, the author examined the effect of Vs, as a pure compound, on mice behavior. Vs content was determined by analyzing VEE using HPLC and was found to be around 2.5%. ICR mice were treated with Vs or bupropion, an agent for major depressant disorder, and subjected to tail suspension test. The results showed an increased immobility time in case of negative control group, while the treatment of bupropion presented an antidepressant effect proved by enhancement of mice mobility. Treatment of Vs at a dose of 2.5 or 5 mg/kg/day significantly decreased the immobility, suggesting that Vs exert antidepressant activity. The author found that the levels of serotonin, noradrenalin, dopamine, and BDNF were significantly increased in brain of mice treat with Vs and Bupropion. The transcriptomic analysis suggested that Vs treatment enhanced several genes involved in cAMP production, calcium signaling, dopamine, and choligergic pathway, while it decreased the expression of genes related to development of depression.The author demonstrated that the treatment of Vs significantly increased the intracellular levels of ATP and calciumin SH-SY5Y cells. From these results, the author concluded thatinduction of relaxation and antidepressant-like effects VEE and Vs, respectively, through modulation of cAMP and calcium.

Furthermore, the author tried to produce stable emulsions using VEE in order to increase Vs conservation time and biological activity.The author determined optimal conditions to formulation of stable VEE-emulsions. Interfacial tension measured for VEE showed a significant decrease, in a concentration-dependent manner, up to 6.74 mN/m for 2% (w/w) solution. Given that the compound is destined to be administrated orally, the author used the oil-in-water (O/W) emulsion to produce stable VEE emulsions. Emulsion's main attribute is to insure a higher bioavailability of bioactive compounds by reducing their degradation in the gastrointestinal system and to increase their stability for long-term storage. Emulsion prepared of VEE and 5% soybean oil was instable, showing an increased droplet size from 0.198 to 7.43 µm after 24h incubation at 5°C. EDTA addition to preparation in order to chelate minerals had no effect on emulsion stability. Then, oleic acid containing 1% of lecithin was used as oil phase instead of soybean oil to increase emulsion stability. Oleic acid was found to increase the viscosity of the preparation which enhances droplets dispersion and stabilizes the emulsion. Also, lecithin, a phospholipid, is a natural emulsifier extracted from soybean that confers stability to micelles. The results showed a high stability of emulsion, with a particle size of 0.440 µm, even after storing it for 15 days at 5°C.

To evaluate the antidepressant effect of VEE emulsions, the author performed tail suspension test. The results showed a decreased immobility time in case of VEE-emulsion treated mice and scores were significantly different from negative control group. The statistical analysis showed no significant difference between bupropion and VEE emulsion, suggesting the antidepressant effect. The immobility time of control emulsion group decreased from day 5, which might be related to involvement of lecithin and/or oleic acid in depression process.

VEE-emulsion was found to increase the expression of dopamine and noradrenaline, confirming the antidepressant-like effect observed *in viv*o. Also, VEE-emulsion increasedthe expression levels of *Camk2n1*, *Gsn*, and *Ttr*.

Taken together, these results showed that VEE induced a relaxant effect of by the increase of Ca^{2+} and cAMP generation. On the other hand, Vs presented antidepressant-like activity through the regulation of dopamine signaling pathways. In order to potentially increase the bioavailability of Vs in VEE, the author established the formulation of stable emulsion using oleic acid-lecithin 1% as oil phase. When administered to mice, VEE-emulsion exerted antidepressant effect. Further studies on molecular mechanism may open the door for potential use of VEE and Vs to treat and/or prevent the depression.

Abstract of assessment result

【Review】

Depression is a disease that causes to the patients a loss of life enjoyment and decreases their ability to accomplish daily life. As it is considered as a major burden worldwide, affecting the lives of 350 million people, the development of antidepressant agent is of great importance. The applicants focused on *Lippiacitriodora*, a medicinal plant, and its chemical components, andexamined the antidepressant effect using tail suspension test and transcriptomic analysis.The applicant found that the high concentration treatment of plant extract may exert the relaxant effect through the regulation of cAMP production and energy metabolism while the low dose of verbascoside, a major component of *L. citriodora*, showed the anti-depressant effect in ICR mice through the upregulation of neurotransmitter and intracellular calcium levels. The applicants also established the formulation of stable emulsion of plant extract using oil-in-water and demonstrated that emulsion of plant extract exhibited the anti-depressant effect in mice. These results revealed the antidepressant effect of *L. citriodora* and implicate its potential use of treatment and/or prevention for depression.

【Result】

The final examination committee conducted a meeting as a final examination on 23October 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Science.