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学位論文題目	Neurogenesis Promoting Effect of Sugarcane (*Saccharum officinarum* L.) Top Extract and Its Cognitive Function Improvement （サトウキビ梢頭部抽出物由来活性成分の神経新生促進を介した認知機能改善効果）

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Abstract of thesis

In chapter 1, the author describes the general introduction of this thesis including background and aims of this study. Decline of cognitive function, including memory loss and learning difficulties, are closely related to aging. The accumulation of microenvironmental changes with the normal aging process generally causes the initial symptoms of the neurodegenerative disease. Although the concerns on aging-related neuronal disease are increased over the world, pharmacological treatments for cognitive decline are currently limited. Alternatively, there is growing interest on the use of dietary interventions and nutritional supplements for prevention of neuronal disease. In this thesis, the author focuses on Sugarcane (*Saccharum officinarum* L.) which is cultivated worldwide for the production of sugar. Although the top of the plant, termed sugarcane top, is mostly wasted because of its low sucrose content, the sugarcane top extract has been reported to have much antioxidant polyphenols. Chemical analysis revealed that 3-*o*-caffeoylquinic acid (3CQA), 5-*o*-caffeoylquinic acid (5CQA), 3-*o*-feruloyquinic acid (3FQA), and isoorientin (ISO) are rich in sugarcane top ethanolic extract (STEE).

In this thesis, the author aims to evaluate the improvement effect of STEE cognitive function and its potential as a novel neutraceutical. Specifically, a multifaceted behavioral, histological, biochemical, and molecular biological evaluation were performed using accelerated-aging mice and *in vitro* neurodevelopmental models. Furthermore, the

identification of polyphenolic constituents related to the biological activity of STEE and the analysis of the mechanism of compound-induced activity were carried out using *in vitro* model.

In chapter 2, the author addressed whether oral administration of polyphenol-rich STEE affects age-related cognitive decline and environmental or biochemical alternations in the brain. Senescence accelerated mouse prone 8 (SAMP8), a pathological aging model, were subjected to Morris Water Maze test after orally administration of STEE. The escape latency of STEE-fed mice was significantly decreased compared with control mice on the 5^{th}, 6^{th}, and 7^{th} day of the test. In the probe test, the time spent in the target quadrant tended to increase in the STEE-fed mice compared with control, suggesting that STEE is effective in restoring cognitive function in SAMP8 mice. To examine the effect of STEE on hippocampal neurogenesis, BrdU-labeled dividing cells in the subgranular zone were quantified. While there was no significant difference of BrdU-positive cells in the treatment group, BrdU-doublecortin (DCX) double positive cells were increased in STEE-fed mice. Furthermore, cortical levels of acetylcholine, dopamine, and norepinephrine were restored by STEE administration in SAMP8 mice. These suggested that increase of newborn neurons in subgranular zone and neurotransmitter levels were involved in the mechanism of improvement of cognitive function by STEE. The author also found that STEE treatment affected the transcription of genes of neurogenesis marker, stem cell marker, and neurotrophin signaling, and discussed the possible relationship of these transcriptional changes and STEE-induced mechanism.

In chapter 3, the author investigated the effect of STEE on the proliferation and differentiation of neural stem cells (NSCs) using *in vitro* model. In human-derived NSCs treated with STEE, marker genes of neuronal and astrocytic differentiations, such as *TUBB3* and *GFAP*, were significantly increased, while markers of oligodendrocyte differentiation and stem cell, including *PDGFA* and *NES*, were downregulated. These results suggested that STEE may induce the loss of stem cell characteristics and the acquisition of transitional forms, and differentiate into neurons or astrocytes. Also, STEE increased the incorporation of BrdU during cell cycles and stimulated the expression of HuC/D proteins, which are neural progenitor markers, suggesting that STEE enhanced cell division and the early phase differentiation of hNSCs. Furthermore, the author found that STEE regulated the expression of differentiation-related transcription factors, including *SCL1*, *HES1*, and *SOX2.* To further investigate the effect of STEE on hNSCs differentiation, changes in the cell population at an almost differentiated stage were analyzed. In hNSCs treated with STEE for 7 days, Tuj1-positive neurons were increased, whereas GFAP-positive astrocytes were not significantly changed. Expansion of the astrocytic process was also observed in STEE-treated hNSCs. These results demonstrated that STEE stimulated neural differentiation, and also affects the morphological expansion of astrocytes. Based on these observations, the author discussed the possible mechanism of STEE on neurogenesis promoting activity as follows: STEE has the effect of inducing and promoting neuronal differentiation from hNSCs by regulating transcription factors, as well as inducing the process expansion of astrocytes differentiated from hNSCs.

In chapter 4, the author investigated the effects of some natural polyphenols as active components of STEE for neuronal differentiation and astrocyte morphogenic maturation in hNSCs. The pro-neurogenic potential of major polyphenolic constituents of STEE, including 3CQA, 5CQA, 3FQA, ISO, and their combinations, were evaluated by measuring the expression levels of neuronal differentiation marker genes. The expression level of *TUBB3* was significantly increased by either STEE or the combination of 3CQA, 5CQA, and ISO, while single treatment of each compound and other combination had no effect on *TUBB3* expression. This result suggested that 3CQA, 5CQA, and

ISO might have synergistic effect on neuronal differentiation in hNSCs. Among these compounds, single treatment of 3CQA or 5CQA decreased cell cycle regulated genes such as *CCND1* and *CCND2*. The two compounds (mono-CQAs) increased phosphorylation of p38, which is a regulator of cell cycle progression and proliferation. These findings suggest that mono-CQAs may regulate cell cycle and inhibit G1 to S phase transition by suppressing cyclin D expression. Furthermore, the mRNA levels of *ASCL1* and *HES1*, which are bHLH factors regulating stem cell fate determination, were significantly increased and decreased, respectively, by the single treatment of ISO. Treatment of ISO also enhanced the phosphorylation of GSK3β, which is a serine/threonine kinase and plays major roles in cell structure formation, cell division and differentiation. These data demonstrated that ISO in STEE directed the fate of differentiating NSCs toward neuronal lineage through regulation of transcription factors and GSK3β activity. The author also found that the mixture of 3CQA, 5CQA, and ISO exhibited mitochondrial activation through the increase of *PPARGCA1*, which is a master regulator of mitochondrial biogenesis. The mixture of these compounds enhanced the phosphorylation of p38 and GSK3β in hNSC-derived immature astrocytes. These data showed that polyphenolic compounds in the STEE can accelerate NSCs differentiation and immature astrocyte maturation through their synergistic effects.

In chapter 5, the author described a general discussion to state the potential of sugarcane top extract for the future application as functional foods. The author also discussed about the relationship of chemical structures of active components and pro-neurogenic effect.

Abstract of assessment result

【Review】

The applicant presents extensive work on the effect of sugarcane top ethanolic extract (STEE) and its chemical constituents on the cognitive dysfunction. *In vivo* study and molecular analysis showed that STEE improved the spatial learning memory in a pathological aging model mice through the regulation of neurogenesis. Specifically, STEE exhibits the promoting effect on neuronal differentiation by regulating the expression of some transcription factors. The applicant also identified natural polyphenols including caffeoylquinic acid derivatives and isoorientin as active components of STEE. These compounds showed the synergistic effect on neuronal differentiation and astrocyte maturation in human neuronal stem cells (hNSCs). The possible roles of each compound in the differentiation of hNSCs were also demonstrated. The effects of STEE and its active components could counteract the age-related decline in neurogenesis and morphological deficits in astrocytes in brain and further prevent the neuronal and synaptic loss, which has been a challenge in treatment of cognitive dysfunction. The findings demonstrated in the thesis add to the knowledge on the potential of sugarcane top extract as a new material of functional foods for the prevention of aging-related neurodegenerative diseases.

【Result】

The final examination committee conducted a meeting as a final examination on 18/1/2022. 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 Food Innovation.