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学位論文題目　Characterization and Identification of Antioxidant and Immunomodulatory Activities of Polysaccharides Extracted from *Helicteres angustifolia* L.

（*Helicteres angustifolia* L.から多糖類の特性及びそれらの抗酸化と免疫活性）

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論　文　の　要　旨
Abstract of thesis

Helicteres angustifolia L. is a traditional medicinal herb widely distributed in China, Japan, and Laos. It has long been used as remedy against various types of illness, such as diabetes, inflammations and fever. Previous works demonstrated that polysaccharide rich fraction of this plant exhibit significant antioxidant activity and play a major role in the immunomodulatory of this plant. However, until now, no further identifications. Thus, proper isolation of native bioactive polysaccharides from *H. angustifolia*, evaluate the antioxidant and immunomodulatory activity both *in vitro* and *in vivo*, and determination of their structural features are the main targets of this study.

The dissertation is divided into 5 chapters.

In chapter 1, the author gave a detailed introduction on the structures and bioactivities of polysaccharides from natural sources, then gave a literature review on the phytochemical composition and pharmacological activities of *H. angustifolia*. Then the author proposed research objectives at the end of this chapter, and the methods and key contents of each chapter were also presented.

In chapter 2, the author used hot water extraction method to extract crude polysaccharide (HACP) from *H. angustifolia*. The *in vivo* immune enhancement ability of HACP was then identified by using 4T1 breast tumor model in BALB/c mice. After orally administering HACP to 4T1 tumor-bearing mice for continuous 15 days, the mice immune response was found significantly ($p < 0.05$) improved. Especially, 300 mg/kg of HACP treatment increased mouse spleen indices, $CD4^+$ and $CD8^+$ ratios about 1.9, 2.0, and 1.4 times compared to the tumor control group. In addition, HACP treatment also enhanced mouse serum TNF-α production in a dose-dependent manner with the highest level (452.7 ± 85.79 pg/mL) achieved at 300 mg/kg group, which was significantly higher than the tumor control group (102.86 ± 48.46 pg/mL). Furthermore, HACP application was found effectively improved the activities of antioxidant enzymes CAT and SOD in the serum of 4T1 tumor-bearing mice, indicating its potent antioxidant activities. Finally, the increased antioxidant activity and immune response resulted in a reduced tumor weight and lung metastasis, with maximum inhibition rates of 67.7% and 13.7%, respectively.

In chapter 3, HACP was purified stepwise successfully with DEAE Sepharose Fast Flow and Sephacry S-400

chromatography. Three major bioactive fractions were then fractionated and purified from HACP, yielding 7.4%, 6.9% and 9.8% of the total amount of HACP, respectively. Physicochemical analysis demonstrated that SPF1-1, SPF2-1, and SPF3-1 were acidic polysaccharides with uronic acids of 12.70 ± 1.80%, 26.92 ± 2.81%, and 58.77 ± 1.64%, respectively. The main sugar component of SPF1-1 was glucose. The main neutral sugars of SPF2-1 were arabinose and glucose, and for SPF3-1 were rhamnose (Rha), arabinose (Ara) and galactose (Gal). And the molecular weight (*Mw*) of SPF1-1, SPF2-1, and SPF3-1 were determined to be 279 kDa, 15.69 kDa, and 13.36 kDa, respectively.

In chapter 4, the author compared antioxidant and immunomodulatory activities of three SPF fractions *in vitro* conditions. Results demonstrated that among the three SPFs, significant high arabinose and relative higher galactose and xylose levels in SPF3-1 were found highly correlating with DPPH radical inhibition effect (maximum inhibition rate of 75%), reducing power (maximum OD value of 0.72) and metal chelating activity (maximum chelating rate of 63%). *In vitro* immunomodulatory assay demonstrated that the fraction SPF3-1 with the highest uronic acid content (58.8%) and lowest molecular weight (13.36 kD) was proven to possess the highest immunomodulatory activities, this observation was attributable to the significant ($p < 0.05$) proliferation (193% at 100 μg/mL) and phagocytic capacity of macrophage cells. In addition, remarkably releases of IL-6 and TNF-α were detected in SPF3-1 treated cells in a dose-dependent manner. Furthermore, SPF3-1 was found to possess obvious induction effect on lymphocytes proliferation, with the highest viability increased to 196% after 100 μg/mL of SPF3-1 treatment for 48 h, indicating its potent lymphocyte activation function. Moreover, 100 μg/mL of SPF3-1 treatment efficiently promoted lymphocytes IL-6 and IFN-γ release (117.3 and 63.6 pg/mL), significantly higher than the control groups (36.8 pg/mL and 21.0 pg/mL).

In chapter 5, conclusions of this study were summarized. All the results proved that polysaccharides from *H. angustifolia* exhibited potent antioxidant and immunomodulatory activity both *in vitro* and *in vivo*. Thus, polysaccharides from *H. angustifolia* could be further developed as new products for medicines or functional foods.

審 査 の 要 旨
Abstract of assessment result

This research was the first time to confirm the antioxidant and immunomodulatory activities of polysaccharides from *H. angustifolia* both *in vitro* and *in vivo* conditions. In this study, the author used breast cancer mouse model to test immune enhancement abilities of HACP, the results obtained were satisfactory as it could significantly increase mouse immune organ indices, spleen lymphocytes $CD4^+$ and $CD8^+$ ratios, serum immune cytokines levels, and reduce 67.7% and 13.7% of tumor progression and lung metastasis. In order to further purify and clarify the structure/activity relationships, the author then used DEAE Sepharose Fast Flow and Sephacry S-400 chromatography to isolate the major bioactive polysaccharide fractions of HACP. Three major fractions SPF1-1, SPF2-1, and SPF3-1 were purified with yields of 7.4%, 6.9% and 9.8% of the total amount of HACP, respectively. Phytochemical analysis indicated that three SPFs were typical pectic polysaccharides which contain similar types of monosaccharide, while differed in their contents. Pharmacological investigation suggested that fraction SPF3-1 with relatively higher arabinose, galactose, xylose, and uronic acid contents, and lower molecular weight was demonstrated to be responsible for stronger antioxidant and macrophages stimulation activities. This study is the extension of our previous study and provides a valuable and practical evidence for new pharmaceutical and functional applications of *H. angustifolia.* In addition, the discussion is excellent, and the structure of thesis is sound. Overall, the contents of the thesis meet the quality of Ph.D. thesis.

The final examination committee conducted a meeting as a final examination on 20 July 2018. 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.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.