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学位論文題目	Study on the Biological Functions of Aronia melanocarpa Fruit Extract （アロニア果実抽出物の生理機能に関する研究）
論文審査委員	主査　教　授　新井　博文 准教授　宮﨑　健輔 准教授　邱　泰瑛 准教授　霜鳥　慈岳 准教授　フォン　チャオフイ

学位論文内容の要旨

Allergy is an excessive immune response to normally harmless environmental substances, such as pollen, house dust mites, and food. Type I allergy is primarily mediated by mast cells and basophils through the cross-linking of allergen specific immunoglobulin E (IgE) bound to the high-affinity IgE receptor (FcεRI). Antigen stimulation activates intracellular signaling pathways, including protein tyrosine phosphorylation and calcium influx, resulting in mast cell degranulation and the release of chemical mediators such as histamine and leukotrienes. These mediators induce allergic symptoms through vasodilation, increased vascular permeability, smooth muscle contraction, and recruitment of inflammatory cells. Therefore, suppression of mast cell activation and chemical mediator release is considered an effective strategy for the prevention and alleviation of allergic diseases.

Aronia melanocarpa (black chokeberry) is a deciduous shrub of the Rosaceae family that is widely cultivated in Hokkaido, Japan. Its fruits are rich in polyphenolic compounds and have been reported to possess antioxidant and anti inflammatory properties. Representative polyphenols in A. melanocarpa include procyanidin B2, cyanidin, chlorogenic acid, epicatechin, quercetin, quercetin glycosides, and rutin. However, the anti-allergic effects of A. melanocarpa fruit and the underlying mechanisms regulating mast cell activation remain unclear. Therefore, this study aimed to investigate the anti-allergic effects of A. melanocarpa fruit extract and its polyphenolic constituents using cultured mast cell models.

Fresh fruits of A. melanocarpa harvested in Hokkaido were extracted with 70% ethanol and fractionated by column chromatography using a Diaion HP-20 column. Total polyphenol content was determined using the Folin–Ciocalteu method, and antioxidant activity was evaluated by the DPPH radical-scavenging assay. Anti allergic activity was examined using RBL-2H3 and PB-3c mast cell lines. Degranulation was evaluated by measuring β-hexosaminidase release, and histamine and leukotriene B4 (LTB4) production were quantified by HPLC.

Intracellular calcium mobilization was analyzed using the fluorescent probe Fluo-4 AM. Protein tyrosine phosphorylation and Syk phosphorylation were evaluated by Western blot analysis, and 5-lipoxygenase (5-LOX) mRNA expression was quantified by RT-qPCR. In addition, representative polyphenolic compounds present in A. melanocarpa were examined to identify the bioactive constituents responsible for the anti-allergic effects.

Among the chromatographic fractions, Fr. 5, eluted with 80% methanol, exhibited the highest total polyphenol content and antioxidant activity. Fr. 5 significantly inhibited antigen-induced β-hexosaminidase and histamine release from RBL-2H3 cells. Furthermore, Fr. 5 suppressed LTB4 production in PB-3c mast cells in a concentration-dependent manner. Mechanistic analyses demonstrated that Fr. 5 inhibited antigen-induced intracellular Ca2+ influx, reduced protein tyrosine phosphorylation and Syk phosphorylation, and significantly suppressed 5-LOX mRNA expression. These results indicate that Fr. 5 suppresses FcεRI-mediated intracellular signaling pathways involved in mast cell activation.

HPLC analysis of Fr. 5 identified chlorogenic acid, procyanidin B2, epicatechin, quercetin, and cyanidin as representative polyphenolic constituents. Eight representative polyphenols, namely procyanidin B2, cyanidin, chlorogenic acid, epicatechin, quercetin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, and rutin, were evaluated for their anti-allergic activities. Among these compounds, quercetin exhibited the most potent inhibitory effects on β-hexosaminidase release, histamine release, and LTB4 production. Quercetin also suppressed Syk phosphorylation, reduced intracellular Ca2+ mobilization, and inhibited 5-LOX mRNA expression, showing effects comparable to those of Fr. 5.

These findings demonstrate that A. melanocarpa fruit extract possesses potent anti-allergic activity through suppression of mast cell activation and chemical mediator release. The underlying mechanism involves inhibition of FcεRI-mediated intracellular signaling pathways, including Syk phosphorylation, intracellular Ca2+ mobilization, and 5-LOX expression. Quercetin was identified as one of the major bioactive polyphenols contributing to these effects. Collectively, these results suggest that A. melanocarpa fruit may serve as a promising functional food material for the prevention and alleviation of type I allergic diseases. Furthermore, the present study provides scientific evidence supporting the value added utilization of A. melanocarpa cultivated in Hokkaido and may contribute to the development of functional foods based on regional agricultural resources.

審査結果の要旨

花粉症をはじめとするⅠ型アレルギーでは、抗原抗体反応により肥満細胞が活性化され、ケミカルメディエーターが放出されることでアレルギー症状が発現する。現在、その抑制は主として薬剤による対症療法に依存しているが、副作用の少ない食品由来成分による症 状緩和が期待されている。アロニア（Aronia melanocarpa）は北海道でも栽培されている果実であるが、その生理機能は十分に解明されていない。本研究では、アロニア果実抽出物の抗アレルギー効果および作用機序を培養細胞を用いて検討した。

その結果、ポリフェノールを豊富に含む画分（Fr.5）は、肥満細胞からのケミカルメディエーター放出を抑制し、その作用には細胞内シグナル伝達の抑制が関与することが示唆 された。また、Fr.5 に含まれるポリフェノールのうち、ケルセチンが抗アレルギー活性に 寄与する主要成分の一つであることが示された。

以上の結果から、アロニア果実由来ポリフェノールは肥満細胞の活性化を抑制し、Ⅰ型アレルギー反応の抑制に寄与する可能性が示された。本研究はアロニアの新たな機能性を科学的に明らかにしたものであり、アロニアの高付加価値化や北海道産農産物を活用した機能性食品の開発に貢献することが期待される。本研究成果は新規性および学術的価値を有し、その内容は査読付国際学術誌 Bioscience, Biotechnology, and Biochemistry に掲載が承認されている。

以上のことから、Ganbayar Ganbold 氏は北見工業大学博士（工学）の学位を授与されるに十分な資格を有するものと認められる。