



Title : Efficacy of Butterfly Pea Anthocyanins for Supporting Microbial Balance and Protecting Intestinal Epithelial Cells from Infection

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ABSTRACT

This research aimed to investigate the chemical stability of anthocyanin extract from butterfly pea (*Clitoria ternatea* L.) and evaluate its biological activities concerning the gastrointestinal tract environments, probiotics growth, and the protection of intestinal epithelial cells from pathogenic infection. This study aimed to evaluate the chemical stability of anthocyanin extract from butterfly pea using UV-Visible absorption spectrum analysis. Consequently, delphinidin-3-glucoside chloride was employed as the standard compound to quantify stability and detect concentration changes under various conditions. The results revealed that simulated digestion by amylase, trypsin, bile salts, and acid were increased the content of delphinidin-3-glucoside chloride comparing to the control group (0.767 mg/ml). After 5 hours of incubation, the extract demonstrated high stability, with the highest contents detected under acidic conditions (2.397 mg/ml) and bile salt (1.974 mg/ml). Moreover, cytotoxicity testing on HT-29 intestinal cells showed that the anthocyanin extract at a concentration of 0.625 mg/ml was non-toxic, with a cell viability rate of 97.16%. Furthermore, the extract at IC₅₀ concentration of 1.25 mg/ml was selected to study the efficacy for stimulating probiotic growth and promoting intestinal cell survival during pathogenic bacteria infection. Regarding probiotics adhesion efficiency, the extract at 0.625 mg/ml showed the greatest potential for enhancing the adhesion of *Lactocaseibacillus paracasei* and *Bifidobacterium animalis* subsp. *lactis* on HT-29 intestinal cells, with adhesion rates of 35.33% and 36.00%, respectively. Additionally, the combinations of anthocyanin extract (0.625 mg/ml) with *L. paracasei* and *B. animalis* subsp. *lactis* were effectively prevented cellular damage from *Escherichia coli* ATCC 25922 infection and increased the survival rate of HT-29 intestinal cells by 134.48% and 179.97%, respectively. Probiotic and pathogenic strains were co-cultivated at a ratio of 1:2 in the

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presence of anthocyanin extract for 24 hours. The co-culture assay results showed that the anthocyanin extract (0.625 and 1.25 mg/ml) had ability to balance the numbers of probiotics (*L. paracasei* and *B. animalis* subsp. *lactis*) and pathogen (*E. coli* ATCC 25922). These findings indicate that anthocyanin extract is stable under acidic and bile salt conditions, effectively supports probiotic adhesion to intestinal epithelial cells, helps maintain microbial balance, and protects the cells from bacterial pathogen infection.

Keywords: Anthocyanin, Butterfly Pea, Microbial Balance, Prebiotics, Probiotics