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

Pattra Jajumpa and Thida Kaewkod★

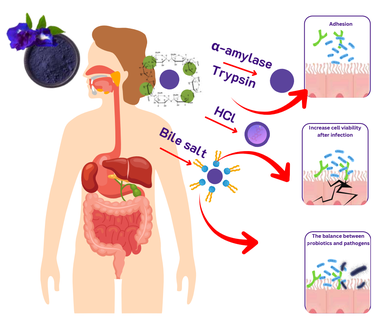
Microbiology, Department of Biology, Faculty of Science, Chiang Mai University, 50200 Thailand

★Email: thida.kaewkod@cmu.ac.th

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 *Lacticaseibacillus 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 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.

Introduction



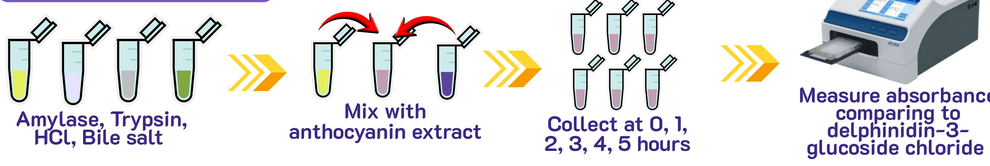
Probiotics are living microorganisms that have a positive impact on health, particularly on the digestive system. Prebiotics are present in plants and can promote probiotics for human health. Anthocyanins are water-soluble pigments, abundantly present in red, purple, and blue colors in many fruits, vegetables, flowers, and leaves. This study investigated the stability of anthocyanin extract from butterfly pea on the digestive processes and their potential as a prebiotic agent to enhance probiotic adhesion and protect intestinal cells against enteric pathogenic bacteria.

Objectives

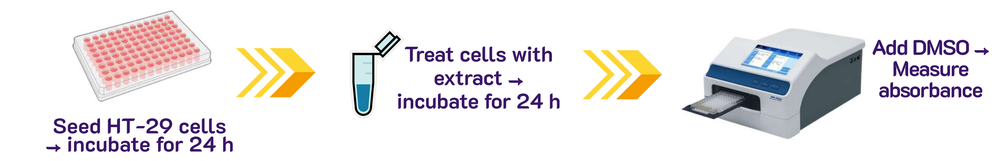
- To study the chemical stability of anthocyanin extract from butterfly pea against degradation by amylase, trypsin, bile salts, and acidic conditions.
- To evaluate the anthocyanin extract for stimulating the adhesion of probiotics to HT-29 colon cells.
- To investigate the survival of HT-29 colon cells when pathogenic bacterial infection and treatment with a combination of anthocyanin extract and probiotics.
- To examine the effects of anthocyanin extract for promoting probiotic growth and inhibiting gastrointestinal pathogens.

Methodology

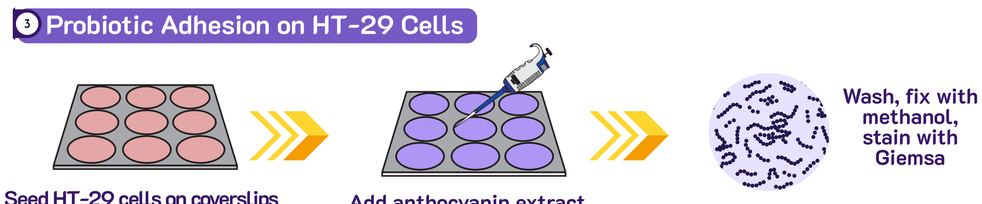
1 Chemical Stability



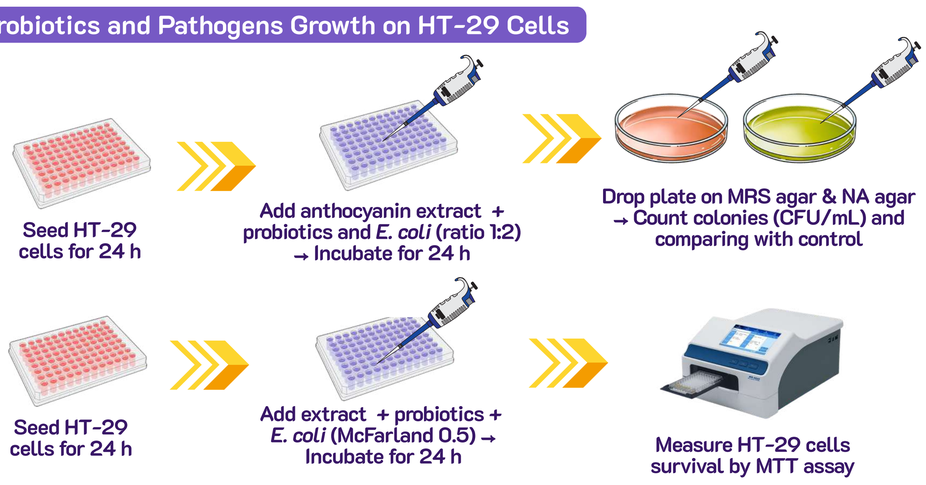
2 Cell Viability (HT-29 Cells)



3 Probiotic Adhesion on HT-29 Cells



4 Probiotics and Pathogens Growth on HT-29 Cells



Results

1 The Chemical Stability of Anthocyanin Extract

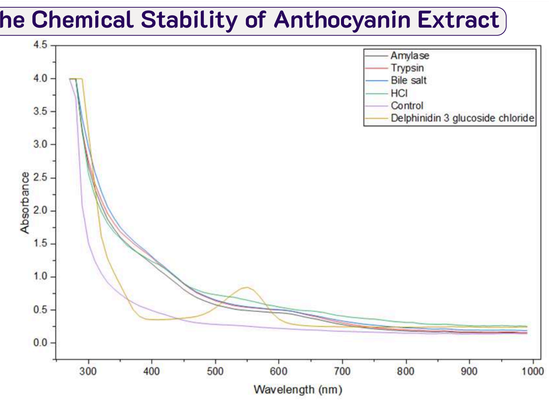


Figure 1 : UV–Vis spectra of delphinidin-3-glucoside from anthocyanin extract after 5 hours of amylase, trypsin, bile salt, and acidic in comparison to delphinidin-3-glucoside chloride standard.

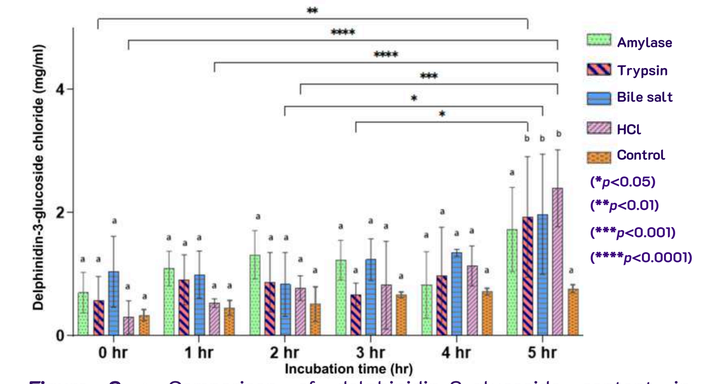


Figure 2 : Comparison of delphinidin-3-glucoside content in anthocyanin extract during digestion with amylase, trypsin, bile salts, and acidic acid over a period of 0, 1, 2, 3, 4, and 5 hours. Different letters (a, b) indicate statistical significance ($p < 0.05$)

2 Cytotoxicity of Anthocyanin Extract on HT-29 Cell Viability

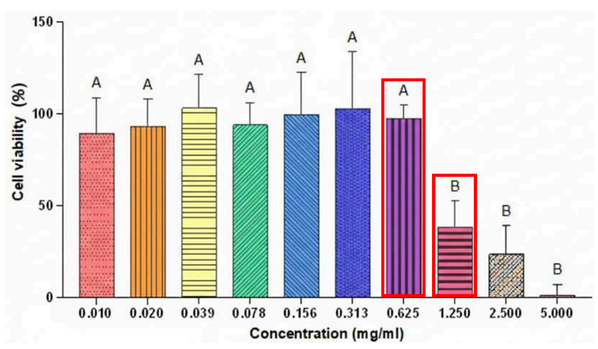


Figure 3: HT-29 cell viability after exposure to various concentrations of anthocyanin extract for 24 hours. Different letters (A, B) indicate highly significant differences ($p < 0.001$)

3 Anthocyanin Extract on Stimulating Probiotics Adhesion to HT-29 Cells

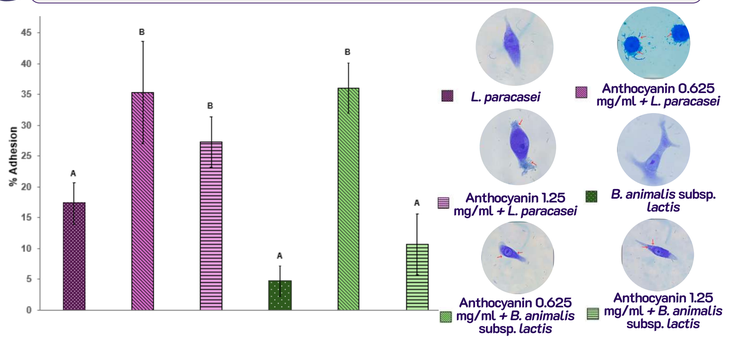


Figure 4: The effects of anthocyanin extract on the stimulation of probiotics adhesion to HT-29 cells. Different letters (A, B) indicate statistically significant differences ($p < 0.05$)

4 Survival of HT-29 Cells Against Pathogenic Infection Under Anthocyanin Extract Combined with *L. paracasei*

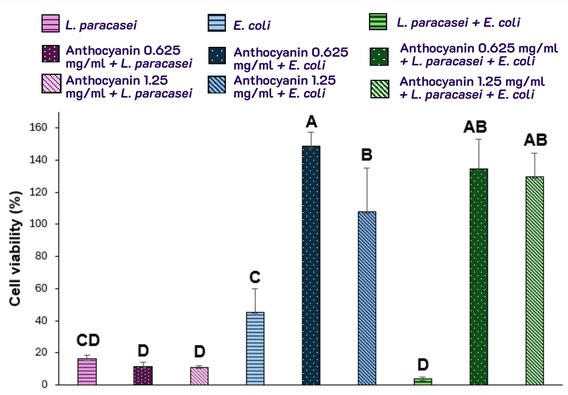


Figure 5 : Survival rates of HT-29 cells following *E. coli* infection under combined with anthocyanin extract and *L. paracasei*. Different letters (A–D) indicate highly significant differences ($p < 0.001$)

5 Survival of HT-29 Cells Against Pathogenic Infection Under Anthocyanin Extract Combined with *B. animalis* subsp. *lactis*

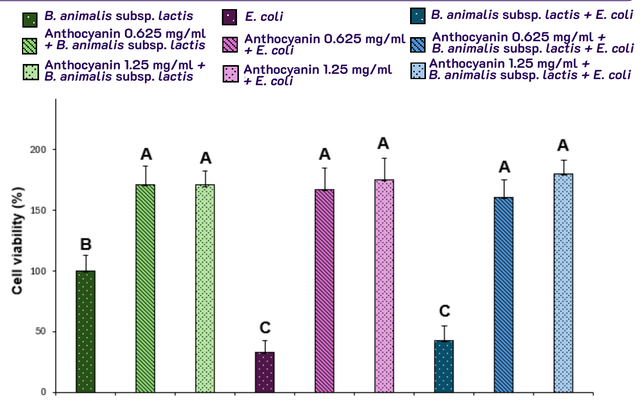


Figure 6 : Survival rates of HT-29 cells following *E. coli* infection under combined with anthocyanin extract and *B. animalis* subsp. *lactis*. Different letters (A–C) indicate highly significant differences ($p < 0.0001$)

6 The Growth of *L. paracasei* and *E. coli* ATCC 25922 in HT-29 Cells and Treatment with Anthocyanin extract

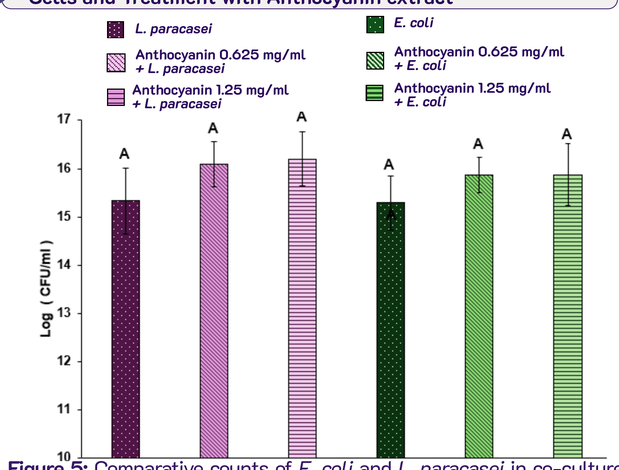


Figure 5: Comparative counts of *E. coli* and *L. paracasei* in co-culture with anthocyanin extract. Different letters indicate statistically significant differences ($p < 0.05$)

7 The Growth of *B. animalis* subsp. *lactis* and *E. coli* ATCC 25922 in HT-29 Cells and Treatment with Anthocyanin extract

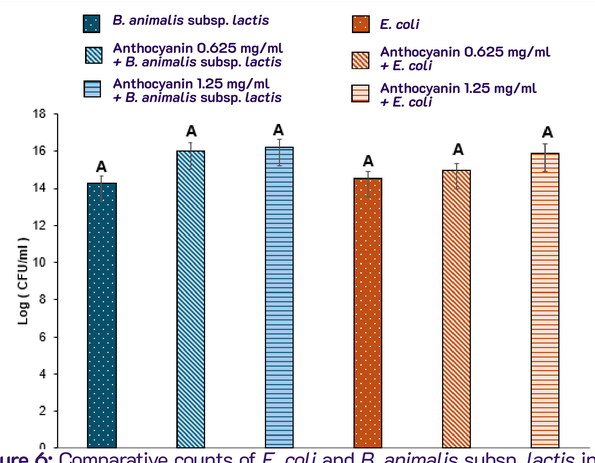


Figure 6: Comparative counts of *E. coli* and *B. animalis* subsp. *lactis* in co-culture with anthocyanin extract. Different letters indicate statistically significant differences ($p < 0.05$)

Conclusion

- Anthocyanin extract from butterfly pea showed maximum stability in acidic conditions (2.397 mg/ml), followed by bile salt exposure (1.974 mg/ml) compared to delphinidin-3-glucoside standard compound.
- The anthocyanin extract at 0.625 mg/ml significantly promoted the adhesion of *L. paracasei* (35%) and *B. animalis* subsp. *lactis* (36%) to HT-29 cells.
- The extract effectively protected HT-29 cells from *E. coli* infection by maintaining cell viability at 161%.
- In co-culture system, anthocyanin extract had ability to balance the numbers of probiotics and pathogen.

References

- Sun, X.-h., Zhou, T.-t., Wei, C.-h., Lan, W.-q., Zhao, Y., Pan, Y.-j., & Wu, V. C. H. (2018). Antibacterial effect and mechanism of anthocyanin rich Chinese wild blueberry extract on various foodborne pathogens. Food Control, 94, 155-161.
- Wallace, T. C., & Giusti, M. M. (2015). Anthocyanins. Advances in Nutrition, 6(5), 620-622.

Acknowledgements

This research was supported by the Fundamental Fund 2026, Chiang Mai University. The authors would like to thank the members of 2711 Microbiology and the Department of Biology, Faculty of Science, Chiang Mai University