Comparative analysis of flavonoid composition and expression of flavonoid biosynthesis enzymes in *Clitoria ternatea* varieties

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Clitoria ternatea is a medicinal plant valued for its flavonoid profile and pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. Although the blue-flowered variety, characterized by anthocyanins such as delphinidin derivatives, has been studied extensively, biochemical and molecular differences among flower colour variants remain less understood. This study aims to compare flavonoid composition and expression of key biosynthetic enzymes: dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonol synthase (FLS) in blue, purple, and white-flowered varieties. Flavonoids were extracted from flowers and leaves and quantified via HPLC using authenticated standards of myricetin, quercetin, kaempferol, luteolin, apigenin, delphinidin, and cyanidin. Gene-specific primers for DFR, ANS, and FLS were designed, and relative expression was analyzed by RT-qPCR using the Livak method, with the white variety as calibrator due to its minimal pigmentation. Reference gene primers were adapted from a validated study and verified against the *C. ternatea* genome (PRJNA1016062; accession KAK7264054.1). Myricetin and kaempferol were more abundant in leaves, whereas quercetin and luteolin were higher in flowers. Quercetin content was highest in the white variety and lowest in the blue. Apigenin predominated in leaves, delphinidin was exclusive to blue flowers, and cyanidin was absent in all samples. DFR was upregulated in blue (18.176-fold) and purple (4.257-fold) relative to white flowers. ANS was upregulated in blue (2.488-fold) but downregulated in purple (0.595-fold) flowers. FLS remained relatively stable (0.765 and 0.883-fold in blue and purple flower varieties respectively) and slightly downregulated across all variants. Due to limited reagents and time, p-values were not generated. Blue flowers exhibit high ANS and DFR expressions, which promotes the production of anthocyanin, particularly delphinidin, responsible for their blue coloration. Purple flowers exhibit moderate DFR but downregulated ANS, indicating alternative pigment or regulatory pathways despite visible pigmentation. White flowers display higher FLS expression, favouring flavonol synthesis over anthocyanins, consistent with their elevated flavonol content, highlighting gene-specific contributions to flower colour variation.

Keywords: *Clitoria ternatea, Flavonoids, Dihydroflavonol 4-reductase, Anthocyanidin synthase, Flavonol synthase*