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# Effect of Precursors on Flavonoid Production in Pegaga Cell Suspension Cultures

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**Abstract**

The present study aimed to investigate the effect of precursors on flavonoid production in cell of the elite C. asiatica accession UPM03. The effect of phenylalanine and tyrosine on enhancing the flavonoid production were examined. The results obtained showed that the intracellular flavonoid content increased by a 11.5-fold and 3.2-fold with the feeding of 60mg/L phenyalanine and 40mg/L tyrosine, respectively. The addition of 60mg/L phenylalanine and 40mg/L tyrosine have successfully produced increased amount of flavonoid in the cell.

## Introduction

In Malaysia, *Centella asiatica* is identified as an important medicinal herb and it is grown commercially. This herbaceous plant has widely been used for wound healing, memory improvement, treatment for mental fatigue, bronchitis, asthma, dysentery, leucorrhoea, kidney problem, urethritis, anti-allergic and anti-cancer purposes (Zainol *et al*., 2003). The different uses claimed for this plant are reported to be mainly due to its high content of secondary metabolites especially flavonoids and triterpenoids (Zheng and Qin, 2007). One of the problems associated with the commercial production of C. asiatica is the current conventional propagation using vegetative cuttings, which is very slow and does not fulfil the demands of herbal industries. Thus, a sustainable supply of the bioactive secondary metabolite has been listed as the most important consideration.

## Materials and Methods

Two precursors, namely phenylalanine and tyrosine were used in this experiment. Both the precursors were filter sterilized using 0.20 µm cellulose acetate minisart (Sartorius, Germany), before adding them separately into the autoclaved media. The supplementation of the sterile precursors was carried out at the time of inoculation of cells. The twelve day old cells were dried at 50°C until the dry weight become constant, prior to grinding using pestle and mortar. The pooled samples (0.1 ± 0.02 g) from each treatment were extracted and flavonoid contents were analyzed as stated in Tan *et al.* (2010). The data were analyzed using the one-way ANOVA. Meanwhile, the mean values were compared by the Duncan's multiple range test at 5 % ($p = 0.05$) significance level, using software SPSS version 11.5 (SPSS Inc. USA).

## Results and Discussion

The addition of phenylalanine in the range of 20 mg/L and 100 mg/L showed negative effects on the cell growth, but it demonstrated a positive effect on the intracellular flavonoid biosynthesis (Figure 1). The control cultures (without any precursor) have higher fresh and dry weight but lower flavonoid content in the cell cultures. This might be due to the addition of precursors that have induced stress to the cell by suppressing the growth and thus promoting the flavonoid synthesis. Tyrosine at 40 mg/L has been shown as an optimal concentration for flavonoid biosynthesis (Figure 2). The content of

flavonoid in cell reached 9.86 mg/g DW, an increase by 3.2-fold. The feedback inhibition of tyrosine seemed to have occurred when the concentration of tyrosine exceeded 40 mg/L.

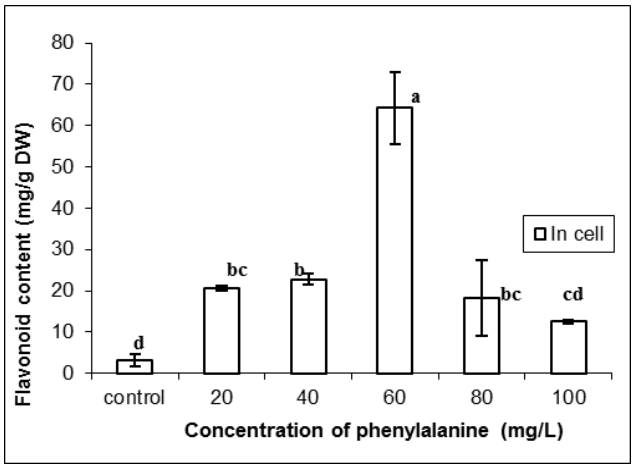


**Figure 1: The effects of different concentrations of phenyalanine on the flavonoid production in the cell of *C. asiatica* accessions UPM03.**

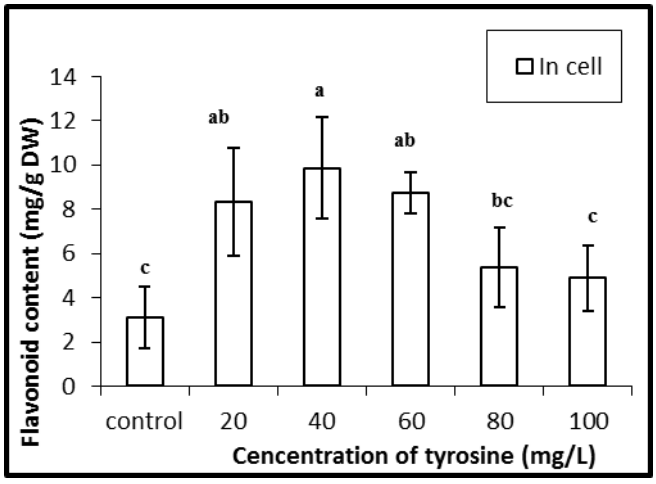


**Figure 2: The effects of different concentrations of tyrosine on flavonoid production in the cell of *C. asiatica* accessions UPM03.**

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