Production of Phenolic Antioxidants in Callus Cultures of *Glycyrrhiza glabra*

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ABSTRACT

Glycyrrhiza glabra is a rich source of phenolic antioxidants licoisoflavone B, licochalcone A and liquirtigenin. These compounds have a high industrial value and exhibit many therapeutic and pharmaceutical activities such as anti-inflammatory, anti-allergic, antitumor and antimicrobial. These compounds occur in minor quantities in the roots of field grown plants and their extraction requires complete uprooting of plant at mature stage, consequently leading to its complete loss. Thus, a protocol has been developed to produce these flavonoids in callus cultures of *Glycyrrhiza glabra* as a viable alternative. The process of solvent extraction of these flavonoids from callus tissue has also been standardized in order to get considerable yield. The research also aims at optimization of conditions for simultaneous extraction of all the three phenolic compounds. Callus cultures were established on MS medium supplemented with 1mg/L NAA and 0.5mg/L BAP, 20mg/L ascorbic acid and 10 mg/L citric acid. Ethanolic extracts of callus were prepared using heat stirred reactor. Optimization was done by L16 orthogonal design of experiment for five factors - solvent concentration, temperature, extraction time, material ratio and number of extraction cycles. The effect of most influencing factors on the yield of flavonoids was studied through response surface methodology. All the three phenolic compounds were successfully produced in callus cultures of *Glycyrrhiza glabra* and their yield was found higher than roots for licochalcone A and liquirtigenin (15 fold and 22.7 fold respectively), while licoisoflavone B was produced in almost similar quantities. The most effective combination of extraction conditions for simultaneously extraction of these phenolic antioxidants was found to be temperature 85°C, 4 hours extraction time, 70% ethanol concentration, 1:30 material ratio and 4 times of extraction cycles. All the three compounds also exhibited high antioxidant potential as evaluated by their DPPH radical scavenging activity of which licochalcone A showed free radical scavenging as high as 78.9%.

Keywords: Phenolic antioxidants Licochalcone A, Liquirtigenin, Licoisoflavone B, *Glycyrrhiza glabra,* callus culture.

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