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Optimization of extraction methods for phenolic and antioxidants activity in *Berberis asiatica* fruits using response surface methodology

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Wild fruits are known to play significant role in preventing free radicals mediated diseases. This property is largely due to the phenolic and other metabolites present in the fruits which are reported to have strong antioxidant activity. Considering to this study was designed to optimize the extraction of phenolic and antioxidants in *Berberis asiatica* fruits using response surface methodology (RSM). Solvent selection was done based on the preliminary experiments and a three-level-five-factor, Central Composite Design (CCD) consisted of 46 experiments was conducted to analyze the effect of extraction temperature (X_1: 30-80°C), extraction time (X_2: 30-90 min), sample to solvent ratio (X_3: 1:10-1:50), pH of the solvent (X_4: 3-5) and solvent concentration (X_5: 20-80%) forextraction of total phenolic content (TPC), total anthocyanin content (TAC), total tannins content (TTC) and antioxidant activity [2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS)]. Results revealed that extraction temperature (X_1), sample to solvent ratio (X_3) and solvent concentration (X_5) significantly influenced response variables and independent variables. The regression coefficient (R^2) was found satisfactory for all the models. The lack of fit was found non-significant ($p>0.05$) for TPC, TAC and TTC indicating that the models could adequately fit the experimental data. Response surface analysis showed that under optimal extraction conditions the phenolic antioxidant extraction maximized. These values are in accordance with the predicted values indicating the success of RSM in optimizing the extraction conditions. This method can be used further for scaling up nutraceutical and pharmaceutical applications and also can be implemented in other fruits of the region for harnessing their potential in commercial sector.

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Overexpression of a tea flavanone 3-hydroxylase gene confers tolerance to salt and *Alternaria solani* stress in transgenic tobacco

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Flavan-3-ols are the major flavonoids present in tea (*Camellia sinensis*) leaves. These are known to have antioxidant and free radical scavenging properties *in vitro*. Flavanone 3-hydroxylase is considered to be an important enzyme of flavonoid pathway leading to accumulation of flavan-3-ols in tea. Expression analysis revealed the up-regulation in transcript levels of *C. sinensis* Flavanone 3-hydroxylase (CsF3H) encoding gene under salt stress. In this study, the biotechnological potential of CsF3H was evaluated by gene overexpression in tobacco (*Nicotiana tabacum* cv. Xanthi). Overexpressed transgenics were found to accumulate more flavan-3-ols and were observed for their tolerance against salt stress and fungus *Alternaria solani* infection. An increased primary root length, number of lateral roots, chlorophyll content, antioxidant enzyme expression and their activities, higher degree of pectin methyl esterification, lower electrolyte leakage and malondialdehyde content may be responsible for salt stress tolerance in CsF3H overexpressed transgenic tobacco plants. The effect of flavan-3-ols on pectin methyl esterification under salt stressed conditions was further validated through *in vitro* experiments in which non-transgenic (wild) tobacco seedlings were exposed to salt stress in presence of flavan-3-ols, Epicatechin and Epigallocatechin. The *in vitro* exposed seedlings showed similar trend of increase in pectin methyl esterification through decreasing PME activity as observed in CsF3H transgenic lines. Taken together, overexpression of CsF3H provided tolerance to salt stress and fungus *A. solani* infection to transgenic tobacco through improved antioxidant system and enhanced pectin methyl esterification.

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