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Analysis of L-citrulline and L-arginine in *Ficus deltoidea* leaf extracts by reverse phase high performance liquid chromatography

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Background: Ficus deltoidea (FD) is one of the native plants widely distributed in several countries in Southeast Asia. Previous studies have shown that FD leaf possess antinociceptive, wound healing and antioxidant properties. These beneficial effects have been attributed to the presence of primary and secondary metabolites such as polyphenols, amino acids and flavonoids. Objective: The aim was to develop a reverse phase high-performance liquid chromatography method with ultraviolet detection that involves precolumn derivatisation with O-phthaladehyde for simultaneous analysis of two amino acids L-citrulline and L-arginine in FD leaf extracts. Materials and Methods: An isocratic elution program consisting of methanol: acetonitrile: Water at 45:45:10 v/v (solvent A) and 0.1 M phosphate buffer pH 7.5 (solvent B) at A: B v/v ratio of 80:20 on Zorbax Eclipse C18 SB-Ag column (250 × 4.6 mm, 5 µm) were used. The flow rate was set at 1 ml/min and detection was carried out at 338 nm with 30 min separation time. Results: Good linearity for L-citrulline and L-arginine was obtained in the range 0.1-1000 μ g/ml at $R^2 \ge 0.998$. The limit of detection and limit of quantification values for both L-citrulline and L-arginine were 1 and 5 µg/ml, respectively. The average of recoveries was in the range 94.94-101.95%, with relative standard deviation (%RSD) less than 3%. Intra- and inter-day precision was in the range 96.36-102.43% with RSD less than 2%. Conclusion: All validation parameters of the developed method indicate the method is reliable and efficient for simultaneous determination of L-citrulline and L-arginine for routine analysis of FD.

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