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IDENTIFICATION AND QUANTIFICATION OF PODOPHYLLOTOXIN FROM PODOPHYLLUM HEXANDRUM BY ESI-LC/MS/MS

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ABSTRACT

An ESI-LC/MS/MS method for the quantification of podophyllotoxin from Podophyllum hexandrum population from Sangla valley of Kinnaur, Himachal Pradesh, India in the Northern Himalayas was developed. Qualification of podophyllotoxin was performed by thin layer chromatography (TLC) with R_f values of 0.85 (leaf) and 0.94 (root) when compared with the standard podophyllotoxin. UV-VIS spectrophotometeric studies showed electronic absorption at λ_{max} value 284 nm. Gradient chromatographic separation of Podophyllotoxin was performed on a YMC-Pak Pro C-18 : 4.6mm × 250 mm, HPLC column, packed with 1.5 µm particles equipped with a 0.7m pre-filter (Supleco), using mobile phase methanol/water (1/1, v/v) and 100% methanol, both containing 0.1% ammonium hydroxide (25%) and 10 m mol ammonium acetate (pH 9). Structural information from the tandem mass spectrometric data compared with those of lignin markers already reported for the herb established the quantitative high yield of pophyllotoxin. The method could be employed in the absence of reference standards for the marker and was particularly useful in view of the scarcity of the chemical standard of podophyllotoxin.

KEYWORDS: Podophyllotoxin; Podophyllum Hexandrun; ESI-LC/MS/MS; Tandem Mass Spectrometry

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