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Short Communication

Isolation and Characterization of Aryltetraline Type Lignan from Roots of *Podophyllum Emodi*

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ABSTRACT

Podophyllotoxin is a bioactive aryltetraline lignan obtained from roots of *Podophyllum emodi* Wall. *P. emodi* has significance in traditional medicine which possess cytotoxic and antitumor activities. Currently column chromatography is preferred method for isolation or purification of podophyllotoxin which is laborous and time consuming activity. The present research deals with the rapid and convenient method for isolation of podophyllotoxin from roots of *P. emodi*. The isolated compound was characterised by TLC, UV, IR, MS and NMR spectral analysis and purity of the isolated compound was ascertained by HPLC analysis.

Keywords: Podophyllotoxin, *Podophyllum emodi*, Isolation, HPLC.

INTRODUCTION

Podophyllotoxin is an bioactive lignan chemically known as l -hydroxy -2-hydroxymethyl -6,7- methylenedioxy -4-(3,4,5-tri-methoxyphenyl) -l,2,3,4-tetrahydronaphthalene -3- carboxylic acid lactone found in the plants of family Berberidaceae, Podophyllaceae, Cupressaceae, Linaceae and Polygalaceae. [(1)] *Podophyllum emodi* Wall. (Family: Berberidaceae) is a perennial herb distributed in higher parts of the Himalayan region and used in traditional medicine due to its cytotoxic and anti-tumor activity. Podophyllotoxin has been reported to be mainly used to prepare active analogues such as etoposide and teniposide. [(2-4)] Plant shows presence of a number of compounds like quercetin, 4-demethylpodophyllotoxin, podophyllotoxin glucoside, 4-dimethyl podophyllotoxin glucoside, kaempferol, picropodophylotoxin,deoxypodophyllotoxin, picropodophylotoxin, isopicropodophyllone, 4-demethyldeoxypodophyllotoxin,α-peltatin and β- peltatin. [(5)]

Podophyllin is a resin of *P.emodi* obtained from methanolic extract of roots which contains upto 32-60% of podophyllotoxin on dry weight basis. [(6,7)] Podophyllotoxin has been isolated from resin by chromatographic separation and purification techniques. Silica gel chromatography helps in eliminating non-essential polyphenolic contaminents in order to achive highest purity podophyllotoxin. [(5)] The literature suggest that High

Performance Liquid Chromatography (HPLC) isolation technique leads to well resolved elution pattern of podophyllotoxin like compounds which required complicated gradient elution system. [8-11] Hence a rationale of this study is to develop rapid and convenient method for isolation of podophyllotoxin from *P. emodi* roots using solvent-solvent extraction method.

MATERIALS AND METHODS

Plant Material: Dried roots of *P. emodi* were procured from Yucca Enterprises, Mumbai, India, which were further authenticated. Voucher specimen (ICT/MNPRL/2014/PE-3) has been deposited at Medicinal Natural Products Research Laboratory, Department of Pharmaceutical Science and Technology, Institute of Chemical Technology, Mumbai, India.

Chemicals: Podophyllotoxin reference standard was procured from Sigma Aldrich, India. All the chemicals used for extraction and isolation were of Laboratory reagent grade and obtained from Rankem Limited, India. All the solvents used for HPLC analysis were of Analytical reagent grade and obtained from Rankem Limited, India.

Instrumentation: UV spectrum was recorded on Jasco V-530 spectrophotometer. IR spectra were recorded on Shimadzu instrument. Mass spectrum was recorded on Micromass Q-TOF/MS Mass Spectrometer (Varian Inc, Peabody, MA, USA). ^{1}H NMR spectra were recorded on a JOEL 400-MHz instrument (Varian Inc, Peabody, MA, USA) with an internal standard of tetramethylsilane.

HPLC analysis was performed with a Jasco (Hachioji, Tokyo, Japan) system consisting of an intelligent pump (PU-1580, PU-2080), a high-pressure mixer (MX-2080-31), a manual sample injection valve (Rheodyne 7725i) equipped with a 20 µL loop and a UV/Vis detector (UV-1575). HPLC column of 250 mm x 4.6 mm internal diameter, 5mm particle, Hibar LiChrocart Purospher star RP-18 (Merck, Darmstadt, Germany) was used. HPLC was performed at ambient temperature and data were analysed using Borwin software.

Method of Isolation: The dried and powdered roots of *P. emodi* (100g) were extracted with methanol (500 mL) in a *Soxhlet* apparatus for 8 h. The extract was filtered and concentrated to 1/4th of its original volume using distillation apparatus to obtained viscous liquid. 500 mL of 5% sodium carbonate (Na_2CO_3) aqueous solution was added to the above extract by sonicating at 40 Hz frequency, 100 W for 15 min, in ultra-sonication bath, to obtain precipitate. The above solution was filtered and the podophyllin precipitate obtained was dried in air. About 3.06 g of podophyllin obtained after drying was extracted with about 80 mL of ethyl acetate using reflux apparatus for 3 h. The extract was filtered and evaporated in pre-weighed evaporating dish on water bath. About 1.79 g of precipitate was obtained which was transferred in flask containing 50 mL 5% aq. Na_2CO_3 solution and refluxed for 1 hr. Na_2CO_3 solution turns yellow at the end of the process and buff color crude podophyllotoxin (about 1.2% of dry root) was obtained. Podophyllotoxin was recrystallized using methanol: chloroform (80:20). TLC and HPLC studies were carried out to determine the purity of the sample while the structure was elucidated and confirmed by UV, IR, MS and [1] H NMR spectral analysis.

RESULTS AND DISCUSSION

The objective of the study was to develop rapid and convenient method for isolation of podophyllotoxin from roots of *P. emodi*. Precipitation of podophyllin was achieved by reported method using distilled water and 5% aqueous solution of Na_2CO_3.

[7] Distilled water yielded mucilagenous precipitate whereas 5% Na_2CO_3 solution gave granular precipitate which was easy to dry and process further. Hence 5% aq. Na_2CO_3 solution was used for precipitation of podophyllin. Ethyl acetate was used for extraction of podophyllotoxin on the basis of good solubility of lignans and limited solubility of polyphenolic compounds in it. Further purification was done by refluxing dried ethyl acetate extract in 5% Na_2CO_3 solution to remove coloured phenolic impurities.

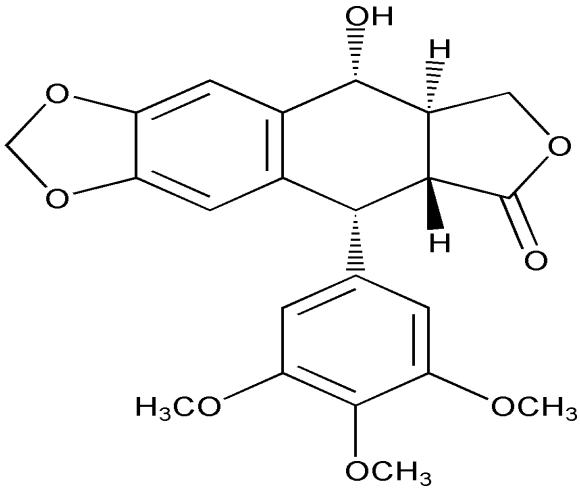

Figure 1: Structure of podophyllotoxin

Thin layer chromatography was performed on pre-coated silica gel plates using toluene: ethyl acetate: acetic acid (15.0:7.5:0.5, v/v/v) as mobile phase, a single band was seen at R_f 0.39 under 254 nm. [12] The UV maxima in methanol were found to be at 292 nm. Melting point was observed in the range of 183-186^{0}C. IR spectrum of the isolated compound showed characteristic peaks at 1762 cm^{-1} (carbonyl) and at 3455 cm^{-1}(hydroxyl). The fragmentation pattern of mass spectroscopy and ^{1}H NMR data of isolated compound was found in accordance with reported values in literature. [13,14] The structure was confirmed by its mass spectrum which showed the molecular ion peak $[M]^-$ at m/z 415.05 (Figure 1). Isocratic elution program of 15 min on the HPLC system with the mobile phase acetonitrile: water containing 0.1% v/v of *o*-phosphoric acid (60:40), showed a peak for isolated podophyllotoxin at Rt of 7.5 min with 98 % purity (Figure 2). [10]

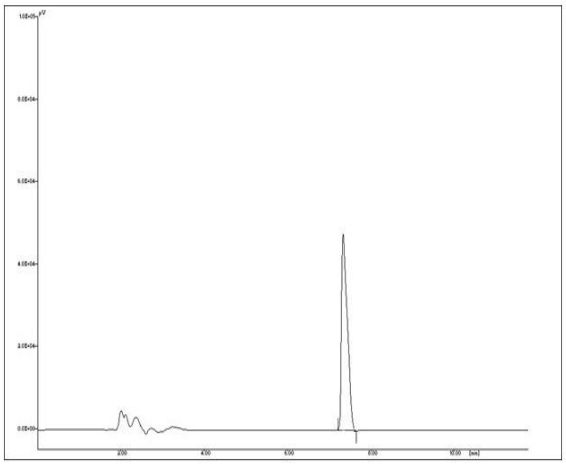

Figure 2: HPLC chromatogram of isolated podophyllotoxin

CONCLUSION

The isolation method developed involved solvent-solvent extraction technique for isolation and purification followed by recrystallization. Since this method is feasible on laboratory scale, it can be further optimized to make it appropriate for large scale isolation of podophyllotoxin.

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