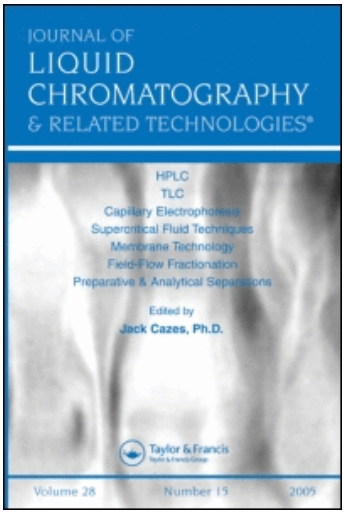

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BOTANICAL SOURCE DIFFERENTIATION OF PODOPHYLLUM RESIN BY HPLC

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ABSTRACT

A high-performance liquid chromatographic method to determine botanical sources of Podophyllum resin via approximate podophyllotoxin content is described. North American resin contains about 10% podophyllotoxin, the Indian variety about 40%. Samples are dissolved in mobile phase solution and analyzed by HPLC using normal-phase chromatography with detection at 280 nm. Estimates of podophyllotoxin content were made using commercially available references.

INTRODUCTION

Podophyllum, as specified by The United States Pharmacopaeia (USP) (1), is the dried rhizome and roots of Podophyllum peltatum L. containing up to 1% podophyllotoxin and 3-6% resin (2). P. peltatum is grown primarily in North America. Podophyllum grown

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in India consists of the rhizome and roots of Podophyllum hexandrum Royale (P. emodi Wall) yielding 1-4% Podophyllotoxin and 6-12% resin (2). Podophyllum resin USP is the percolated alcohol extract of Podophyllum which is concentrated and precipitated from acidified water. The dried resin from P. peltatum contains about 10% podophyllotoxin, while the Indian variety yields a resin with about 40% podophyllotoxin (2). Extracted resins solutions are used in topical solutions for the treatment of venereal and other warts. The British Pharmaceutical Codex (BPC) (2) lists monographs for Podophyllum (peltatum), Indian Podophyllum and resin prepared from both. BPC specifies that the resin labels state the botanical source. The USP (1) excludes Indian Podophyllums and resins prepared from them. The USP specifies a differentiation test which relies on Indian resin forming a gel in alcoholic potassium hydroxide solution. Differentiation by this test has not always been conclusive, particularly if the Indian resin is low in podophyllotoxin. An HPLC method is reported which distinguishes the botanical source of podophyllum resin via podophyllotoxin content differences.

EXPERIMENTAL

Apparatus and Reagents - A conventional isocratic HPLC was used. The apparatus was equipped with: a silica column [We used LiChrospher, Si 100, (E. Merck)]; UV detection at 280 nm; and a Rheodyne 20 µl injection valve. Mallinckrodt HPLC grade solvents

were used for the mobile phase solution prepared as follows: dilute 100 ml of anhydrous methanol, 40 ml of tetrahydrofuran, and 10 ml of glacial acetic acid to 1000 ml with hexane, mix and degas.

Reference Materials - Podophyllum resin extracted from P. peltatum (Code P-8503 Sigma Chemical, St. Louis, MO) and podophyllotoxin (Practical, Sigma Code P2765, approximately 75% pure was used. Recently introduced Code P-4405, approximately 98% pure, would also suffice).

Procedure - Reference podophyllotoxin solution was prepared by accurately weighing approximately 0.04 g of reference material into a 100-ml volumetric flask, then dissolving and diluting with mobile phase solution. Sample solutions consisted of about 0.1 g of resin, accurately weighed and similarly dissolved in 100 ml of mobile phase solution. Ultrasonic agitation was necessary to aid dissolution. Test solutions were filtered through 0.5 μm Teflon filters before injection. Into an appropriately equilibrated HPLC, at a flow rate of 2 ml/min, test solutions were injected and the chromatograms recorded for 15 minutes. The retention for podophyllotoxin is approximately 4 minutes.

RESULTS AND DISCUSSION

Figure 1 displays representative chromatograms of our podophyllotoxin reference and resin from North American and Indian sources. Based on the podophyllotoxin reference containing

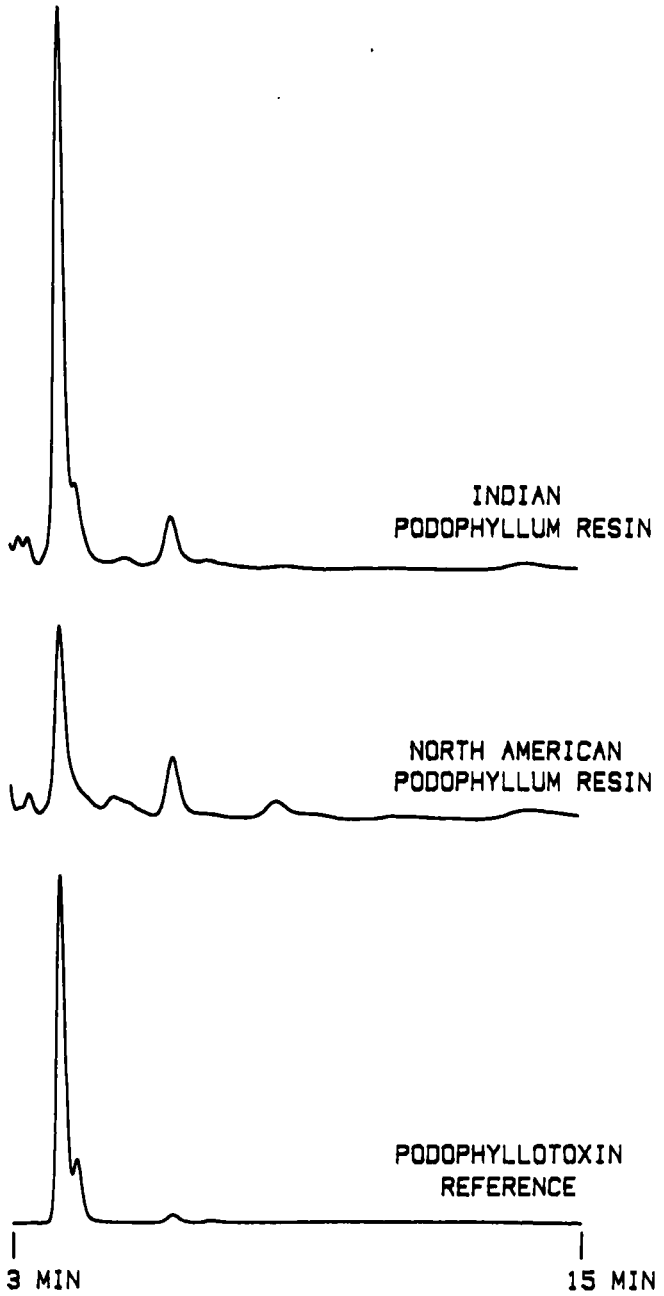

FIGURE 1: Representative chromatograms for podophyllotoxin reference material and extracted Podophyllum resin from the two sources indicated. Podophyllotoxin is the major peak at about 4 minutes.

approximately 75% toxin, we calculated that the Sigma P. peltatum resin contained approximately 11% podophyllotoxin. We analyzed several extracted resin samples from uncertain sources. Based on our method and computations by referral to the known toxin, we concluded that a group which assayed 8, 12, 18, and 15% podophyllotoxin was P. peltatum (North American). A second grouping with assay values of 42, 34, 36, and 44% was derived from P. hexandrum (Indian).

Treppendahl and Jakobsen (3) describe an involved preparative chromatographic procedure for isolating podophyllotoxin which was used as a standard for an HPLC assay procedure. Our purpose was to define a rapid qualitative procedure to determine the botanical source of podophyllum resin independent of the dubious gel formation test or supplier claims. The assay procedure by Treppendahl and Jakobsen (3) was too lengthy for our purposes.

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