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A Simple Method for Isolation of Plumbagin from Roots of *Plumbago rosea*

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Abstract

Plumbagin, the major active compound and a potential biomarker characteristically found to be present in different Plumbaginales, is isolated by an austere and efficient method involving column chromatography. A literature survey suggests that the roots of *Plumbago rosea* Linn. are the richest source of plumbagin. Chemically identified as a naphthoquinone, the compound is claimed to sublime at 90°C. This disposition of the compound correlates with the implication of cold maceration employed in isolation methodology for extraction of root powder with acetone. Plumbagin, being hydrophobic and insoluble in water, was precipitated out by addition of water to the acetone extract. The filtered residue was taken in chloroform, and the concentrated chloroform extract, when subjected to column chromatography, yielded plumbagin (1.65%), elution being carried out with *n*-hexane:ethyl acetate (92:8). The identity of the compound was confirmed by melting point data, UV, IR, and mass spectral data reported in the literature. The purity of the compound was further analyzed by subjecting the compound to HPTLC studies.

Keywords: Plumbagin, *Plumbago rosea*.

Introduction

Plumbago rosea Linn. (syn. *Plumbago indica* Linn.), commonly known as red chitrak of the family Plumbaginaceae, is a highly reputed Indian medicinal plant mentioned in Ayurvedic literature. It is a perennial subscandent shrub frequently grown in gardens as an ornamental plant for its showy bright-red flowers. The plant is widely distributed in the tropics, more specifically in Southern India. It is reported as wild or indigenous to Sikkim and Khasi hills (Anonymous, 1976; Drury, 1982). Plumbagin, an active principle, is contained in this plant; the roots of this plant have been quite well-known in India for a very long time. It is believed to be useful in dyspepsia, piles, skin diseases (leprosy, leucoderma), cancer, rheumatoid arthritis, and dysmenorrhea. The roots have been largely used as an abortifacient in indigenous practice (Satyavati et al., 1987).

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is a yellow crystalline substance present in the roots of *P. zeylanica*, *P. rosea*, *P. capensis* (syn. *P. auriculata* Thunb.), and *P. europea*, belonging to the family Plumbaginaceae (Kirtikar & Basu, 1975). Plumbagin contains a 2-methyl-1,4-naphthoquinone skeleton (i.e., an aromatic methyl *p*-quinone moiety). The compound is steam volatile and sublimes at 90°C (John & Maria, 1962). Earlier exhaustive phytochemical studies on the genus *Plumbago* have resulted in the isolation of several naphthoquinones, naphthalenones, naphthoquinonoids and their derivatives, aliphatics, amino acids, flavonoids, and sitosterol, campesterol, stigmasterol, and hydrocarbons (Dinda & Saha, 1989, 1990; Dinda et al., 1998, 1999). In view of the fact that plumbagin has been screened for a number of major pharmacological activities, viz., antitumor (Umadevi et al., 1999), anticoagulant (Santhakumari et al., 1978), antifertility (Kini et al., 1997), antimalarial (Nakornchai et al., 1995), hyperglycemic (Olagunju et al., 1999), hypolipidemic (Sharma et al., 1991), and antimicrobial (Krishnaswamy & Purushothaman, 1980), the current study was undertaken to facilitate the isolation of the active constituent, plumbagin, applying simple methodology and convenient techniques. Many co-workers have reported the isolation

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procedure for plumbagin in the past. Efforts have been made in this task to demonstrate an easier, economical, feasible, and effective method.

Materials and Methods

Plant material

Fresh, fully grown plants of *P. rosea* were procured from Eastern India, Kolkata, in the month of September. The authenticity of the plant was established, and the voucher specimen (135) was deposited in the Pharmacognosy and Phytochemistry Department, L. M. College of Pharmacy, Ahmedabad. The roots of the plant were separated, washed, and shade-dried for a week at room temperature, powdered to 60 mesh, and stored in an airtight container until further use.

Chemicals

All solvents and reagents used for the current study were of analytical grade (AR).

Extraction and isolation

Root powder (100 g) was subjected to cold maceration (refrigerator 2–8°C) for 3 days using acetone (500 ml × 5). The acetone extract was concentrated under reduced pressure to a volume of 70 ml. Plumbagin, being water-insoluble, was precipitated by addition of 450 ml of distilled water. The precipitate was filtered under vacuum, washed, and taken in chloroform (A).

The filtrate (mother liquor) remaining after vacuum filtration was further extracted with chloroform (B). The chloroform extracts (A) and (B) were pooled and concentrated, which left an oily brownish residue (7.4 g).

The oily residue was taken in 7 ml of chloroform and loaded onto a glass column (60 × 3 cm) using silica gel (140 g) as stationary phase. Gradient elution was performed using *n*-hexane containing increasing amounts of ethyl acetate. The fractions, each of 10-ml elutes, were collected and monitored simultaneously on a thin-layer chromatography (TLC) plate using silica gel as a stationary phase and *n*-hexane:ethyl formate (9:1) as a mobile phase. The fractions eluted with *n*-hexane:ethyl acetate (92:8) showing only one spot on TLC [*n*-hexane:ethyl formate (9:1), R_f:0.37] were pooled and evaporated to dryness at 25 ± 2°C, yielding 1.65 g of fine crystalline orange needles of plumbagin.

General

The melting point was recorded on a Büchi capillary melting point apparatus (Switzerland). For TLC experiments, precoated plates of silica gel 60 F_{254} (E. Merck) were used and spotting was done with a CAMAG LINOMAT IV Automatic TLC spotter (Switzerland). For purity assessment of the isolated compound and for recording the UV spectrum of the compound, the plates were scanned on a CAMAG TLC scanner 3 at 254 nm. UV absorption spectrum of the sample in methanol was also recorded on a UV/Vis spectrophotometer (JASCO model 7850, Germany). IR spectrum was recorded on a BUCK SCIENTIFIC IR Spectrophotometer (model 500, Germany). Atmospheric pressure ionization with ion spray mass spectra of molecular ions was obtained on a PE SCIEX API 165 MS with a WATERS LC (Applied Biosciences, USA).

Results and Discussion

The yield of plumbagin was found to be 1.65 g. Plumbagin turns red in color on reaction with alkali; the test is therefore indicative of the identity of plumbagin in TLC studies (Tumminkatti, 1928).

Characterization of isolated plumbagin

Melting point

The melting point of plumbagin was recorded to be 78°C, which matched with the data given in the literature (Tumminkatti, 1928).

TLC analysis

The crystals of plumbagin after dissolving in acetone were spotted on precoated plates of silica gel 60 F_{254}. Among the different solvent systems employed, in the mobile phase *n*-hexane:ethyl formate (9:1), plumbagin resolved at R_f 0.37. The spot of plumbagin showed a yellow coloration in daylight, gave a faint quenching in UV (long wave) and magenta pink color with a 10% alcohol KOH solution. The purity of the compound was further confirmed by recording a chromatogram and a UV absorption spectrum of the compound developing on a TLC plate in the solvent system containing *n*-hexane:ethyl formate (9:1) on a CAMAG TLC Scanner 3. The TLC chromatogram showed a single peak scanned at 254 nm. Further, a UV absorption spectrum, recorded on CAMAG TLC Scanner 3 at start, middle, and end positions of the band for purity of the sample, completely overlapped, and gave two peaks of absorption maxima at 260 nm and 425 nm (cc. λ_{max} in MeOH = 253, 403) (Sankaram & Siddhu, 1971).

Spectral analysis

UV: λ_{max} in methanol = 253, 406 nm (JASCO UV/Vis spectrophotometer), λ_{max} = 260 nm, 425 nm (CAMAG TLC Scanner 3).

IR (KBr): 3577 cm^{-1} (OH stretching), 1647 cm^{-1} (ketone), 751.7 cm^{-1} (aromatic C–H stretching), 1609 cm^{-1} (C=C in aromatic system).

Mass (M^{+1}): 189 (cc. mol. wt. 188, *Merck Index*, 1989), other prominent peaks were observed at 161, 121, 104, and 74.

The physical, chemical and spectroscopic data of plumbagin was found to be as reported in the literature.

Conclusions

Plumbagin, with its versatile and significant pharmacological activities, is an important phytoconstituent. The current study was intended as a part of developing simplified protocols for isolation of highly reputed drugs having herbal origin. The proposed method of isolation is very simple and nowhere involves the use of complicated laboratory techniques. This novel method is justified and found to be appropriate for isolating plumbagin, as the yield of compound procured is fairly high. Plumbagin, being sublimating in nature, demands a very careful and sensitive approach. The project emphasizes this fact and thus overcomes the possibility of alleviating the yield. The current method is a suitable alternative for the older methods of isolation involving steam distillation, which are comparatively tedious and technically difficult. Remarkable results obtained in the project with respect to quality and quantity of compound are suggestive of a promising approach in isolating the compound.

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