Development and validation of a new HPTLC method for quantification of conophylline in Tabernaemontana divaricata samples obtained from different seasons and extraction techniques: Insights into variation of pancreatic lipase inhibitory activity

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ABSTRACT

Tabernaemontana divaricata (L.) R. Br. Ex Roem. & Schult (Apocynaceae) is an important indole alkaloid rich Indian medicinal plant with conophylline being a potential lead in the treatment of diabetes mellitus and obesity. Seasonal variations and extraction techniques are two major variables that play a crucial role in the extractive yield of phytochemical constituents. Till date, there are no reports on quantification of conophylline from the leaves of T. divaricata of Indian origin, and its variation due to season and extraction techniques. The present study reports for the first time, development and validation of a new HPTLC method for the quantification of conophylline. A resolved peak of conophylline was achieved in the chromatogram with mobile phase of chloroform: methanol (90:10% v/v), when developed on a 15 cm length TLC plate. Further, the leaf samples of T. divaricata were collected during the months of August, November, February and May, and subjected individually to different extraction techniques viz., ultrasonic extraction, hot percolation and cold maceration. A total of 12 alkaloid rich fractions were obtained, and the extractive value of conophylline was found to be highest in August sample, when subjected to ultrasonic extraction (35.57 mg per 1 g of alkaloid rich fraction). Pancreatic lipase inhibitory activity of the alkaloid rich fractions was in correlation to their respective conophylline content (Pearson’s $r = -0.7152$) with potent activity exhibited by August sample, that was obtained by ultrasonic extraction (IC$_{50} = 7.86 \mu$g/mL).

1. Introduction

Plant based natural products represent a vast reservoir of bioactive molecules that have the potential to treat various diseases and disorders (Cragg and Newman, 2013; Newman and Cragg, 2012). Isolation of these bioactive molecules is preceded by collection and extraction of plant material, that are important but more often unrecognized in natural product based drug discovery. Seasonal variations and extraction techniques are two major variables that have great impact on the quality of herbs and herbal products. The effect of seasons on the metabolic profile of plants can be understood from the fact that some seasons produce high extractive yields of chemical constituents while some do not (Manika et al., 2013; Singh et al., 2010). Similarly, the choice of a right extraction technique plays an important role in producing a quality extract, ultimately contributing to the desired pharmacological effect. Conventional extraction techniques most commonly used at laboratory and industrial scale include hot percolation and cold maceration. However, these techniques are tedious, less extractive, time consuming, unsafe, and expensive. Recently, ultrasonic extraction has gained more interest over these conventional methods, wherein an excellent extraction of the phytochemical constituents can be achieved even at room temperature, reducing the risk of their thermal degradation (Azmir et al., 2013; Cravotto et al., 2005; Mason et al., 2011).

Tabernaemontana divaricata (L.) R. Br. Ex Roem. & Schult (Apocynaceae) is a tropical evergreen shrub widely grown in many Southeast Asian countries including India, Sri Lanka, Malaysia, and Thailand. It is commonly referred to as Crepe Jasmine, and is cultivated as a common garden plant. It grows well in sunny weather/partial shade; on alkaline, clay, sand, acidic, and loam soils. The plant exists in...
two distinct varieties, the single-flower variety and the double flower variety (Fig. 1). Widely reported for its use in traditional systems of medicine, *T. divaricata* is a rich source of indole alkaloids and has been investigated for wide range of pharmacological activities (Pratchayasakul et al., 2008). Further, previous literature has highlighted the potential of *T. divaricata* leaves against metabolic disorders viz., diabetes mellitus and obesity. The hot aqueous decoction of the leaves is consumed by the tribal communities of Assam for the treatment of diabetes mellitus (Namsa et al., 2011). Moreover, the methanol extract of the aerial parts of *T. divaricata* has been investigated for its pre-clinical anti-obesity effect (Kanthial et al., 2012).

In our previous study, the crude methanol extract of *T. divaricata* leaves exhibited potential pancreatic lipase (PL) inhibitory activity with an IC₅₀ value of 12.73 μg/mL. Further investigation resulted in the identification of conophylline (1) as a potent PL inhibitor (IC₅₀ = 3.31 μM), comparable to that of orlistat (IC₅₀ = 0.99 μM), the only approved drug for obesity (Nightingale, 1999; Sridhar et al., 2017a). According to Dr. A. S. Sandhu, Department of Natural Products, NIPER Pilani (Pilani Campus), located at 28.36°N 75.58°E and 285 m above the sea level. Mature large-sized leaves (7–10 cm in length and 5–6 cm in width) of *T. divaricata* (single variety) were collected during 15th day of November 2013, February 2014, May 2014, and August 2014, which represented the respective seasons of Pilot (BSS No. 10).

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Considering the above facts, the present study was aimed a) to develop and validate a new High Performance Thin Layer Chromatography (HPTLC) method for the quantification of conophylline (1) in the leaves of *T. divaricata*; b) to understand the role of seasonal variations and extraction techniques on the extractive yield of conophylline (1), and its correlation to pancreatic lipase inhibition.

2. Materials and methods

2.1. Chemicals and reagents

Conophylline (1) was obtained from the natural products repository of Laboratory of Natural Drugs, Department of Pharmacy, Birla Institute of Technology and Science, Pilani (BITS Pilani). Methanol and chloroform (Merck, India) used in mobile phase were of HPLC grade. Silica gel G60 F₂₅₄ TLC plates (E. Merck, USA) were used for chromatogram development. Porcine PL (Type II) and 4-nitrophenyl butyrate used for PL inhibition assay were procured from Sigma-Aldrich (Sigma-Aldrich, USA). Tris buffer and Sodium chloride (Sisco Research Laboratories, India) used for assay were of molecular biology grade. All other chemicals and solvents (analytical grade) were used without further purification.

2.2. Plant material

The leaf samples of *T. divaricata* (single flower variety) were collected from the botanical garden of BITS Pilani (Pilani Campus) located at 28.36°N 75.58°E and 285 m above the sea level. Mature large-sized leaves (7–10 cm in length and 5–6 cm in width) of *T. divaricata* were collected during 15th day of November 2013, February 2014, May 2014, and August 2014, which represented the respective seasons of Pilani i.e., Winter, Spring, Summer, and Rainy. The plant was authenticated by Dr. A. S. Sandhu, Department of Natural Products, NIPER (S.A.S. Nagar). The leaf samples were dried under shade at room temperature (≈ 25°C), subjected to pulverization, and passed through sieve (BSS No. 10).

2.3. Procedure for extraction and preparation of alkaloid rich fractions (ARF)

The dried leaf samples (30 g each) were individually subjected to three different extraction techniques viz. Cold maceration (72 h, room temperature), Hot percolation (24 h, 60°C), and Ultrasonic extraction (1 h, ≈ 25°C) using methanol (100 mL) as solvent. A total of 12 crude methanol extracts were obtained from a combination of four leaf samples of *T. divaricata*, each subjected separately to three extraction techniques. The respective ARFs were prepared from the crude methanol extracts as per the procedure performed in our previous study (Sridhar et al., 2017a). Briefly, the methanol extracts were acidified to pH 4 using 5% HCl, to convert the alkaloids into their respective salt derivatives. This aqueous phase was extracted with chloroform.
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