Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 497–502

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simultaneous determination of three steroidal glycoalkaloids in *Solanum xanthocarpum* by high performance thin layer chromatography

Karuna Shanker[a], Shalini Gupta[a], Pooja Srivastava[a], Santosh K. Srivastava[a], Subash C. Singh[b], Madan M. Gupta[a,*]

[a] Analytical Chemistry Department, Central Institute of Medicinal and Aromatic Plants, Council of Scientific and Industrial Research (CSIR), Lucknow 226015, India
[b] Botany and Pharmacognosy Department, Central Institute of Medicinal and Aromatic Plants, Council of Scientific and Industrial Research (CSIR), Lucknow 226015, India

ARTICLE INFO

Article history:
Received 5 May 2010
Received in revised form 6 August 2010
Accepted 18 September 2010
Available online 29 September 2010

Keywords:
Steroidal glycoalkaloids
Solasonine
Solamargine
Khasianine
Solanum xanthocarpum
HPTLC-fingerprint

ABSTRACT

A new high-performance thin-layer chromatographic (HPTLC) method has been developed for the simultaneous quantitation of three bioactive steroidal glycoalkaloid (SGA) markers, solasonine (SN), solamargine (SM) and khasianine (KN) in the plant *Solanum xanthocarpum*. Extraction efficiency of targeted SGAs from plant matrix using methanol and acidified methanol were studied using percolation, ultrasonication and microwave techniques. The separation was achieved on silica gel $60F_{254}$ TLC plates using chloroform–methanol–water as mobile phase. The quantitation of SGAs was carried out using the densitometric reflection/absorption mode at 520 nm after post chromatographic derivatization using Dragendorff's reagent. The method was validated for peak purity, precision, accuracy, robustness, limit of detection (LOD) and quantitation (LOQ). Method specificity was confirmed using retention factor (R_f), Vis spectral correlation and electrospray ionization mass spectra (ESI-MS) of marker compounds in the sample track.

1. Introduction

Steroidal glycoalkaloids (SGAs) are the secondary metabolites produced by solanaceous plants and possesses potential food safety concerns [1,2]. Some of the solanaceous plants are being used in Indian-system of medicine after processing with specific purification processes [3]. *Solanum xanthocarpum* Schrad & Wendl (Syn. *S. virginianum*/*S. surattense*; Family – Solanaceae), commonly known as '*Kantakari*' is prickly diffuse bright green perennial herb which grow mostly in dry places as a weed on roadsides and waste lands of India and other South-East Asian countries [4,5]. *S. xanthocarpum* is an important ingredient of generic Ayurvedic formulation "*Dashamularishta*", and its each part is used in several others medicinal formulations such as *Kantakari ghrta*, *Kantakari avaleha*, *Kantakari kalpa*, *Vyaghri taila*, *Vyaghriharitaki*, *Kanakasava*, *Nidigdhadi* and *Kvatha*. Its decoction is used in "*Chyawanprash – A Rejuvenating Ayurvedic tonic*" as mucolytic and expectorant agent to cure the *Kaphaj Dosha* related to allergic bronchitis, bronchial asthma, common cold, anthelmintic, antipyretic, laxative, anti-inflammatory and antiasthmatic [6–8].

Antispasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic, cytotoxic [9], hypoglycemic [10], bronchodilator [11], activities have also been reported. Several secondary metabolites such as steroidal alkaloids like solanocarpine [12], solanocarpidine, solasonine (SN) [7] solamargine (SM) [13] and steroidal glycosides [14] are reported from the plant. Biological investigations of SM and SN showed significant cytotoxicity against the several human cell lines and skin tumor [15,16]. Human intake of high doses of other solanaceae steroidal glycoalkaloids has led to acute intoxication, in severe cases coma and death [1,2,17], but no adverse effect of *S. xanthocarpum* is reported so far.

Absence of chromophore in SGAs makes their detection a major challenging problem in the assay of a biological sample [18,19]. Detection after derivatization on a TLC plate is a simple and rapid option for such compounds. Among various analytical techniques, high-performance thin-layer chromatography (HPTLC) in particular appears to be suitable for phytomolecules of varying nature and provides a rational approach in the authentication and quality assessment of crude medicinal herbs and their formulations [20–22]. Earlier reports on quality analysis of *S. xanthocarpum* involve complex sample preparation steps, require long analysis time, use of gradient elution or involve tedious pre-chromatographic derivatization to form an ion-paring complex followed by LC–UV detection [23]. Acid dye based TLC method provide the quantitation of only one alkaloid and also involves addition

* Corresponding author. Fax: +91 522 2342666.
E-mail address: guptammg@rediffmail.com (M.M. Gupta).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.09.025

step of ion pair complex formation of solasodine prior to detection [24].

Our continued interest on the development of rapid HPTLC method for quality assessment of medicinal plants [20–22] led us to develop an analytical procedure for quality assessment of *S. xanthocarpum*. The objective of present study was to optimize, develop and validate a rapid, sensitive and accurate HPTLC method for the simultaneous determination of three potentially toxic steroidal glycoalkaloids in aerial part of *S. xanthocarpum*.

2. Materials and methods

2.1. Plant materials

The aerial parts of wildly grown *S. xanthocarpum* plants were collected in the month of June 2009, at the flowering stage from the research farm of CIMAP, Lucknow. Voucher specimen (CIMAP No. 12470) is deposited in the Botany and Pharmacognosy Department of the Institute. The plant material was powdered, packed in airtight container and stored at 20 °C until analysis.

2.2. Chemicals and reference compounds

All reagents and solvents used were either of analytical or HPLC grade (E. Merck Ltd., Mumbai, India). Prior to use, the solvents were filtered through a 0.45 μm membrane (Millipore, Billerica, MA, USA). Pre-coated TLC silica gel $60F_{254}$ aluminum plates were purchased from Merck (Darmstadt, Germany). The sorbents silica gels, used for vacuum liquid chromatography (VLC) and flash chromatography were procured from Qualigens Fine Chemicals, Mumbai, India. The standard compounds, solasonine (SN), solamargine (SM) and khasianine (KN) were isolated (purity >99% using area normalization method of HPLC–MS) and characterized by spectral analysis in our laboratory.

2.3. Apparatus

Vario system, TLC Scanner, winCATS-III, Reprostar-3, twin trough chamber, immersion device-III, TLC plate heater (Camag, Muttenz, Switzerland) were used for digital image scanning, method development and validation. Ultrasonic extraction was performed in ultrasonic bath (Oscar Micro clean-109, Mumbai, India). Microwave assisted extraction (MAE) experiments were performed with microwave oven (Whirlpool, New Delhi, India) with programmable heating power and pulse radiations (controlled temperature). Shimadzu (Japan) LC–MS was used for ESI-MS spectra which consisted of LC-20AD solvent delivery pumps, a DGU-$20A_5$ degasser, a CTO-20A column oven and a SPD-M 20A photodiode array detector and a mass spectrometer LCMS-2010EV. The 300 MHz NMR (Avance, Bruker, Switzerland) was used to record spectra in CD_3OD with tetramethyl silane (TMS) as internal standard.

2.4. Extraction and isolation of SGAs from S. xanthocarpum

Air dried and finely powdered aerial parts of *S. xanthocarpum* (1.3 kg) were exhaustively extracted with 3 × 10 l of methanol in a percolator at room temperature (25 ± 2 °C). The pooled extract was evaporated to dryness in vacuum at 40 °C. From the material thus obtained the SGAs were extracted with 10× 200 ml of 2% aqueous HCl solution as water-soluble hydrochloride salts. The combined acidic extract was defatted with 3× 500 ml hexane. The defatted acidic extract was basified under cooling with 10% sodium hydroxide solution up to pH 12 and extracted with *n*-butanol saturated with water (4× 500 ml). The pooled butanol extract was washed with water and evaporated under vacuum to afford a dark brown residue (9.7 g).

Table 1
Extraction efficiency of different solvents for chemical marker (SGAs) from the aerial parts of *S. xanthocarpum* on plant dry weight basis.

Techniques[a]/solvents	Amount of compound quantified (%, w/w)		
	Solasonine (SN)	Solamargine (SM)	Khasianine (KN)
Cold percolation			
Methanol	0.100 ± 0.005	0.209 ± 0.009	0.076 ± 0.004
Acidified methanol	0.108 ± 0.005	0.229 ± 0.012	0.082 ± 0.005
Hot extraction			
Methanol	0.108 ± 0.005	0.237 ± 0.011	0.082 ± 0.004
Acidified methanol	0.115 ± 0.007	0.338 ± 0.017	0.085 ± 0.005
Soxhlet extraction			
Methanol	0.090 ± 0.006	0.368 ± 0.013	0.085 ± 0.004
Acidified methanol	0.110 ± 0.009	0.442 ± 0.022	0.090 ± 0.005
Ultrasonic extraction			
Methanol	0.102 ± 0.010	0.257 ± 0.012	0.085 ± 0.005
Acidified methanol	0.166 ± 0.007	0.443 ± 0.010	0.088 ± 0.005
Microwave extraction			
Methanol	0.105 ± 0.006	0.241 ± 0.011	0.082 ± 0.006
Acidified methanol	0.168 ± 0.008	0.449 ± 0.020	0.110 ± 0.007

[a] The experimental conditions are described in Section 2.5.

For the isolation of SGAs, 7.4 g of the butanol extract was subjected to vacuum liquid chromatography (VLC, 7.5 cm × 7.5 cm, Silica gel H, 125 g). Gradient elution was carried out with $CHCl_3$, $CHCl_3$:MeOH and MeOH in increasing polarity. On the basis of TLC monitoring of a total of 296 fractions using Merck (Darmstadt) silica gel $60F_{254}$ plates and visualization with Dragendorff's reagent, 3 g material from fractions 257–280 was selected for further fractionation by flash chromatography using glass column (3 cm × 23 cm) packed with silica gel (230–400 mesh). After four subsequent fractionation steps 16 mg of KN, 60 mg of SM and 20 mg of SN were obtained. The structure elucidation of SN, SM and KN (Fig. 1) was carried out with the help of ^{1}H NMR, ^{13}C NMR, 2-dimensional NMR experiments (HSQCGP and HMBC) and mass (ESI-MS) spectroscopic data, which were in complete agreement with the reported data [25].

2.5. Standard stock solutions and sample preparation

Standard stock solutions of compounds SN and SM were prepared as 5.0 mg/ml and of KN 15 mg/ml in methanol. Working stocks for calibration studies were prepared by dilution using Hamilton syringe (Bonaduz, Switzerland). The dried and milled aerial parts of *S. xanthocarpum* were extracted with acidified methanol (1% AcOH) by cold percolation (3× 15 ml, 10 h extraction time at room temperature); hot extraction (3× 15 ml, 30 min extraction time at 50 °C); soxhlet (50 ml, 2 h extraction time); ultrasonication (3× 5 ml, 15 min extraction time); microwave (3× 5 ml, at 650 W, 50 °C for 3 min extraction time) assisted extraction separately (1.0 g plant in each case). The extracts were evaporated to dryness and re-dissolved in 1.0 ml of methanol and centrifuged at 10,000 rpm for 10 min. The supernatants were pre-filtered with 0.45 μm and used for HPTLC analysis. The result of extraction efficiency of solvents and suitability of extraction techniques are summarized in Table 1.

2.6. Chromatographic procedure

Chromatography was performed on preactivated HPTLC plates (10cm × 10 cm or 20cm × 10 cm). The plates were washed with methanol and activated at 120 °C for 20 min. Standard and sample solutions were applied in the form of band at 15 mm from both the lower and left edge with 20 mm space between two bands using a 100 μl syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was carried out in pre-saturated (optimized to