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Liquid chromatography–mass spectrometry-based quantification of steroidal glycoalkaloids from *Solanum xanthocarpum* and effect of different extraction methods on their content

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ABSTRACT

A new liquid chromatography–mass spectrometry (LC–MS)-based method coupled with pressurized liquid extraction (PLE) as an efficient sample preparation technique has been developed for the quantification and fingerprint analysis of *Solanum xanthocarpum*. Optimum separations of the samples were achieved on a Waters MSC-18 XTerra column, using 0.5% (v/v) formic acid in water (A) and acetonitrile (ACN):2-propanol:formic acid (94.5:5:0.5, v/v/v) (B) as mobile phase. The separation was carried out using linear gradient elution with a flow rate of 1.0 mL/min. The gradient was: 0 min, 20% B; 14 min, 30% B; 20 min, 30% B; 27 min, 60% B and the column was re-equilibrated to the initial condition (20% B) for 10 min prior to next injection. The steroidal glycoalkaloids (SGAs) which are the major active constituents were isolated as pure compounds from the crude methanolic extract of *S. xanthocarpum* by preparative LC–MS and after characterization were used as external standards for the development and validation of the method. Extracts prepared by conventional Soxhlet extraction, PLE and ultrasonication were used for analysis. The method was validated for repeatability, precision (intra- and inter-day variation), accuracy (recovery) and sensitivity (limit of detection and limit of quantitation). The purpose of the work was to develop a validated method, which can be used for the quantification of SGAs in commercialized *S. xanthocarpum* products and the fingerprint analysis for their routine quality control.

1. Introduction

Saponins are one of the most widely distributed groups of natural products in the plant kingdom. They can be broadly divided into two groups based on whether the aglycone is of a triterpene or steroidal type. Triterpene saponins are the more widespread class, while steroidal saponins are mainly found in families of monocotyledons. SGAs represent the major class of saponins that are present in the genus *Solanum*. Due to their biological activities, SGAs are very interesting compounds for the biochemists and analytical chemists. They are assumed to play an important role in protection of the plant from the abiotic and biotic stress. The SGAs aglycones consist of a common non-polar steroid unit and either indolizidine or oxa-azaspirodecane basic portion. They have a polar tri- or tetrasaccharide moiety attached to the C-3 position of the aglycone. This overall complex nature of SGAs posses problem during their isolation (due to the formation of silicates on silica gel) and quantitation [1].

SGAs are also usually difficult to detect by HPLC–UV since they lack a strong UV chromophore [2]. Generally they are monitored at lower UV wavelengths ranging from 200 to 210 nm. This problematic detection of SGAs by UV absorbance has encouraged the development of HPLC-evaporative light scattering detection (ELSD) and HPLC–MS methods, resulting in an increasing number of publications using these techniques [3–5]. HPLC–MS methods have largely been concerned with the analysis of the saponins in commercially important plant extracts [6], such as ginsenosides in *Panax ginseng* [7,8], soyasaponins in *Glycine max* [9], saponins in species of *Glycyrrhiza* [10]. However, the studies of steroidal saponins using HPLC–MS are very few and these have been restricted mainly on the saponins in *Ruscus* spp., *Tribulus terrestris* and *Solanum tuberosum* [11–16].

Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) commonly known as Yellow Berried Nightshade (syn: kantakari), is a commonly used Ayurvedic medicine for treatment of asthma and bronchitis. It is officially included in the Ayurvedic Pharmacopoeia of India and is used in various Ayurvedic formulations. It is a prickly diffuse bright green perennial herb, found throughout India, mostly in dry places as a weed on roadsides and waste lands [17]. The fruits are glabrous, globular berries, green and white strips

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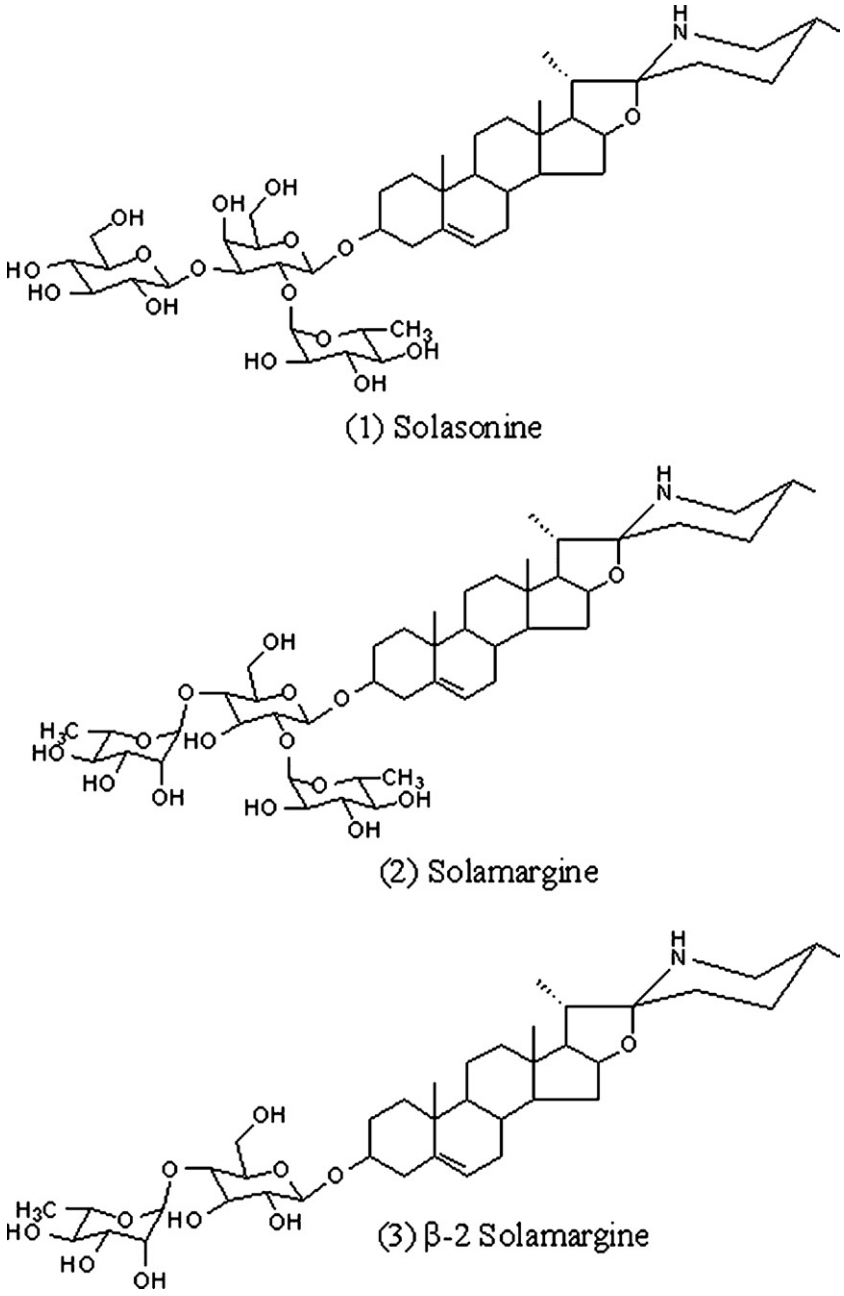

Fig. 1. Chemical structures of (1) solasonine, (2) solamargine and (3) β-2 solamargine.

when young, but yellow when mature [18]. The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic and aphrodisiac activities [19]. The fruit paste is applied externally to the affected area for treating pimples and swellings [20]. The anti-spasmodic, anti-tumor, cardiotonic, hypotensive, anti-anaphylactic and cytotoxic activities have also reported [21–23]. The fruits are reported to contain several SGAs like solanacarpine [24], solanacarpidine, solasonine [25] and solamargine [26] (Fig. 1). Other constituents like caffeic acid [26] coumarins like aesculetin and aesculin [27], steroids like carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol are reported from the fruits [28,29].

To our knowledge no LC–MS procedure for analysis/quantification of SGAs in *S. xanthocarpum* has been reported. Most of reports on SGAs quantitation are reported for the different species of potatoes [14,30]. Literature is full of reports on quantification of Solasodine (the aglycones) for *S. xanthocarpum* from different regions [26,31,32]. Also there are no reports on effect of different extraction procedures on SGAs content. Therefore, the aim of the current research was to develop a simple validated LC–MS protocol for preparative scale isolation and quantification of the SGAs in methanolic extract of *S. xanthocarpum* (mainly containing fruits) coupled with the study of effect of different extraction methods on their contents. Another aim of the study was to develop finger print for routine analysis of the plant material. Different routine methods like Soxhlet extraction and sophisticated methods like ultrasonification and pressurized liquid extraction (PLE) (procedure utilizing solvent under high pressure and temperature in a controlled manner, on material placed in