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ORIGINAL ARTICLE

High-performance thin layer chromatography based assay and stress study of a rare steroidal alkaloid solanopubamine in six species of *Solanum* grown in Saudi Arabia

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Abstract The present study describes a method developed for quantification and stability study of a rare steroidal alkaloid solanopubamine (SPN) in aerial parts of six different species of genus *Solanum* extracted with two different solvents. The *Solanum* species selected for investigation include *S. schimperianum* (SS), *S. villosum* (SV), *S. coagulans* (SC), *S. glabratum* (SG), *S. incanum* (SI) and *S. nigrum* (SN). The estimation of SPN was done by a validated high-performance thin layer chromatography method. The developed chromatographic system was found to give a sharp spot for solanopubamine at $R_f = 0.39 \pm 0.01$. The steroidal alkaloid SPN was observed to be present only in extracts of aerial parts of *S. schimperianum*. The sensitivity of developed method produced 40 ng and 115 ng band^{-1}, respectively as LOD and LOQ values. The percentage yield of SPN in aerial

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parts of *S. schimperianum* extracted by ethanol (95%) only and a mixture of ethanol and ammonium hydroxide (6:4) was found to be 1.03 w/w and 2.09 w/w, respectively. Stability studies of SPN exhibited the maximum (100%) degradation in an alkaline environment and H_2O_2 treated samples and 61.4% in acidic conditions. The SPN was found to be significantly stable against UV exposure, photo-oxidation and at room temperature while 13.83% and 57.88% destruction has been observed when exposed to dry heat at 40 °C and 60 °C, respectively.

1. Introduction

Solanaceae is one of the therapeutically important plant families possessing a broad spectrum of chemical entities and always been an attractive target for the researchers. *Solanum* being the largest genera of family Solanaceae possessing around 1700 species distributed throughout the world. In Saudi Arabia, the genus *Solanum* is represented by about 16 species found in West and Southwest areas of the country (Chaudhary, 2001; Collenette, 1999). Solanopubamine (SPN) (Fig. 1), chemically named as 3β-amino-5α, 22αH, 25βH-solanidan-23β-ol, is a rare steroidal alkaloid and till now reported only in aerial parts of two species of *Solanum*. The first reporting of Solanopubamine was done by Kumari et al., 1985 in *S. pubescence* followed by Al-Rehaily et al., 2013 in *S. schimperianum*. *Solanum* is a medicinally useful genus and particularly known for therapeutically active alkaloids. Ethyl acetate extract of leaves of *Solanum pubescens* exhibits significant anti-inflammatory activity (Niyogi et al., 2012). The plant is also used as anti-lice (Hemamalini et al., 2011a), anthelmintic (Hemamalini et al., 2011b), antioxidant (Peddi et al., 2013), anticonvulsant, sedative (Suvarchala et al., 2013), antibacterial (Haseebur et al., 2014), gastroprotective (Hemamalini et al., 2011c), and hepatoprotective agent (Pushpalatha and Ananthi, 2012. The literature survey revealed that *S. schimperianum* possesses potent antitrypanosomal (Abdel-Sattar et al., 2009), antibacterial (Al-Oqail et al., 2012) and antifungal activity (Al-Rehaily et al., 2013).

There are a variety of compounds reported in *S. pubescens* and some of them are myricetin methyl ethers (Kumari et al., 1984), solanopubamine (Kumari et al., 1985), and solanopubamides A & B (Kumari et al., 1986). The compounds reported from *S. schimperianum* are solanopubamine (Al-Rehaily et al., 2013), esculetin, astragalin, isoquercetin, kaempferol 3-diglucoside or 3-diglucosidekaempferol, rutin, solamargine and solamarine (Coune and Denoel, 1975; Angenot, 1969); lupeol, β-sitosterol, β -sitosterol glucoside, oleanolic acid, teferidin, teferin, ferutinin, 5-hydroxy-3,7,4'-trimethoxyflavone, retusin and kaempferol-3-***O***-β-D-glucopyranoside (Al-Oqail et al., 2012). Steroidal alkaloids are well known for their therapeutic potential as antifungal (Chen et al., 1998), antiviral (Wang et al., 2010), antiestrogen (Chang et al., 1998) and antitumor (Kupchan et al., 1965; Lee et al., 2004; Nin et al., 2009) activities. During combination chemotherapy for treating multidrug resistant cancer with the conventional cytotoxic drugs, steroidal alkaloids are used as chemosensitizers (Lavie et al., 2001).

The clinical importance of steroidal alkaloids has drawn our interest to explore the presence of SPN and its quantification in six species of *Solanum* collected from Abha region of Saudi Arabia. These species include *S. schimperianum* (SS), *S. villosum* (SV), *S. coagulans* (SC), *S. glabratum* (SG), *S. incanum* (SI) *and S. nigrum* (SN). Authors did not find literature related to the analytical and stability aspects of SPN. In recent years, high-performance thin-layer chromatography (HPTLC) gained much popularity for its analytical interventions in herbal drugs and formulations. This popularity is due to some of its special features such as simple, rapid, economic and multimarker assessment capability (Siddiqui et al., 2015a). HPTLC technique is a multipurpose analytical tool and can be used for analyzing a broad range of chemical compounds. Formulations such as pharmaceuticals, cosmetics, and dietary supplements may also be analyzed for their contents and efficacy (Siddiqui et al., 2015b). This study was performed to explore SPN in some other species of *Solanum* as well as its quantification in *S. schimperianum*.

2. Materials and methods

2.1. Materials

The aerial parts of six *Solanum* species viz. *S. schimperianum* (SS), *S. villosum* (SV), *S. coagulans* (SC), *S. glabratum* (SG), *S. incanum* (SI) and *S. nigrum* (SN) with voucher specimen numbers 15,038, 15,032, 15,101, 15,043, 15,102 and 15,149 respectively were collected from Abha region of Saudi Arabia in March, 2013, and identified by Dr. Mohammed Yusuf, Taxonomist, Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia. Specimens of the plant were deposited in the departmental herbarium.

2.2. Apparatus and reagents

Standard solanopubamine was obtained after isolation from aerial parts of *S. schimperianum* and characterized by UV,

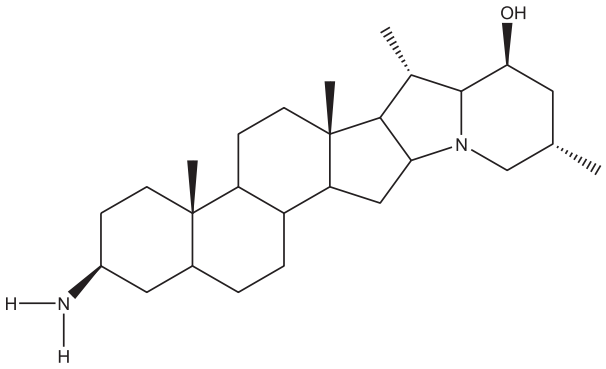

Figure 1 Chemical structure of solanopubamine.