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Quantitative estimation of steroidal drug solasodine in wild species of *Solanum* by reversed-phase HPLC method

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Abstract
An improved reversed phase HPLC method for the quantification of Solasodine, an alternative precursor for the commercial production of steroid drug. The comparative analysis of solasodine in eight wild species of the genus *Solanum* was achieved via UFLC, SHIMADZU equipped with 254 nm. The 30 µL of filtered methanolic extracts was injected into Supelco reverse phased C18 analytical column at a flow rate of 1 mL/min at 30°C and the effluent was monitored by UV detection at 205 nm. The solvent system was linear gradient of water and methanol from 40 to 70% for 25 min. Linear calibration curve was obtained between the amount of solasodine injected and the area under the peak over the concentration range of 0.1-1.0 mg mL^{-1} of solasodine with a correlation coefficient of 0.9999. The limit of detection (LOD) was found to be 0.012 µg mL^{-1}. The limit of quantification (LOQ) value was found to be 0.035 µg mL^{-1}. The method allows quantification of solasodine in plant tissue samples of *S. diphyllum, S. incanum, S. nigrum, S. sisymbrifolium, S. surattense, S. torvum, S. verbascifolium* and *S. vellosum.* The maximum concentration of solasodine found in *S. sisymbrifolium* 0.494 mg gm^{-1} and minimum in *S. incanum* 0.341mg gm^{-1}.

Keywords: Reverse-phase HPLC, solasodine, *Solanum*, wild

Abbreviations

Introduction
Solasodine, a steroidal alkaloid obtained primarily from Solanaceous plants, has been employed as an alternative to diosgenin (Chand 1991) [1] for the commercial synthesis of steroid drugs. These two compounds share the characteristic that they can be readily converted into 16-dehydropregnenolone acetate, a key intermediate in the synthesis of steroid drugs, e.g., progesterone, cortisone. Solasodine has been reported to occur in relatively high concentrations in a number of *Solanum* species (Jaggi and Kapoor 1994) [10]. Their study is a topic of great interest for botanists and pharmaceutical industries interested in novel molecules as potential drugs.

The use of HPLC as a quantitative assay for solasodine has also been attempted (Crabbe and Fryer 1980) [2] but previous attempts were limited by low sensitivity due to short, broad, asymmetric peaks. HPLC quantification of solasodine following derivatization improved the sensitivity but did not allow the recovery of solasodine for further use (Drewes *et al*. 1992) [4].

Although there are several analytical methods for the determination of steroidal glycoalkaloids and their aglycones, such as GC–MS (Laurila *et al*. 1999) [13], LC–MS (Eanes *et al*. 2008) [6], MALDI–TOF/MS (Eanes *et al*. 2008) [6], HPLC–UV and HPTLC (Eanes *et al*. 2008; Paul *et al*. 2008; Skarkova *et al*. 2008; Shanker *et al*. 2011) [6, 21, 26, 24], but there is no report on the Comparative quantitative estimation of Solasodine in eight species of genus *Solanum*. Solasodine does not have a conjugated double bond in its structure. The nitrogen is protonated and forms complexes that are extractable into organic solvent like chloroform. Liquid-liquid extraction is one of the most versatile techniques for sample matrix separation. It has been applied to various analytical fields. However, manual extractions present a series of drawbacks such as high consumption of sample and toxic organic solvents, low sampling frequency and loss of analyte through manipulation. In this study the use of high performance liquid chromatography (HPLC) has been recognized as a developed method for the separation and quantitative analysis of solasodine. Because solasodine lacks conjugate double bonds in its structure, detection of solasodine in the ultraviolet range is not fully convincing. It would be more appropriate if the ʎ max of solasodine could be enhancing to ensure a more effective detection. Hence, an attempt was made to introduce the use of high performance liquid chromatography (HPLC) for the separation and qualitative analysis of solasodine (Hunter *et al*. 1980; Osman and Sinden 1989) [8, 17].

Some of the solanaceous plants, namely *S. diphyllum, S. incanum, S. nigrum, S. sisymbrifolium, S. surattense, S. torvum, S. verbascifolium* and *S. vellosum* are found wildly most promising, because of its potential as an alternative precursor for the industrial production of certain steroid drugs and its moderately high value quantification is an important component of a strategy to improve yield. Therefore, our interest focused on assessing the comparative analysis for determination of bioactive solasodine compound in fruits of all eight *Solanum* species.

Experimental

Plant Material

Green *Solanum* berries were picked from wild plant. The plant specimens were identified with the help of different floras (Dastur 1970; Jaeger 1985; Khanna *et al*. 1999; Mabberley 2008; Mishra and Verma 1992; Raizada 1976; Saini *et al*. 2010; Singh 1996; Hooker 1872-96; Duthie 1903-1920) [3, 9, 11, 14, 16, 22, 23, 25, 7, 5]. The plant species are preserved in the form of herbarium of Botany Department of Udai Pratap (Autonomous) College, Varanasi.

Sample preparation

The berries of plant samples (1.0 g) were individually picked up from each treatment and were crushed with 10 ml of methanol: H_2O (1:1) in a mortar and pestle and left overnight at room temperature. Clear supernatants obtained by filtering through sterilized Whatman No. 1 filter paper were and mixed with an equal volume of ethyl acetate. The mixture thus obtained was shaken vigorously in a separating funnel to collect the ethyl acetate fractions. The residue was extracted for a second time and the ethyl acetate fractions were pooled with the previous extract. The fractions were then removed under vacuum on the rotatory evaporator (Eyela N–N series, Tokyo, Japan). Dried samples were suspended in 1.0 mL of high-performance liquid chromatography (HPLC) grade methanol by vortexing, followed by filtration through a 0.22 µm membrane filter (Merck).

Preparation of sample solution

One-gram aerial parts of each of the eight *Solanum* species were extracted thrice with methanol (20 mL) in an ultrasonic extractor (30 min, 45 ^{0}C, Microclean-109 bath; Oscar, Mumbai, India). The pooled extracts were separately evaporated to dryness under vacuum at 40 ^{0}C and the residues were dissolved separately in 1mL methanol and filtered through 0.45 mm membrane (Millipore).

Preparation of standard solution

Standard stock solutions of solasodine (Fig.1) was prepared separately at a concentration of 1.0 mg/mL in methanol. A serial dilution for each stock solution was made at concentrations of 0.1-0.8 mg/mL by adding methanol.

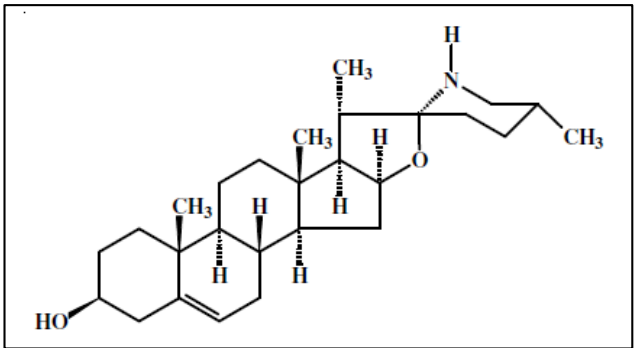

Fig 1: Structure of solasodine

HPLC analysis

The extraction and HPLC analysis of the steroid alkaloid solasodine was followed by the method applied by Shilpha *et al*. (2014). The quantitative determination of solasodine was achieved via UFLC, SHIMADZU equipped with two LC-10 pumps and an UV detector SPD-10A. The extracts were filtered through 0.22 µm membrane filter and an aliquot of 30 µL was injected into Supelco reverse phased C18 analytical column (25×4.6 mm, 5µm) at a flow rate of 1 mL/min at 30◦C and the effluent was monitored by UV detection at 205 nm. The solvent system was a linear gradient of water (A) and methanol (B) from 40 to 70% for 15 min; 100 % B for 5 min and then from 100 to 0% B for 5 min followed by washing for 26 min (Table 1). Samples were identified by comparing retention time (RT) of peaks obtained in samples with that of standard and quantity was calculated in mg g^{-1} by comparing the area of the peak thus obtained with that of standard.

Table 1: Gradient programming for the separation of Solasodine from the MeOH extract of different *Solanum* species

Time (min)	Water concentrate (%) (pump A)	Methanol concentrate (%) (pump B)	Flow rate (mL/min)
0-5.59	60	40	1
5-14.59	30	70	1
15-19.59	0	100	1
20-24.59	100	0	1
25-26	100	0	1

Chemicals

Solasodine standard (99%) was purchased from Sigma Chemical Co. (Merck). All solvents used for chromatographic purposes were of HPLC grade.

Method validation

Method validation was performed on parameters such as linearity, limit of sensitivities, selectivity, precision, accuracy, recovery and robustness as per ICH (ICH-Q2 (R1), 2005) guidelines. All the data were evaluated using standard statistical packages for Windows and Graph Pad Prism 4.0 (Graph Pad Software Inc., La Joll, CA, USA).

Results and discussion

The results obtained from analysis of high performance liquid chromatography (Fig 5) showed that solasodine content in methonolic extract of fruits of *S. diphyllum, S. incanum, S. nigrum, S. sisymbrifolium, S. surattense, S. torvum, S. verbascifolium* and *S. vellosum* was 0.356, 0.341, 0.472, 0.494, 0.446, 0.396, 0.416 and 0.465 mg/gm respectively (Table 4). The maximum concentration of solasodine was found in *S. sisymbrifolium* 0.495 mg/gm whereas, the minimum solasodine content in *S. incanum* was 0.341 mg/gm (Fig. 4).

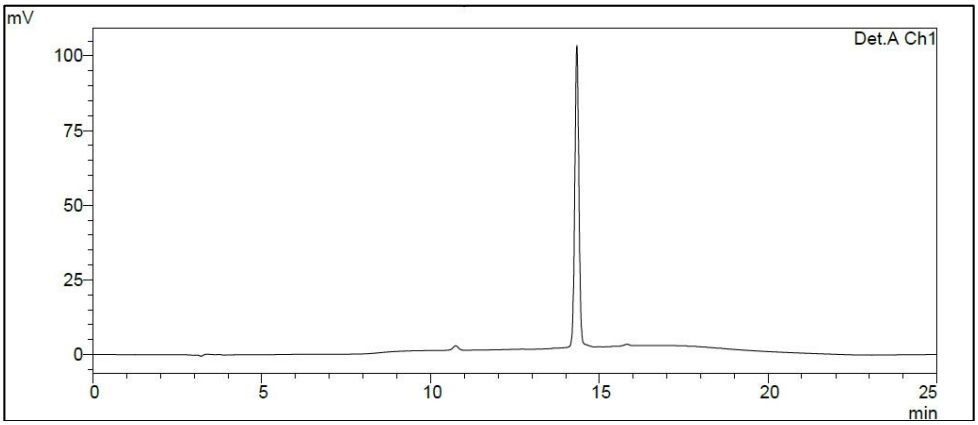

Fig 2: Chromatogram of Solasodine standard (at concentration 0.1 mg mL^{-1})

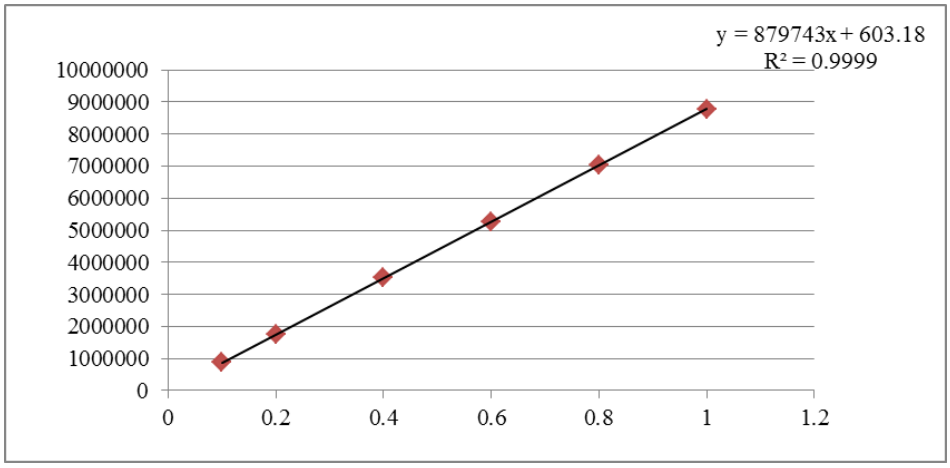

Fig 3: Linear calibration curve of solasodine

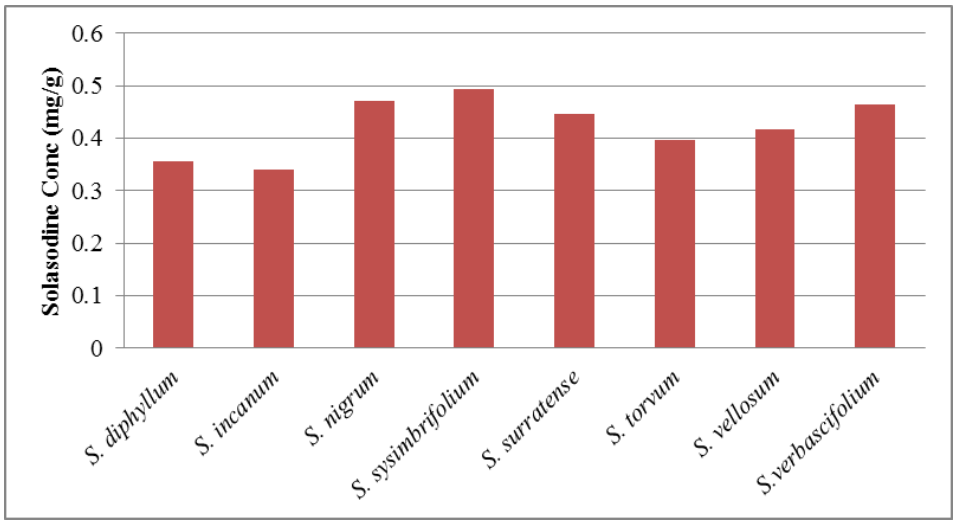

Fig 4: Solasodine content in different *Solanum* species

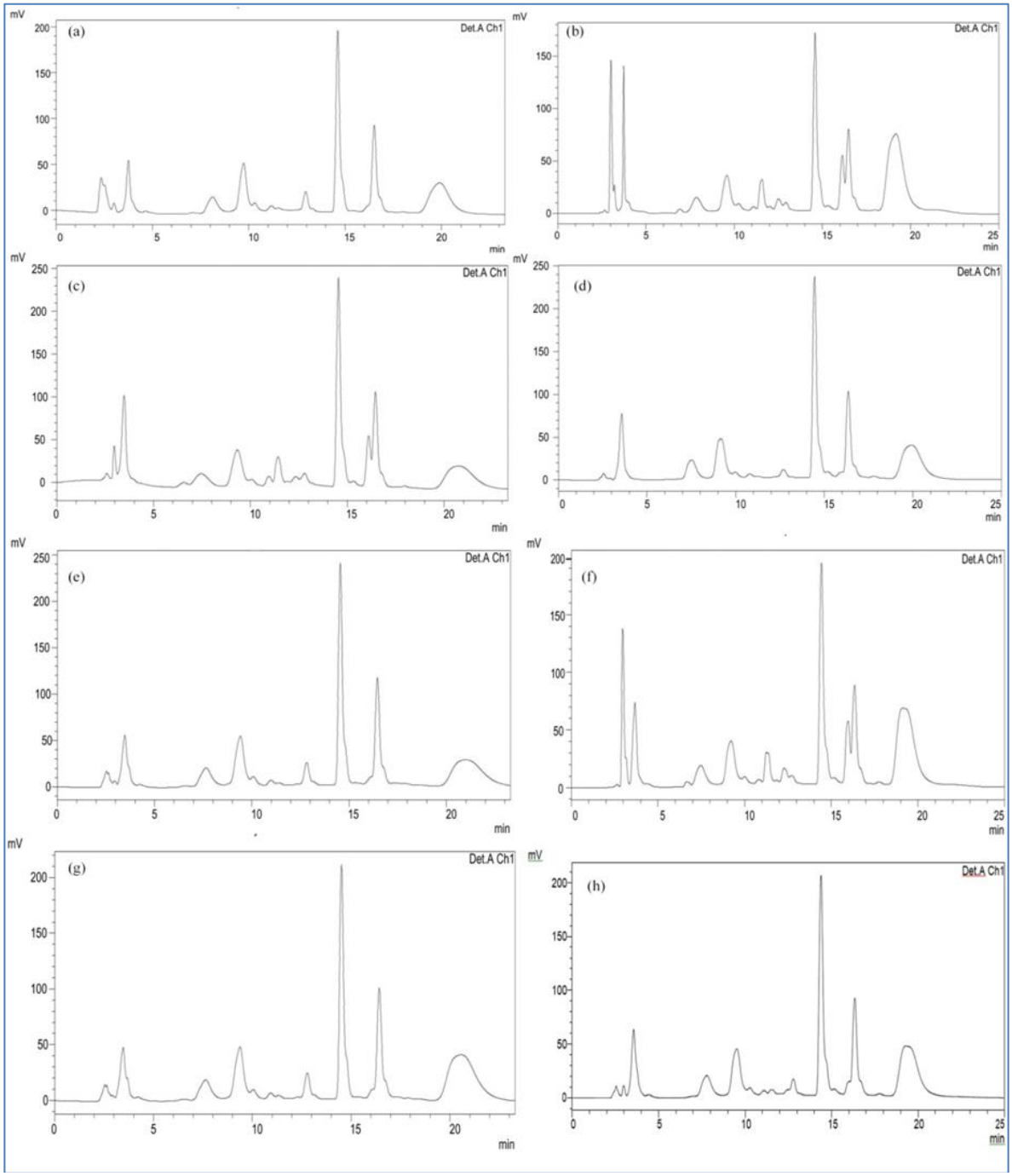

Fig 5: HPLC chromatogram of the methanolic extract of different *Solanum* species. (a). *S. diphyllum*, (b). *S. incanum*, (c). *S. nigrum*, (d). *S. sisymbrifolium*, (e). *S. surratense*, (f). *S. torvum*, (g). *S. vellosum*, (h). *S. verbascifolium*

Optimisation of separation conditions

Since solasodine is a weak basic, and therefore, polar compound, interaction with silanol groups on the bonded phase occurs easily, resulting in peak tailing (Fig. 2). The use of a base-deactivated C-18 Alltima column was introduced in an attempt to decrease peak tailing. This column is packed with abase-deactivated silica (decreased free silanol concentration) that is less active toward polar compounds (Steffeck *et al.,* 1995) [27] and therefore provides a more symmetrical peak shape, the retention time can be shortened. Optimization of the mobile phase focused upon the composition and pH. Methanol: water mixtures were found to be more effective than acetonitrile: water or acetonitrile: methanol mixtures in the separation of solasodine from other components in the tissue samples (Kittipongpatana *et al.* 1999) [12]. The reported HPLC methods were performed using the mobile phase at pH 7.0 to obtain reproducible separations. Solasodine shows a maximum UV absorbance at 205 nm. The sensitivity of the detector was limited in the methanol-containing mobile phase because methanol has a UV cut off at 205 nm as well. With the shorter retention time in the acidic mobile phase, it was possible to decrease the methanol percentage in the mobile phase to increase the detection sensitivity.

Linearity of calibration graph

The linearity of responses to solasodine was determined. Calibration curve was obtained by using the least-square linear regression analysis of the studied solasodine peak area (y) *versus* analyte concentration (x). Each concentration was tested in triplicate. Linear calibration curve was obtained over the concentration range of 0.1-1.0 mg mL^{-1} of solasodine (Fig 3). The standard solution was injected into the HPLC system. Linear calibration graph over the concentration range 0.1-1.0

mg mL^{-1} of solasodine was obtained with a correlation coefficient of 0.9999.

Limit of detection and limit of quantification

The detection limit of the method was investigated by injecting standard solution of solasodine into the HPLC column. The limit of detection (LOD) was found to be 0.011 mg mL^{-1}. It is calculated three times of the standard deviation. The limit of quantification (LOQ) value was found to be 0.035 mg mL^{-1}. This value was calculated directly from the calibration graph. It is defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision, LOQ may be expressed as: LOQ=10SD/S where S is the slope of calibration graph and SD is standard deviation.

Precision and accuracy

The intra- and interday precision (expressed in terms of %RSD was observed in the range of 0.32–0.98% and 0.33–1.13%, respectively, which demonstrated good precision of the method. The low values of %RSD (< 2%) reflect the good precision of the method. The accuracy of the proposed method was expressed as the recovery of standard compounds added to the pre-analysed samples. The recovery was found to be in the range of 98.8–101.4%, indicating good accuracy of the method. These results are summarised in Table 2.

Table 2: Intra- and Inter day precision (%RSD) of methods for the Solasodine (n=6)

Amount added (mg/mL)	Amount found (mg/mL)	% RSD	Recovery (%)
Intraday (n=6)			
0.1	0.098	0.40	98.81
0.2	0.201	0.68	100.76
0.4	0.397	0.45	99.28
0.6	0.606	0.98	101.06
0.8	0.794	0.32	99.37
1	1.001	0.52	100.11
Inter day (n=6)			
0.1	0.099	0.33	99.55
0.2	0.203	0.48	101.27
0.4	0.398	0.63	99.63
0.6	0.614	1.13	102.38
0.8	0.798	0..74	99.78
1	1.015	0.88	101.48

Recovery

The three different concentrations diluted from the stock solution were added to an extract with a known content of Solesodine. The recovery (R) was calculated as R = (C found-C sample)/C added, where C found is the concentration in a spiked sample, C sample is the concentration in the sample prior to spiking and C added is the concentration of added standard (Table 2).

Robustness

Robustness of the method was determined by measuring the effect of small and deliberate changes in the analytical parameters on retention time and peak area of test compounds. The parameters taken into consideration were mobile phase composition, flow rate and temperature. Only one parameter was changed at a time while the others were kept constant. The relative standard deviations of retention time and peak area counts of Solosodine were calculated for each parameter. The standard deviations (%RSD) of retention time and peak area counts were calculated for each parameter and were less than 2 (%RSD>2). The lower RSD% confirmed the robustness of the method (Table.3).

Table 3: Robustness testing for the Solasodine (n=3)

Variable	Retention time % RSD	Peak area % RSD
Mobile phase composition	0.505604	1.163094
Flow rate	0.789004	1.321516
Column temperature	0.281288	1.657895

Quantification of steroidal glycosides

The methanolic extract showed the presence of Solasodine as major constituent in eight *Solanum* species, was confirmed by comparison of their retention times and overlaying of UV spectra with those of standard compounds. The percentage contents of Solasodine in eight *Solanum* species are summarised in Table 4.

Table 4: Content of steroidal glycosides: Solasodine in different *Solanum* species

Plant name	Solasodine (mg/g)
S. diphyllum	0.356
S. incanum	0.341
S. nigrum	0.472
S. sisymbrifolium	0.494
S. surratense	0.446
S. torvum	0.396
S. vellosum	0.416
S. verbascifolium	0.465

Conclusion

With the increasing use of steroids drugs for the treatment of asthma inflammatory disorders, tumors, sex hormone imbalances, and as oral contraceptives and the discovery the solasodine can be easily converted into 16-dehydropregnenolone acetate, a key intermediated in the synthesis of steroid drug; it is appropriate that the search be made for high solasodine yielding plant species (Maiti 1979).

Herbal medicines are used for the treatment of various disorders and gaining popularity both in developing as well as developed countries due to its fewer side effects. Many traditional medicines are derived from medicinal plants, minerals and organic matter. According to the World Health Organization (WHO) more than 21000 plants are used for medicinal purposes in the world (Patel *et al*. 2011a; Patel *et al*. 2011b) [18, 19]. Due to presence of diverse phytoconstituents, many medicinal plants have excellent pharmacological actions and could lead to the development of new classes of possibly safer agents for the treatment of disease. Plants have been used as a source of drugs and many of the currently available drugs *have been derived directly or indirectly from them (Patel *et al*. 2011) [20]. Only a few herbs and bioactive chemical moiety have attracted the interest of scientists and have been put forward for investigations. Solasodine may have all these actions and could be used for the treatment of various complications in the near future.

In this study, we found that the solasodine content of *S. sisymbrifolium* is maximum and minimum in *S. incanum*. Our findings highlight the importance of wild neglected flora also need a well-documented clinical trial and more laboratory work to justify their pharmacological actions and toxicity for safe and effective treatment.

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Conflict of interest statement

We declare that we have no conflict of interest.

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