ORIGINAL ARTICLE

Screening, detection, and quantification of solasodine in *Solanum pubescens* Willd. by reversed-phase high-performance liquid chromatography method

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Abstract

Objective: The aim of the study is to extract the solasodine with different solvents from leaf and stem bark of *Solanum pubescens* and to screen, detect, and quantify using reversed-phase high-performance liquid chromatography (RP-HPLC) methods. **Methods:** Standard solasodine marker compound and five different solvent extracts made through Soxhlet extraction from leaf and stem bark of *S. pubescens* were injected (10 µl) to HPLC with C_{18} reversed-phase column, gradient solvent eluent system, and photo-diode array detector (DAD) under ultraviolet absorbance at 205 nm with flow rate of 1.2 ml/min. a simple formula is adopted to quantify the assay % of solasodine. **Results:** Standard solasodine marker was detected at a retention time (RT) 21.59 min with the peak area of 5245605 at a wavelength of 205 nm. Among the ten extracted samples, solasodine was detected in leaf methanol extract (RT 21.81 min) and hydro-alcohol leaf extract (RT 21.82 min) with the peak area of 191694 and 246023, respectively. The quantified assay % of solasodine was highest in leaf hydro-alcohol extract (1.857%) followed by leaf methanol extract (1.447%). In the remaining eight extracts, solasodine was not detected. **Conclusion:** The present study findings are the first report with accuracy and simple assay method for extraction, screening, detection, and quantification of solasodine using RP-HPLC from *S. pubescens*.

Key words: Glycoalkaloid, gradient solvent, reversed-phase high-performance liquid chromatography, *Solanum pubescens*, solasodine

INTRODUCTION

In the genus, *Solanum* of *Solanaceae* member found to have rich steroidal glycoalkaloids (SGAs), which is an important group of plant secondary metabolites, and more than 100 different types of glycoalkaloids have been isolated from more than 350 *Solanum* species.[1,2] Solasodine occurs as aglycone part of glycoalkaloids, which is a nitrogen analog of saponins. It has C27 cholestane skeleton and can be readily converted to 16-dehydropregnenolone which is a key intermediate in the synthesis of steroidal drugs such as progesterone and cortisone.[3] It is also a potential moiety to be used as a substitute for diosgenin in the semi-synthetic production of steroidal hormones in pharmaceuticals. SGA from *Solanaceae* plants has become increasingly important as the starting material for the production of steroidal hormones.[4] Solasodine was explored in many *Solanum* species extensively, which was initially estimated by adopting colorimetric and spectrophotometric methods and high concentration was reported.[5,6] However, these methods were reported as non-specific and destructive, and the results represent solasodine along with its glycosides solamargine, solasonine, and solasodine.[7] High-performance liquid chromatography (HPLC)-based qualitative[8,9] and quantitative[10-14] method of assay for solasodine was reported from many *Solanum* species, but detection and quantification

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of solasodine from *Solanum pubescens* are not yet attempted. In the present investigation, an attempt is made to develop an accurate, sensitive, reproducible, rapid, and simplified quantification method using reversed-phase (RP)-HPLC (RP-HPLC) for solasodine from *S. pubescens*. In this plant, for the 1st time, we have reported an improved and simplified RP-HPLC-based assay method over the methods reported earlier for separation, detection, and quantification of solasodine from *S. pubescens*.[11,15]

MATERIALS AND METHODS

Plant Material Collection and Identification

The study plant, *S. pubescens* was collected from the hilly areas of Chitradurga, Karnataka, India, located at 13.95°N 76.62°E. The botanical identity of the plant was confirmed by referring Phytographia[16] and further authenticated by Prof. L. Rajanna, Chairperson, Department of Botany, Bangalore University, Bangalore, Karnataka, India. The plant herbarium (voucher number: IDSGH-43) is deposited and maintained in the Department of Botany, Indavara Dodda Siddalinge Gowda Government College, Chikkamagaluru, Karnataka, India. All the chemicals, solvents, and water used in this study are HPLC grade and procured from Merck, India.

Sample and Standard Solasodine Preparation for HPLC Analysis

The matured leaf and stem bark of *S. pubescens* were dried and powdered, and Soxhlet extractions for each sample were made separately using five different solvents. Samples were labeled from serial number 1 to 10 in the following order such as leaf methanol extract, bark hydro-alcohol extract, leaf *n*-hexane extract, leaf chloroform extract, bark ethyl acetate extract, bark *n*-hexane extract, leaf ethyl acetate extract, bark methanol extract, bark chloroform extract, and leaf hydro-alcohol extract. For HPLC analysis, all the samples were prepared at a concentration of 25 mg of extract dissolved in 10 ml of methanol in 10 ml volumetric flasks. Standard marker compound solasodine was procured from Gesra Labs India Pvt. Ltd., Chennai, Tamil Nadu, India, and prepared 10 mg/10 ml concentration using methanol. Samples and standard solutions were sonicated for 15 min to remove air bubbles, made up to 10 ml in a volumetric flask, and filtered through 0.25 µ filters.

HPLC Solvents

Solvent A was made as follows: Orthophosphoric acid (0.5 ml) and potassium dihydrogen phosphate (136 mg) were dissolved in 900 ml of water and made up to 1000 ml. Solvent B was acetonitrile. Solvents were filtered through 0.25 µ filter and degassed in ultrasonicator for 15 min.

HPLC Gradient Conditions

The HPLC gradient condition program for solasodine screening and detection is given in Table 1.

RP-HPLC Conditions

Shimadzu HPLC (LC 2030C3D) with photo DAD and lab solution software was used to develop chromatogram. The column conditions are like Phenomenex Luna C_{18}, 5.0 µ (250 × 4.6 mm), reverse phase with ultraviolet (UV) detector at 205 nm and flow rate is 1.2 ml/min with sample injection volume 10 µl. Samples and standard analyses were repeated three times, and the values shown in this study are mean ± SD (standard deviation) of three reproducible experiments.

Sample Assay Formula

(Sample area/standard area) × (Standard dilution/sample dilution) × Purity of Standard (99%).

RESULTS

In the RP-HPLC analysis, all the ten extracted samples and standard marker were screened, detected, and quantified using reversed-phase C_{18} column [5.0µ (250 × 4.6 mm)] coupled with a UV detector at 205 nm for solasodine. Solasodine was detected at RT 21.59 min with the peak area of 5245605 [Table 2 and Figure 1a]. Among the ten samples screened, the solasodine was detected only in the leaf methanol extract (peak area 191694) and hydro-alcohol leaf extract (peak area 246023) at RT 21.81 and 21.82 min, respectively [Table 2 and Figure 1b and c]. Solasodine assay % of samples and standard was calculated; the standard solasodine was 99%, and 1.447% was in methanol leaf extract (sample 01), and in hydro-alcohol leaf extract (sample 10), it was 1.857%. In the remaining eight extracts (Sample number 2–9), solasodine was not detected.

Table 1: Concentrations of solvents A and B in the high-performance liquid chromatography gradient condition and its duration

Solvent A concentration (%)	Solvent B concentration (%)	Run time/ duration (min)
95	5	00.01
80	20	10.00
55	45	20.00
40	60	25.00
10	90	30.00
55	45	35.00
95	5	40.00
95	5	45.00
Stop		45.01

Table 2: High-performance liquid chromatography analysis data of standard and ten different solvent extracts made from leaf and stem bark of *Solanum pubescens*

Sample no. and name	Retention time (Min)	Peak Area	Sample wt (mg)	Sample dilution (ml)	Assay (%) (mean±SD)
Standard solasodine	21.59	5245605	10	10	99.00
Sample 1	21.81	191694	25	10	1.447±0.09
Sample 2	NA	-	25	10	-
Sample 3	NA	-	25	10	-
Sample 4	NA	-	25	10	-
Sample 5	NA	-	25	10	-
Sample 6	NA	-	25	10	-
Sample 7	NA	-	25	10	-
Sample 8	NA	-	25	10	-
Sample 9	NA	-	25	10	-
Sample 10	21.82	246023	25	10	1.857±0.06

-Indicates not detected. *Values are mean±SD (standard deviation) of three reproducible experiments

DISCUSSION

Solasodine's wide pharmaceutical applications made researchers to develop many methods and test for the isolation and quantification from plant materials using spectroscopy, thin-layer chromatography, immunostaining using monoclonal antibodies, high-performance thin-layer chromatography, HPLC, capillary electrophoresis, gas chromatography, and others.[3,17] In the present study, RP-HPLC-based solasodine investigation was focused on *S. pubescens* Willd. because it was not attempted in this plant so far and it is widely used by the local medicinal practitioners for treating different diseases such as whooping cough,[18] headache, menstrual pain, rheumatoid arthritis, tuberculosis, and ulcers.[19] In this plant, many research reports have been found on pharmacological studies such as antidiabetic activity,[20] anti-inflammatory activity,[21] antidiarrheal activity,[22] antinociceptive activity,[19] anticonvulsant and sedative effects,[23] and antibacterial activity.[24] Similarly, many investigations have proved that solasodine from *Solanum* species has diuretic, anticancer, antifungal, cardiotonic, antispermatogenetic, antiandrogenic, immunomodulatory, antipyretic, and other effects on the central nervous system.[1]

In the study plant, leaf and bark were subjected to Soxhlet extraction using different solvents such as methanol, *n*-hexane, chloroform, ethyl acetate, and hydro-alcohol then 25 mg of extract dissolved in 10 ml of HPLC grade methanol, and the entire sample was screened for solasodine using RP-HPLC method under UV 205 nm. Among the sample extracts screened, the solasodine was detected only in leaf, and highest percentage of solasodine was quantified in the hydro-alcohol extract (1.857%) while it was 1.447% in the methanolic extract [Table 2 and Figure 1a-c]. A similar methodology was adopted[13] for quantification of solasodine in the callus and suspension cultures of *S. xanthocarpum,* and Solasodine was highest in stem callus (0.062% w/v) followed by the natural stem (0.048% w/v) and its suspension cultures (0.027% w/v). In the present study, the Soxhlet method of extraction of solasodine and screening by RP-HPLC method with the gradient solvents yielded very high solasodine (1.857%) in the leaf hydro-alcohol extract from *S. pubescens* which we report as precision, accurate, and robust. Similar reports were made on quantification of solasodine content in the field grown leave extracts (0.0798 mg/g) and in *in vitro* callus extracts (0.142 mg/g and 0.1162 mg/g) produced using plant growth regulators.[25] In the present investigation, the yield of solasodine content quantified from *S. pubescens* was high using RP-HPLC method and it is precise, accurate, and reproducible over the previous reports without any callus production through plant tissue culture methods. Solasodine shows a maximum UV absorbance at 205 nm[11] which was adopted in the current methodology, and furthermore, we used the photo DAD for the estimation of solasodine as described in which quantitative determination of solasodine was made in aerial parts of *S. nigrum* Linn.[26] Recently, similar studies on estimation of curcumin and piperine[27] using RP-HPLC and method development and validation for analysis of deoxyarbutin by HPLC[28] were reported. The accurate and fast HPLC method was adopted to separate and determine the SGAs (solasonine, solanine, chaconine, and solamargine) and the SGA aglycones (SGAAs) solasodine from *Solanum* extracts with the suitable mobile phases combined with the gradient elution.[15] They also mentioned that to separate the SGAs and SGAAs in the *Solanum* extract, a most efficient eluent tritely ammonium dihydrogen phosphate was used, and a simple gradient elution mobile phase modifier acetonitrile is still required. In the present investigation, solasodine was screened using RP-HPLC system with photo DAD, under UV absorbance 205 nm with high efficient gradient solvent systems with solvent A – ortho phosphoric acid and potassium dihydrogen phosphate and solvent B – acetonitrile and quantified using simple assay formula with accuracy and precision.

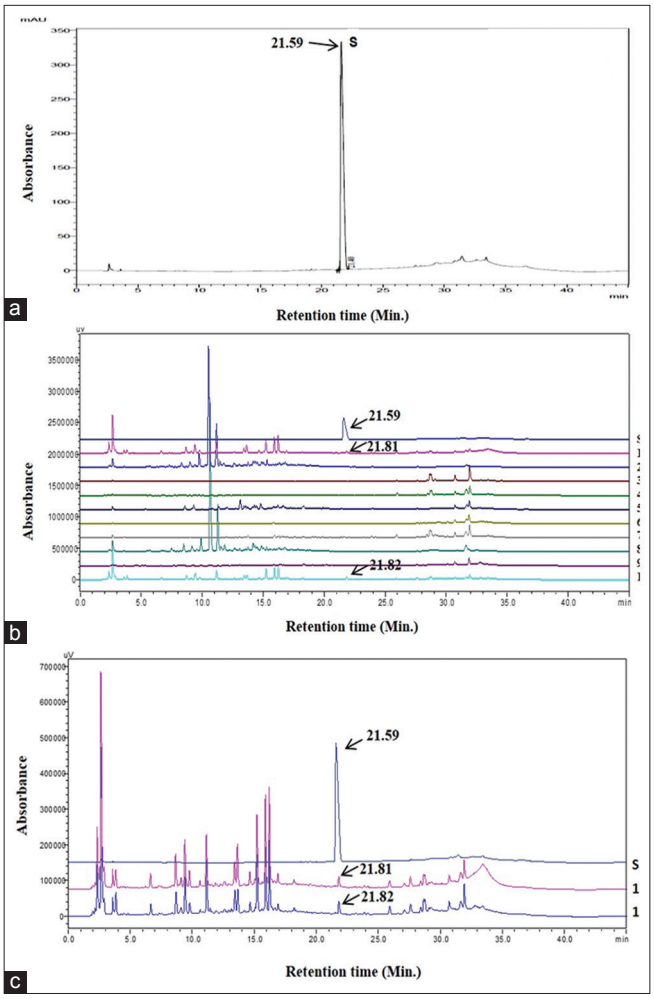

Figure 1: (a) High-performance liquid chromatography (HPLC) chromatogram of standard solasodine; (b) HPLC chromatogram of standard solasodine (S) with all the ten extracted samples (1-10), 1 – leaf methanol extract, 2 – bark hydro-alcohol extract, 3 – leaf *n*-hexane extract, 4 – leaf chloroform extract, 5 – bark ethyl acetate extract, 6 – bark *n*-hexane extract, 7 – leaf ethyl acetate extract, 8 – bark methanol extract, 9 – bark chloroform extract, and 10 – leaf hydro-alcohol extract; (c) A portion enlarged HPLC chromatogram of standard solasodine (S) with standard samples 1 and 10

CONCLUSION

The first report with precision, accuracy, robustness, reproducibility, and simple assay method was developed for extraction, detection, and quantification of solasodine in the leaf and stem bark of *S. pubescens* using RP-HPLC technique with a suitable mobile phase gradient elution system and photo DAD under UV absorbance at 205 nm. Highest solasodine was detected and quantified in the leaf hydro-alcohol extract (1.857%). Hence, the present investigation established a method for separation, detection, and quantification of solasodine from *S. pubescens* using RP-HPLC technique. We infer that the leaf of *S. pubescens* is a potential source of solasodine, and its pharmacological application studies are under progress.

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REFERENCES

1. Patel K, Singh RB, Patel DK. Medicinal significance, pharmacological activities, and analytical aspects of solasodine: A concise report of current scientific literature. J Acute Dis 2013;2:92-8.
2. Sun F, Li S, He D, Cao G, Ni X, Tai G, *et al.* Effects of glycoalkaloids from *Solanum* plants on cucumber root growth. Phytochemistry 2010;71:1534-8.
3. Pankaj KP, Tushar PB, Rizwan ZP, Vijay LM. A simple technique for rapid quantitative determination of solasodine from cultured hairy roots of *Solanum surattense*. J Herb Med Toxicol 2008;2:7-10.
4. Solouki M, Hoshyar H, Ramroudi M, Tavassoli A. Comparison and evaluation of steroid alkaloid solasodine on *in vivo* and *in vitro* cultures of *Solanum surattense* Burm L. Afr J Microbiol Res 2011;5:3810-14.
5. Jaggi RK, Kapoor VK. Solasodine its occurrence in nature and production. J Sci Ind Res 1994;43:34-42.
6. Chand R. Simplified method for estimation of solasodine from the berries of *Solanum laciniatum* ait. Ind J Pharm Sci 1991;53:20-1.
7. Subroto MA, Doran PM. Production of steroidal alkaloids by hairy roots of *Solanum aviculare* and the effect of gibberellic acid. Plant Cell Tissue Organ Cult 1994;38:93-2.
8. Hunter IR, Walden MK, Heftmann E. High-performance liquid chromatography of *Solanum* and veratrum alkaloids. J Chromatogr A 1980;198:363-6.
9. Osman SF, Sinden SL. High-performance liquid chromatographic analysis of *Solanum* steroidal alkaloids. J Chromatogr A 1989;479:189-93.
10. Crabbe PG, Fryer C. Rapid quantitative analysis of solasodine, solasodine glycosides and solasodiene by high-pressure liquid chromatography. J Chromatogr A 1980;187:87-100.
11. Kittipongpatana N, Porter JR, Hock RS. An improved high performance liquid chromatographic method for the Quantification of solasodine. Phytochem Anal 1999;10:26-31.
12. Suthar AC, Mulani RM, Gulati V. A HPTLC method for estimation of solasodine and diosgenin in *Solanum xanthocarpum* Schrad. and Wendl. Nat Prod Indian J 2008;4:108-12.
13. Rasheeduz Z, Parisa H. Quantification of solasodine in callus and suspension cultures of *Solanum xanthocarpum* schrad. and Wendl. by RP-HPLC. Int J

Pharm Phytophram Res 2015;7:206-14.
14. Tayade S, Patil D. Solasodine alkaloid extraction from leaves of *Solanum xanthocarpum*. Int J Adv Res Sci Eng 2017;6:226-30.
15. Madeha HH, Ali SH, Fadhil MA. Reversed phase HPLC method for separation and identification of steroidal glycoalkaloids (SGAs) from (*Solanum*) extract. Indian J Public Health Res Dev 2018;9:1197-1.
16. Phytographia: 5. t. 3. (1794); Hook. f., Flora of British India 4: 230, Gamble, 2, 936, (1883).
17. Trivedi P, Pundarikakshudu K. Novel TLC densitometric method for quantification of solasodine in various *Solanum* species, market samples and formulations. Chromatographia 2007;65:239-43.
18. Reddy KN, Reddy CS, Trimurthulu G. Ethnobotanical survey on respiratory disorders in Eastern Ghats of Andhra Pradesh, India. Ethno Bot Lealf 2006;10:139-48.
19. Sumalatha P, Hemamalini K, Shwetha R, Reddy UV. Antinociceptive screening of methanol extract of *Solanum pubescens*. Int J Pharm 2013;4:149-51.
20. Hemamalini K, Krishna VR, Bhargav A, Reddy UV. Hepato protective activity of *Tabebuia rosea* and *Solanum pubescens* against paracetamol induced hepatotoxicity in rats. Asian J Pharm Clin Res 2012;5:153-6.
21. Niyogi P, Raju NJ, Reddy PG, Rao BG. Formulation and evaluation of anti-inflammatory activity of *Solanum pubescens* wild extracts gel on albino wister rats. Int J Pharm 2012;2:484-90.
22. Hemamalini K, Bhargav A. Antidiarrheal activity of methanolic extracts of leaves of *Solanum pubescens* willd. Int J Pharma Toxicol 2013;3:43-5.
23. Kiranmai AS, Hemamalini K, Reddy UV. Anticonvulsant and sedative effects of leaf extract of *Solanum pubescens*. Int J Pharm Sci Res 2013;4:1424-7.
24. Rahman H, Mahmood R, Rahman N, Haris M. Antibacterial activity of *Solanum pubescens* an ethnomedicinal plant from South Western region of Andhra Pradesh. Int J Appl Sci Biotechnol 2014;2:501-9.
25. Yogananth N, Bhakyaraj R, Chanthuru A, Parvathi S, Palanivel S. Comparative analysis of solasodine from *in vitro* and *in vivo* cultures of *Solanum nigrum* Linn. Kath Univ J Sci Eng Tech 2009;5:99-03.
26. Desai S, Tatke P, Gabhe S. A new validated HPLC-DAD method for estimation of solasodine from *Solanum nigrum* Linn. extracts and formulations. Nat Prod J 2014;4:1-5.
27. Kuber BR. Method development and validation for estimation of curcumin and piperine by RP-HPLC. Int J Appl Pharm 2018;11:216-21.
28. Musfiroh MM, Fauzi A. Method development and validation for analysis of deoxyarbutinin anhydrous emulsion system using high-performance liquid chromatography. Int J Appl Pharm 2019;11:172-5.

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