Original Article

Simultaneous determination of vasicine and vasicinone by High-performance liquid chromatography in roots of eight *Sida* species

M. D. Subramanya, Sandeep R. Pai[1], Gireesh M. Ankad[2], Harsha V. Hegde[2], Subarna Roy[2], S. L. Hoti[2]

Department of Dravyaguna, BMK Ayurveda Mahavidyalaya, Belagavi, [1]Department of Biotechnology, Amity Institute of Biotechnology, Amity University Mumbai, Maharastra, [2]Herbal Medicine Division (BIC), ICMR – National Institute of Traditional Medicine, Belagavi, Karnataka, India

Abstract

Introduction: *Sida* L. is a medicinally important genus widely used in conventional systems of medicine in India. **Aim:** The present study aims toward simultaneous determination of two bioactive compounds vasicine and vasicinone in root extracts of eight *Sida* spp. from Western Ghats, India. **Materials and Methods:** Determination of vasicine and vasicinone was undertaken in methanolic root extracts (10% w/v) of *Sida acuta*, *Sida cordata*, *Sida cordifolia*, *Sida rhombifolia*, *Sida spinosa*, *Sida indica*, *Sida retusa* and *Sida mysorensis* by high performance liquid chromatography (HPLC) method. The standards were prepared with the concentration of mg/mL. Data were expressed as mean values of three reading and relative standard deviations. The separation was achieved on a Waters, Nova-Pack, C18 (250 mm × 4.6 mm, 5 µ) column, with acetonitrile - 0.1 M phosphate buffer-glacial acetic acid (15: 85: 1, v/v/v) as solvent system at a flow-rate of 1.0 mL/min. The effluent was monitored using ultraviolet detection at a wavelength of 300 nm. **Results:** Both calibration curves of standard showed good linear regression ($R^2 > 0.994$). The limit of detection and the limit of quantification for vasicine was 0.110 and 0.333 µg/mL and for vasicinone was 0.059 and 0.179 µg/mL respectively. The vasicine content was highest in *S. cordifolia* (9.891 ± 0.495 µg/100 mg) and vasicinone content was rich in *S. cordata* (33.013 ± 1.651 µg/100 mg.) The content of vasicinone was higher than vasicine. **Conclusion:** HPLC method provides simple, accurate, and reproducible quantitative analysis for simultaneous determination of vasicine and vasicinone. Among the selected *Sida* species, *S. cordifolia* and *S. cordata* were found to be rich in the vasicine and vasicinone contents, respectively.

Keywords: High performance liquid chromatography, *Sida* spp, vasicine, vasicinone

Introduction

Genus *Sida* L. (Malvaceae) comprises of 200 species distributed throughout the world, out of which 17 species are confined to India.[1] The plant is well documented in ancient Indian system of medicine "*Ayurveda*." *Bala* is an important plant belonging to *Karpasa kula,*[2] used as *Rasayana*[3] to treat various ailments such as *Vatavyadhi*[4] and *Pradara*.[5] Even though species belonging to genus *Sida* are well known for their medicinal properties, especially in *Ayurveda*, the issue in their correct botanical identity persists. *Bhavaprakasha Nighantu* mentions four varieties of *Bala* (*Balachatushtaya*)[6] while *Dhanvantari Nighantu* mentions five varieties (*Panchabala*).[7] In general, as per the classics, *Bala* is *Sida cordifolia* L., *Mahabala* is correlated to two plants, namely, *Kshetrabala* (*Sida rhombifolia* L.) and *Sahadevi* (*Vernonia cinerea* L.). Similarly, the name *Atibala* is attributed to *Abutilon indicum* L. and *Nagabala* indicate three plants, i.e. *Bhumibala* (*Sida veronicaefolia* L.), *Kantakinibala* (*Sida spinosa* L.) and *Gudasharkara (Grewia hirsuta* Vanb.).[8] Other classical references mentioned *Rajabala* or *Bruhat Nagabala* as *Sida acuta* Burm. F.[9] There are different of opinion regarding botanical identities of *Bala*, which are still controversial.[10]

It is known for various pharmacological activities such as hepatoprotective, anti-arthritic, gonorrheal, end also as an immune enhancer.[11] Several *Sida* species have been

Access this article online

Quick Response Code:

Website: www.ayujournal.org

DOI: 10.4103/ayu.AYU_49_16

Address for correspondence: Dr. Gireesh M. Ankad, Herbal Medicine Division (BIC), ICMR – National Institute of Traditional Medicine, Belagavi - 590010, Karnataka, India.
E-mail: drgirishankad@gmail.com

How to cite this article: Subramanya MD, Pai SR, Ankad GM, Hegde HV, Roy S, Hoti SL. Simultaneous determination of vasicine and vasicinone by High-performance liquid chromatography in roots of eight *Sida* species. AYU 2016;37:135-9.

investigated for their phytochemical constituents and have reported many compounds using various analytical techniques.[12-16] The previous study suggested, that *Sida* species contain polyphenols and possess antioxidant activity.[17] Reports suggested high performance thin layer chromatography (HPTLC) estimation of ephedrine, a major active principle from *Sida* spp.,[18] optimization and validation of high performance liquid chromatography (HPLC) and HPTLC method for quantification of vasicine (IUPAC name: [3R]-1,2,3,9-tetrahydropyrrolo [2,1-b] quinazolin-3-ol; Mol. Formula: $C_{11}H_{12}N_2O$; Mol. Wt.: 188.226) and vasicinone (IUPAC name: 3S-hydroxy-2,3-dihydro-1H-pyrrolo [2,1-b] quinazolin-9-one; Mol. Formula: $C_{11}H_{10}N_2O_2$; Mol. Wt.: 202.209) two other major compounds reported in roots of two *Sida* species (*viz*. *S. cordifolia* and *S. acuta*).[12] Vasicine is a light sensitive compound and when exposed to light, it is auto-oxidized to vasicinone.[19,20] Both vasicine and vasicinone are chief alkaloids known to possess interesting biological activities.[21-23] Vasicine and vasicinone are quinazoline alkaloids known to possess bronchodilatory activity; the first was reported to be more potent and also showed anti asthmatic activity.[24,25]

Hence, the present study aims at quantifying vasicine and vasicinone and also to identify the highest yielding species from roots of 8 *Sida* species (*viz*. *S. acuta* Burm. F., *S. cordata* Boiss., *S. cordifolia* L., *S. rhombifolia* L., *S. spinosa* L., *S. indica* L., *S. retusa* L., and *S. mysorensis* Wt. and Arn.) collected from Western Ghats, India.

Materials and Methods

Eight selected *Sida* species were collected from Belagavi region of Western Ghats, India during September, 2013. Plants were identified, authenticated, and voucher specimens were deposited at ICMR - NITM, Belagavi (KA), India for future reference (*S. cordata* Boiss., *S. spinosa* L., *S. rhombifolia* L., *S. acuta Burm*. F., *S. cordifolia* L., *S. indica* L., *S. mysorensis* Wt. and Arn., *S. retusa* L.; Voch. Nos.: RMRC 475, 477, 479, 484, 938, 939, 970, and 971, respectively). All the solvents *viz*. methanol, water, acetonitrile, and glacial acetic acid were of HPLC grade (Fischer Scientific, Mumbai, India). Vasicine (>95% pure) and vasicinone (>95% pure) were procured from, Natural Remedies, Bengaluru, India.

Roots of the eight species were washed, dried, ground to fine powder, and was stored in air tight containers until further use. Continuous shaking extraction was performed on an orbital shaker (Orbitek, India) by subjecting 10 g of dried plant materials in 100 mL of methanol in 250 mL Erlenmeyer flask at 150 rpm, overnight at ambient temperature. Samples were filtered through Whatman filter paper no. 1, to obtain a residue. The filtrate was passed through 0.45 µ nylon filters before analysis to remove impurities from the extract and to avoid damaging the column. The extracts were diluted to 10% (w/v) for HPLC analysis.

A previously described solvent system[12] was employed during the present study. The separation was achieved on a Waters, Nova-Pack, C18 (250 mm × 4.6 mm, 5 µ) column, with acetonitrile - 0.1 M phosphate buffer-glacial acetic acid (15:85: 1, v/v/v) (pH 4.0 by phosphoric acid) as solvent system at a flow-rate of 1.0 mL/min. The effluent was monitored using ultraviolet detection at a wavelength of 300 nm. The mobile phase was filtered through 0.45 µm nylon filter before use.

Standard stock solution of vasicine and vasicinone (5 mL) were prepared with the concentration of mg/mL. The concentrations of both the standards were made from 0.1 to 40 µg/mL with 7 point calibration. The system suitability test was assessed by triplicate injections of the standard solutions at a particular concentration. The peak areas were used to evaluate repeatability of the proposed method, and their peaks were analyzed for resolution.

Data were expressed as mean values of three reading and relative standard deviations. The statistical analyses were performed using Microsoft Excel 2007 software.

Results

Vasicine and vasicinone were determined quantitatively using HPLC analysis in roots of eight, *Sida* species from Western Ghats, India. The identification of these compounds in extracts was achieved by comparing retention time of authentic standards. Chromatograms of 2 standards and comparative report of representative samples are depicted in Figures 1-4. The method of extraction and HPLC conditions were unchanged throughout the study.

Different concentrations of vasicine and vasicinone were detected at 300 nm using HPLC system. Profiles with retention times of 3.722 ± 0.318 (vasicine) min and 6.272 ± 0.265 min (vasicinone) were obtained as the output of standard injection by HPLC analysis. Seven point calibration curves of both the standards within the concentration range of 0.1– 40 µg/mL were constructed

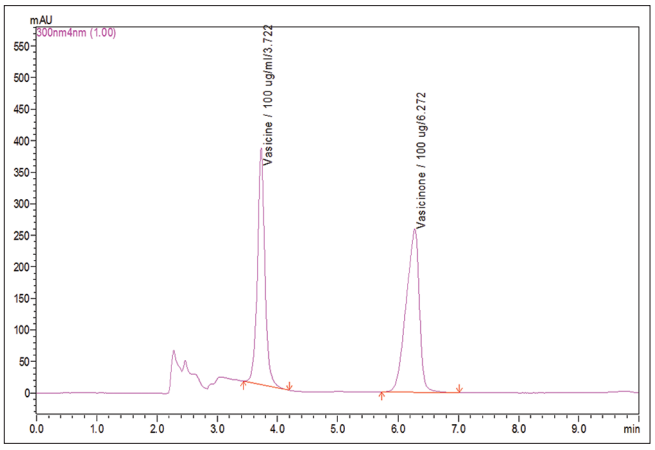

Figure 1: Simultaneous detection of Vasicine and Vasicinone standards (100 µg/mL)

with the coefficient of determination (R^2) above 0.994. The regression equation showed a significant relationship between peak areas and concentrations and this equation was used to estimate content from samples. The results of the regression equations were $y = 17449x + 24238$ (vasicine) and $y = 19946x + 11986$ (vasicinone), a linear relationship between peak area and concentration was established. Limit of detection and limit of quantification as determined by signal to noise ratio for vasicine was 0.110 and 0.333 µg/mL and for vasicinone was 0.059 and 0.179 µg/mL, respectively.

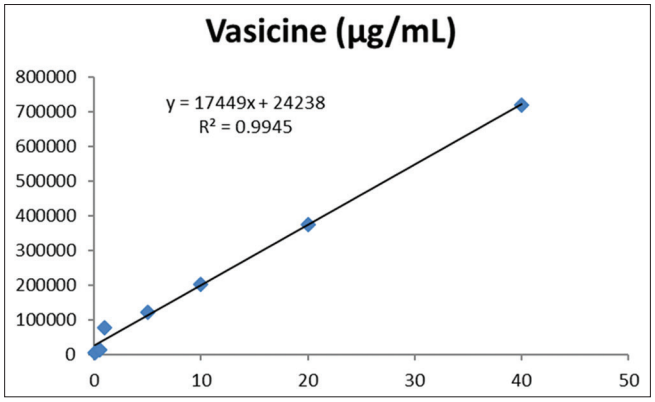

Figure 2: Seven point calibration of vasicine

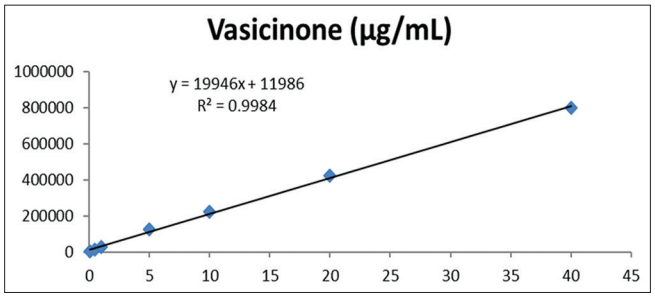

Figure 3: Seven point calibration of vasicinone

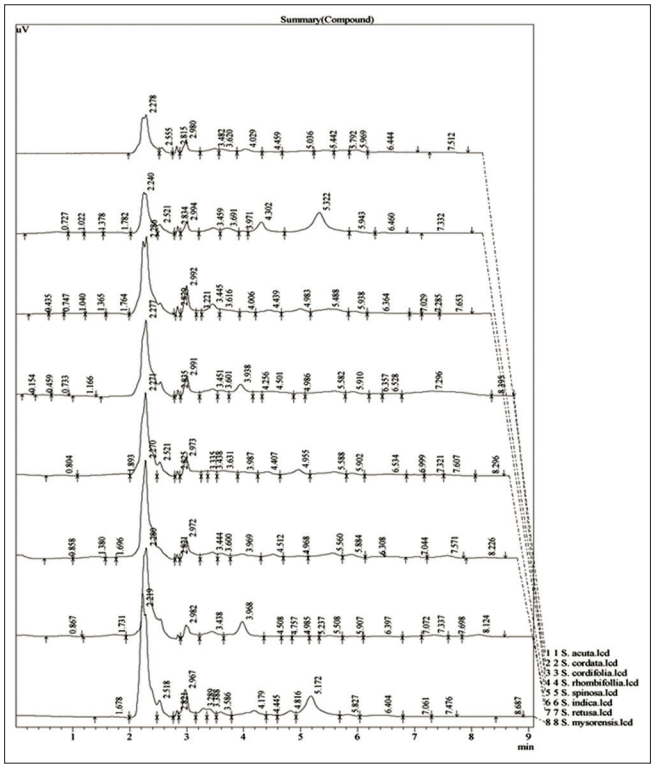

Figure 4: Comparative chromatograms of 8 *Sida* species

The contents (µg/100 mg) were calculated using standard calibration curves [Table 1]. Simultaneous determination of both the standards from roots of eight *Sida* spp. varied from 3.194 ± 0.160–9.891 ± 0.495 µg/100 mg (vasicine), and 02.328 ± 0.116–33.013 ± 1.651 µg/100 mg (vasicinone).

Discussion

The highest vasicine content was determined in *S. cordifolia* and vasicinone in *S. cordata*. It was also interesting to note that the retention times for detection of both the compounds were less than the earlier reports.[12] The results were in accordance with the earlier reports[12] of *S. acuta* and *S. cordifolia* using HPLC analysis, wherein it has been reported the similar content of vasicine (10.00 µg/100 mg) in *S. cordifolia* and vasicinone (2.30 µg/100 mg) in *S. acuta*. However, the content of vasicinone in *S. cordifolia* (6.00 µg/100 mg) was lower and vasicine in *S. acuta* (8.00 µg/100 mg) was higher than the present study [Table 1]. It is well evident from the earlier studies on medicinal plants that the variation in chemical makeup may be attributed to change in the area of collection and climatic factor.[26-28] Furthermore, it is evident that vasicine and vasicinone are the chief marker compounds of *Adhatoda vasica (Syn. Adhatoda zeylanica)*.[29] Vasicine and vasicinone content as determined in methanolic extracts of *A. vasica* leaves were 0.1200% and 0.0340%, respectively. However the content of vasicine in *Sida* spp. during the present study was lower than that reported earlier in *A. vasica* and vasicinone content in *S. cordata* [Table 1] was almost equal to that mentioned by Srivastava *et al*. in *A. vasica*.[30]

Conclusion

Conclusively, HPLC method provides simple and reproducible quantitative analysis for simultaneous determination of vasicine and vasicinone. Vasicinone was available in higher amount as compared to vasicine in *Sida* species. *S. cordifolia* and *S. cordata* were the species with a higher amount of vasicine and vasicinone, respectively. The study helps to select the plant species with higher content of these compounds required for its maximum therapeutic potential.

Acknowledgment

The authors are indebted to Director-in-Charge, ICMR, Belagavi for providing lab facility and support, Mr. Bhoopal Talawar, MTS (Technical) and Mr. Jotiba Palekar, Lab attendant (BIC Project) ICMR-NITM, Belagavi for their assistance in collection and processing plant material.

Table 1: Content of vasicine and vasicione as determined by high performance liquid chromatography in roots of eight species of *Sida*

Species	Vasicine			Vasicinone		
	RT*	Area (%)	Content**	RT*	Area (%)	Content**
Sida acuta	3.482	6.676	4.178 ± 0.209	5.44	4.525	2.699±0.135
Sida cordata	3.459	5.7471	6.514 ± 0.326	5.322	27.9420	33.013±1.651
Sida cordifolia	3.445	6.7466	9.891 ± 0.495	5.488	8.4570	11.768±0.588
Sida rhombifolia	3.451	4.8929	9.198 ± 0.460	5.582	7.4457	13.492±0.675
Sida spinosa	3.438	2.6219	3.194 ± 0.160	5.588	6.0274	8.616±0.431
Sida indica	3.444	5.3671	7.891 ± 0.395	5.560	5.2728	7.374±0.369
Sida retusa	3.438	6.5838	9.777 ± 0.489	5.508	1.9744	2.328±0.116
Sida mysorensis	3.388	2.6565	4.425 ± 0.221	5.172	15.0663	28.247±1.412

Values in table represents mean of 3 HPLC injection ± SD. HPLC: High performance liquid chromatography, SD: Standard deviation, RT: Retention time, *minutes, **µg/100 mg

Financial support and sponsorship

Drugs have been collected from wild source, the lab facility was utilized from ICMR-NITM, Belagavi.

Conflicts of interest

There are no conflicts of interest.

References

1. Sivarajan VV, Pradeep KA. Malvaceae of Southern Peninsular India: A Taxonomic Monograph. New Delhi: Daya Publishing House; 1996. p. 12.
2. Sharma PV. Dravyaguna Vijnana. Vol. 2, Reprint ed. Varanasi: Chaukhambha Bharathi Academy; 2005. p. 734-38.
3. Trikamji Y, editor. Sushruta Samhita of Sushruta, Chikitsa Sthana. 4th ed. Varanasi: Chaukhamba Orientalia; 1980. p. 499.
4. Trikamji Y, editor. Charaka Samhita of Agnivesha, Chikitsa Sthana. 1st ed Ch. 28, Ver. 148, Varanasi: Chaukhambha Prakashan; 2001. p. 623.
5. Tripati I, editor. Chakradatta with Vaidayaprabha, Chakrapanidatta. 5th ed. Ch. 61, Ver. 9, Varanasi: Chaukhambha Sanskrit Bhawan; 1983. p. 504.
6. Mishra B, Vaisya R, editors. Bhavaprakasha Nighantu of Bhavamishra, Guduchyadi Varga. 11th ed., Vol 1. Varanasi: Chaukhambha Sanskrit Bhawan; 2004. p. 366-68.
7. Sharma PV, Sharma G, editors. Dhanvantari Nighantu, Guduchyadi Varga, Ver. 121, 2nd ed. Varanasi: Chaukhamba Orientalia; 1998. p. 66.
8. Mishra B, Vaisya R, editors. Bhavaprakasha Nighantu of Bhavamishra. Guduchyadi Varga. 11th ed., Vol. 1. Varanasi: Chaukhambha Sanskrit Bhavan; 2004. p. 366-72.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 1, 2nd ed. Dehradun: International Book Distributors; 1999. p. 305-18.
10. Vaidya B. Some Controversial Drugs in Indian Medicine, 1st ed. Varanasi: Chaukhamba Orientalia; 1982. p. 213-18.
11. Levekar GS, Kailash C, Yelne MB, Dhar BP, Joseph GV, Mangal AK, *et al*. Database of Medicinal Plants. Vol 8. New Delhi: Central Council for Research in Ayurveda and Siddha, Govt. of India; 2007. pp. 42-50.
12. Dhalwal K, Shinde VM, Mahadik KR. Optimization and validation of reverse phase HPLC and HPTLC method for simultaneous quantification of Vasicine and Vasicinone in *Sida* Species. J Med Plant Res 2010;4:1289-96.
13. Chaves OS, Gomes RA, Tomaz AC, Fernandes MG, das Graças Mendes L Jr., de Fátima Agra M, *et al.* Secondary metabolites from *Sida rhombifolia* L. (*Malvaceae*) and the vasorelaxant activity of cryptolepinone. Molecules 2013;18:2769-77.
14. Sutradhar RK, Rahman AK, Ahmad MU, Saha K. Alkaloids of *Sida cordifilia* L. Indian J Chem 2007;46:1896-900.
15. Chena C, Chaob L, Panc M, Liaob Y, Chang C. Tocopherols and triterpenoids from *Sida acuta*. J Chin Chem Soc 2007;54:41-45.
16. Prakash A, Varma RK, Ghosal S. Alkaloid constituents of *Sida acuta*, *S. humilis*, *S. rhombifolia* and *S. spinosa*. Planta Med 1981;43:384-88.
17. Subramanya MD, Pai SR, Upadhya V, Ankad GM, Bhagwat SS, Hegde HV. Total polyphenolic contents and *in vitro* antioxidant properties of eight *Sida* species from Western Ghats, India. J Ayurveda Integr Med 2015;6:24-28.
18. Khatoon S, Srivastava M, Rawat AK, Mehrotra S. HPTLC method for chemical standardization of *Sida* species and estimation of the alkaloid ephedrine. J Plan Chromatogr 2005;18:364-67.
19. Brain KR, Thapa BB. HPLC determination of Vasicine and Vasicinone in *A. vasica* Nees. J Chromatogr 1983;258:183-88.
20. Chowdhuri BK, Hirani SK. HPLC study of the photochemical oxidation of Vasicine and its analogues. J Chromatogr 1987;390:439-43.
21. Gupta OP, Sharma ML, Ghattak BJ, Atal CK. Pharmacology investigation of Vasicine and Vasicinone - The alkaloids of *A. vasica*. Indian J Med Res 1977;66:680-91.
22. Chopra RN, Ghosh S. Preliminary note on the pharmacological and therapeutics of *A. vasica* (Basak). Ind Med Gaz 1925;60:354-55.
23. Bhide MB, Naik PY, Ghooi RB. Comparative studies on the pharmacological evaluation of Vasicine and Vasicinone. Bull Haffkine 1976;4:43-50.
24. Patil S, Ojha R, Kaur G, Nepali K, Aggarwal S. Estimation of seasonal variation of two major pyrrolo [2, 1-b] quinazoline alkaloids of *Adhatoda vasica* by HPLC. Nat Prod J 2013;3:30-34.
25. Nepali K, Sharma S, Ojha R, Dhar KL. Vasicine and structurally related quinazolines. Med Chem Res 2013;22:1-15.
26. Pai SR, Nimbalkar MS, Pawar NV, Patil RP, Dixit GB. Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothapodytes nimmoniana* (Grah.) Mabb. Int J Pharma Bio Sci 2010;1:1-17.
27. Pai SR, Pawar NV, Nimbalkar MS, Kshirsagar PR, Kolar FK, Dixit GB. Seasonal variation in content of camptothecin from the bark of *Nothapodytes nimmoniana* (Grah.) Mabb. using HPLC analysis. Pharmacognosy Res 2013;5:219-23.
28. Pawar NV, Pai SR, Nimbalkar MS, Dixit GB. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. Food Chem 2011;126:1330-36.
29. Dash RP, Chauhan BF, Anandjiwala S, Nivsarkar M. Comparative pharmacokinetics profile of Vasa Swaras with Vasicine and Vasicinone. Chromatographia 2010;71:609-15.
30. Srivastava S, Verma RK, Gupta MM, Singh SC, Kumar S. HPLC determination of Vasicine and Vasicinone in *Adhatoda vasica* with photo diode array detection. J Liq Chromatogr Relat Technol 2001;24:153-59.

हिन्दी सारांश

आठ सीडा प्रजातियों की जड़ों में उच्च प्रदर्शनतर लिक्विड क्रोमैटोग्राफी द्वारा वासीसिन और वासीसिनोन का निर्धारण

एम डी सुब्रमण्या, संदीप आर. पै, गिरेश एम अंकद, हर्ष व्ही हेगडे, सुबर्ण रॉय, एस एल होती

सीडा जाति एक महत्वपूर्ण औषध है जो कि व्यापक रूप से आयुर्वेद और अन्य पारंपरिक चिकित्सा के प्रणालियों में विभिन्न रोगों के इलाज के लिए इस्तेमाल किया जाता है। वर्तमान अध्ययन का लक्ष्य भारत के पश्चिमीघाट में पाए जाने वाले आठ सीडा प्रजाति के जड़ो से वासीसिन और वासीसिनोन का एच.पी.एल.सी. विधि द्वारा एक साथ निर्धारण करना है। सीडा के आठ प्रजातियों नामतः, सीडा एक्यूटा, एस. कॉर्डेटा, एस. कॉर्डिफोलिया, एस. रोम्बिफोलिया, एस. स्पीनोसा, एस. इंडिका, एस. रेटुसा और एस. मायसोरेंसिस की जड़ो का मिथेनोलिक अर्क का अध्ययन किया गया। प्राप्त जानकारी को तीन अंकों के औसत संबंधी मानक विचलन के आधार पर व्यक्त किया गया। एसीटोनाइट्राइल- 0.1 M फॉस्फेट ग्लेसियल एसीटिक एसिड (15:85:1, v/v/v) को विलयन प्रणाली के रूप में प्रयोग कर 0.1 ml/ min प्रवाह की दर पर वार्टरस, नोवा पैक c18 (150 x 4.6 mm, 5/4) कॉलम पर पृथकीकरण किया गया तथा प्रवाह को पराबैगनी 300 nm तरंगदैर्ध्य पर देखा गया । दोनों ही मानक के अंशाकन वक्र में उचित रेखीय प्रतिगमन पाया गया (R^2>0.994)। सीडा कॉर्डिफोलिया में वासीसिन (9.891±0.495 µg/100mg) तथा सीडा कॉर्डेटा में वासिसिनोन (33.013±1.651 µg/100mg) अधिक पाया गया। इससे यह निष्कर्ष निकलता है कि एच पी एल सी पद्धति जो कि एक सामान्य, अचूक तथा पुनरुत्पादनीय परिमाणात्मक समीक्षा है, तथा इसके द्वारा वासीसिन तथा वासिसिनोन का निर्धारण किया जा सकता है। चयन की गई प्रजातियों में से सीडा कॉर्डिफोलिया तथा सीडा कॉर्डेटा में क्रमशः अधिक वासीसिन तथा वासिसिनोन प्राप्त हुये।