Phytochemical screening and HPTLC fingerprinting of different parts of *Solanum indicum* L.: A dashmool species

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Abstract

The objective of the study is to screen phytochemicals and develop chemical fingerprints of medicinally important *Solanum indicum* L. The powdered pant materials of leaf, fruit, stem and roots of *S. indicum* were extracted in methanol by soxhlet apparatus. Extracts were subjected to phytochemical screening and HPTLC fingerprints were developed. For development of fingerprints Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1) was used as mobile phase. Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and terpenoids in all the plant parts. Steroids were found present in leaves, fruits and roots whereas tannins were detected in leaves only. HPTLC fingerprinting of methanolic extracts of all plant parts has shown several peaks with different Rf values and peak areas. Phytoconstituents investigated have been described to have tremendous medicinal values in literature. HPTLC fingerprints would be helpful in identification, authentication and quality control of this species.

Keywords: phytochemical screening, chemical fingerprints, caffeic acid, *S. indicum*, HPTLC

Introduction

Medicinal plants have been used as herbal drugs since times immemorial. All plant parts (leaves, flowers, stem, roots, seeds, bark etc) are used as herbal drugs in particular or in combinations of each other. According to WHO, approximately 80% of world population are still relying on traditional system of medicines to cure their diseases in various forms such as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol [1, 2]. Due to being safe and effective, the world market for herbal medicines is growing at the rate of 7-15% annually [3, 4]. Standardization of the herbal raw materials is the need of the hour to make the Indian branded drugs most reliable. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical characters. Hence the modern methods describing the identification and quantification of active chemical constituents in the plant material may be helpful for proper standardization of herbs and their formulations [5-7]. World Health Organization (WHO) has also emphasized on the quality assurance of medicinal plants using modern sophisticated techniques and applying suitable standards [8, 9].

High Pressure Thin Layer Chromatography (HPTLC) has emerged as a simple, versatile, accurate, cost effective, rapid and reliable tool for identification, quantification and standardization of herbal materials [5, 10]. Chromatographic fingerprints generated through HPTLC can be visualized and stored as electronic images [11].

*Solanum indicum* L. is commonly known as Birhata or Badi Kateri or Indian night shade and belongs to the family Solanaceae. It is an erect undershrub of 0.30 to 1.8 m in height (Fig. 1) and found throughout warmer parts of India, Asia and Africa upto an elevation of 1.5 m [12]. The national demand of *S. indicum* is 500-1000 MT per annum [13]. Due to high demand and overexploitation, the herb has become rare in Madhya Pradesh [14, 15]. All plant parts viz. berries, leaves, roots, seeds and stem of this species have been utilized in traditional system of medicine and are useful in various diseases such as bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness and pruritis [16-19]. The plant has been documented in Chinese folk medicine as anti-inflammatory and wound-healing agents and as an analgesic for toothache, rhinitis and breast cancer [20]. The species is among the ten medicinal plants whose roots are principally employed in preparation of Dashmularishta, a well-established ayurvedic drug used in the treatment of fatigue, oral sores...
and gynecological disorders [21]. The basic ingredient of Dashmoolarishta is utilized in the manufacture of over hundred ayurvedic drugs [22]. This study aimed to screen the phytochemicals, to develop chemical fingerprints of leaves, fruits, stem and roots of *S. indicum* using HPTLC technique which can be utilized for quality standardization of this species. The presence of caffeic acid, a phenolic acid was also checked in the plant samples.

**Fig 1: Solanum indicum L.**

### Material and Methods

#### Collection of plant material

Leaves, fruits, stem and roots (Fig. 2 A, B, C, D) of this species were collected from Tamia range of West Chhindwara Forest Division. The herbarium of plant specimens was deposited in Biodiversity and Sustainable Management Division of Tropical Forest Research Institute, Jabalpur (Identification no. 1761).

#### Chemicals

Caffeic acid (Fig. 3) was purchased from Sigma Aldrich, India. All chemicals and solvents used were of AR grade.

#### Processing and extraction of plant material

The different plant parts of above said species were separated, packed in jute bags and brought to the laboratory. These were washed thoroughly in running water to remove soil and other foreign particles. The stem and roots were cut into small pieces. All plant parts of *S. indicum* were dried in shade and subsequently dried in a hot air oven at 40 °C for 48 hours. All the plant parts were powdered using grinder and the powdered plant materials were used for making extracts. 100 mg of powdered plant materials of leaves, fruits, stem and roots were separately soaked overnight in 25 ml of methanol. Different extracts were filtered and filtrates were used for qualitative phytochemical analysis.

**Fig 2: S. indicum A. Leaves B. Fruits C. Stem D. Roots**

### Results

The phytochemical characters of leaves, fruits, stem and roots of *S. indicum* are summarized in Table 1. The results showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and terpenoids in all the plant parts. Steroids were detected in leaves, fruits and roots whereas tannins in leaves only.

HPTLC profiles of methanolic extracts of leaves, fruits, stem and roots of *S. indicum* under visible light, UV 254 nm and 366 nm were recorded and presented in Fig. 3. The corresponding chromatograms of HPTLC profiles of extracts obtained after densitometric scanning at 330 nm are given as Fig. 4 which showed several peaks of various phytochemicals. Number of peaks observed in chromatogram of all extracts along with their Rf values, maximum height and area % are defined in Table 2. It can be observed in Table 2 that HPTLC chromatogram of stem extract revealed 9 peaks with maximum Rf values in the range of 0.04 to 0.84 (Table 2, Fig. 4, Track 2), leaf extract showed 13 peaks with Rf values in the range of 0.06 to 0.98 (Table 2, Fig. 4, Track 3), fruit extract revealed 7 peaks with Rf values in the range of 0.04 to 0.98 (Table 2, Fig. 4, Track 4). The presence of caffeic acid, a phenolic acid was also checked in the plant samples.

**Fig 3: Chemical structure of caffeic acid**

### Phytochemical screening of plant extracts

The preliminary phytochemical screening of methanolic extracts of selected plant parts was carried out according to the methods described by Edeoga (2005) [23], Harborne (1998) [24], Sofawara (1993) [25] and Trease and Evans (1989) [26].

#### Preparation of caffeic acid solution

A solution of 0.1 mg/ml of caffeic acid in methanol was prepared to compare the presence of caffeic acid in different crude extracts.

#### Development of chemical fingerprints using HPTLC

Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1) mobile phase was standardized for better resolution of peaks. 10 µL of each solution and 3 µL of caffeic acid solution were applied in the form of bands of width 8 mm using a 100 µL CAMAG syringe on 10 x 10 cm aluminum packed TLC plate prewashed with methanol and coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd., Darmstadt, Germany) with the help of a 100 µL Hamilton syringe and LinomatV applicator attached to CAMAG HPTLC system, which was programmed through WinCATS software. Samples loaded TLC plate was developed by the ascending technique using 10 ml of standardized mobile phase in a CAMAG twin-through glass chamber (10 cm x 10 cm) saturated with mobile phase and covered with a stainless-steel lid. The developed plate was dried by hot air to evaporate solvents from the plate and kept in photo – documentation chamber and captured the images at 254 and 366 nm. The image of plate was also taken in visible light. Densitometric scanning was then performed with a CAMAG TLC Scanner4 equipped with WinCATS software at λ<sub>max</sub> = 330 nm using deuterium and tungsten light source. Rf values, peak tables and HPTLC chromatograms were recorded.
0.83 (Table 2, Fig. 4, Track 4) and root extract revealed 9 peaks with Rf values in range of 0.04 to 0.84 (Table 2, Fig. 4, Track 5). Caffeic acid appeared at Rf 0.47 in all extracts. The presence of caffeic acid in samples was confirmed by comparing the absorption spectra at start, middle and end position (Fig. 5).

**Table 1: Phytochemical characters of leaves, fruits, stem and roots of *S. indicum***

| S. No. | Phytochemical constituents | Plant parts | Methanol extract |
|--------|---------------------------|-------------|-----------------|
| 1.     | Alkaloids                 | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |
| 2.     | Cardiac glycosides        | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |
| 3.     | Flavonoids                | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |
| 4.     | Phenols                   | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |
| 5.     | Saponins                  | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |
| 6.     | Steroids                  | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | -               |
|        |                           | Roots       | +               |
| 7.     | Tannins                   | Leaves      | +               |
|        |                           | Fruits      | -               |
|        |                           | Stem        | -               |
|        |                           | Roots       | -               |
| 8.     | Terpenoids                | Leaves      | +               |
|        |                           | Fruits      | +               |
|        |                           | Stem        | +               |
|        |                           | Roots       | +               |

(+) = detected and (-) = not detected

**Fig 3:** HPTLC fingerprint profiles of methanolic extracts of different parts of *S. indicum*. A. Visible light, B. 254 nm, C. 366 nm (Track 1: Caffeic acid, Track 2: Stem extract, Track 3: Leaf extract, Track 4: Fruit extract, Track 5: Root extract)
Fig 4: HPTLC chromatograms of caffeic acid (Track 1), methanolic extracts of stem (Track 2), leaves (Track 3), fruits (Track 4) and roots (Track 5) of *S. indicum* showing peaks of phytochemicals at 330 nm.
Table 2: Peak list and Rf values of HPTLC chromatograms of methanolic extracts of stem, leaves, fruits and roots of *S. indicum* at 330 nm

| Plant parts | Peak | Max Rf | Max Height (AU) | Area (AU) | Area (%) |
|-------------|------|--------|----------------|-----------|----------|
| Caffeic acid | 1    | 0.47   | 808.4          | 34262.6   | 85.99    |
|             | 2    | 0.38   | 222.4          | 8380.5    | 11.89    |
|             | 3    | 0.47   | 122.3          | 4390.6    | 6.23     |
|             | 4    | 0.32   | 243.2          | 7370.1    | 10.04    |
|             | 5    | 0.47   | 8488.8         | 10.41     |          |
| Stem        | 1    | 0.04   | 692.7          | 20298.7   | 28.80    |
|             | 2    | 0.13   | 146            | 4281.6    | 6.08     |
|             | 3    | 0.27   | 235.5          | 10863.3   | 15.41    |
|             | 4    | 0.38   | 222.4          | 8380.5    | 11.89    |
|             | 5    | 0.47   | 122.3          | 4390.6    | 6.23     |
|             | 6    | 0.52   | 122            | 4307.6    | 6.11     |
|             | 7    | 0.63   | 151.3          | 6357.8    | 9.02     |
|             | 8    | 0.67   | 147.5          | 4306.5    | 6.11     |
|             | 9    | 0.84   | 131.8          | 7286.4    | 10.34    |
| Leaves      | 1    | 0.06   | 808.2          | 43635.2   | 31.85    |
|             | 2    | 0.16   | 239.8          | 8323.5    | 6.07     |
|             | 3    | 0.26   | 280.3          | 12662.6   | 9.24     |
|             | 4    | 0.38   | 185.9          | 7752.2    | 5.66     |
|             | 5    | 0.47   | 300.7          | 10786     | 7.87     |
|             | 6    | 0.52   | 298.4          | 9254.5    | 6.75     |
|             | 7    | 0.58   | 297.8          | 7558.3    | 5.52     |
|             | 8    | 0.62   | 444            | 12871     | 9.39     |
|             | 9    | 0.67   | 628.2          | 19912.2   | 14.53    |
|             | 10   | 0.80   | 60.9           | 1596.6    | 1.17     |
|             | 11   | 0.84   | 45.9           | 1147.5    | 0.84     |
|             | 12   | 0.94   | 17.1           | 326.9     | 0.24     |
|             | 13   | 0.98   | 66.6           | 1196      | 0.87     |
| Fruits      | 1    | 0.04   | 654.9          | 27292.7   | 39.38    |
|             | 2    | 0.23   | 140            | 6928      | 10.00    |
|             | 3    | 0.36   | 118.4          | 5599.9    | 8.08     |
|             | 4    | 0.47   | 130            | 7632.3    | 11.01    |
|             | 5    | 0.57   | 279.2          | 12124     | 17.49    |
|             | 6    | 0.70   | 115.9          | 7414.5    | 10.70    |
|             | 7    | 0.83   | 50.7           | 2311.2    | 3.33     |
| Roots       | 1    | 0.04   | 743.8          | 15741.5   | 19.31    |
|             | 2    | 0.08   | 552            | 20091.1   | 24.64    |
|             | 3    | 0.18   | 109.2          | 3594.1    | 4.41     |
|             | 4    | 0.27   | 268.6          | 10986.5   | 13.48    |
|             | 5    | 0.39   | 185.4          | 7211.5    | 8.85     |
|             | 6    | 0.47   | 243.2          | 7370.1    | 9.04     |
|             | 7    | 0.56   | 111.5          | 5971.2    | 7.32     |
|             | 8    | 0.68   | 62.6           | 2069.3    | 2.54     |
|             | 9    | 0.84   | 166.8          | 8488.8    | 10.41    |

Fig 5: Spectra overlay of CA standard and test samples, scanned at 330 nm
Chemical fingerprinting emerged as an effective tool to resolve problems in standardization of plant-based drugs. With the help of chemical fingerprinting, species, strain and geographical origin of plants can be delineated [42]. HPTLC fingerprinting is proved to be a better, linear, precise and accurate method for herbal identification, authentication, quality standardization and characterization of medicinal plant species [10]. HPTLC profiles of methanolic extracts of leaf, fruit, stem and root of S. indicum were developed in order to find out various chemical moieties which will be helpful in further isolation and structure elucidation of active compounds [43]. There profiles will also be useful in quality control and standardization of herbal preparations. The developed chromatograms will be specific with standardized solvent system Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1), v/v) and Rf values. Caffeic acid, an important phenolic compound found effective in various chronic diseases was also investigated in all selected plant parts of S. indicum. A large number of biological activities have been reported for caffeic acid and its phenethyl ester in the literature such as strong antioxidant, antimitogenic, anti-allergic, immunomodulatory, anti-inflammatory and anti-carcinogen activities both in-vitro and in-vivo [44]. The presence caffeic acid in plant parts of S. indicum is adding towards its therapeutic potential and utilization in Ayurvedic preparations.

Conclusion
It can be concluded from the study that methanolic extracts of leaves, fruits, stem and roots of S. indicum are rich source of phytoconstituents which are conferred with huge therapeutic values. On the basis of present investigation, further studies can be planned in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. Besides, HPTLC fingerprint images would be helpful in identification and authentication of this dasshoom species. These fingerprints will serve as biochemical markers to distinguish between authentic drug and adulterants, thus will be of utmost importance for quality control purpose.

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References
1. Anand SP, Jayakumar E, Jayachandran R, Nandagobalan V, Doss A. Direct organogenesis of Passiflora foetida L. through Nodal Explants. Plant Tissue Culture and Biotechnology 2012;22(1):87-91.
2. Julsing KM, Quax JW, Kayser O. The Engineering of Medicinal Plants: Prospects and Limitations of Medicinal Plant Biotechnology. In: Medicinal Plant Biotechnology (eds Oliver K. and Wim J.Q.) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2007.
3. Warude D, Patwardhan B. Botanicals: Quality and regulatory issues. Journal of Scientific & Industrial Research 2005;64:83-92.
4. Anonymous. Report of the inter-regional workshop on intellectual property rights in the context of traditional medicine, Bangkok Thailand 2000.
5. Gunalan G, Saraswathy1 A, Vijayalakshmi K. HPTLC fingerprint profile of Bauhinia variegata Linn. Leaves. Asian Pacific Journal of Tropical Disease 2012, S21-S25.
6. Palanisamy Hariprasad, Natesan Ramakrishnan. Chromatographic finger print analysis of Rumex vesicarius L. by HPTLC Technique. Asian Pacific Journal of Tropical Biomedicine 2012, 1-2.
7. WHO. Quality Control Method for Medicinal Plant Material. Geneva 1998, 1-15.
8. Sharma P, Kaushik S, Jain A, Sikarwar SM. Preliminary phytochemical screening and HPTLC fingerprinting of Nicotiana tabacum leaf. Journal of Pharmacy Research 2010;3(5):1144-1145.
9. Vasudevan H. DNA fingerprinting in the standardization of Herbs and Neutaceuticals. The Science Creative Quarterly 2009, 4.
10. Subramanian S, Ramakrishnan N. Chromatographic finger print analysis of Naringi crenulata by HPTLC technique. Asian Pacific Journal of Tropical Biomedicine 2011, S195-S198.
11. Johnson M, Mariswamy Y, Gnaraj WE. Chromatographic finger print analysis of steroids in Aerva lanata L by
HPTLC technique. Asian Pacific Journal of Tropical Biomedicine 2011;1:428-433.

12. Hasan RU, Prabhat P, Shaafat K, Khan R. Phytochemical investigation and evaluation of antioxidant activity of fruit of *Solanum indicum* L. International Journal of Pharmaceutical Sciences and Research 2013;5(3):237-42.

13. National Medicinal Plants Board, Ministry of Ayush, Government of India. https://nmpb.nic.in/medicinal_list. December 2020.

14. Kotak N. A comparative appraisal of Brihati (*Solanum indicum* L.) and its Substitutes W.S.R. to its pharmacognostical physicochemical and pharmacological profile. Institute for Postgraduate Teaching and Research in Ayurveda, Gujrat Ayurved University (Jamnagar) 2008-09.

15. Joshi PR, Patel BR. Pratinitdi dryava and its adaptation in current scenario - A bird’s eye view. Research in Pharmacy 2012;2(2):21-6.

16. Publications and Information Directorate, CSIR. The Wealth of India - Raw Materials, New Delhi (India) 1986.

17. Bhakta T. Common Vegetables of the Tribals of Tripura. Agartala (India): Tripura Tribal Research Institute 2004.

18. Bhattacharya AS, Chiranjivi Banaushadhi. 2nd reprint. Kolkata: Ananda Publishers 1982, 3.

19. Sharma V, Hem K, Seth A, Maurya SK. *Solanum indicum* Linn. An ethnopharmacological, phytochemical and pharmacological review. Current Research Journal of Pharmaceutical and Allied Sciences 2017;1(2):1-9.

20. Yin HL, Li JH, Li B, Chen L, Li J, Tian Y et al. Two new coumarins from the seeds of *Solanum indicum*. Journal of Asian Natural Products Research 2014;16(2):153-7.

21. Yadav AK, Yadav D, Shanker K, Verma RK, Saxena AK, Gupta MM. Flavone Glycoside Based Validated RP-LC Method for Quality Evaluation of Prishniparni (*Uraria picta*). Chromatographia 2009;69(7-8):653-8.

22. Pathak JM, Krishnamurthy R, Chandorkar MS, Gulkari VD, Rajendra G. Identification of high yielding genotypes of Dashmool Shalparni (*Desmodium gangeticum*) drug plant and its cultivation under high density planting. Indian Journal of Horticulture 2005;62(4):378-84.

23. Edeoga HO, Okwu DE, Mbaebie BO. African Journal of Biotechnology 2005;4(7):685-688.

24. Harborne JB. Phytochemical methods, London. 3rd ed., Chapman and Hall, Ltd 1998, 1-302.

25. Sofowora A. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; Screening Plants for Bioactive Agents 1993, 134-156.

26. Trease GE, Evans WC. A textbook of Pharmacognosy, 13 Bacilliiree Tinall Ltd, London 1989.

27. Kantamreddi VSSN, Lakshmi YN, Kasapu VVVS. Preliminary phytochemical analysis of some important Indian plant species. International Journal of Pharmacy and Biological Sciences 2010;1(4):351-358.

28. Sagwan S, Rao DV, Sharma RA. Phytochemical evaluation and quantification of primary metabolites of *Maytenus emarginata* (Wild.) Ding Hou. Journal of Chemical and Pharmaceutical Research 2010;2(6):46-50.

29. Rajurkar NS, Gaikwad K. Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris*. Journal of Chemical and Pharmaceutical Research 2012;4(1):365-374.

30. Robert AM. Encyclopedia of Physical Science and Technology - Alkaloids, 3rd edition 2002.

31. Tadzabia K, Maina HM, Maitera ON, Ezekiel JS. Evaluation of phytochemical and elemental contents of *Haematostaphis barteri* leaves and stem bark in Hong local government area of Adamawa state, Nigeria. Journal of Chemical and Pharmaceutical Research 2013;5(9):150-156.

32. Sharma HL, Sharma KK. Drug therapy of heart failure. In: Principle of pharmacology, 1 ed, Hyderabad, Paras publishers 2007, 314-325.

33. Duthie GG, Duthie SJ, Kyle AM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition Research Reviews 2000;13:79-106.

34. Okwu DE. Evaluation of chemical composition of indigenous species and flavouring agents. Global Journal of Pure and Applied Sciences 2001;8:455-459.

35. Hayashi T, Maruyama H, Hatton K, Hazeki O, Yamasaki K, Tanaka T. Ellagitannins from *Lagerstroemia speciosa* as activators of glucose transport in fat cells. Planta Medica 2002;68(2):173-175.

36. Asl MN, Hosseinzadeh H. Review of pharmacological effects of *Glycyr rhiza* sp. and its bioactive compounds. Phytotherapy Research 2008;22:709-24.

37. Rupasinghe HP, Jackson CJ, Poya V, Berardo CD, Bewley JD, Jenkinson J. Oyasapogenol A and B distribution in soyabean (*Glycine max* L.) Merr in relation to seed physiology, genetic variability and growing location. Journal of Agricultural and Food Chemistry 2003;51:5888-5894.

38. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJK. Anti-ulcer activity of crude alcoholic extract of *Toona ciliata* (heartwood). Journal of Ethnopharmacology 2006;19:425-428.

39. Saxena HO, Soni A, Mohammad N, Choubey SK. Phytochemical screening and elemental analysis in different plant parts of *Uraria picta* Desv. A Dashmool species. Journal of Chemical and Pharmaceutical Research 2014;6(5):756-760.

40. Liu F, Kim JK, Li Y, Liu XQ, Li J, Chen X. An extract of *Lagerstroemia speciosa* L. has insulin-like glucose uptake–stimulatory activity and adipocyte differentiation–inhibitory activities in 3T3-L1 cells. The Journal of nutrition 2001;131(9):2242-7.

41. Luo J, Cheung J, Yevich EM, Clark JP, Tsai J, Lapesra P et al. Novel terpenoid-type quinones isolated from *Pycnanthus angolensis* of potential utility in the treatment of type 2 diabetes. Journal of Pharmacology and Experimental Therapeutics 1999;288(2):529-34.

42. Jeba RC, Rameshkumar G. Comparison of bioactive ingredients in *Ocimum* species. International Journal of Applied Biology and Pharmaceutical Technology 2013;4(3):1-8.

43. Varghese S, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Phytochemical screening and HPTLC finger printing analysis of *Citrus limon* (Thunb.) seed. Journal of Acute Disease 2013;2(2):122-126.

44. Saxena HO, Pawar G. Total phenolic and caffeic acid contents in roots of *Solanum indicum* L. from different agroclimatic regions of Madhya Pradesh state of India. Indian Journal of Pharmaceutical Education and Research 2019;53(2S):s164-s169.