Original Article

Total Phenolic and Caffeic Acid Contents in Roots of *Solanum indicum* L. from Different Agroclimatic Regions of Madhya Pradesh State of India

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ABSTRACT

Introduction: *Solanum indicum* L. is one of the species of Dashmularishta, a well-established ayurvedic medicine used in the treatment of fatigue, oral sores and gynecological disorders. The present investigation dealt with the estimation of total phenolic and caffeic acid contents in roots of *S. indicum* from different agroclimatic regions of Madhya Pradesh. **Materials and Methods:** The roots were collected from 8 places of 7 agroclimatic regions of Madhya Pradesh following purposive sampling. Phenolic content was determined with spectrophotometric method and caffeic acid content was estimated using HPTLC device. **Results:** The roots from Indore (Malwa plateau) and Balaghat (Chhattisgarh plains) contained maximum (0.859%) and minimum (0.595%) phenolic content respectively. Similarly, the highest content of caffeic acid was detected in the roots (0.0198%) collected from Chhindwara and Betul (Satpura plateau) and lowest content (0.0084%) in roots of Amarkantak (Northern Hill's Zone of Chhattisgarh). **Conclusion:** The present investigation revealed the highest phenolic content in root samples of Indore belonging to Malwa plateau and maximum content of caffeic acid in root samples of Chhindwara and Betul belonging to Satpura plateau agroclimatic regions. The populations of Chhindwara and Betul can be considered as superior chemotypes in terms of caffeic acid content and can be *in-situ* as well as *ex-situ* conserved for future.

Key words: *Solanum indicum*, Roots, Phenols, Caffeic acid, Agroclimatic regions, Superior chemotypes.

INTRODUCTION

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 and found throughout warmer parts of India, Asia and Africa upto an elevation of 1.5 m.[1] The national demand of *S. indicum* is 500-1000 MT per annum.[2] Due to high demand and overexploitation, the herb has become rare in Madhya Pradesh.[3,4] 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.[5-8] 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.[9] 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.[10] The basic ingredient of Dashmularishta is utilized in the manufacture of over hundred ayurvedic drugs.[11] The pharmacological potential of a plant

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lies in its active chemical ingredients, also called secondary metabolites and high content of active ingredients provides superior chemotypes. Madhya Pradesh state is endowed with rich and diverse flora and is divided into 11 agro-climatic regions. Active ingredients being the secondary metabolites are often influenced by the environmental and edaphic factors and vary from one region to another.[12] Hence, the standardization of herb is the need of the hour. WHO and modern herbal pharmacopoeia lay a strong emphasis on the need for quality standardization of herbs with respect to their active ingredients.[13-15] Phenolics are the most common and widely distributed plant phytoconstituents (Figure 1).[16-18] These possess broad spectrum of bioactivities such as antioxidant, anti-allergic, anti-carcinogenic or anti-mutagenic, anti-inflammatory, antibacterial, anti-fungal, anti-viral etc.[19-25] Many research reports supported the phenolic compounds as an essential health promoting agents.[26,27] The present investigation dealt with the estimation of total phenolic and caffeic acid contents in roots of *S. indicum* collected from different agroclimatic regions of Madhya Pradesh to find out the variations and superior chemotypes.

MATERIALS AND METHODS

Collection and Processing of Plant Material

Roots of *S. indicum* were collected from 8 places of 7 agroclimatic regions following purposive sampling as per availability in the state. These were washed thoroughly in running water to remove soil and other foreign particles. The roots were cut into small pieces and dried in shade and subsequently in a hot air oven at 40°C for 48 hr. Dried materials were powdered using grinder and stored in polythene bags for further phytochemical analysis. GPS readings of collection sites were recorded. The herbarium of plant specimen was deposited in Tropical Forest Research Institute, Jabalpur and identification no. 1761 was obtained.

Quantification of Total Phenolic Content

Total phenols were determined by Folin Ciocalteau method.[28] 0.5 gm of powdered sample in 10 times volume of 80% ethanol was grinded using a motor and pestle. The homogenate was then centrifuged at 10,000 rpm for 20 min. The supernatant was then evaporated to dryness. The residue was dissolved in a known volume of distilled water. 0.2 ml of this sample was then taken in test tube and volume made up to 3 ml with distilled water. 0.5 ml of Folin Ciocalteau reagent was then added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added, mixed thoroughly, placed in boiling water for exactly 1 min, cooled and absorbance was taken at 650 nm against blank. The standard graph was prepared by using different concentrations of catechol. Percentage of phenol was calculated from the following formula:

$$\text{Percentage of Phenol} = \frac{\text{Amount of standard}}{\text{O.D. of standard}} \times \frac{\text{O.D. of sample}}{\text{Weight of sample}} \times \frac{\text{Total volume makeup}}{\text{Volume taken}} \times 100$$

Quantification of caffeic acid using HPTLC

Preparation of extracts

2.5 gm dried and finely powdered root samples were taken in conical flasks containing 50 ml of 2N HCL and heated for 30 min over a boiling water bath, cooled and filtered. The filtrate was transferred to a separating funnel and extracted with 75 ml (50: 25) of diethyl ether. The combined diethyl ether layers were washed two times with distilled water, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated and concentrated extract was dissolved in 2 ml of methanol for analysis.

Preparation of standard solution

Caffeic acid purchased from Sigma Aldrich India, was used as chemical standard. Standard solution of 0.1 mg/ml concentration was prepared for calibration graph.

Standardization of solvent system for HPTLC

A number of solvent systems were tried, but the satisfactory resolution of caffeic acid in the extracts was obtained in the solvent combination of Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1).

Sample application

10 µl of each sample was spotted in triplicate in the form of bands of width 8 mm using a 100 µl CAMAG syringe on 20 x 10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd., Darmstadt, Germany) with the help of LinomatV applicator attached to CAMAG HPTLC system, which was programmed through winCATS software. Different volumes of standard solution such as 4, 6, 8, 10 and 12 µl (Corresponding to 40, 60, 80, 100 and 120 ng respectively of caffeic acid) were applied on HPTLC plate in five tracks.

Development of chromatograms

20 ml of mobile phase Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1) was used per chromatography run. The linear ascending was carried out in a twin glass chamber (20 cm x 10 cm dimension) saturated with mobile phase.

Detection of spots

The developed HPTLC plate was dried by hot air to evaporate solvents from the plate and kept in photo –

documentation chamber. Images of plates were captured under UV light at 254 and 366 nm respectively. Densitometric scanning was then performed with a CAMAG TLC Scanner 4 equipped with winCATS software at λ_{max} = 330 nm using deuterium and tungsten light source. The slit dimensions were 6.00 x 0.45 mm. Respective peak areas were recorded and a calibration curve was prepared by plotting the peak areas vs. concentrations of caffeic acid standard applied. Amount of caffeic acid in plant extracts was calculated by using the calibration curve of standard.

Statistical Analysis

Data were statistically analyzed. Each experiment was carried out in triplicate and the results are expressed as Mean ± SD (n=3). ANOVA was applied to check the results as significant and non – significant.

RESULTS AND DISCUSSION

The collection sites along with, agroclimatic regions and GPS locations are given in Table 1. Total phenolic and caffeic acid contents in roots of *S. indicum* collected from 8 sites of 7 agroclimatic regions are given in Table 2. Total phenolic contents in root samples varied from 0.859% (Indore, Malwa plateau) to 0.595% (Balaghat, Chhattisgarh plains) (Figure 2). Similarly, caffeic acid content in root samples differed from 0.0198% (Chhindwara and Betul, Satpura plateau) to 0.0084% (Amarkantak, Northern Hill's Zone of Chhattisgarh) (Figure 3). Thus, the maximum phenolic content (0.859%) was found in the root samples of Indore belonging to Malwa plateau agroclimatic region and maximum caffeic acid content (0.0198%) was found in the root samples of Chhindwara and Betul belonging to Satpura plateau agroclimatic region. Significant variations in total phenol and caffeic acid contents were found among the root samples collected from various agroclimatic regions of Madhya Pradesh state of India. Estimates of total phenolic and caffeic acid contents did not exhibit positive co-relation between themselves because total phenols represent a group of phenolic compounds and it may be concluded that root samples of different places may contain other phenolic compounds also along with caffeic acid. Besides, variations in secondary metabolite concentrations due to abiotic and stress factors viz. temperature, altitude, soil, rainfall, humidity, drought, light intensity, high salinity, supply of water, minerals, freezing temperatures and CO_2 have been described by many researchers from time to time.[29-35] The stress conditions triggered accumulation of secondary metabolites which help the plants to adapt according to environment and in overcoming stresses.[36] In our earlier studies, we found out the variations in colchicine, lupeol and rhoifolin contents in *Gloriosa superba*, *Hemidesmus indicus* and *Uraria picta* respectively in Madhya Pradesh state.[12,13,37] In other studies also, various authors

Table 1: Collection of *S. indicum* Roots from Different Agro-Climatic Regions of Madhya Pradesh.

Agro-climatic regions	Places of collection	GPS Location
Kymore Plateau and Satpura Hills	Seoni	N 22°02'20.4" E 79°25'48.1"
Chhattisgarh plains	Balaghat	N 21°57'21.6" E 80°25'21.9"
Central Narmada Valley	Hoshangabad	N 22°26'28.0" E 78°25'05.3"
Malwa Plateau	Indore	N 22°23'38.7" E 75°39'28.3"
Satpura Plateau	Chhindwara	N 22°20'40.5" E 78°39'57.4"
	Betul	N 21°51'31.2" E 77°56'07.9"
Northern Hill Zone of Chhattisgarh	Amarkantak	N 22°40'59.1" E 81°45'07.7"
Vindhyan Plateau	Sehore	N 22°47'00.1" E 77°37'13.2"

Table 2: Quantification of Phenols and Caffeic Acid in Roots of *S. indicum*.

Agroclimatic regions	Places	Total phenols (%±SD)	Caffeic acid (%±SD)
Kymore Plateau and Satpura Hills	Seoni	0.802±0.02	0.0196±0.003
Chhattisgarh plains	Balaghat	0.595±0.01	0.0170±0.006
Central Narmada Valley	Hoshangabad	0.769±0.01	0.0132±0.002
Malwa Plateau	Indore	0.859±0.01	0.0164±0.001
Satpura Plateau	Chhindwara and Betul	0.738±0.09	0.0198±0.002
Northern Hill's Zone of Chhattisgarh	Amarkantak	0.618±0.05	0.0084±0.002
Vindhyan Plateau	Sehore	0.726±0.05	0.0085±0.005

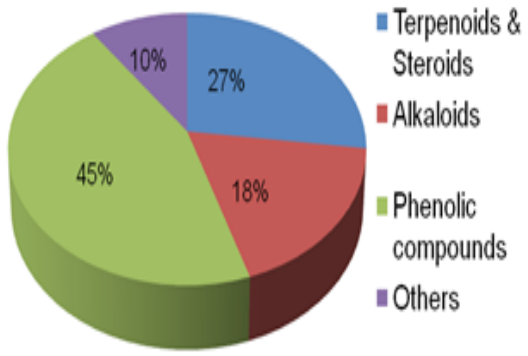

Figure 1: Perentage Distribution of Phytochemicals in Plants.

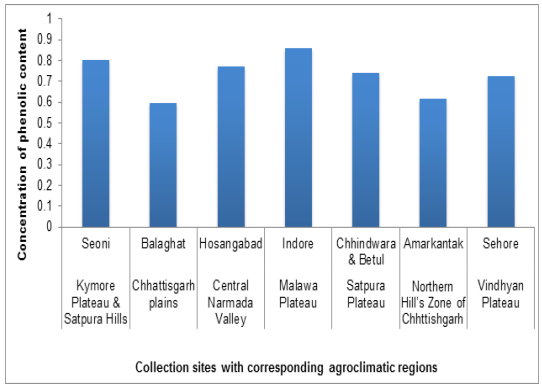

Figure 2: Total Phenolic Content in Roots of *S. indicum*.

reported the variations in respective active chemical ingredients of *Terminalia arjuna*, *Embelia tsjeriam-cottam* and *Andrographis paniculata* in Madhya Pradesh state and the results showed significant variations among the plant samples of different locations.[38-40] Our present study is also in the same line of earlier studies. Caffeic acid and its phenethyl ester confer strong antioxidant, antimitogenic, anti-allergic, immuno-modulatory, anti-inflammatory and anti-carcinogen activities both *in-vitro* and *in-vivo*.[41-46] Hence, its presence in roots of *S. indicum* is validating the therapeutic potential and utilization of this plant in Ayurvedic system of medicine.

CONCLUSION

It emerged from the study that population of *S. indicum* in Chhindwara and Betul belonging to Satpura plateau agroclimatic region contained the maximum caffeic acid content, hence, these populations can be considered as superior chemotypes in terms of active chemical ingredient caffeic acid. The superior chemotypes of this commercially important but rare species can be *in-situ* as well as *ex-situ* conserved for their utilization as elite material in pharmaceutical industries, mass propagation and other research needs.

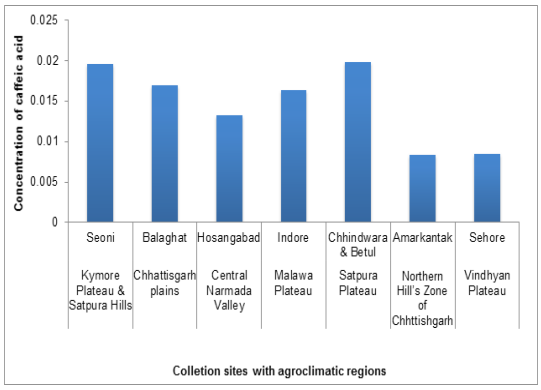

Figure 3: Caffeic Acid Content in Roots of *S. indicum*.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS

L.: Linn; **HPTLC:** High Performance Thin Layer Chromatography; **m:** Meter; **WHO:** World Health Organization; **°C:** Degree centigrade; **hr:** Hour; **GPS:** Global Positioning System; **gm:** Gram; **rpm:** Round per minute; **min:** Minute; **ml:** Milliliter; **Na_2CO_3:** Sodium carbonate; **nm:** Nano meter; **N:** Normality; **HCL:** Hydrochloric acid; **mg:** milligram; **µl:** Microliter; **cm:** Centimeter; **ng:** Nanogram; **UV:** Ultraviolet; **λ_{max}:** Wavelength maximum; **vs.:** Versus; **mm:** Millimeter; **CO_2:** Carbon dioxide; **ICFRE:** Indian Council of Forestry Research and Education.

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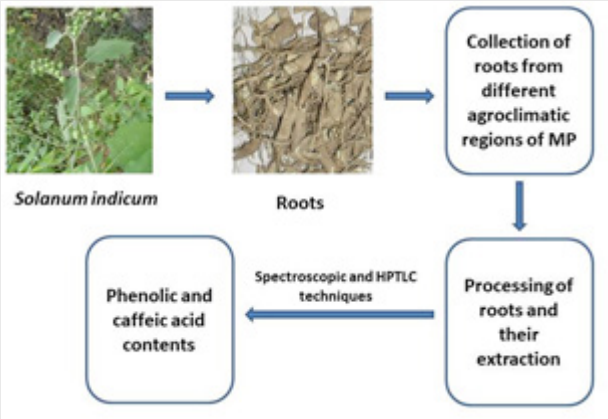
PICTORIAL ABSTRACT



SUMMARY

- The roots of *Solanum indicum* were collected from different agroclimatic regions of Madhya Pradesh.
- Roots were processed and powdered. Root powders were extracted in suitable solvents.
- Phenolic and caffeic acid contents were determined using spectrophotometric and HPTLC methods respectively.
- Study showed the significant variations in phenolic and caffeic acid contents among the root samples of different agroclimatic regions.

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Dr. Hari Om Saxena received his M.Sc. from University of Lucknow, Lucknow (Uttar Pradesh) and PhD from Rani Durgavati Vishwavidyalaya, Jabalpur (Madhya Pradesh). His research interests include harvesting and post harvesting practices of Non Wood Forest Produce (NWFPs), their conservation, domestication and phytochemical analysis using sophisticated instruments.

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