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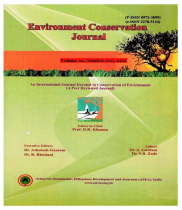

Comparative analysis of phenolic content in *Solanum indicum* L. harvested from different locations of Madhya Pradesh state of India

Hari Om Saxena ✉
Tropical Forest Research Institute, Jabalpur (M.P.), India
Samiksha Parihar
Tropical Forest Research Institute, Jabalpur (M.P.), India
Ganesh Pawar
Tropical Forest Research Institute, Jabalpur (M.P.), India
G. Rajeshwar Rao
Tropical Forest Research Institute, Jabalpur (M.P.), India
Ved Ram Sahu
Tropical Forest Research Institute, Jabalpur (M.P.), India
Pranab Dhar
Tropical Forest Research Institute, Jabalpur (M.P.), India

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ABSTRACT

Phenolics are well distributed secondary metabolites in the plant kingdom and cure various ailments in human beings. In the present study, total phenolic (TP) and caffeic acid (CA) contents in fruits, leaves and stem of *Solanum indicum* species collected from different locations of Madhya Pradesh state of India were studied. Results showed the following trend for TP and CA contents; fruits> leaves> stem. Fruits (28.52±0.29) from Chhindwara & Betul area (Satpura plateau agroclimatic region) contained maximum TP content trailed by leaves (16.29±0.07) and stem (11.79±0.03) belonging to Amarkantak area (Northern Hill's Zone of Chhattisgarh agroclimatic region). CA content was observed maximum in fruits (0.0192±0.00) followed by leaves (0.0187±0.01) and stem (0.0154±0.01) of Seoni area (Kymore Plateau & Satpura Hills agroclimatic region). The variation in the populations will be helpful for *in-situ* as well as *ex-situ* conservation of this reagionally threatened dashmool species and its further utilization in Ayurvedic formulations.

Introduction

Nature has created a full storehouse with remedies to heal humanity's ailments. Natural drugs in the form of herbs/ plants provide us remedies to treat incurable illnesses without any toxic consequences. Due to their efficacy, minimal side effects in clinical experience and relatively low cost, there is a growing interest in herbal remedies. Even when their biologically active compounds are unknown, herbal drugs or extracts are widely prescribed (Namsa *et al.,* 2009; Sharma *et al.,* 2017; Pandeya *et al.,* 2013; Sharma *et al.,* 2017; Arunachalam *et al.,* 2009). *Solanum indicum* L. (common name: Birhata or Badi Kateri or Indian nightshade; family: Solanaceae) is an important ingredient of "Dashmoolarishta", a well-established drug of the Indian system of medicine used for fatigue, oral sores, and gynecological disorders (Yadav *et al.,* 2009). *S. indicum* is reported as a rare drug (Joshi and Patel, 2015). The national demand for *S. indicum* is 500-1000 MT per annum (Anon, 2021). All plant parts of this species are utilized for curing various diseases such as bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness, and pruritis (Anon, 1986; Bhakta, 2021; Bhattacharya, 1982; Sharma *et al.,* 2017). In addition, the plant has been documented in Chinese folk medicine as an anti-inflammatory, wound-healing agent and an

Corresponding author E-mail: *hariomsaxena81@gmail.com*
Doi: https://doi.org/10.36953/ECJ.2021.22348

analgesic for toothache, rhinitis, and breast cancer (Yin *et al.,* 2014). The quality and efficacy of medicinal plants/ raw materials depend on their biologically active ingredients which produce specific physiological action on the human body (Joshi and Uniyal, 2008; Akinmoladun *et al.,* 2007). These active ingredients often vary from region to region and localities to localities according to the environmental, edaphic and seasonal factors. Hence, the correct identification of medicinal plants and quality assurance in terms of active components are critical for ensuring reproducible quality, safety, and efficacy of herbal medications to tackle global public health concerns (Kushwaha *et al.,* 2010; Goldman, 2001; Straus, 2002; Drew and Myers, 1997). WHO and modern herbal pharmacopoeia strongly emphasized on scientific validation of medicinal plants with respect to their active ingredients (Kaushik *et al.,* 2010 and Vasudevan, 2009). Madhya Pradesh state of India is enriched with diverse flora and comprises different agroclimatic regions based on its climatic factors, i.e. temperature, rainfall, soil conditions etc. Various medicinal plants viz., *Terminalia arjuna* (Pandey and Kori, 2009), *Embelia tsjeriam-cottam* (Pandey and Ojha, 2011), *Andrographis paniculata* (Mishra *et al.,* 2010), *Hemidesmus indicus* (Saxena *et al.,* 2017), *Gloriosa superba* (Saxena *et al.,* 2017), *Plumbago zeylanica* (Saxena *et al.,* 2016), *Uraria picta* (Saxena *et al.,* 2016) etc have been studied to find out variations in secondary metabolite concentrations in the state.Phenolic compounds are well known for numerous biological activities such as anti-oxidant, anti-allergic, anti-carcinogenic or anti-mutagenic, anti-inflammatory, anti-bacterial, anti-fungal, anti-viral etc. These are also reported as essential health-promoting agents (Saxena and Pawar, 2019). The present study focuses on assessing the variations in total phenolic (TP) and caffeic acid (CA) contents in fruits, leaves, and stem of *S. indicum* collected from different locations belonging to seven different agro-climatic regions of Madhya Pradesh state. CA and its phenyl esters comprised of a number of biological activities such as anti-oxidant, anti-mitogenic, anti-allergic, immuno-modulatory, anti-inflammatory and anti-carcinogen activities (Ilya *et al.,* 2013; Bhimani *et al.,* 1993; Sudina *et al.,* 1993; Natarajan *et al.,* 1996; Jaiswal *et al.,* 1997; Michaluart *et al.,* 1999; Prasad *et al.,* 2011), hence, CA was estimated in the plant parts as marker compound.

Material and Methods

Chemicals and reagents

Standard CA (98%) arranged from Sigma Aldrich, India. Aluminum packed TLC plates precoated with silica gel 60F254 (20×20 cm, 0.2 mm layer thickness) arranged from E. Merck Ltd (Darmstadt, Germany). All the chemicals and reagents used in the present work were of analytical grade.

Collection and processing of plant material

Solanum indicum is a rare plant species in Madhya Pradesh. During field survey and through information received from the secondary sources, it was found available only at eight locations of seven agroclimatic regions of the state. Therefore, different plant parts (fruits, leaves, and stem) of this species were collected from eight locations i.e. Seoni, Balaghat, Hoshangabad, Indore, Chhindwara & Betul, Amarkantak and Sehore following purposive sampling (Figure 1). These parts were washed thoroughly in running water to remove soil and other foreign particles. Plant parts were dried in shade and powdered using the grinder and stored in airtight polythene bags for further phytochemical analysis. GPS readings of collection sites were recorded. Herbarium of plant specimen was deposited in Tropical Forest Research Institute, Jabalpur (Identification number 1761).

Quantification of TP content

TP content was quantified by the Folin Ciocalteau method (Singleton and Rossi, 1965; Madhavan, 2015). 0.5 g of a powdered sample in 10 times volume of 80% ethanol was crushed using a motor and pestle. Homogenate was then centrifuged at 10,000 rpm for 20 mins. Supernatant was then evaporated to dryness. Residue was dissolved in a known volume of distilled water. 0.2 ml of this sample was then taken in a test tube, and volume made up to 3 ml with distilled water. 0.5 ml of Folin Ciocalteau reagent was then added. After 3 mins, 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. TP content was determined from the linear equation of a standard curve of catechol and expressed as mg of catechol equivalent per g of dry extract weight (mg CE/g).

Estimation of CA content

The CA content was estimated by the method previously reported (Saxena and Pawar, 2019).

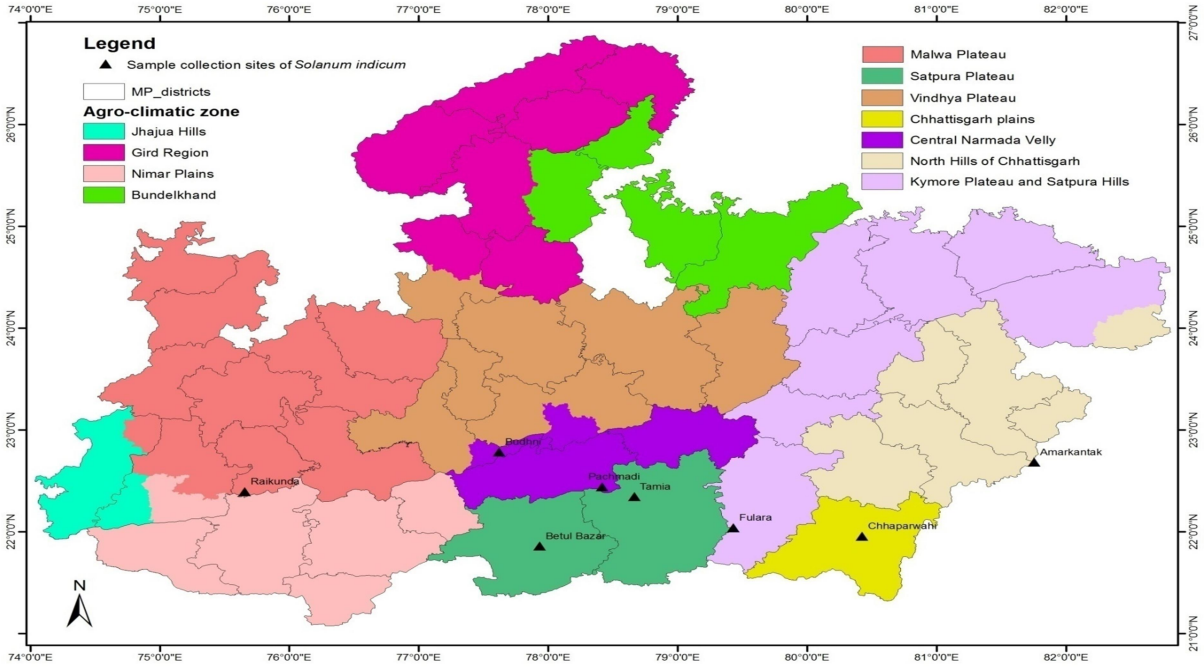


Figure 1: Collection sites of *S. indicum* in Madhya Pradesh state

Preparation of standard solution

A stock solution of 0.1mg/ ml concentration of standard CA was prepared for preparing the calibration curve.

Preparation of plant extracts

2.5 gm dried and finely powdered fruit, leaf and stem samples were taken separately in conical flasks containing 50 ml of 2N HCl and heated for 30 minutes 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 the concentrated extract was dissolved in 2 ml of methanol for analysis.

High Perfornace Thin Layer Chromatography analysis

Each sample (10 µl volume) was applied in triplicate in 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 with the help of LinomatV applicator attached to CAMAG HPTLC system, programmed through winCATS software. 4, 6, 8, 10 and 12 µL (corresponding to 400, 600, 800, 1000 and 1200 ng, respectively) of CA standard solution were applied on TLC plate in five tracks. Mobile phase used for the chromatographic separation was Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1). TLC plate was run in a twin glass chamber (20 cm x 10 cm dimension) saturated with mobile phase. The developed plate was dried by hot air to evaporate solvents and kept in photo – documentation chamber. Images of plates were taken under UV light. Densitometric scanning of the plates was performed with a CAMAG TLC Scanner 4 equipped with winCATS software at λmax = 330 nm using deuterium and tungsten light source. Respective peak areas were recorded and calibration curves were plotted by using peak areas vs. concentrations of CA standard. These calibration curves were used for quantification of CA content in plant extracts.

Statistical analysis

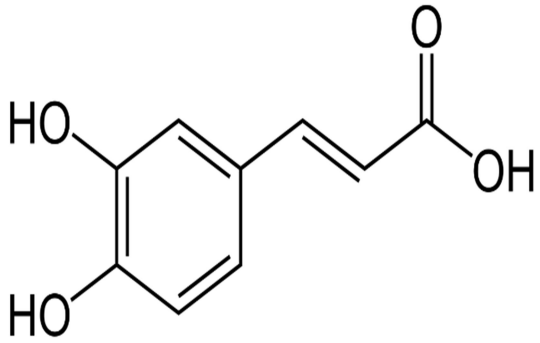
Each experiment was carried out in triplicate, and results are expressed as Mean ± SD (n=3). Data were subjected to descriptive statistics and analysis of variance using Windostat Ver 9.1 Software (Indostat, Hyderabad, India).

Results and Discussion

Results showed significant variations in TP and CA contents within the selected plant's parts of *S. indicum* and between the seven agroclimatic

regions of Madhya Pradesh state. Values of TP and CA content was described in Table 1. TP content in fruits, leaves and stem varied from 6.88±0.01 - 28.52±0.29, 3.44±0.00 - 16.29±0.07 and 3.19±0.01 - 11.79±0.03 mg CE/g, respectively. Besides, CA content varied from 0.0108±0.01 - 0.0192±0.00%, 0.0086±0.00 - 0.0187±0.01% and 0.0061±0.00 - 0.0154±0.01% in fruits, leaves and stem respectively. TP and CA in different plant parts showed the following trend - fruits> leaves> stem. Moreover, fruits from Satpura Plateau, leaves and stem of Northern Hill's Zone of Chhattisgarh agroclimatic regions contained a maximum concentration of TP as 28.52±0.29, 16.29±0.07 and 11.79±0.03 mg CE/g, respectively. Kymore Plateau & Satpura Hill agroclimatic region was observed to comprise maximum content of CA for all three plant parts, i.e. fruits (0.0192±0.00%), leaves (0.0187±0.01%) and stem (0.0154±0.01%). Among all plant parts, fruits from the Satpura plateau held the highest value of TP (28.52±0.29 mg CE/g) and fruits from the Kymore Plateau & Satpura Hills agroclimatic region was detected to contain the maximum value of CA (0.0192±0.00%).

Figure 2: Chemical structure of caffeic acid



From the results, it is evident that TP and CA contents in fruits, leaves and stem of *S. indicum* varied significantly. Variation in secondary metabolite concentrations from climatical zone to zone is largely due to various environmental factors such as temperature, altitude, soil, rainfall, humidity, drought, light intensity, high salinity, supply of water, minerals, freezing temperatures and CO_2 which trigger the accumulation of secondary metabolites for adaptation and overcoming stresses as reported by various workers (Garg *et al.,* 1999; Morison and Lawlor, 1999; Pothitirat and Gritsanapan, 2006; Payyavula *et al.,* 2012; Anandaraj *et al.,* 2014; Sandeep *et al.,* 2015; Akula and Ravishankar, 2011). In the same line, Szakiel *et al.,* (2011) described the influence of environmental biotic factors on the content of saponins in medicinal plants. Since TP represents all types of phenolics found in plant including CA, the estimates of TP and CA did not show a positive co-relation. In our earlier study, we investigated variations of TP and CA contents in roots of *S. indicum* (Saxena and Pawar, 2019) and the present work is the extended part of the earlier study. The results showed that the fruits of *S. indicum* harvested from the Satpura Plateau region contain the highest amount of TP. Leaves and stems collected from Northern Hill's Zone of Chhattisgarh region have greater TP content than leaves and stem of other regions. This observation shows that fruits should be harvested from Satpura plateau region for preparation of medicines. In contrast, if the medicine prepared from leaves and stem, these should be collected from the Northern Hill's Zone of Chhattisgarh region. Furthermore, extraction of CA from fruit, leaves and stem of *S. indicum* should be done from Kymore Plateau & Satpura Hills region only because it has the highest caffeic acid content in comparision to other regions.

Conclusion

S. indicum is a commercially important medicinal plant species. The study revealed that TP and CA contents among *S. indicum* populations varied significantly due to altered environmental factors. Since, CA is one of the important phenolic acids comprised of a number of pharmacological activities, it can be concluded that the germplasm of Kymore Plateau & Satpura Hills agroclimatic region contains the highest CA content and thus, of best quality and superior chemotypes too. These superior chemotypes may be *in-situ* and *ex-situ* conserved and exploited further for their utilization in various ayurvedic formulations. Moreover, the results of the present investigation will help in proper management, conservation and improvement programmes of *S. indicum.*

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Table 1: TPC and CAC in fruits, leaves and stem of *S. indicum* L. (n = 3).

S. No.	Agroclimatic regions of Madhya Pradesh state of India	Collection sites	Fruits		Leaves		Stem	
			TPC (mg CE/g dry extract wt)	CAC (%)	TPC (mg CE/g dry extract wt)	CAC (%)	TPC (mg CE/ g dry extract wt)	CAC (%)
1	Kymore Plateau &Satpura Hills	Seoni	8.13±0.03	0.0192±0.00	13.41±0.05	0.0187±0.01	5.07±0.00	0.0154±0.01
2	Chhattisgarh plains	Balaghat	25.25±0.01	0.0152±0.00	4.46±0.01	0.0124±0.00	3.19±0.01	0.0061±0.00
3	Central Narmada Valley	Hoshangabad	16.41±0.02	0.0146±0.00	5.73±0.00	0.0086±0.00	3.61±0.00	0.0068±0.00
4	Malwa Plateau	Indore	17.04±0.00	0.0126±0.00	5.38±0.00	0.0141±0.00	3.63±0.02	0.0151±0.01
5	Satpura Plateau	Chhindwara & Betul	28.52±0.29	0.0189±0.00	13.30±0.00	0.0144±0.01	6.98±.01	0.0100±0.00
6	Northern Hill's Zone of Chhattisgarh	Amarkantak	6.88±0.01	0.0108±0.01	16.29±0.07	0.0119±0.01	11.79±0.03	0.0090±0.00
7	Vindhyan Plateau	Sehore	18.64±0.00	0.0183±0.00	3.44±0.00	0.0135±0.00	4.66±0.00	0.0067±0.01
C.D. at 5% (within the characters among the agroclimatic regions)			0.036	0.006	0.072	N/A	0.042	0.003

TPC: total phenolic content; CE: catechol equivalent; CAC: caffeic acid content

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