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ORIGINAL CONTRIBUTION

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Variation for caffeic acid and phenolic content in different plant parts of *Solanum xanthocarpum* Schrad. and Wendl. – a commercially important dashmool species

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Abstract

Background: Environmental factors have profound effect on quantity vis-a-vis quality of phytochemicals in medicinal plants. *Solanum xanthocarpum* Schrad. and Wendl. is among the 10 dashmool species which is utilized in more than hundreds of Ayurvedic preparations including 'Dashmoolarishta'. Phenolics are the pharmacologically valuable compounds. Therefore, the present study was undertaken to assess the total phenolic (TP) and Caffeic acid (CA) contents in four different plant parts i.e., leaves, fruits, stem and roots of *S. xanthocarpum* sampled randomly from different locations of Madhya Pradesh, a central Indian state.

Methods: Plant samples were collected from 99 places of 29 districts falling in 11 agroclimatic regions of Madhya Pradesh through random sampling. UV-VIS spectrophotometer and HPTLC were used to determine TP and CA contents, respectively. Phytochemical screening was carried out using standard methods.

Results: Preliminary phytochemical screening indicates the presence of alkaloids, cardiac glycosides, flavonoids, phenols, steroids and terpenoids in all plant parts. Quantification of TP and CA contents revealed that both varied significantly between agroclimatic zones as well as within plant parts of *S. xanthocarpum*. Results revealed that among analysed plant parts, roots and stem harbored highest content of CA while fruits and leaves had the highest TP content. Among agroclimatic regions, accessions of Satpura plateau can be considered rich in CA and TP contents for fruits (0.030%; 28.70 mg CE/g), leaves (0.058%; 27.90 mg CE/g) and roots (0.161%; 5.17 mg CE/g). For stem, highest CA (0.100%) and TP (13.23 mg CE/g) contents were observed in samples of Malwa Plateau and Central Narmada Valley, respectively.

Conclusion: We conclude that agroclimatic regions have significant effect on studied phytochemicals and Satpura plateau agroclimatic zone may be targeted for conservation and sustainable utilization of this valuable dashmool species if the target plant parts are fruits, leaves and roots. While, Malwa Plateau and Central Narmada Valley zones may be targeted for stem. Further, fruits and roots may be utilized for extraction of TP compounds and CA respectively.

Keywords: *Solanum xanthocarpum*, Leaves, Fruits, Stem, Roots, Phytochemicals, Agroclimatic regions

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Background

Solanum xanthocarpum Schrad. and Wendl., commonly known as Kantakari or Yellow Berried Night Shade, is a perennial herb of Solanaceae family. It is one of the dashmool species having an important place among medicinal herbs since ancient times. It is distributed to plains and lower hills of India and is abundantly available in Madhya Pradesh, a central Indian state [1].

All plant parts of this species are useful and reported to have medicinal properties [2]. Fruits of this herb are the source of solasodine, a valuable natural precursor of several commercial steroidal drugs such as corticosteroids, antifertility drugs, anabolic steroids and sex hormones [3, 4]. Fruits have also been reported to contain several medicinal properties like anthelmintic, antipyretic, anti-inflammatory, antitumor, cytotoxic, anti-asthmatic, antispasmodic, antidiabetic, hypotensive [5–7]. Flowers, fruits and stem are prescribed for relief during burning sensation in the feet [8]. Paste of leaves are used to relieve body or muscle pain; while its juice mixed with black pepper is advised for rheumatism [9]. Roots of the plant are used in formulation of "Dashmoolarishta", a well-established ayurvedic drug of Indian system of medicine utilized for treating general fatigue, oral sores and various gynecological disorders [10–13]. Due to various medicinal properties, annual demand of this herb is approximately 500–1000 MT per annum [14].

Phytochemical and pharmacological studies proved that *S. xanthocarpum* is rich in steroids, flavanoids, phenolics, coumarins and major one includes CA, lupeol, carpesterol, solanocarpine, solasonine, solamargine, and diosgenin [15]. CA (3, 4-dihydroxycinnamic acid) is one of the most commonly found phenolics in a wide range of medicinal plants and found effective in treatment of a number of chronic diseases [16, 17]. In *S. xanthocarpum*, CA was first identified in the berries [18] and in roots [19]. Other plant parts of this commercially important medicinal herb were not assessed for this valuable compound.

Considering vast medicinal importance of this species, present investigation was undertaken to assess agroclimatic region wise variability exist in CA and TP contents in *S. xanthocarpum*. Additionally, variations in CA and TP were also assessed in different plant parts of this herb. Our hypothesis was that climatic factors have profound effect on the quantity of phytochemicals which also vary in plant parts of the species.

Methodology

Chemicals and solvents

All the chemicals and solvents used in the study were of analytical grade and chromatography grade. Standard Caffeic acid (98%) was purchased from Sigma Aldrich, India. Aluminum packed thin layer chromatography (TLC) plates precoated with silica gel 60 F254 (20 × 20 cm, 0.2 mm layer thickness) were purchased from E. Merck Ltd. (Darmstadt, Germany).

Collection and authentication of plant material

Different plant parts (fruits, leaves, roots and stem) of this species were collected from 99 locations of 29 districts belonging to 11 agroclimatic regions by following random sampling (Fig. 1). From each agroclimatic region 9 samples were collected on random basis. For confirmation of the species, herbarium of collected specimens was prepared and get authenticated from the Biodiversity and Sustainable Division of Tropical Forest Research Institute, Jabalpur (Identification no. 1760).

Processing of plant materials

Plant parts were separated and brought to the laboratory. These were washed thoroughly in running water to remove soil and other foreign particles. Stem and roots were cut into small pieces. All samples were dried in shade and powdered. Equal amount of nine samples of all plant part collected from each agroclimatic region were pooled separately and was made for making extracts and chemical analysis.

Phytochemical screening

One hundred milligram of dried and powdered plant material, each of stem, leaves, fruits and roots of *S. xanthocarpum* was soaked overnight in 25 ml of different solvents namely water, methanol, ethanol, petroleum ether, chloroform, diethyl ether and ethyl acetate. Different extracts were filtered and filtrates were used for qualitative phytochemical screening following standard methods [20, 21].

Determination of Total Phenolic (TP) content

TP content was determined by Folin-Ciocalteau method [22, 23]. A quantity of 0.5 g of powdered sample was taken in a motor and pestle and grinded in 10 times volume of 80% ethanol. The homogenate was then centrifuged at 10,000 rpm of 20 min. The supernatant was then evaporated to dryness. The residue was dissolved in a 20 ml of distilled water. Zero point two millilitre of sample was then taken in test tube and volume made up to 3 ml with distilled water. Zero point five millilitre of Folin-Ciocalteau reagent was then added. After 3 min, 2 ml of 20% sodium carbonate solution was added to each tube, 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.

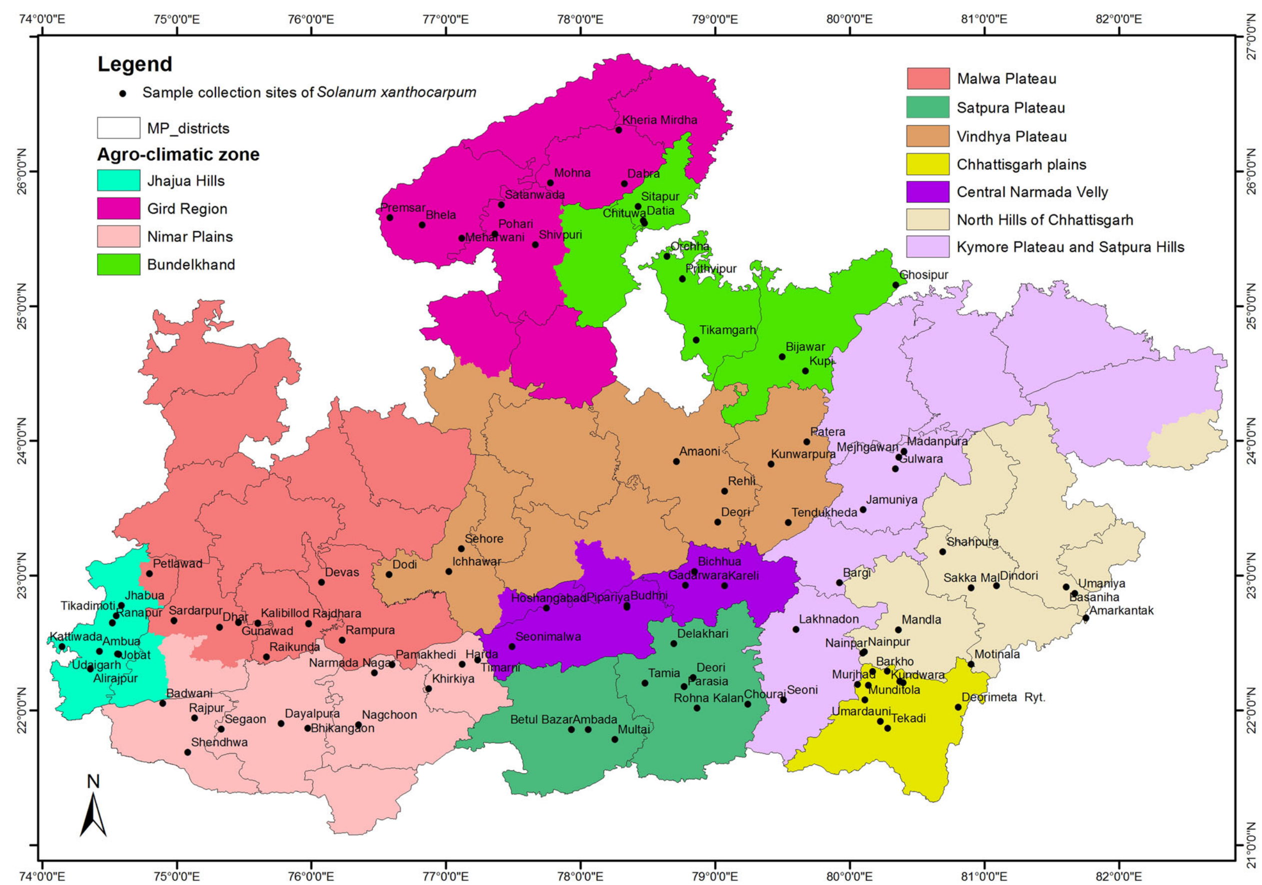

Fig. 1 Mapping for collection sites of *Solanum xanthocarpum*

High Performance Thin Layer Chromatography (HPTLC) densitometric determination of CA content

Ten microliter of each test sample in triplicate and various volumes of standard CA (4, 6, 8, 10 and 12 μl corresponding to 40, 60, 80, 100 and 120 ng respectively of CA per spot) were applied on HPTLC plate. Plates were analyzed and the concentration of CA in each extract was calculated as per given method [24].

Data interpretation and comparative studies

Data was subjected to descriptive statistics and analysis of variance using Windostat Ver 9.1 Software (Indostat, Hyderabad, India).

Results

Critical perusal of the results of phytochemical screening of fruits, leaves, roots and stem of *S. xanthocarpum* (Table 1) revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, steroids and terpenoids in all the plant parts while saponins were present in fruits and leaves only. Tannins were not detected in any plant part.

Analysis revealed significant variations for CA and TP content within and between agroclimatic zones as well as among plant parts of *S. xanthocarpum* collected from 11 different agroclimatic regions of Madhya Pradesh state. Estimates of CA and TP contents in leaves, fruits, roots and stem of *S. xanthocarpum* are summarized in Table 2. TP content in fruits, leaves, roots and stem collected from 11 agroclimatic regions varied from 7.63–28.70, 7.02–27.90, 2.17–5.40 and 3.41–13.23 mg CE/g, respectively. Fruits, leaves, roots of Satpura plateau and stem of Central Narmada Valley were found to contain higher TP content i.e., 28.70, 27.90, 5.17 and 13.23 mg CE/g, respectively. Whereas, fruits and leaves of Nimar valley, roots of Grid zone and stem of Vindhya plateau were found to contain lower TP content i.e., 7.63, 7.02, 2.17 and 3.41 mg CE/g, respectively. On overall comparison, fruits of Satpura plateau region contained highest TP content (28.70 mg CE/g) whereas roots of Grid zone contained the lowest (2.17 mg CE/g).

With regard to CA content, it varied from 0.004–0.031%, 0.002–0.078%, 0.002–0.161% and 0.011–0.100% in fruits, leaves, roots and stem respectively. Fruits of

Table 1 Phytochemical screening of different parts of *Solanum xanthocarpum* Schrad. and Wendl

S. No.	Phytochemical constituents	Plant parts	Aqueous extract	Methanol extract	Ethanol extract	Petroleum Ether extract	Ethyl Acetate extract	Diethyl Ether extract	Chloroform extract
1.	Flavanoids	Stem	–	+	+	–	–	–	–
		Leaves	–	–	–	+	–	–	+
		Fruits	–	+	+	+	–	–	+
		Roots	–	–	–	–	–	–	–
2.	Terpenoids	Stem	–	+	+	–	+	+	+
		Leaves	–	+	+	–	+	+	+
		Fruits	–	+	+	+	+	+	+
		Roots	–	+	+	+	+	+	+
3.	Steroids	Stem	–	–	+	–	+	–	+
		Leaves	+	+	+	+	+	+	+
		Fruits	–	+	+	+	+	+	+
		Roots	–	+	+	+	+	+	+
4.	Saponins	Stem	–	–	–	–	–	–	–
		Leaves	+	+	+	–	–	–	–
		Fruits	+	+	–	–	–	–	–
		Roots	–	–	–	–	–	–	–
5.	Tannins	Stem	–	–	–	–	–	–	–
		Leaves	–	–	–	–	–	–	–
		Fruits	–	–	–	–	–	–	–
		Roots	–	–	–	–	–	–	–
6.	Alkaloids	Stem	+	+	+	–	–	–	–
		Leaves	+	+	+	–	–	–	–
		Fruits	+	+	+	–	–	–	–
		Roots	+	+	+	–	–	–	–
7.	Cardiac glycosides	Stem	–	–	+	+	+	+	–
		Leaves	+	+	+	–	+	+	–
		Fruits	–	+	+	+	–	+	+
		Roots	–	+	+	+	–	+	–
8.	Phenols	Stem	–	+	+	–	+	–	–
		Leaves	–	+	+	–	+	–	–
		Fruits	–	+	+	–	+	–	–
		Roots	–	+	+	–	+	–	–

(+) = detected and (–) = not detected

Chhattisgarh plains, leaves of Bundelkhand zone, roots of Satpura Plateau and stem of Malwa Plateau contained higher content as 0.031%, 0.078%, 0.161% and 0.100%, respectively while fruits of Vindhya Plateau, leaves of Central Narmada Valley, roots of Grid zone, stem of Vindhya plateau and Central Narmada Valley had lower CA content as 0.006%, 0.002%, 0.002% and 0.011% respectively. Among all plant parts, roots samples of Satpura Plateau recorded highest CA content (0.161%). Representative HPTLC profiles, HPTLC densitometric 3D image and spectral pattern of tracks of 03 samples applied in triplicate and five tracks of different concentrations of CA standard are given as Figs. 2, 3 and 4 respectively.

Discussion

Phytochemical screening helps in isolating and characterizing the chemical constituents present in the plant extracts and the knowledge of the chemical constituents of plants is desirable to understand herbal drugs, their preparations and finally in discovering the actual value of folkloric remedies. These phytochemicals (alkaloids, cardiac glycosides, flavonoids, phenols, steroids, terpenoids and

Table 2 TPC and CAC in fruits, leaves, roots and stem of *S. xanthocarpum*. Schrad. and Wendl

S. No.	Agroclimatic regions of Madhya Pradesh state of India	Fruits		Leaves		Roots		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 (%)	TPC (mg CE/ g dry extract wt)	CAC (%)
1	Kymore Plateau & Satpura Hills (KPSH)	25.37 ± 0.13	0.012 ± 0.01	22.73 ± 0.01	0.049 ± 0.00	2.30 ± 0.01	0.043 ± 0.01	4.40 ± 0.01	0.037 ± 0.00
2	Chhattisgarh plains (CP)	23.83 ± 0.01	0.031 ± 0.02	21.80 ± 0.01	0.011 ± 0.00	5.40 ± 0.01	0.040 ± 0.00	5.03 ± 0.49	0.022 ± 0.00
3	Central Narmada Valley (CNP)	24.20 ± 0.01	0.004 ± 0.00	23.63 ± 0.01	0.002 ± 0.00	2.93 ± 0.01	0.005 ± 0.00	13.23 ± 0.01	0.011 ± 0.01
4	Malwa Plateau (MP)	26.80 ± 0.02	0.008 ± 0.00	23.07 ± 0.02	0.003 ± 0.00	2.87 ± 0.01	0.050 ± 0.00	8.93 ± 0.01	0.100 ± 0.01
5	Satpura Plateau (SP)	28.70 ± 0.02	0.030 ± 0.01	27.90 ± 0.05	0.058 ± 0.02	5.17 ± 0.03	0.161 ± 0.00	10.83 ± 0.07	0.077 ± 0.01
6	Grid Zone (GZ)	25.20 ± 1.50	0.015 ± 0.00	24.93 ± 0.01	0.009 ± 0.00	2.17 ± 0.01	0.002 ± 0.00	5.37 ± 0.11	0.032 ± 0.00
7	Northern Hill's Zone of Chhattisgarh (NHZC)	26.53 ± 0.01	0.015 ± 0.00	23.67 ± 0.03	0.008 ± 0.00	2.63 ± 0.04	0.003 ± 0.00	5.20 ± 0.11	0.050 ± 0.02
8	Vindhya Plateau (VP)	8.61 ± 0.07	0.006 ± 0.00	14.29 ± 0.23	0.012 ± 0.00	2.96 ± 0.03	0.038 ± 0.00	3.41 ± 0.02	0.011 ± 0.01
9	Bundelkhand Zone (BZ)	9.44 ± 0.06	0.011 ± 0.01	18.29 ± 0.11	0.078 ± 0.01	3.50 ± 0.03	0.052 ± 0.01	3.50 ± 0.03	0.051 ± 0.03
10	Nimar Valley (NV)	7.63 ± 0.18	0.015 ± 0.00	7.02 ± 0.19	0.009 ± 0.00	2.74 ± 0.02	0.044 ± 0.06	9.12 ± 0.08	0.044 ± 0.00
11	Jhabua Hills (JH)	9.39 ± 0.05	0.028 ± 0.00	20.45 ± 0.03	0.007 ± 0.00	4.15 ± 0.02	0.032 ± 0.01	3.50 ± 0.07	0.072 ± 0.01
	C.D. at 5% (within the characters among the agroclimatic regions)	0.120	0.013	0.180	0.010	0.038	0.036	0.110	0.019

TPC total phenolic content, *CE* catechol equivalent, *CAC* caffeic acid content

saponins) were reported to have a number of biological activities and protect humans from most of the chronic diseases [25]. Our results of phytochemical screening are in agreement with the earlier findings [26–28]. Similar variations in TP content in different plant parts of *S. xanthocarpum* were also noticed in previous studies [28, 29].

Estimates of CA and TP did not exhibit positive relationship because TP represents all types of phenolic compounds found in plants along with CA. Besides, environmental factors such as temperature, altitude, soil, rainfall, humidity, drought, light intensity, high salinity, supply of water, minerals, freezing temperatures and CO_2 also affects concentrations of secondary metabolites [30–35]. Stressed conditions are well known to trigger the accumulation of secondary metabolites which help the plants to adapt and overcome stresses [36]. Similar variations in phytochemicals in *Solaum indiucm* [37], *Uraria picta* [38], *Gloriosa superba* [39] and *Hemidesmus indicus* [40] sampled

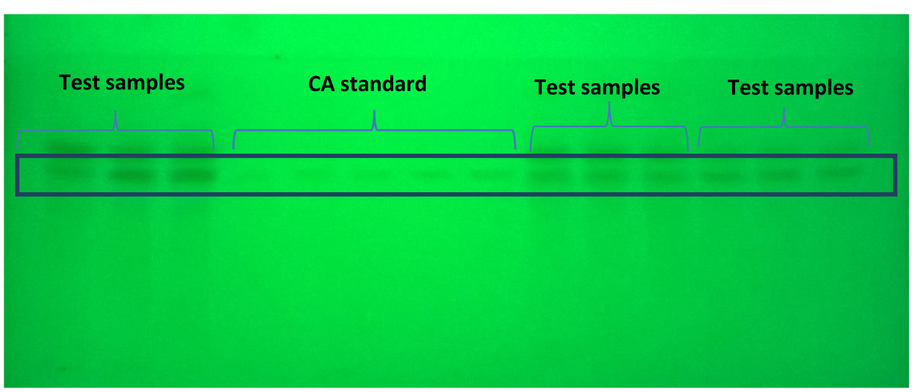

Fig. 2 HPTLC profiles of test samples and caffeic acid standard

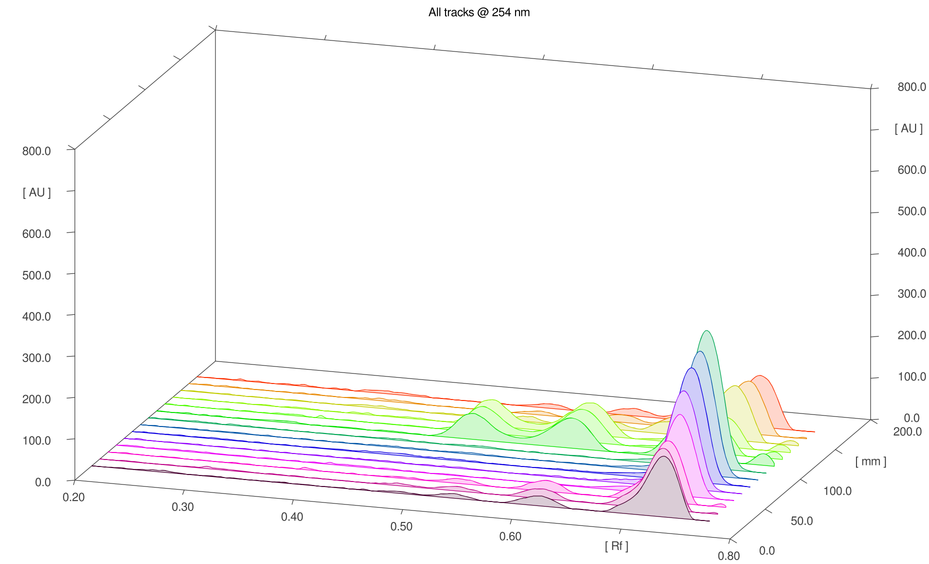

Fig. 3 HPTLC densitometric 3D image of tracks on HPTLC plate

from different agro-climatic regions of central India were also observed.

Analysis of variance revealed that estimates of CA and TP varied significantly within the plant parts i.e., fruits, leaves, stem, roots samples and also among the agroclimatic regions. This indicates the importance of selection of plant parts used for commercial exploitation as well as revealed the environmental effect on yield of chemical constituents. However, trend is not consistent for TP and CA contents in different plant parts across the agroclimatic regions. CA and its phenyl esters were reported to have strong biological activities such as antitumor activity in-vivo and in-vitro both, anti-platelet activity, acute pneumonitis, neuro protective, antioxidant, anti-microbial, antidepressant, anxiolytics, anti-inflammatory, analgesics, anti-cancer, potent collagen antagonist, anti-hypertensive, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, anti-hyperglycemic etc [41–47].

Hence, quantification of CA in all plant parts of *S. xanthocarpum* added value to its pharmacological

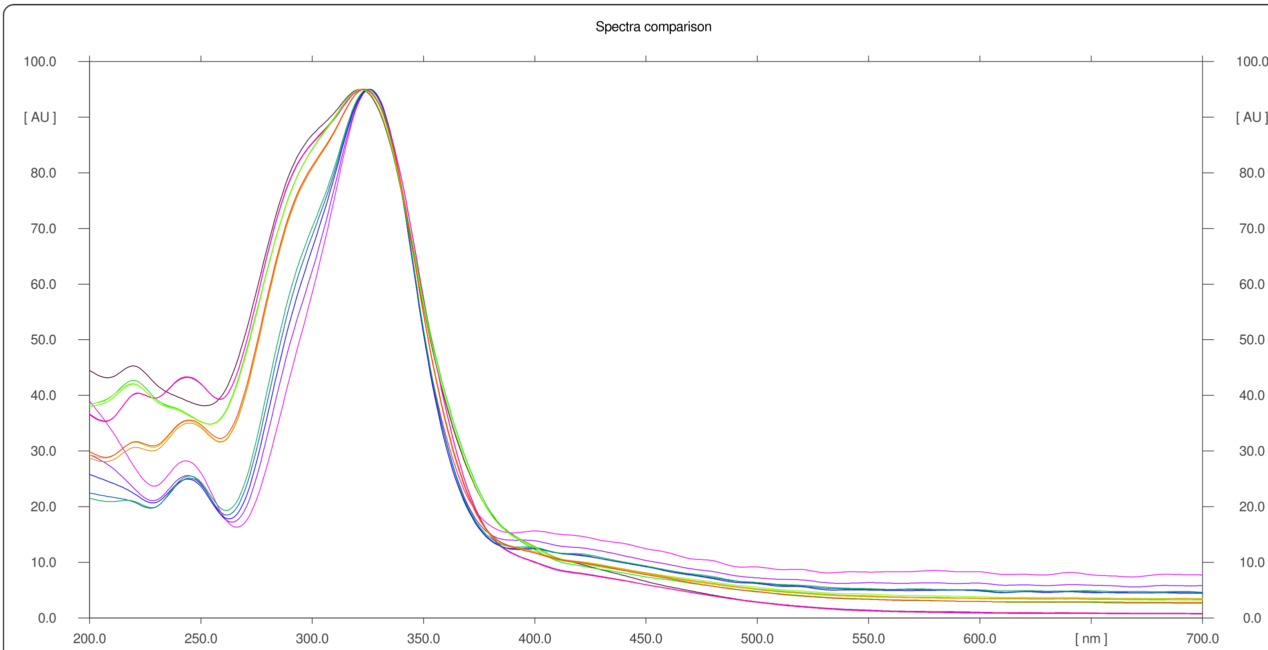

Fig. 4 Overlain spectrum of test samples with CA standard, scanned at 330 nm

potential to utilize it in Ayurvedic formulations. Present investigation on *S. xanthocarpum* will help in its collection from the appropriate locations for sustainable utilization and undertaking conservation programmes.

Conclusion

Present work is the first comprehensive investigation presenting variations in TP and CA contents in plant parts of *S. xanthocarpum* belonging to different agroclimatic regions of Madhya Pradesh state of India. We conclude that samples from Satpura plateau region of state contains highest TP and CA content. Findings of the present study will help in efficient utilization as well as conservation cum improvement programmes of *S. xanthocarpum.*

Abbreviations

TP: Total phenolic; CA: Caffeic acid; AR: Analytical reagent; min: minutes

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Authors' contributions

HOS planned and executed the work and prepared the manuscript. SP and NM edited the manuscript. GP performed experiments in the laboratory. All the authors have read and approved the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors declare that they have no competing interests.

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