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Geographical differentiation of phytochemical content among different accessions of Tinospora cordifolia grown in different regions of India

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ABSTRACT

The recent changes in global climate have resulted notable effects on plant phenology. The present study has considered the impact of climate change on phenology and phytoconstituents of *Tinospora cordifolia* plant accessions found in three different northern-western regions of India. The changes were analysed at regular intervals of climatic conditions such as; soil condition, precipitation and humidity. Phenological monitoring was done in terms of plant's rooting percentage. Phytochemical studies were carried out through estimation of total alkaloidal content followed by high performance thin layer chromatography (HPTLC) analysis of major alkaloidal marker compound, berberine estimation. The results of the study revealed variations in phenological as well as phytochemical parameters. The GH-5 accession of the plant of Bhopal region was found to have higher rooting percentage of 98.51. The total alkaloidal content of the plant was found to be 12.4 % and maximum amount of berberine was found to be 254.60 ng/gm of the extract in different accessions of *Tinospora cordifolia* grown at higher altitudes of Bhopal region. The assessment of the phytochemical content carried out at varying altitudes can help in selecting the elite genotype for the commercial cultivation of the species and to determine the possible therapeutic value of the plant.

INTRODUCTION

The biogenesis of the secondary metabolites in flora is controlled genetically, as well as by different biotic and abiotic stresses [1]. In different ecological niches, plants behave differently in terms of biochemical aspects so as to adapt to their environment. This broad range of environmental factors across altitudes affects the chemical composition and ultimately, the survival of medicinal plants in such regions. Hence the stress conditions affect the secondary metabolites or the so called active ingredients produced by the plants that are considered as the basis for their medicinal activity [2]. Plants at various altitudes can adapt by avoiding and overcoming the stress conditions by means of various physiological and biochemical mechanisms, including the evolution of a resistance-conferring genotype, or by improvement of genes which can produce ecologically adapted phenotypes or can have a different response related to their resistance to these stress conditions which depends mainly on the morphology, anatomy and life cycle [3].

Tinospora (Manispermaceae) is a climber distributed throughout tropical and subtropical India [4] and only three species of the plant is found in the Indian subcontinent. The three species include: *Tinospora cordifolia, Tinospora malabarica* and *Tinospora crispa*. *Tinospora cordifolia* is widely distributed throughout all the tropical regions of the country. However, in the northwestern regions, especially Jammu and Himachal Pradesh, it is in abundance. The plant is well known for its adaptogenic, immunomodulatory activity [5-7].

The stem of the plant is one of the main constituent of several Ayurvedic/herbal preparations that are used in general debility, dyspepsia, fever, and urinary diseases. It is bitter, stomachic, diuretic, stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood, and cures jaundice [8]. The extract of the plant stem is useful in the management of skin disorders [9-10]. Dry bark of *Tinospora cordifolia* has anti-spasmodic, antipyretic, anti-allergic, anti-inflammatory and antileprotic properties. The roots of the plant are a powerful emetic and are used for visceral obstructions [11-12]. Several organic molecules with diverse structures, such as alkaloids, terpenoids, glycosides, sterols, lactones, and fatty acids, have been reported from this herb that include,

tinosporaside, berberine, cordifol, cordioside and magnoflorine. Prompted by the facts above and in continuation of our search, berberine is found to be a most important alkaloid fraction of *Tinospora cordifolia* responsible for many of the above mentioned therapeutic actions [13-15].With the importance of the plant in the traditional and folklore systems of medicine in mind and in continuation of our research on herbal drugs, it was decided to carry out studies to explore those accessions of *Tinospora cordifolia* grown in different eco-geographical regions of northern-western India (Rohtak, Jaisailmer and Bhopal), which shows variation in the timing of biological events (phenology) and content of phytoconstituents being affected by changes in climatic conditions.

To support this type of study, a sensitive and specific assay technique was needed for the quantitation of berberine in different accessions of the plant collected from different locations. These studies were carried out with the high-performance thin layer chromatography (HPTLC) method. The qualitative as well as quantitative HPTLC analysis under standard conditions provides chromatograms, which are very useful for controlling the quality of phyto-pharmaceuticals [16]. HPTLC was thus chosen as well-suited analytical tool for this study to fulfil the objective of conceiving project.

MATERIALS & METHODS

Plant material

Tinospora cordifolia plant accessions were cultivated at three different locations having different geographical co-ordinates, rainfall, altitude and soil conditions (Table 1). Herbal Garden, Maharshi Dayanand University (Rohtak District, Haryana), Professor T.S. Moorti Research Farm (Ubaidullaganj, Madhya Pradesh) and Central Arid Zone Research Institute, CAZRI (Jaisailmer, Rajasthan) were selected as different farming sites. For broad-based study, all of the cultivars for cultivation in three locations were procured from Chaudhary Charan Singh Hisar Agriculture University (CCSHAU), Hisar.

Two to three ploughing and harrowing was done before the rains for bringing the soil to a fine tilth. Farm yard manure was applied, mixed and then the field was levelled. The planting was done at the same time in all three regions. Fresh stem cuttings of the plant were sown in well prepared nursery beds using poly bags (Fig. 1) Transplanting method was preferred for better quality and export purpose. The nursery bed was prepared by thorough mixing with compost and sand. The nursery was raised in the first week of July 2014. Seeds were sown just before the onset of monsoon and covered thinly using sand. After the manure was incorporated in the soil, ridges were prepared as per layout (Fig. 2). Transplantlig was done in the month of July, 2014. Two weedings were done every month to keep the field free from weeds. The crop was irrigated lightly twice a week. Light shower after transplantation ensured better establishment.

Phenological observations

Different phenophases such as appearance of Ist planlet, appearance of full plantlet and percentage germination were recorded from the tagged individuals in each population throughout the growing season. The data were recorded over a whole growing period. Healthy and disease free plant of *Tinospora cordifolia* was collected from different study sites.

Phytochemical analysis

The experiment for determining percentage total alkaloidal content in *Tinospora cordifolia* was performed as per standard method described by Dey *et al.,* 2012 [17].

Quantitative analysis of berberine

A validated HPTLC method (details not discussed here) was used for estimation of berberine content in different accessions of *Tinospora cordifolia.* Around 20 g of air dried sample (stem cuttings) was grounded to pass through 20 mesh SS sieve and 5 g from it was accurately weighed and refluxed with 50 ml of methanol for about 2 h. The resulting solutions were filtered, redissolved in methanol (25 ml) and the final volumes were made up to 50 ml. A 0.1 mg/ml solution of the berberine reference standard was prepared in methanol as a stock solution. These solutions were used for further HPTLC analysis as per the procedure.

A Camag HPTLC system equipped with a sample applicator Linomat V, twin rough plate development chamber, TLC scanner and integration software WINCATS 4.0 was used. An aluminum plate (10 x 10 cm) precoated with silica gel 60F 254 (E. Merck) was used as an adsorbent. Methanol, acetic acid and water in the ratio of 8:1:1 were used as a mobile phase. The solvent was

Table 1. : Geographical coordinates of the selected sites

S. No.	Study site	Altitude m Asl (meters above sea level)	Geographical co-ordinates	Average rainfall (mm) SW (South west monsoon June-September)	Soil condition
1.	Rohtak	245	28.8909°N, 76.5796° E	478.3	Sandy loam soil
2.	Bhopal	427	23.2500°N, 77.4167° E	1154.2	Deep soil
3.	Jaisailmer	264	26.9200°N, 70.9000° E	146.9	Deep Yellowish brown sandy soil

Source: Department of Agriculture & Co-operation, Goverment of India

Table 2. : Phenological observation of *Tinospora cordifolia* at different locations

Plant	Plant Species	Days to appearance of Ist plantlet*	Days to full growth of plantlets*	Rooting percentage*
Tinospora cordifolia Rohtak Region	NBPGR-1	15	33	95.60
	GH-2	14	31	94.69
	GH-3	14	32	89.20
	GH-4	14	31	83.21
	GH-5	15	31	91.23
Tinospora cordifolia Bhopal Region	NBPGR-1	15	32	97.29
	GH-2	16	32	92.86
	GH-3	16	32	97.83
	GH-4	16	31	94.33
	GH-5	17	34	98.51
Tinospora cordifolia Jaisailmer Region	NBPGR-1	17	34	70.37
	GH-2	17	37	65.44
	GH-3	16	36	65.76
	GH-4	17	34	62.89
	GH-5	17	34	72.30

*Values are expressed as mean (n=5)

allowed to run up to 80 mm and the chromatograms were scanned at 366 nm. The test sample (8 µl) was shaken well and applied to an HPTLC plate along with 2, 3, 4, 5 and 6 µl of standard berberine. The plate was developed up to 80 mm under chamber saturation condition. After air-drying the solvent, the plates were scanned at 366 nm in flourosecence reflectance mode.

RESULTS

Phenology

It was observed that the growth pattern of the species goes on increasing as altitude increases with moderate temperature conditions (Table 2).

Phytochemical analysis & comparative estimation of berberine

The total alkaloidal content was found to be 12.4%. Based on presence of high content of total alkaloids, a novel validated HPTLC (details not discussed in this paper) method was developed in order to qualitatively and quantitatively determine the major alkaloid moiety; berberine content of different accessions of plant *Tinospora cordifolia* collected from three regions (five cultivars from each region) of north-western India. A typical HPTLC chromatogram (Fig. 3) was obtained which exhibits an R_f value (0.63) for berberine. The amount of berberine present was determined using the calibration curve plotted between concentration and area of standard (Table 3). The regression equation was found to be, Y= 2857.423 + 215.790X.

Phytoconstituent berberine was present in all the sampled populations, but their quantity varied significantly across these populations. Quantitative estimation of phytochemical was performed by the validated HPTLC method. It was observed during the present study that the quantity of berberine increased with increase in altitude of the studied populations. Low quantity of berbeine was observed at Jaisailmer (264 mAsl) and higher at Bhopal (427 mAsl. Bhopal being at higher altitudes (427 mAsl) revealed higher amount of phytochemical content due to moderate temperature & rainfall conditions.

DISCUSSION

The changed phenology may have consequential effects on production of chemicals in plants. Regional and seasonal or climatic variations are reported in a number of medicinal plants [18]. The season also had its impact on the quantity and/or quality of active principles and secondary metabolites in medicinal plants [19].

Recent studies revealed that climate change affects the chemical composition of plants also survival of some medicinal plants in higher altitude region. Particularly, temperature can affect secondary metabolite and other compound that plant produces [20-21]. Several studies examined the effects of

Table 3. : Total alkaloidal and berberine found in each sample (per gram of extract)

Plant- *Tinospora cordifolia* Rohtak Region	Sample ID	Total alkaloidal content (%)	Berberine content (ng/gm of extract)
Sample 1	NBPGR 1	10.33	145.04
Sample 2	GH 2	10.17	146.9
Sample 3	GH 3	9.45	152.12
Sample 4	GH 4	12.02	191.19
Sample 5	GH 5	11.12	151.34
Plant- *Tinospora cordifolia* Jaisailmer Region	Sample ID		Berberine content (ng/gm of extract)
Sample 6	NBPGR 1	6.40	145.44
Sample 7	GH 2	8.76	146.67
Sample 8	GH 3	6.92	141.73
Sample 9	GH 4	8.10	136.80
Sample 10	GH 5	8.43	152.92
Plant- *Tinospora cordifolia* Bhopal Region	Sample ID		Berberine content (ng/gm of extract)
Sample 11	NBPGR 1	10.12	176.00
Sample 12	GH 2	10.12	172.29
Sample 13	GH 3	11.79	155.04
Sample 14	GH 4	10.92	147.33
Sample 15	GH 5	12.40	254.60

Figure 1. : Nursery raising of different accessions of *Tinospora cordifolia*

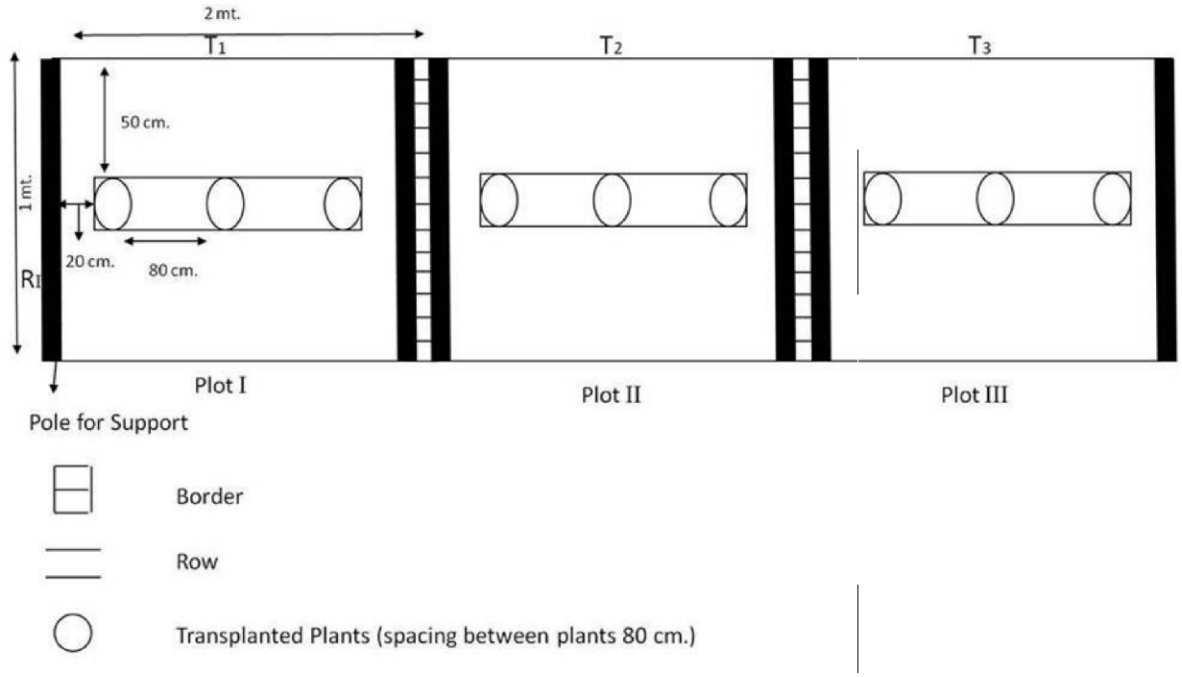

Figure 4. : Zoomed planting plan of *Tinospora cordifolia* for each plot with row to row and plant to plant spacing

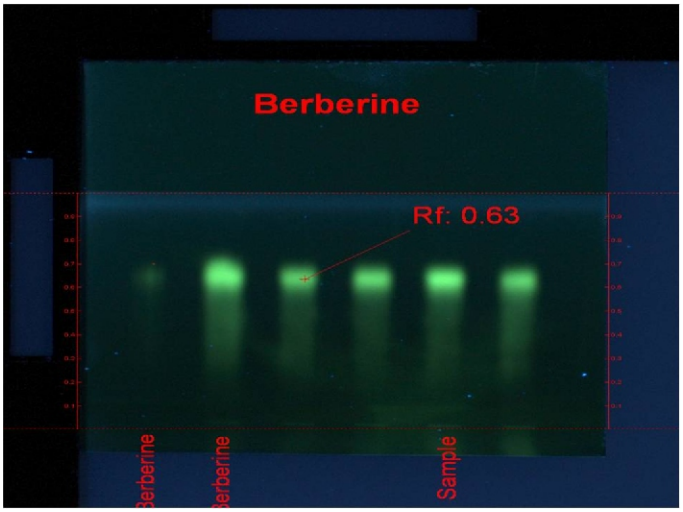

Figure 3. : HPTLC chromatogram plate under fluorescence reflectance (Std. plate)

increased temperature on production of secondary metabolites, but most of these studies showed contradictory results [22]. Some studies reported that secondary metabolites increases in response to elevated temperatures [23] while others report that they decreases [24-25]. As such the responses of secondary chemicals to increased temperature are less understood. The authors highlighted that the effect of climate change factors on plant secondary metabolites appears to be plant species specific as well as dependent upon the type of constituent. Therefore, studies on climate responses in medicinally important plants grown in different altitude regions needs greater attention to understand the underlying phenomenon.

CONCLUSION

Tinospora cordifolia grown at varying altitudes results in intraspecific variations among phonological and phytochemical behaviour. The scientific information generated through this study can be used further to access the best suited climatic conditions for further industrial use.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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