RESEARCH PAPER

Altitudinal Variation in the Antioxidant Potential of *Tinospora cordifolia* (Willd.) Hook. F. & Thomson Stem Extracts from Himachal Pradesh, Western Himalaya: A DPPH– and ABTS–Based Comparative Study

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ABSTRACT
Tinospora cordifolia (Menispermaceae), the Ayurvedic Rasayana herb Guduchi, is widely used across the Himalayan foothills, yet how growing altitude shapes its free-radical scavenging capacity is not well resolved. Stem material was collected from four populations along a 328–1,453 m altitudinal transect in Una, Hamirpur, and Mandi districts of Himachal Pradesh (coded CP01–CP04 corresponding to Amb, Bhota, Sundernagar, and Karsog respectively), and methanolic Soxhlet extracts were evaluated for antioxidant activity using DPPH and ABTS radical scavenging assays, with IC_{50} values (mean ± SEM) determined by an independent testing laboratory. In the DPPH assay, Sundernagar (1,140 m; IC_{50} = 5.694 ± 0.01 µL mL^{-1}) and Bhota (867 m; IC_{50} = 7.592 ± 0.03 µL mL^{-1}) outperformed the ascorbic acid standard (IC_{50} = 7.844 ± 0.02 µg mL^{-1}), while Karsog (1,453 m; 13.77 ± 0.03 µL mL^{-1}) and Amb (328 m; 15.84 ± 0.02 µL mL^{-1}) did not. In the ABTS assay, all four populations outperformed the ascorbic acid standard (IC_{50} = 20.19 ± 0.02 µg mL^{-1}), with potency ranked Sundernagar (3.161 ± 0.06) > Bhota (3.399 ± 0.01) > Karsog (3.527 ± 0.03) > Amb (5.355 ± 0.02 µL mL^{-1}). The two assays agreed on an identical overall potency ranking : Sundernagar > Bhota > Karsog > Amb. Only the lowest-altitude population (Amb) was consistently the least potent in both assays; the highest-altitude population (Karsog) was not the most potent in either assay, and the relationship between altitude and antioxidant potency was not monotonic. These findings support Sundernagar- and Bhota-sourced *T. cordifolia* stem as comparatively potent raw material for antioxidant-oriented pharmaceutical and nutraceutical formulation, while indicating that altitude alone does not reliably predict antioxidant potency across these four populations, highlighting the need for a larger population sample to clarify the relationship.

Keywords: ABTS assay, Altitudinal gradient, Antioxidant activity, DPPH assay, Guduchi, IC_{50}, *Tinospora cordifolia*, Western Himalaya
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INTRODUCTION

The Himalayan mountain system is one of the most extraordinary repositories of plant diversity on the planet, harbouring an estimated 8,000 species of higher plants, of which roughly 1,748 carry recognised medicinal value in traditional healing systems (Samant et al., 1998; Kala et al., 2006). Himachal Pradesh's elevation gradient, from the sub-tropical Shivalik foothills near 350 m to Trans-Himalayan cold deserts above 6,500 m, compresses an unusual range of climatic zones and vegetation types into a narrow geographic belt, making the state a natural laboratory for chemotaxonomic and pharmacognostic study (Singh & Rawat, 2000; Chandra & Nautiyal, 2016).

Among the medicinal plants populating this gradient, *Tinospora cordifolia* (Menispermaceae), known as Guduchi or Giloy, is of particular scientific and commercial interest. It is a large, glabrous, deciduous climbing shrub occurring from warm moist deciduous forests to cooler mixed-oak belts (Sinha et al., 2004; Dhama et al., 2016), and is one of the three botanical pillars of Ayurveda's Rasayana category, alongside Emblica officinalis and Terminalia chebula (Singh et al., 2003; Patel & Mishra, 2011).

The stem, the primary drug material in both Ayurvedic practice and modern nutraceutical manufacture, contains a chemically diverse set of bioactives: protoberberine and quaternary alkaloids (berberine, palmatine, tembetarine, magnoflorine), diterpenoid furanolactones (columbin, chasmanthin, palmatosides), phenolic glycosides, sesquiterpenoids, and polysaccharides (Grover et al., 2002; Upadhyay et al., 2010; Singh et al., 2026; Balkrishna et al., 2024), and this phytochemical diversity has made metabolomic profiling and standardisation of raw material an active area of quality-control research for the species (Islam et al., 2024) underlying a broad pharmacological profile spanning antioxidant, anti-diabetic, hepatoprotective, cardioprotective, and antimicrobial activity (Gupta et al., 2024). The radical-scavenging capacity of these extracts is

generally attributed to polyphenolic and flavonoid constituents capable of donating hydrogen atoms or electrons to neutralise free radicals (Singh et al., 2003).

Altitude is a proxy for a bundle of correlated stressors, falling temperature, reduced atmospheric pressure, intensified UV-B, and shifting soil chemistry, any of which could in principle modulate secondary metabolite biosynthesis (Korner, 1999; Wink, 2010). UV-B radiation rises by roughly 10–12% per 1,000 m gained in the Himalaya (Blumthaler et al., 1997) and is a known trigger for the phenylpropanoid pathway producing UV-absorbing, radical-scavenging flavonoids (Akula and Ravishankar, 2011), and the Optimal Defence Theory predicts, on general grounds, greater investment in chemical defence at higher, more resource-limited elevations (Rhoades, 1979). Some Himalayan species show potency or metabolite content that broadly tracks elevation in this way (Rana et al., 2020; Khantwal et al., 2025), but the relationship is not universal: even within a single species, altitude-linked compound classes do not always vary monotonically with elevation, and site-specific factors such as microclimate, soil, and host association can outweigh altitude itself (Khantwal et al., 2026). A recent review of climatic effects on Himalayan plant metabolites likewise concludes that the direction and magnitude of altitude effects on phenolic and flavonoid accumulation can be species- and tissue-specific rather than a fixed rule (Jangpangi et al., 2025). Whether *T. cordifolia* stem follows a simple altitude-potency relationship, or instead reflects the more site-dependent pattern seen in some other Himalayan taxa, has not previously been tested directly.

The DPPH and ABTS radical scavenging assays remain the two most widely used in vitro antioxidant methods in plant pharmacognosy (Brand-Williams et al., 1995; Re et al., 1999). The present study characterises DPPH and ABTS IC_{50} values, determined by an independent testing laboratory, for *T. cordifolia* stem extracts collected from four populations across a 328–1,453 m altitudinal transect in Himachal Pradesh, to examine whether antioxidant potency varies consistently with altitude of origin, or instead reflects population-specific differences not fully explained by elevation, across two independent assays.

2. Materials and Methods

2.1 Study sites and sample coding. Stem material was collected from four localities spanning an altitudinal gradient across Una, Hamirpur, and Mandi districts of Himachal Pradesh, in the Western Himalayan foothill-to-mid-hill belt (Table 1). The four sites were deliberately chosen to span the principal vegetation and climatic zones through which *T. cordifolia* naturally occurs in this region: Amb (328 m, Una district) lies within the sub-tropical Shivalik zone, characterised by dry mixed-deciduous scrub forest and warm, semi-arid conditions for much of the year; Bhota (867 m, Hamirpur district) and Sundernagar (1,140 m, Mandi district) occupy the mid-elevation subtropical-to-subtemperate transition zone, with mixed broadleaf forest cover; and Karsog (1,453 m, Mandi district) represents the lower subtemperate zone bordering mixed oak-conifer forest. The four sites are geographically distinct, separated from one another by straight-line distances of roughly 18–90 km, reducing the likelihood that shared local microclimate or a single contiguous population could account for the observed differences. Site selection followed three criteria: (i) confirmed natural, wild occurrence of *T. cordifolia* climbing on a host tree, with no visible evidence of recent cultivation, transplantation, or nursery origin; (ii) minimal visible anthropogenic disturbance (grazing, lopping, or roadside pollution load) at the collection point; and (iii) safe, consistent accessibility to permit sampling within the same narrow collection window across all four sites. Geographic coordinates for each site (Table 1) were recorded in the field using a handheld GPS device at the point of collection. Each collection site was assigned a laboratory sample code for testing (Table 1).

Table 1. Collection sites, altitude, and corresponding laboratory sample codes.

Sample code	Location	District	Altitude (m)	Latitude	Longitude
CP01	Amb	Una	328	31.68° N	76.35° E
CP02	Bhota	Hamirpur	867	31.78° N	76.35° E
CP03	Sundernagar	Mandi	1,140	31.53° N	76.88° E
CP04	Karsog/Balichowki	Mandi	1,453	31.68° N	76.95° E

2.2 Plant material and authentication. Healthy, mature, morphologically uniform stem segments (10–15 cm long, 1–2 cm diameter) were harvested from three individual vines at each site during the active growing season (November–December 2024). Material was surface-cleaned in the field, transported in labelled polyethylene bags, and voucher specimens were deposited in the Departmental Herbarium (accession CPU-2024/S4). Taxonomic identity was authenticated by the Department of Botany, Career Point University, Hamirpur.

2.3 Extraction. Air-dried, powdered stem material was extracted with methanol using a Soxhlet apparatus to obtain the working extract for each population, which was submitted to an independent testing laboratory under the codes shown in Table 1.

2.4 Antioxidant assays. DPPH radical scavenging activity was calculated as

$$\% \text{ RSA} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

following the principle established by Brand-Williams et al. (1995). ABTS radical cation scavenging activity was calculated by the analogous formula using the

ABTS control and sample/test absorbance, following Re et al. (1999). Ascorbic acid was used as the reference standard in both assays, tested over 0–50 µL mL⁻¹; sample extracts were tested over a broader concentration range (0–50 µL mL⁻¹) to accommodate their comparatively unbound potency relative to the purified standard. Extract IC_{50} values are reported in µL mL⁻¹ (volume basis), while the standard's are in µg mL⁻¹ (mass basis); the two are not directly interconvertible. IC_{50} values (the concentration required for 50% radical inhibition) were determined by the testing laboratory and are reported as mean ± SEM.

3. Results

Antioxidant activity of *T. cordifolia* stem extracts from the four altitudinal populations was evaluated using two independent radical scavenging assays, DPPH and ABTS, alongside the ascorbic acid reference standard. IC_{50} values, representing the extract concentration required to neutralise 50% of the target radical, were used as the primary measure of potency in both assays, with a lower IC_{50} indicating stronger antioxidant activity. Table 2 summarises the IC_{50} values (mean ± SEM) obtained for all four populations and the standard across both assays, and Figure 1 presents these values graphically for direct visual comparison. Across both assays, extract potency varied considerably with collection site, and the two assays showed general agreement in the relative ordering of populations, an observation examined more closely in Section 3.3. The following subsections report the DPPH (3.1) and ABTS (3.2) results in detail, followed by the relationship between potency and altitude of origin (3.3).

Table 2. DPPH and ABTS IC_{50} values (mean ± SEM) of *T. cordifolia* stem extracts (µL/mL) and the ascorbic acid standard (µg/mL), as reported by the testing laboratory.

Sample code	Site (altitude)	DPPH IC_{50}	ABTS IC_{50}
—	Ascorbic acid (standard)	7.844 ±0.02 (µg/mL)	20.19 ± 0.02 (µg/mL)
CP01	Amb (328 m)	15.84 ± 0.02 (µL/mL)	5.355 ± 0.02 (µL/mL)
CP02	Bhota (867 m)	7.592 ± 0.03 (µL/mL)	3.399 ± 0.01 (µL/mL)
CP03	Sundernagar (1,140 m)	5.694 ± 0.01 (µL/mL)	3.161 ± 0.06 (µL/mL)
CP04	Karsog (1,453 m)	13.77 ± 0.03 (µL/mL)	3.527 ± 0.03 (µL/mL)

3.1 DPPH radical scavenging activity. IC_{50} ranged from 5.694 ± 0.01 µL mL⁻¹ (Sundernagar) to 15.84 ± 0.02 µL mL⁻¹ (Amb). Sundernagar (1,140 m) and Bhota (867 m) returned IC_{50} values lower than the ascorbic acid standard (7.844 ± 0.02 µg mL⁻¹), indicating antioxidant potency exceeding the standard. Karsog (1,453 m) and Amb (328 m) returned higher IC_{50} values than the standard, indicating lower potency. Potency ranking: Sundernagar > Bhota > Ascorbic acid > Karsog > Amb.

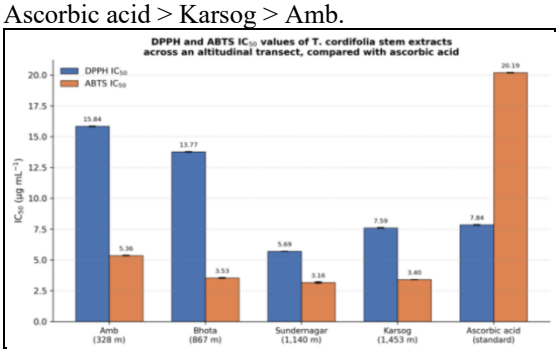
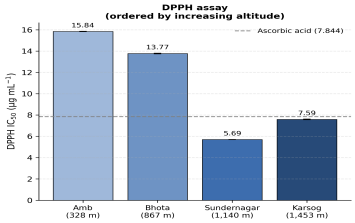


Figure 1. Comparative DPPH and ABTS IC_{50} values (µg mL⁻¹ for ascorbic acid standard; µL mL⁻¹ for extracts, mean ± SEM) of *Tinospora cordifolia* stem extracts from four altitudinal populations (Amb, Bhota, Sundernagar, Karsog)

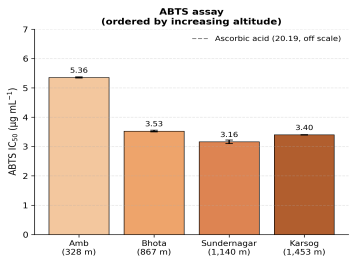
3.2 ABTS radical cation scavenging activity. IC_{50} ranged from 3.161 ± 0.06 µL mL⁻¹ (Sundernagar) to 5.355 ± 0.02 µL mL⁻¹ (Amb). All four populations returned IC_{50} values well below the ascorbic acid standard (20.19 ± 0.02 µg mL⁻¹). Sundernagar showed the strongest ABTS radical-scavenging activity among all populations tested, followed by Bhota, Karsog, and Amb. Potency ranking: Sundernagar > Bhota > Karsog > Amb > Ascorbic acid.

3.3 Relationship with altitude. The two assays agreed on an identical overall potency ranking among the four populations: Sundernagar (1,140 m) > Bhota (867 m) > Karsog (1,453 m) > Amb (328 m), with Amb consistently the least potent extract in both assays. However, this ranking does not track altitude in a simple, consistent way. Amb, the lowest-altitude site, was reliably the least potent population in both assays, consistent with an altitude-related trend. But Karsog, the highest-altitude site (1,453 m), was not the most potent population in either assay;



(a)

instead it ranked third, behind both Sundernagar (1,140 m) and the lower-altitude Bhota (867 m). The most potent population, Sundernagar, was not the highest-altitude site sampled. Taken together, these results do not support straightforward monotonic relationship between altitude and antioxidant potency across the four populations studied; altitude alone does not reliably predict relative potency once population identity is accounted for.



(b)

Figure 2. DPPH (a) and ABTS (b) IC_{50} values (µg mL^{-1} for ascorbic acid standard; µL mL^{-1} for extracts, mean ± SEM) of *T. cordifolia* stem extracts, with populations arranged in order of increasing collection altitude (Amb 328 m → Bhota 867 m → Sundernagar 1,140 m → Karsog 1,453 m).

Unlike a simple altitude-driven pattern, the two mid-to-high-altitude sites (Bhota, Sundernagar) outperformed the highest-altitude site (Karsog) in both assays, while only the lowest-altitude site (Amb) was consistently least potent.

4. Discussion

The agreement between two mechanistically distinct assays on an identical potency ranking, Sundernagar > Bhota > Karsog > Amb, strengthens confidence that this ranking reflects a genuine property of the extract chemistry rather than an assay-specific artefact. However, this ranking complicates a simple altitude-driven explanation: while Amb, the lowest-altitude population, was consistently the least potent, consistent with reduced chemical defence investment at lower elevation (Rhoades, 1979), the highest-altitude population, Karsog (1,453 m), was not the most potent in either assay. It was instead outperformed by both Sundernagar (1,140 m) and Bhota (867 m), the latter more than 500 m lower in elevation. This pattern departs from a straightforward reading of the Optimal Defence Theory prediction that chemical defence investment should increase monotonically with elevation-linked resource limitation (Rhoades, 1979), and from the expectation that UV-B-driven flavonoid biosynthesis (Blumthaler et al., 1997; Akula & Ravishankar, 2011) should scale simply with altitude of origin.

That said, non-monotonic, site-dependent patterns of this kind are not unusual in Himalayan phytochemical surveys: a 2026 altitudinal survey of Aesculus indica bark across the Garhwal Himalaya likewise found that phytochemical composition tracked elevation-linked compound classes (phenolic esters, triterpenoids, phytosterols) without a simple linear trend, and attributed this partly to local edaphic variation (Khantwal et al., 2026). This could reflect site-specific factors beyond altitude alone, such as microclimate, soil composition, host-tree association, or sun exposure, that were not controlled for in this study, and underscores that altitude functions here as a proxy for, rather than a direct cause of, the environmental conditions that drive secondary metabolite accumulation. A recent literature review of climatic effects on Himalayan plant metabolite production similarly concludes that altitude-linked temperature and radiation stress are consistent drivers of phenolic and flavonoid accumulation across multiple genera, though the magnitude and even the direction of the effect can be species- and tissue-specific (Jangpangi et al., 2025). With only four populations, the small sample size limits confidence in any altitude relationship formally; a larger set of populations spanning a denser altitudinal series, together with site-level environmental measurements, would be needed to determine whether a genuine altitude effect exists once confounding site factors are accounted for.

4.1 Pharmaceutical and human health relevance. Excess reactive oxygen species contribute to the pathophysiology of a wide range of chronic human conditions, including diabetes, cardiovascular disease, hepatic injury, and several cancers, and dietary or supplemental antioxidants are widely investigated as adjuncts in managing this oxidative burden. *T. cordifolia* extracts are documented to exert antioxidant effects alongside anti-diabetic, hepatoprotective, cardioprotective, and immunomodulatory activity (Gupta et al., 2024), partly through modulation of pathways such as Nrf2 that govern the body's own antioxidant defences (Ghuge et al., 2025).

The present findings support a practical sourcing recommendation based on population identity rather than altitude alone: raw stem material from Sundernagar and Bhota is both more potent than material from Karsog and Amb, and in the DPPH assay specifically, more potent than the ascorbic acid reference standard itself, making these two populations stronger candidate raw material sources for antioxidant-oriented Guduchi formulations than either Karsog or Amb. Guduchi stem is traditionally consumed and formulated as Swarasa (fresh expressed juice), Kwatha (aqueous decoction), Churna (dried powder), and Satva (a starch-rich sedimented extract), in addition to modern standardised capsules and hydro-alcoholic extracts (Kumar et al., 2023), and Ayurvedic processing steps such as repeated Bhavana (levigation) have been shown to further potentiate the biological activity of Guduchi churna relative to unprocessed powder (Kumar et al., 2023). From a drug-delivery standpoint, the phenolic/flavonoid-rich fraction responsible for the activity documented here is a reasonable candidate for encapsulation or nanoformulation approaches already explored to improve the stability and bioavailability of *T. cordifolia* extract antioxidants (Masilamani et al., 2023).

4.2 Limitations. IC_{50} values were obtained as summary statistics from the testing laboratory; raw per-concentration % inhibition data and raw triplicate absorbance readings were not available for independent verification or dose-response curve fitting, and total phenolic/flavonoid content was not separately quantified. Metabolomic profiling

approaches capable of resolving this compositional variation across populations have been reviewed for *T. cordifolia* specifically and represent a logical next step for confirming which compound classes drive the altitude-linked potency differences observed here (Islam et al., 2024). Site-specific microclimatic and edaphic factors that may confound the altitude relationship were not measured. With only one population per altitude class, the present design cannot separate altitude effects from other site-specific or individual-population factors, which likely explains the non-monotonic pattern observed.

5. Conclusion

Laboratory-verified DPPH and ABTS IC_{50} values for *T. cordifolia* stem extracts from four populations across a 328–1,453 m altitudinal transect in Himachal Pradesh show consistent agreement between the two assays on an overall potency ranking: Sundernagar (1,140 m) > Bhota (867 m) > Karsog (1,453 m) > Amb (328 m). Only the lowest-altitude population (Amb) was consistently the least potent in both assays; the highest-altitude population (Karsog) was not the most potent in either assay, indicating that antioxidant potency does not increase monotonically with altitude across the populations sampled. The two best-performing populations, Sundernagar and Bhota, exceeded the ascorbic acid standard's potency in the DPPH assay; all four populations exceeded it in the ABTS assay. These results support Sundernagar and Bhota as preferred raw-material sources for antioxidant-oriented pharmaceutical and nutraceutical formulation on the basis of population identity, while indicating that altitude of origin alone is not a reliable predictor of antioxidant potency in this species, and that a larger, denser altitudinal sampling series with environmental covariates is needed to resolve the underlying drivers.

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